BIOLOGY OF WASTEWATER TREATMENT

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Second Edition

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ICP

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Preface to the Second Edition

Since writing the first edition of *Biology of Wastewater Treatment* the wastewater industry has changed quite dramatically. While the basic concepts remain the same, the processes and the industry that design, build and operate treatment systems have all radically altered. So why has wastewater technology changed so much since 1990? In Europe the introduction and rapid implementation of the Urban Wastewater Treatment Directive has to be a major factor. Nutrient removal, especially biological phosphorus removal, is now commonplace. This in turn has forced us back to the use of the original batch reactor designs for activated sludge. The large increase in sludge production has required the development of integrated disposal strategies linked with better recovery and reuse technologies. The rapid expansion of wastewater treatment is allowing manufacturers to experiment with new innovative designs and processes, and for the first time in nearly half a century new sewage treatment plants are being built rather than existing plants merely being upgraded or retrofitted. Privatisation in the UK has also been hugely influential bringing into play the often-conflicting factors of cost, especially operational cost, and accountability. Better regulation and control in all countries, coupled with better process management has resulted in better treatment overall. The concept of sustainability has also become an important factor, although it is still to have any real influence on long-term design or planning. Growing urbanization, climate change, and new analytical techniques that are constantly allowing us to identify new pollutants and understand the fate of others during treatment and subsequently in receiving waters, have all significantly influenced the wastewater industry. However, many fear that wastewater treatment will

eventually reach crisis point where existing technologies will prove to be too expensive and energy dependent to be able to satisfy all the needs of a modern society. Also, long-term planning is difficult with legislation and regulation constantly changing. So now is the time to stand back and take a new look at the whole concept of the wastewater cycle from production at the household level through to treatment. Our highly diluted wastewaters, heavily contaminated with metals, pharmaceutical drugs, oestrogen mimicking compounds, more varied and dangerous pathogens, and an alarmingly wide range of trace organic compounds is simply too difficult to treat effectively in a manner that is going to be sustainable. Rather than developing better and more efficient process designs we need to start by looking at the basic concepts of treatment and redesign the system as though starting from scratch. For, example new separation technologies and water reuse at the household level is reducing wastewater loadings. New advances with in-sewer treatment have been very successful in reducing organic loads to treatment plants and at the same time creating a more treatable wastewater entering the wastewater treatment plant. Localised treatment plants rather than centralized systems are now thought to be more efficient. Removing pollutants at source rather than at the treatment plant is making effluents and sludges in particular less hazardous. What is clear is that wastewater treatment will have to become a joint venture between all the stakeholders, with every person having to take some responsibility for their waste.

I have tried to retain as much of the original text as possible, but due to the rapid changes that have occurred over the past decade then considerable revision was necessary. All sections have been updated with many expanded to reflect the new importance or popularity of processes. There is also a new chapter on sustainability.

It is often forgotten by environmentalists, and the public in general, what an important role wastewater treatment plays in protecting both the environment and the health of the public. Without it there would be no development and growth, without it our environment and our very lives would be at risk. It is a huge credit to all those involved in the industry that this vital service is carried out in such a discreet and professional manner. For all those of you who have made it your career, thank you. For those who would like to, then welcome and I hope that you will also find it equally as rewarding and exciting as I have.

Nick Gray
TCD
January 2004

‘To you it’s just crap, to me it’s bread and butter.’
Spike Milligan
Recollections of the latrine orderly

1

How Nature Deals with Waste

1.1. Introduction

1.1.1. *The wastewater problem*

Each day, approximately $1 \times 10^6 m^3$ of domestic and $7 \times 10^6 m^3$ of industrial wastewater is produced in the UK. This, along with surface runoff from paved areas and roads, and infiltration water, produces over $20 \times 10^6 m^3$ of wastewater requiring treatment each day. To cope with this immense volume of wastewater there were, in 1999, some 9260 sewage treatment works serving about 95% of the population (Water UK 2001). The size of these plants varies from those serving small communities of < 100, to plants like the Crossness Sewage Treatment Works operated by Thames Water which treats the wastewater from over 1.7 million people living in a 240 km^2 area of London.

In terms of volume or weight, the quantity of wastewater treated annually in the UK far exceeds any other product (Table 1.1) including milk, steel or even beer (Wheatley 1985), with vast quantities of wastewater generated in the manufacture of most industrial products (Fig. 1.1). The cost of wastewater treatment and pollution control is high, and rising annually, not only due to inflation but to the continuous increase in environmental quality that is expected. During the period 1994–1999, the ten main water companies in England and Wales invested £16.55bn into its services. Over half of this was on wastewater provision. In the year 1998/1999, £1.9bn was spent on new wastewater treatment plants alone as compliance with the European Union Urban Wastewater Treatment Directive continues. The industry is extremely large, with the income for these water companies for

Table 1.1. The quantity of sewage treated in the UK far exceeds the quantity of other industrial products processed. Comparative values are based on 1984 sterling values (Wheatley 1985).

Product	Tonnes/annum ($\times 10^6$)	Price (£/tonne)
Water as sewage	6500	0.10
Milk	16	25
Steel	12	300
Beer	6.6	280
Inorganic fertilizer	3.3	200
Sugar	1.0	350
Cheese	0.2	1300
Baker's yeast	0.1	460
Citric acid	0.015	700
Penicillin	0.003	45000

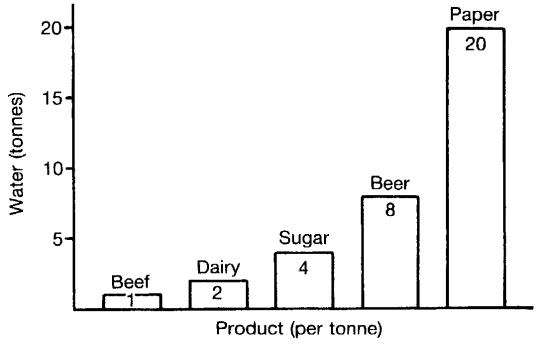


Fig. 1.1. Tonnes of water required in the manufacture of some products that produce organic effluents.

1998/1999 in excess of £6,000m with operating costs approaching £4,000m (Water UK 2001).

There are two fundamental reasons for treating wastewater: to prevent pollution, thereby protecting the environment; and, perhaps more importantly, protecting public health by safeguarding water supplies and preventing the spread of water-borne diseases (Sec. 2.1).

The safe disposal of human excreta is a pre-requisite for the supply of safe drinking water, as water supplies can only become contaminated where disposal is inadequate. There are many infectious diseases transmitted in

excreta, the most important being the diarrhoeal diseases cholera, typhoid, and schistosomiasis. The faeces are the major source of such diseases with few infections, apart from schistsosomiasis, associated with urine. Among the most common infectious water-borne diseases are bacterial infections such as typhoid, cholera, bacillary dysentery, and gastro-enteritis; viral infections such as infectious hepatitis, poliomyelitis, and various diarrhoeal infections; the protozoal infections cryptosporidiosis, giardiasis, and amoebic dysentery, and the various helminth infections such as ascariasis, hookworm, and schistosomiasis (bilharzia). Although the provision of clean water supplies will reduce the levels of infection in the short term, in the long term it is vital that the environment is protected from faecal pollution (Feachem and Cairncross 1993; Mara 1996). Adequate wastewater treatment and the disinfection of water supplies has effectively eliminated these water-borne diseases from developed countries, but they remain endemic in many parts of the world, especially those regions where sanitation is poor or non-existent (Chap. 9). In developed countries where there are high population densities, such as the major European cities, vast quantities of treated water are required for a wide variety of purposes. All the water supplied needs to be of the highest quality possible, although only a small proportion is actually consumed. To meet this demand, it has become necessary to utilise lowland rivers and groundwaters to supplement the more traditional sources of potable water such as upland reservoirs (Gray 1997). Where the water is reused on numerous occasions, as is the case in the River Severn and the River Thames Sec. 10.2.2, adequate wastewater treatment is vital to ensure that the outbreaks of waterborne diseases that were so prevalent in the eighteenth and nineteenth centuries do not reoccur (Chap. 9).

In terms of environmental protection, rivers are receiving large quantities of treated effluent while estuaries and coastal waters have vast quantities of partially or completely untreated effluents discharged into them. Although in Europe, the Urban Wastewater Treatment Directive has caused the discharge of untreated wastewater to estuarine and coastal waters to be largely phased out. Apart from organic enrichment endangering the flora and fauna due to deoxygenation, treated effluents rich in oxidised nitrogen and phosphorus can result in eutrophication problems. Where this is a particular problem, advanced or tertiary wastewater treatment is required to remove these inorganic nutrients to protect rivers and lakes (Sec. 2.4). Environmental protection of surface waters is therefore a major function of wastewater treatment. In 1998, 30% of all rivers surveyed in England and Wales (12,241 km) were classified as having doubtful, or worse, quality (i.e. class D, E and F using the Environment Agency General

Table 1.2. The river quality in England and Wales based on the Environment Agency GQA systems.

	River length (%) in each quality grade						
	A	B	C	D	E	F	Total km
Chemical GQA							
1988–1990	17.7	30.1	22.8	14.4	12.7	2.3	34161
1993–1995	26.8	32.7	21.3	10.2	8.1	0.9	40227
1994–1996	27.1	31.5	21.2	10.4	8.8	1.0	40804
Biological GQA							
1990	24.0	31.6	21.6	9.8	7.3	5.7	30001
1995	34.6	31.6	18.4	8.1	5.4	1.9	37555
Nutrient GQA							
1990	8.0	17.7	10.2	13.1	28.0	22.9	23003
1993–1995	14.7	22.6	11.0	13.1	27.3	11.0	34864

Quality Assessment (GQA) chemical classification system) (Environment Agency 1998; Gray 1999; Water UK 2001) (Table 1.2). As in Ireland, there is an increasing trend in eutrophication of surface waters (EPA 2000). The cost of rehabilitating rivers, as was seen with the River Thames in the period 1960–1980, is immense. The River Mersey for example, now Britain's most polluted river, will cost an estimated £3,700m over the next quarter of a century to raise to a standard suitable for recreation (Department of the Environment 1984).

1.1.2. *Legislation*

Environmental legislation relating to wastewater treatment and receiving water quality is based largely on quality standards that are related to suitability of water for a specific use, the protection of receiving waters, or emission limits on discharges. Standards are usually mandatory with maximum permissible concentrations based on health criteria or environmental quality standards. Table 1.3 lists the key Directives concerning the aquatic environment that govern legislation in countries (Member States) comprising the European Union. The principal Directives are those dealing with Surface Water (75/440/EEC), Bathing Waters (76/160/EEC), Dangerous Substances (76/464/EEC; 86/280/EEC), Freshwater Fish (78/659/EEC), Ground Water (80/68/EEC), Drinking Water

Table 1.3. EU Directives concerning inland waters by year of introduction.

1973
Council Directive on the approximation of the laws of the Member States relating to detergents (73/404/EEC)
Council Directive on the control of biodegradability of anionic surfactants (73/405/EEC)
1975
Council Directive concerning the quality required of surface water intended for the abstraction of drinking water in the Member States (75/440/EEC)
1976
Council Directive concerning the quality of bathing waters (76/160/EEC)
Concil Directive on pollution caused by certain dangerous substances discharged into the aquatic environment (76/464/EEC)
1977
Council decision establishing a common procedure for the exchange of information on the quality of surface in the Community (77/795/EEC)
1978
Council Directive on titanium oxide waste (78/178/EEC)
Council Directive on quality of fresh waters needing protecting or improvement in order to support fish life (78/659/EEC)
1979
Council Directive concerning the methods of measurement and frequencies of sampling and analysis of surface water intended for the abstraction of drinking water in the Member States (79/869/EEC)
Council Directive in the quality required for shellfish wates (79/923/EEC)
1980
Council Directive on the protection of ground water against pollution caused by certain dangerous substances (80/68/EEC)
Council Directive on the approximation of the laws of the Member States relating to the exploitation and marketing of natural mineral waters (80/777/EEC)
Council Directive relating to the quality of water intended for human consumption (80/778/EEC)
1982
Council Directive on limit values and quality objectives for mercury discharges by the chlor-alkali electrolysis industry (82/176/EEC)
Council Directive on the testing of the biodegradability of non-ionic surfactants (82/883/EEC)
Council Directive on the monitoring of waste from the titanium oxide industry (82/883/EEC)
1983
Council Directive on limit values and quality objectives for cadmium discharges (83/513/EEC)
1984
Council Directive on limit values and quality objectives for discharges by sectors other than the chlor-alkali electrolysis industry (84/156/EEC)
Council Directive on limit values and quality objectives for discharges of hexachlorocyclohexane (84/491/EEC)
1985
Council Directive on the assessment of the effects of certain public and private projects on the environment (85/337/EEC)

Table 1.3. (*Continued*)

1986
Council Directive on the limit values and quality objectives for discharge of certain dangerous substances included in List I of the Annex to Directive 76/464/EEC (86/280/EEC)
1987
Council Directive on the preventation and reduction of environmental pollution by asbestos (87/217/EEC)
1988
Council Directive amending Annex II to the Directive 86/280/EEC on limit values and quality objectives for discharges of certain dangerous substances included in List I of the Annex to Directive 76/464/EEC (88/347/EEC)
1990
Council Directive amending Annex II to the Directive 86/280/EEC on limit values and quality objectives for discharges of certain dangerous substances included in List I of the Annex to Directive 76/464/EEC (90/415/EEC)
1991
Council Directive concerning urban waste water treatment (91/271/EEC)
Council Directive concerning the protection of waters against pollution caused by nitrates from agricultural sources (91/676/EEC)
1992
Council Dirrective on pollution by waste from the titanium oxide industry (92/112/EEC)
1996
Council Directive on integrated pollution prevention control (96/61/EEC)
1998
Council Directive on the quality of water intended for human consumption (98/83/EEC)
2000
Council Directive establishing a framework for community action in the field of water policy (00/60/EC)

(80/778/EEC), Urban Waste Water Treatment (91/271/EEC), Nitrates (91/676/EEC), Integrated Pollution Prevention Control (96/61/EEC), and Water Framework (00/60/EEC). The Directive controlling sewage sludge disposal to agricultural land (86/278/EEC) is discussed in Chap. 8. In most Directives both guide (G) and imperative, or mandatory, (I) values are given. The G values are those which Member States should be working towards in the long term. In most cases, nationally adopted limit values are the I values although occasionally more stringent values are set.

The Dangerous Substances Directive (76/464/EEC) requires licensing, monitoring and control of a wide range of listed substances discharged to the aquatic environment. List I (Black List) substances have been selected mainly on the basis of their toxicity, persistence and potential for bioaccumulation. Those that are rapidly converted into substances that are biologically harmless are excluded. List II (Grey List) substances are considered to be less toxic, or the effects of which are confined to a limited area

Table 1.4. List I and List II substances defined by the EU Dangerous Substances Directive (76/464/EEC).

List no. 1 (‘*black list*’)
Organohalogen compounds and substances which may form such compounds in the aquatic environment
Organophosphorus compounds
Organotin compounds
Substances, the carcinogenic activity of which is exhibited in or by the equatic environment (substances in List 2 which are carcinogenic are included here)
Mercury and its compounds
Cadmium and its compounds
Persistent mineral oils and hydrocarbons of petroleum
Persistent synthetic substances
List no. 2 (‘*grey list*’)
The following metalloids/metals and their compounds:
Zinc, copper, nickel, chromium, lead, selenium, arsenic, antimony, molybdenum, titanium, tin, barium, beryllium, boron, uranium, vanadium, cobalt, thalium, tellurium, silver
Biocides and their derivatives not appearing in List 1
Substances which have a deleterious effect on the taste and/or smell of products for human consumption derived from the aquatic environment and compounds liable to give rise to such substances in water
Toxic or persistent organic compounds of silicon and substances which may give rise to such compounds in water, excluding those which are biologically harmless or are rapidly converted in water to harmless substances
Inorganic compounds of phosphorus and elemental phosphorus
Non-persistent mineral oils and hydrocarbons of petroleum origin
Cyanides, fluorides
Certain substances which may have an adverse effect on the oxygen balance, particularly ammonia and nitrites

which is dependent on the characteristics and location of the water into which they are discharged (Table 1.4). Member States are in the process of establishing environmental quality standards (EQS) for surface and ground waters. These will be used as maximum permissible concentrations in waters receiving discharges containing such compounds (Table 1.5).

Water policy in the EU has recently been rationalized into three key Directives: Drinking Water (80/778/EEC), Urban Waste Water Treatment (91/271/EEC), and the Water Framework Directive (2000/60/EEC).

The Water Framework Directive (2000/60/EEC) brings together the existing Directives on water quality of surface fresh water, estuaries, coastal waters and ground water. It covers all aspects of aquatic ecology and water quality, including the protection of unique and valuable habitats, the protection of drinking water resources and the protection of bathing waters. It achieves this by managing all water resources within River Basin

Table 1.5. Environmental quality standards for List I and List II substances in England and Wales (Environment Agency 1998).

List I substances	Statutory EQS[a] (μg/l)	Number of discharges
Mercury and compounds	1	752
Cadmium and compounds	5	2196
Hexachlorocyclohexane (all isomers)	0.1	123
DDT (all isomers)	0.025	15
DDT (pp isomers)	0.01	1
Pentachlorophenol	2	88
Carbon tetrachloride	12	51
Aldrin	0.01	35
Dieldrin	0.01	58
Endrin	0.005	37
Isodrin	0.005	7
Hexachlorobenzene	0.03	20
Hexachlorobutadience	0.1	14
Chloroform	12	73
Trichloroethylene	10	48
Tetrachloroethylene	10	51
Trichlorobenzene	0.4	31
1,2-dichloroethane	10	87

[a]Standards are all annual mean concentrations

List II substances	Operational EQS[a] (μg/l)	Measured as
Lead	10	AD
Chromium	20	AD
Zinc	75	AT
Copper	10	AD
Nickel	150	AD
Arsenic[b]	50	AD
Boron	2000	AT
Iron	1000	AD
pH	6.0–9.0	P
Vanadium	20	AT
Tributyltin[b]	0.02	MT
Triphenyltin[b]	0.02	MT
PCSD	0.05	PT
Cyfluthrin	0.001	PT

Table 1.5. (*Continued*)

List II substances	Operational EQS[a] (μg/l)	Measured as
Sulcofuron	25	PT
Flucofuron	1	PT
Permethrin	0.01	PT
Atrazine and simazine[b]	2	A
Azinphos-methyl[b]	0.01	A
Dichlorvos[b]	0.001	A
Endosulphan[b]	0.003	A
Fenitrothion[b]	0.01	A
Malathion[b]	0.01	A
Trifluralin[b]	0.1	A
Diazinon	0.01	A
Propetamphos	0.01	A
Cypermethrin	0.0001	A
Isoproturon	2.0	A

A = annual average, P = 95% of samples, D = dissolved, T = total, M = maximum.
[a]Standards quoted for metals are for the protection of sensitive aquatic life at hardness 100–150 mg/l $CaCO_3$, alternative standards may be found in DoE circular 7/89.
[b]Standards for these substances are from the Surface Waters (Dangerous Substances) (Classification) Regulations 1997, Sl 2560 in which case these are now statutory.

Districts for which management plans will be drawn up using environmental quality standards (EQSs) (Table 1.5). The Directive sets clear monitoring procedures and lists specific biological, hydromorphological and physico-chemical parameters to be used for rivers, lakes, estuaries and coastal waters. For each of these resource groups, definitions of high, good and fair ecological quality are given for each specified parameter.

The Urban Waste Water Treatment Directive (91/271/EEC) makes secondary treatment mandatory for sewered domestic waste waters and also all biodegradable industrial (e.g. food processing) waste waters. Minimum effluent standards have been set at BOD 25 mg l^{-1}, COD 125 mg l^{-1} and suspended solids 35 mg l^{-1}. Those receiving waters that are considered to be at risk from eutrophication are classified as sensitive so that discharges require more stringent treatment to bring nutrient concentrations of final effluents down to a maximum total phosphorus concentration of 2 mg l^{-1} for P and a total nitrogen concentration of 10–15 mg l^{-1} for N (Table 1.6). Due to the cost of nutrient removal, the designation of receiving waters as sensitive has significant cost implications for Member States.

Table 1.6. The Urban Wastewater Treatment Directive (91/271/EEC) sets discharge limits for wastewater treatment plants. Values for total phosphorus and nitrogen only apply to discharges $> 10,000$ population equivalents (PE) discharging to surface waters classed as sensitive (e.g. those subject to eutrophication).

Parameter	Minimum concentration	Minimum percentage reduction
BOD_5	25 mg O_2 l^{-1}	70–90
COD	125 mg O_2 l^{-1}	75
Suspended solids	35 mg l^{-1}	90
Total phosphorus	1 mg P l^{-1}[a]	80
	2 mg P l^{-1}[b]	80
Total nitrogen	10 mg N l^{-1}[a]	70–80
	15 mg N l^{-1}[b]	70–80

[a]10000–100000 PE.
[b]>100000 PE.

Strict completion dates have been set by the Commission for the provision of minimum treatment for waste waters entering freshwater, estuaries and coastal waters. For example, full secondary treatment (Sec. 2.1) including nutrient removal for all discharges to sensitive waters with a population equivalent (PE) >10,000 must be completed by the end of 1998. By 31 December 2005 all waste waters from population centres <2,000 PE discharged to freshwaters, and <10,000 PE to coastal waters must have sufficient treatment to allow receiving waters to meet environmental quality standards, while populations centres larger than these require secondary treatment (Fig. 1.2). The Directive also requires significant changes in the disposal of sewage sludge including:

(i) That sludge arising from waste water treatment shall be reused whenever possible and that disposal routes shall minimise adverse effects on the environment
(ii) Competent authorities shall ensure that before 31 December 1998, the disposal of sludge from waste water treatment plants is subject to general rules (i.e. Codes of Practice) or legislation
(iii) The disposal of sludge to surface waters by dumping from ships or discharge from pipelines or other means shall be phased out by 31 December 1998
(iv) That the total amount of toxic, persistent or bioaccumable material in sewage sludge is progressively reduced

This wide scoping legislation is considered in more detail in Chap. 8. The disposal options for sewage sludge are further limited if it contains

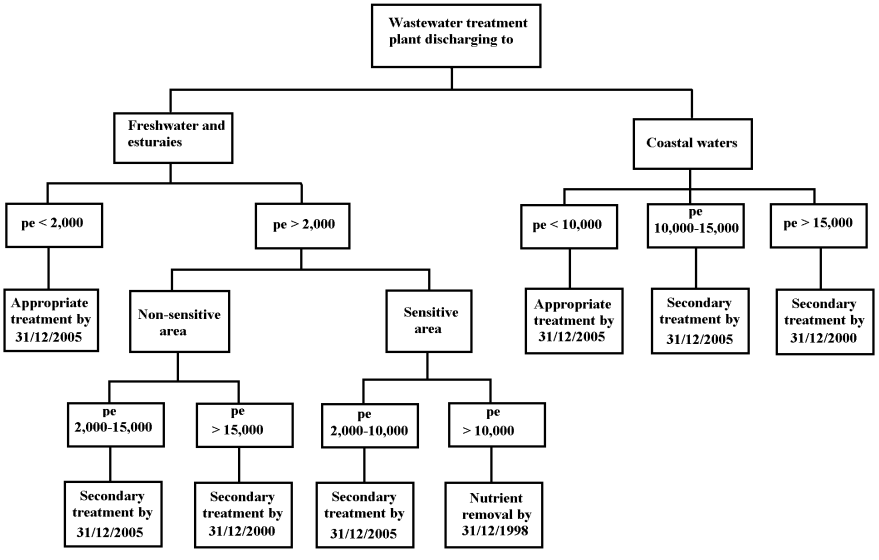


Fig. 1.2. The implementation of the EU Urban Wastewater Directive, with dates for compliance by Member States.

metals or listed substances which may categorise it as a hazardous waste under the EU Directive on Hazardous Waste (91/689/EEC).

Industrial effluents have in the past been a major cause of pollution. The discharge of industrial effluents is generally governed by two objectives: (1) the protection of environmental water quality, and (2) the need to protect sewers and wastewater treatment plants (Table 1.7). To meet these objectives, discharge standards are required that are a compromise between what is needed to protect and improve the environment and the demands of industrial development. Most industrialists accept that the application of the *best practical technology* (i.e. effluent treatment using the best of current technology to meet local environmental requirements at the lowest financial cost) is a reasonable way to comply with the effluent discharge standards set. However, where discharges contain dangerous or toxic pollutants which need to be minimised, then the application of the *best available technology* is required (i.e. effluent treatment using the best of current technology to minimise local environmental change, especially the accumulation of toxic materials, where financial implications are secondary considerations). Where effluent standards are necessary that are even unobtainable using the best available technology, then of course industries can no longer continue at that location.

Table 1.7. Typical effluent standards for discharges to sewers (Gledhill 1986).

Parameter	Standard	Reasons
pH	6 to 10	Protection of sewer and sewage works fabric from corrosion.
Suspended solids	200–400 mg l^{-1}	Protection from sewer blockages and extra load on sludge disposal system.
BOD_5	No general limit	Local authorities would be concerned with large loads on small sewage works and balancing of flows may be required in order not to overload treatment units.
Oils/fats/grease	100 mg l^{-1}	Prevention of fouling of working equipment and safety of men. Soluble fats, etc. can be allowed at ambient temperature.
Inflammables, hydrocarbons, etc.	Prohibited	Prevention of hazards from vapours in sewers.
Temperature	43°C	Various reasons — promotes corrosion, increases solubility of other pollutants, etc.
Toxic metals	10 mg l^{-1}	Prevention of treatment inhibition. The soluble metal is more toxic and different metals can be troublesome. Total loads with a limit on soluble metals more realistic.
Sulphate	500–1000 mg l^{-1}	Protection of sewer from sulphate corrosion.
Cyanides	0–1 mg l^{-1}	Prevention of treatment inhibition. Much higher levels can also cause hazardous working conditions due to HCN gas accumulation in sewer.

The integrated pollution prevention and control (IPPC) Directive (96/61/EEC) was adopted in September 1996. Integrated pollution prevention and control is a major advance in pollution control in that all discharges and environmental effects to water, air and land are considered, together with the *Best Practicable Environmental Option* (BPEO) selected for disposal. In this way, pollution problems are solved rather than transferred from one part of the environment to another. In the past, licensing of one environmental media (i.e. air, water or land) created an incentive to release emissions to another. Integrated pollution prevention and control also minimises the risk of emissions crossing over into other environmental media after discharge (e.g. acid rain, landfill leachate). There is only one licence issued under IPPC covering all aspects of gaseous, liquid, solid waste and noise emissions, so that the operator only has to make one application as well as ensuring consistency between conditions attached to the licence in relation to the different environmental media. In Europe, IPPC applies to the most complex and polluting industries and substances (e.g. large chemical works, power stations, etc.). In England and Wales, the Environment

Agency issues guidance for such processes to ensure that the BPEO is carried out. The aim of IPPC is to minimise the release of listed substances and to render substances that are released harmless using *Best Available Techniques Not Entailing Excessive Cost* (BATNEEC). The objective of the guidance notes is to identify the types of techniques that will be used by the Agency to define BATNEEC for a particular process. The BATNEEC identified is then used as a base for setting emission limit values (ELVs). Unlike previous practice in the identification of BATNEEC, emphasis is placed on pollution prevention techniques such as cleaner technologies and waste minimisation rather than end-of-pipe treatment. Other factors for improving emission quality include in-plant changes, raw material substitution, process recycling, improved material handling and storage practices. Apart from the installation of equipment and new operational procedures to reduce emissions, BATNEEC also necessitates the adoption of an ongoing programme of environmental management and control which should focus on continuing improvements aimed at prevention, elimination and progressive reduction of emissions.

The selection of BATNEEC for a particular process takes into account (i) the current state of technical knowledge, (ii) the requirements of environmental protection, and (iii) the application of measures for these purposes which do not entail excessive costs, having regard to the risk of significant environmental pollution. For existing facilities, the Agency considers (i) the nature, extent and effect of the emissions concerned, (ii) the nature and age of the existing facilities connected with the activity and the period during which the facilities are likely to be used or to continue in operation, and (iii) the costs, which would be incurred in improving or replacing these existing facilities in relation to the economic situation of the industrial sector of the process considered. Thus, while BATNEEC guidelines are the basis for setting licence emission standards, other factors such as site-specific environmental and technical data as well as plant financial data are also taken into account. In Ireland, similar IPPC licensing procedures are operated by the Environmental Protection Agency (EPA 1994), and like the Environment Agency in England and Wales, public registers of all licences are maintained.

The introduction of the polluter which pays charging system throughout Europe and the USA is an attempt to achieve such environmental objectives, at least in terms of the cost to the community, by reinforcing the philosophy that the polluter is responsible for all aspects of pollution control in relation to its own effluent (Deering and Gray 1987). Two distinct types of charges exist: *effluent charges* are levied by local authorities for

discharges directly to surface waters, whereas *user charges* are levied for the use of the authority's collective treatment system (Table 1.16). By charging industry for treating their effluents in terms of strength and volume, it encourages them to optimise production efficiency by reducing the volume and strength of their effluent. Most important of all, such charging systems ensure that effluent disposal and treatment costs are taken into account by manufacturers in the overall production costs, so that the cost of the final product reflects the true cost of production (Deering and Gray 1986).

Wastewater treatment is not solely a physical phenomena controlled by engineers, it also involves a complex series of biochemical reactions involving a wide range of micro-organisms. The same micro-organisms that occur naturally in rivers and streams are utilised, under controlled conditions, to rapidly oxidise the organic matter in wastewater to innocuous end products that can be safely discharged to surface waters. Compared with other industries which also use micro-organisms, such as brewing or baking, wastewater treatment is by far the largest industrial use of micro-organisms using specially constructed reactors. As treatment plants that were constructed during the early expansion of wastewater treatment in the late nineteenth and early twentieth centuries now near the end of their useful lives, it is clear that the opportunities for the biotechnologists to apply new technologies, such as genetic manipulation combined with new reactor designs, to pollution control are enormous (Chap. 10). In the future, cheaper, more efficient, and more compact processes will be developed, with the traditional aims of removing organic matter and pathogens to prevent water pollution and protect public health replaced with a philosophy of environmental protection linked with conservation of resources and by-product recovery (Chap. 11).

Natural scientists, whether they are trained as microbiologists, biochemists, biologists, biotechnologists, environmental scientists or any other allied discipline, have an important role in all aspects of public health engineering. They already have a significant function in the operation and monitoring of treatment plants, but their expertise is also needed in the optimisation of existing plants and in the design of the next generation of wastewater treatment systems.

1.2. Nature of Wastewater

Although there has been a steady increase in the discharge of toxic inorganic and organic materials, it is still the biodegradable organic wastes

that are the major cause of pollution of receiving waters in Britain and Ireland (Gray and Hunter 1985; DETR 1998; Environment Agency 1998, 1999; EPA 2000). Organic waste originates from domestic and commercial premises as sewage, from urban runoff, various industrial processes and agricultural wastes. Not all industrial wastes have a high organic content that is amenable to biological treatment, and those with a low organic content, insufficient nutrients, and which contain toxic compounds, require specific chemical treatment, such as neutralisation, chemical precipitation, chemical coagulation, reverse osmosis, ion-exchange, or adsorption onto activated carbon (Table 1.8) (Casey 1997).

This book concentrates on non-toxic wastewaters. It is these that are of particular interest to the biologist and biotechnologist in terms of reuse, conversion, and recovery of useful constituent materials. Primarily sewage containing pathogenic micro-organisms is considered, although other wastewaters, such as agricultural wastes from intensive animal rearing and silage production, food processing wastes, and dairy industry wastes are also briefly reviewed.

1.2.1. *Sources and variation in sewage flow*

The absolute minimum quantity of wastewater produced per person (per capita), without any excess water, is 4 litres per day. At this concentration, the wastewater has a dry solids content in excess of 10%. However, in most communities that have an adequate water supply this minimum quantity is greatly increased. In those countries where technology and an almost unlimited water supply has led to the widescale adoption of water-consuming devices — many of which are now considered to be standard, if not basic, human requirements — the volume of wastewater produced has increased by a factor of 100 or more. Flush toilets, baths, showers, automatic washing machines, dishwashers and waste disposal units all produce vast quantities of diluted dirty (grey) water with a very low solids content and all requiring treatment before being discharged to surface waters. For example, a flush toilet dilutes small volumes of waste matter (< 1 litre) to between 10 or 30 litres each time it is used. Domestic sewage is diluted so much that it is essentially 99.9% water with a dry solids content of less than 0.1%. Conventional sewage treatment aims to convert the solids into a manageable sludge (2% dry solids) while leaving only a small proportion in the final effluent (0.003% dry solids).

The total volume of wastewater produced per capita depends on the water usage, the type of sewerage system used and the level of infiltration.

Table 1.8. Main chemical and biological unit processes employed in wastewater treatment.

Process	Description
Chemical unit processes	
Neutralisation	Non-neutral waste waters are mixed either with an alkali (e.g. NaOH) or an acid (e.g. H_2SO_4) to bring the pH as close to neutral as possible to protect treatment processes. Widely used in chemical, pharmaceutical and tanning industries
Precipitation	Dissolved inorganic components can be removed by adding an acid or alkali, or by changing the temperature, by precipitation as a solid. The precipitate can be removed by sedimentation, flotation or any other solids removal process
Ion-exchange	Removal of dissolved inorganic ions by exchange with another ion attached to a resin column. For example Ca and Mg ions can replace Na ions in a resin, thereby reducing the hardness of the water
Oxidation reduction	Inorganic and organic materials in industrial process waters can be made less toxic or less volatile by subtracting or adding electrons between reactant (e.g. aromatic hydrocarbons, cyanides, etc.)
Biological unit processes	
Activated sludge	Liquid waste water is aerated to allow micro-organisms to utilise organic polluting matter (95% reduction). The microbial biomass and treated effluent are separated by sedimentation with a portion of the biomass (sludge) returned to the aeration tank to seed the incoming waste water
Biological filtration	Waste water is distributed over a bed of inert medium on which micro-organisms develop and utilise the organic matter present. Aeration occurs through natural ventilation and the solids are not returned to the filter
Stabilisation ponds	Large lagoons where waste water is stored for long periods to allow a wide range of micro-organisms to break down organic matter. Many different types and designs of ponds including aerated, non-aerated and anaerobic ponds. Some designs rely on algae to provide oxygen for bacterial breakdown of organic matter. Sludge is not returned
Anaerobic digestion	Used for high strength organic effluents (e.g. pharmaceutical, food and drink industries). Waste water is stored in a sealed tank which excludes oxygen. Anaerobic bacteria breakdown organic matter into methane, carbon dioxide and organic acids. Final effluent still requires further treatment as has a high BOD. Also used for the stabilisation of sewage sludge at a concentration of 2–7% solids

The volume of wastewater varies from country to country depending on its standard of living and the availability of water supplies (Table 1.9). Generally, the volume and strength of the sewage discharged in a particular country can be predicted fairly accurately. For example, the mean daily volume of wastewater, excluding industrial waste but including infiltration,

Table 1.9. Specific water consumption in Europe (IWSA 1995).

	Household and small businesses		Industry and others		Total	
	1980	1993	1980	1993	1980	1993
Austria	155	170	100	92	255	262
Belgium	104	120	59	37	163	157
Denmark	165	155	96	74	261	229
France	109	157	58	58	167	215
Germany[1]	137	136	74	41	211	177
Hungary	110	121	107	63	217	184
Italy	211	251	69	78	280	329
Luxembourg	183	178	76	83	259	261
Netherlands	142	171	37	32	179	203
Norway	154	180	247	340	401	520
Spain	157	210	58	90	215	300
Sweden	195	203	120	73	315	276
Switzerland	229	242	163	120	392	362
United Kingdom	154	—[2]	100	—[2]	254	331

[1]Includes former GDR.

[2]UK values not available in this format.

produced per capita in England is 180 l d^{-1}, compared 230 l d^{-1} in Ireland and 250 l d^{-1} in Scotland. The equivalent volume of sewage produced in the USA is on average 300 l per capita per day (100 US gallons d^{-1}). The amount of wastewater produced per capita can be estimated quite accurately from the specific water consumption.

The variation in volume depends on a number of variables including the amount of infiltration water entering the sewer. The higher volume of wastewater produced in Scotland is primarily due to the widescale use of a larger flushing cistern, 13.6 l compared with 9.0 l in England and Wales, although other factors also contribute to this variation. Guidelines from the Department of the Environment in England and Wales stipulate that all new cisterns manufactured after 1993 should have a maximum flushing volume of 7.5 l. However, the reliance of water closets which function on a siphon rather than a valve to release water restricts the minimum operational volume to between 4–5 l (Pearse 1987). The Building Research Establishment (1987) highlights the potential water saving from the adoption of new cistern designs and suggests the need for new British Standards.

Comparative studies were carried out using a 'standard turd', which is a 43 mm diameter ball of non-absorbent material with a relative density of 1.08, and with a cohesive shear strength, coefficient of friction, and adhesive properties very close to the real thing.

In rural areas, where water is drawn from boreholes or from small community water schemes, water may be at a premium, so the necessary conservation of supplies results in reduced volumes of stronger sewage. Occasionally, the water pressure from such rural supplies is too low to operate automatic washing machines or dishwashers and results in an overall reduction in water usage and subsequent wastewater discharge.

In the home, wastewater comes from three main sources. Approximately a third of the volume comes from the toilet, a third from personal washing via the wash basin, bath, and shower, and a third from other sources such as washing up, laundry, food and drink preparation (Tables 1.10 and 1.11). Outside the home, the strength and volume of wastewater produced per capita per day will fluctuate according to source, and this variation must be taken into account when designing a new treatment plant. For example, the flow per capita can vary from 50 l d^{-1} at a camping site to 300 l d^{-1} at a luxury hotel (Table 1.11). More detailed tables of the volume of wastewater produced from non-industrial sources, including the strength of such wastewater, are given by Hammer (1999) and also by Metcalf and Eddy (1991).

The diluted nature of wastewater has led to the development of the present system of treatment found in nearly all the technically-developed countries, which is based on treating large volumes of weak wastewater. In less developed communities, the high solids concentration of the waste

Table 1.10. Comparison of the percentage consumption of water for various purposes in a home with an office; indicating the source and make-up of wastewater from these types of premises (Mann 1979).

Home (sources)	Total water consumed (%)	Office (sources)	Total water consumed (%)
WC flushing	35	WC flushing	43 } 63
Washing/bathing	25	Urinal flushing	20 }
Food preparation/drinking	15	Washing	27
		Canteen use	9
Laundry	10	Cleaning	1
Car washing/garden use	5[a]		

[a]May not be disposed to sewer.

Table 1.11. Daily volume of wastewater produced per capita from various non-industrial sources (Mann 1979).

Source category	Volume of sewage (litres/person/day)
Small domestic housing	120
Luxury domestic housing	200
Hotels with private baths	150
Restaurants (toilet and kitchen wastes per customer)	30–40
Camping site with limited sanitary facilities	80–120
Day schools with meals service	50–60
Boarding schools: term time	150–200
Offices: day work	40–50
Factories: per 8 hour shift	40–80

Table 1.12. Comparison of the concentration of various compounds reported in urban runoff with precipitation, strictly surface runoff from roads and with combined sewer overflow (Pope 1980). All units are in mg l^{-1} unless specified. Those marked with † are in mg kg^{-1} and ‡ in kg curb km^{-1}.

Parameter	Reported concentration range (mg l^{-1})			
	Precipitation	Road/street runoff	Urban runoff	Combined sewer overflow
COD	2.5–322	300	5–3100	93–2636
BOD	1.1	25–165	1–700	15–685
Total solids	18–24	474–1070	400–15322	150–2300
Volatile total solids	—	37–86	12–1600	—
Suspended solids	2–13	11–5500	2–11300	20–1700
Volatile suspended solids	6–16	100–1500	12–1268	113
Settleable solids	—	—	0.5–5400	—
Total dissolved solids	—	66–33050	9–574	—
Volatile dissolved solids	—	1630	160	—
Conductance (μmho cm^{-1})	8–395	10000	5.5–20000	—
Turbidity (JTU)	4–7	—	3–70	—
Colour (Pt-Co units)	5–10	—	5–160	—
Total organic carbon	1–18	5.3–49	14–120	—
Total inorganic carbon	0–2.8	—	1.17	—
Oils/hydrocarbons	—	28–400	0–110	—
Phenols	—	—	0–10	—

Table 1.12. (*Continued*)

Parameter	Reported concentration range (mg l^{-1})			
	Precipitation	Road/street runoff	Urban runoff	Combined sewer overflow
Total nitrogen N	0.5–9.9	0.18–4.0	1.1–6.2	4.0–63.3
Organic N	0.1–0.32	0.18–3.23	0.1–16	1.5–33.1
Inorganic N	0.69	—	1.0	—
Ammonia N	0.01–0.4	1–2	0.1–14.0	0.1–12.5
Nitrate N	0.02–5.0	0.31–2.62	0.1–2.5	—
Nitrite N	0–0.1	—	0-1.5	—
Total phosphorus	0.001–0.35	0.3–0.7	0.09–4.4	1.0–26.5
Hydrolysable phosphorus	0.8–0.24	—	0.1–10	—
Aldrin	—	—	'trace'	—
Dieldrin	0.003†	6.8×10^{-6}‡	'trace'	—
p,p'-DDD	—	18.9×10^{-6}‡	—	—
p,p'-DDD	—	17.2×10^{-6}‡	—	—
Heptachlor	0.04†	—	'trace'	—
Lindane	—	—	'trace'	—
PCB	—	311×10^{-6}‡	—	—
Bromide	—	—	5	—
Chloride	0.1–1.1	4–70000	2–25000	—
Cadmium	0.013–0.056	0.002–0.01	0.006–0.045	—
Chromium	0.023–0.08	0.018–1.0	0.01–27.0	—
Copper	0.06–0.48	0.007–2.55	0.041–0.45	—
Iron	0–3.05	5–440	0–5.3	—
Lead	0.024–10.4	1–113	0.01–14.5	—
Mercury	—	0.029	—	—
Nickel	—	0.02–1.5	—	—
Zinc	0.02–4.9	1–15	0.01–5.23	—
Total coliform (ml^{-1})	—	—	240–99100	—
Total coliform (organisms km^{-1})	—	15.9×10^{10}	—	—
Faecal coliform (ml^{-1})	—	—	5500–11200	—
Faecal coliform (organisms km^{-1})	—	0.9×10^{10}	—	—
Faecal streptococcus (ml^{-1})	—	—	120–20000	—

makes it difficult to move to central collection and treatment sites, while the more diluted wastewater flows easily through pipes, and can be transported easily and efficiently via a network of sewers to a central treatment works. In isolated areas or underdeveloped countries, human waste is normally treated on-site, due to its smaller volume and less fluid properties (Feachem and Cairncross 1993; Mara 1996).

The collection and transport of sewage to the treatment plant is via a network of sewers. Two main types of sewerage systems are used, combined and separate. Combined sewerage systems are common in most towns in Britain. Surface drainage from roads, paved areas, and roofs are collected in the same sewer as the foul wastewater and piped to the treatment works. This leads to fluctuations in both the volume and the strength of sewage due to rainfall, and although the treatment works is designed to treat up to three times the dry weather flow of wastewater (DWF), problems arise if the rainfall is either heavy or continuous. During such periods, the wastewater becomes relatively diluted and the volume too great to be dealt with by the treatment works. Excess flow is, therefore, either directly discharged to a watercourse as storm water or stored at the treatment works in storm water tanks. The stored wastewater can be circulated back to the start of the treatment works once capacity is available. However, once the tanks become full, and then the settled wastewater passes into the river without further treatment where the watercourse, already swollen with rainwater, can easily assimilate the diluted wastewater because of the extra dilution now available.

A separate sewerage system overcomes the problem of fluctuations in sewage strength and volume due to rain, by collecting and transporting only the foul wastewater to the treatment works, and surface drainage is discharged to the nearest water course. Such systems are common in new towns in Britain and are mandatory in Canada and the USA. This type of sewerage system allows more efficient and economic treatment works to be designed as the variation in the volume and strength of the wastewater is much smaller and can be more accurately predicted. A major drawback with separate systems is that the surface drainage water often becomes polluted. All stormwater is contaminated to some degree because of contact during the drainage cycle: it passes over paved areas along roadside gullies to enter the sewer via a drain with a gully pot, which catches and removes solids that might otherwise cause a blockage in the sewer pipe (Bartlett 1981). The quality of urban runoff is extremely variable and biochemical oxygen demand (BOD) values have been recorded in excess of 7,500 mg l^{-1} (Mason 1991; Lee and Bang 2000). It is the first flush of storm water

that is particularly polluting as it displaces the anaerobic wastewater, rich in bacteria, that has been standing in the gully pots of the roadside drains since the last storm (Butler and Memon 1999). The runoff from roads is rich in grit, suspended solids, hydrocarbons including polycyclic aromatic hydrocarbons (Krein and Schorer 2000), heavy metals, pesticides such as the herbicide atrazine (Appel and Hudak 2001), and, during the winter, chloride from road-salting operations. Surprisingly, it also contains organic matter, not only in the form of plant debris such as leaves and twigs, but also dog faeces (Table 1.12). It has been estimated that up to 17 g m^{-2} y^{-1} of dog faeces are deposited onto urban paved areas and that the dog

Table 1.13. Chemical characteristics of treated effluents from three UK sewage treatment plants.

Constituent[a]	Source		
	Stevenage	Letchworth	Redbridge
Total solids	728	640	931
Suspended solids	15		51
Permanganate value	13	8.6	16
BOD	9	2	21
COD	63	31	78
Organic carbon	20	13	
Surface-active matter			
Anionic (as Manoxol OT)	2.5	0.75	1.4
Non-ionic (as Lissapol NX)			0.4
Ammonia (as N)	4.1	1.9	7.1
Nitrate (as N)	38	21	26
Nitrite (as N)	1.8	0.2	0.4
Chloride	69	69	98
Sulphate	85	61	212
Total phosphate (as P)	9.6	6.2	8.2
Sodium	144	124	
Potassium	26	21	
Total hardness	249	295	468
pH value	7.6	7.2	7.4
Turbidity (ATU)[b]			66
Colour (Hazen units)	50	43	36
Coliform bacteria (no./ml)	1300		3500

[a]Results are given in mg l^{-1}, unless otherwise indicated.

[b]Absorptiometric turbidy units

population of a city the size of Manchester will produce an organic load equivalent to the human population of a small town of 25–30,000 people. In New York, the dog population deposits over 68,000 kg of faeces and 405,000 l of urine onto the streets each day, much of which is washed by storm water into local streams and rivers (Feldman 1974). The degree of contamination of urban runoff during a specific storm depends on: (i) the intensity and duration of the rainfall; (ii) the length of the preceding dry period, which controls the build up of pollutants on roads and in the quality of water stored in gully pots and gutters; (iii) seasonal variations that occur in the rainfall pattern and temperature which affects the degradation of organic matter; including leaf fall and the use of grit and salt during the winter, and (iv) the effectiveness of local authorities to clean roadside gullies and gully pots (Helliwell 1979). Unlike drainage from land, runoff from roads and paved areas is very rapid due to the short length of surface water sewers. The contaminated wastewater, therefore, reaches the receiving watercourse very quickly and before the dry weather flow has increased, so that any pollutants entering will receive minimum dilution. Where there is an accidental or deliberate spillage of chemicals or noxious wastes on roads, or in private yards, serious pollution of receiving waters is bound to occur. However, with combined sewerage systems such spillages can be confined at the treatment works and recovered or treated before reaching the watercourse (Sec. 2.1.1). During storm events, it is possible for combined sewers in particular to become overloaded, leading to the operation of sewer overflow systems. Combined sewer overflows (CSOs) discharge a mixture of wastewater and surface runoff that causes severe pollution in receiving waters (Balmforth 1990; Field *et al.* 1994). Extensive work has been undertaken to reduce the number of storm water overflows within sewer networks and to reduce the amount of storm water entering the sewer by using interception systems such as swales, percolation areas, porous roads and wetlands (Field *et al.* 1994; Debo and Reese 1995; Shutes *et al.* 1997; Sieker 1998; Adams and Papa 2000).

It is common, in both separate and combined sewers, for water not discharged as wastewater to enter the sewer via joints and cracks in the pipework. Infiltration water is normally from ground water sources and can be especially high during periods of rainfall. Few estimates of the extent of the problem are available, although some studies have found infiltration to be as high as 80% of the total volume in badly deteriorated sewers. In the USA, it is estimated that a mean value is 70 $m^3 d^{-1}$ per km of sewer (30,000 US gallons per day per mile of sewer) (Clarke *et al.* 1971), although Grace (1979) recorded mean values some 50% less. As groundwater is generally

very clean, infiltration has the effect of diluting the strength of wastewater and at the same time increasing the volume requiring treatment.

The flow rate of wastewater to treatment works is extremely variable, and although such flows follow a basic diurnal pattern, each treatment works tends to have a characteristic flow pattern. This pattern is controlled by such factors as: the time taken for sewage to travel from households to the treatment works, which is itself a function of sewer length; the degree of infiltration; the presence of stormwater and the variability in the water consumption practices of communities (Gower 1980). Industrial inputs obviously have a profound effect on flow rates, and industrial practices such as discharging wastes after 8-hour shifts can completely alter the expected normal flow pattern to a treatment plant. The basic flow pattern for a domestic wastewater treatment plant is shown in Fig. 1.3 with the minimum flow normally occurring in the early hours of the morning when water consumption is lowest and the flow consists largely of infiltration water. Flow rate rapidly increases during the morning when peak morning water consumption reaches the plant, followed by a second peak in the

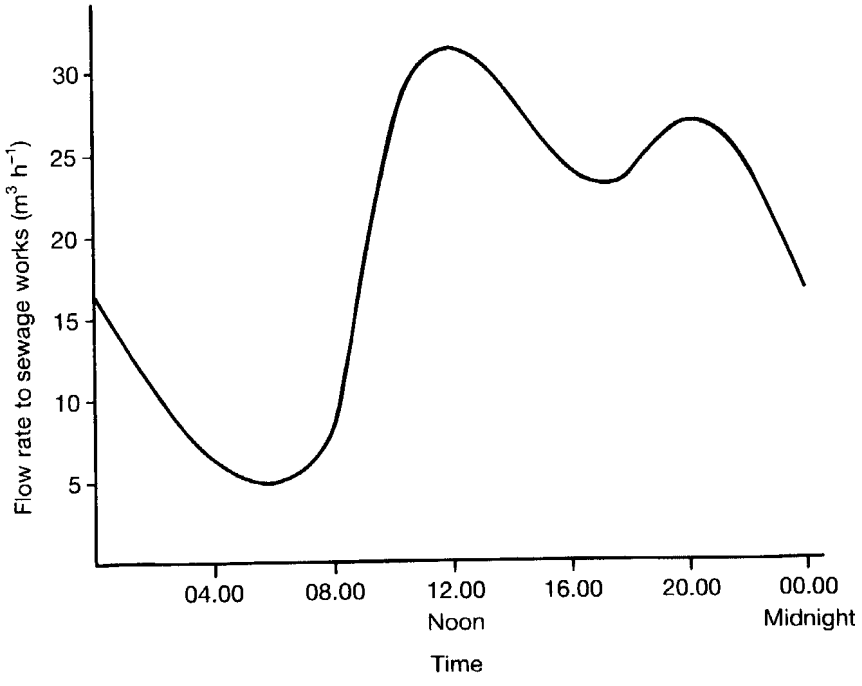


Fig. 1.3. Example of the hourly variation in flow to a sewage treatment plant.

early evening. When infiltration, storm water, and the water used for non-sewered purposes such as garden use, are removed from a basic model of consumption and discharge, then the water supplied is essentially equivalent to the wastewater discharged to the sewer (Lenz 1983a). Thus, the wastewater discharge curve, as measured at the sewage treatment works, will closely parallel the water supply curve, as measured at the waterworks, with a lag of several hours.

Infiltration and storm water tend to distort the basic shape of the hydrograph of diurnal flow. Infiltration, while increasing the total daily volume, does not alter its characteristic shape. Storm water, however, can alter the shape of the hydrograph by hiding peaks and troughs or adding new peaks as the rainfall causes rapid increases in the flow. Hourly fluctuations are less clear in large catchments due to the diversity of activities taking place during the 24-hour period and the presence of industry. The variable distance of households from the treatment works normally results in the hydrograph of the diurnal pattern becoming flattened and extended so that only one trough and one peak is seen daily (Clark *et al.* 1977; Escritt and Haworth 1984). Many problems at small to moderate sized treatment works are associated with the diurnal variation in flow, which is especially serious at the smallest works where often there is no flow at all during the night. Smaller variations of the average daily flow rate are recorded at treatment works serving large catchments (50–200%) compared with smaller communities (20–300%) (Painter 1958; Water Pollution Control Federation 1961). Many works overcome the problem of flow variation by using flow balancing, where the wastewater is stored at times of high flow and allowed to enter the works at a constant rate, or by recirculating treated final effluent during periods of low flow.

Variation between weekday flows is negligible, except in those areas where the household laundry is done on specific days. However, with the advent of automatic washing machines this practice has become largely extinct. With changing work patterns, many homes are now only occupied at night and on the weekends, leading to changes in diurnal and daily flow characteristics. Also, automatic washing of household laundry and dishes is increasingly done at night to take advantage of cheaper off-peak electricity tariffs. Although summer discharges normally exceed winter flows by 10–20%, up to 20–30% in the USA, seasonal variations in flow are due mainly to variation in population, as is the case at holiday resorts, schools, universities, and military camps. Other seasonal variations in flow are due to infiltration, which is linked to rainfall pattern and groundwater levels, and seasonal industrial activities such as food processing.

1.2.2. *Composition of sewage*

Wastewater is defined as domestic (sanitary) or industrial (trade). Domestic wastewater comes exclusively from residences, commercial buildings, and institutions such as schools and hospitals, while industrial wastewater comes from manufacturing plants. Inevitably, large towns and cities have a mixture of domestic and industrial wastewaters which is commonly referred to as municipal wastewater, and normally includes effluents from the service industries such as dairies, laundries, and bakeries, as well as a variety of small factories. It is unusual for modern municipal treatment plants to accept wastewater from major industrial complexes, such as chemical manufacturing, brewing, meat processing, metal processing, or paper mills, unless the treatment plant is specifically designed to do so. The practice in all European countries is now for water authorities to charge industry for the treatment and disposal of their wastewater. Thus, the current trend is for industry to treat its own waste in specifically designed treatment plants. In many cases, it is not cost-effective for an industry to provide and operate its own treatment plant, although most industries partially treat their waste to reduce the pollution load before discharge to the public sewer, in order to reduce excessive treatment charges.

It is of prime importance for the designer and operator of a treatment plant to have as much knowledge of the composition of the wastewater to be treated as possible. This is particularly important when new or additional wastes are discharged to existing plants. A full analysis of the wastewater will, for example:

(i) determine whether pretreatment is required;
(ii) determine whether an industrial waste should be treated alone or with sewage and, if so, in what proportions;
(iii) determine whether an industrial waste would attack the sewer;
(iv) permit a better selection of the most appropriate treatment process;
(v) allow an assessment of the toxicity or disease hazards;
(vi) provide indication of the resultant degree of eutrophication or organic enrichment in the form of sewage fungus in the receiving water (i.e. impact assessment); and
(vii) an assessment of the recoverable or reusable fractions of the wastewater.

Although there is considerable similarity in the basic content of sewage, the precise volume and characteristics will vary not only from country to country because of climatic conditions and social customs, but also within

Table 1.14. Volume and composition of human faeces and urine (Gloyna 1971).

	Faeces	Urine
Moist weight per capita per day	135–270 g	1.0–1.3 kg
Dry weight per capital per day	35–70 g	50–70 g
Moisture content	66–80%	93–96%
Organic matter content (dry basis)	88–97%	65–85%
Nitrogen (dry basis)	5.0–7.0%	15–19%
Phosphorus (as P_2O_5) (dry basis)	3.0–5.4%	2.5–5.0%
Potassium (as K_2O) (dry basis)	1.0–2.5%	3.0–4.5%
Carbon (as dry basis)	40–55%	11–17%
Calcium (CaO) (dry basis)	4–5%	4.5–6.0%

individual countries due to supply water characteristics, water availability, population size, and the presence of industrial wastes. Data on wastewaters is normally limited to BOD_5 (the five day biochemical oxygen demand test), COD (chemical oxygen demand), suspended solids, and ammonia, while a fuller characterisation of the wastewater being treated is rare (Tables 1.13 and 3.11). Analysis of wastewater composition can be done directly by laboratory examination of the sewage itself or indirectly by predicting the composition by examination of the gross components. Details of the composition of human faeces and urine are available (Table 1.14) although details of other household wastes which are more variable are less well-known, therefore, more direct methods of wastewater characterisation are preferred. Surprisingly, little is known of the composition of sewage and few specific studies have been carried out. Casanova *et al.* (2001) carried out a detailed study of the chemical and microbial characteristics of the wastewater generated by a single family home comprising two adults in Arizona in the USA. The wastewater is significantly weaker in terms of BOD and suspended solids compared with domestic wastewater treated at a central works. The wastewater contained high densities of total coliforms 8.03×10^7 CPU 100 ml^{-1}, faecal coliforms 5.63×10^5 CPU 100 ml^{-1}, faecal streptococci 2.38×10^2 CPU 100 ml^{-1}, and *Pseudomonas aeruginosa* 1.99×10^4 CPU 100 ml^{-1}. Legislation is setting even tighter controls on effluent quality, especially in terms of nutrients and listed substances (Table 1.4). More attention is now being paid to eliminating these at source rather than providing expensive and often energy intensive end of pipe solutions (Chap. 11). Some individual components of sewage which causes

specific problems have been studied. For example, total phosphorus and nitrogen in eutrophication studies, detergents causing foaming, and indole in the control of odours.

Sewage is a complex mixture of natural inorganic and organic materials with a small proportion of man-made substances. The main source of pollution in sewage is human excreta with smaller contributions from food preparation, personal washing, laundry, and surface drainage. The chemical and physical nature of wastewaters can be further complicated by the inclusion of industrial wastes which are composed of strong spent liquors from main industrial processes and comparatively weak wastewaters from rinsing, washing and condensing.

The reason why sewage composition is normally measured in terms of BOD_5, COD, suspended solids, and ammonia content is because it is from these basic determinants that its polluting strength is assessed. Most charging systems are based on the Mogden formula, which uses these basic determinants. Other variables occasionally measured under specific circumstances, such as total phosphorus if the final effluent is discharged to inland lakes, or heavy metals if the sludge is to be subsequently used for agriculture. Charges are calculated from separate costs for reception, conveyance treatment and disposal actually given to the trade effluent. The basic formula is:

$$C = R + V + (Ot/Os)B + (St/Ss)S$$

where C is the total charge in pence (sterling) per 1000 litres of trade effluent, R is the reception and conveyance cost per 1000 litres, V the volumetric and primary treatment costs per 1000 litres, Ot the COD of trade effluent after one hour quiescent settlement (mg l^{-1}), Os the COD of average strength settled sewage (mg l^{-1}), B the cost of biological oxidation of settled sewage, St the total suspended solids of the trade effluent (mg l^{-1}), Ss the total suspended solids of average strength settled sewage (mg l^{-1}), and S the treatment and disposal cost of primary sludge per 1000 litres of sewage.

Some companies have modified the basic Mogden formula to incorporate additional factors. These are Bv or Vb, which is the additional volume charge if there is biological treatment (p m^{-3}), M is cost for treatment and disposal where the effluent goes to a sea outfall (p m^{-3}), and Md or Vm a supplement to M where the effluent goes to a designated long sea outfall (p m^{-3}). This gives a modified Mogden formula:

$$C = R + V([V + Bv] \text{ or } Vm \text{ or } M) + (Ot/Os)B + (St/Ss)S$$

Table 1.15. Trade charging formula employed in the UK during 1999/2000. Details of abbreviations are given in the text except: $Vb = V + Bv$; $Vm = M$; Mo = monitoring costs; and P = the cost per m^3 of the preliminary treatment required for foul sewage.

Water service company	Charging
Anglian	$C = R + (V \text{ or } Vb \text{ or } Vm \text{ or } M) + (Ot/Os)B + (St/Ss)S$
Dwr Cymru	$C = R + V \text{ or } Vb + (Ot/Os)B + (St/Ss)S$
Northumbrian	$C = R + V + (Ot/Os)B + (St/Ss)S$
North West	$C = R + V + M + B_1 + (Ot/Os)B_2 + (St/Ss)S$
Severn Trent	$C = R + V + (Ot/Os)B + (St/Ss)S$
Southern	$C = R + (V \text{ or } Vb \text{ or } Vm) + (Ot/Os)B + (St/Ss)S + M$
South West	$C = R + V \text{ (or } Vm) + (Ot/Os)B + (St/Ss)S$
Thames	$C = R + V + (Ot/Os)B + (St/Ss)S$
Wessex	$C = R + V + (Ot/Os)B + (St/Ss)S$
Yorkshire	$C = R + P(Ot/Os)B + (St/Ss)S$
North of Scotland	$C = R + V + (Ot/Os)B + (St/Ss)S$
East of Scotland	$C = R + V + (Ot/Os)B + (St/Ss)S + Mo$
West of Scotland	$C = R + V + (Ot/Os)B + (St/Ss)S$
Northern Ireland	$C = R + V + (Ot/Os)B + (St/Ss)S$

Specific company trade effluent charging formulae are given in Table 1.15, and charges in Table 1.16. Other variables are occasionally measured under specific circumstances, such as total phosphorus if the final effluent is disposed of to inland lakes or heavy metals if the sludge is to be subsequently used for agriculture.

The strength of sewage varies widely and depends on such factors as per capita water usage, infiltration, surface and storm water, and local habits. The water usage in the USA is at least three times greater than in Britain, which is why American sewage is usually weaker. Although the per capita production of organic matter is essentially the same in the USA and Britain, the difference in water consumption results in a raw sewage BOD_5 of between 100–700 mg l^{-1} (with a mean BOD_5 of 320 mg l^{-1}) in Britain (Painter 1971). The use of garbage grinders or disposal units, so that household kitchen waste is disposed to the sewer rather than the refuse bin, results in a 30% increase in wastewater BOD_5 and 60% increase in the suspended solids. The concentration of nitrogen in domestic wastewater is directly related to the BOD_5, with about 40% of the total nitrogen in solution as ammonia. Proteins and urea undergo deamination releasing ammonia as the wastewater flows to the treatment plant. The longer the

Table 1.16. British trade effluent tariffs 2000–2001.

Water & sewerage companies	Minimum charge £	R p/m^3	V p/m^3	Bv p/m^3	M p/m^3	B p/kg	S p/kg	Regional strengths Os mg/l	Ss mg/l
Anglian — Green	167.00	13.49	21.12	4.08	11.31	44.05	30.99	419	402
— Orange	167.00	12.58	19.69	3.80	10.55	41.08	28.92	419	402
— Blue	167.00	12.35	19.32	3.75	10.35	40.34	28.35	419	402
— Industrial	167.00	9.53	14.93	2.89	8.01	31.16	21.92	419	402
Dŵr Cymru[1]	112.50	17.29	19.68	8.18	11.77	25.55	26.41	500	350
North West	120.00	11.20	9.00	1.30	8.60	25.50	29.20	247	232
Northumbrian	230.00	18.66	9.15	—	—	33.20	37.72	386	187
Severn Trent[2]	86.60	12.53	11.81	—	—	20.11	15.35	351	343
South West	149.00	35.80	32.99	—	6.01	78.09	70.96	744	489
Southern	180.00	24.26	17.70	2.87	15.37	51.70	31.25	452	512
Thames[3]	71.00	6.71	8.25	—	—	24.95	35.87	445	336
Wessex	170.00	22.65	14.65	—	7.57	29.77	37.29	802	313
Yorkshire[4]	202.00	20.48	20.22	—	12.13	21.94	36.00	905	314

Notes:

[1]Dŵr Cymru has a reduced charge R of 11.24p/m^3, where the volume discharged is $> 100,000$m^3 per annum. A fixed charge of £6,050 also applies to discharges $> 100,000$m^3.

[2]Severn Trent Water has a banded charge, $R \leq 49,999$m^3 charged at the standard rate of 12.53p/m^3, then $\geq 50,000$m^3 to $< 250,000$m^3 is charged at 11.49p/m^3 and $\geq 250,000$m^3 at 9.23p/m^3.

[3]Thames Water has a large user trade effluent tariff for customers with an annual bill > £58,000. This includes a fixed charge based on meter size, an annual charge of £10,000 and reception and treatment charges of R = 5.34p/m^3, V = 6.56p/m^3, B = 19.86p/m^3 and S = 28.55p/m^3.

[4]Yorkshire Water has a banded charge, $R \leq 50,000$m^3 charged at the standard rate of 20.48p/m^3, then $> 50,000$m^3 to $\leq 250,000$m^3 is charged at 11.32p/m^3 and $> 250,000$m^3 at 7.72p/m^3. The M charge is calculated as 60% of the V charge.

Table 1.17. Comparison of typical chemical composition of raw wastewaters from the USA and UK.

Parameter	USA (mg l^{-1})	UK (mg l^{-1})
pH	7.0	7.2
BOD	250	326
COD	500	650
TOC	250	173
Total solids	700	—
Suspended solids	220	127
Total nitrogen	40	66
Organic nitrogen	25	19
Ammonia nitrogen	25	47
Nitrite	0	0
Total phosphorus	12	15
Oraganic phosphorus	2	3
Inorganic phosphorus	10	12

sewage is held in the sewer, the greater will be the release of ammonia. The per capita nitrogen production in the United Kingdom is 5.9 g N per day (Painter 1958) which is essentially the same as the American figure (Babbitt 1947). The per capita production of phosphorus is about a third of the weight of nitrogen produced, about 1.4 kg per capita per year. Of this, up to 70% comes from polyphosphate builders used in synthetic detergents (Table 1.17). Detergent polyphosphate builders are slowly being replaced by alternative phosphorus free builders such as zeolites.

The amount of organic matter produced per capita each day expressed in terms of BOD_5 is also known as the population or person equivalent. Population equivalent (PE), expressed in kg BOD_5 per capita per day, is determined as:

$$PE = \frac{\text{mean flow (l)} \times \text{mean } BOD_5 \text{ (mg } l^{-1})}{10^6}$$

Population equivalent is often used in the design of treatment plants, and the volume and strength of industrial wastewaters are normally expressed in terms of equivalent population. In the UK, the PE of domestic sewage is equivalent to 0.055 kg BOD_5 per capita d^{-1}. This ranges from 0.045 kg for an entirely residential area to 0.077 kg for a large industrial city. American figures are similar for domestic sewage, being 0.052 kg for separate sewers and 0.063 kg for combined sewers. However, the recognised design figures

in the USA and Canada are 0.077 kg BOD_5 and 0.10 kg suspended solids. Apart from BOD_5 and suspended solids, it is also common to quote total nitrogen or total phosphorus in terms of PE.

The PE of an industrial wastewater in Britain is calculated using the relationship:

$$\text{PE} = \frac{\text{mean flow } (\text{m}^3\text{d}^{-1}) \times \text{mean BOD}_5(\text{mg l}^{-1})}{0.055 \times 10^3}$$

Similar to flow, the strength and composition of sewage changes on an hourly, daily, and seasonal basis. However, it is the diurnal variation that is usually the greatest. A similar diurnal pattern of sewage strength is formed, in terms of BOD_5 and suspended solids, as occurs with flow (Fig. 1.3). The peak in BOD occurs in the mid morning but, as with the flow, the actual time depends on the length of the sewers and the nature of the area served. The strength of sewage in large cities, with very long and complex sewerage systems, does not fluctuate as widely as it does in smaller catchments with maximum values occurring between 10 p.m. and 6 a.m. (Painter 1971). There is a wide diurnal variation in BOD_5 strength. This variation is also reflected by similar fluctuations in the concentrations of the various carbohydrates and fatty acids that largely make up the biodegradable carbon fraction (Painter 1958). Peaks also occur in the concentrations of ammonia and urea, occurring in the morning and late at night, reflecting the habits of the local population served. However, only the morning peak is generally discernible.

Strength varies from day to day due to the dilution effect of surface and storm water. The daily fluctuation in flow and strength is much less at treatment plants fed by a separate sewerage system. Wastewater volumes are greater on Mondays than any other day in the USA (Heukelekian and Balmat 1959), while the concentration of detergents in the UK has been reported as being higher on Mondays than the rest of the week (Eden and Truesdale 1961). However, this data was collected when automatic washing machines were not widely available and the household laundry for the week was done by necessity on a specific day, usually Monday, so it is unlikely that this trend is still discernible today. Seasonally, sewage strength does not vary significantly, although periods of drought and excessive rainfall can affect the dilution ratio in combined sewerage systems due to reduced infiltration and storm water. In the summer, bacterial concentrations in the wastewater reach a maximum, as do virus concentrations (Painter 1971).

Wastewater can only be treated biologically if sufficient nitrogen and phosphorus are present. Normally, there is a surplus of these nutrients in

sewage for biological needs but it is necessary to assess the treatability of wastewaters by checking the ratio of carbon to nitrogen and phosphorus (C:N:P). The optimum C:N:P weight ratio for biological treatment is 100:5:1 (100 mg l^{-1} BOD_5, 5 mg l^{-1} N, 1 mg l^{-1} P). The C:N:P ratio for raw sewage is approximately 100:17:5 and 100:19:6 for settled sewage, both containing abundant nutrients for microbial growth. The nitrogen requirement of the micro-organisms in the biological unit of a treatment plant is satisfied if the ratio of carbon, measured as BOD_5, to nitrogen equals or is less than 18:1. Even at C:N ratios > 22:1 removal still occurs, but much less efficiently.

Micro-organisms require much lower levels of phosphorus compared with nitrogen so the phosphorus requirement will be met if the C:P ratio is less than 90–150:1. Above 150:1, there is an increasing loss of efficiency (Porges 1960, Hattingh 1963, Komolrit, *et al.* 1967). The exact C:N:P ratio for optimum biological growth depends on the biological process and the form in which the nutrients are available in the wastewater. Where wastes fail to meet the C:N:P criteria, it becomes necessary to add nutrients in order to ensure that biological oxidation occurs. This is often carried out by mixing nutrient deficient wastes with sewage in the correct proportions (Sec. 3.3.2). Wastewaters from the brewing and canning industries are particularly deficient in nitrogen and phosphorus, therefore nutrient addition is required if optimum carbonaceous oxidation is to be achieved. An example of nutrient deficient wastewater is cited by Jackson and Lives (1972) who found that the BOD removal during the treatment of a cider effluent using low-rate percolating filtration was increased from 92 to 99% when the nitrogen and phosphorus balance was corrected by the addition of an inorganic supplement.

Physical properties

Those who have not actually come into contact with raw sewage often harbour rather strange ideas as to what it looks and smells like. By the time it reaches the sewage treatment plant the vast majority of the large solids, such as faeces and paper, have broken up into very small particles. Thus, apart from a small quantity of floatable material, raw sewage is a rather turbid liquid with visible particles of organic material that readily settle out of suspension. The colour is normally grey to yellow-brown, according to the time of day. However, if all the oxygen has been used up during transit in the sewer then the wastewater becomes anaerobic or septic and takes on a much darker colour, and in extreme cases turns black. Municipal

wastewaters receiving industrial wastes containing dyes will take on the colour of the dye present, and at treatment plants receiving effluents from the textile industry in particular, the raw wastewater undergoes spectacular and frequent colour changes. Under ultraviolet light, domestic sewage has a characteristic coloured fluorescence which is due to a variety of minor constituents present in household detergents.

Generally, domestic wastewater has a musty or earthy smell which is not at all offensive, although pungent odours can be produced if the wastewater becomes anaerobic. Certain industrial wastes and contaminants in surface runoff do have distinctive odours. Like a wine connoisseur, the ability of the operator to develop a discerning nose can be extremely helpful in identifying potential problems within the treatment plant and to identify changes in the composition of the sewage entering the plant. The odours produced are usually caused by gases produced by the decomposition of various fractions of the organic matter present in the wastewater. The commonest odour encountered is the smell of rotten eggs caused by hydrogen sulphide, which is produced by anaerobic bacteria reducing sulphate to sulphide. Table 1.18 lists the major categories of odours encountered at sewage treatment plants, although the quantification of such odours for use as a control variable has proved extremely difficult (American Public

Table 1.18. Some characteristic odours produced by compounds present in wastewaters. These degradation products can be categorised into two main groups, either degradation products of nitrogenous or sulphurous compounds. There are other odourous compounds such as those associated with chlorine and phenolic wastes.

Compounds	General formulae	Odour produced
Nitrogenous		
Amines	CH_3NH_2, $(CH_3)_3N$	Fishy
Ammonia	NH_3	Ammoniacal, pungent
Diamines	$NH_2(CH_2)_4NH_2$, $NH_2(CH_2)_5NH_2$	Rotten flesh
Skatole	$C_8H_5NHCH_3$	Faecal, repulsive
Sulphurous		
Hydrogen sulphide	H_2S	Rotten eggs
Mercaptans	CH_3SH, $CH_3(CH_2)_3SH$	Strong decayed cabbage
Organic sulphides	$(CH_3)_2S$, CH_3SSCH_3	Rotten cabbage
Sulphur dioxide	SO_2	Pungent, acidic
Other		
Chlorine	Cl_2	Chlorine
Chlorophenol	$Cl.C_6H_4OH$	Medicinal, phenolic

Health Association *et al.* 1983; Metcalf and Eddy Inc. 1991). Some food processing wastewaters produce extremely strong odours, especially during treatment and storage (Gerick 1984; Gray 1988). For example, sugar beet wastewater undergoes partial anaerobic breakdown within the process and subsequently on storage in lagoons, with the production of a variety of odours. The major odours come from the volatile fatty acids that comprise most of the organic fraction of the effluent. The odour threshold concentrations for the volatile acids produced during treatment of sugar beet are 24.3 ppm for acetic acid, 20.0 ppm propionic acid, 0.05 ppm iso-butyric acid, 0.24 ppm butyric acid, 0.7 ppm iso-valeric acid, and 3.0 ppm for valeric acid. Therefore, by measuring the volatile acid concentration and dividing it by the appropriate odour threshold concentration, a measure of the odour production or concentration known as the odour number can be calculated (Gray 1988). Other odours associated with sugar beet processing include trimethylamine which has a fishy odour and organic sulphides which produce a strong odour of rotting cabbage, as do the thiol compounds methyl mercaptan (CH_3SH) and ethyl mercaptan (C_2H_5SH), both of which have very low odour thresholds of 0.0011 ppm and 0.00019 ppm respectively (Shore *et al.* 1979). A detailed list of odour threshold values has been compiled by Fazzalari (1978).

The temperature of sewage is normally several degrees warmer than the air temperature, except during the warmest months, due to the specific heat of water being much greater than that of air. Sewage temperatures are normally several degrees warmer than the water supply and because of its high conductivity rarely freezes in temperate climates. In the UK, the temperature of raw sewage ranges from 8–12°C in winter to 17–20°C in the summer (Painter 1971). Comparative studies have shown that the variability in the temperature of settled sewage, as it enters the percolating filtration stage at a treatment works in South Yorkshire, is 30–50% less than that recorded for the air temperature, a total range of 11.5°C and 30.4°C respectively. Both the sewage and air temperatures followed similar seasonal patterns, reaching maximum and minimum temperatures during the same periods. However, while the mean daily air temperature varied annually, the mean daily temperature of the sewage remained constant at 12.4°C (Gray 1980). In hotter climates domestic sewage can be much warmer, and in India sewage temperatures of 28–30°C are not unusual (Kothandaraman *et al.* 1963).

The pH of sewage is usually above 7 with the actual value depending largely on the hardness of the supply water. Although extreme values are occasionally encountered, soft water catchments generally have a pH range

of 6.7–7.5 (modal pH = 7.2) and hard water catchments a range of 7.6–8.2 (modal pH = 7.8) (Painter 1971).

Total solids (i.e. the weight of matter remaining after a known volume of wastewater has been evaporated at 105°C) is a commonly used wastewater variable in the USA. It provides a simple characterisation of the wastewater with which the theoretical performance of unit treatment processes can be predicted. Total solids can be classified as either suspended or filterable depending on whether solids will pass through a standard filter (Department of the Environment 1972).

The standard filter used in Britain is a Whatman GF/C filter paper, the GF referring to its glass fibre structure, which has a pore size of 1.0 μm. Therefore, all the filterable solids have a particle size of < 1.0 μm. The suspended solids fraction ranges from colloidal particles < 1.0 μm up to recognisable gross matter. A portion of the suspended solids fraction is settleable, which is measured by measuring the volume of solids that settle out of suspension over a 60 minute period under quiescent conditions. An Imhoff cone, which is an inverted 1 litre conical flask, is used for this purpose and provides a useful estimate of the solids removal and sludge production during primary sedimentation. The filterable fraction contains colloidal and dissolved material. The colloidal solids are particulate ranging from 1 nm–1 μm in size, which are too small to be removed by gravity settlement. An assessment of colloidal solids can be made by measuring the light-transmitting properties of the wastewater, the turbidity, as colloidal matter scatters and absorbs light. The dissolved fraction is made up of both organic and inorganic molecules that are in solution.

Each of these major categories of solids is comprised of both organic and inorganic material, and the ratio of each can be measured by burning off the organic fraction in a muffle furnace at 600°C (Allen 1974). Certain salts are also destroyed by heating, although only magnesium carbonate is decomposed at this temperature being transformed to magnesium oxide and carbon dioxide at 350°C. The major inorganic salt in domestic sewage is calcium carbonate, but this remains stable up to 825°C. The percentage of each solids fraction in a wastewater depends on the chemical composition of the sewage. However, Painter (1971) separated the particulate solids in domestic sewage as approximately 50% settleable (> 100 μm diameter), 30–70% supra-colloidal (1–100 μm) and the remaining 17–20% as colloidal (1 nm–1 μm). However, a more detailed breakdown of the proportion of particular solids fractions in domestic wastewater and the organic strength of each fraction is shown in Table 1.19.

Table 1.19. The organic strength in terms of chemical oxygen demand (COD) of the various solids fractions of sewage (adapted from Rickert and Hunter 1971).

	Total solids		Organic content		COD	
Solids fraction	mg l^{-1}	%	mg l^{-1}	%	mg l^{-1}	%
Settleable	74	15	59	25	120	29
Supra-colloidal	57	11	43	18	87	21
Colloidal	31	6	23	9	43	10
Soluble	351	68	116	48	168	40
Total	513	—	241	—	418	—

Organic properties

Organic matter comprises of carbon, hydrogen and oxygen with nitrogen frequently present. Sulphur, phosphorus, and iron are only occasionally present. In medium strength sewage, 75% of the suspended solids and 40% of the filterable solids fractions are organic. In settled sewage, Painter (1983) estimates that 50% of the organic carbon and between 35–50% of the organic nitrogen is in solution. While three-quarters of the organic carbon can be attributed to the major organic groups carbohydrates, fats, proteins, amino acids and volatile acids, the remainder is made up of other organic molecules such as hormones, vitamins, surfactants, antibiotics, hormonal contraceptives, purines, pesticides, hydrocarbons and pigments. Many of the synthetic organic molecules are non-biodegradable while others are only decomposed biologically at very slow rates. The organic constituents of suspended solids and the filterable fraction of sewage are very different (Table 1.20). Carbohydrates are the largest group in solution in British sewage, with non-volatile and volatile acids, free and bound amino acids and anionic detergents all major constituents. Urea is a major component of urine but is hydrolysed so rapidly to ammonia that it is only found in very fresh sewage. The composition of sewage changes rapidly on storage due to bacterial action with the sugars in particular quickly converted to organic acids. Fats are the major organic constituents in the suspended solids fractions, and together with carbohydrates and proteins account for 60–80% of the organic carbon present.

Most of the naturally occurring amino acids, carbohydrates and organic acids are found in sewage. Of the carbohydrates, glucose, sucrose and lactose are the major ones with smaller proportions of galactose, fructose, xylose and arabinose. Together they account for 90–95% of all the carbohydrate

Table 1.20. Organic constituents of domestic sewage (Painter 1983).

Constitutent	In solution		In suspension	
	Concentration (mg l^{-1})	Proportion as C of total C in solution (%)	Concentration (mg l^{-1})	Proportion as C of total C in suspension (%)
Fats	—	—	140	50
Carbohydrates	70	31.3	34	6.4
Free and bound amino acids	18	10.7	42	10
Volatile acids	25	11.3	12.5	2.3
Non-volatile acids	34	15.2		
Detergents (ABS)	17	11.2	5.9	1.8
Uric acid	1	0.5	—	—
Creatine	6	3.9	—	—
Amino sugars	—	—	1.7	0.3
Amides	—	—	2.7	0.6
Organic carbon				
by direct analysis	90	100	211	100
by addition of above constituents	75.6	84.1	151	71.4

present which is equivalent to 50–120 mg l^{-1}. A diurnal variation in carbohydrate concentration and composition is evident, and although glucose accounts for over 50% of the total carbohydrate content in composite samples, sucrose concentration is greater than glucose in the afternoon. The ratio of hexose to pentose is between 10 and 12. The non-soluble high molecular weight carbohydrates such as starches, cellulose and wood fibre are restricted to the suspended solids fraction resulting in a low hexose to pentose ratio (2.0–2.6) and a concentration of 30–38 mg l^{-1}.

In wastewater terminology, fats is a general term as is lipids or grease, to describe the whole range of fats, oils, and waxes discharged to the sewer. They are among the more stable organic compounds and are not easily degraded biologically. The major source of fats is from food preparation and to a lesser extent excreta, the major sources being butter, lard, margarine, vegetable fats and oil, meat, cereals, nuts and certain fruit. Fats are only sparingly soluble in water and so are only an important component of the suspended fraction of the wastewater, contributing up to 50% of the total carbon present. Normal concentration ranges for fats in domestic wastewater are between 40–100 mg l^{-1}, although this figure is normally higher than that recorded for American sewages. Fats are broken down by hydrolytic action to yield fatty acids and a wide variety of free fatty acids have been reported from sewage, including all the saturated ones from C_8 to C_{14} as well as C_{16}, C_{18} and C_{20}. The major acids include palmitic, stearic and oleic which form between 75–90% of those present. Full details of the fat content of domestic sewage are given by Painter (1971).

Acetic acid is the major volatile acid found in sewage, being recorded at concentrations between 6–37 mg l^{-1}, and together with propionic, butyric and valeric acids make up 90% of the total volatile acidity in wastewater. The acidity of sewage rapidly increases on storage at the expense of sugars and if high concentrations are recorded in fresh sewage, anaerobosis should be suspected. Non-volatile soluble acids are present at concentrations between 0.1–1.0 mg l^{-1}, the commonest being glutaric, glycolic, lactic, citric benzoic and phenyllactic acids.

Proteins are a comparatively important source of carbon in wastewater although they are less important than soluble carbohydrate or fats in suspension. Protein is the principal constituent of all animal and to a lesser extent plant tissue, so waste from food preparation and excreta are both rich in protein such as casein from milk or albumen and gelatine from animal tissue and bone. Apart from containing carbon, hydrogen and oxygen, proteins also contain a fairly high proportion of nitrogen, which is consistent at about 16%. Proteins, apart from urea, are the chief source of nitrogen

in wastewater and supply up to 80% of the total organic nitrogen present. Proteins are made up of long chains of amino acids connected by peptide bonds, and are readily broken down by bacterial action to form free amino acids, fatty acids, nitrogenous compounds, phosphates and sulphides. In wastewater, the free amino acids generally account for < 5 mg N l^{-1}, although this can occasionally be higher, while bonded amino acids in the form of peptides or protein account for between 4–15 mg N l^{-1}.

Apart from amino acids, urea is also a major source of nitrogen in sewage, providing between 2–16 mg N l^{-1}. Urea is most abundant in fresh sewage as it is rapidly converted to ammonia under both aerobic and anaerobic conditions. The rate of conversion to ammonia has been estimated at 3 mg N l^{-1} per hour at 12°C in stored samples (Painter 1958).

Not all biodegradable organic matter found in sewage can be classified into one of the major categories, and some natural compounds are in fact combinations of carbohydrates, proteins and fats such as lipoproteins and nucleoproteins. Of the organic matter in wastewater, 20–40% is essentially non-biodegradable within the treatment plant. However, the actual proportion of non-biodegradable material is normally very small. Fractions such as lignins and cellulose are only slightly degraded due to the limited time for decomposition within the treatment plant, and the absence of many of the specific micro-organisms required for decomposition in normal sewage.

Wastewater can contain an enormous range of specific compounds. It is estimated that some 50,000 dangerous chemicals are currently used within the EU, of which some 4,500 are potential List I substances (76/464/EEC). Therefore, where industrial wastewaters are discharged to sewer, they require careful characterisation and pretreatment as necessary (Howard 1990, 1991; EPA 1998). Although chemicals with a low vapour pressure, high adsorptivity onto solids, or a high solubility in water, are unlikely to vaporise and so become concentrated in the air space within the sewer, those chemicals showing the opposite characteristics are likely to vaporise and possibly cause explosions. Oestrogen mimicking compounds are currently causing much concern, as they have been shown to disrupt the endocrine systems of humans and wildlife (Dempsey 1998; Fawell and Chipman 2001). These compounds exhibit a wide chemical diversity and are defined more by their biological function than their composition. Natural animal oestrogen hormones (e.g. oestrone, 17ß - oestradiol) and synthetic oestrogen (ethinyl oestradiol) are the most oestrogenically active chemicals found in wastewater. Other Oestrogen mimicking compounds include: organochlorine pesticides (e.g. PCBs), organotins and dioxins. Alkylphenol ethoxylates (APEs) which are widely used in industrial detergents, paint formulations and metal

finishing are also common. Some 18,000 tonnes of APEs are used annually in the UK; phytoestrogens and phytosterols which are naturally found in certain trees and plants and are strongly associated with wood pulp effluents; and finally, some phthalates which are ubiquitous in the environment. They are used as plasticers in adhesives, food wrapping and packaging. Bisphenol-A is a plastic manomer used in food and drink packaging, including the protective coating in food cans.

Sewage contains a diverse range of organisms which originate not only from faeces, but also from soil and water. They include viruses, bacteria, fungi, protozoans and a variety of other groups of organisms. Many of these organisms are pathogenic to man and for this reason are discussed fully in Chap. 9.

Inorganic properties

There is a substantial inorganic component in sewage, especially compounds continuing sodium, calcium, potassium, magnesium, chlorine, sulphur (as sulphates and other forms), phosphates, bicarbonates, and ammonia. Traces of heavy metals are also found. The inorganic content of domestic wastewater depends on the geology of the catchment from which the water supply originated, (natural water dissolves minerals from the surrounding rocks and soil of the area), and on the nature of the polluting material itself. This is vividly illustrated by comparison of sewages from a soft and hard water area (Table 1.21). In hardwater catchments, the calcium, sodium, and chloride, ions are significantly more concentrated in the supply water, which is reflected in the resulting wastewater. Domestic wastewater contains a very wide range of inorganic salts and trace elements, including all those necessary for biological growth and activity. When trace elements are limited (e.g. Ca, Mg, Fe), then biological treatment efficiency will be reduced (Sec. 3.3.2). Among the major ions in wastewater which are worthy of further discussion are chloride, nitrogen, and phosphorus.

Chloride is found naturally in water due to leaching, but it also originates from a wide variety of agricultural, industrial and domestic sources. While infiltration by groundwater contaminated with salt-water into the sewer is a major source of chloride and sulphate in some areas, a major seasonal source at treatment plants served by combined sewerage systems is from road runoff during salting operations in icy weather. In hardwater areas, the widespread use of water softeners can result in significant increases in the wastewater chloride concentrations. Without these additional sources, sewage normally contains between 30–100 mg l^{-1} of chloride:

Table 1.21. Concentration of major inorganic constituents of domestic sewage (Painter 1971).

Constituent	Whole sewage USA Soft water area (mg l^{-1})	Settled sewage UK Hard water area (mg l^{-1})
Cl	20.10	68.00
Si	3.90	—
Fe	0.80	0.80
Al	0.13	—
Ca	9.80	109.00
Mg	10.30	6.5
K	5.90	20.00
Na	23.00	100.00
Mn	0.47	0.05
Cu	1.56	0.2
Zn	0.36	0.65
Pb	0.48	0.08
S	10.30	22.00
P	6.60	22.00

human excreta contains 6 g Cl per capita per day and urine contains 1% chloride. As chloride is not removed to any great extent by conventional treatment, the detection of higher concentrations in surface waters may indicate that they are being used for wastewater disposal.

Nitrogen and phosphorus are both essential nutrients for plant growth. Nitrogen is also essential for the synthesis of protein and so biological growth generally. In fresh wastewater, nitrogen is primarily present as proteinaceous matter and urea. This organic nitrogen is rapidly decomposed by bacterial action in the case of proteins or by hydrolysis in the case of urea to ammonia, the concentration of which in wastewater is indicative to some extent of its age. Ammonia N exists in aqueous solution as either the ammonium ion (NH_4^+) or as ammonia (NH_3), depending on the pH of the wastewater. At pH values of > 7, the equilibrium of the reaction:

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^-$$

is displaced to the left so that ammonia predominates and at pH values < 7, equilibrium moves to the right and ammonium predominates (Fig. 1.4). Organic nitrogen is normally measured separately from ammonia, although occasionally they are expressed together as the kjeldhal nitrogen. The normal concentration range of nitrogen in settled sewage in Britain is 41–53 mg

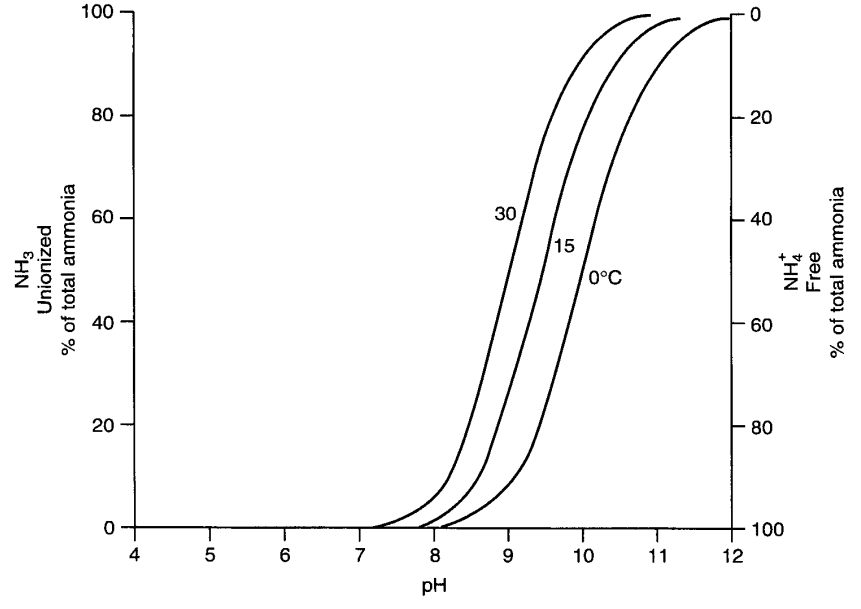


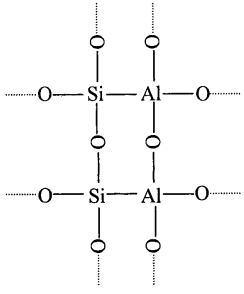
Fig. 1.4. The general variation between the proportion of unionised to free ammonia at varying pH and temperatures (Chapman 1996).

N l^{-1} as ammonia, 16–23 mg N l^{-1} as organic nitrogen and 57–76 mg N l^{-1} as kjeldhal nitrogen (Painter 1971). The oxidised forms of ammonia, nitrite and nitrate, are normally absent from fresh sewage being products of the biological oxidation processes within the treatment plant. Therefore, as the total nitrogen includes all chemical forms of nitrogen, the kjeldhal nitrogen can be assumed to be equivalent to the total nitrogen in raw and settled sewage.

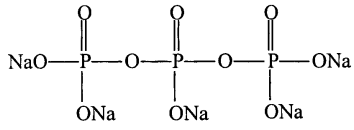
Phosphorus is present in sewage in three distinct forms, as orthophosphate, polyphosphate, and organic phosphate. Organic phosphate is a minor constituent of sewage and like the polyphosphates requires further decomposition to the more assimilable orthophosphate form, which is normally fairly slow. About 25% of the total phosphorus in settled sewage is present as orthophosphates, such as PO_4^{3+}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4, which are available for immediate biological metabolism. Therefore, in terms of utilisation both in the treatment plant and subsequently in receiving waters, it is the inorganic phosphate concentration that is important rather than the total phosphorus concentration. After secondary treatment, about 80% of the total phosphorus in a final effluent is in the orthophosphate form. Average phosphorus concentrations in sewage range from 5–20 mg P l^{-1}

as total phosphorus, of which 1–5 mg P l^{-1} is the organic fraction and the rest inorganic.

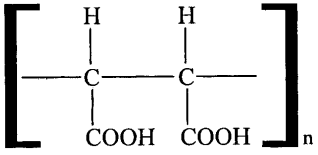
Since 1965, legislation in the USA and a voluntary ban in Britain has seen a steady reduction in the use of 'hard' or non-biodegradable alkyl-benzene-sulphonate (ABS) detergents in favour of 'soft' biodegradable linear-alkyl-sulphonate (LAS) detergents. The ABS detergents were responsible for persistent foaming problems at both sewage treatment plants and in receiving waters (Klein 1972b). Detergents are made up of a number of compounds, each with a specific function during the washing process (Broze 1999). All detergents vary in their specific formulation, although all generally contain the basic functional groups of compounds: surfactant (e.g. linear alkyl benzene sulphonate) 3–15%; builder (e.g. sodium tripolyphosphate) 0–30%; ion-exchanger (e.g. zeolite A) 0–25%; antiredeposition agent (e.g. polycarboxylic acids) 0–4%; bleaching agent (e.g. sodium perborate) 15–35%; bleach stabilizer (e.g. phosphonate) 0.2–1.0%; foam booster (e.g. ethanolamide) 1–5%; enzyme (e.g. protease) 0.3–1.0%; optical brightener (e.g. pyrazolan derivatives) 0.1–1.0%; corrosion inhibitor (e.g. sodium silicate) 2–7%; and a fragrance 0.05–0.3%. However, to increase the washing ability of LAS detergents, 'builders' in the form of sodium tripolyphosphate (STPP), are included. Their function is to remove hardness, which interferes



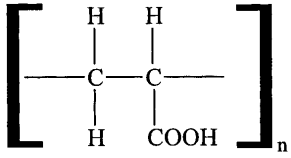
[Zeolite]



[Sodium tripolyphosphate]



[Polymaleic]



[Polyacrylic acid]

Fig. 1.5. Chemical structures of common detergent builders.

with the action of the surfactant, buffer the water to ensure optimum alkaline washing conditions, and to emulsify soils and prevent their redeposition. Sodium tripolyphosphate is made from phosphoric acid, neutralised with caustic soda or soda ash to make either monosodium orthophosphate (NaH_2PO_4) or disodium orthophosphate (Na_2HPO_4). The molecular water is then removed in a kiln to yield STPP ($Na_5P_5O_{10}$) (Fig. 1.5) (Morse *et al.* 1994). These particular phosphates are highly unstable and readily break down to super-phosphates within the treatment plant, which has resulted in growing problems of high phosphate concentrations in the final effluents being discharged to surface waters. In order to reduce the level of phosphates in the environment, certain countries have introduced legislation to control the use of STPP in detergent formulations. For example, the Irish Government have implemented a four year plan to phase out the use of phosphate-based detergents by the end of 2002 (DOELG 1999). However, phosphate comes from a variety of sources (Table 1.22) and it is unlikely that replacing detergent phosphates will solve eutrophication on its own (Morse *et al.* 1995). In Ireland, almost 75% of phosphorus inputs to lakes and rivers come from agriculture (EPA 2000). Initially, organic builders were used to replace STPP, the most efficient being nitrilotriacetic acid (NTA), polyacrylic acid (PAA), polymaleicacid (PMA) and polycarboxylic

Table 1.22. Phosphate sources in Europe in 1992 as the percentage from each source and total in tonnes per annum (Morse *et al.* 1993).

Source (%)	Human	Detergents	Livestock	Fertilisers	Industry	Background	Total
Member State							
Austria	20	10	36	16	6	12	13
Belgium	26	11	43	7	8	5	13
Denmark	12	11	55	11	5	6	15
Finland	18	9	17	15	3	38	9
France	18	15	31	19	6	11	106
Germany	28	3	44	12	6	7	97
Greece	21	7	18	34	5	15	17
Ireland	9	7	49	24	2	9	15
Italy	35	2	26	18	8	11	56
Netherlands	23	3	57	9	5	3	24
Portugal	24	14	27	16	7	12	15
Spain	19	16	18	26	7	14	72
Sweden	21	10	15	14	7	33	14
UK	24	19	29	14	8	6	82
EU Total	23	11	32	17	7	10	548

acids (PCAs) (Hunter *et al.* 1988) (Fig. 1.5). Although initially thought to be a benign alternative to STPP, problems about the toxicity of these organic builders were quickly identified (Anon 1994; Rand 1995). For that reason, zeolites have been widely adopted as an alternative builder to STPP, and are used in conjunction with polycarboxylates and sodium carbonate. Zeolite A ($Na{-}2OAl_2O_3 \cdot 2SiO_2 \cdot 4.5H_2O$) has a three dimensional framework of AlO_4 and SiO_4 tetrahedra linked by the oxygen atoms they share (Fig. 1.5). Its internal surface area is many times larger than the external surface area, allowing for greater sequestration of hardness ions than initially appears possible. However, it appears that zeolite based detergents are less efficient than STPP based detergents, with more of the former needed each wash to obtain the same performance as the latter detergent formulation (Wilson and Jones 1995). Zeolites increase the solids concentration in sewage sludge by up to 30% and improve activated sludge settling qualities; although, filterability deteriorates and the aluminium concentration increases. Overall zeolites do not appear to affect treatment efficiency or encourage filamentous bacterial development (Piirtola *et al.* 1998), although some environmental concerns have been raised (Wilson and Jones 1995).

Sulphur is another essential element in the metabolism of all organisms. However, most micro-organisms only require small amounts of the element which is used in the synthesis of the amino acids cysteine and methionine, which are found in protein. Nearly all the requirement for sulphur comes from sulphate. Trace quantities of all the metals necessary for biological growth are present in sewage. Those metals which are particularly toxic in excessive concentrations and which are common in sewage are nickel, lead, chromium, cadmium, zinc, and copper. Vacker *et al.* (1967), Heukelekian and Balmat (1959), and Painter (1958) have provided mean values of the most important metals in sewage and mean values using this data, which were collected from very different catchments, have been given by Gould (1976). This data, and metal concentrations collected from a domestic sewage treatment works in South Yorkshire, are compared in Table 1.23. The data from South Yorkshire has much lower concentrations of chromium and copper as it is a purely domestic sewage, however the high iron and lead concentrations are due to mine water infiltration and runoff from the nearby M1 motorway respectively. There has been a recent increase in the use of antiseptic creams and hair shampoos which are rich in zinc and this has led to a significant increase in the zinc concentration of sewage.

Sewage remains aerobic as long as it is not permitted to stand. Normal dissolved oxygen concentrations in flowing sewage are usually in the order of

Table 1.23. Variation of metals found in domestic sewage.

Metal	Gould (1976) (mg l^{-1}) (mean)	Gray (1980) (mg l^{-1}) (range)
Cd	0.02	0.00–0.05
Co	< 0.02	0.00–0.01
Cr	0.4	0.00–0.1
Cu	0.88	0.00–0.16
Fe	0.8	0.15–1.30
Mn	0.2	0.01–0.02
Ni	< 0.02	0.00–0.33
Pb	0.25	0.01–1.78
Zn	0.50	0.05–0.84

1–2 mg l^{-1}. However, long retention times in sewers should be avoided and, where this is not possible, then re-aeration, normally by oxygen injection, should be included in sewer design. Hydrogen sulphide should not be present in sewers if they are properly vented. However, if blockages occur or the flow falls to below 0.52 ms^{-1} then anaerobic conditions can develop and hydrogen sulphide be given off (Sec. 1.3.1).

1.2.3. *Other wastewaters*

The other wastewaters rich in organic materials and so readily degraded biologically are the agricultural and food processing wastes. Of particular importance at present, and this varies according to agricultural practice and manufacturing processes, are wastes from intensive animal rearing, silage production, food processing, and the dairy industry.

Animal wastes

Specialisation and the development of new methods in agriculture has led to the intensification of animal rearing and a departure from the traditional farming practice of returning all wastes back to the land as fertilizer, which avoided pollution and treatment. The adoption of intensive farm practices can lead to enormous numbers of animals being kept in comparatively small areas. For example, Lynch and Poole (1979) cite an American situation where 35,000 cattle were kept in a feed lot of less than one square mile, whereas some farm animals such as pigs and poultry are now raised

almost exclusively indoors in specially constructed units. Sewage is comparatively weak compared to most animal wastes which have very high BOD_5 concentrations (Table 1.24). The characteristics of animal wastes has been extensively reviewed by Evans *et al.* (1978; 1980). Most farm animals produce large quantities of waste each day compared to man, and in terms of mean population equivalents based on the BOD_5 where a man = 1.0, then a cow = 16.4, a horse = 11.2, a pig = 3.0, a sheep = 2.45, and a chicken = 0.014 (Gloyna 1971). Another problem is that the waste has a high solids content and thus, unlike sewage, is not a liquid but either a semi-liquid or semi-solid (Fig. 1.6). It is, therefore, difficult to handle or pump unless dewatered or diluted respectively. For many farmers, the limiting factor in the development of intensive animal rearing is the disposal of the increased amounts of animal waste. In Britain, the population of farm animals in 1978 was approximately 3 million cows, 9 million cattle, 7 million pigs and 130 million poultry. Their waste in terms of population equivalents was of the order of 30 million, 50 million, 17 million and 13 million respectively, which is almost twice the organic load produced by the human population in Britain (Weller and Willetts 1977). It is neither feasible nor desirable to discharge this quantity or type of waste to the public sewer. First, because of the vast volume of dilution water required to reduce the BOD to treatable levels by conventional wastewater treatment methods, and secondly because of the cost of increasing the treatment capacity of existing works by 200%. The strength of animal wastes compared to sewage and other agricultural wastewaters (Table 1.24) must be seen in relation to the dilution of the effluent. The daily volume of effluent produced by the major categories of animals being dairy cow 0.0445 m^3, beef cattle 0.0198 m^3, sow 0.0117 m^3, fat pig 0.0049 m^3, and poultry 0.0001 m^3 (Gowan 1972). A particular problem with animal wastes is the enhanced metal concentrations that are often present. Concentrated feeds used for fattening pigs in intensive rearing units contain high concentration of metals, especially copper and zinc. Research on a range of commercial pig foods has shown that these metals are present at concentrations ranging from 116–233 ppm dry weight for copper and 194–300 ppm for zinc. However, little of the metals is retained by the animals with between 70–80% of the copper and 92–96% of the zinc being excreted (Table 1.25). Effluents from intensively reared stock which are fed concentrates, and in particular pigs, will inevitably contain high concentrations of metals (Priem and Maton 1980), as well as other additives such as antibiotics.

Table 1.24. Average volume, strength, and nutrient content of animal wastes.

Animal	Volume of waste per adult animal ($m^3\ d^{-1}$)	COD ($mg\ l^{-1}$)	BOD ($mg\ l^{-1}$)	N ($kg\ tonne^{-1}$)	P ($kg\ tonne^{-1}$)	K ($kg\ tonne^{-1}$)	Moisture content (%)
Cow	0.0500	150000	16100	11.1	4.5	13.4	87
Pig	0.0045	70000	30000	8.9	4.5	4.5	85
Poultry	0.0001	170000	24000	38.0	31.3	15.6	32–75[a]

[a]Depending on housing.

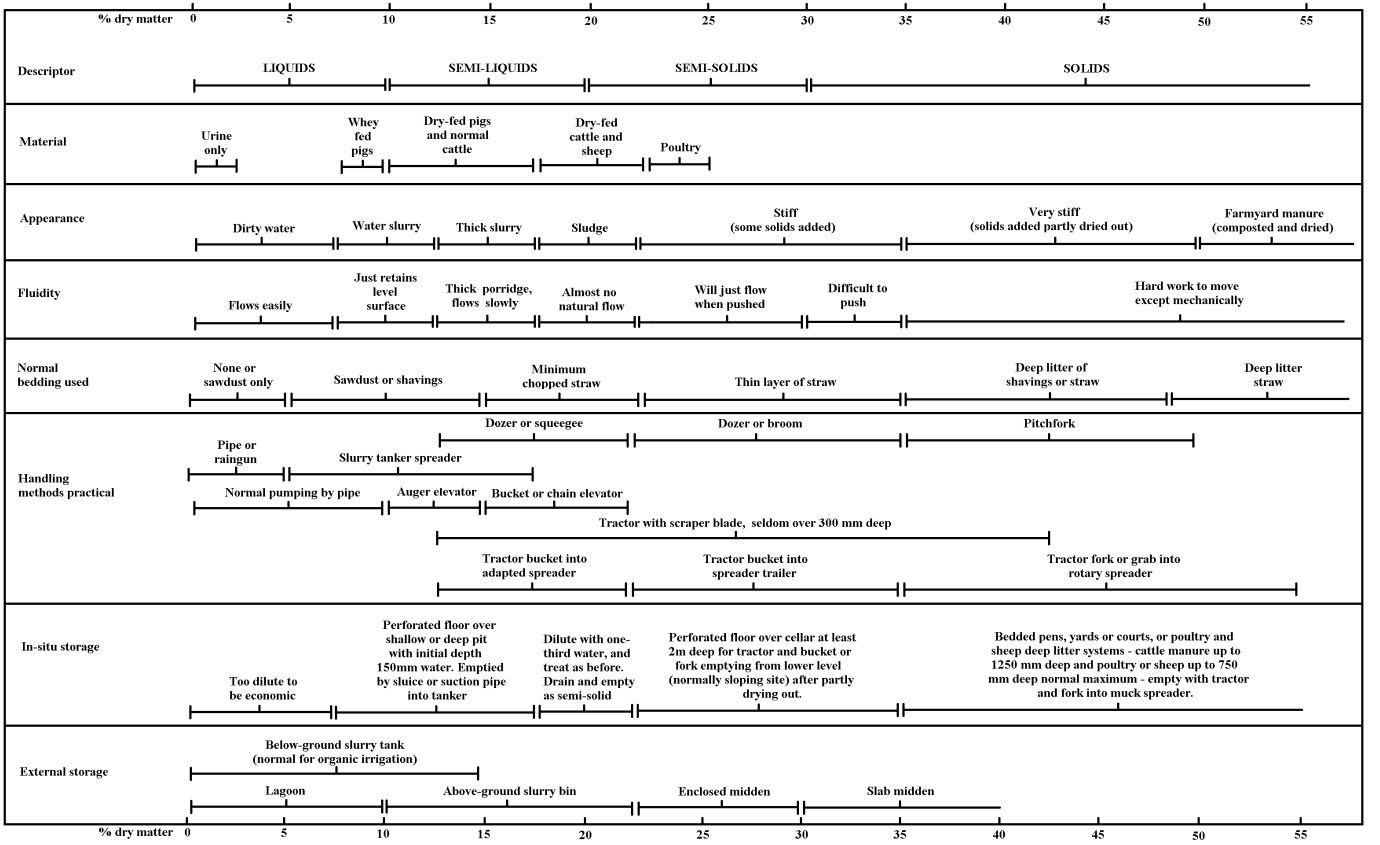


Fig. 1.6. Definition, handling, and storage of livestock effluent (Weller and Willets 1977).

Table 1.25. Average composition of the liquid manure from intensively reared pigs fed on commercial food concentrates rich in metals. Feed A, B and C contained 116, 233 and 189 ppm dry weight of copper and 194, 300 and 260 ppm dry weight of zinc respectively (adapted from Preim and Maton 1980).

	A		B		C	
	$\bar{x}$	S.D.	$\bar{x}$	S. D.	$\bar{x}$	S.D.
Dry matter (%)	16.2	1.19	15.9	0.75	14.6	2.14
Ash in dry matter (%)	23.9	2.20	24.6	1.79	25.6	2.48
BOD (mg l^{-1})	41807	1293	41967	2504	35546	5253
COD (mg l^{-1})	163539	26051	154026	15867	143178	19997
P (mg l^{-1})	3548	546	3491	360	3294	838
Ammonia nitrogen (mg l^{-1})	6606	1343	6351	1097	5774	1209
Kjeldhal nitrogen (mg l^{-1})	10345	1272	9998	1000	9083	1367
Cu (ppm in dry matter)	416	44.1	859	68.2	754	66.3
Zn (ppm in dry matter)	851	74.5	1385	107.4	1180	31.2
As (ppm in dry matter)	8.50	0.83	10.93	1.76	11.13	0.87
Se (ppm in dry matter)	0.63	0.05	1.57	0.17	1.08	0.08

Silage liquor

Ensiling is a large-scale microbial process in which cut grass is degraded anaerobically so that the complex cellulose component is broken down into simpler organic acids, preserving the grass as food for cattle by raising the pH. The effluent (silage liquor) from the clamp in which the grass is stored is a mixture of surface water and plant juices from the ensiled herbage. It has a pH of 4.5 or less and is composed mainly of organic acids, in particular lactic, acetic, propionic, and butyric, which are very readily broken down biologically at a rate about four times faster than sewage, thus making it up to 1,000 times more potent as a pollutant (Patterson 1981). Thus, with an average BOD of 30,000–80,000 mg l^{-1} (Beck 1989), discharges of the acidic liquor to watercourses lead to whole-sale destruction of all aerobic life. Silage liquor is also rich in nitrogen and can contain up to 2.5 g of nitrogen per litre of liquor (Weller and Willetts 1977). The dry matter content of silage liquor ranges from 4–10% (mean value of 6.5%). The average composition of the liquor as a percentage of the dry matter is crude protein 25.0%, ash 22.0%, lysine 1.0%, calcium 2.2%, phosphorus 1.0%, nitrogen-free extract 53.0%, lactic acid 25.0%, and volatile fatty acids 5.5% (Patterson 1980). The amount of liquor produced by silage is closely related to moisture content of the grass at the time of ensiling with the

quantity of effluent per tonne of silage being 360–450 l^{-1} at 10–15% dry matter, 90–225 l at 16–20%, and less than 90 l at dry matters of 25% and over (Gibbons 1968). The variation in the volume of effluent produced is also dependant on rainfall. Thus, the percentage of agricultural related water pollution incidents due to silage liquor varies from 14% in a dry year to 25% in a wet year (Haigh 1994). Between 1995 and 1998, there were on average 153 silage liquor related pollution incidents annually (range 114–234) in England and Wales (Environment Agency 1999). Acidic additives, used to preserve the silage, can increase the volume of liquor produced by up to 25%, although finely chopping silage does not have an effect on effluent production. While the volume of liquor can be greatly reduced by wilting the grass in the field before ensiling, occasionally wilting is not possible due to time or weather making the disposal of liquor a serious problem. It is not generally advisable to dispose of these effluents to the public sewer as small sewage works can be put out of action due to the resulting toxic shock. Such wastes are generally stored with the animal wastes in slurry tanks or stored in specially constructed acid-resistant tanks (O'Donnell *et al.* 1995a,b) and returned to the land when possible (Burford 1976). Some research has been undertaken on feeding fresh silage liquor to pigs (Patterson and Kilpatrick 1991), although this has not been widely adopted. Approximately 42.3×10^6 tonnes of forage crops were cut for silage in the UK during 1998, producing an estimated 2.12×10^9 litres of liquor (MAFF 1999).

Dairy industry

Milk production has steadily grown over the past 30 years such that the dairy industry has now become the major agricultural processing industry in Europe.

Wastewater originates from two major processes, from the fluid milk itself at reception and bottling plants but more importantly at the processing plants that produce butter, cheese, evaporated and condensed milk, milk powder and other milk products. Milk itself has a BOD_5 of 100,000 mg l^{-1} and washings from plants producing butter and cheese can have a BOD_5 ranging between 1,500–3,000 mg l^{-1}. Dairy wastes are dilutions of whole milk, separated milk, butter milk and whey. They are high in dissolved organic matter mainly in the form of the proteins (3.8%) casein and albumin, fat (3.6%) and lactose (4.5%) but low in suspended solids except for the fine curd found in cheese wastes. Nitrogen and phosphorus are also present, originating mainly from milk proteins (Guillen-Jimenez *et al.* 2000). Apart from whey, derived from the manufacture of cheese which is acidic, most

dairy wastes are neutral or slightly alkaline but have a tendency to become acidic quite rapidly due to the fermentation of lactose to lactic acid. The average composition of milk, milk by-products and cheese wastes are given in Table 1.26. Details of the various processes used in the dairy industry, with specific reference to wastewater production, are given by Nemerow and Agardy (1998).

Food processing industries

Waste from food processing is similar in nature to the food itself. Some processes give rise to large volumes of weakly polluted effluents such as vegetable washing water, which only contains soil and small amounts of organic matter. More concentrated wastewaters come from processes that either prepare the food or transform it in some way, such as the blanching of vegetables or pickling of meat. Generally, these wastes are rich in organic matter and normally contain sufficient nitrogen, phosphorus and trace elements for biological growth. The volume and strength of wastewater from food processing greatly depends on the type of process, the size and age of the plant as well as the season.

Cannery wastewaters are essentially the same as domestic kitchen waste. The waste originates from trimming, culling, juicing and blanching fruit and vegetables. The wastewaters are high in suspended solids, colloidal and dissolved organic matter, the main components being starch and fruit sugars. For example, 85–90% of the organic waste from a pineapple cannery is sugar in the form of sucrose (Painter 1971). Details of these wastes are summarised by Nemerow and Agardy (1998). Sugar beet waste is also comprised of sugars, 95% of which is sucrose with raffinose making up most of the remainder, although the waste is particularly low in nitrogen and phosphorus. The sugars are leached from cut and damaged surfaces into the transport and wash-water circuits, so that the BOD of these wastes can be as high as 8,000–10,000 mg l^{-1} (Table 1.27). The accumulated sugars are rapidly catabolised in the circuits to short chain aliphatic carboxylic acids, principally acetic, propionic, and butyric acids, so that the wastewater requiring treatment is comprised almost exclusively of these acids. However, at low pH concentrations offensive odours from volatile fatty acids and sulphides can be generated, and sufficient lime (CaO) must be added to maintain circulating water at a neutral pH (Shore *et al.* 1984; Gray 1988). Brewery and distillery wastewaters are high in dissolved solids which contain nitrogen and fermented starches and their products. Fermentation wastes and in particular spent yeast is extremely concentrated with

Table 1.26. Composition and organic strength of milk products and associated waste products (Nemrow 1978).

Characteristics	Whole milk (mg l^{-1})	Skim milk (mg l^{-1})	Buttermilk (mg l^{-1})	Whey (mg l^{-1})	Process wastes (mg l^{-1})	Separated whey (mg l^{-1})
Total solids	125000	82300	77500	72000	4516	54772
Organic solids	117000	74500	68800	64000	2698	49612
Ash solids	8000	7800	8700	8000	1818	5160
Fat	36000	1000	5000	4000		
Soluble solids					3956	54656
Suspended solids					560	116
Milk sugar	34000	46000	43000	44000		
Protein (casein)	38000	39000	36000	8000		
Total organic nitrogen					73.2	1300
Free ammonia					6.0	31
Na					807	648
Ca					112.5	350
Mg					25	78
K					116	1000
P					59	450
BOD_5	102500	73000	64000	32000	1890	30100
Oxygen consumed	36750	32200	28600	25900		

Table 1.27. Comparative strengths of wastewaters from food-processing industries.

	BOD (mg l^{-1})	COD (mg l^{-1})	PV (mg l^{-1})	Suspended solids (mg l^{-1})	pH	Population equivalent per m^3 of waste
Brewery	850	17000	—	90	4–6	14.2
Cannery						
citrus	2000	—	—	7000	Acid	33.3
pea	570	—	—	130	Acid	9.5
Dairy	600–1000	—	150–250	200–400	Acid	10.0–16.7
Distillery	7000	10000	—	Low	—	116.7
Farm	1000–2000	—	500–1000	1500–3000	7.5–8.5	16.7–33.3
Silage	50000	—	12500	Low	Acid	833.3
Potato processing	2000	35000	—	2500	11–13	33.3
Poultry	500–800	600–1050	—	450–800	6.5–9.0	8.3–13.3
Slaughterhouse	1500–2500	—	200–400	800	7	25.0
Sugar beet	450–2000	600–3000	—	800–1500	7–8	7.5–33.3

the BOD (2,000–15,000 mg l^{-1}), total nitrogen (800–900 mg l^{-1}) and phosphate (20–140 mg l^{-1}), almost entirely present in the dissolved or colloidal fractions with the suspended solids content rarely in excess of 200 mg l^{-1}. Slaughterhouse and meat packing wastewaters are strong and unpleasant, being comprised of faeces and urine, blood washings from carcasses, floors and utensils, and the undigested food from the paunches of slaughtered animals. These wastewaters are high in dissolved and suspended organic matter, in particular proteins and fats, high in organic nitrogen and grease, as well as pathogens.

The strengths and volumes of wastewaters from the main food-processing industries are summarised in Table 1.27.

1.3. Micro-organisms and Pollution Control

Micro-organisms have a number of vital functions in pollution control. It is the microbial component of aquatic ecosystems that provides the self-purification capacity of natural waters in which micro-organisms respond to organic pollution by increased growth and metabolism (Sec. 1.4.1). It is essentially the same processes which occur in natural waters that are

utilised in biological treatment systems to treat wastewater. Apart from containing food and growth nutrients, wastewater also contains the micro-organisms themselves, and by providing a controlled environment for optimum microbial activity in a treatment unit or reactor, nearly all the organic matter present can be degraded (Chap. 3). Micro-organisms utilise the organic matter for the production of energy by cellular respiration and for the synthesis of protein and other cellular components in the manufacture of new cells. This overall reaction of wastewater treatment can be summarised as:

$$\text{Organic matter} + O_2 + NH_4^{2+} + P \longrightarrow \text{New cells} + CO_2 + H_2O$$

Similar mixed cultures of micro-organisms are used in the assessment of wastewater and effluent strength by the biochemical oxygen demand test (BOD_5), in which the oxygen demand exerted by an inoculum of micro-organisms growing in the liquid sample is measured over five days to give an estimate of the oxidisible fraction in the wastewater (Sec. 1.4.2). Many diseases are caused by waterborne micro-organisms, a number of which are pathogenic to man. The danger of these diseases being transmitted via wastewater is a constant threat to public health (Chap. 9). Therefore the use of micro-organisms, such as *Escherichia coli*, as indicator organisms to assess the microbial quality of water for drinking, recreation and industrial purposes, as well as in the assessment of wastewater treatment efficiency is an essential tool in pollution control (Sec. 9.2).

1.3.1. *Nutritional classification*

In wastewater treatment, it is the bacteria that are primarily responsible for the oxidation of organic matter. However, fungi, algae, protozoa (collectively known as the Protista) and higher organisms all have important secondary roles in the transformation of soluble and colloidal organic matter into biomass, which can be subsequently removed from the liquid by settlement prior to discharge to a natural watercourse. In order to function properly, the micro-organisms involved in wastewater treatment require a source of energy and carbon for the synthesis of new cells as well as other nutrients and trace elements. The micro-organisms are classified as either heterotrophic or autotrophic according to their source of nutrients. Heterotrophs require organic matter both for energy and as a carbon source for the synthesis of new micro-organisms, whereas autotrophs do not utilise organic matter but oxidise inorganic compounds for energy and use carbon dioxide as a carbon source.

Heterotrophic bacteria, which are also referred to as saprophytes in older literature, utilise organic matter as a source of energy and carbon for the synthesis of new cells, respiration and mobility. A small amount of energy is also lost as heat during energy transfer reactions. The heterotrophs are subdivided into three groups according to their dependence on free dissolved oxygen.

Aerobes require free dissolved oxygen in order to decompose organic material:

$$\text{Organics} + \text{O}_2 \xrightarrow{\text{aerobic micro-organisms}} \text{Aerobic micro-organisms} + \text{CO}_2 + \text{H}_2\text{O} + \text{energy}$$

Like all microbial reactions it is autocatalytic, that is the micro-organisms that are required to carry out the reaction are also produced. Aerobic bacteria predominate in natural watercourses and are largely responsible for the self-purification process (Sec. 1.4.1). They are also dominant in the major biological wastewater treatment processes such as activated sludge and percolating filtration. Aerobic processes are biochemically efficient and rapid in comparison with other types of reactions, producing by-products that are usually chemically simple and highly oxidised such as carbon dioxide and water.

Anaerobes oxidise organic matter in the complete absence of dissolved oxygen by utilising the oxygen bound in other compounds such as nitrate:

$$\text{Organics} + \text{NO}_3 \xrightarrow{\text{anaerobic micro-organisms}} \text{Anaerobic micro-organisms} + \text{CO}_2 + \text{N}_2 + \text{energy}$$

or sulphate:

$$\text{Organics} + \text{SO}_4^{2-} \xrightarrow{\text{anaerobic micro-organisms}} \text{Anaerobic micro-organisms} + \text{CO}_2 + \text{H}_2\text{S} + \text{energy}$$

Anaerobic bacterial activity is found in freshwater and estuarine muds rich in organic matter and in the treatment works in the digestion of sludge. Anaerobic processes are normally biochemically inefficient and generally slow, giving rise to chemically complex by-products which are frequently foul-smelling (Chap. 7). The end products of proteins, carbohydrates, and fats which have undergone microbial breakdown under anaerobic and aerobic conditions are summarised in Table 1.28. Facultative bacteria use free dissolved oxygen when available but in the absence of oxygen are able to

Table 1.28. End-products of the aerobic and anaerobic microbial breakdown of the major organic substrates found in sewage (Berthouex and Rudd 1977).

Substrates	+	Enzymes of micro-organisms	→	Representative end-products (Anaerobic conditions)	Representative end-products (Aerobic conditions)
Proteins and other organic nitrogen compounds		Enzymes of micro-organisms		Amino acids	Amino acids
				Ammonia Hydrogen sulphide Methane Carbon dioxide	Ammonia→nitrites→nitrates Hydrogen sulphide→sulphuric acid Alcohols, Organic acids → CO_2+H_2O
				Hydrogen Alcohols Organic acids Phenols Indol	
Carbohydrates		Enzymes of micro-organisms		Carbon dioxide Hydrogen Alcohols Fatty acids Neutral compounds	Alcohols, Fatty acids → CO_2+H_2O
Fats and related substances		Enzymes of micro-organisms		Fatty acids + glycerol Carbon dioxide Hydrogen Alcohols Lower fatty acids	Fatty acids + glycerol Alcohol, Lower fatty acids → CO_2+H_2O

gain energy anaerobically and so are known as facultative aerobes. An example of a facultative bacterium is *E. coli*, a common and important coliform, this and other such bacteria are common in both aerobic and anaerobic environments and treatment systems. Often, the term obligate is used as a prefix to these categories of heterotrophic bacteria to indicate that they can only grow in the presence (obligate aerobe) or absence of oxygen (obligate anaerobe).

Using these basic reactions as guides, it is possible to write balanced equations for the utilisation of the organic substrate and the synthesis of new micro-organisms. For example, using glucose as the organic substrate and the formulae $C_5H_7O_2N$ to represent the composition of the organisms, equations for respiration and the production of energy for cell maintenance and synthesis can be written:

$$\text{Respiration: } C_6H_{12}O_6 + O_2 \longrightarrow 6CO_2 + H_2O + \text{energy}$$

while the equation for synthesis of new micro-organisms is:

$$\text{Synthesis: } C_6H_{12}O_6 + NH_4^+ \longrightarrow C_5H_7O_2N + 3H_2O + H^+$$

These two 'half-reactions' can be combined to give the basic organic transformation reaction brought about by aerobic micro-organisms in biological wastewater treatment plants and which is discussed in more detail in Sec. 3.1.

$$C_6H_{12}O_6 + 0.5NH_4^+ \longrightarrow C_5H_7O_2N + 3.5CO_2 + 5H_2O + O.5H^+$$

Bacteria are comprised of 80% water and 20% dry matter. Of the dry matter, 90% is organic and the remainder is inorganic. Hoover and Porges (1952) used the equation $C_5H_7O_2N$ to describe the organic fraction of bacteria in wastewater with 53% of the weight of the organism assumed to be organic carbon. More comprehensive equations have been formulated to describe the chemical composition of bacteria, for example the one used by Mara (1974) which takes into account the phosphorus content of bacterial cells, $C_{60}H_{87}O_{25}N_{12}P$. The remaining 10% of the cells are comprised of phosphorus (50%), sulphur (15%), sodium (11%), calcium (9%), magnesium (8%), potassium (6%), and iron (1%). As all these inorganic elements are required for microbial growth, any deficiency will result in growth limitation or inhibition.

The amount of energy biologically available per unit of organic matter broken down by heterotrophs depends on the oxygen source used. The

greatest yield of energy comes from the use of dissolved oxygen in oxidation, while least energy is from strict anaerobic metabolism. With a mixed culture of micro-organisms, as is found in wastewater treatment, the micro-organisms seek the greatest energy yield in order to achieve maximum synthesis. This is illustrated by the microbial activity which occurs when an organically enriched water is put into a closed container. At first, aerobic and facultative bacteria will decompose organic matter, gradually depleting the dissolved oxygen. After all the dissolved oxygen is exhausted, the facultative bacteria continue to use oxygen bound as nitrate and sulphate. At this state, other facultative and anaerobic bacteria begin to break down the organic matter to organic acids and alcohols which produce least energy:

$$\text{Organics} \longrightarrow \text{Organic acids} + CO_2 + H_2O + \text{energy}$$

If methane forming bacteria are present, then the anaerobic digestion process is completed by converting the organic acid to methane and carbon dioxide:

$$\text{Organic acids} \longrightarrow CH_4 + CO_2 + \text{energy}$$

Autotrophic bacteria cannot utilise organic matter, instead they oxidise inorganic compounds for energy and use carbon dioxide or carbonate as a carbon source. There are a number of autotrophs in the aquatic ecosystem, however only the nitrifying, sulphur and iron bacteria are particularly important in wastewater oxidation. The nitrifying bacteria oxidise ammonia nitrogen in a two step reaction, initially to nitrite, which is unstable, and finally to nitrate.

$$NH_4^+ + \text{oxygen} \xrightarrow{\textit{Nitrosomonas}} NO_2^- + \text{energy}$$

$$NO_2^- + \text{oxygen} \xrightarrow{\textit{Nitrobacter}} NO_3 + \text{energy}$$

The reaction occurs in secondary treatment units although it is very sensitive to environmental conditions, occurring most efficiently at low organic loadings and warm temperatures (Sec. 3.5.2).

In sewers, hydrogen sulphide is given off by sulphate reducing bacteria if the wastewater becomes anaerobic. The slightly acidic gas is absorbed into condensation water which collects on the top or crown of the sewer or on the side walls. Here, sulphur bacteria, which are able to tolerate pH levels of 1.0 oxidise the hydrogen sulphide to strong sulphuric acid using atmospheric oxygen:

$$H_2S + \text{oxygen} \xrightarrow{\textit{Thiobacillus}} H_2SO_4 + \text{energy}$$

The sulphuric acid reacts with the lime in the concrete to form calcium sulphate, which lacks structural strength. Gradually the concrete pipe can be weakened so much that it eventually collapses. Crown corrosion is particularly a problem in sewers which are constructed on flat gradients, in warm climates, in sewers receiving heated effluents, with wastewaters with a high sulphur content or in sewers which are inadequately vented. Corrosion-resistant pipe material such as vitrified clay or PVC plastic, prevents corrosion in medium size sewers, but in larger diameter sewers where concrete is the only possible material, corrosion is reduced by ventilation which expels the hydrogen sulphide and reduces condensation. In exceptional circumstances, the wastewater is chlorinated to prevent sulphate-reducing bacteria forming hydrogen sulphide or the sewer is lined with a synthetic corrosion-resistant coating.

Not all species of iron bacteria are strictly autotrophic, however, those that are can oxidise inorganic ferrous iron to the ferric form as a source of energy:

$$\mathrm{Fe^{2+}(ferrous) + oxygen} \xrightarrow{\textit{Leptothrix}} \mathrm{Fe^{3+}(ferric) + energy}$$

The bacteria are filamentous and deposit oxidised iron ($Fe(OH)_3$) in their sheath. They occur mainly in iron rich mine wastewaters but can also occur in biological wastewater treatment units. For example, they are common in percolating filters which treat domestic effluents receiving infiltration water from coal mining areas and so are rich in iron (Gray 1980). If the domestic water supply contains dissolved iron, the bacteria can become established in water pipes, forming yellow or reddish-brown slimes and tainting the water as the mature bacteria die.

Autotrophs derive energy from either sunlight (photosynthetic) or from inorganic oxidation-reduction reactions (chemosynthetic). Chemoautotrophs do not require external sources of energy but utilise the energy from chemical oxidation, while phototrophs require sunlight as an external energy source:

$$\mathrm{CO_2 + H_2O} \xrightarrow{\text{Photosynthetic micro-organisms}} \mathrm{Phototrophs + O_2}$$

Free dissolved oxygen is essential for the aerobic processes of heterotrophic and autotrophic bacteria. When aerobic organisms utilise organic nutrients, they consume dissolved oxygen at the same time. Each molecule of glucose, which is the basic building block of all carbohydrates, requires six molecules of oxygen for complete conversion to carbon dioxide and water by aerobic

bacteria:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{bacteria}} 6CO_2 + 6H_2O$$

There is also a considerable oxygen demand during the nitrification of nitrogenous compounds by autotrophic nitrifying bacteria:

$$2NH_4 + +3O_2 \xrightarrow{\text{bacteria}} 2NO_2 + 4H^+ + H_2O$$

$$2NO_2 + O_2 + 2H^+ \xrightarrow{\text{bacteria}} 2NO_3^- + 2H^+$$

If the dissolved oxygen is not replaced, then aerobic growth will eventually stop when the oxygen is exhausted, allowing only the slow anaerobic processes to continue. Microbial activity is not only oxygen-limited in the case of aerobic micro-organisms, it is also restricted by the availability of adequate supplies of carbon, nutrients such as nitrogen and phosphorus, trace elements and growth factors. It is the actual composition of micro-organisms that controls the nutrient requirements of organisms, and as proteins are composed mainly of carbon, nitrogen and smaller amounts of phosphorus, it is these three elements which are essential for microbial growth. The requirements of carbon, nitrogen and phosphorus by microbial cultures in wastewater treatment processes is expressed as a ratio (C:N:P) and if the waste is deficient in any one of these basic components, complete utilisation of the wastewater cannot be achieved.

Many inorganic ions, mainly metals, are required to ensure that bacterial enzymatic reactions can occur. Therefore, trace amounts of calcium, magnesium, sodium, potassium, iron, manganese, cobalt, copper, molybdenum and many other elements are required. These are found in adequate amounts in sewage, as are growth factors such as vitamins. However, if any of these materials are deficient or absent, then microbial activity will be restricted or may even stop (Jefferson *et al.* 2001).

The mixed microbial cultures found in biological wastewater treatment units degrade and subsequently remove colloidal and dissolved organic substances from solution by enzymatic reactions. The enzymes are highly specific, catalysing only a particular reaction and are sensitive to environmental factors such as temperature, pH, and metallic ions. The major types of enzyme-catalysed reactions in wastewater biochemistry are:

Oxidation	the addition of oxygen or the removal of hydrogen;
Reduction	the addition of hydrogen or the removal of oxygen;
Hydrolysis	the addition of water to large molecules which results in their breakdown into smaller molecules;

Deamination the removal of an NH_2 group from an amino acid or amine; and

Decarboxylation the removal of carbon dioxide.

Microbial energetics, metabolism, population and community dynamics are fully explored in Chap. 3.

1.4. Microbial Oxygen Demand

It is important to know how much oxygen will be required by micro-organisms as they degrade organic matter present in wastewater for two reasons: (a) to ensure that sufficient oxygen is supplied during wastewater treatment so that oxidation is complete and (b) to ensure receiving waters do not become deoxygenated due to the oxygen demand of these micro-organisms, which results in the death of the natural fauna and flora. The amount of organic matter that a stream can assimilate is limited by the availability of dissolved oxygen. This is largely determined by the rate oxygen is utilised by microbial oxidation and the rate at which it can be replaced by reaeration and other processes.

1.4.1. *Self purification*

The term self-purification is defined as the restoration, by natural processes, of a river's natural clean state following the introduction of a discharge of polluting matter. In natural river systems, organic matter is assimilated by a number of processes which include sedimentation which is enhanced by mechanical and biological flocculation, chemical oxidation, and the death of enteric and pathogenic micro-organisms by exposure to sunlight. Of course, the assimilative capacity of rivers, i.e. the extent to which the river can receive waste without significant deterioration of some quality criteria, usually the dissolved oxygen concentration, varies according to each river because of available dilution, existing quality and the rate of the self-purification capability (Benoit 1971). The most important process in self-purification is biochemical oxidation, i.e. the aerobic breakdown of organic material by micro-organisms. Biodegradable organic matter is gradually eliminated in rivers due mainly to bacterial action, by methods very similar to those occurring in wastewater treatment. Complex organic molecules are broken down to simple inorganic molecules in a process requiring oxygen. In this process of self purification, it is the attached micro-organisms, collectively known as periphyton, that are normally responsible for the greatest

removal. The suspended micro-organisms, that are mainly supplied with the discharge, are less important in the removal of organic material. However, although the decomposition of organic waste by micro-organisms is advantageous, the process does remove oxygen from solution, and in order to prevent the destruction of the natural fauna and flora, aerobic conditions must be maintained.

Water at normal river temperatures holds very little oxygen compared to the air. In the atmosphere, gas molecules diffuse or move from an area of high concentration to an area of low concentration. In the same way, oxygen molecules diffuse through the air–water interface into the water where they become dissolved. At the same time, oxygen is diffusing in the opposite direction, but when the volume of oxygen diffusing in either direction per unit time is equal, then the water is said to be in equilibrium and therefore saturated with oxygen (100%). The solubility of oxygen depends on three major factors: pressure, temperature and the concentration of dissolved minerals (salinity). A decrease in atmospheric pressure causes a decrease in oxygen; therefore, streams at high altitudes have less oxygen at saturation concentration at a standard temperature than a lowland stream (Table 1.29). Al-

Table 1.29. Correction factor for changes in dissolved oxygen concentration due to pressure.

Altitude (m)	Pressure (mm)	Factor	Altitude (m)	Pressure (mm)	Factor
0	760	1.00	1300	647	1.17
100	750	1.01	1400	639	1.19
200	741	1.03	1500	631	1.20
300	732	1.04	1600	623	1.22
400	723	1.05	1700	615	1.24
500	714	1.06	1800	608	1.25
600	705	1.08	1900	601	1.26
700	696	1.09	2000	594	1.28
800	687	1.11	2100	587	1.30
900	676	1.12	2200	580	1.31
1000	671	1.13	2300	573	1.33
1100	663	1.15	2400	566	1.34
1200	655	1.16	2500	560	1.36

Divide the oxygen saturation concentration by the correction factor to give the adjusted saturation concentration at the specific elevation. For example, the saturation concentration of freshwater at 12°C is 10.8 mg l^{-1}. For an altitude of 600 m this is divided by the correction factor 1.08 to give the satiration concentration of water at 600 m of 10.0 mg l^{-1}

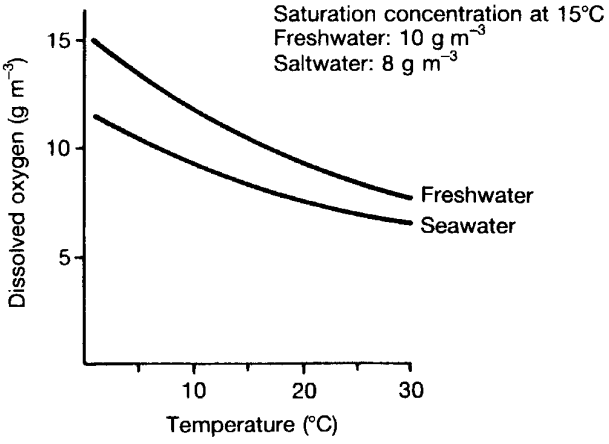


Fig. 1.7. Variation of saturation concentration of dissolved oxygen with temperature at a pressure of 1 atmosphere.

though this is of little practical significance in surface waters in the British Isles, this effect is important in a number of wastewater treatment processes where increased solubility of oxygen is achieved by increasing the pressure within the treatment reactor. Thus, it is standard to express the solubility of oxygen at 1 atmosphere of pressure. Freshwater at 1 atmosphere of pressure at 20°C contains 9.08 g of oxygen per m^3 (gm^{-3}=mg l^{-1}) and as the temperature increases, the saturation concentration (the maximum amount of oxygen that can dissolve into water) decreases (Fig. 1.7). The concentration of dissolved salts lessens the saturation concentration of oxygen which is why seawater has a lower saturation concentration than freshwater at various temperatures and pressures.

Although the dissolved oxygen concentration is affected by factors such as temperature, BOD_5, and salinity, oxygen depletion is prevented primarily by reaeration, although other sources of oxygen, such as photosynthesis, may also be important under certain conditions. It is important to know how quickly oxygen dissolves into water, and this depends to a large extent on the concentration of oxygen already in solution in relation to the saturation concentration, i.e. the oxygen deficit. For example, water containing 7 gm^{-3} of oxygen but with a saturation concentration of 9 gm^{-3} has an oxygen deficit of 2 g m^{-3}. The oxygen concentration can become supersaturated, up to 200%, under conditions of agitation at waterfalls and weirs. Supersaturation can also occur on bright sunny days due to photosynthesis when algal growth is abundant (Fig. 1.8). In both cases, the oxygen

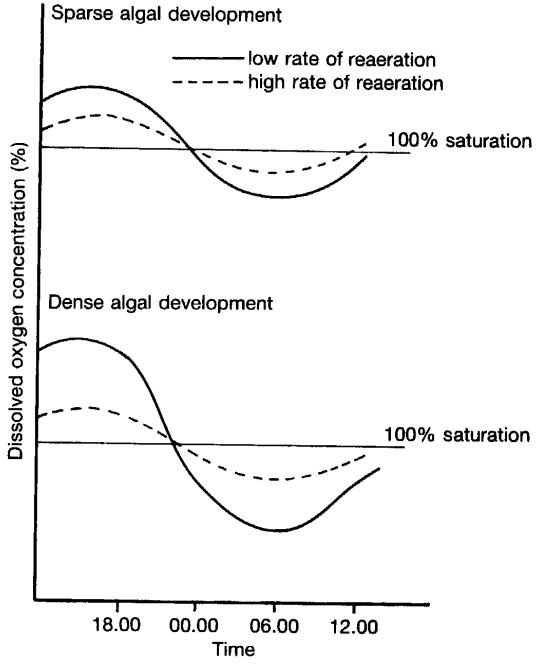


Fig. 1.8. Diurnal variation in dissolved oxygen over a 24 hour period in a river with macrophytes and algae present.

concentration will quickly return to equilibrium by the excess oxygen being lost to the atmosphere by diffusion. In general terms, the greater the organic load to the river, the greater the response in terms of microbial activity, resulting in a larger demand for the available dissolved oxygen.

Reaeration

Oxygen diffuses continuously over the air–water interface in both directions. In the water, the concentration of oxygen will eventually become uniform due to mixing or in the absence of mixing by molecular diffusion. The rate of diffusion is proportional to the concentration gradient which has been described by Flick's Law as:

$$\frac{dM}{dt} = K_{\mathrm{d}} A \frac{dC}{dx}$$

where M is the mass transfer in time t (mass–transfer rate), K_d the diffusion coefficient, A the cross-sectional area across which transfer occurs, C the concentration, and x the distance of transfer (concentration gradient).

If a uniform concentration gradient is assumed, then:

$$\frac{dM}{dt} = K_{\mathrm{d}} A \frac{(C_{\mathrm{s}} - C_{\mathrm{t}})}{x}$$

where C is the concentration at saturation (C_{s}) and after time t (C_{t}).

The equation can be solved as:

$$C_{\mathrm{t}} = C_{\mathrm{s}} - 0.811(C_{\mathrm{s}} - C_0)(e^{-K_{\mathrm{d}}} + (1/9)e^{-9K_{\mathrm{d}}} + (1/25)e^{-25K_{\mathrm{d}}} + \cdots)$$

where C_0 is the concentration after time 0 and

$$K_{\mathrm{d}} = \frac{K_{\mathrm{d}} \pi^2 t}{4x^2}$$

The diffusion coefficient K_{d} can be expressed in $\mathrm{mm}^2\ \mathrm{s}^{-1}$ or $\mathrm{cm}^2\ \mathrm{s}^{-1}$. The K_{d} for oxygen in water at 20°C is $1.86 \times 10^{-3}\ \mathrm{mm}^2\ \mathrm{s}^{-1}$.

Aeration in time or distance can be expressed:

$$\frac{dC_{\mathrm{t}}}{dt} = K_2(C_{\mathrm{s}} - C_{\mathrm{t}})$$

Integrating with limit $C_{\mathrm{t}} = C_0$ at $t = 0$

$$\int_{C_{\mathrm{o}}}^{C_{\mathrm{t}}} \frac{dC_{\mathrm{t}}}{C_{\mathrm{s}} - C_{\mathrm{t}}} = K_2 \int_0^t dt$$

i.e.

$$\log_e \frac{(C_{\mathrm{s}} - C_{\mathrm{t}})}{(C_{\mathrm{s}} - C_0)} = -K_2 t\,.$$

If D_{t} and D_0 are the dissolved oxygen deficit at times t and 0 respectively, and K_2 is the reaeration constant:

$$\log_{\mathrm{e}} \frac{D_{\mathrm{t}}}{D_0} = -K_2 t$$

thus,

$$D_{\mathrm{t}} = D_0 e^{-k_2 t}\,.$$

A more useful parameter than the reaction constant (K_2) is the exchange coefficient f. The exchange coefficient, also known as the entry or exit coefficient, is the mass of oxygen transferred across unit area of interface in unit time per unit concentration deficit.

$$f = \frac{K_{\mathrm{d}}}{x}$$

$$\frac{dM}{dt} = fA(C_{\mathrm{s}} - C_{\mathrm{t}})$$

and if a finite volume of water (V) is assumed, then:

$$\frac{dC_t}{dt} = \frac{dM}{dt}\frac{1}{V} = \frac{fA}{V}(C_s - C_t)$$

i.e.

$$\frac{dC_t}{dt} = K_2(C_s - C_t)$$

where

$$K_2 = f\frac{A}{V}$$

$$f = K_2\frac{V}{A} = K_2 h$$

where V is the volume of water below interface, A is the area of the air–water interface, and h is the mean water depth.

The exchange coefficient f is expressed in units of velocity (mm h^{-1}) and at 20°C in British rivers it can be estimated by the formulae:

$$f = 7.82 \times 10^4 U^{0.67} H^{-0.85}$$

where U is the water velocity (ms^{-1}) and H the mean depth (mm). Typical values for f range from 20 for a sluggish polluted lowland river to over 1,000 for a turbulent unpolluted upland stream. Values for the exchange coefficient for various aeration systems expressed in cm h^{-1} have been collated by Klein (1972b) and summarised in Table 1.30.

A rise in temperature can increase the rate of reaeration and vice versa. The reaeration rate constant (K_2) can be related to temperature (T) by:

$$K_{2(T)} = K_{2(20)} 1.047^{(T-20)}$$

In general terms, an increase in temperature of 1 degree will result in an increase in the exchange coefficient f by about 2%.

A number of physical factors affect reaeration. The transfer of oxygen at the air–water interface results in the surface layer of water becoming saturated with oxygen. If the water is turbulent as is the case in upland streams, the saturated surface layer will be broken up and mixing will ensure that reaeration is rapid. When no mixing occurs, as in a small pond to take an extreme example, then oxygen has to diffuse throughout the body of the water. In some cases, the diffusion rate may be too slow to satisfy the microbial oxygen demand so that anaerobic conditions may occur at depth. In rivers, velocity, depth, slope, channel irregularity and temperature will all affect the rate of reaeration. To increase the rate of aeration and speed up

Table 1.30. Typical values for the exchange coefficient f (Klein 1972b).

Aeration system	f(cm h^{-1})
Stagnant water	0.4–0.6
Water flowing at 0.4 m per min in a small channel	
water polluted by sewage	0.4
clean water	0.5
Water flowing at 0.6 m per min in a channel	1
Polluted water in dock and tidal basin	1–3
Sluggish polluted river (Sincil Dike)	2
Sluggish clean water about 51 mm deep	4
Thames Estuary under average conditions	5.5
Water flowing at 10.06 m per min in a small channel	7.5
The open sea	13
Water flowing at 14.94 m per min in a channel	30
Turbulent Lakeland beck	30–200
Water flowing down a 30° slope	70–300

the self purification process, weirs can be built below discharges. Floating aerators have been employed on rivers during periods of high temperature when the initial point has fallen dangerously low. More sensitive rivers, containing salmonid fish, have been protected by pumping pure oxygen into the river at times of particular stress. This technique has been developed specifically to control deoxygenation caused by accidental discharges of pollutants (Anon 1979). The use of a compressor with a perforated rubber hose has also been successfully employed. In emergencies, for example where a deoxygenated plug of water is moving downstream, the local fire brigade has been able to prevent total deoxygenation by using the powerful pumps on their tenders to recirculate as much water as possible, with the water returned to the river via high-pressure hoses. The main advantage of this method is that the fire crews can make their way slowly downstream, keeping abreast of the toxic plug. Thames Water utilise a barge capable of injecting 10 tonnes of pure oxygen per day into the River Thames to prevent deoxygenation. The barge is primarily for use in the estuary where the discharge of storm water from the combined sewers of London during periods of heavy rainfall reduces the dissolved oxygen concentration of the water to dangerously low levels. The oxygen is processed on board by a pressure swing absorption plant, although liquid oxygen stored in special

tanks can also be used, and injected into the water via a Vitox® injection system (Sec. 5.3.4). The barge is ideal for use in large rivers where it can quickly move to threatened areas (Griffiths and Lloyd 1985).

The oxygen sag curve

When an organic effluent is discharged into a stream, it exerts a biochemical oxygen demand with the processes of oxygen consumption and atmospheric reaeration proceeding simultaneously. Although other processes such as photosynthesis, sedimentation, and oxidation of the bottom deposits can also affect oxygen concentration, oxygen consumption and reaeration are the primary processes affecting oxygen status.

In many cases, the oxygen demand will initially exceed the reaeration rate, so the dissolved oxygen concentration will fall downstream of the outfall (discharge point). The rate of diffusion across the air–water interface is directly proportional to the oxygen deficit and if the rate of consumption lowers the oxygen concentration, the oxygen mass transfer rate will increase. At some point downstream, the rate of reaeration and consumption become equal and the oxygen concentration stops declining. This is the critical point of the curve where the oxygen deficit is greatest (D_c) and the dissolved oxygen concentration is lowest (Fig. 1.9). Thereafter, reaeration predominates and the dissolved oxygen concentration rises to approach saturation. The characteristic curve which results from plotting dissolved

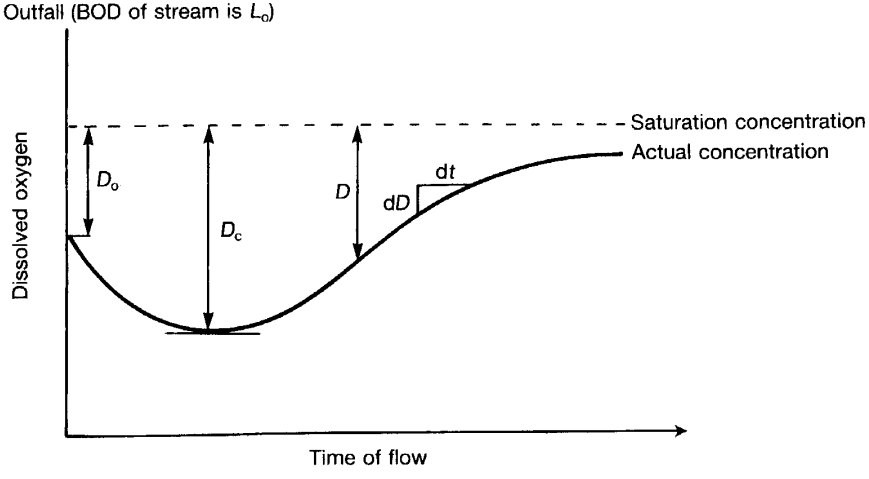


Fig. 1.9. Dissolved oxygen sag curve.

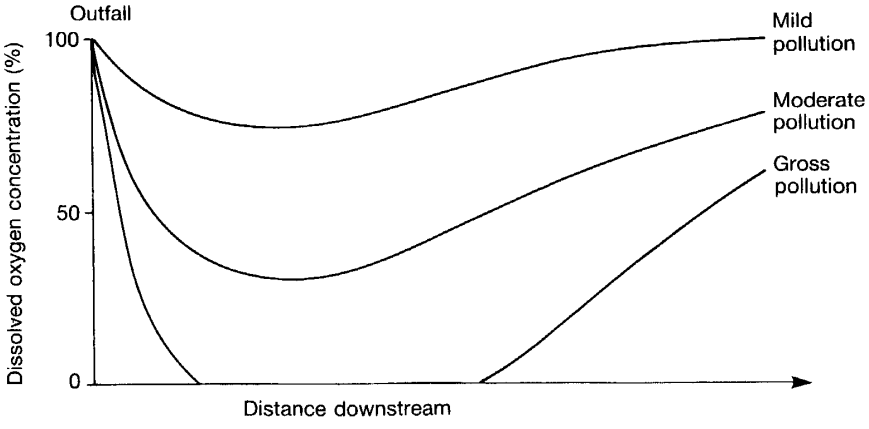


Fig. 1.10. The effect of an organic discharge on the oxygen content of river water.

oxygen against time or distance downstream is known as the oxygen sag curve. The long tail associated with the recovery phase of the curve is due to the rate of mass transfer of oxygen. As the river's dissolved oxygen concentration recovers, the oxygen deficit is reduced and as the rate of mass transfer is proportional to the oxygen deficit, the rate of reaeration slows, thus extending the curve. For example, water containing 10 gm^{-3} but with a saturation concentration of 12 gm^{-3}, has an oxygen deficit of 2 gm^{-3}. Since the rate of diffusion is directly proportional to the oxygen deficit, if the same water now contained only 4 gm^{-3} and so had an oxygen deficit of 8 gm^{-3}, the oxygen would diffuse four times faster.

The shape of the curve remains more or less the same, except that the critical point will vary according to the strength of the organic input. It is possible for the dissolved oxygen concentration to be reduced to zero and an anaerobic or septic zone to be formed (Fig. 1.10). De-oxygenation is generally a slow process, so the critical point may occur some considerable distance downstream of the outfall. The degree of de-oxygenation not only depends on the strength of the discharge, but also on dilution, BOD of the receiving water, nature of the organic material in terms of availability and biodegradability, temperature, reaeration rate, dissolved oxygen concentration of the receiving water and the nature of the microbial community of the river.

The oxygen sag curve can be expressed mathematically for idealised conditions in terms of the initial oxygen demand, the initial dissolved oxygen concentration in the river and the rate constants for oxygen consumption (K_1) and reaeration (K_2). These mathematical formulations were derived

by Streeter and Phelps (1925) when working on the Ohio River. This large river had long uniform stretches between pollution discharges, also relatively little photosynthesis, so the only major factors affecting the oxygen status were oxygen consumption and reaeration. They considered that the rate of biochemical oxidation of the organic matter was proportional to the remaining concentration of unoxidised organic material, typified by the first-order reaction curve (Fig. 3.7).

Assuming first-order kinetics, the oxygen demand with no aeration can be represented as:

$$\frac{dL_\mathrm{t}}{dt} = -K_1 L_\mathrm{t}$$

$$L_\mathrm{t} = L_0 - D_\mathrm{t} \cdot d(L_0 - D_\mathrm{t}) = -dD_\mathrm{t}$$

thus,

$$\frac{dD_\mathrm{t}}{dt} = K_1 L_\mathrm{t}$$

Reaeration with no oxygen demand:

$$\frac{dC_\mathrm{t}}{dt} = K_2(C_\mathrm{s} - C_\mathrm{t})$$

therefore,

$$\frac{d(C_\mathrm{s} - C_\mathrm{t})}{dt} = K_2 D_\mathrm{t}$$

thus,

$$\frac{dD_\mathrm{t}}{dt} = -K_2 D_\mathrm{t}$$

It is possible to express both demand and aeration in terms of change in the oxygen deficit (dD_t/dt). Thus, for simultaneous oxygen demand and reaeration:

$$\frac{dD_\mathrm{t}}{dt} = K_1 L_\mathrm{t} - K_2 D_\mathrm{t}$$

where D is the dissolved oxygen deficit at time $t(D_\mathrm{t})$, L is the ultimate BOD at time $t(L_\mathrm{t})$ or initially (L_0), K_1 the BOD reaction rate constant, and K_2 the reaeration rate constant.

Provided that oxygen is not a limiting factor, the oxygen demand is not dependent on the oxygen deficit. Thus, by substituting L_t according to the

equation:

$$L_t = L_0 e^{-K_1 t}$$

$$\frac{dD_t}{dt} = K_1 L e^{-K_1{}^t} - K_2 D_t \,.$$

When this equation is integrated with limit $D_t = D_0$ when $t = 0$

$$D_t = \frac{K_1 L_0}{(K_2 - K_1)} \{e^{-K_1 t} - e^{-K_2 t}\} + D_0 e^{-K_2 t}$$

which is the well known Streeter and Phelps equation.

By changing to base 10 ($K = 0.4343k$):

$$D_t = \frac{K_1 L_0}{(K_2 - K_1)} \{10^{-K_1 t} - 10^{-K_2 t}\} + D_0 10^{-K_2 t} \,.$$

The minimum dissolved oxygen concentration (the critical point), which occurs at maximum oxygen deficit D_t when

$$\frac{dD_t}{dt} = 0 \,,$$

is given by:

$$\frac{dD_t}{dt} = 0 = L_1 L_0 - K_2 D_c$$

thus,

$$D_c = \frac{K_1}{K_2} \cdot L_e^{-K_1 t_c}$$

therefore,

$$t_c = \frac{1}{K_2 - K_1} \cdot \log_e \left\{ \frac{K_2}{K_1} \left[1 - \frac{(K_2 - K_1) D_0}{K_1 L_0} \right] \right\}$$

where the critical (maximum) deficit (D_c) occurs at time t_c.

Both K_1 and K_2 in the model are assumed to be constant. However, although K_1 is measured by running a BOD determination in the laboratory, it may vary with time. The K_2 value will vary from reach to reach within the river and must be measured in the field. Both these constants are temperature functions and so temperature effects must be taken into consideration. For domestic sewage, K_1 approximates to 0.1 at 20°C while K_2, which is mainly a function of turbulence, can be assessed using the equation developed by O'Connor and Dobbins (1958):

$$K_2 = \frac{(K_d U)^{1/2}}{H^{3/2}}$$

where K_2 is the reaeration coefficient (base e) per hour, K_d the diffusion coefficient of oxygen into water, U the velocity of flow and H the depth. Approximate values can be obtained. Low values represent deep, slow-moving rivers and high values shallow fast-flowing upland streams. In reality, K_2 is at best a crude estimate, and often an assumed value which can have severe effects on the predictive estimate. The measurement of K_1 is even more critical.

Boyle (1984) has examined the effects of biological films (including sewage fungus growths) on BOD decay rates (K_1) in rivers. He cites several examples where the dissolved oxygen sag below outfalls could only be modelled if K_1 was assumed to be an order of magnitude greater than expected. Clearly, the decay rate is enhanced by the presence of a biological film on submerged surfaces, although the decay rate was dependent on both nutrient concentration and water velocity. For example, Boyle and Scott (1984) quote K_1 values for a small English river receiving papermill waste and supporting sewage fungus of between 3.9–4.2 d, although other workers, mainly in New Zealand, have recorded BOD rate coefficients of up to 10.56 d.

The oxygen sag curve can be more accurately assessed by providing a third point. This is provided by the point of inflexion, where the net rate of aeration is at a maximum, when $(d^2D_t/dt^2) = 0$ then:

$$t_i = \frac{1}{(K_2 - K_1)} \log_e \left\{ \left(\frac{K_2}{K_1} \right)^2 \left[1 - \frac{(K_2 - K_1)D_0}{K_1 L} \right] \right\}$$

where the inflexion deficit (D_i) occurs at time t_i.

It is now possible to plot the oxygen sag curve and to predict the minimum oxygen concentration downstream of a point discharge of organic waste such as sewage (Fig. 1.9).

Although the Streeter and Phelps model provides an extremely useful basis for the study of the sequence of events which occur in an organically polluted river, it must be applied with care, particularly to rivers where conditions change frequently, where there is appreciable photosynthesis, deposition of debris and sediment, or discharges of inhibitory or toxic substances. The model is only valid for a single pollution discharge and where there is no dilution from tributaries. Where these occur, the river must be split up into discrete sections according to changes in flow or discharge, so that each section can be treated as an individual case and the model applied. The output data from one section provides the input data for the next, and in this way the entire river system can be covered to provide

an overall calculation. This type of model is the basis of predictive water quality models with many other variables, such as benthic and nitrogenous oxygen demand, salinity and temperature also included (Forster *et al.* 1985).

In Ireland, An Foras Forbartha (AFF) have developed a deterministic, steady state model based on a series (approximately 20) of integrated differential equations (McGarrigle 1984). Like the Streeter Phelps model, the principle outputs from the AFF model are dissolved oxygen and BOD. However, like most other river models, it makes the following assumptions: (i) no longitudinal dispersion, (ii) effluent is mixed immediately and uniformly across the section of river, (iii) coefficients are uniform and constant throughout a reach, and (iv) that flow is steady and one-dimensional. Although none of these assumptions is completely justified, they are not likely to lead to significant errors in the predictions made by the model. The factors influencing the dissolved oxygen and BOD which are considered in the model are summarised in Table 1.31 and how they interrelate in the flow diagram in Fig. 1.11. Although this model has been widely applied to clean sections of river with some success, problems arise in more organically polluted sections. The BOD decay rate is cited as a particular problem in the prediction of downstream BOD and dissolved oxygen with this model (AFF 1984). They observed that in the field, the BOD value drops much more quickly than can be predicted using a theoretical laboratory constant. The same problem was observed by Boyle (1984). For example, in the River Suir, the BOD dropped from 80 mg l^{-1} to 20 mg l^{-1} over a distance of just 1700 m in under 80 minutes. The measured laboratory decay constant of 0.000139 d, which appears very low, only accounted for a fraction of this drop in BOD. Clearly, the rapid assimilation by sewage fungus growths must also be incorporated into model equations.

There are a relatively small number of computer simulation models for pollutants in surface waters as compared with those commercially available for simulating pollutant behaviour in groundwaters, estuaries, or coastal waters. Those used most widely have been developed by the US Environmental Protection Agency (USEPA). The most widely used programme is the *Enhanced Stream Water Quality Model* (*QUAL2E*). This powerful model is applicable to well mixed, dendritic streams. It can simulate nutrient cycles, algal production, benthic and carbonaceous oxygen demand, atmospheric reaeration, and their effect on the dissolved oxygen balance. It can predict the concentrations of up to 15 water quality parameters and is primarily intended as a planning tool for developing total maximum daily loads (TMDLs) to surface waters. In conjunction with field sampling, it can

Table 1.31. Principle factors affecting the dissolved oxygen and BOD that are accounted for in the River Suir Ecological Model (AFF 1984).

Factors influencing dissolved oxygen directly
1. BOD decay
2. Atmospheric interchange
3. Plant respiration
4. Plant photosynthesis
5. Mud respiration
Factors influencing BOD
1. Temperature
2. Dissolved oxygen availability
3. Settlement
4. Resuspension
Factors influencing atmospheric interchange of dissolved oxygen
1. Temperature
2. Depth
3. Velocity
4. Nature of water surface
5. Weirs, waterfalls
6. Dissolved oxygen
Factors influencing extent of plant respiration
1. Plant biomas
2. Dissolved oxygen
3. Temperature
Factors influencing effect of mud respiration
1. Current velocity
2. Temperature
3. Dissolved oxygen
4. Water depth
Factors influencing plant photosynthesis
1. Plant biomass
2. Light intensity
3. Temperature
4. Depth
Factor influencing nitrification
1. Ammoniacal nitrogen concentration

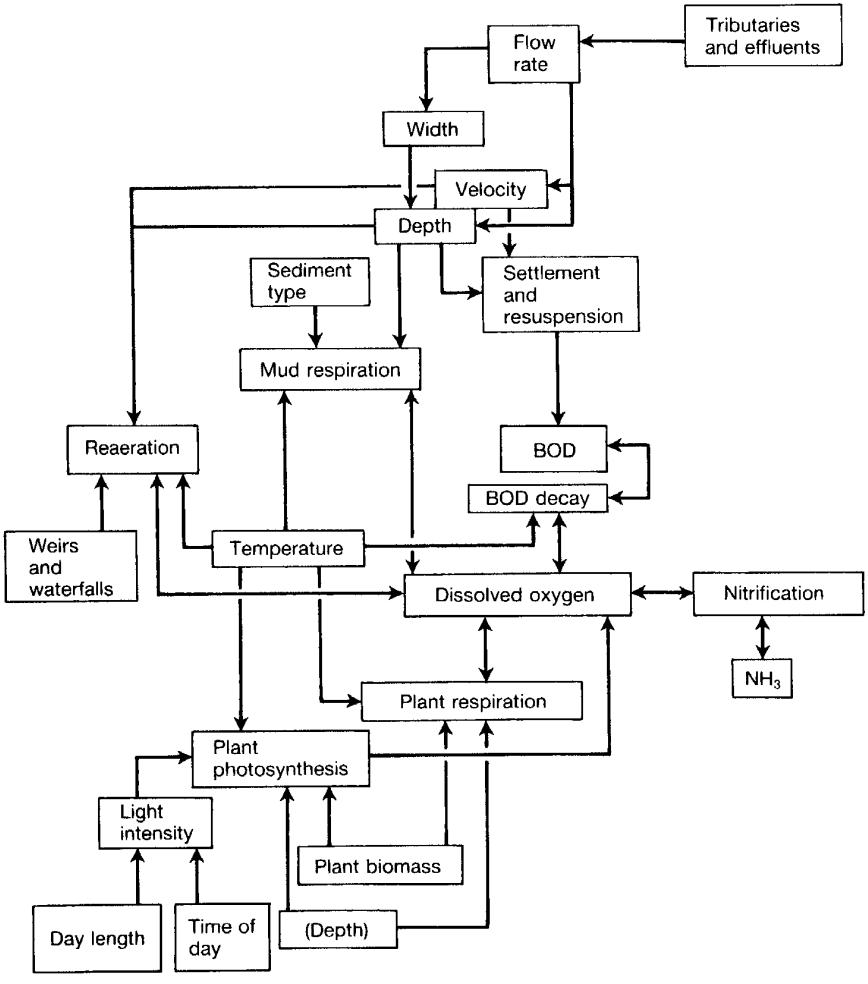


Fig. 1.11. Flow chart of the River Suir Ecological Model (AFF 1984).

be used to identify the magnitude, and quality characteristics, of non-point sources. There are three other important USEPA models. The *Water Quality Analysis Simulation Program* (*WASP5*) models the fate and transport of a wide range of contaminants in surface waters. The *Storm Water Management Model* (*SWMM*) allows the movement of water and pollutants from surface runoff via the stormwater network to the receiving water to be simulated. Finally, the *Hydrological Simulation Program–FORTRAN (HSPF).* This program integrates stream water quality modelling with watershed

hydrology modelling, simulating the effects of runoff on receiving water and sediment quality. This is a powerful model requiring extensive climatic data such as continuous rainfall data, temperature, solar intensity etc.

A final word of caution on the use of the Streeter and Phelps model. The model assumes that the flow does not vary over time, that the organic matter is distributed uniformly across the stream's cross-section, and that there is no longitudinal mixing. The effects of algae and bottom sediments are not considered in the equation. In reality, however, the dissolved oxygen sag curve can be affected by other factors apart from microbial oxygen demand and reaeration rate. Among those worthy of further consideration are photosynthesis with the addition of oxygen during the day and the uptake of oxygen by plant respiration at night, benthic oxygen demand, the removal of oxygen by gases released from the sediments and the release of soluble organic material from the sediments which has an oxygen demand, and finally, the input of oxidisable material from surface water. These inputs and the dissolved oxygen are constantly being redistributed within the water column by longitudinal mixing. Some of these factors can be easily predicted and so built into the existing model, whereas other factors are less quantifiable.

Microbial interactions

The microbial response to organic enrichment in streams and rivers is essentially the same as those which occur in the biological unit processes at wastewater treatment plants. In natural waters, these responses occur longitudinally, often occurring over many miles, whereas in treatment units these changes are accelerated, occurring over a much shorter distance and time basis within a single or series of reactors.

The rate of biological oxidation of organic waste is a time–temperature function, with the concentration of available oxygen decreasing as the temperature rises. It is possible to describe the changes that occur during the self-purification process over a time basis. A river polluted with organic matter responds in a characteristic way. This has been categorised into a number of stages or zones: a polluted zone, a recovery zone (which is normally split in two, depending on the rate of oxidation) and a recovered or clean zone (Fig. 1.12). The best known descriptive classification of the degree of pollution is the Saprobrien system, which uses four terms to describe these zones: *polysaprobic* (p), the heavily polluted or septic zone; the *mesosaprobic* zone of recovery and active oxidation, which is split up into the *alpha-mesosaprobic* (α) zone of heavy pollution and the *beta-mesosaprobic*

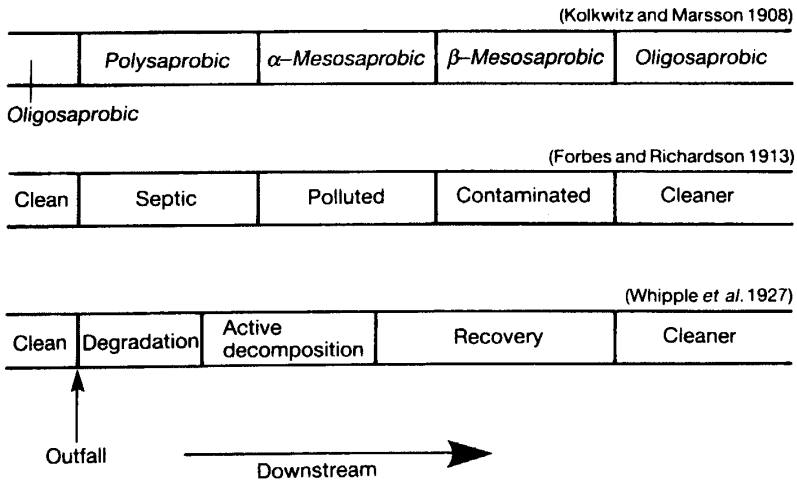


Fig. 1.12. Summary of early descriptive classifications of the various zones of a polluted river.

(β) zone of moderate pollution. The final area is the *oligosaprobic* (o), the zone of very slight pollution and of complete oxidation.

Immediately below an outfall the density of bacteria rapidly increases in response to the increase in available organic substrate with direct counts of up to 36×10^6 ml^{-1} common (Fig. 1.13). Most of these bacteria are suspended with $< 10\%$ attached to surfaces (Edwards and Owens 1965), but as the effluent proceeds downstream, attached growths of bacteria and fungi dominate, utilising the breakdown products of polysaccharides and producing a thick growth which covers the entire surface of the bottom substrate. Sewage fungus takes a number of macroscopic growth forms and can form fronds, cotton-wool-like growths, or gelatinous growths in rivers depending on the flow rate and the form of carbon substrate available. This high level of heterotrophic activity exerts a huge oxygen demand which may exceed the available oxygen, causing deoxygenation, but also rapidly utilises the available BOD. As bacterial breakdown of proteinaceous compounds continues, ammonia and phosphorus are released which increases in concentration downstream. As degradation proceeds, less suitable carbon substrate is left for heterotrophic activity which subsequently declines and is eventually replaced by algae. Stimulated by the high nitrogen and phosphorus concentration, filamentous algae are first to colonise the stream and complete the oxidation of organic nitrogen to nitrate. There is a discernible change in the microbial population as the organic matter

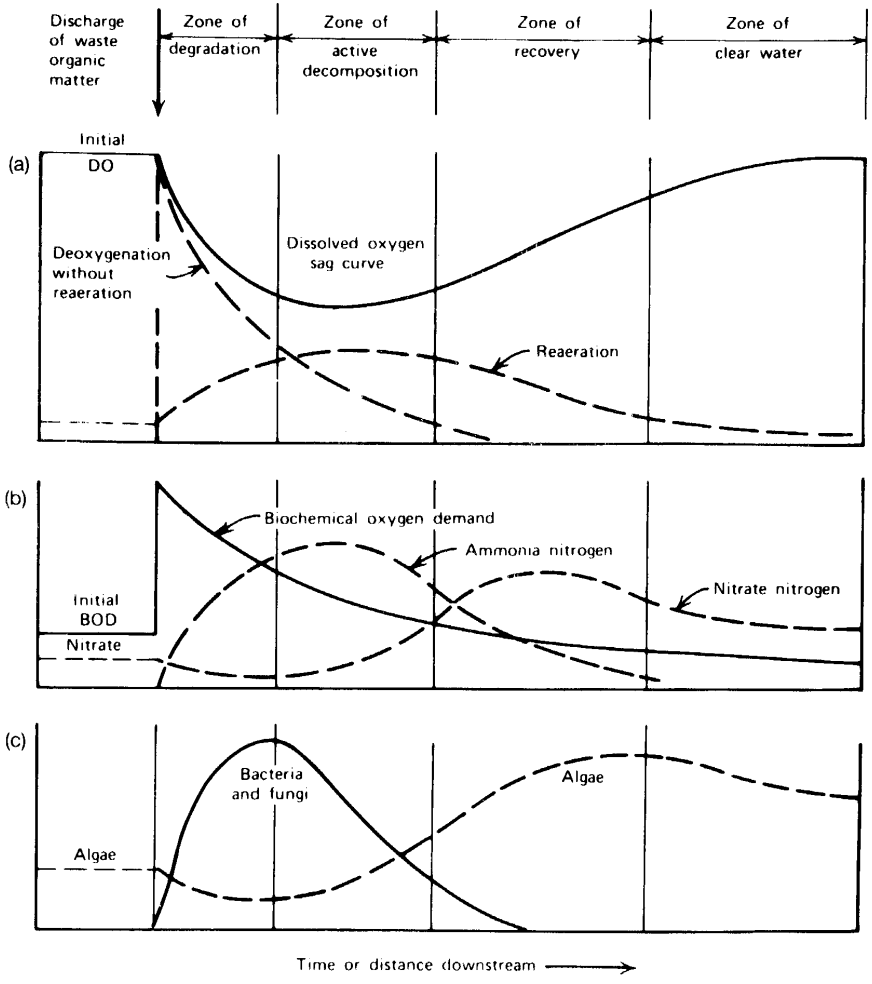


Fig. 1.13. General effects of organic pollution in streams (Hammer 1977).

is oxidised (Fig. 1.14). Bacteria decrease downstream of the outfall due to natural death and predation by ciliate protozoa. Further downstream as the protozoan population becomes food limited, due to low bacterial numbers, the rotifers and crustaceans increase. The rotifers and crustaceans not only feed on the ciliates, but are able to utilise the remaining bacteria due to the reduced competition from the ciliates. Within the stream, the pathogenic bacteria naturally die off rapidly, although predation by protozoans is also an important factor.

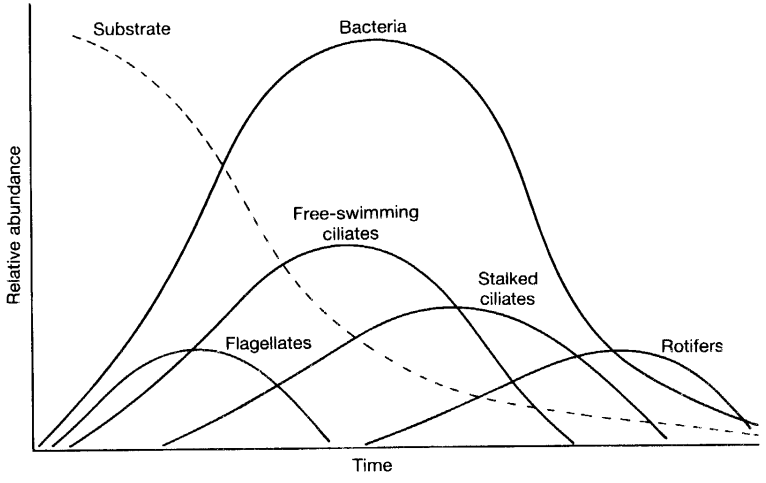


Fig. 1.14. Relative abundance of micro-faunal groups relative to remaining substrate. Bacteria thrive and finally become prey of ciliates, which in turn are food for rotifers and crustaceans.

The conditions which characterise each zone are summarised below:

Polysaprobic zone: This zone occurs directly below the discharge point and rapid breakdown occurs so that anaerobic conditions can occur depending on the organic load and the assimilative capacity of the receiving water. The higher degradation stages of the proteins are present, partly as peptines, polypeptids, oligopeptides, and peptides, but degradation can extend to amino acids. Chemically, this zone is characterised by the presence of albumens, polypeptides and carbohydrates, with hydrogen sulphite, ammonia, and carbon dioxide being produced as end products of anaerobic digestion. Physically, the water has a dirty-grey colour, a faecal or mouldy smell, and is turbid due to enormous quantities of bacteria and colloids present. The bottom of the watercourse can be covered with a black digesting sludge and the reverse sides of stones will be coloured black by a coat of iron sulphide. Most autotrophic organisms are missing, although bacteria will be abundant reaching densities of $> 10^6$ ml^{-1}. If the organic matter is sewage, then *E. coli* will be abundant although absent if the organic waste is from an industrial or vegetable processing source. Other micro-organisms are scarce with some blue-green algae, flagellate protozoa, and amoebae present.

Alpha-mesosaprobic zone: Although the level of pollution is still heavy, recovery begins in this zone as oxidation processes speed up. There are no anaerobic sediments and there is more oxygen available to allow aerobic

oxidation to proceed. There is a high concentration of breakdown products such as amino acids and their degradation products, mainly fatty acids. Physically, the water is grey in colour with mouldy smells produced due to residues of protein and carbohydrate fermentation. The oxygen status is still $< 50\%$ saturation but never falls to zero. This is the zone of most active microbial activity and while bacterial density has fallen and is $< 10^5$ ml^{-1}, filamentous bacteria and fungi are common, often resulting in sewage fungus growths developing. Few algae are present and both flagellate (*Bodo* spp.) and ciliate protozoa (*Paramecium* spp. and *Colpidium* spp.) are common.

Beta-mesosaprobic zone: This is still a zone of active oxidation, although the level of pollution has been reduced significantly. Degradation products such as amino acids, fatty acids and ammonia are found in low concentrations, although ammonical compounds are abundant. The water has plenty of available oxygen which never falls below 50% saturation, although diurnal variations in dissolved oxygen are possibly due to photosynthesis. Degradation no longer affects the oxygen status of the water so much. The water is physically cleaner, being only slightly turbid, and is free from odour and any discolouration. The bacterial concentration is always $< 10^5$ ml^{-1} and bacteria are no longer the dominant organism with filamentous algae, such as *Cladophera* (blanket weed), dominating. The protozoa are dominated by the ciliates with stalked species (Peritichia) abundant.

Oligosaprobic zone: All the waste products have now been broken down to stable organics and inorganic salts. The dissolved oxygen concentration is normally 100%, although if algae is still present there may be some diurnal fall. The water is clear, odourless and colourless, with bacterial density < 100 ml^{-1}. Filamentous algae is largely replaced by macrophytes and mosses, although diatoms and a few green or blue-green algae may be present (Hawkes 1972).

The changes occurring during self-purification by the microbial component of the river are summarised in Fig. 1.13. Not all the zones may be present below an outfall, for example, if there is sufficient assimilative capacity available in the river system, then only the β mesosaprobic conditions occur before the river returns to its natural oligosaprobic state.

Dispersed bacteria, present either as individual cells or as small suspended flocs, and free-living protozoa in rivers and streams are essentially the same micro-organisms responsible for biological wastewater treatment in mixed reactors such as activated sludge. Although attached micro-organisms such as bacterial and fungal slimes, stalked protozoans and algae are similar to those growing on fixed-film reactors, e.g. percolating filters.

Table 1.32. Percentage of outbreaks of heterotrophic slime (sewage fungus) caused by specific effluent sources or mixtures of effluents in Irish rivers ($n = 148$).

%	Effluent source
16.2	Farms
14.9	Agricultural industries
14.2	Domestic sewage
8.8	Domestic sewage and agricultural industries
7.4	Waste-tips
6.8	Domestic sewage and industrial
6.1	Industrial
2.7	Domestic sewage and farms
2.0	Industrial and agricultural industries
0.7	Other

Sewage fungus

Sewage fungus growths are predominately heterotrophic communities which lie between the autotrophic-heterotrophic and the heterotrophic-phototrophic continuum. The term *sewage fungus* was devised by Butcher (1932) who felt that such growths were generally associated with sewage and formed fungus-like growths. This term is rather misleading as sewage fungus is not only associated with sewage (Table 1.32) but with all organic effluents. Also, as fungi are rarely a major component of such growths, the term heterotrophic slime would seem to be more descriptive and appropriate. Slime-forming organisms are probably part of the normal flora of all rivers, and these attach themselves to any suitable and stable material to form visible macroscopic slimes only when there are significant amounts of readily assimilable organic nutrients to serve as growth substrate. Slimes are complex assemblages of micro-organisms: filamentous bacteria, fungi, zoogloeal bacteria, protozoa, and occasionally algae (Table 1.33). A grazing population of protozoans, rotifers, and macro-invertebrates are supported by feeding off the slime. In Ireland, three slime-forming organisms are frequently found forming these growths in rivers, two *bacteria Sphaerotilus* natans and *Zoogloea* spp., and a fungus, *Leptomitus lacteus* (Gray 1982, 1987) (Sec. 3.3.1) (Table 1.33).

Sphaerotilus natans is a filamentous bacterium made up of Gram-negative non-sporing rod-shaped cells with rounded ends, each 1–4 μm

Table 1.33. The occurrence of the commonest slime-forming organisms expressed as a percentage of the total sites examined in the UK (Curtis and Harrington 1971) and Ireland.

	UK			Ireland		
Organism	Dominant	Secondary	Total	Dominant	Secondary	Total
Sphaerotilus natans	52.1	37.1	89.2	52.8	23.3	76.1
Zoogloea spp.	58.5	34.0	92.5	11.1	43.3	54.4
Beggiatoa alba	6.3	21.4	27.7	5.5	23.3	28.8
Carchesium polypinum	6.3	10.1	16.4	2.8	0	2.8
Geotrichium candidum	4.4	3.1	7.5	0	0	0
Flavobacterium sp.	3.1	37.1	40.2	0	0	0
Leptomitus lacteus	3.1	0.6	3.7	22.2	3.3	25.5
Fusarium aquaeductuum	1.9	0	0	5.5	0	5.5
Stigeoclonium tenue	3.1	7.6	10.7	0	3.3	3.3

$\times$ 4–10 μm in size, enclosed within a sheath of varying thickness. It uses simple sugars such as glucose, maltose, sucrose, fructose, and mannose, although amino acids, glycerol, and even organic acids can be utilised if sugars are not available. Its nitrogen requirement can be satisfied, using organic nitrogen sources in the form of amino acids or short chain peptides, while inorganic nitrogen can be used only if vitamin B_{12} is also available. Calcium is required to develop the sheath which is a protein-polysaccharide-lipid complex. The capsule is a simple polysaccharide, the composition of which varies with the nutrient regime. The cells contain globular inclusions, which are food reserves of poly-ß-hydroxybutrate, which can make up to 40% of the dry weight of the cell. A high C:N ratio normally results in an increased formation of food reserves (Fig. 1.15). The bacterium is a strict aerobe and is restricted by dissolved oxygen concentrations of < 1 mg l^{-1}. It can exert an enormous demand for oxygen in the river, requiring 10–20 times more oxygen than the equivalent biomass of macrophytes. It has a wide temperature tolerance, being found in waters between 4–40°C, although its optimum growth rate occurs between 25–30°C. *Sphaerotilus natans* requires a minimum water velocity of 0.05 m s^{-1} to ensure oxygen and nutrient transfer, but at velocities > 0.6 m s^{-1} the growth is scoured away. The pH for growth varies between 6.8–9.0, but in more acidic waters slimes are dominated by fungi. *Sphaerotilus natans* tends to form slimy fronds in rivers, whereas zoogloeal bacteria are restricted to slow flowing waters and form more gelatinous growths which are easily broken up and washed away.

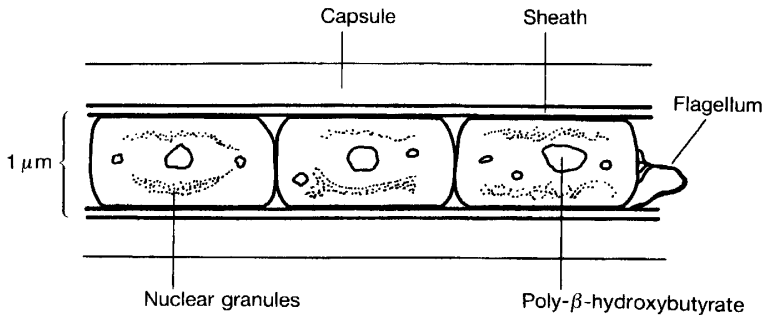


Fig. 1.15. Diagrammatic section through a filament of *Sphaerotilus natans*.

Zoogloea is not well defined taxonomically, but includes *Zoogloea ramigera*, *Pseudomonas* spp. and zoogloeal forms of *S. natans*. Zoogloeal bacteria comprise of Gram-negative non-sporing cells (0.5–1.0 μm $\times$ 1.0–3.0 μm) not arranged in filaments but embedded in a gelatinous matrix, forming lobed and unlobed spherical masses (Curtis 1969; Gray 1982). They have the same nutritional requirements as *S. natans* (Sec. 3.3.1). In Ireland, the fungus *Leptomitus lacteus* is a major slime-forming organism. It has a macroscopic growth form similar to that of *S. natans* except less slimey in texture as no external mucilage is produced. Unlike *S. natans*, it does not form fronds but long characteristic streamers, composed of overlapping spherical cotton-wool like growths. This *Phycomycete* is non-septate (no cell walls) but has constrictions at irregular intervals along the hyphae. Spherical plugs, made out of cellulin, move along the length of the hyphae between constrictions, blocking the constricted gaps and preventing movement of cellular material along the hyphae. The plugs are composed of a polysaccharide which remain in the slime matrix, even after the fungal mycelium has degraded, leaving a high density of the spherical plugs mixed with the remaining slime. The fungus requires a high dissolved oxygen concentration and grows preferentially at acid pH values. Sugars do not support growth and it proliferates in wastes rich in acetate, most low-molecular weight fatty acids, and is especially associated with waste-tip leachates, dairy wastes, and paper pulp wastes (Gray 1985). *Leptomitus lacteus* is found in moderate to fast-flowing water and normally dominates growths upstream of *S. natans*. (Fig. 1.16).

It is difficult to quantify the effects of outbreaks of heterotrophic slime in rivers, although a survey by Gray and Hunter (1985) found that the major effect was damaging to the amenity value of the river, and actual damage to fish was restricted to 40% of the outbreaks. Severity of the

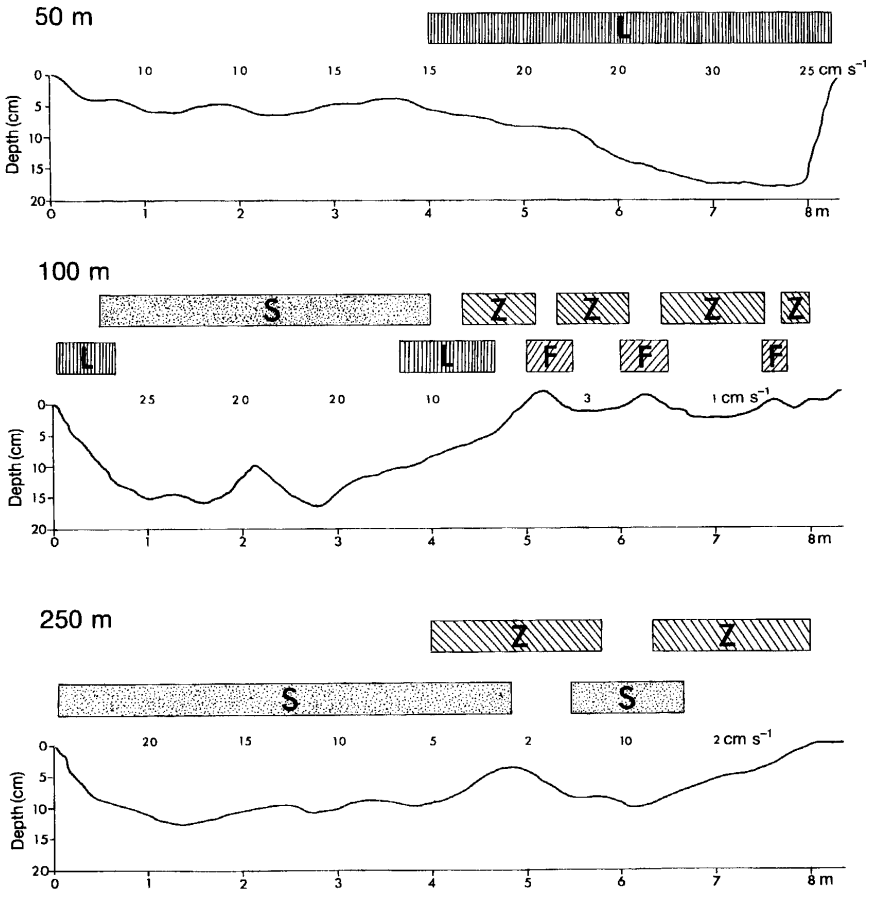


Fig. 1.16. Transects across the River Big in Co. Louth at 50 m, 100 m, and 250 m below the effluent outfall. Complete mixing of the effluent with the receiving water occurred at 70 m. The diagram shows the depth profile across the river (cm), the water velocity at 1 m intervals along each transect (cm s^{-1}) and the dominant slime-forming organism present. L, *Leptomitus lacteus*; S, *Sphaerotilus natans*; Z, zoogloeal bacteria; F, *Fusarium aquaeductuum*.

problem is related to the length of outbreaks with damage to fish stocks and the problem of sloughed flocs increasing with length. The percentage of sites where slimes caused no adverse effects decreased with increasing length (Table 1.34).

The length of river affected by slime growth can be extensive and in Ireland, outbreaks are generally greater than in England and Wales, with 55.1% of outbreaks < 0.8 km in length and 31.4% > 1.6 km in Ireland,

Table 1.34. The influence of effluent source, total length, and duration of heterotrophic slimes (sewage fungus) on the severity of effects measured in Irish rivers.

		Effects of slime outbreaks						
	n	Appearance and amenity (%)	Smell and de-oxygenation (%)	Smell only (%)	De-oxygenation only (%)	Sloughed flocs (%)	Damage to fish (%)	None (%)
Effluent source								
Farm	26	92.3	46.2	3.9	7.8	11.5	34.6	11.5
Agricultural industry	27	92.6	48.1	7.4	7.4	55.6	66.7	7.4
Industrial	14	100.0	57.1	28.6	42.9	35.7	42.9	7.1
Domestic sewage	53	84.9	50.9	11.3	5.7	22.6	22.6	17.0
Waste-tip	4	50.0	25.0	25.0	0	0	25.0	25.0
Length of outbreak								
0–20 m	11	72.7	27.3	9.0	0	0	9.0	36.4
20–100 m	14	78.6	21.4	0	0	14.3	21.4	28.6
100–500 m	21	85.7	57.1	9.5	19.1	14.3	19.1	14.2
0.5–1 km	13	100.0	53.9	0	15.4	30.8	46.2	0
1–5 km	18	77.8	50.0	38.9	22.2	38.9	55.6	5.6
5 km +	12	100.0	50.0	16.7	0	50.0	75.0	0
Duration								
Permanent	21	85.5	66.7	23.8	19.1	38.1	47.6	9.5
Spring	7	100.0	57.1	0	0	14.3	28.6	0
Summer	27	88.9	48.2	7.4	11.1	22.2	40.7	18.5
Autumn	12	91.7	41.7	16.7	0	41.7	50.0	16.7
Winter	11	100.0	54.6	18.2	0	54.6	72.7	0

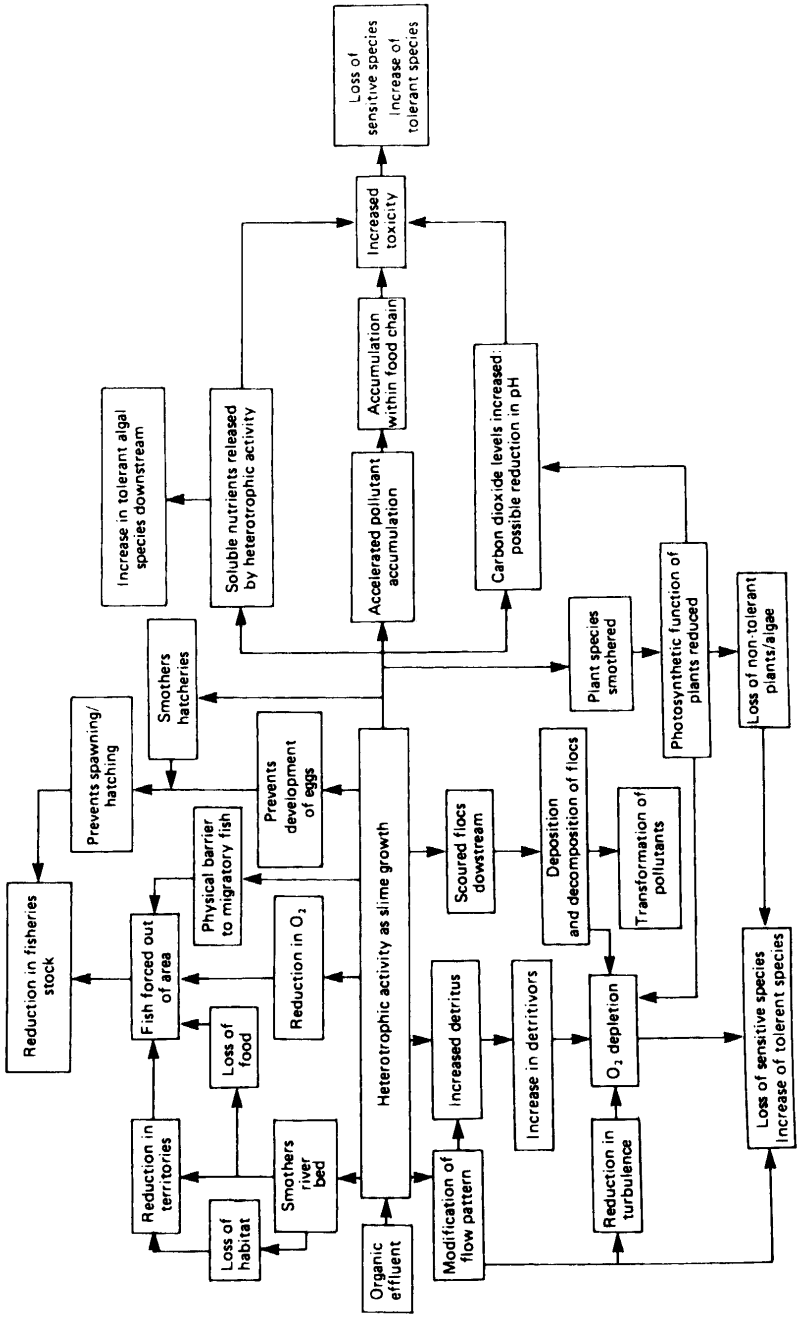


Fig. 1.17. Summary of the major pathways and subsequent effects of heterotrophic slimes in river ecosystems.

compared with 73.5 and 15.5% respectively in England and Wales (Curtis 1972; Gray and Hunter 1985). Between 1982 and 1983, 34% of outbreaks in Ireland were longer than 1 km and 13.5% in excess of 5 km, reaching a maximum length of 29 km in the River Barrow below a sugar beet factory. Large outbreaks have been recorded elsewhere in the world, for example, a 64 km outbreak on the River Altamaha in Georgia, USA, caused by a Kraft process waste (Phaup and Gannon 1967). Heterotrophic slimes can have direct or indirect effects on the river ecosystem and these are summarised in Fig. 1.17. Such slimes can remove soluble carbohydrate extremely rapidly from solution. Starch and intermediate polymers are not removed by the slime, but short-chain carbohydrates (mono to pentasaccharides) are readily utilised. Under ideal conditions, the biomass of slime produced is proportional to the concentration of organic carbon in the effluent, when this is in the form of glucose or acetate (Curtis 1972). The rate of removal of organic carbon is also directly proportional to the slime biomass at a rate of 0.3g C g^{-1} dry wt. of slime d^{-1}, although less than 20% of the organic carbon is incorporated into the slime (Curtis *et al.* 1972). Although slimes are effective in removing nutrients from solution, this does not involve a complete removal from the river as nutrients can be released back into solution by the slime. The economic coefficient (i.e. the ratio of pollutant incorporated into slime to the total amount removed from solution) of river slimes varies from 60% at the time of colonisation, falling to 11% as heavy slimes build up (Curtis 1969).

It is difficult to estimate the severity of a sewage fungus outbreak and normally only total length or presence/absence of outbreaks are used. A knowledge of the position of the recovery zone below the sewage fungus outbreak allows the exact length of river affected to be determined. Also, maximum oxygen demand exerted by the slime will occur upstream of the recovery zone and so it is important to know where the recovery zone occurs. If the degree of pollution increases, then the recovery zone will be pushed further downstream and vice versa. The resultant eutrophication zone may be as extensive as the sewage fungus outbreak, causing as many problems. Rapid and accurate plotting of the development of the various zones is therefore very useful. Gray (1987) has used three indices to evaluate sewage fungus growth in rivers and streams.

The first index allows the degree of recovery to be determined at any particular site along the watercourse (Table 1.35). This identifies exactly where the site is in relation to the recovery curve (Fig. 1.13) and whether the situation is improving or getting worse.

The whole width of the river should be examined for 2–5 m upstream.

Table 1.35. Index to determine the degree of recovery in rivers containing sewage fungus.

Heterotrophs	Score	Phototrophs
Not visible on hand-held boulders	1.	Not visible on hand-held boulders
Visible on hand-held boulders	2.	Visible on hand-held boulders
Present as clearly visible colonies on river bed	3.	Present as clearly visible colonies on river bed
Covering many surfaces	4.	Covering many surfaces
Covering most surfaces	5.	Covering most surfaces
Covering all surfaces	6.	Covering all surfaces

Degree of recovery = Heterotrophic rating − Phototrophic rating

Where:

$+5 \longleftarrow \quad 0 \quad \longrightarrow -5$

Heterotrophic Recovery zone Phototrophic

Table 1.36. Descriptive index of the degree of heterotrophic and phototrophic growths in rivers.

1.	Heterotrophs only
2.	Heterotrophs dominant: some algal growth visible
3.	Heterotrophs dominant: algal growth common
4.	Heterotrophs dominant: algal growth abundant
5.	Heterotrophs and phototrophs equally abundant
6.	Phototrophs dominant: sewage fungus abundant
7.	Phototrophs dominant: sewage fungus common
8.	Phototrophs dominant: small colonies of sewage fungus
9.	Phototrophs only
10.	Discrete colonies of phototrophs only

Always work upstream so that the area under examination is not damaged. Remember the fronds of sewage fungus will be broken off by your feet and could be mistaken for sloughed material downstream. The index is calculated by estimating the degree of heterotrophic growth and subtracting the estimate for phototrophic growth. This gives a rating for the river which, if positive, indicates heterotrophic activity predominating (i.e. there is an ample supply of organic matter to support sewage fungus growth), whereas a negative rating indicates a high level of algal growth (i.e. reduced organic matter but abundant organic and inorganic forms of nitrogen). The closer to zero the rating is then the closer you are to the recovery zone.

The second index is purely a descriptive index which allows a rapid estimation of the degree of recovery (Table 1.36). It is less sensitive than the previous index but is also useful for comparing the situation at individual sites over a long time period, as the length of the various zones will vary according to factors such as organic load, river flow, temperature, and, where used, pulsing frequency of effluent discharge.

Once a sewage fungus outbreak has been identified, then the severity of the growth can be estimated by using the third index (Table 1.37). Where deep rivers are studied, then examination will be restricted to the banks only and the degree of cover section of the index omitted and the index scored out of a total of nine. When very small or shallow streams are examined, it may be necessary to modify the sloughed floc section of the index to simply present (1) or absent (0) and the index scored out of ten. Experiments have shown that the standing crop of sewage fungus is directly related to the rank obtained using the index. It is not possible to give exact

Table 1.37. Index of sewage fungus development in rivers and streams (0, lowest; 12, highest).

Score	0	1	2	3
Cover	Occasional rare 0–1%	Common 1–20%	Frequent 20–40%	Abundant > 40%
Frond length	Thin film only no fronds visible	Visible fronds formed	Short–medium fronds > 50 mm	Medium–long fronds > 100 m
Sloughed flocs	None	Occasional (small particles)	Common (small–medium particles)	Heavy (medium–large particles)
Surface mats	No	Small area	Large area	—
Algae	Present	Absent	—	—

biomass values for particular ranks as this differs for each river and effluent source. However, plotting this association, the oxygen demand on the river exerted by the sewage fungus can be predicted when required, or an estimation made of the total biomass of sloughed flocs being released downstream. A review on heterotrophic slimes has been prepared by Gray (1985).

1.4.2. *Biochemical oxygen demand*

1.4.2.1. *The test*

The most important effect that organic wastes can cause in receiving waters is a reduction in the dissolved oxygen concentration, which is normally due to the microbial breakdown of the organic matter present. It is possible to determine the theoretical oxygen demand of a specific compound in wastewaters from the stoichiometry of its oxidative breakdown, although it is impossible to calculate the oxygen demand in this way for complex wastes such as domestic sewage. In order to determine the gross oxygen demand that will be exerted in a river or a wastewater treatment plant, a test is required that will estimate the amount of oxygen needed to oxidise all the compounds present, both the major and minor components of the waste.

Although the total organic carbon (TOC) content of the waste could be measured using a carbon analyser, it is more useful in terms of predicting effects in watercourses to measure the oxygen demand which will be exerted by these wastes on the watercourse. There are two widely used measures of oxygen demand: chemical oxygen demand (COD) which measures the organic content in terms of biodegradable and non-biodegradable compounds, and the biochemical oxygen demand (BOD) test, which measures the biodegradable fraction of the wastewaters by monitoring the assimilation of organic material by aerobic micro-organisms over a set period of time under strictly controlled conditions (ISO 1989a). The COD test employs a potassium dichromate reflux with concentrated sulphuric acid, using silver sulphate (Ag_2SO_4) as catalyst and mercuric sulphate ($HgSO_4$) to complex any chlorides present which could interfere with the reaction. The sample is refluxed for 2 hours in an acidified potassium dichromate solution of known strength so that the amount of oxidisable organic matter in the sample is proportional to the potassium dichromate consumed in the oxidation reaction. The excess dichromate is titrated in the ferrous ammonium sulphide to calculate the amount of dichromate consumed. Although nearly all organic compounds are oxidised by this procedure, some aromatic compounds, such as benzene, pyridine, and toluene are either unaffected or

only partially oxidised during the test (ISO 1989b). The COD will always be higher than the BOD as the former includes substances that are chemically oxidised as well as biologically oxidised. The ratio of COD:BOD provides a useful guide to the proportion of organic material present in wastewaters that is biodegradable, although some polysaccharides such as cellulose can only be degraded anaerobically and so will not be included in the BOD estimation. The COD:BOD relationship varies from 1.25 to 2.50 depending on the waste being analysed. The ratio increases with each stage of biological treatment as biodegradable matter is consumed but non-biodegradable organics remain and are oxidised in the COD test. The relationship remains fairly constant for specific wastes, although the correlation is much poorer when the COD values are < 100 mg O_2 l^{-1} (Aziz and Tebbutt 1980). This correlation can be expressed by the simple linear regression equation:

$$\mathrm{COD} = a \times \mathrm{BOD}_5 + b$$

where a and b are constants, the values of which depend on the wastewater. For domestic wastewater:

$$\mathrm{COD} = 1.64 \times \mathrm{BOD} + 11.36 \text{ (Ademoroti 1986)}.$$

The Biochemical Oxygen Demand Test (BOD), often incorrectly but rather accurately referred to as the Biological Oxygen Demand Test, is a laboratory simulation of the microbial self-purification process occurring in rivers. The test measures the amount of oxygen consumed in five days at a temperature of 20°C by the biological oxidation of any biodegradable organic material present. The oxygen is consumed by the micro-organisms, mainly bacteria, via respiration and metabolism. The organic matter is broken down to carbon dioxide, although some of it is incorporated into cellular material or oxidised for energy. If the sample contains large amounts of organic matter, the micro-organisms will require proportionately larger volumes of oxygen in order to degrade it. The amount of dissolved oxygen consumed does, however, depend on temperature and the duration of the test. Originally the test was carried out at 18.3°C (65°F) for 5 days, the reason being that British rivers do not have a flow time to the sea in excess of 5 days and have a mean summer temperature of 18.3°C. Thus, the use of these values ensured that the maximum possible oxygen demand which could occur under British conditions would be measured for each sample.

Not all the substrate within the BOD bottle will be oxidised to CO_2, some will be converted to new cells. Thus, if a simple organic source like

glucose is oxidised both chemically and biologically, there will be a discrepancy. For example, the COD test will predict an oxygen consumption of 192 g O_2 per mole of glucose compared with only 150 g O_2 per mole using the BOD test. Thus, the BOD test does not give a measure of the total oxidisable matter present in wastewaters because of the presence of considerable quantities of carbonaceous matter resistant to biological oxidation. However, it does indicate the potential possessed by a wastewater for de-oxygenating a river or stream. The test also provides a useful theoretical example of the oxygen balance in aquatic ecosystems, thus allowing a clearer understanding of the role of micro-organisms in oxygen-food limited environments (Stones 1981).

Complete breakdown of even the most biodegradable wastes can take several weeks, so during the 5-day test, only a proportion of the organic material will be broken down. Some organic materials, such as cellulose, can remain virtually unaffected by aerobic micro-organisms, only being broken down anaerobically. When the organic fraction has been aerobically broken down as completely as possible, the oxygen consumed is termed the ultimate BOD or ultimate oxygen demand. The test can incorporate two discrete oxygen demands forming the characteristic BOD curve (Fig. 1.18). The basic curve represents the carbonaceous material which can take up to 3 weeks to be fully degraded at 20°C. The second source of oxygen demand comes from the nitrogenous material present (nitrification). In raw wastewaters, nitrification only becomes a significant source of oxygen demand after 8–10 days, while in partially treated effluents, nitrification can

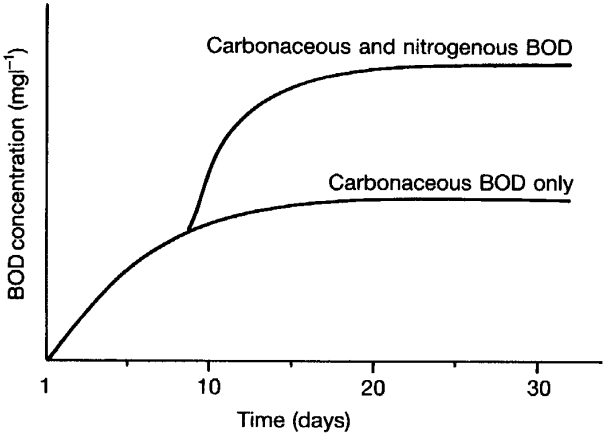


Fig. 1.18. The BOD curve showing carbonaceous and nitrogenous oxidation.

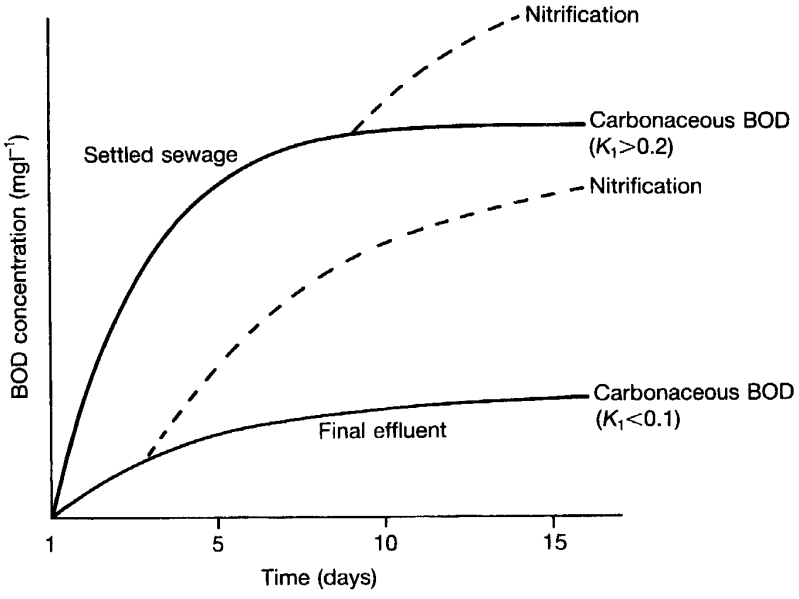


Fig. 1.19. Comparison of the BOD curves obtained using settled sewage and treated wastewater.

dominate the oxygen demand after just a few days (Fig. 1.19).

The standard 5-day BOD test (BOD_5) measures only the readily assimilable organic material present in a wastewater. However, the BOD_5 gives a far more reliable estimation of the possible oxygen demand that a waste will have on a river than the COD test, as the latter also measures the more refractory (non-biodegradable) compounds. Because of the similarity between the self-purification process and wastewater treatment process, the BOD test has been widely used as a measure of organic strength of river water and effluents. The low capital cost, unlike TOC analysers, and low running costs of the test have ensured that it remains popular even today, some 90 years after its introduction by the Royal Commission on Sewage Disposal in 1913, although a similar test was being used as early as 1868 (Phelps 1944). Today the BOD test is in use throughout the world, although there are many problems associated with its use, most of them associated with the way it is carried out (Sec. 1.4.2.2). The test is used for numerous purposes, including assessing the quality of river water, the strength of wastewaters, the assimilative capacity of receiving waters, and the effect of effluent discharges on receiving waters, as well as being used in the design and operation of treatment processes.

Stoichiometry

With domestic wastewaters, only 60–70% of the total carbonaceous BOD is measured within 5 days at 20°C (BOD_5), and only the most biodegradable fraction utilised. For most materials, an incubation period of about 20 days (BOD_{20}) is required for complete breakdown, even though some more recalcitrant organic compounds, such as certain polysaccharides, will not have been degraded even then. The test is essentially the oxidation of carbonaceous matter:

$$C_xH_yO_z \longrightarrow CO_2 + H_2O$$

However, whereas this first stage may be the only component of the BOD curve, often a second stage is present, i.e. nitrification. The oxidation of nitrogenous matter proceeds as:

$$NH_3 \longrightarrow NO_2^- \longrightarrow NO_3^-$$

Glucose is used as a reference for the BOD test and is also useful for examining the stoichiometry of the test. Glucose is completely oxidised as:

$$\underset{\text{glucose}}{C_6H_{12}O_6} + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

For complete oxidation, a glucose solution of 300 mg l^{-1} concentration will require 320 mg l^{-1} of oxygen at 20°C. However, using the standard 5-day BOD_5 test, only 224 mg of oxygen is utilised with complete oxidation taking longer than 5 days. Thus, the BOD_5 only measures part of the total oxygen demand of any waste, and in this case:

$$\frac{BOD_5}{BOD_{20}} = \frac{224}{300} = 70\%$$

Kinetics

The BOD has been traditionally modelled as a continuous first order reaction (Sec. 3.1.3), so that the rate of breakdown of carbonaceous material is proportional to the amount of material remaining. In this type of reaction, the rate of breakdown is at first rapid when the organic content is high, but gets progressively slower as the organic material is utilised. This can be expressed as:

$$\frac{dL}{dt} = -K_1 L$$

where K_1 is the BOD reaction rate constant and L the ultimate BOD (carbonaceous only). This integrates to:

$$L_\mathrm{t} = L_0 e^{-K_1 \mathrm{t}}$$

where the initial BOD (L_0) is L_t after time t. The amount of oxygen consumed during the BOD test period (Y) is:

$$Y = L_0 - L_\mathrm{t}\,.$$

Thus,

$$Y = L_0(1 - e^{-K_1 \mathrm{t}})$$

or using base 10:

$$Y = L_0(1 - 10^{-K_1 \mathrm{t}})\,.$$

Thus, for a test where 65% of the carbonaceous material is broken down within the five days, K_1 will equal 0.223 d^{-1}. Thus, the removal rate is approximately 20% per day. Therefore, 95% removal will take 13 days and 99% removal 21 days, although adherence to the relationship between K_1 at base e and base 10 is:

$$K_e = 2.303 K_{10}\,.$$

It is convention to quote K_1 to the base 10. The rate constant K_1 varies according to the quantity and nature of the organic matter present, the temperature and the type of micro-organisms in the wastewater. This can be best illustrated by considering the way in which micro-organisms utilise the available organic material present. Essentially, two reactions take place within a BOD bottle; a rapid synthesis reaction in which there is a rapid consumption of oxygen due to the high concentration of available organics, which is characteristic of raw wastewaters or effluents high in low molecular weight carbohydrates, followed by a slower endogenous metabolism (Fig. 1.20). In treated effluents, most of the organics originally present in the wastewater have been removed and oxygen is consumed at the lower endogenous rate. Therefore, the greater the rate of reaction due to the concentration of assimilable organic material, the larger the K_1 value. The average BOD rate constant at 20°C ranges from 0.04–0.08 for rivers with low pollution, 0.06–0.10 for biologically treated effluents, 0.12–0.22 for partially treated effluents, and those using high-rate systems, to 0.15–0.28 for untreated wastewaters. It is possible for samples with different reaction rates to have the same BOD_5 (Fig. 1.21).

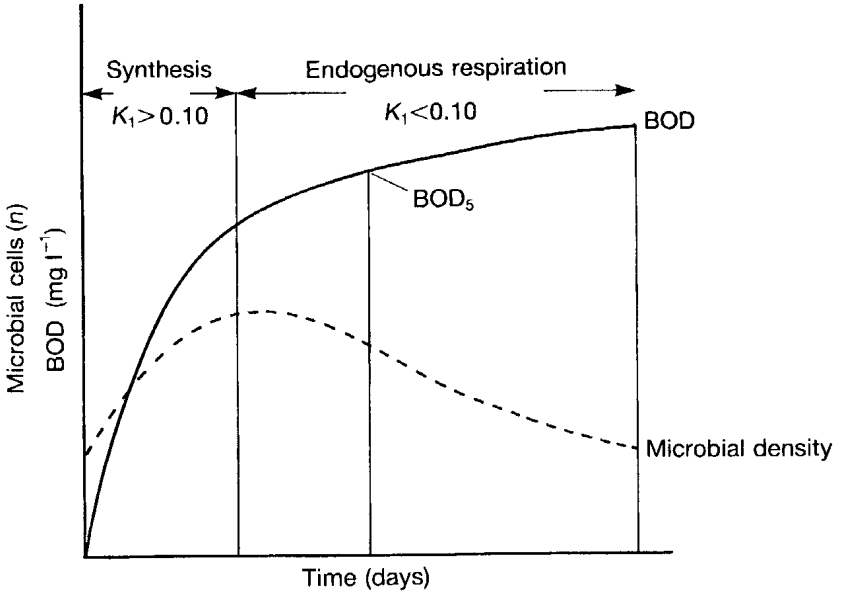


Fig. 1.20. Microbial reactions that occur in the BOD bottle.

The rate constant K_1 and the ultimate BOD (L) are traditionally calculated using graphical methods such as non-linear fitting and linear fitting of modified expressions of the BOD equation. However, the graphical method of Thomas (1950) is still the most widely used. The main methods are compared by Cutrera *et al.* (1999).

Of all the available methods of calculating the BOD constants, the Thomas method is perhaps the simplest. The procedure is based on the function:

$$\left(\frac{t}{y}\right)^{1/3} = (2.3K_1L)^{-1/3} + \frac{K_1^{2/3}}{3.43L^{1/3}} \cdot t$$

where y is the BOD exerted in time t, K_1 the reaction rate constant (base 10) and L the ultimate BOD.

This equation forms a straight line with $(t/y)^{1/3}$ plotted as a function of time t. The slope $K_1^{2/3}/(3.43\ L)^{1/3}$ and the intercept $(2.3K_1L)^{-1/3}$ of the line of best fit of the data is used to calculate K_1 and L.

Using the form $Z = a + bt$ for the straight line where $Z = (t/y)^{1/3}$, $a = (2.3K_1L)^{-1/3}$, and $b = K_1^{2/3}/(3.43\ L)^{1/3}$:

$$K_1 = 2.61(b/a)$$

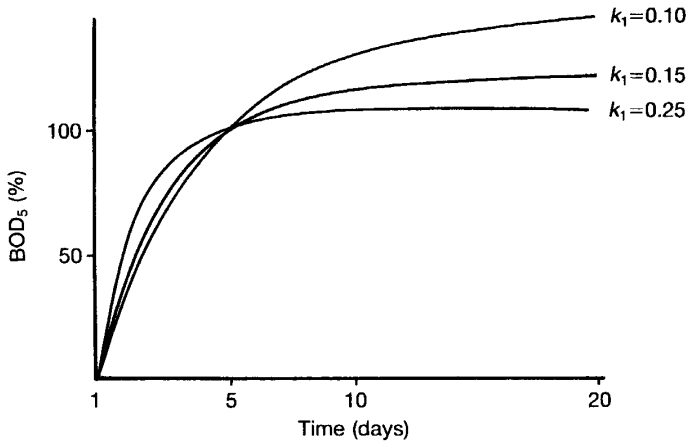


Fig. 1.21. The effects of various rate constants in the calculation of the same BOD. The resultant BOD is expressed as a percentage (Tebbutt 1983).

$$L = \frac{1}{2.3K_1a^3}\,.$$

For example, over a 10-day period the BOD was measured every second day. From this data $(t/y)^{1/3}$ can be calculated:

$t(\mathrm{d}^{-1})$	2	4	6	8	10
$y(\mathrm{mg\ l}^{-1})$	14	22	27	30	32
$\left(\frac{t}{y}\right)^{1/3}$	0.523	0.567	0.606	0.644	0.679

The graph of $(t/y)^{1/3}$ is plotted against t (Fig. 1.22) and from this, the intercept a can be measured ($a = 0.481$) and slope b calculated:

$$\text{slope } b = (0.042/2) = 0.021\,.$$

From these values the rate reaction rate K_1 and the ultimate BOD (L) can be estimated:

$$K_1 = 2.61(b/a) = 2.61\frac{(0.021)}{0.481} = 0.114$$

$$L = 1/2.3K_1a^3 = \frac{1}{2.3(0.114)(0.481)^3} = 34.3\ \mathrm{mg\ l}^{-1}$$

Although the kinetics of the BOD test have been modelled as a first-order reaction, it has been argued that the BOD process is so complex that it cannot be adequately described solely by the first-order reaction

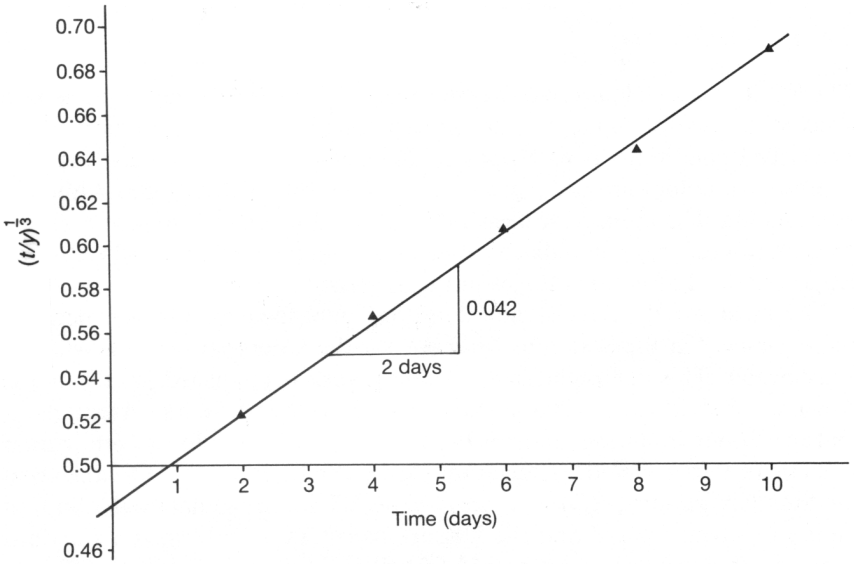


Fig. 1.22. Determination of the BOD constant K_1 (reaction rate constant) and L (the ultimate BOD) from BOD data using the Thomas method.

equation (Young and Clark 1965; Landine 1971; Stones 1981, 1982; Adrian *et al.* 1999). This is fully discussed in Sec. 3.1.3.

1.4.2.2. *Methodology*

The BOD_5 is defined universally as the mass of dissolved oxygen required by a specific volume of liquid for the process of biochemical oxidation under prescribed conditions over 5 days at 20°C in the dark. The result is then expressed as milligrams of oxygen per litre of sample (HMSO 1983). Although three standard methods have been published for the test, each follows a similar procedure with only minor discrepancies regarding the strength and volume of reagents added at various stages in the analysis.

The most widely used standard is that published as 'Oxygen Demand (Biochemical)' in the Standard Methods for the Examination of Waste and Wastewater. This comprehensive reference work is published jointly by the American Public Health Association, American Water Works Association and the Water Pollution Control Federation (American Public Health Association 1985). The International Organization for Standardisation (ISO) is a world-wide federation of national standard bodies of which Ireland and the United Kingdom are members. It published a standard

procedure for the determination of BOD (ISO:5815) in 1983, entitled *Water Quality — Determination of Biochemical Oxygen Demand after 'n' days (BOD_n) — Dilution and Seeding Method*, which was updated in 1989 (ISO 1989a). Finally, a new British standard method, *Biochemical Oxygen Demand (1981)* published by the Department of the Environment as part of their series 'Methods for the Examination of Wastes and Associated Materials' (HMSO 1983). The methodology described below is based on this method and further discussion of the test can be found in that standard method.

The 5-day incubation period for the BOD test has limited its use as an active wastewater operational parameter, where rapid information is required. For that reason, total organic carbon (TOC) has been widely adopted for controlling organic loading to treatment plants, especially in industrial situations where loadings are unpredictable due to variable batch production. Biosensors have been utilised in the development of rapid BOD measurement (Sec. 10.4.4). A biosensor comprises a biological sensing element that produces a signal when exposed to a specific analyte. The sensing element may be immobilised micro-organisms, an enzyme, antibody or even nucleic acid. The signal produced by the biological sensing element is converted to an electrical signal by a transducer (Praet *et al.* 1995; Burlage 1997). A range of immobilised micro-organisms have been used including the bacteria *Bacillus subtilis* (Tan and Qian 1997; Qian and Tan 1998), and *Pseudomonas putida* (Chee *et al.* 1999), the yeasts *Axula adeninivorans* (Reidel *et al.* 1998; Chan *et al.* 2000) and *Trichosporon cutaneum* (Marty *et al.* 1997), mixtures of activated sludge micro-organisms (Liu *et al.* 2000) and micro-organisms from manufactured seeds used for BOD analysis (Tan and Wu 1999).

Karube and Tamiya (1987) produced a BOD biosensor using the immobilised yeast *Trichosporon cutaneum*. The biosensor is based on a dissolved oxygen electrode with a platinum cathode and aluminium anode in saturated potassium chloride solution. The yeast cells are immobilised onto a porous membrane that is placed under the outer Teflon membrane of the electrode trapping the yeast cells between the two membranes. In this way, the immobilised cells cause a decrease in current as they consume oxygen. The sensor has an effective BOD range of 3–60 mg l^{-1}, and is closely correlated with standard BOD_5 determinations. Reidal *et al.* (1990) also used *T. cutaneum* but immobilised in polyvinyl alcohol. This biosensor is able to give a BOD analysis within 30 seconds, remains stable for up to 48 days, and shows a good correlation with standard BOD_5. The measurement of BOD by biosensors is affected by inhibitory substances such as heavy metals,

just like the standard BOD_5 (Qian and Tan 1999). The use of biosensor technology in BOD determinations has been reviewed by Normura *et al.* (1998) (Sec. 10.4.4).

Sample preparation

It is only in a small number of cases that dilution and seeding will not be necessary. Dilution can only be omitted when the BOD of the sample is < 4 mg l^{-1}; and seeding is not necessary if the sample already contains adequate numbers and a suitable diversity of acclimatised micro-organisms. Although these conditions exist in some treated effluents and most river waters, if doubt exists then a series of dilutions and a seed should be used. In practice, it is difficult to know whether a sample does contain suitable micro-organisms, so there is a growing tendency to seed all samples, regardless. Samples need to be analysed as quickly as possible, preferably within 2 hours. If this is not possible, the organic decomposition must be inhibited as a significant proportion of the available organic substrate could be oxidised giving a low BOD value. For example, samples stored at 20°C for 4 and 22 hours resulted in decreases in the BOD value of 14 and 22% respectively (American Public Health Association 1985). Chemical inhibition will obviously interfere with the test as well, so samples should be stored at between 2–4°C and be analysed ideally within 6 hours but never more than 24 hours after collection. Influences of the methods and period of storage on the BOD have been reviewed by Ranchet *et al.* (1981). They found that freezing samples, as recommended in the ISO standard, depressed the BOD results. If samples are frozen, seeding with bacteria acclimatised to 20°C must be carried out to replace those destroyed by the low temperature. The storage period is also critical and if it exceeds 24 hours, then samples must be discarded. In the case of composite samples collected using a 24 hours sampler, the container must be kept as near to 4°C as possible during collection, and all the samples must be analysed within 24 hours of the last aliquot being collected (Water Pollution Research Laboratory 1967). There is some evidence to suggest that diluted samples can be successfully stored at 4°C for up to 4 days without any effect on the BOD. It appears that the low density in the sample after dilution remains so low at the reduced temperatures as to have little impact on the substrate in solution (Tyers 1988).

As chlorine and chloramines severely inhibit microbial activity, tap water is unsuitable for use as dilution water. Until fairly recently clear natural waters, especially groundwaters, were used for diluting samples in the BOD

test. However, the variability in nutrient content had a significant effect on the microbial activity of the micro-organisms, with nutrients often limiting full microbial oxidation. This has been overcome by the introduction of a standard synthetic mineral nutrient dilution water. Freshly prepared distilled or deionized water is used, although distilled water from a copper still should not be used as residual copper concentrations in excess of 0.01 mg l^{-1} can inhibit bacterial activity. By adding small amounts of chemicals to the distilled water, a dilution water with a standard pH, reasonable buffering capacity and salinity, and sufficient inorganic nutrients to support microbial activity can be produced. The chemicals are added in the form of a phosphate buffer solution to provide the phosphorus requirement and to maintain an optimum pH of 7.2; potassium, sodium, calcium, and magnesium salts which are essential nutrients for the growth and metabolism of micro-organisms; and finally ferric chloride, magnesium sulphate and ammonium chloride to provide iron, sulphur and nitrogen. Together these solutions should be added to the dilution water to give a BOD:N:P ratio of 60:3:1. Four stock solutions are made and 1 ml of each is added to each litre of dilution water prepared, in the following order: ferric chloride (0.0124% m/V), calcium chloride (2.75% m/V), magnesium sulphate (2.5% m/V) and phosphate buffer solution (pH 7.2). The HMSO standard dilution water differs from the ISO and the US standard in that a 50% lower concentration of ferric chloride is used and a 10% higher magnesium sulphate concentration is used. The former is to reduce the possibility of bacterial inhibition. The dilution water is saturated with oxygen and stabilised before use, with the temperature maintained at 20°C (Sec. 1.4.2.3).

When samples are diluted, then it is vital that the dilution water used has a very low oxygen demand. Unseeded dilution water can be used so long as it has an oxygen demand of < 0.3 mg O_2 l^{-1}. High dilution water BOD is usually caused by a combination of factors such as the use of dirty glassware or storage vessels; glassware containing trace amounts of detergents, the presence of volatile organic materials in the distilled water, and the topping up of old dilution water with freshly prepared dilution water. There is much dissatisfaction in this maximum oxygen demand standard for dilution water, especially as it is extremely difficult to measure a BOD of < 0.3 mg l^{-1}. Also, as a period of 5-day incubation is required to establish the BOD of the dilution water, it is not possible to check the suitability of the dilution water before use, so if the BOD has exceeded the 0.3 mg l^{-1} maximum, then that set of BOD analyses will have to be discarded (Fitzmaurice and Gray 1987a). If dilution water is constantly exceeding the limit, then it can be stored at 20°C in the dark long enough for it to satisfy its own

BOD. However, if this is done, a small amount of seed is required to ensure an oxygen uptake of 0.1 mg O_2 l^{-1} and a nitrification inhibitor should be added to preserve the ammonia and prevent the growth of nitrifying bacteria. When seeded, the dilution water should have an oxygen demand of < 0.5 mg O_2 l^{-1}, although the ISO and US standards allow the BOD to be < 1.0 mg O_2 l^{-1}. In an inter-laboratory study of 23 state and semi-state water laboratories in Ireland, Fitzmaurice (1986) found that the BOD of dilution water seeded with a dehydrated proprietary seed ranged from 0.2–2.1 mg l^{-1}. Five laboratories exceeded the ISO standard of 1.0 mg l^{-1} and of those recording a BOD of < 1.0 mg l^{-1} the mean value was 0.51 mg l^{-1}. The fact that 13 of the 23 laboratories failed to reach the more stringent level of 0.5 mg l^{-1} may indicate that this figure may, in practice, be too low. Stover and McCartney (1984) addressed the problem of high blank dilution values and formulated a seed correction factor. A sample of unseeded dilution water and several dilutions of the seed material are incubated along with the samples under test. After the incubation period, a plot of dissolved oxygen depletion versus ml of seed added is made. This results in a straight line (Fig. 1.23). Simple linear regression is applied to the results with the intercept on the y-axis, at zero seed concentration, corresponding to the unseeded dilution water BOD. The slope of the line corresponds to the dissolved oxygen depletion of 1 ml of seed. The sum of these two correction factors is then substituted in the calculation formulae for (B_0–B_n). This method provides a dilution water correction and a seed correction as separate and independent factors.

There are a number of methods of diluting samples. The least satisfactory is pipetting the sample directly into the BOD bottle and then filling it with dilution water. The problem with this approach is that the volume of the BOD bottles is never exactly 150 or 250 cm^3, and varies enough to significantly affect the final BOD when large dilutions have been employed. Also, as the top is placed into position, some of the sample will be lost, and as it is not possible to completely mix the sample before placing the stopper, the loss of sample and dilution water will most likely be unproportional, thus affecting the final dilution. The most widely adopted method is the jug technique. Here the sample and dilution water are mixed in a graduated cylinder using a plunger-type mixing rod, so as not to entrain air in the sample, and then transferred into the BOD bottles. A development of this technique is the automated mixing chamber, developed at Aston University, which is perhaps the most efficient method of ensuring adequately diluted and mixed samples. The apparatus consists of a glass aspirator with a tap, a separating funnel, a three-way large bore stopcock

Sample Description		Seed (direct)		
Day	5-4-80	Wednesday	0	5
10	Bottle No.	DO	9.0	8.8
		Accm. Depl.		
0	Dilution	H_2O Corr.		
0	Sample Vol.	Seed Corr.		
	Factor	Net Depl.		0.2
		BOD		
11	Bottle No.	DO	9.0	8.5
		Accm. Depl.		
0	Dilution	H_2O Corr.		
1	Sample Vol.	Seed Corr.		
	Factor	Net Depl.		0.5
		BOD		
12	Bottle No.	DO	8.9	6.4
		Accm. Depl.		
0	Dilution	H_2O Corr.		
5	Sample Vol.	Seed Corr.		
	Factor	Net Depl.		2.5
		BOD		
13	Bottle No.	DO	8.7	3.7
		Accm. Depl.		
0	Dilution	H_2O Corr.		
10	Sample Vol.	Seed Corr.		
	Factor	Net Depl.		5.0
		BOD		

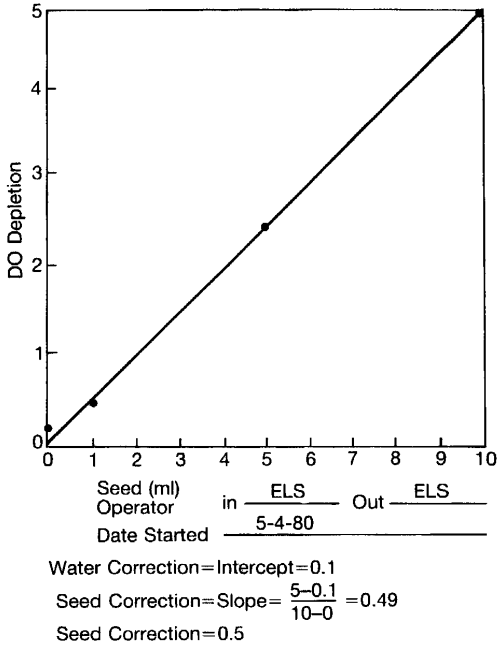


Fig. 1.23. An example of the graphical method used for correcting oxygen depletion due to dilution water and microbial seed used in the BOD test (Strover and McCartney 1984).

assembly and associated tubing (Fig. 1.24). Seeded or unseeded dilution water is stored in the aspirator which acts as a reservoir, and flows under gravity through the three-way stopcock into a graduated separating funnel. The dilution water enters at the base of the funnel, thus avoiding the entrainment of air, and is allowed to partially fill the funnel (25%) before the flow is stopped by closing the stopcock. The required amount of sample is added by pipetting it through the neck and allowing it to flow down the side of the funnel. The dilution water is then allowed to flow into the funnel up to the required volume, usually 600 cm^3, to fill two 250 cm^3 BOD bottles. The contents of the funnel are mixed using a plunger-type mixing rod to ensure no air is entrained and the flow diverted via the free leg of the three-way stopcock to rinse and fill the BOD bottles.

If the Winkler method is used (see below), two BOD bottles must be prepared for each sample. However, if the electrode method is used for oxygen analysis, only one bottle is required as it can be used for both the determination of the initial and final dissolved oxygen concentrations (Fitzmaurice and Gray 1987a,b).

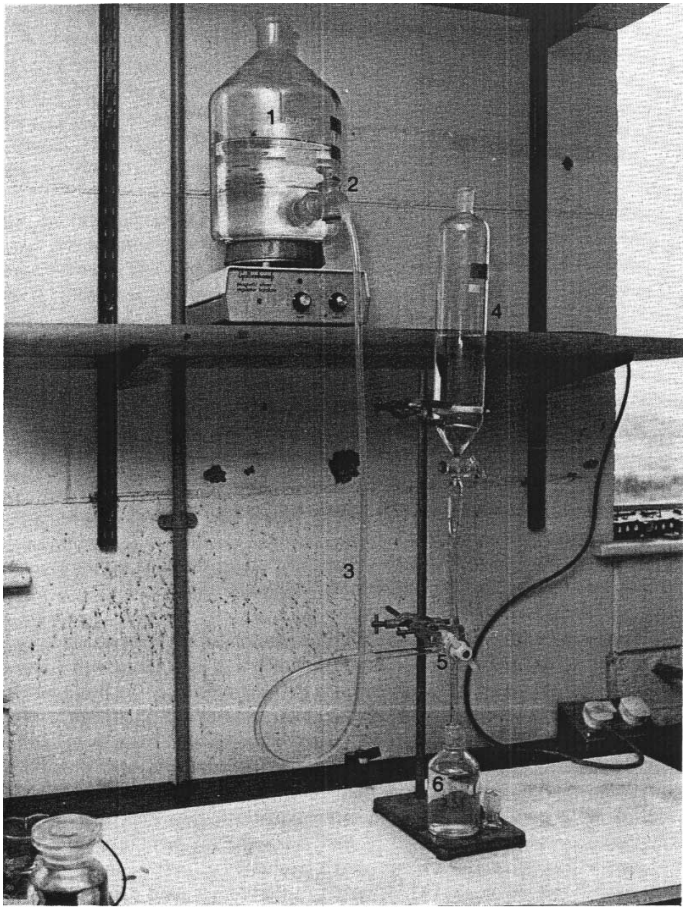


Fig. 1.24. BOD mixing chamber: 1, dilution water reservoir (5 or 10 litre aspirator bottle); 2, glass stopcock assembly with key; 3, tubing; 4, graduated cylinder separating funnel (1 litre); 5, three-way T-form glass stopcock; 6, BOD bottle (250 ml).

Measuring the oxygen concentration

There are two standard methods for determining the dissolved oxygen concentration in the BOD test, a titimetric procedure and a membrane electrode method. The classical wet chemistry iodometric technique developed by L.W. Winkler in 1888 is still widely used today albeit in modified forms. It is a titimetric procedure based on the oxidising property of dissolved oxygen and is usually referred to as the Winkler method. Due to the variability of the chemical composition of natural waters and wastewaters, the iodometric method has always been prone to chemical interference. To overcome

the more common interfering substances, a number of modifications of Winkler's original method have been developed. For example: the permanganate modification used in the presence of ferrous iron, the alum flocculation modification used in the presence of suspended solids, the copper sulphate-sulphamic acid flocculation modification developed for use with activated sludge mixtures and the azide modification which is used in the presence of nitrite. It is this last modification that is recommended for the analysis of dissolved oxygen in sewage, effluents and river waters. Complete details of this method are given elsewhere (HMSO 1983) and only a résumé of the azide modification of the Winkler method is given here.

The basis of the method is the production of a white precipitate of manganous hydroxide which reacts with the dissolved oxygen to form a brown hydroxide of manganese in higher valency states. The sample is then acidified with sulphuric acid, which in the presence of iodide, liberates free iodine equivalent in amount to the original concentration of dissolved oxygen. The iodine is titrated with a standard solution of thiosulphate. The end point of the titration can be determined electrometrically but is normally detected visually using soluble starch as an indicator. Five reagents are required for these reactions: manganous sulphate solution to produce the manganous hydroxide precipitate; alkali-iodide-azide reagent provides the iodide concentration and the azide counteracts any interference due to the presence of nitrites; sulphuric acid to acidify the manganese hydroxide precipitate; starch solution to detect the end point of the titration and sodium thiosulphate is the titrant used to measure the free iodine concentration. The analysis begins with the addition of 2.0 ml of manganous sulphate and 2 ml of alkali-iodide-azide solutions to the sample within the BOD bottle. The stopper is replaced and the content of the bottle mixed vigorously producing a precipitate which is allowed to settle to the lower half of the bottle with a clear supernatant discernible. Then 4.0 ml of sulphuric acid solution is added to the sample, which is repeatedly mixed to ensure the precipitate is fully dissolved. A portion of the acidified solution (100–200 ml) is transferred to a conical flask and titrated with sodium thiosulphate using starch as an indicator. The end point of the titration is reached at the first disappearance of the blue colouration, with any recolouration ignored. One percent starch glycollate has been shown to give a sharper and more reliable end point (Vogel 1978). If 0.0125 M solution thiosulphate titrant is used to titrate 200 ml of the acidified solution, then the amount used is equivalent to the dissolved oxygen concentration of the sample. In cases where the dissolved oxygen measurement is not part of BOD analysis, a correction

factor is applied to compensate for the displacement of a small portion of the sample caused by the addition of the reagents.

The more recent membrane electrode method is based on the rate of diffusion of molecular oxygen across a permeable membrane. A modified oxygen electrode is used to measure the dissolved oxygen in the BOD test. The electrode is usually of the polarographic type and is manufactured to a size capable of being inserted into the standard BOD bottle with a wide neck. In order to contain the small volume of sample which is displaced as the electrode is inserted into the BOD bottle, a special funnel is supplied which forms a seal at the neck of the BOD bottle and contains the displaced liquid in a chamber above the neck. A fixed magnetic stirring bar is attached to the end of the funnel to provide the necessary flow across the electrode. The advantages of this method over the Winkler method include its speed of measurement; simplicity; less chance of errors in measurement; that it can be used for continuous monitoring of uptake; allows K_1 values to be calculated without the need of many replicate bottles; that only a single bottle is required and that it is not susceptible to interfering substances as is the iodometric method. However, it is subject to interferences, particularly from gases which undergo reduction at the same potential as oxygen, such as nitrous oxide, chlorine, nitric oxide, hydrochloric acid, and formaldehyde as well as the presence of hydrogen sulphide.

Each day before use, the electrode must be calibrated using single point calibration at both high and low dissolved oxygen concentrations. The calibration should be repeated as frequently as practicable, but especially at the end of the day. Checks can be made by analysing split samples of air saturated and deoxygenated samples of distilled water using the iodometric method as a reference by which the electrode is calibrated. There are several ways of preparing oxygen-free water (HMSO 1983; APHA 1985), but a particularly successful one is to deoxygenate distilled water by boiling and then bubbling with oxygen-free nitrogen overnight before use. Once calibrated, the electrode can be inserted into the BOD bottle. The mixing mechanism is then switched on and a period of at least 60 s allowed to elapse before the oxygen concentration is read. The electrode is carefully removed to allow the displaced liquid retained by the collar to drain back into the bottle, and if the oxygen consumption is to be measured over more than five days, the volume of the BOD bottle is made up using fresh dilution water if necessary and the stopper replaced. The electrode is rinsed with distilled water and it is then ready to measure the next sample. The linearity of response of the electrode will alter over a long period, so it should be checked at monthly intervals by plotting the dissolved oxygen concentrations, in BOD

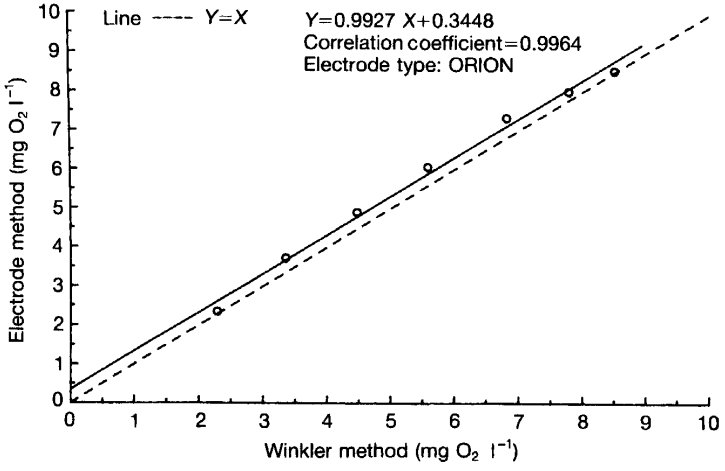


Fig. 1.25. Oxygen electrode calibration test showing a small fixed bias in response (Fitzmaurice 1986).

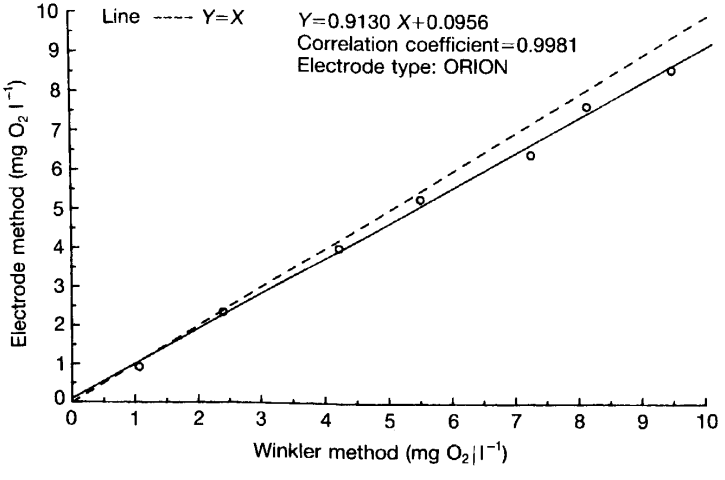


Fig. 1.26. Oxygen electrode calibration test showing a small relative bias in response (Fitzmaurice 1986).

bottles, over as wide a range as possible, measured by the Winkler method against the electrode method. A small fixed bias will have no effect on the BOD calculation (Fig. 1.25) while a relative bias, which is either increasing or decreasing with increasing sample concentration will severely affect the calculation of BOD (Fig. 1.26) (Fitzmaurice 1986).

Manometric repirometers are used primarily to measure oxygen uptake rate. In its basic form, a measured volume of test substance is stirred in a partially filled bottle which is connected to a closed-end mercury manometer. Oxygen consumption is measured by observing the change in level in the mercury column of the manometer. Any carbon dioxide that evolves into the bottle atmosphere is absorbed by alkali which is held in a small cup within the bottle cap (Kilroy and Gray, 1995; Çeçen and Yangin 2000, 2001). A more sophisticated type of respirometer, the WTW Oxitop®, has been designed specifically for BOD_5 analysis. The system has all the advantages and simplicity of a manometric method (i.e. no separate dissolved oxygen analysis), without the use of mercury which poses serious health and safety problems. Each bottle is fitted with a special head (top) that incorporates a sensitive micro-electronic pressure sensor. This records the reduction in oxygen by measuring the pressure difference within the sealed bottle. The head also houses an integrated data logger and timer so that daily measurements are made and stored. The BOD results are read directly from a LED display on the head, with daily readings also available. Therefore, a complete record of BOD over 5 days can be obtained without disturbing the sample. The bottles come with special platforms that incorporate magnetic stirrers for each bottle in units of 2, 6, or 12. For larger laboratories, different heads are used which use an infra-red interface to communicate to a central hand held control unit that records the data and can even graph the results from each bottle. The control unit can manage up to 120 bottles at the same time. The system can measure BOD concentrations over a wide range (0–10,000 mg l^{-1}) with no dilution of the sample required, making it extremely simple and the results very repeatable.

The BOD calculation

Two forms of BOD calculation are used. For undiluted, unseeded samples:

$$\mathrm{BOD_n} = (\mathrm{D_0} - \mathrm{D_n})\mathrm{mg\ O_2\ l^{-1}}$$

where D_0 and D_n are the dissolved oxygen concentrations before and after n days incubation respectively. For diluted samples, seeded or unseeded:

$$\mathrm{BOD_n} = f\left[(D_0 - D_n) - \frac{(f-1)}{f}(B_0 - B_n)\right]\mathrm{mg\ O_2\ l^{-1}}$$

where B_o and B_n are the dissolved oxygen concentrations of the seed control (blanks) before and after n days respectively and f is the dilution factor.

Thus, $([f-1]/f)(B_o - B_n)$ is the oxygen demand of the seed, but the dilution factor correction $([f-1]/f)$ becomes insignificant with dilutions in excess of 1:100. If a chemical inhibitor is added to the dilution water to suppress nitrification, this should be stated when expressing the result. For example, if allythiourea is used, the result should be expressed as $BOD_{(ATU)_n}$.

1.4.2.3. *Factors affecting the test*

Temperature

As bacterial activity is a function of temperature, the BOD test is temperature dependent. Although the ultimate BOD (L_0) is slightly affected, because oxidisability increases with temperature, in practice the temperature only affects the rate of oxidation (K_1) and not the amount of waste oxidised, therefore the ultimate BOD will always be the same regardless of the temperature at which the test is performed. Although not widely used, the breakdown process can be accelerated within the BOD bottle by incubating at higher temperatures. The time lapse of 5 days between sample preparation and the result is a severe limitation and with a 5-day working week, this means that in practice BOD analyses cannot be commenced on Mondays or Tuesdays without incurring overtime attendance. The time restraint also means that the use of the BOD for process control and effluent monitoring purposes is meaningless. To overcome these limitations, rapid BOD tests have been developed. The most popular of these based on raising the incubation temperature resulting in increased bacterial activity. For example, there is close agreement between the 5-day BOD test at 20°C and a 2.5-day test at 35°C (Fig. 1.27), allowing the BOD test to be completed within a working week, and the Ministry of Health (1936) published tables of BOD_5 at 20°C and BOD_3 at 27°C for a range of effluents with differences rarely exceeding 5%. Good correlations between BOD_2 at 37°C and BOD_5 at 20°C were achieved by Orford and Matusky (1959) and Robbins (1961) suggested a BOD_1 at 37°C for effluent treatment plant control. However, it is advisable to calibrate particular effluents at the two different temperatures first. In warmer climates, 30°C is a more appropriate temperature at which to carry out the BOD test, as the naturally occurring bacteria are acclimatised to this higher temperature and a BOD_3 at 25–30°C is commonly used in tropical countries. However, the adoption of a single 20°C standard over 5 days means that BOD results are comparable internationally.

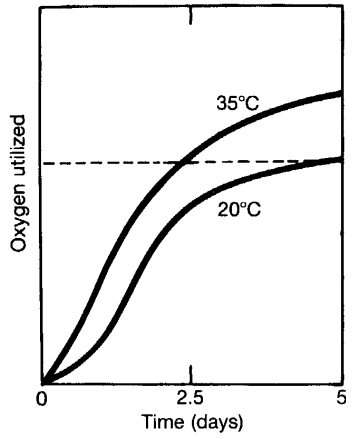


Fig. 1.27. Association between the 2.5-day BOD test at 35°C and the standard 5-day BOD test at 20°C (Mara 1974).

The reaction rate constant (K_1) in the BOD test increases with temperature according to Vant Hoff's law:

$$K_{1(T)} = K_{1(20)} \cdot \theta_T^{(T-20)}$$

where θ_t is the temperature coefficient with values between 1.047 to 1.135 and the reaction rate constant (K_1) measured at temperatures T°C and 20°C. The value of θ depends on the temperature and its mean value is 1.047 (Streeter and Phelps 1925), which essentially means that the speed of reaction increases by 4.7% for each 1°C rise in temperature. The mean value proposed by Streeter and Phelps is, however, inaccurate at low temperatures, so two values of θ are used: 1.135 between 4–20°C and 1.056 over the temperature range of 20–30°C.

Once the incubation temperature has been decided, every attempt must be made to ensure that the exact temperature is maintained over the incubation period, which includes preheating the dilution water to the incubation temperature. A 1°C deviation from the 20°C incubation period can produce an error of up to 5% over the five days. The effect of temperature on BOD stoichiometry and oxygen uptake rate has been reviewed by Flegal and Schroeder (1976).

Dilution

It is vital that some dissolved oxygen remains after incubation to ensure that the oxygen assimilation during the test can be calculated. If the waste

is too strong, then all the dissolved oxygen will be utilised before the 5-day incubation period has elapsed. In contrast, if the waste is too dilute, then only a small proportion of the dissolved oxygen will have been used, which leads to any analytical errors in determining the oxygen becoming excessively significant. As a general guide, if the BOD_5 is < 7 mg l^{-1}, no dilution is required; however, if it exceeds 9 ml^{-1}, all the dissolved oxygen will be depleted after 5 days, resulting in a zero value on completion of the test. This means that the BOD_5 calculation cannot be worked out and the result will be limited to > 9 mg l^{-1} only. Thus, apart from river waters, most samples require dilution. Dilution of samples should be done outside the BOD bottle and the dilution water itself should be capable of sustaining bacterial growth. It should contain a mixture of salts including nitrogen, phosphorus, sulphur and iron, as well as a range of trace elements, have a neutral pH and contain sufficient ions to give an ionic strength to the water which favours microbial growth. It is important that it contains as little organic material as possible so that it does not exert a significant BOD in its own right, and of course it must be aerated for 40 minutes before use to ensure supersaturation and then left to stand for a further 30 minutes to ensure all the excess oxygen is released (i.e. equilibriate) and that 100% saturation has been achieved. The water is normally preheated to 20°C and constantly stirred.

The dilution required depends on the actual BOD of the original sample. The most accurate BOD estimation will be obtained when between 35–50% of the dissolved oxygen is utilised. If the dissolved oxygen remaining in the bottle falls to below 1 mg l^{-1}, aerobic breakdown is inhibited, resulting in a misleading result. Choosing the correct dilution is vital to the successful operation of the test. For example, if the original sample has a BOD_5 of 250 mg l^{-1} then a x50 dilution is required, which will give a predicted BOD_5 value for the diluted sample of 5 mg l^{-1}. However, if only a x20 dilution is used, then the diluted sample will have a BOD_5 of 12.5 mg l^{-1}, and as this is greater than the available oxygen in the BOD bottle, where the maximum is 9.8 mg l^{-1} at 20°C, all the available dissolved oxygen will be used within the five days so that no result can be calculated. Thus, if the approximate BOD value of a sample is not known, a range of dilutions should be used to cover all the most likely ranges, and the dilution resulting in between 35–50% dissolved oxygen utilisation used to calculate the BOD. Typical dilution ranges are given in Table 1.38. For non-river samples, the most efficient dilution ranges to use are 1:20 (5%), 1:50 (2%) and 1:100 (1%), which will ensure an accurate estimation of the BOD_5 over a range of 40–700 mg l^{-1}. Each dilution gives a certain degree of overlap with the

Table 1.38. Recommended dilution factors for the determination of BOD (ISO 1983).

Expected BOD (mg $O_2 l^{-1}$)	Dilution factor	Report to nearest mg $O_2 l^{-1}$	Applicable to
3–6	1–2	0.5	R
4–12	2	0.5	R, E
10–30	5	0.5	R, E
20–60	10	1	E
40–120	20	2	S
100–300	50	5	S, C
200–600	100	10	S, C
400–1200	200	20	I, C
1000–3000	500	50	I
2000–6000	1000	100	I

R, river water; *E* biologically treated domestic effluents; *S*, clarified domestic effluents; *C*, raw domestic effluents; *I*, heavily contaminated industrial effluents.

1:20 dilution causing the 40–180 mg l^{-1} range, 1:50 the 100–350 mg l^{-1} range and 1:100 the 200–700 mg l^{-1} BOD range.

Microbial influences on the test

The BOD test is a microbial growth system and so it is important to make certain that a suitable microbial community is present in order to ensure that the test proceeds efficiently. Four factors can have a significant effect on the BOD result: (i) a low initial bacterial density, (ii) the use of unacclimated bacteria, (iii) the presence of nitrifying bacteria and finally, (iv) the presence of algae (Mara 1974).

Most effluent samples contain sufficient bacteria to allow biological oxidation to proceed immediately. However, if the density of bacteria is initially low, there may be a delay before a sufficient population of bacteria have developed to allow oxidation to proceed at its optimum rate (Fig. 1.28). If samples have less than 10^3 bacteria per ml, then seeding will be necessary. In practice, all samples should be seeded. Settled sewage, is generally used as acclimated to the BOD test temperature for 24 to 36 hours, seed with 1–2 ml added to each litre of dilutant or in the case of undiluted samples, per litre of sample tested. This provides a mixture of micro-organisms capable of metabolising a range of substances that may be present. The effluent

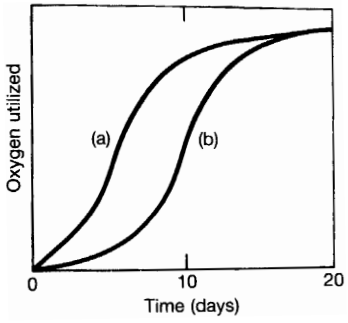


Fig. 1.28. Effect of initial bacterial population on the BOD test. The BOD curves obtained using (a) normal initial population of acclimatised bacteria, and (b) low initial bacterial population or an unacclimatised seed (Mara 1974).

from the biological treatment unit can also be used, and like settled sewage, it should be settled for at least 1 hour at 20°C before use. Another source of seed is river water, especially below effluent outfalls, and where it is impossible to obtain seed from one of these sources, garden soil can be used. About 100 g of soil is added to 1 litre of water, well mixed and allowed to stand for 10 minutes; 10 ml of the supernatant is then diluted with water to 1 litre and used as seed in the usual way. Proprietary seeds are also available, which are made up of a number of bacterial and fungal species. They are supplied in a capsule containing the correct weight of dried organisms that when mixed with 1 litre of water will provide a seed of standard quality (Sec. 10.4.3). As the microbial quality and diversity of settled sewage is very variable, the advantages of a standard seed are obvious. Manufactured seeds have been shown to produce more repeatable test results than other seeds (Fitzmaurice and Gray 1989; Kumar *et al.* 1999; Manoharan *et al.* 2000). However, the sample must provide a reasonable nutritional balance to allow the micro-organisms to thrive. Some industrial wastewaters may have a limiting range of nutrients which will only support a restricted range of micro-organisms, while others may be toxic and completely inhibit microbial activity. For example, phenol wastewaters are toxic to normal micro-organisms and so a BOD test will give a zero result as no dissolved oxygen is utilised. Thus, it is important to use specially adapted microbial cultures, which can be purchased or collected from biological treatment units treating phenol wastes in order to obtain a BOD value. It is possible to produce your own acclimatised microbial culture for seeding difficult industrial wastes, although it is time consuming. A 1-litre plastic or glass bottle is filled three-quarters full with settled wastewater and aerated using

a small aquarium aerator. Starting with small amounts, the industrial effluent is added to the system over a period of several weeks. As the wastewater becomes cloudy, this indicates that the culture is acclimated. Alternatively, instead of settled wastewater, activated sludge can be used with the final settled effluent being used as the acclimatised seed. It is not only industrial wastes which are nutritionally deficient. Many biodegradable wastes from the food processing and drink manufacturing industries are deficient in either nitrogen or phosphorus, which need to be supplemented, normally via the dilution water, otherwise degradation proceeds more slowly, producing a low BOD value. By removing protozoa which prey on the bacteria, by filtering the seed, it is possible to increase the oxidation rate by allowing the concentration of bacteria to rapidly increase, thus allowing carbonaceous oxidation to be completed more rapidly, thereby allowing a shorter incubation period to be used. Le Blanc (1974) found that compared to the standard BOD_5, samples seeded with protozoan-free inocula produced more reproducible BOD results after just 2 days of incubation. So far this rapid BOD method has not been widely adopted.

In the oxidation of ammonia by nitrifying bacteria, considerable quantities of oxygen can be utilised, which can represent a significant fraction of the total oxygen demand of a wastewater. For example, it is normal for the nitrogenous fraction to account for two or even three times more than the carbonaceous fraction in the BOD test. Considerable oxygen is required to oxidise ammonia, as can be seen from the stoichiometry:

$$2NH_4 + 3O_2 \xrightarrow{Nitrosomonas} 2NO_2 + 4H^+ + 2H_2O + \text{energy}$$

$$2NO_2 + O_2 \xrightarrow{Nitrobacter} 2NO_3 + \text{energy}$$

with the overall reaction:

$$NH_4 + 2O_2 \longrightarrow NO_3 + 2H + H_2O + \text{energy}$$

Theoretically, 3.43 g of molecular oxygen is required by *Nitrosomonas* to oxidise 1 g of ammonia to nitrite and a further 1.14 g of molecular oxygen by *Nitrobacter* to oxidise 1 g of nitrite to nitrate. However, small amounts of nitrogen are assimilated as cell material during synthesis and this amount must be subtracted from the theoretical requirement. Montgomery and Bourne (1966) calculated the oxygen equivalent of the assimilated ammonia at 0.20 g and nitrite at 0.02 g. From these values, the following equation can be used to predict the extent of nitrogenous oxygen

demand (NOD) in the BOD test:

$$\text{NOD} = 3.23 \times \text{increase in nitrite-N} + 4.35 \times \text{increase in nitrate-N}$$

Thus, a partially nitrified effluent containing 20 mg l^{-1} of ammonia would exert a NOD in the order of 80 mg O_2 l^{-1}. The extent of nitrification in the BOD test is more easily measured by incubating a parallel set of samples, one with and one without nitrification suppressed, the difference being the NOD. Nitrification only occurs when ammonia and nitrifying bacteria are present in sufficient concentration and numbers, and nitrification inhibitors are absent. In non-nitrified effluents, only ammonia is present and the density of nitrifying bacteria is extremely low. Nitrifying bacteria multiply very slowly, with a doubling time of 2–6 days (Downing *et al.* 1969), therefore nitrification generally occurs towards the end of the carbonaceous oxidation phase in the BOD test. Generally, it will be upwards of 10 days before nitrification begins to exert an oxygen demand. In partially nitrified effluents, both ammonia and nitrifying bacteria will be abundant, therefore nitrification exerts a high oxygen demand after about 5 days that will be far in excess of the carbonaceous oxygen demand (Figs. 1.18 and 1.19). This produces a problem in interpreting the BOD of sewage before and after treatment and raises the question that is often posed by wastewater treatment plant operators: 'Should nitrification be included in the measurement of BOD when sewage treatment processes are based on the removal of organic material only?' Normally, the nitrogenous oxygen demand that occurs in the BOD test is much greater than what will occur in natural water, with greatest nitrification occurring in natural waters during the summer months. Thus, in general, nitrification should be suppressed during the test using an inhibitor, so only the carbonaceous demand is measured. This is now standard practice both in the UK and the USA (National Water Council 1978; Carter 1984). Two inhibitors are widely used, allythiourea (ATU) or 2-chloro-6-(trichloromethyl) pyridine (TCMP) added to either the dilution water or the sample. A dosage rate of 0.5 mg l^{-1} ATU prevents the onset of nitrification for a period of up to nine days with no effect on carbonaceous oxidation, and unlike thiourea, ATU does not interfere with the azide modification of the Winkler method. This dosage rate of ATU exerts an average oxygen (iodine) demand of 0.06 mg O_2 l^{-1}. ATU only inhibits *Nitrosomonas* and does not inhibit nitrification by *Nitrobacter*, but the second stage of nitrification rarely proceeds in the absence of the first. Although ATU is recommended by HMSO (1983), the US standard recommends the use of TCMP for inhibiting nitrification.

Originally developed for the fertilizer industry to prevent the leaching of nitrogen based fertilizers through the soil, a TCMP concentration of 10 mg l^{-1} will effectively inhibit nitrification without affecting carbonaceous oxidation for a much longer period than ATU (Young 1973). Numerous other chemicals can inhibit nitrification and these have been listed by Richardson (1985). Mara (1974) suggests exceptions when nitrification should be taken into account in the overall determination of the BOD exerted on receiving waters. These are: (i) when the river temperature is greater than about 20°C; (ii) when the effluent is discharged into an estuary; (iii) when the effluent is discharged into a river which has a flow time in excess of five days from the point of discharge to the sea; and (iv) when effluent flow contributes more than 50% of the total river flow. The US Environmental Protection Agency has recommended the use of an approximation to calculate the Ultimate Oxygen Demand (L_o) from the carbonaceous oxygen demand ($BOD_{(ATU)}$):

$$L_o = (1.5 \times BOD_{(ATU)}) + (4.6 \times NH_3\text{-N})$$

Although it is possible for carbonaceous oxidation and nitrification to occur simultaneously, with the resultant BOD a mere composite of the two reactions, nitrification normally begins some time after carbonaceous oxidation has started, resulting in the characteristic two stage BOD curve (Fig. 1.18).

The second stage reaction (nitrification) can be described mathematically as:

$$Y_2 = L_n(1 - e^{-K_n t})$$

so that the overall two stage BOD curve can be expressed as:

$$Y = L_o(1 - e^{-K_1 t}) + L_n(1 - e^{-K_n t})$$

where L_o is the ultimate oxygen demand and L_n the ultimate nitrogenous demand, K_1 is the rate constant for carbonaceous demand and K_n for nitrogenous demand. The rate constant K_n is usually less than the rate constant for carbonaceous material (K_1) and has been approximated by the Water Research Centre for river water taken from the Thames estuary as:

$$K_n = 0.0317(1.017)^t \text{ d}^{-1}.$$

Thus, at 20°C $K_n = 0.044$.

The presence of algae in samples can cause significant problems. Normally, the production of oxygen by algae is prevented by incubating BOD

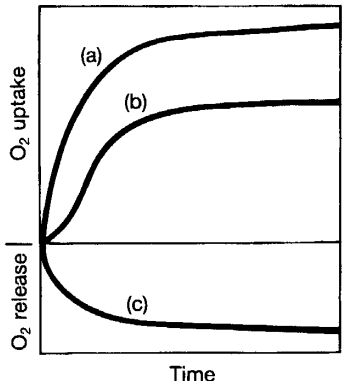


Fig. 1.29. Effect of algae on the BOD curve: (a) containing algae incubated in the dark; (b) filtered sample containing no algae; (c) oxygen is released when sample contains algae and is incubated in the light (Mara 1974).

bottles in the dark. However, like other aerobic micro-organisms, algae respire even in the dark and so exert an oxygen demand. Although their overall contribution is normally slight, samples containing high algal populations, such as highly eutrophic lake waters or samples from oxidation ponds, will have significant oxygen demands exerted by the algae. That will distort the BOD value and so provide an inaccurate measure of the biodegradable fraction in the sample. In these cases, it is essential that the algal cells are removed by filtration through a Whatman GF/C filter. The effect of algae on the BOD is summarised in Fig. 1.29.

In the dark, algal cells only survive for a short time after which they die and may contribute to the organic content of the sample, thus increasing the BOD. The BOD is unrepresentative of the deoxygenation processes occurring in eutrophic lakes or other systems where algae is abundant, as no estimation is made of the benefits of reaeration via photosynthesis.

Suspended solids and turbulence

The suspended solids content of wastewaters, especially those from the food processing industries, are likely to be composed of both a biodegradable and non-biodegradable organic matter. These wastewaters normally require high dilutions for BOD analysis and it is difficult to ensure that the small sub-sample used is representative of the wastewater. Therefore, the presence of suspended solids can lead to erroneous results. Another problem with suspended solids is found during incubation when the solids will settle to the bottom of the BOD bottle causing stratification of the dissolved

oxygen concentration, being greater in the top half than that in the lower half. Mixing of the sample during incubation will equalise the dissolved oxygen concentration and is widely employed in respirometric BOD apparatus; however, the resultant turbulence in the BOD bottle may break up the solid particles into a more readily usable substrate with a consequent increase in BOD_5. Ali and Bewtra (1972) found that the average increase in the BOD_5 due to mixing ranged from 7% for a synthetic wastewater to 44% for final effluents. They suggest that turbulence around the bacterial cells increases the rate of material transport into the cell and the rate of removal of by-products accumulating on the cell membrane. Turbulence also increases the contact between the bacterial cells and the substrate, thereby increasing the rate of assimilation (Fitzmaurice 1986). The optimum mixing speed during incubation is in the range of 300–400 rpm (Morrissette and Marvinic 1978), while higher speeds cause the flocs to shear and increases the rate of CO_2 production with a consequent reduction in pH which may cause bacterial inhibition.

Filtering the sample through Whatman GF/C filter paper removes the problem of interference of the BOD test by suspended solids. However, this will nearly always result in a significant reduction in the BOD and a possible change in the K_1 constant (Fig. 1.30). Filtered samples are used as a measure of soluble BOD.

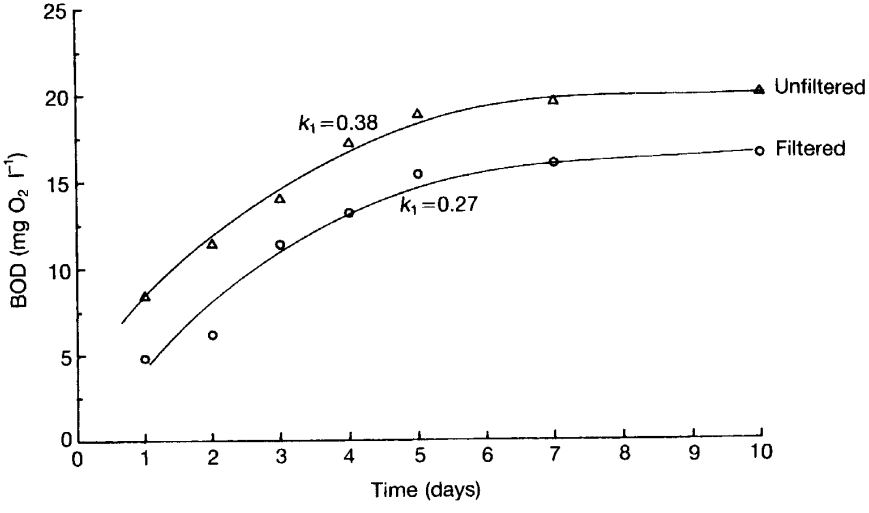


Fig. 1.30. Comparison of the BOD curves for a sample of filtered and unfiltered treated domestic effluent (Fitzmaurice 1986). Where BOD (unfiltered), 18 mg O_2 l^{-1}; BOD (filtered), 15 mg O_2 l^{-1}; suspended solids, 7 mg l^{-1}.

Aeration

Achieving the correct dissolved oxygen concentration in the bottle before the test commences is often difficult. If the sample is being diluted with 100% saturated dilution water, then there is no problem. However, there must be at least 7 mg l^{-1} of dissolved oxygen initially available for the BOD_5 test, and undiluted samples may need to be aerated prior to commencement of incubation. Saturation of individual samples can be achieved by aerating or shaking, but they must be left to stand for 20 minutes to allow the excess air to be released. Over-aerating, using supersaturated dilution water or shaking a partially filled BOD bottle, will all result in excess oxygen being released after the test has commenced, causing gross errors in the test. The presence of algae can cause oxygen to be released so all BOD bottles should be incubated in the dark. Anaerobic samples have a high instantaneous oxygen demand so pre-aeration is vital, even when diluted. In such samples, aerobic micro-organisms may take some time to become established, so seeding is recommended.

Inhibitory and toxic wastes

Various chemical compounds present in wastewaters are toxic to micro-organisms. At high concentrations, these compounds will kill the micro-organisms and at sub-lethal concentrations, their activity can be

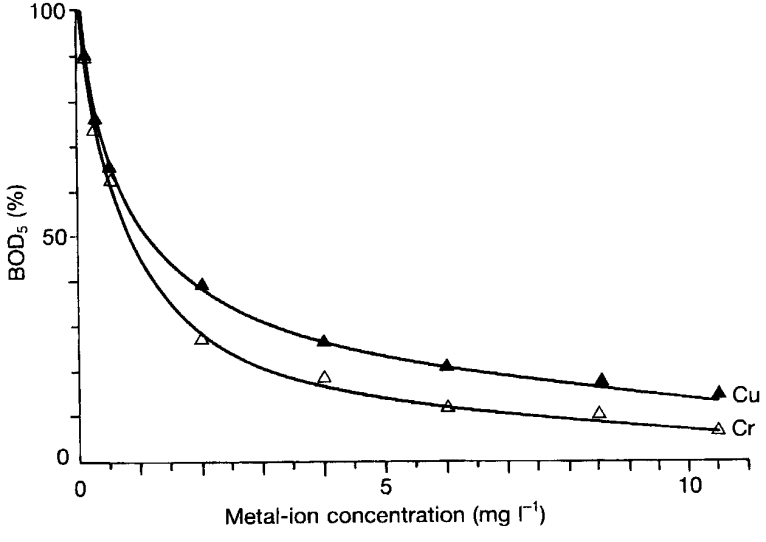


Fig. 1.31. The effect of metal-ion concentration, using copper and chromium, on BOD.

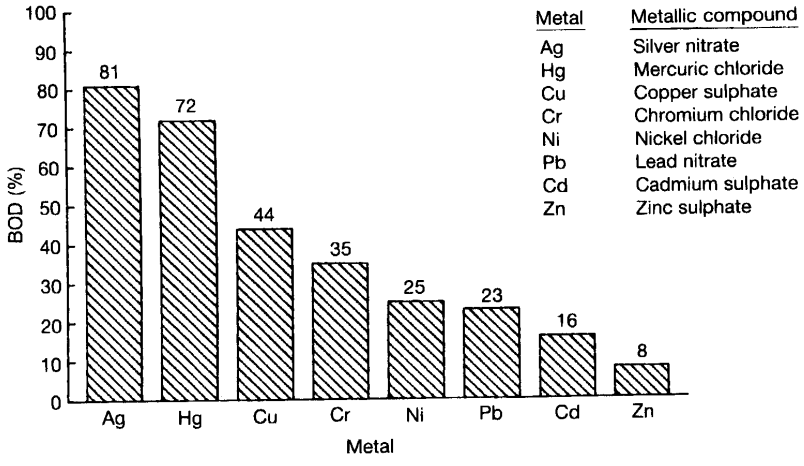


Fig. 1.32. BOD_5 inhibition (%) caused by 1 mg l^{-1} of selected metal compounds during the BOD test of a 1:100 dilution of settled domestic sewage (adapted Stones 1979).

significantly altered (Mittal and Ratra 2000). Non-tolerant bacteria may be unable to degrade wastewaters containing toxins to the same extent as they would in the absence of the toxin, resulting in a depressed BOD value. Much work has been done on heavy metals with the BOD_5 severely suppressed by even small concentrations (1–2 mg l^{-1}) of Cu or Cr (Fig. 1.31). Stones (1979) measured the percentage suppression caused by 1 mg l^{-1} of selected heavy metals, dosed in the form of inorganic salts, on the BOD of domestic sewage (Fig. 1.32). The results showed metal toxicity was in the order of Ag > Hg > Cu > Cr > Ni > Pb > Cd > Zn. Although not considered a heavy metal, ferrous iron in concentrations exceeding 1 mg l^{-1} will also interfere with the BOD test. The ferrous iron reacts with the oxygen in the dilution water producing falsely high BOD results. However, the concentration of heavy metals which causes total inhibition of bacterial activity in the BOD test tends to be very high, far in excess of those normally encountered in either domestic wastewaters or river samples. Mittal and Ratra (2000) found that the addition of < 0.5 mg l^{-1} of Pb and < 0.75 mg l^{-1} of Al and Zn ions increased the expected BOD concentration. The authors gave inhibition data for Al, Co, Cu, Hg, Ni, Pb, and Zn over a range of concentrations from 0.1 mg l^{-1} to 50.0 mg l^{-1}. Berkun (1932) found that 6 mg l^{-1} of mercuric chloride, 40 mg l^{-1} of copper sulphate and 30 mg l^{-1} of potassium dichromate were required to completely inhibit bacterial activity on a sample of glucose. The inhibitory effect of chloride and free ammonia on the test was examined by Çeçen and Yangin (2001).

Toxicity is usually suspected when the BOD increases with increasing dilution. Also, as chemical (COD) and instrumental (TOC) methods are unaffected by the presence of toxins, they can be used to check if the BOD is being depressed. A ratio of COD or TOC:BOD should be established for a specific wastewater and this then used to check for inhibition. For example, the normal COD:BOD ratio for domestic sewage is 2:1, so if the ratio was found to be $>$ 4:1, the presence of toxic compounds in the sewage should be suspected as a possible cause. Toxicity in effluents can be overcome by pretreatment. Toxic metals can be complexed with chelating agents such as EDTA or precipitated out of solution. Volatile compounds and residuals of chlorine can be reduced by allowing the sample to stand for several hours or by gentle agitation, while high concentrations of chlorine residuals can be neutralised by sodium sulphite. Reseeding is required after samples have been dechlorinated. Acidic samples should be neutralised to pH 6.5–7.5 using sodium hydroxide and acidic samples using sulphuric acid. In both cases, neutralisation should not dilute the sample by the addition of reagents, by more than 0.5% The most effective way to overcome the presence of inhibitory or toxic compounds in wastewaters is to use an acclimatised seed.

1.4.2.4. *Sources of error*

Any analytical determination will inevitably include some errors and the fact that the BOD test is biological in action, depending on active aerobic micro-organisms, is another possible source of error. However, with practice, duplicate results can be within 5%, and certainly should not exceed 8–10%.

The residual BOD in the dilution water is measured by carrying out blank tests using duplicate bottles containing no sample at all. The dilution water blanks are treated in the same way as samples and this residual BOD_5 value subtracted from the overall BOD value. The BOD of the dilution water will be significantly increased by seeding and a separate determination of seeded dilution water is necessary. In order to ensure that this residual BOD does not affect the overall reaction within the BOD bottle, it should not exceed 0.2 mg l^{-1} for unseeded and 0.5 mg l^{-1} for seeded dilution water.

Probably the most common source of error in the test is the measurement of the oxygen concentration. It should be remembered that the determination of the BOD of a single unknown sample will involve a minimum of eight oxygen determinations, which includes three dilutions and one blank. Therefore, the chances of making an error that is carried through to the

final calculation are large. There are three widely used methods of determining the dissolved oxygen concentration in the BOD test. The Winkler method is a chemical titration method, while electrodes which incorporate stirrers are also now widely used. Manometric methods have always been used in respiration studies, but recently a mercury free manometric system for BOD analysis has been introduced which is very simple to use and does not require any manual determination of oxygen concentration, resulting in fewer analytical steps, which minimises any introduction of error. In the chemical method, high concentrations of suspended solids can adsorb iodine and give low oxygen value, and in these cases settlement, filtration, or flocculation may be required. The starch-iodine titration requires skill and experience, with the recognition of the correct end-point important. Whereas the acid-titration ensures that the BOD bottles are kept clean, the use of an electrode or a manometric system requires the bottles to be acid-rinsed inbetween use. Apart from the obvious problems of calibrating the electrode, it is necessary to establish standardised measuring techniques, for example, employing fixed stirring speeds and taking readings after a specific period of stirring. In the newer manometric systems, such as Oxitop®, these problems are largely overcome by the use of standard apparatus.

Among the most frequently cited sources of error are poor analytical and laboratory technique; inadequate preparation of dilution water; using contaminated glassware and sampling bottles; incorrect dilution and poor mixing of samples; failure to use seeds and to pretreat samples when necessary; utilisation of more than 50% of the dissolved oxygen in the second bottle over the incubation period; poor titration technique and in particular the end-point determination; infrequent and poor calibration of dissolved oxygen electrodes and meters; inefficient incubation both in allowing exactly 120 hours (5 days) and ensuring the temperature is 20°C. When incubation periods are less than five days, it is possible to apply a correction factor which will allow a rough approximation of what the BOD_5 would have been. Simply the BOD_n value is multiplied by the correction factor:

$$BOD_5 = K^1(BOD_n)$$

where $K^1 = 1.58$ for BOD_2, 1.243 for BOD_3 and 1.10 for BOD_4. These correction factors were computed by determining the BOD_2, BOD_3, BOD_4 and BOD_5 of different strengths of domestic and industrial wastewater. Plots were made of the BOD results versus wastewater strength. The slope of each line was computed by linear regression and the ratio of BOD_2, BOD_3 and BOD_4 slopes to the BOD_5 slope is the K^1 value (Ademoroti

Table 1.39. Inter-laboratory BOD precision test results using the membrane electrode (E) and Winkler methods (W) expressed in mg O_2 l^{-1} (Fitzmaurice and Gray 1987a).

Test Level	1		2		3	
Method	E	W	E	W	E	W
Expected BOD_5	40	40	100	100	400	400
Mean BOD	41.2	35.6	196.1	168.4	356.6	336.4
Maximum result	51.0	52.0	225.0	239.5	429.0	515.0
Minimum result	33.5	18.0	181.5	102.0	302.5	173.5
Range	17.5	34.0	43.5	137.5	122.5	341.5
Standard deviation	6.0	10.8	15.0	48.0	39.3	109.6
Repeatability	4.9	9.3	19.6	23.8	34.5	46.3
Reproducibility	17.2	24.1	45.1	148.7	113.9	306.3

1984). This technique can be successfully applied to wastewaters for which specific K^1 values have been determined as a rapid BOD technique.

Basic analytical technique should be checked periodically using inter-laboratory harmonisation studies and more frequently by using standard samples of known BOD strength (Committee for Analytical Quality Control 1984). A useful test is a mixture of 150 mg l^{-1} glucose and 150 mg l^{-1} glutamic acid, seeded with fresh settled sewage. This should give a BOD_5 of 218 ± 11 mg l^{-1}. An error of up to 5% is acceptable, even using this standard solution, however, the greater the error the poorer the analytical technique.

Fitzmaurice and Gray (1987a) carried out an inter-laboratory precision test between 23 Irish water pollution laboratories. They measured the repeatability (within laboratory precision) and reproducibilty (between laboratory precision) (BSI 1979, 1987) of the BOD test at three test levels using sterile synthetic solutions of glucose and glutamic acid representing expected concentrations of 40, 200 and 400 mg O_2 l^{-1} (Table 1.39).

The results showed that the membrane electrode method is more precise than the Winkler method at each of the test levels. They suggested that the poor performance recorded for the Winkler method was probably due to the influence of random errors caused by poor quantitative techniques.

The azide modification of the Winkler titrametric procedure involves ten steps before a dissolved oxygen result can be calculated. Apart from the preparation of the reagents and the titrant, these steps involve the

addition of reagents to the BOD bottle; the transfer of the sample from the BOD bottle to a titration flask via a graduated cylinder; the filling of the burette with the titrant; the titration of the sample; the addition of the indicator; the visual detection of the end-point in the titration; the reading of the burette and the calculation of the dissolved oxygen concentration. The transfer of reagents and samples using various types and sizes of volumetric glassware and the filling/reading of burettes are all common sources of random errors. The volume of sample titrated is very important as the loss of iodine during transfer from the BOD bottle to the titration flask may result in negative bias of up to 2% (DoE 1980, 1983). For this reason, it is recommended that a large sample volume, > 200 ml, should be titrated. The rate at which the titrant is added to the sample may introduce a significant random error as the colour change from blue to colourless is very rapid. A final drop of 0.05 ml is sufficient to affect the end point in the titration. Therefore, it is very important to add the titrant very slowly after the addition of the starch indicator. The occurrence of small random errors in the Winkler method tends to become significant when the results are used in the calculation of BOD because of the multiplicative factor introduced by high sample dilutions. With so many sources of random error, the precision of the Winkler method is dependent upon the skill of the analyst, who should have a firm understanding of the principles and procedures of good quantitative techniques. This skill can only be acquired by frequent analysis, but unfortunately the Winkler method tends to be used by the laboratories with a low turnover of BOD determinations.

The precision of the membrane electrode method was comparable to the results of studies conducted by the Environmental Protection Agency (1978) in the USA. In contrast to the Winkler method, the membrane electrode method only involves two steps and is a much faster technique requiring half the sample volumes used in the Winkler method. After setting up the electrode and checking its linearity of response, the procedure only involves the insertion of the electrode into the same BOD bottle both before and after incubation. A direct reading in mg O_2 l^{-1} is obtained after a response time of around 60 seconds. Provided the calibration check is carried out regularly and the membrane is maintained, there is little chance of significant errors occurring in the analysis. Even if the electrode has a fixed positive or negative bias, the result of the BOD test remains unaffected because the BOD calculation is based on the depletion of dissolved oxygen over a given period rather than the actual concentration of dissolved oxygen in the sample at any instant.

Table 1.40. The analytical acceptability of the membrane electrode (E) and Winkler (W) methods for determining BOD as measured in the inter-laboratory BOD precision test (Fitzmaurice and Gray 1987a).

Test Level	1		2		3	
Analytical method	E	W	E	W	E	W
Total error (%)	33	65	17	64	31	71

The criterion for judging the acceptability of analytical methods as developed by McFarren *et al.* (1970) was applied to the results for both analytical methods used in the BOD precision test. The percentage total error for both methods at each test level is calculated and based on the result, the analytical methods are divided into the following categories:

(1) Excellent: Total Error $< 25\%$.
(2) Acceptable: Total Error $> 25\% < 50\%$.
(3) Unacceptable: Total Error $> 50\%$.

The analytical acceptability of the membrane electrode and Winkler methods as measured in the inter-laboratory BOD precision test are tabulated in Table 1.40, which shows that the membrane electrode method is rated as excellent for test level 2 and acceptable for test levels 1 and 3 while the Winkler method is rated as being unacceptable for each of the test levels.

The BOD test is still widely used as a parameter in the measurement and control of water pollution. BOD tests are frequently used to assess the degree of pollution in prosecutions; to check compliance with effluent discharge licences; to determine the deoxygenating effects of effluents discharged to rivers and streams; to determine (in conjunction with other parameters) charges for effluent treatment and to classify the quality of rivers. BOD results that do not achieve an acceptable level of precision are meaningless in court cases, cause disputes between dischargers and regulatory authorities, and risk misclassifying the quality of rivers. Fitzmaurice and Gray concluded that there is no justification for poor precision due to bad quantitative techniques. Where the constituents of the sample cause precision problems, other parameters such as TOC or COD should be used, but in no case should the BOD test be performed in isolation to other parameters.

Specific actions to avoid the commonest sources of error in the BOD test identified by Fitzmaurice and Gray are summarised below:

Glassware

- All glassware should be cleaned with an acidic iodide/iodine wash solution irrespective of the method used to determine dissolved oxygen.
- Volumetric flasks should be used in preference to graduated cylinders for preparing sample dilutions.

Sample dilution

- Freshly prepared distilled water from an all-glass water still should be used.
- Nutrient solutions should be prepared monthly and stored in the dark at all times.
- Dilution factors should be chosen by reference to Table 1.38.
- Where the expected BOD concentration of a sample is unknown, COD analysis should be carried out to determine the optimum dilution factor.

Seeding

- A dehydrated microbial seed should be used in preference to seed from a biological effluent treatment process.
- The composition of seed from effluent treatment plants is rarely, if ever, determined before use and tends to be very variable both within and between plants. On the other hand, manufactured seed contains homogeneous microbial cultures, is easy to prepare and produces more repeatable BOD results than seed from biological effluent treatment plants. The universal use of such seeds should, in theory, eliminate a significant variable from inter-laboratory BOD analysis (Fitzmaurice and Gray 1989). However, there is a need for more research and development on such products to ensure standard species composition and density of micro-organisms between batches.

The Winkler method

- Where the Winkler method is used on an infrequent basis or by inexperienced personnel, factory prepared volumetric solutions of sodium thiosulphate titrant should be used.
- Automatic dispensers should be used to add the other reagents to the BOD bottles. This reduces the hazards associated with strong alkaline and acidic solutions and avoids cross contamination of reagents.

- Sodium starch glycollate (0.5% m/V) is more stable than soluble starch powder and should be used as the indicator solution (DoE 1980).
- Automatic zero burettes with a reservoir are both more convenient and faster than ordinary burettes. Their use also considerably reduces the problem of contamination of standard solutions.

The membrane electrode method

- Prior to the recording of any BOD measurements, the electrode should be calibrated at both high and low dissolved oxygen concentrations by reference to the Winkler method. This type of calibration check is preferable to air calibration checks which are recommended by some manufacturers.
- The linearity of response of the electrode should be checked at monthly intervals.

Sample incubation

- Only standard BOD bottles should be used for incubation. The well in the bottle neck should be filled with dilution water and Parafilm® should be wrapped tightly around the neck of the bottle, totally enclosing the glass stopper and the water seal.
- Reagent bottles with polypropylene stoppers should never be used for incubating BOD samples.
- The temperature in the incubator should be checked by placing a water filled BOD bottle coupled with a thermometer into the centre shelf of the incubator.

BOD calculation

- Both the initial and final dissolved oxygen concentrations should be recorded to two significant figures and the standard BOD formula, which incorporates the seed correction factor, should be used to calculate the BOD.

Further reading

General: Johnston *et al.* 1991; Metcalf and Eddy Inc. 1991; EPA 1994; Johnstone and Horan 1994; Polevoy 1996; Environment Agency 1998; Rendell 1999; EPA 2000.

Sewage composition: Hunter and Heukelekian 1965; Loehr 1968; Painter 1971; Rickert and Hunter 1971; Ligman *et al.* 1974; Metcalf and Eddy Inc. 1991; Henze *et al.* 1995.

Sewerage: Bartlett 1981; Read and Vickridge 1997.

Infiltration and urban runoff: Torno *et al.* 1986; Field *et al.* 1994; Debo and Rees 1995; Bretot *et al.* 1999; Adams and Papa 2000.

Agricultural wastewaters: Hobson and Robertson 1977; Taiganides 1977; Gasser 1980; Beck 1989.

Food processing wastewaters: Dickinson 1974; Nemerow 1979; Nemerow and Agardy 1998.

Volume and flow-rate: Geyer and Lentz 1964; Hubbell 1962; Metcalf and Eddy Inc. 1991; Hammer and Hammer 2001.

Micro-organisms: Mara 1974; Lynch and Poole 1979; Bitton 1999.

Self purification: Benoit 1971; Klein 1972; Hynes 1971; Welch 1980; Nemerow 1991.

Biochemical oxygen demand: Young *et al.* 1981; HMSO 1983; Carter 1984; American Public Health Association *et al.* 1985; Fitzmaurice and Gray 1987a,b; Clesceri *et al.* 1998; Cutera *et al.* 1999.

2

How Man Deals with Waste

2.1. Basic Treatment Processes

The aims of wastewater treatment are to convert the waste materials present in wastewaters into stable oxidised end products which can be safely discharged to inland or coastal waters without any adverse ecological effects; to protect public health; to ensure wastewater is effectively disposed of on a regular and reliable basis without nuisance or offence; to provide an economical method of disposal; and more recently, to recycle and recover the valuable components of wastewater. A wastewater treatment plant is a combination of separate treatment processes or units designed to produce an effluent of specified quality from a wastewater (influent) of known composition and flow rate. The treatment plant is also usually required to process the separate solids to a suitable condition for disposal. The amount of treatment required depends largely on the water quality objectives for the receiving water and also the dilution available. It has been the general practise in the past to base treatment plant design on the production of a Royal Commission Standard effluent which has a BOD_5 of 20 and a suspended solids concentration of 30 mg l^{-1}. This effluent quality was considered safe to discharge so long as there was at least an eight-fold dilution by clean water, with a $BOD_5 < 2$ mg l^{-1}, available in the receiving watercourse. The 20:30 standard, as it is known, has led to the final effluent quality of treatment plants to be normally quoted in this manner, such as 10:10 or 5:10, with the first value generally referring to the BOD_5 and the second to the suspended solids concentration in mg l^{-1}. More relaxed standards apply to effluents discharged to estuarine, coastal or marine waters, as the available dilution is vast.

The dilution based approach to effluent disposal has been superceded by the introduction of the Urban Waste Water Treatment Directive (91/271/EEC). Secondary treatment is now mandatory for all inland discharges and those to estuaries with a population equivalent (PE) > 2,000, and for discharges with a PE > 10,000 to coastal waters regardless of assimilative capacity (Fig. 1.2). New minimum treatment standards of 25:35 have been set with minimum limits for effluent nitrogen and phosphorus concentrations, depending on PE, for discharges to receiving waters classified as sensitive (Table 1.6). This has been fully explained in Sec. 1.1.

Consent conditions for discharges are set according to downstream river users and existing assimilative capacity. River quality objectives are the recognised uses to be made of each stretch of river, which may range from salmonid fisheries requiring a $BOD_5 < 4$ mg l^{-1} and an ammonical nitrogen concentration < 0.5 mg l^{-1}, to minor watercourses where the objective is merely to prevent a nuisance developing so that a BOD < 20 mg l^{-1} and an ammonical nitrogen concentration of < 10 mg l^{-1} are acceptable. Water quality criteria have been set locally, nationally and internationally in order to achieve specific water quality objectives (Table 2.1). Within the European Union, water quality criteria are based on a number of specific directives, most notably the Freshwater Fish Directive (78/649/EEC) which specifies 14 physico-chemical parameters in order to maintain water quality suitable for either salmonid or cyprinid fish, and the Surface Water Directive

Table 2.1. General river water quality criteria in terms of $BOD_{(ATU)}$ and ammonical nitrogen concentration (Warn 1982a).

Type of use	Water quality criteria (95-percentile)	
	$BOD_{(ATU)}$ (mg l^{-1})	Ammoniacal nitrogen (mg l^{-1})
Fisheries		
F1: trout, dace, perch, roach	4	0.5
F2: perch, roach, bream	6	1.0
F3: roach, bream	9	2.0
Public water supply direct	6	1.0
Public water supply (impounded)	9	2.0
High amenity	9	2.0
General amenity	12	5.0
No recognised river use; minor watercourses where the objective is merely to prevent nuisance developing	20	10.0

(75/440/EEC) which specifies mandatory levels of potable water treatment for three different categories of water quality based on 46 parameters. These, and other important directives covering water quality (Table 1.3), are to be incorporated into the new EU Water Framework Directive (00/60/EEC). This requires all water resources within a river basin (i.e. a catchment) to have a management plan. This means that all receiving waters within a single river basin will have clearly defined water quality objectives (Gray 1999).

Calculation of consent conditions are based on the mass-balance equation:

$$T = \frac{FC + fc}{F + f}$$

where F is the river flow upstream of the discharge; C is the concentration of pollutant in the river upstream of the discharge, f is the flow of the discharge, c is the concentration of pollutant in the discharge and T is the concentration of pollutant downstream of the discharge.

Thus, the permitted concentration of a pollutant for a discharge can be calculated by re-arranging the mass-balance equation:

$$c = \frac{T(F + f) - CF}{f}$$

where T is now the water quality criteria set for that stretch of river, that is the safe concentration required to be achieved in the river downstream of the discharge (Warn 1982a).

Wastewater treatment is essentially a mixture of settlement and biological or chemical unit processes. Little real purification takes place as the action of the treatment is one of separation of the suspended and soluble nutrients from the water by adsorption onto particles large enough to be removed from suspension by settlement. The concentrated particles form a sludge that is to be processed and disposed, which can itself be a major problem, especially at smaller plants. Treatment plants are assembled from combinations of unit processes, and as the range of available unit processes is large, (Table 2.2) by using a suitable combination of the available processes, it is possible to produce a final effluent of a specified quality from almost any type of influent wastewater (Fig. 2.1). Unit treatment processes can be classified into five stages:

(1) *Preliminary treatment*: the removal and disintegration of gross solids, the removal of grit and the separation of storm water. Oil and grease are also removed at this stage if present in large amounts.

Table 2.2. Summary of major unit processes available in wastewater treatment.

Group	Specific unit processes
Solids conversion	Biochemical precipitation including activated sludge and biofiltration
	Chemical precipitation
	Chemical oxidation/reduction
	Biochemical oxidation/reduction
	Combustion
Solids conditioning	Chemical coagulation
	Mechanical flocculation
Screening	Macro-screening
	Fine-screening
	Ultrafiltration
	Reverse osmosis
	Electrodialysis
Sedimentation	Plain sedimentation (gravity settling)
	Fluidised bed sedimentation
	Dissolved gas flotation
	Centrifugation
Filtration	Sand filtration
	Grass plot irrigation
	Membrane filtration
Ion-exchange	Softening
	Demineralisation
	Metal recovery
	Phosphate, nitrate, ammonia removal
Absorption	Activated carbon removal of surfactants, trace organics, etc.
Gas transfer	Aeration to bring oxygen into solution or strip supersaturated gases such as carbon dioxide, ammonia, etc.

(2) *Primary (sedimentation) treatment*: the first major stage of treatment following preliminary treatment, which usually involves the removal of settleable solids which are separated as sludge.
(3) *Secondary (biological) treatment*: the dissolved and colloidal organics are oxidised in the presence of micro-organisms.
(4) *Tertiary treatment*: further treatment of a biologically treated effluent to remove BOD_5, bacteria, suspended solids, specific toxic compounds or nutrients to enable the final effluent to comply with a standard more stringent than 20:30 before discharge.
(5) *Sludge treatment*: the dewatering, stabilisation and disposal of sludge (Institution of Water Pollution Control 1975; Rae 1998).

The quality of the final effluent required, the nature of the wastewater, and its volume all influence the unit processes selected in the design of a wastewater treatment plant. Since the introduction of the EU Urban

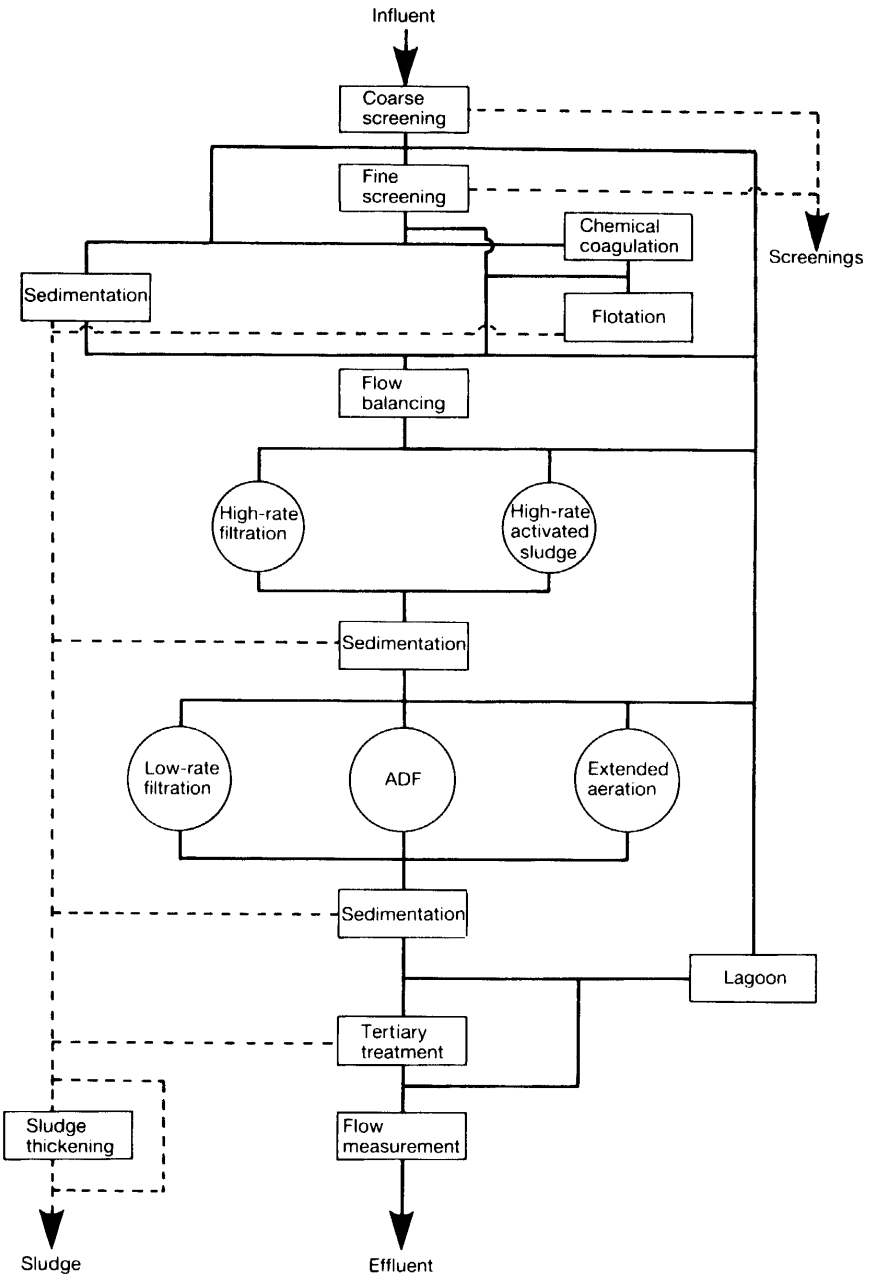


Fig. 2.1. Common process operations in wastewater treatment.

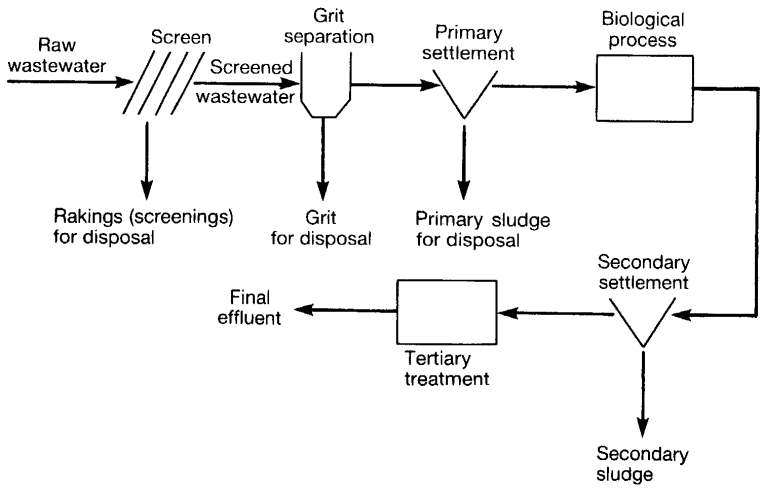


Fig. 2.2. General layout of wastewater treatment showing primary, secondary, and tertiary treatment stages.

Waste Water Treatment Directive, nearly all plants now have secondary treatment while those discharging to sensitive receiving waters have tertiary or advanced treatment stages. All wastewater treatment plants are based on the generalised layout shown in Fig. 2.2.

2.1.1. *Preliminary treatment*

Screens

Raw sewage enters the plant containing a variety of coarse solids that need to be removed prior to treatment so that pumps are not damaged and pipework is not blocked. Screens come in a variety of designs and sizes (Table 2.3). Most plants employ simple screens comprising vertical rows of steel bars which can be curved, vertical straight or inclined straight in design, to physically remove such solids from the influent wastewater. Screens are in four sizes, coarse, fine, very fine, or micro. In domestic wastewater plants, it is normal to use a coarse screen with apertures of between 19 to 25 mm. Coarse screens with even larger apertures, > 50 mm, often precede the main 25 mm screen at municipal plants to remove larger debris such as pieces of wood; while fine screens are normally constructed out of mesh (e.g. spiral sieve screen) and are installed after grit removal. The use of fine screens is generally limited to certain industrial and food processing treatment plants. Floating solids, rags, paper, wood and plastic are all caught on the screens

Table 2.3. Typical size range of screens used in wastewater treatment.

Category	Spacing (mm)	Application
Coarse screens	> 10	Removal of large material
Fina screens	1.5–10	Used as substitute for primary sedimentation for extended aeration plants
Very fine screens	0.2–1.5	Used as substitute for primary sedimentation Used in conjunction with series of larger screens
Micro-screens	0.001–0.3	Effluent polishing. Treatment of inert quarry washings

which are either raked manually, in which case the screen will be angled at 60° to the flow to facilitate cleaning, or if the flow exceeds approximately 1000 m^3d^{-1}, then mechanically operated rakes are used. Mechanical raking systems operate either on a time basis or by depth using some form of water level detector (e.g. float switch) because as the solids are retained, thereby blocking the screen, the flow is impeded so that the level of the wastewater downstream of the screen rises. At a small works, a rough design figure for manually-raked screens is 0.14 m^2 of submerged areas per 1000 population. This figure assumes that the screen will be cleaned frequently and if this is not possible, then the area is increased accordingly. Flow velocity is important for the successful operation of screens; above 0.9 m s^{-1}, the trapped solids are scoured from the screen while below 0.3 m s^{-1}, grit deposition occurs within the screen chamber (Institute of Water Pollution Control 1984).

The materials removed from screens are generally called screenings or rakings, and because they contain material such as gross faecal solids and sanitary towels, they are very unpleasant and unhygienic to handle. Between 0.01 and 0.03 m^3d^{-1} of screenings are produced per 1000 population, although the exact quantity depends on the aperture of the bars or mesh. For example, the volume of screenings produced at a treatment plant serving a population of 100,000 would be 0.9 m^3 h^{-1} using a screen with an aperture of 10 mm, 0.6 m^3h^{-1} at 25 mm and 0.25 m^3h^{-1} at 45 mm. With a moisture content of 69–85%, screenings weigh between 600–1000 kg m^{-3} and so can be difficult to handle. Careful disposal is essential and the main options are incineration or landfill. It is unwise to store such material uncovered as gulls will readily feed on the waste which could result in contamination of supply reservoirs used by the gulls as roosting sites (Gray 1979) (Sec. 9.4.1). If buried, screenings must be covered with soil immediately at a sufficient depth to prevent birds and rats from being attracted. Various dewatering and compaction equipment is used at

larger plants, which is much cleaner and reduces the volume of screenings to be disposed. These units can be attached to automated bagging units to make this unpleasant task even easier (Institute of Water Pollution Control 1984).

Many plants use macerators, comminutors, or disintegrators to finely chop up the coarse solids so that they can be dealt with by the normal treatment system, mainly by settlement. Whereas some plants macerate the screenings returning them to the inlet, other plants macerate the whole flow. However, if the latter option is employed then macerators must be placed after coarse screens and grit removal. Maceration increases the organic loading to the plant so extra treatment capacity must be provided. The macerated screenings are co-settled with raw sludge in the primary sedimentation tank.

Grit separation

After screening and/or maceration, the wastewater is termed screened or macerated sewage. Although it no longer contains gross solids, it does contain grit which is not only mineral aggregate but a mixture of silt, sand and gravel, as well as fragments of metal, glass and even dense plastic. Grit needs to be removed from the influent wastewater to prevent pump damage and silting.

The density of grit is much greater than wastewater, so there is a tendency for it to settle whenever the rate of flow falls below 0.3 m s^{-1}. This can occur in sewers if the gradient is inadequate or if flows are low, such as might happen during the night. Therefore, the quantity of grit arriving at the treatment plant will vary according to the flow conditions in the sewerage system. Particularly large quantities of grit can be flushed to treatment plants after periods of dry weather when grit accumulates in roadside gully pots and in the sewers which is then displaced by a heavy storm. Grit originates mainly from surface drainage, and thus is a problem of combined sewerage systems only, coming from road construction and especially from newly constructed housing estates where the roads have not been properly surfaced. Sandy material can enter damaged and cracked sewers from the ground water if the water table is above the level of the sewer. Some industrial processes such as vegetable washing can also produce enormous quantities of silt. The normal quantity of grit in domestic wastewater ranges between 0.005 to 0.05 m^3 per 1000 m^3.

Detritors and vortex separators (hydrocyclones) are the most commonly used systems, and both are based on sedimentation (Institute of Water

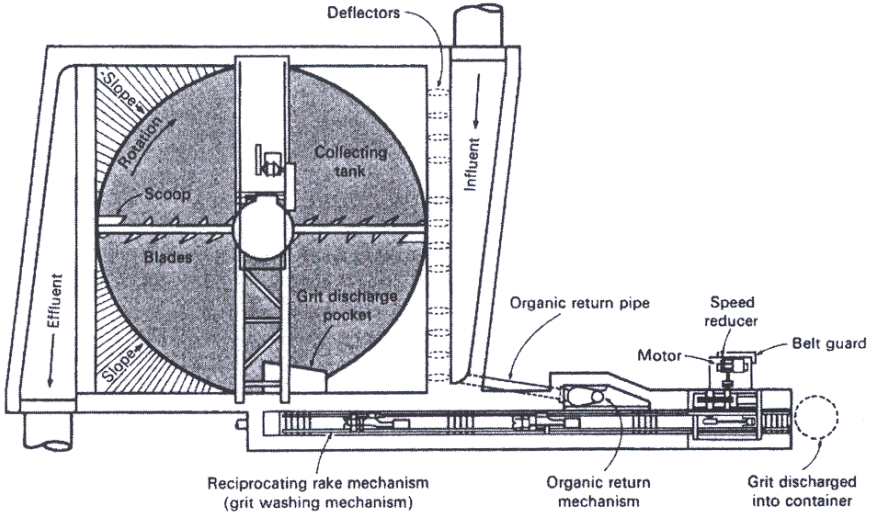
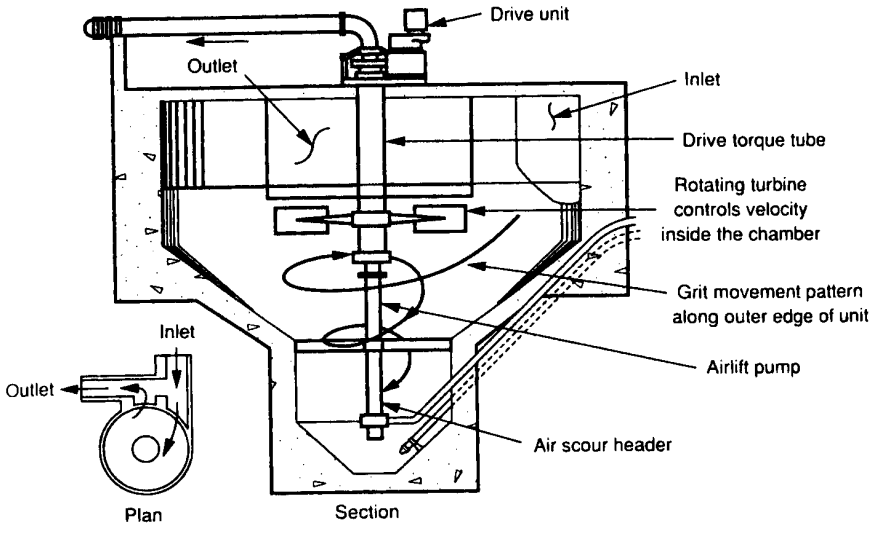


Fig. 2.3. Outline design of a detritor or square horizontal-flow grit chamber (Metcalf and Eddy Inc. 1991).

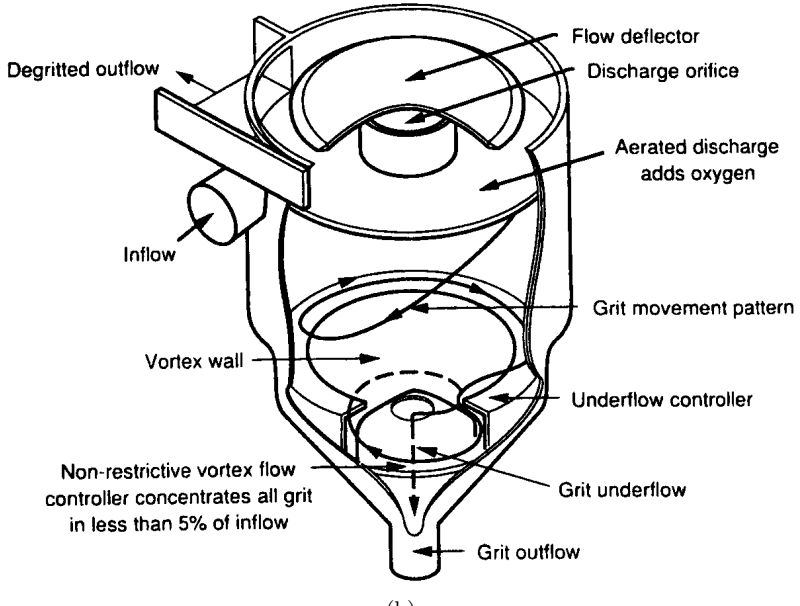
Pollution Control 1984; Metcalf and Eddy Inc. 1991; Hammer and Hammer 2001).

Detritors are high-rate, shallow settlement tanks designed to remove particles of 0.2 mm diameter with a specific gravity of 2.65 and a settling velocity of 0.02 m s^{-1}. It is a square tank with a wide inlet channel with vertical pivoted deflector baffles angled to ensure an even distribution of flow across the tank. Retention time within the tank is only 30–60 seconds with the settled grit removed continuously to a peripheral sump by a rotary scraping mechanism (Fig. 2.3). The velocity of the wastewater is maintained at 0.3 m s^{-1} to ensure that only the dense particles are removed and that all the organic solids remain in suspension. The material removed by the detritor is washed in the classifier (cleansing channel) which is an inclined ramp up which a reciprocating rake pushes the grit against a counter flow waste water. The grit collects in a disposal unit from where it is landfilled.

Vortex separators such as the Pista grit trap are very compact. Waste water enters a vortex shaped tank tangentially and the separation of the grit by centrifugal force is assisted by a rotating paddle. The grit is flung against the outer wall of the tank and falls to the lower chamber where it is removed by air lift pump to a drainage area for disposal (Fig. 2.4)



(a)



(b)

Fig. 2.4. Grit separation using vortex-type systems: (a) a Pista grit separator manufactured by Smith and Loveless Ltd., and (b) a teacup unit manufactured by Eutek Ltd. (Metcalf and Eddy 1991).

Grit contains between 15–30% organic matter, which at the larger plants will be reduced by washing using a cyclone-type washer. Even after washing, grit will still contain about 15% organic matter and so must be removed to land-fill sites. For example, grit removed in the shallow square tank of the detritor has the organic matter removed by an inclined cleansing channel where the reciprocating action of the inclined rake pushes the grit slowly up the channel against a counter flow of wastewater which removes much of organic matter present.

Dissolved air flotation

Suspended particles that have a specific gravity less than water are removed by allowing them to float to the surface and then be removed by a mechanical skimming device. Although this is normally done passively during primary sedimentation, it can be assisted by using special units that employ dissolved air or nitrogen. Compressed air is fed into the wastewater and fully mixed. Very small bubbles are formed that attach to the solid, increasing their buoyancy, and lifting them out of the water to form a thick surface layer of solids known as float. Dissolved air flotation (DAF) units are used primarily to remove emulsified fats, oils and grease (FOG) although they are employed to remove suspended solids and other contaminants from a wide range of other wastewaters e.g. food and animal production and processing, industrial processes including mining, hydrocarbon oils and emulsions etc. (Edzwald 1995; Galil and Wolf 2001; Liu and Lien 2001). DAF units are almost square in section with a little more length than breadth. Effluent is recycled to a pressure vessel where it is saturated with air (Fig. 2.5). It is then added and fully mixed with the incoming wastewater where, under atmospheric pressure, the dissolved gas comes out of solution forming minute bubbles that rise to the surface taking all the FOG with them. A thick micro-bubble bed is formed at the front of the tank, 30–50 cm in depth, the thickness of which decreases linearly along the tank becoming only 10–20 cm in thickness at the outlet end (Fig. 2.6). The float is removed by a mechanical scrapper that pushes the solids into a collection chamber. There is an underflow baffle in front of the back wall of the tank to allow the clarified effluent to escape from the flotation area. The flow rate is < 10 m h^{-1}, with conventional DAF units operating at between 5–7 m h^{-1}. The flow rate is optimised by increasing the depth of the tank to ensure an angle of flow some 30–45° from the horizontal. This ensures that the bubble bed remains tight below the water surface throughout the flotation area (Kiuru 2001).

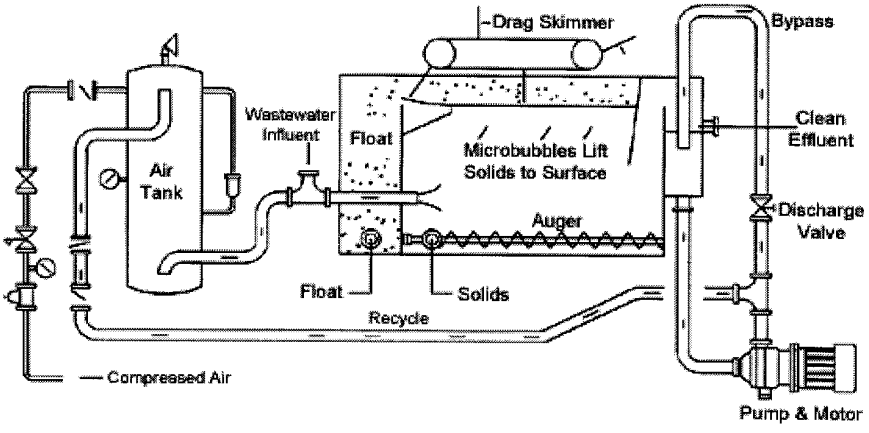


Fig. 2.5. Cross-section through a conventional dissolved air flotation unit.

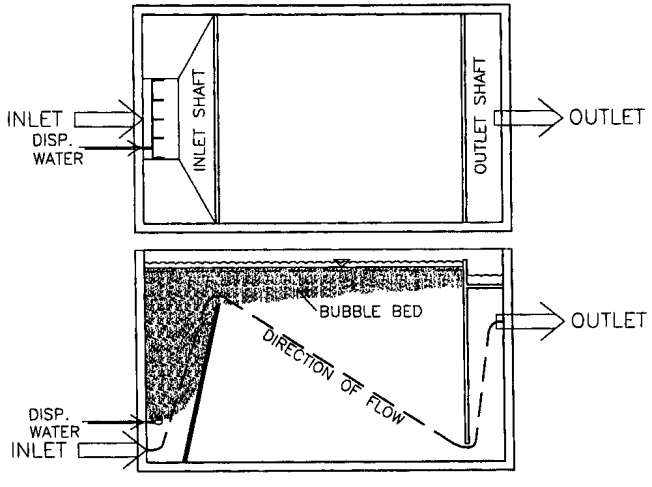


Fig. 2.6. Action of a conventional dissolved air flotation operated at a flow rate of 5–7 m h^{-1}. Reproduced with kind permission of Dr. H.J. Kiuru.

Chemical flocculants (e.g. polyacrylamide, iron and aluminium salts) are used to enhance DAF performance. Where the float is to be used either for animal or human consumption, then natural organic flocculants such as chitosan, carrageenan, lignosulphonic acid, or their derivatives, are used. The use of flocculants has allowed DAF systems to replace conventional sedimentation tanks in some industrial applications. Both pathogens and micro-pollutants, such as PCB and PAH, have a high affinity for particulate

matter. Dissolved air flotation can be used as either a preliminary or tertiary step to help remove such material (ϕ degaard 2001).

Storm water

When it rains, the normal concentration of the wastewater is reduced according to the volume of surface water entering the sewerage system. This diluted wastewater is known as either storm water or storm sewage. Wastewater treatment plants are not designed to provide secondary treatment for all storm water entering during wet weather, so only a proportion of the flow arriving at the plant will receive full treatment — the rest will be stored and treated later. The design of treatment plants is based on dry weather flow (DWF) 'which is the average daily flow to the treatment plant during seven consecutive days without rain following seven days during which the rainfall did not exceed 0.25 mm in any one day' (Institute of Water Pollution Control 1975). Plants have been designed to be able to fully treat three times the dry weather flow (3 DWF) with the excess stored in extra settlement tanks known as storm water tanks. The maximum flow to the plant is equivalent to twice this flow 6 DWF and at this loading rate, the excess wastewater is discharged directly to the receiving water without any treatment. The separation of flow is achieved by overflow weirs set at the appropriate heights situated downstream of the preliminary treatment units so that the gross solids can be removed or macerated and grit removed before storage in the storm tanks or direct discharge.

The storm tanks provide a buffer between the excess wastewater and the watercourse. When a storm occurs, the surface runoff reaches the treatment plant faster than it is able to significantly increase the flow of the watercourse. Thus, it is important that the diluted wastewater, which cannot be treated at the plant, should not be discharged until the flow in the water courses has increased sufficiently to produce adequate dilution for the storm water in order to prevent pollution. The first flush of storm water is usually the most polluted as the sediment in the sewers and roadside gully pots is usually flushed out. Therefore, the first flush is able to be stored in the storm tanks which have a capacity equivalent to 6 hours at DWF. Storm water tanks are almost identical to primary settlement tanks in design and have two functions: (1) to store as much storm-diluted sewage as possible so that it can be returned for full treatment after the influent flow rate has returned to normal; and (2) to remove as much of the suspended solids and associated BOD_5 as possible from the storm water, that cannot be stored and which overflows to the watercourse. The storm water tanks can be

used for emergency storage of sewage contaminated by toxic or dangerous substances resulting from spillages.

The 3 and 6 DWF systems were superseded by a more specific formula that takes into account the main factors more efficiently (Ministry of Housing and Local Government 1970). The maximum flow to a plant is estimated as:

$$(PG + I + E) + 1.36P + 2E \text{ m}^3 \text{ d}^{-1}$$

where P is the population, G the average domestic waste consumption (m^3 hd^{-1} d^{-1}), which is generally considered to be 0.18 m^3 hd^{-1} d^{-1}, although research has put it as low as 0.12 m^3hd^{-1} d^{-1} (National Water Council 1982), I the infiltration (m^3 d^{-1}) and E the industrial effluent (m^3 d^{-1}). The DWF is represented by $(PG + I + E)$ in this formula, so before the storm water flow operates, the domestic sewage must be diluted by 1.36 m^3 per person and the industrial effluent by a factor of two. The maximum flow to receive full secondary treatment is expressed as:

$$3\ PG + I + 3E$$

the infiltration component remaining constant.

2.1.2. *Primary treatment*

Primary treatment or sedimentation is the process by which settleable solids are removed from the screened sewage by passing it through a specially constructed tank at such a velocity that the solids settle out of suspension by gravity. The settleable solids collect at the base of the tank where they are removed as primary sludge. The retention time in sedimentation tanks is usually a minimum of 2 hours at 3 DWF and when operated correctly, can significantly reduce the organic loading to the secondary treatment stage. Comparing the unit processes, sedimentation is far cheaper than biological treatment in terms of unit removal of pollution and for that reason, the majority of plants have incorporated primary sedimentation into their design. Removal of suspended solids is due to a number of processes occurring simultaneously within the sedimentation tank such as flocculation, adsorption, and sedimentation. Maximum removal efficiencies can be as high as 70% for suspended solids and 40% for BOD_5. The overflow from primary sedimentation tanks is known as settled sewage and contains fine suspended solids and dissolved material which passes onto the next treatment stage (Fig. 2.2).

There are a variety of tank designs which are fully described in Sec. 2.2.

2.1.3. *Secondary treatment*

Secondary treatment is a biological process where the settled sewage enters a specially designed reactor where under aerobic conditions, organic matter in solution is oxidised or incorporated into cells. The reactor provides a suitable environment for the microbial population to develop and as long as oxygen and food, in the form of settled sewage, are supplied then the biological oxidation process will continue. Biological treatment is primarily due to bacteria which form the basic trophic level in the reactor food chain. The biological conversion of soluble material into dense microbial biomass has essentially purified the wastewater and all that is subsequently required is to separate the micro-organisms from the water by settlement. Secondary sedimentation is essentially the same as primary sedimentation except that the sludge is comprised of biological cells rather than gross solids (Sec. 2.2).

There are two main types of reactors, those where the micro-organisms are attached to a fixed surface (e.g. percolating filter) and those where the micro-organisms mix freely within the wastewater (e.g. activated sludge). In the latter, the biological cells which are separated after secondary settlement are returned to the reactor to maintain a high microbial density in order to maintain maximum microbial breakdown of the wastewater (Figs. 2.7 and 2.8). The secondary treatment phase may comprise of other biological systems, both aerobic or anaerobic, or incorporate a mixture of several systems. Biological treatment is further explored in Section 2.3 and the individual processes reviewed in Chapters 4 to 7.

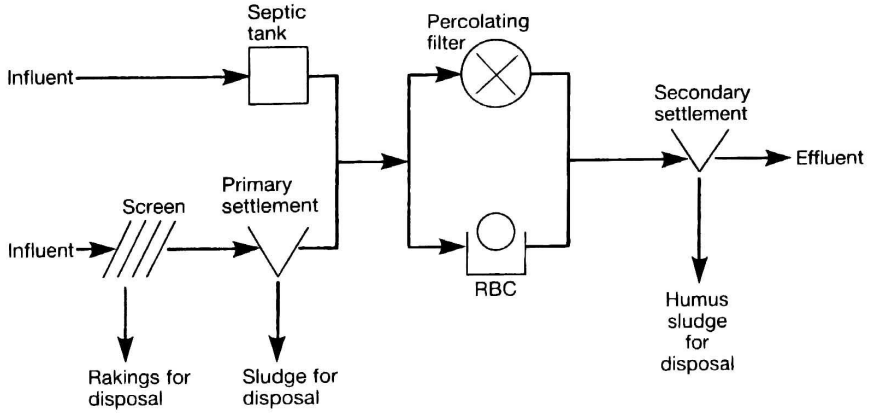


Fig. 2.7. Schematic layout of sewage treatment by fixed film reactor.

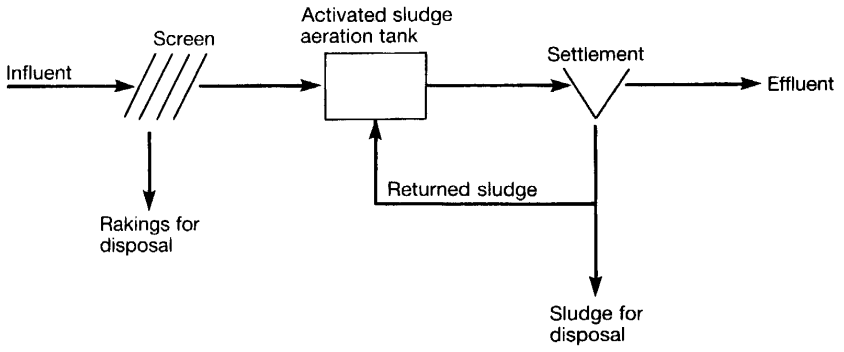


Fig. 2.8. Schematic layout of sewage treatment at a small activated sludge plant.

2.1.4. *Tertiary treatment*

Primary and secondary treatment will normally be able to produce a 20:30 effluent, often even better, but when the dilution available in the receiving water is inadequate and the water quality is already under stress, then further treatment of the secondary effluent will be necessary. Tertiary treatment processes, or polishing processes as they are often referred to, are normally filtration or straining devices, although the various processes now available are extremely varied. They can be used to reduce the BOD_5 or suspended solids concentration, eliminate bacteria or pathogens, remove the nutrients nitrogen and phosphorus, and meet the more stringent conditions that are unobtainable by using biological treatment only (Sec. 2.4).

2.1.5. *Examples of treatment plants*

Nearly all text books on wastewater treatment give specific examples of treatment plants. For example, Mason (1981) describes the Crossness treatment plant which serves 1.7 million Londoners and treats an average of 580,000 m^3 of sewage daily. However, the majority of treatment plants are much smaller than this, serving populations of less than 25,000 PE. In Ireland, most treatment plants built in the past 30 years have been based on extended aeration activated sludge systems, mainly of the oxidation ditch type and to a lesser extent on surface-aerated activated sludge using vertical shaft aeration (Sec. 5.3). In such plants, the primary sedimentation tank is often omitted with the extra organic loading taken into account when designing the capacity of the secondary treatment unit (Fig. 2.9).

The Osberstown wastewater treatment plant in Co. Kildare (Ireland) serves a population equivalent (pe) of 80,000 with a design flow (3 DWF) of

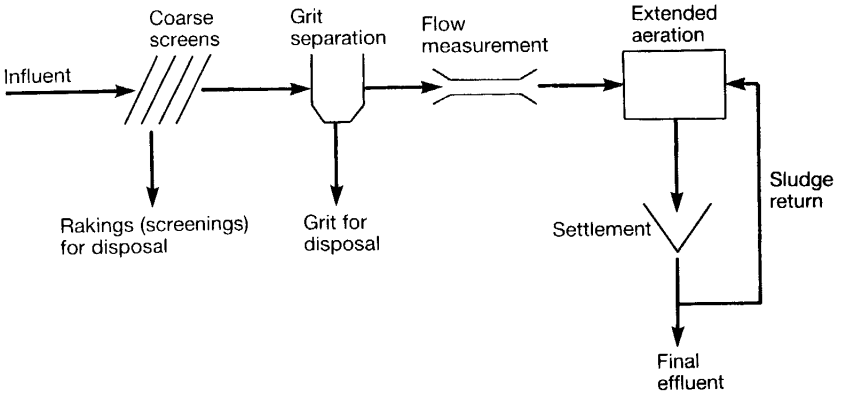


Fig. 2.9. Schematic layout of an extended aeration (oxidation ditch) activated sludge plant for populations of less than 25,000 where primary sedimentation is omitted.

53,000 m^3d^{-1} and an organic loading of 4,800 kg BOD_5 d^{-1}. The effluent is discharged into the River Liffey, a designated EU salmonid river classified as sensitive under the Urban Wastewater Treatment Directive. The discharge consent for the treatment plant, on a 95 percentile basis, is 15 mg l^{-1} BOD_5, 125 mg l^{-1} COD, 35 mg l^{-1} suspended solids, 25 mg N l^{-1} total nitrogen, and 0.9 mg P l^{-1} total phosphorus.

The incoming wastewater is raised to give enough hydraulic head to flow through the plant by gravity using four variable speed submersible pumps in a circular precast concrete shaft (Fig. 2.10). The raw wastewater passes through 6 mm fine screens with automatic cleaning. The screenings are collected via a launder trough, washed, dewatered, and bagged for disposal off site. Grit is then removed by vortex separators with the grit washed and transferred by screw conveyor to a dedicated skip for disposal to landfill. The entire preliminary treatment area is housed with each unit process sealed and any odours produced ducted to a peat biofilter odour removal plant. Flows in excess of 3 DWF are collected as storm water in two storm water (sedimentation) tanks with storm water pumps to return the settled wastewater back to the inlet for treatment. Primary treatment is by two primary sedimentation tanks followed by activated sludge biological treatment. This is carried out in four sequencing batch reactors using the CASS^{TM} process (Sec. 5.3.4). Ferric sulphate/chloride is dosed into the aeration basins to assist phosphorus removal by chemical precipitation. The effluent is then piped to the receiving water where it is discharged into the river via submerged outlets at river bed level. Approximately 8,000 kg of dry solids is thickened, digested and dewatered to sludge cake each day. Sludge

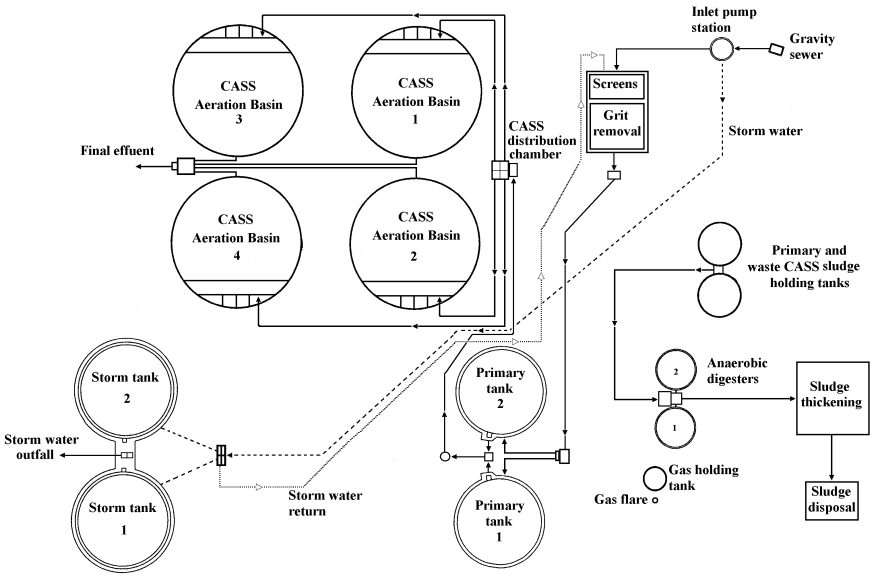


Fig. 2.10. Schematic layout of Osbertstown Wastewater Treatment Plant, Naas, Co. Kildare, Ireland. Reproduced with kind permission of Kildare County Council.

from outside the plant is screened and then stored in a primary buffer tank where it is mixed with primary and secondary sludge. The blended sludge is thickened to 6% dry solids using two drum thickeners before being fed into two reinforced concrete anaerobic digestion tanks, 1,300 m^3 each, that have a separate gas holder for the methane produced. The sludge retention time is 20 days. The digesters are heated (35°C) by dual fuel boilers that run on either digester biogas or fuel oil. Surplus biogas is used to feed two 80 kW CHP (combined heating and power) units which operate on duty/standby/assist basis. The digested sludge is drawn off into two digested sludge holding tanks with 15 days storage capacity. The sludge is subsequently dewatered to 20% dry solids by two belt presses with the pressed cake pumped directly to covered skips for disposal to landfill. All odour sources within the sludge buildings, sludge tanks and skips are ducted to the peat biofilter odour removal plant.

The plant is highly automated with numerous sensors and on-line monitoring systems. The whole plant is computer operated including alarm systems and even remote telemetry systems that provide data on incoming wastewater flows from the sewerage network.

Further reading

General: British Standards Institute 1983; Metcalf and Eddy Inc. 1991; Hammer and Hammer 2001.

Preliminary processes: Institute of Water Pollution Control 1984; Metcalf and Eddy Inc. 1991.

2.2. Sedimentation

Sedimentation is the most widely used unit operation in wastewater treatment. The terms sedimentation and settling are the same and can be used interchangeably. Sedimentation is the process by which the suspended solids, which are heavier than water and commonly referred to as the settleable fraction, are removed from wastewater by allowing the particles to gravitate to the floor of a tank to form a sludge under near quiescent conditions. The process is used in primary treatment to remove settleable organic and inorganic material to reduce the organic load to the secondary treatment processes. It is also used for secondary sedimentation, to remove material converted to settleable solids during the biological phase of treatment, such as the removal of humus from filter effluents and the recovery of activated sludge.

2.2.1. *The settlement process*

The way in which particles settle out of suspension is a vital consideration in the design and operation of sedimentation tanks, as well as other unit processes. There are four types of settling: type I or discrete, type II or flocculant, type III or hindered which is occasionally referred to as zonal settling, and type IV or compression settlement. Where particles are dispersed or suspended at a low solids concentration, then type I or II settlement occurs. Types III or IV settlement only occurs when the solids concentration has increased to such an extent that particle forces or particle contact affects normal settlement processes. During settlement, it is common to have more than one type of settling occurring at the same time and it is possible for all four to occur in a settlement tank simultaneously.

Type I or discrete particle settling

Discrete particles settle out of a dilute suspension as individual entities. Each particle retains its individual characteristics and there is little

tendency for such particles to flocculate, so settlement remains solely a function of fluid properties and particle characteristics. Settling of heavy inert particles such as grit and sand particles is an example of type I settlement.

Type I settlement is analysed by means of the classic laws of sedimentation formed by Newton and Stokes. Newton's law yields the terminal particle velocity by equating the gravitational force of the particle to the frictional resistance or drag. The rate of settlement of discrete particles varies with the diameter of the particle, the difference in density between the particles and fluid in which it is suspended and the viscosity, as shown by Stokes Law. The theory of settlement is fully explained elsewhere (Clark *et al.* 1977; White 1978; Metcalf and Eddy Inc. 1991).

Under quiescent conditions, discrete particles settle out at their terminal velocity, which will remain constant provided the fluid temperature does not alter. A knowledge of this velocity is fundamental in the design of sedimentation tanks and in the evaluation of their performance. A sedimentation tank is designed to remove all particles which have a terminal velocity equal to or greater than V_c. The selection of V_c will depend on the specific function of the tank and on the physical characteristics of the particles to be removed. The rate at which clarified water is produced in a sedimentation tank is given by

$$Q = V_{\mathrm{c}} A \ (\mathrm{m^3 d^{-1}})$$

where V_{c} is the terminal velocity of the particle and A is the surface area of the settling chamber. Therefore, in theory, the rate of clarification by type I settlement is independent of depth (Dick 1976). The overflow rate or surface loading, which is the average daily flow in cubic metres per day divided by the total surface area of the sedimentation tank in square metres and so equivalent to the terminal settling velocity, is the basis of sedimentation tank design:

$$V_{\mathrm{c}} = \frac{Q}{A} = \text{overflow rate } (\mathrm{m^3 m^{-2} d^{-1}})$$

Effluent weir loading is also a widely used design criterion and is equal to the average daily overflow divided by the total weir length, being expressed as cubic metres of effluent flowing over per metre of weir per day ($\mathrm{m^3 m^{-1} d^{-1}}$).

Modern sedimentation tanks are operated on a continuous-flow basis. Therefore the retention time of the tank should be long enough to ensure that all particles with the desired velocity V_{c} will settle to the bottom of the tank. Retention time is calculated by dividing the tank volume by the

influent flow:

$$T = 24\frac{V}{Q}\ (\mathrm{h})$$

where T is the retention time in hours, V the tank volume (m^3) and Q the mean daily flow (m^3d^{-1}). The depth of a sedimentation tank is taken as the water depth at the side wall, measuring from the tank bottom to the top of the overflow weir. This excludes the additional depth resulting from the slightly sloping bottom in both rectangular and circular sedimentation tanks. The terminal velocity, tank depth and retention time are related as:

$$V_\mathrm{c} = \frac{D}{T}(\mathrm{ms}^{-1})$$

where D is the depth (m).

Type II or flocculant settling

Type II particles in relatively dilute solutions do not act as discrete particles but coalesce or flocculate with other particles during gravitational settlement. Subsiding particles coalesce with smaller particles falling at lower velocities to form larger particles which then settle faster than the parent particle. Settlement of solids in the upper layer of both primary and secondary sedimentation tanks are typical of type II settlement, and flocculation is particularly important in the separation of the sludge in the activated sludge process where rapid separation is required, so that the sludge can be returned to the aeration basin. The degree of flocculation is dependent on the opportunity for particle contact, which increases as the depth of the settling tank increases. Thus, the removal of suspended material depends not only on the clarification rate (Q) but also on depth, which is a major difference between type I and type II settlement. Surface overflow rate, the velocity gradients in the system, the concentration of particles and the range of particle sizes are also important considerations in flocculant settling. As there is no adequate mathematical relationship to determine the effect of flocculation on sedimentation, the effects of all these variables can only be determined by settling column analysis.

Settling column analysis is carried out in a cylinder of the same depth as the proposed tank, and with a minimum width of 150 mm to minimise wall effects. Sample ports spaced at 0.5 m intervals allow samples to be taken for analysis from all depths. The wastewater is poured rapidly into the column and mixed to ensure that there is a uniform distribution of particles throughout the column at the beginning of the analysis. Care must

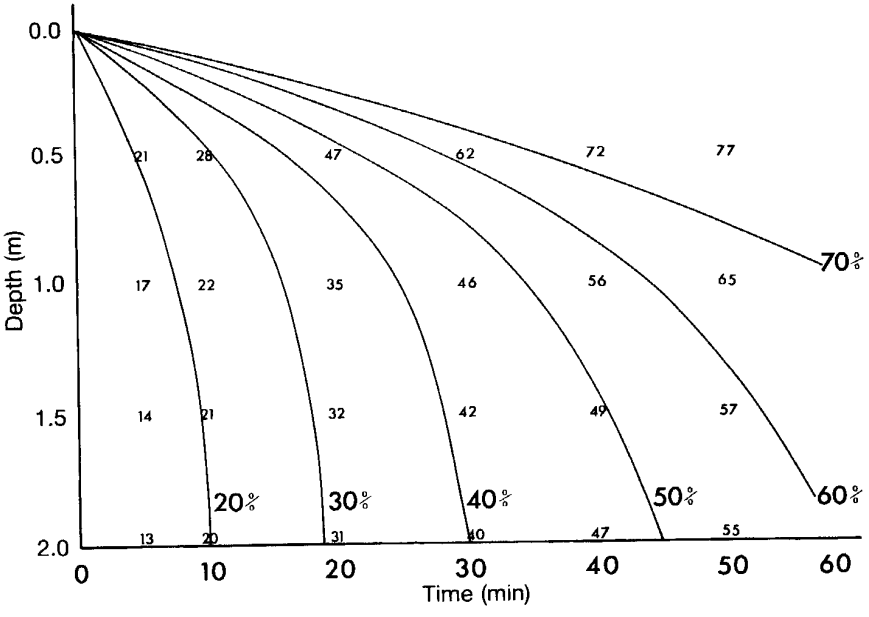


Fig. 2.11. Iso-concentration curves for solids removal from a flocculant suspension. The numbers plotted represent the percentage removal of solids based on settling column analysis.

also be taken to ensure that uniform temperature is maintained throughout the column to avoid convection currents affecting the settlement rate. Water samples are taken from the ports at regular intervals and are analysed for suspended solids. The percentage removal from the original suspended solids concentration of the wastewater is plotted against time and depth which allows curves of equal percentage removal, iso-concentration curves, to be plotted (Fig. 2.11). These curves allow depth and retention time to be compared so providing the optimum design and operating conditions for particular wastes. The degree of flocculation is indicated by the slope of the iso-concentration curve, increasing as the slope increases. The overall removal of solids can be estimated by reading values directly from the plotted curves. For example, in Fig. 2.7 the percentage removal after 25 minutes retention is 40% at 1 m and 36% at 2 m. The curves also represent the settling velocities of the particles for specific concentrations in a flocculant suspension where V_c = depth/retention time. Thus, after 25 minutes, 40% of the particles in suspension at 1 m will have average velocities of $\geq 6.7 \times 10^{-4}$ m s^{-1} and 36% $\geq 1.3 \times 10^{-3}$ m s^{-1} at 2 m.

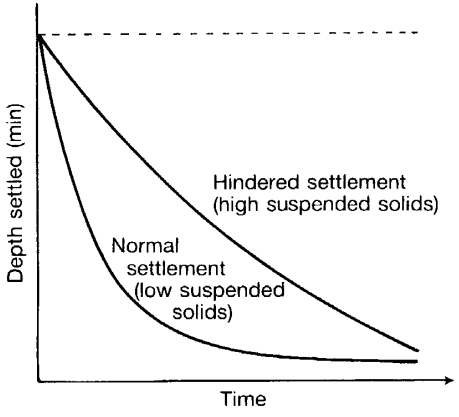


Fig. 2.12. Comparison of the rate of settlement of solids for a wastewater with a high suspended solids concentration showing hindered settlement compared with a normal wastewater containing a low suspended solids concentration.

Type III or hindered (zonal) settling

In dilute suspensions, particles settle freely at their terminal velocity until they approach the sludge zone when the particles decelerate and finally become part of the sludge. In concentrated suspensions (> 2000 mg l^{-1} of suspended solids), hindered settlement occurs. Due to the high concentration of particles, there is a significant displacement of liquid as settlement occurs which moves up through the interstices between particles reducing their settling velocity and forming an even more concentrated suspension (Fig. 2.12). The particles are close enough for inter-particulate forces to hold them in fixed positions relative to each other so that all the particles settle as a unit or blanket. A distinct solids–liquid interface develops at the top of the blanket with the upper liquid zone relatively clear and free from solids. This type of settling is normally associated with secondary settlement after a biological unit, in particular, the activated sludge process. As settlement continues, a compressed layer of particles begins to form at the base of the tank, where the particles are in physical contact with one another. The solids concentration increases with depth and this gradation of solids is even apparent throughout the hindered settling zone from the solids–liquid interface to the settling-compression zone.

Hindered settling and compression can be clearly demonstrated using a graduated cylinder, by measuring the height of the interface between the settling particles and the clarified liquid at regular intervals (Fig. 2.13(i)). This is known as a batch settling test and can be used to plot a settlement

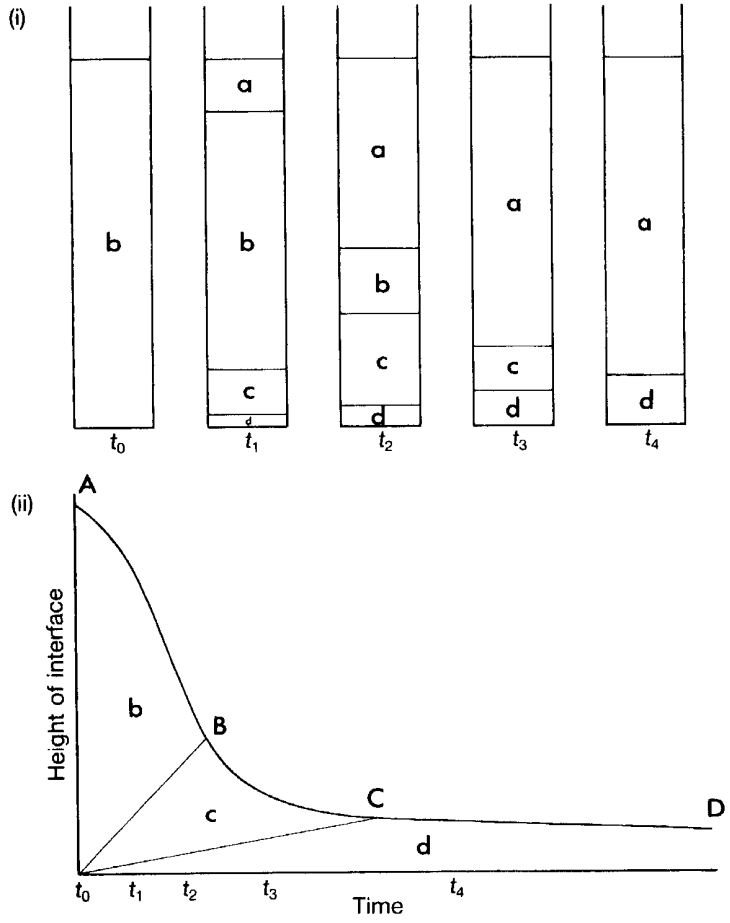


Fig. 2.13. (i) The development of discernible settlement zones at various times during the settling test on a flocculant suspension. Where (a) is the clarified effluent, (b) the wastewater with solids at its initial concentration and distributed evenly throughout its volume, (c) the zone where particle concentration is increasing and compaction is prevented by constant water movement up through the sludge blanket due to water being displaced in the lower zone, and (d) where the particles have compacted. (ii) The data derived from the settling test is used to plot a settlement curve. Where A–B shows hindered settling of the solids–liquid interface, B–C deceleration as there is a transition between hindered and compressive settling, and C–D, which is compression.

curve (Fig. 2.13(ii)). On the curve, A–B shows hindered settling of the solid–liquid interface, B–C deceleration as there is a transition between hindered and compressive settling and finally C–D which represents compression. Further settlement in the compression zone is due to physical compression

of the particles. The surface area required in a continuous-flow system designed to handle concentrated suspensions depends upon the clarification and thickening capabilities of the system. Batch settling tests are used to estimate these factors using the method developed by Talmadge and Fitch (1955).

Type IV or compressive settling

Consolidation of particles in the compressive zone is very slow as can be seen in Fig. 2.13(ii). With time, the rate of settlement decreases as the interstices between the particles become progressively smaller until, eventually, the release of liquid from the zone is inhibited. The particles are so concentrated that they are in physical contact, forming a thick sludge. Further settling is only possible by the weight of the new particles physically compressing the sludge layer. Gentle agitation enhances further compaction of the sludge by breaking up the flocs and releasing more liquid. Similarly, stirring has also been shown to increase the rate of settlement in the hindered zone (Dick 1967; Dick and Ewing 1967).

Compression normally occurs in sludge thickening tanks where specifically designed stirrers are used to encourage consolidation, or in the bottom of deep sedimentation tanks.

2.2.2. *Design of sedimentation tanks*

Sedimentation tanks have two functions, the removal of settleable solids to produce a clarified effluent and the concentration of solids to produce a handleable sludge. The design of a sedimentation tank takes both of these functions into consideration and the eventual size will depend on whichever function is limiting. For example, in the design of tanks for activated sludge settling, the limiting factor is usually sludge thickening. The criteria for sizing sedimentation tanks are the overflow rate, tank depth, and retention time. If wastewater was only composed of discrete particles, then primary sedimentation tanks would be constructed as shallow as possible to optimise removal efficiency, as the rate of type I settlement is independent of depth. However, wastewater also contains some flocculant particles and so the depth of primary sedimentation tanks is roughly of the same depth as secondary tanks, which normally have a minimum depth of 2 m. Although increasing the depth does not increase the surface loading rate, it does, however, increase the retention time which allows a greater degree of flocculation and so enhances settlement. In practice, retention time should be

used as the primary design criterion instead of surface loading or over-flow rate. However, whether either retention time or surface loading rate is used, the other is normally kept at an accepted value. Furthermore, because the depth varies only between narrow limits, the choice of criterion has had little influence in practice on the dimensions of the tanks constructed (White 1978).

Three designs of sedimentation tank are widely used at wastewater treatment works. These are the rectangular horizontal-flow, circular radial-flow, and upward flow units.

Rectangular horizontal-flow tanks are commonly used for primary sedimentation and are particularly favoured at larger works, including coastal sites where the wastewater receives primary treatment only, before discharge to the sea via a long outfall pipe. The length of rectangular tanks is usually 2.5–4.0 times the width, which is limited by the availability of mechanical scrappers which rarely exceed 24 m. Depth can vary from 2.5 m but is normally 3.0–3.5 m. The size, location and choice of rectangular tanks depend on such considerations as site layout and structural economy. The design of such a tank is shown in Fig. 2.14. Baffles deflect the incoming sewage, producing an even flow in the tank and preventing high velocity streams within the body of liquid which could cause short-circuiting of the liquid and hydraulic disturbances (Clements 1966). The flow through the tanks is rarely laminar, and although the unit has been designed to provide an optimum velocity of particles of 5 mm s^{-1}, eddies and reversals in flow result in greater local velocities (Fig. 2.15). The majority of solids settle at the inlet end of the tank from where sludge is removed. The tanks gently

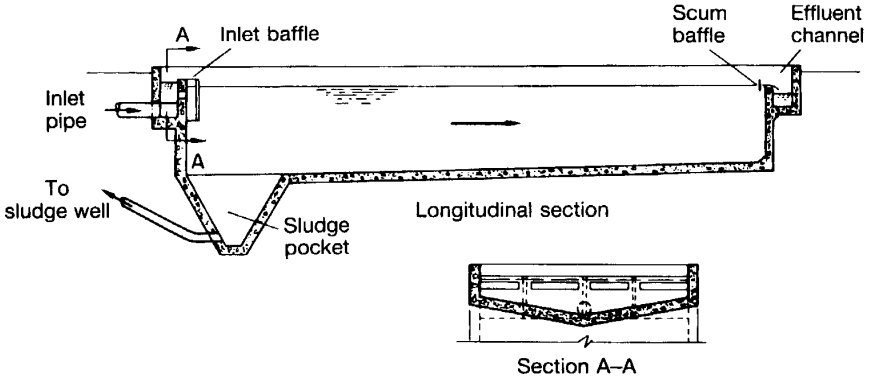


Fig. 2.14. Sections through a horizontal-flow rectangular sedimentation tank. The scrapping machinery is omitted (White 1978b).

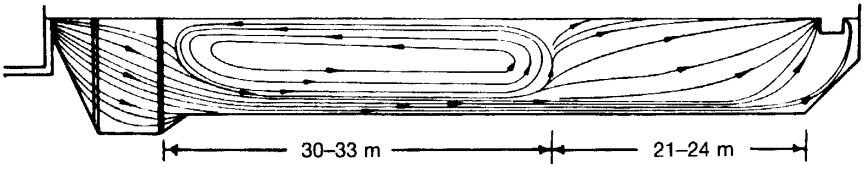


Fig. 2.15. Example of the pattern of flow in a horizontal-flow tank measured using a radioisotope ^{82}Br (Institute of Water Pollution Control 1980).

slope (1:100) towards the inlet end where the sludge is scrapped mechanically and collects in a sump to consolidate. The sump also provides a degree of storage for sludge so that sludge withdrawal can be balanced. Sludge is withdrawn from the sump under hydrostatic head by opening the valve in the sludge well. The floor of the tank is swept at regular intervals by scrapper blades that push the sludge towards the sump. In the UK, blades are normally attached to a moving bridge which scans the width of the tank, whereas in the USA, flight scrappers, which are multiple blades attached to a continuous belt or chain, are commonly used. Scum baffles at each end of the tank prevent floating material passing over the weir into the effluent channel. Skimmer bars attached to the moving bridge or the exposed returning blades on the flight scrappers, push the floating material to the end of the tank where it is collected in a channel which scans the width of the tank. The inlet and outlet weirs are usually the full width of the channel to ensure as little turbulence within the tank as possible. Surface loadings of 30 $m^3m^{-2}d^{-1}$ and a weir loading of 300 $m^3m^{-1}d^{-1}$ are normal. The major advantage of rectangular tank design is its compact nature. However, such units have a restricted weir length.

Radial-flow sedimentation tanks are circular in plan and a typical design is shown in Fig. 2.16. The normal diameter for such tanks ranges between

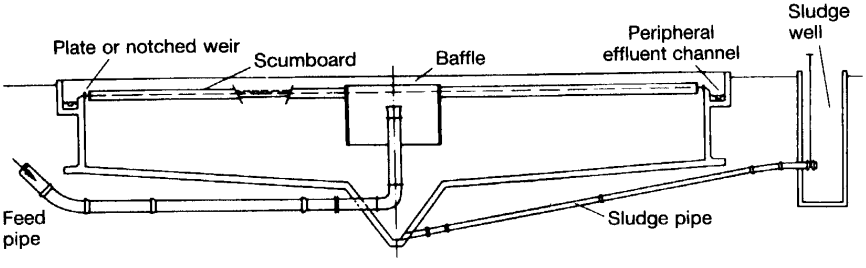


Fig. 2.16. Cross-section of a radial-flow circular sedimentation tank. The scraping machinery and centre supports are omitted (White 1978b).

l5 and 30 m, although they can be significantly smaller (5 m) or larger (50 m). They are the same depth as rectangular tanks, although circular tanks used as secondary clarifiers tend to be smaller in diameter than those used for primary sedimentation. The wastewater is piped under the floor of the tank and up a centrally placed vertical pipe which ends in a bell mouth just below the liquid surface. A deep cylindrical baffle plate prevents the flow from streaming across the surface and induces a gentle radial flow. Although the flow is nearly horizontal, there is a slight upward rise and due to the radial design, the velocity decreases outwards. Settlement occurs as the wastewater moves up and out, with an optimum upward velocity > 1.5 m s^{-1}, minimum sidewall depth of 2 m, and a surface loading of 30 $m^3m^{-2}d^{-1}$. The overflow weir runs around the periphery of the tank giving low weir loadings of < 220 $m^3m^{-1}d^{-1}$. As with rectangular tanks, complex flow patterns can develop within the tanks which can adversely effect settlement. The clarified effluent passes over a peripheral weir which is often protected by a plate or notched weir. The sludge is swept into a central sump for collection and consolidation by blades supported from a rotating radial bridge. As with rectangular tanks, sludge withdrawal is by hydrostatic pressure. Floating solids migrate slowly to the edge of the tank and are prevented from passing above the peripheral weir with the clarified effluent by a scumboard set in front of the weir. The skimmer attached to the rotating bridge concentrates the floating scum which is discharged into a mechanically operated scum box that drains into the sludge well. Advantages of circular tank design include long weir length and simpler scrapping mechanisms compared with rectangular designs, but they are not so compact. Also, the installation and maintenance costs of circular tanks are lower for small to medium sized treatment plants.

Upward flow sedimentation tanks are compact units used primarily at small treatment plants. Settlement occurs by the upward movement of the wastewater causing flocculation to increase the size of the particles. Thus, the wastewater moves through a sludge blanket where the particles are settling slowly in the opposite direction to the flow. These deep tanks are either conical (45° slope) or pyramidal (60° slope) in cross-section with an upward velocity > 1.5 m h^{-1} (Fig. 2.17). There is no scraper and the sludge that collects at the base of the tank is removed by hydrostatic pressure. Such tanks are difficult and expensive to construct, so their application is limited.

The design of sedimentation tanks is dealt with in more detail elsewhere (Metcalf and Eddy Inc. 1991; IAWQ 1997; Gray 1999).

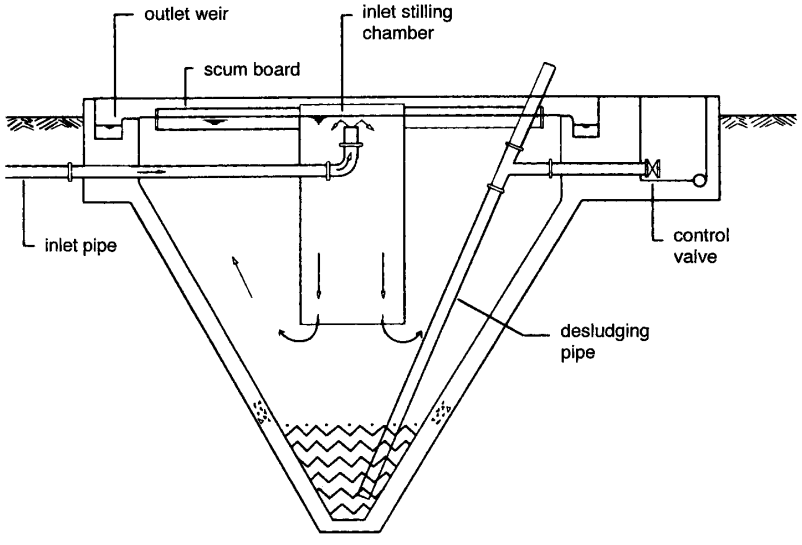


Fig. 2.17. Upward-flow primary sedimentation tank (Casey 1997).

Primary sedimentation

Primary sedimentation tanks clarify sewage by flotation as well as settlement. Any suspended matter which is less dense than water such as oil, grease or fat will rise to the surface and form a scum. Skimmer blades attached to the scrapper bridge push the scum across the surface of the tank to where it is discharged into a special sump or a mechanically operated skimming device which decants the scum from the surface. Although primary sedimentation tanks are designed to remove both floating and settleable material, special flotation tanks may be required before the primary settlement stage if large amounts of floatable material are present in the wastewater (Sec. 2.1). There is little difference between the efficiency of horizontal or radial-flow units for primary settlement, and selection is normally based on constructional costs or plant configuration. For example, a circular tank of the same volume and surface area as a rectangular tank usually costs less to build (White 1978). However, if a group of tanks are required, then rectangular tanks are normally used with common dividing walls which are not only cheaper to construct than individual circular tanks, but also take up considerably less space. Except at very small rural plants, there is always more than one primary sedimentation tank to allow for maintenance, with the flow divided equally between the tanks. Primary sedimentation tanks are designed to remove between 50–70% of

the suspended solids and 25–40% of the BOD of the raw wastewater. Effectiveness depends on the nature of the wastewater and in particular, the proportion of soluble organic material present, which can severely affect BOD removal efficiency. Some industrial wastes may contain higher proportions of settleable organic matter resulting in BOD removals at the primary sedimentation stage in excess of 60–70%. Overflow rates vary between 16–32 $m^3m^{-2}d^{-1}$, with 24 $m^3m^{-2}d^{-1}$ the commonly used value for design. The accumulated sludge density is influenced by the overflow rate forming a dense sludge at loadings of < 24 $m^3m^{-2}d^{-1}$ with the scrappers enhancing consolidation, or a dilute sludge at > 32 $m^3m^{-2}d^{-1}$ as the extra hydraulic movement inhibits consolidation. At higher overflow rates, the turbidity of the effluent will increase, especially during periods of maximum flow. Turbid effluents from primary tanks are also caused by excessive weir loadings, which should not exceed 120 $m^3m^{-1}d^{-1}$ for plants receiving $< 4,000$ m^3d^{-1} or 180 $m^3m^{-1}d^{-1}$ at flows of $> 4,000$ m^3d^{-1} if solids are not to be carried over the final effluent weir. Over storage of sludge, especially if wasted activated sludge has been discharged to the primary sedimentation tank for settlement, can result in anaerobic bacterial activity in the sludge layer. Gas is produced and becomes trapped in the solids, making them more buoyant, expanding the sludge blanket, reducing consolidation and reducing the solids concentration of the sludge. In severe cases, gas production can become extremely active with unpleasant odours being produced, solids rising and floating to the tank surface, and the effluent turning darker and eventually grey-black in colour.

Secondary sedimentation

Secondary sedimentation tanks separate the microbial biomass produced in the biological treatment unit from the clarified effluent. The biological growth or humus which is flushed from fixed film reactors of the percolating filter type comprises of well oxidised particles, mainly in the form of dense microbial film, and also living invertebrates and invertebrate debris, which settle readily. The volume of sludge produced is much less compared with the activated sludge process (Table 8.1). The sludge will form a thin layer at the base of the sedimentation tank, rarely exceeding 0.3 m in depth, if the tank is regularly desludged twice a day. The overflow rate can be up to 32 $m^3m^{-2}d^{-1}$ with similar weir loadings and depth as a primary sedimentation tank.

Settlement of activated sludge is more variable as the flocs are light and more buoyant than solids from percolating filters, which reduces their

settling velocities. This is made worse by denitrification at the centre of the tiny sludge flocs which results in small bubbles being produced which buoy up the clusters of particles. This results in a thick sludge blanket which can often take up half of the tank volume at peak flows and is normally between 0.8 and 1.0 m in depth. Therefore, a rapid and continuous method of sludge removal is necessary. This is achieved by not having a central sump but instead, a floor slope of 30° to the horizontal, which allows the sludge to be quickly and continuously withdrawn. Another common method of removal is to have numerous uptake tubes attached to the scrapper, which forces the sludge up and out by the hydrostatic pressure. Rapid withdraw is achieved across the entire bottom of the tank, which ensures that the retention time of solids settling at the periphery of the tank is no greater than at the centre, reducing the age of the floc and the chance of gas production resulting in reduced settleability. Sludge removed by this method encourages a vertical, rather than horizontal, movement in the tank which encourages flocculation and increases sludge density. Other problems causing reduced settleability of activated sludge effluents are discussed in Sec. 5.2.

The volume of effluent from a primary sedimentation tank is equivalent to the influent, as the volume of sludge withdrawn from the tank is negligible compared with the total volume of wastewater. However, in a secondary settlement tank receiving activated sludge, the influent is equivalent to the effluent plus the returned sludge. In this case, the overflow of effluent is used for design purposes and not the influent which includes recirculation. Secondary and primary sedimentation tanks are similar in design, except that special attention is paid to the large volume of flocculant particles in the liquid. The capacity of tanks receiving activated sludge effluents is usually based on a retention time of l.5–2.0 hours at the maximum flow rate. The maximum rate of flow will be 3 DWF plus 1 DWF for returned sludge which will give a DWF retention time of 6–8 hours which is the same capacity as a primary sedimentation tank. A retention time of 2–3 hours at maximum flow rate for primary sedimentation is adequate to allow almost all settleable solids to settle to the bottom of the tank, but is not long enough for septic conditions to result. However, at flows below DWF, septicity of the sludge can occur due to the extended retention time.

2.2.3. *Performance evaluation*

The efficiency of sedimentation tanks is normally evaluated in terms of suspended solids and BOD removal. Examples of typical performance data for horizontal and radial-flow primary sedimentation tanks are given in

Table 2.4. The performance of horizontal flow primary sedimentation tanks (Institute of Water Pollution Control 1980).

		Primary tanks							
Sewage treatment works	Average daily flow (tcm)	No.	Length (m)	Width (m)	Average depth (m)	Ratio of length to width	Weir overflow rate* ($m^3\ m^{-1}\ d^{-1}$)	Surface loading* ($m^3\ m^{-2}\ d^{-1}$)	Retention period* (h)
Cambridge	40.2	2	33.9	16.3	1.8	2.1	305	8.4	5.0
Darlington	13.4	2[a]	47.0	12.8	2.8	3.7	530	11.2	6.2
Kew	32.0	11	31.1	9.1	1.8	3.3	320	10.3	5.0
High Wycombe	25.2	11	41.3[b]	11.3[b]	1.3[b]	3.7[b]	205	17.6	6.4
Nottingham (Stoke Bardolph)	154.1	6	91.4	34.1	1.9	2.7	750	8.2	5.6
Oldham	49.2	8	39.0	11.0	2.0	3.6	545	14.1	3.4
Oxford	12.5	3	45.7	15.2	3.3	3.0	275	6.0	13.1
Oxford	16.3	2	45.7	15.2	3.3	3.0	535	11.7	6.7
Rotherham (Aldwarke)	27.5	2	93.0	12.0	1.8	7.8	1150	12.3	3.7
Scunthorpe	11.3	4[e]	16.0	8.0	2.7	2.0	370	22.5	2.9

Table 2.4. (*Continued*)

	Suspended solids			BOD			Sludge production		Sludge dry solids	
	Crude sewage (mg l^{-1})	Settled sewage (mg l^{-1})	Removal (%)	Crude sewage (mg l^{-1})	Settled sewage (mg l^{-1})	Removal (%)	Average ($m^3 d^{-1}$)	1 hd^{-1} d^{-1}	%	Volatile content (%)
Cambridge	260	135	49	210	150	29	202 (B)	1.7	3.8	72
Darlington	295	195	34	280	215	24	137[d] (B)	1.5	5.9	64
Kew	310	150	51	210	160	24	264 (AL)	3.2	3.7	75
High Wycombe	300	115	62	270	150	44	54 (BL)	0.9	6.6	76
Nottingham (Stoke Bardolph)	240	140	42	310	220	30	1714 (AL)	3.7	2.2	72
Oldham	305	130	57	290	160	44	110[d] (ABL)	0.7	6.8	63
Oxford	390	105	73	350	180	49	103 c	2.2	5.3	73
Oxford	415	125	70	315	150	52	108 c	2.3	4.0	79
Rotherham (Aldwarke)	200	80	61	215	145	32	224 (AL)	2.3	3.8	73
Scunthorpe	310	150	51	325	207	36	61 (ABL)	1.1	6.6	—

[*]Based on average daily flow.

[a]Two tanks (out of three) in operation.

[b]Average of all tanks.

[c]Same tanks but under different operating conditions.

[d]Sludge from horizontal flow tanks and radial flow tanks is combined.

[e]Consists of 4 hoppers and 4 tanks constructed as one unit.

A — includes surplus activated sludge.

B — includes sludge from biological filters.

L — includes works' liquors.

Table 2.5. The performance of radial flow primary sedimentation tanks (Institute of Water Pollution Control 1980).

		Primary tanks						
Sewage treatment works	Average daily flow (tcm)	No.	Diameter (m)	Side wall depth (m)	Ratio of radius to side wall depth	Weir over-flow rate* ($m^3\ m^{-1}\ d^{-1}$)	Surface loading* ($m^3\ m^{-2}\ d^{-1}$)	Retentaion period* (h)
Barnsley Lundwood	15.6	6	16.7	3.0	2.4	52	11.8	7.0
Bournemouth								
(Holdenhurst)	8.6	2	24.4	2.7	4.5	56	9.2	7.9
(Kinson)	8.9	2	18.3	2.1	4.4	77	16.9	3.5
Darlington	14.3	2	27.5	2.1	6.6	83	12.0	6.0
Hogsmill Valley	9.8	4	30.5	3.2	4.8	108	15.0	6.7
Malling	4.5	2	19.8	2.4	4.1	38	7.3	9.7
Oldham	17.0	1	33.5	3.3	5.1	161	19.4	5.2
Oxford	23.2	8	19.5	4.2	2.3	47	9.7	7.8

Table 2.5. (Continued)

	Suspended solids			BOD			Sludge production		Sludge dry solids	
	Crude sewage (mg l^{-1})	Settled sewage (mg l^{-1})	Removal (%)	Crude sewage (mg l^{-1})	Settled sewage (mg l^{-1})	Removal (%)	Average (m^3 d^{-1})	1 hd^{-1} d^{-1}	%	Volatile content (%)
Barnsley Lundwood	370	150	60	400	250	38	114 (BL)	1.5	4.6	78
Bournemouth										
(Holdenhurst)	420	125	71	305	150	50	59 (AL)	1.3	4.1	78
(Kinson)	275	135	51	265	155	41	45 (AL)	1.1	5.5	78
Darlington	295	140	53	280	180	36	137† (B)	1.5	5.9	64
Hogsmill Valley	305	100	67	275	150	45	327 (AL)	1.75	4.4	75
Malling	490	135	72	405	170	58	46	1.5	5.6	75
Oldham	165	95	43	195	115	41	110 (ABL)	0.7	6.9	63
Oxford	405	100	75	370	175	53	86	1.0	5.3	73

*Based on average daily flow.

†Sludge from radial flow tanks and horizontal flow tanks is combined.

A — Includes surplus activated sludge.

B — Includes sludge from biological filters.

L — Includes works' liquors.

Tables 2.4 and 2.5. The suspended solids of primary settled sewage is normally in excess of 100 mg l^{-1} and the percentage removal of suspended solids is between 40–70% for horizontal-flow and 50–75% for radial-flow tanks (Institute of Water Pollution Control 1980). Although the dry solids content of the sludge depends on the skill of the operator, it is normally within the range of 3.0–6.5%. The efficiency of secondary sedimentation tanks is linked to the efficiency of the biological unit and depends on the settleability of the microbial biomass formed and, as stated above, this is particularly critical in the separation of activated sludge from the treated wastewater. The sludge volume index (SVI) and sludge density index (SDI) are long established measures of activated sludge settling ability. Both are obtained by allowing a sludge sample to settle under standardised conditions. The SVI is measured by filling a 1 litre graduated cylinder with mixed liquor from the activated sludge aeration basin and allowing it to stand undisturbed for 30 minutes. The volume of settled sludge is then read in millilitres. The suspended solids concentration is determined using a sample of the mixed liquor from the aeration basin, which is known as the mixed liquor suspended solids (MLSS) concentration, and the SVI expressed as the volume in millilitres occupied by 1 gram of settled suspended solids:

$$\text{SVI} = \frac{V \text{ X } 1000}{\text{MLSS}} (\text{ml g}^{-1})$$

where V is the volume of settled sewage and the MLSS is the mixed liquor suspended solids (mg l^{-1}). The SDI = 100/SVI. A high SVI indicates a poor settleability and, in general, a sludge with an SVI $>$ 120 has poor settling properties. A good sludge would have an SVI $<$ 80 and a very good one around 50. These indices are easily and rapidly performed on site and are routinely used by operators to check the condition of activated sludge (Sec. 5.2.1). They are not used for design purposes. A more accurate measure of settleability, which is used for research and design studies, is the stirred specific volume index (SSVI). This is measured in a special settling column 0.5 m deep and about 0.1 m in diameter with settlement impeded by a wire stirrer rotating at 1 revolution per minute (Fig. 2.18). This test reproduces the non-ideal situation in settling tanks, whereas the SVI is measured under complete quiescence (White 1976). The use of the SSVI test is becoming more widely used for normal operational management as the SVI has been shown to have serious limitations. For example, marked changes in settleability which have been reflected by the SSVI have not been detected by the SVI (Johnstone *et al.* 1979, 1980, Rachwal *et al.*

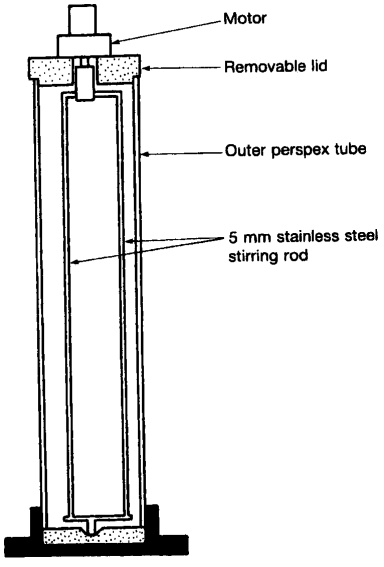


Fig. 2.18. The standard SSVI settling apparatus developed at the Water Research Centre (UK).

1982). Also, the SVI can vary in an inconsistent way with the initial concentration of suspended solids (White 1982). Rachwal *et al.* (1982) have also demonstrated that SVI has a much more limited working range than SSVI due to the independence of SSVI on solids concentration. At high solids concentration, the settled volume of the sludge, as measured in an unstirred 1 litre cylinder, increases at approximately the same rate as the increase in MLSS, thus suppressing the SVI. They found that by comparing sludges with the same settleability as measured by the SSVI (SSVI = 80–85 ml g^{-1}), the equivalent SVI's ranged from 80–290 ml g^{-1}. By plotting SVI against solids concentration, a steady rise in SVI is discernible up to a solids concentration of 4 g l^{-1}. Above this concentration, there is virtually no settlement, indicating that 4 g l^{-1} is the maximum limit of the test (Fig. 2.19). The wider working range of SSVI compared with SVI is clearly illustrated by plotting the percentage volume, occupied by sludges with the same settleability (SSVI = 80–85 ml g^{-1}) after 30 minutes settlement in the respective cylinders, against solids concentration. The SVI curve ends at just below 4 g l^{-1} with virtually no settlement above 4 g l^{-1}. The relationship between settled volume in the SSVI cylinder and solids concentration is very close to linear (Fig. 2.20). Activated sludge plants are designed to operate at an optimum MLSS, so it is convenient to quote

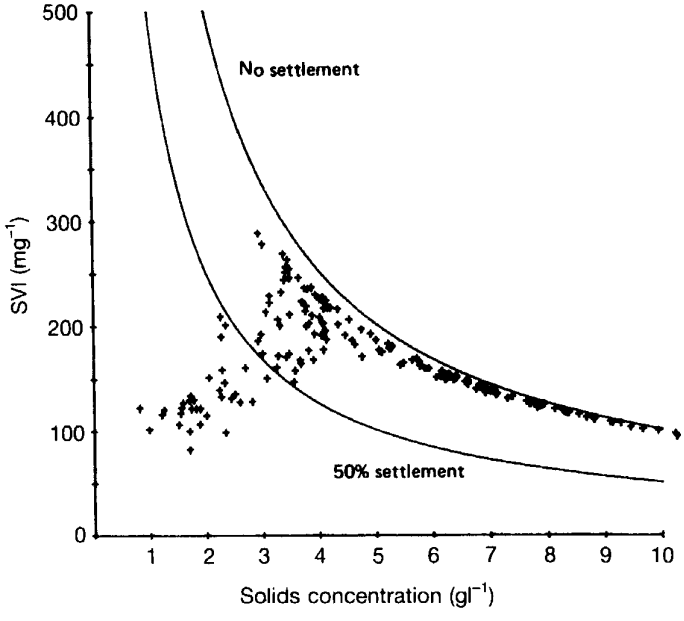


Fig. 2.19. SVI plotted against solids concentration for a sample of sludges with the same settleability as measured by the SSVI (Rachwal *et al.* 1982).

the SSVI at this concentration (i.e. normally at 3.5 g l^{-1}). This is done by carrying out an SSVI determination on both the aeration tank mixed liquor and the returned activated sludge (RAS) which will have a much higher MLSS concentration. The results are plotted (SSVI against MLSS) and a line fitted allowing the SSVI to be quoted at the design MLSS (e.g. $SSVI_{3.5}$).

The diluted sludge volume index is increasingly used in place of the standard SVI as it overcomes the problems with sludges that have a high MLSS concentration. Several 1 litre graduated cylinders are required for this index. The mixed liquor is taken and a series of two-fold dilutions using secondary settled effluent are made up. Thus, the first graduated cylinder contains 1000 ml of mixed liquor, the second 500 ml mixed liquor made up to 1000 ml using final effluent, the third 250 ml made up to 1000 ml using final effluent, and so on. The mixed liquor in the graduated cylinders is fully mixed using a plunger (e.g. mixing for a standard time of 30–60 seconds) and then allowed to settle for 30 min. The volume of settled sludge (V) is then measured in ml as in the standard SVI test. The DSVI is calculated from the equation below using the dilution at which the settled sludge

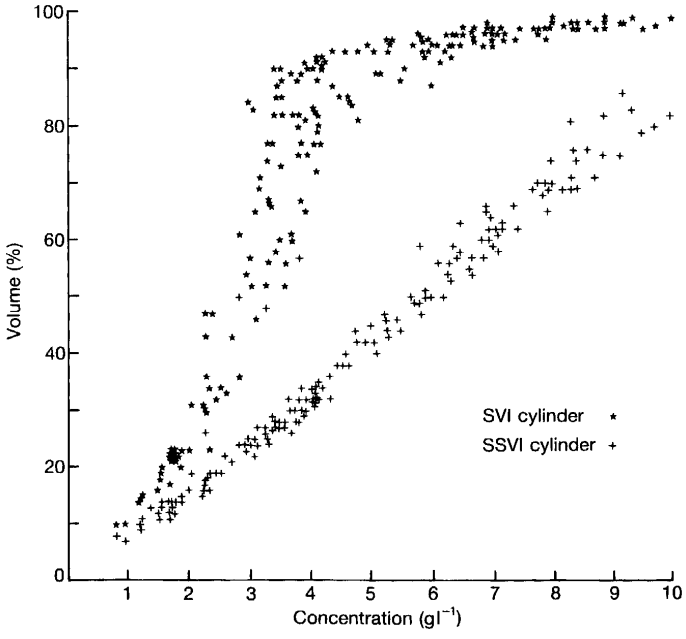


Fig. 2.20. Comparison of the working range of SVI with SSVI. The percentage volume of sludges with the same settleability (SSVI 80–85 ml g^{-1}), after settlement for 30 minutes in the respective cylinders, is plotted against concentration (Rachwal *et al.* 1982).

volume (V) is less than, but closest to 200 ml (i.e. $V < 200$ ml):

$$\text{DSVI} = \frac{V \times 2^n}{\text{MLSS}} \text{ ml g}^{-1}$$

where n is the number of two-fold dilutions required; V the volume of settled sludge in ml l^{-1}; MLSS the mixed liquor suspended solids concentration in the *undiluted sample* and expressed in g l^{-1}. The values obtained are interpreted in the same way as a normal SVI. Sludge settleability in relation to biological processes is discussed further in Chap. 5.

The level of the sludge blanket in an activated sludge sedimentation tank can vary quite dramatically over 24 hours (Fig. 2.21), especially if the flow is not well balanced. The level of the sludge blanket can be very important, especially if the settleability of the sludge particles is low and there is a risk that sludge particles could be discharged with the clarified effluent. Sludge level detectors are available based on optical or ultrasonic attenuation (Fig. 2.22). Such instruments can not only detect the level or follow the rise and fall of the sludge-water interface, but also measure the concentration or density of the sludge, allowing computers to continuously

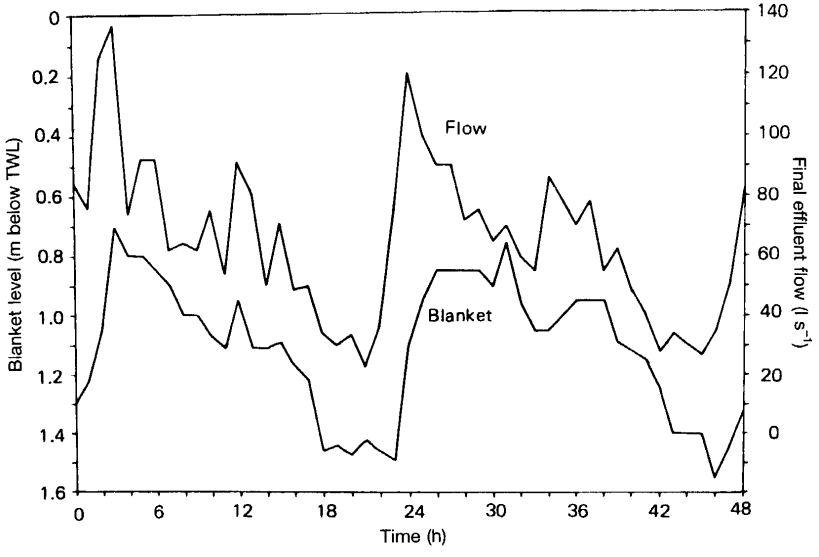


Fig. 2.21. Example of the variation in sludge blanket level in a final sedimentation tank with flow. The tank is receiving the effluent from a carrousel-type activated sludge unit (Rachwal *et al.* 1982).

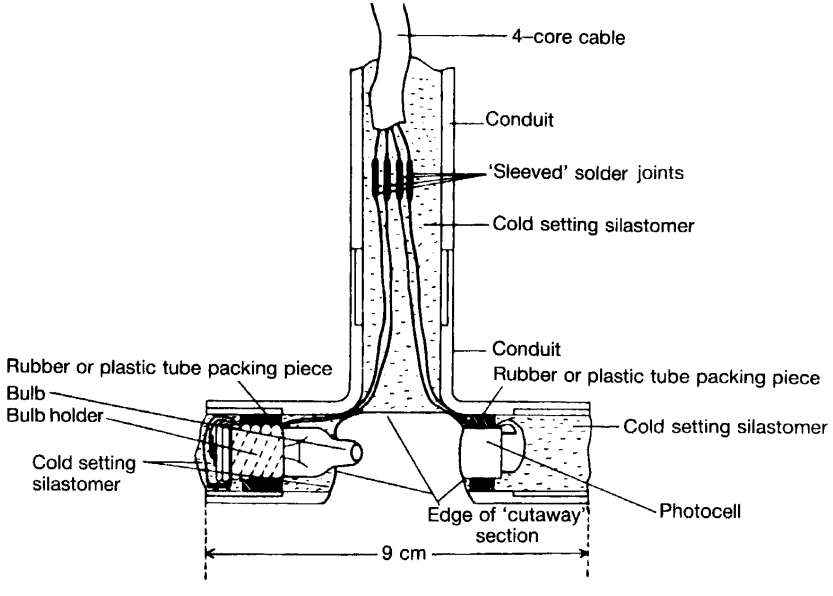


Fig. 2.22. The design of a simple photoelectric sludge-level detector (Institute of Water Pollution Control 1980).

calculate an adjust sludge wastage and return rates. Equipment is available to reduce the variation in the level of the sludge blanket by linking the rate of return to the rate of incoming sewage flow.

The pattern of flow and the retention period in sedimentation tanks can be measured using chemical and radioactive tracers. The range of available tracers has been reviewed by the Institute of Water Pollution Control (1980). However, the tracer most commonly employed is lithium chloride, although any element which is easily detected and is not already present in large or irregular concentrations in sewage can be used. Sodium chloride has also been employed using conductivity to measure the concentration of the salt (Gray 1981). Where the quantity of a chemical tracer required is excessively large, then radioactive tracers such as 82Br are used. The advantage of radioactive tracers is that they can be easily and continuously monitored without the necessity of taking water samples for laboratory analysis. Dyes are only used for a quick, semi-quantitative assessment of flow pattern as they can be followed visually. The flow pattern can also be studied using float techniques (Clements and Price 1972) or temperature differentials (Cox 1971).

Further reading

General: White 1978; Institute of Water Pollution Control 1980; Metcalf and Eddy Inc. 1991; Casey 1997; Rendell 1999.
Design: Dick 1976; White 1976; IAWQ 1997.
Theory: Dick 1976; White and Allos 1976; Scottish Development Department 1980; Metcalf and Eddy Inc. 1991.
Settleability tests: White 1975; Rachwal *et al.* 1982.
Operation and maintenance: Institute of Water Pollution Control 1980.

2.3. Secondary (Biological) Treatment

After preliminary and primary treatment, the wastewater will still contain significant amounts of colloidal and dissolved material that needs to be removed before discharge to a watercourse. Secondary treatment biologically converts this unsettleable material into biological cells which can then be removed by settlement using sedimentation tanks similar to those used for primary sedimentation (Sec. 2.2).

Methods of purification in secondary treatment units are similar to the *self-purification process* that occurs naturally in rivers and streams, and

involves many of the same organisms. Removal of organic matter from settled wastewaters is carried out by heterotrophic micro-organisms, predominantly bacteria but also occasionally fungi. The micro-organisms breakdown the organic matter by two distinct processes, biological oxidation and biosynthesis, both of which result in the removal of organic matter from solution. Oxidation or respiration results in formation of mineralised end products that remain in solution and are discharged in the final effluent, while biosynthesis converts the colloidal and soluble organic matter into particulate biomass (new cells) which can then be removed by settlement. If the food supply, in the form of organic matter, becomes limiting, then the microbial cell tissue will be endogenously respired (auto-oxidation) by the micro-organisms to obtain energy for maintenance. All three processes occur simultaneously in the reactor and can be expressed stoichiometrically as:

Oxidation:

$$\underset{\text{(organic matter)}}{\text{COHNS}} + O_2 + \textit{bacteria} \rightarrow CO_2 + NH_3 + \text{other end-products} + \text{energy}$$

Biosynthesis:

$$\underset{\text{(organic matter)}}{\text{COHNS}} + O_2 + \textit{bacteria} \rightarrow \underset{\text{(new cells)}}{C_5H_7NO_2}$$

Auto-oxidation:

$$\underset{\text{(bacteria)}}{C_5H_7NO_2} + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O + \text{energy}$$

In natural waters, soluble organic matter is principally removed by oxidation and biosynthesis, but in the intensified microbial ecosystem of the biological treatment plant, adsorption is perhaps the major removal mechanism, with material adsorbed and agglomerated onto the dense microbial mass. The adsorptive property of the microbial biomass is particularly useful as it is also able to remove from solution non-biodegradable pollutants present in the waste-water, such as synthetic organics, metallic salts and even radio-active substances. The degree to which each removal mechanism contributes to overall purification depends on the treatment system used, its mode of operation, and the materials present in the wastewater.

In nature, heterotrophic micro-organisms occur either as thin films (periphyton) growing over rocks and plants, or over any stable surface, or as individual or agglomerations of organisms suspended in the water. These

natural habitats of aquatic heterotrophs have been utilised in wastewater treatment to produce two very different types of biological units, one using attached growths and the other suspended microbial growths. The design criteria for secondary treatment units are selected to create ideal habitats to support the appropriate community of organisms responsible for the purification of wastewater, so attached and suspended microbial growth systems will require fundamentally different types of reactors. Both treatment systems depend on a mixed culture of micro-organisms, but grazing organisms are also involved so that a complete ecosystem is formed within the reactor, each with distinct trophic levels. In its simplest form, the reactor food chain comprises:

$$\text{Heterotrophic bacteria and fungi} \rightarrow \text{holozoic protozoa} \rightarrow \text{rotifers and nematodes} \rightarrow \text{insects and worms} \rightarrow \text{birds}\,.$$

Due to the nature of the reactor, suspended growth processes have fewer trophic levels than attached growth systems (Fig. 2.23). These man-made ecosystems are completely controlled by operational practice, and

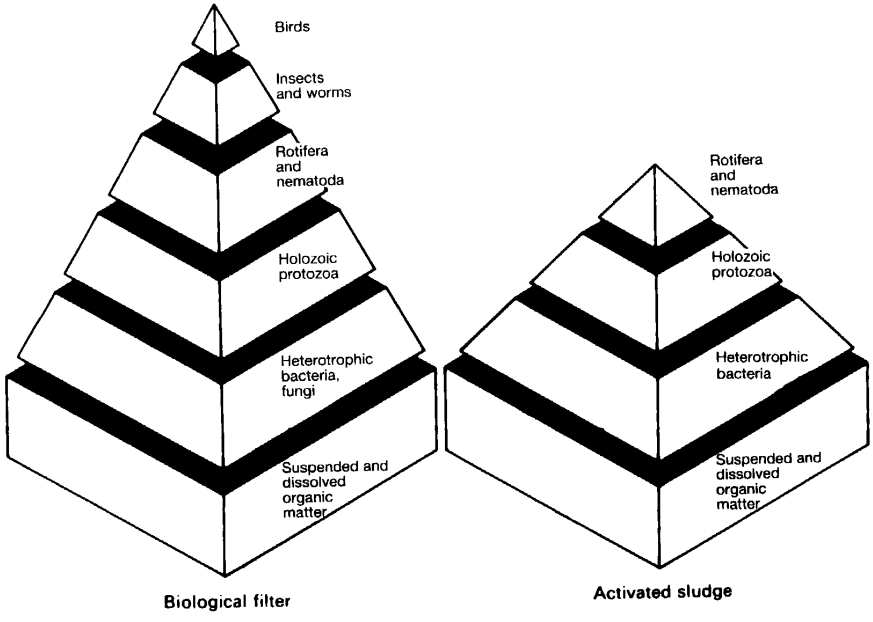


Fig. 2.23. Comparison of the food pyramids for a percolating filter and activated sludge treatment systems (Wheatley 1985).

are limited by food (organic loading) and oxygen (ventilation or aeration) availability.

Chemical engineers have been able to manipulate the natural process of self-purification, and by supplying ideal conditions and unlimited opportunities for metabolism and growth, have intensified and accelerated this biological process to provide a range of secondary wastewater treatment systems. However, a number of basic criteria must be satisfied by the design. In order to achieve a rate of oxidation well above that found in nature, a much denser biomass in terms of cells per unit volume must be maintained within the reactor. This will result in an increased oxygen demand which must be met in full, so as not to limit the rate of microbial oxidation. Essentially, this is done by increasing the air–water interface. The wastewater containing the polluting matter must be brought into contact for a sufficient time with a dense population of suitable micro-organisms and with excess oxygen, to allow oxidation and removal of unwanted material to the desired degree. Finally, inhibitory and toxic substances must not be allowed to reach harmful concentrations in the reactor.

The main methods of biological treatment rely on aerobic oxidation. To ensure that oxidation proceeds quickly, it is important that as much oxygen as possible comes into contact with the wastewater so that the aerobic micro-organisms can break down the organic matter at maximum efficiency. Secondary treatment units of wastewater treatment plants are designed to bring this about. Oxidation is achieved by three main methods.

(1) by spreading the sewage into a thin film of liquid with a large surface area so that all the required oxygen can be supplied by gaseous diffusion (e.g. percolating filter);
(2) by aerating the sewage by pumping in bubbles of air or stirring vigorously (e.g. activated sludge); and
(3) by relying on algae present to produce oxygen by photosynthesis (e.g. stabilisation pond).

In systems where the micro-organisms are attached, a stable surface must be available. Suitable surfaces are provided by a range of media such as graded aggregate or moulded plastic and even wooden slats, retained in a special reactor, on which a dense microbial biomass layer or film develops. These reactors are generally categorised as fixed-film reactors, the most widely used type being the percolating or trickling filter. Organic matter is removed by the wastewater as it flows in a thin layer over, and to some extent, through the biological film covering the static medium. Oxygen is provided by natural ventilation which moves through the bed of medium

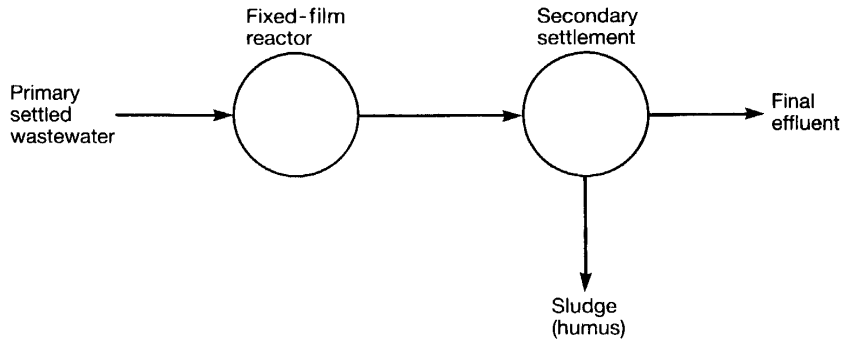


Fig. 2.24. Schematic layout of a fixed film reactor.

via the interstices supplying oxygen to all parts of the bed. The oxygen diffuses into the thin layer of wastewater to the aerobic micro-organisms below (Chap. 4). The final effluent not only contains the waste products of this biological activity, mainly mineralised compounds such as nitrates and phosphates, but also particles of displaced film and grazing organisms flushed from the medium. These are separated from the clarified effluent by settlement, and the separated biomass disposed as secondary sludge (Chap. 8) (Fig. 2.24). In suspended growth processes, the micro-organisms are either free-living or flocculated to form small active particles or flocs which contain a variety of micro-organisms including bacteria, fungi, and protozoa. These flocs are mixed with wastewater in a simple tank reactor, called an aeration basin or tank, by aerators that not only supply oxygen but also maintain the microbial biomass in suspension to ensure maximum contact between the micro-organisms and the nutrients in the wastewater. The organic matter in the wastewater is taken out of solution by contact with the active suspended biomass (Chap. 5). The purified wastewater is displaced from the reactor by the incoming flow of settled wastewater and contains a large quantity of micro-organisms and flocs. The active biomass is separated from the clarified effluent by secondary settlement, but if the biomass was disposed of as sludge, the concentration of active biomass in the aeration basin would rapidly fall to such a low density that little purification would occur. Therefore the biomass, called activated sludge, is returned to the reactor to maintain a high density of active biological solids ensuring a maximum rate of biological oxidation (Fig. 2.25). Excess biomass, not required to maintain the optimum microbial density in the reactor, is disposed of as surplus sludge (Chap. 8). Suspended growth processes are more intensive than attached growth systems and are able to treat up to ten

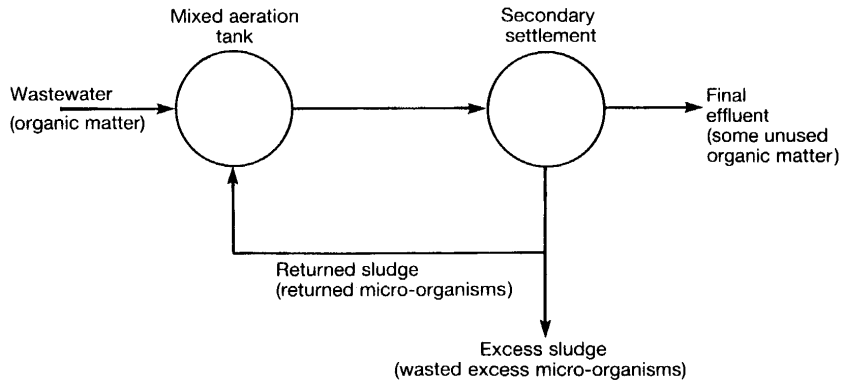


Fig. 2.25. Schematic layout of a mixed reactor (activated sludge) with sludge return.

times more effluent per unit volume of reactor, making them much cheaper in terms of capital costs. However, suspended growth systems are more difficult to operate and maintain. They have much higher operating costs due to mixing, aeration, and pumping sludge from the settlement tank back to the reactor, and also produce comparatively large quantities of surplus biomass as sludge.

Biological wastewater treatment differs from more traditional fermentation processes, such as the production of bakers yeast, in a number of important ways. Primarily, wastewater treatment is aimed at removing unwanted material, whereas the commercial fermentation processes are all production systems. These production fermentations use highly developed, specialised strains of particular micro-organisms to synthesise the required end-product while in wastewater treatment a broad mixture of micro-organisms are used. These are largely self-selecting, with nearly all the organisms which can contribute to substrate removal welcomed. Unlike commercial fermenters, wastewater reactors are not aseptic and because production fermenters require highly controlled conditions, they are more complex and comparatively more expensive than those used in wastewater treatment.

Secondary treatment processes are extensively reviewed in chapters 4 to 7.

2.4. Tertiary and Advanced Treatment

Most biological treatment plants are designed to produce a 20:30 effluent when loaded correctly, with extended aeration processes normally

producing an even better effluent. However, treatment plants are all affected by a wide range of environmental variables and so it is not possible to rely on a specific level of treatment all of the time. Furthermore, as the demand for water increases, it is necessary to produce effluents to even higher standards in order to protect receiving water quality. This is particularly important if receiving water is extracted for domestic or industrial supply downstream of the effluent outfall, or if there is inadequate dilution. In order to meet standards more stringent than 20:30 on a reliable basis, some form of tertiary treatment is required. Most tertiary treatment methods remove suspended solids, and also the associated BOD_5 and pathogens. A removal of 10 mg l^{-1} of suspended solids from a normal sewage effluent is likely to reduce the BOD_5 by up to 3 mg l^{-1}. Methods that remove nutrients such as ammonia, nitrates, phosphates or other soluble material are more strictly termed as advanced treatment methods. Such techniques are used in eutrophication control, water reuse for potable or industrial supply, and perhaps most widely in desalination. There is confusion between tertiary and advanced treatment, but the former removes fine suspended matter while the latter removes soluble components from secondary treated wastewaters. So, in essence, both can be classed as the third stage in the treatment process (Institute of Water Pollution Control 1987a).

2.4.1. *Tertiary treatment*

Tertiary treatment is a method of improving good quality effluents, not for converting poor effluents to an acceptable standard. The processes are expensive and so cannot be considered as a method of uprating substandard treatment plants. It is often more cost-effective to use other methods rather than incorporating tertiary treatment to protect water quality, such as augmenting low river flow, the direct oxygenation of the river when necessary, or the adoption of a more efficient treatment plant design. Tertiary treatment methods are predominantly physical in action, relying largely on flocculation, settlement, and filtration. Among the commoner methods are: prolonged settlement; irrigation onto grassland; constructed wetlands; straining through a fine mesh or filtration through media, such as sand or gravel.

Lagoons

If sufficient land is available, shallow lagoons are the most efficient tertiary treatment process available (Potten 1972). The lagoons allow further

Table 2.6. Comparison of the performance and relative cost of commonly used tertiary treatment units (Foster 1985). Based on 1984 costs.

Method	Percentage removal			Approximate cost (£ per 1000 m^3)
	Suspended solids	BOD	Coliform bacteria	
Grass plots	70	50	90	0.38
Shallow lagoons (detention 3–4 d)	40	40	70	0.29
Deep lagoons (detention 17 d)	80	65	99	0.54
Slow sand filters	60	40	50	1.92
Rapid gravity sand filters	80	60	30	0.67
Upward flow sand filters	70	55	25	0.46
Microstrainers	70	40	15	0.75
Upward flow gravel bed clarifier	50	30	25	0.63

settlement, and as the retention time is much longer than secondary sedimentation tanks, flocculation and settlement of some of the remaining suspended material will occur (Sec. 6.3.2). Lagoons, also known as clarification lakes or maturation ponds, provide a combination of settlement and biological oxidation depending on the retention time. At short retention times ($<$ 60h), purification is mainly by flocculation and settlement with suspended solids removals of 30–40% normal. There is a marked improvement in performance at longer retention times (14–21d) with 75–90% removals of suspended solids, 50–60% BOD_5 and 99% coliform removals possible (Table 2.6). Fish (1966) reported BOD_5 removals in a single lagoon of 29% at 52 h and 70% at 84 h retention. At longer retention times, algae develop which can be discharged with the final effluent, resulting in a raised suspended solids and BOD_5, and so offsetting the potential advantage gained by the increased settlement. Phosphate removal is linked to algal biomass and at the Rye Meads Treatment Works, phosphate removal varied seasonally from a maximum removal of 73% in May to a minimum of 2% in January. The removal of nitrate is unaffected by algal biomass (Fig. 2.26) (Department of the Environment 1973). Research has shown that in eutrophic lagoons, fish can be reared very rapidly (White and Williams 1978), and that by harvesting fish commercially, the cost of effluent treatment can be largely offset (Wert and Henderson 1978). Of all the tertiary treatment processes, lagoons, under ideal conditions, provide the most efficient bacteriological removals, except for grass plots. They are particularly useful for protecting receiving waters used as a raw water supply, for water-contact

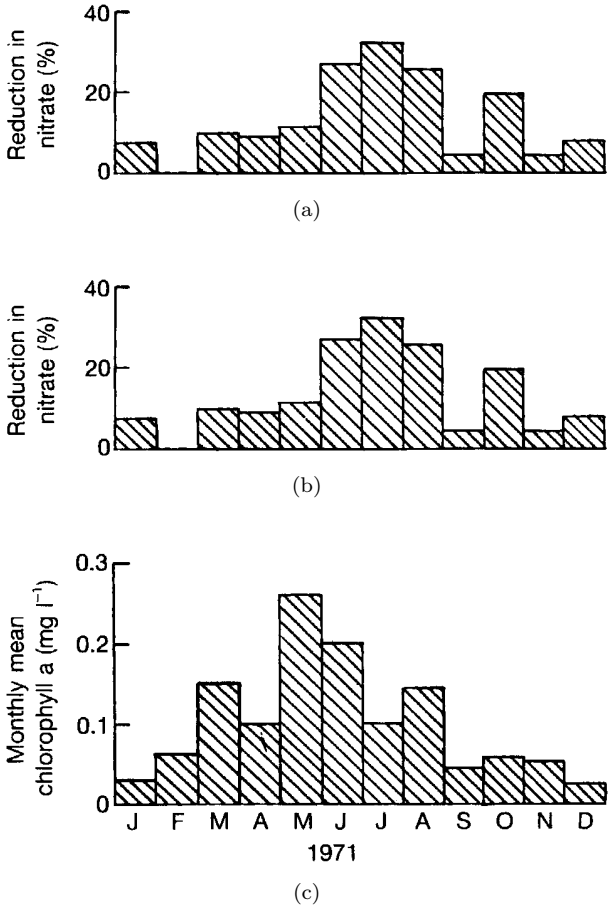


Fig. 2.26. Seasonal cycles in the removal of phosphate and the growth of algae in maturation lagoons after 10 days retention (Institute of Water Pollution Control 1974).

recreation, and for the protection of shell-fisheries. An optimum depth of lagoons is about 1 m in the UK, which will restrict growth of macrophytes, and at a retention time of < 60 h will provide maximum treatment efficiency without giving rise to excessive algal growths. The lagoon is a buffer between the treatment plant and the river, with retention times optimised by using baffles to prevent short-circuiting. Scumboards will also be required to prevent the loss of sludge which may rise from the bottom of the lagoon as anaerobic breakdown progresses. Loading rates depend on the efficiency of the secondary treatment process, but if a 20:30 effluent is produced, then a maximum loading of between 3500–5000 $m^3ha^{-1}d^{-1}$ can

be used. If the effluent quality entering the lagoon is worse than 20:30, then the loading rate must be reduced accordingly down to 1500 $m^3ha^{-1}d^{-1}$. If lagoons are small, it is advisable to have at least two in series rather than one of the same capacity. A greater buffering effect, as well as improved nitrification, is obtained with several well mixed lagoons (Fig. 2.27). The use of maturation ponds is fully examined in Sec. 6.3.2.

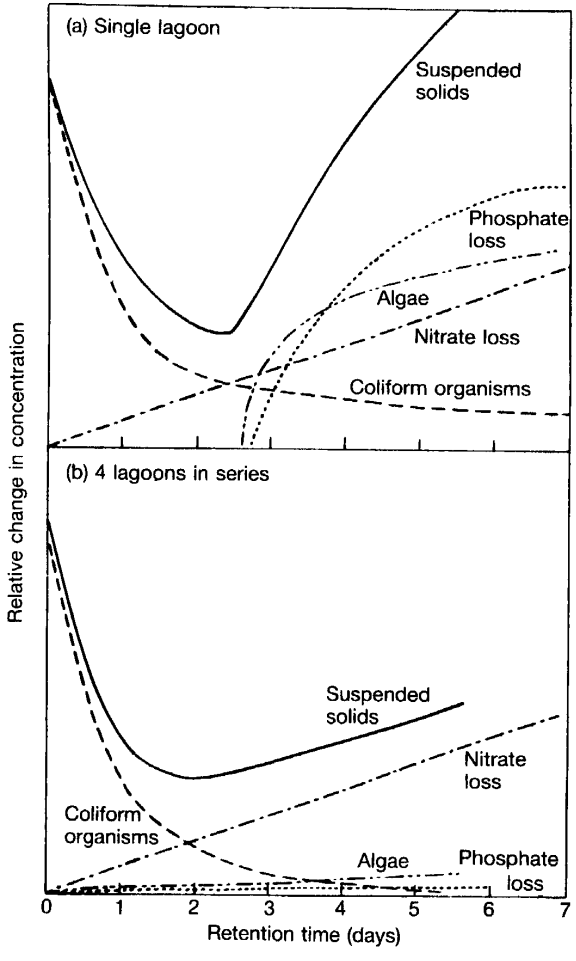


Fig. 2.27. Effect of retention time on performance in (a) a single lagoon 1 m deep and (b) four lagoons in series also 1 m deep when operated in a temperate climate. Phosphate removal and algal growth were measured from April to September (Institute of Water Pollution Control 1974).

Grass-plot irrigation

Land irrigation on grass plots is probably the most economical method of tertiary treatment and is especially suitable for treatment plants serving small communities. Effluent is distributed evenly over grassland via channels or spray guns. The plot is graded to a slope of between 1:60 to 1:100 to ensure that the effluent percolates downhill where it is collected in a channel. Except in very porous soil, sites should be under-drained to prevent water-logging and to encourage growth of grass. The suspended solids are largely retained in the soil and some of the nutrients present are used for plant growth. Maximum removals of 80% suspended solids and up to 70% of BOD_5 are possible, with enterobacteria greatly reduced (Table 2.6). At least two plots are needed, preferably more, as the ground needs to be used intermittently to allow full drainage and subsequent mowing. There is no minimum or maximum size of plots, but a recorded range of 0.1 to 3.0 ha is reported by the Institute of Water Pollution Control (1987a). Loadings to grass plots are expressed in terms of volume of wastewater applied per unit area of land per day. This figure should be based on the total land area available including those plots not currently in use. Loadings range from 2,000–5,000 $m^3ha^{-1}d^{-1}$ depending on climatic conditions (rainfall) and soil structure. If the secondary effluent fails to reach the 20:30 standard, then the loading rate to the grassland must be reduced.

Short grasses are preferable, although the sowing of special grass mixtures does not appear worthwhile. The growth of grass irrigated with wastewater is prolific due to the nutrients present and so the grass plot needs to be mown regularly to a height of about 5 cm to discourage weeds. It is usually best to remove the cuttings from the plot, and there is some indication that they can be safely used as silage. However, the area involved is usually too small to be of interest to farmers. Although most of the suspended solids removed by the plot are degraded in the soil, the plot will require periodic renovation. This is done by allowing the plot to fully drain and dry off, and then harrowing, rotavating or even ploughing the land before regrading and reseeding. As in lagoons, mosquitoes can breed on grass-plots if the humidity is high, allowing a serious fly nuisance to develop, so care must be taken not to overload plots. Regrading is also important, as if the slope becomes too steep, channelling occurs which causes erosion and usually an increase in the suspended solids concentration of the final effluent. Land treatment options are discussed in detail in Sec. 6.1.

Constructed wetlands

There is a wide variety of wetland systems available for tertiary treatment of domestic wastewaters. In the UK, the horizontal flow reed bed is the most widely employed (Fig. 6.15). They consist of shallow excavations lined with an impermeable membrane, filled with gravel and planted with a suitable reed species (e.g. *Phragmites australis*). Stone gabions are used at the inlet and outlet to ensure equal distribution of effluent. Severn and Trent Water (UK) have installed such reed bed systems at many (> 200) of their small treatment plants, and using a bed area of 0.7–1.0 $m^2 pe^{-1}$, they are achieving final effluents < 5 mg BOD l^{-1} and 10 mg TSS l^{-1} (Green and Upton 1995). The design and operation of constructed wetlands is considered in detail in Sec. 6.2.3 and is reviewed by Kadlec *et al.* (2000).

Microstrainers

Suspended solids in secondary wastewater can be efficiently removed by passing it through a fine mesh screen made from stainless steel. Such screens have been widely adopted for tertiary treatment of wastewaters since their introduction in 1948. Because of the small apertures that make up the mesh, the screen rapidly becomes blocked when used with treated effluents, and the screens require almost constant backwashing. This can only be achieved by fixing the mesh around the periphery of a rotating drum which is open at one end. Drums range from 1–3 m in diameter and 0.3–4.5 m in length. The drum is fixed horizontally allowing the wastewater to enter the drum to about two thirds of its depth so that the effluent passes laterally through the sides of the drum under a small hydraulic head (150 mm) out into a collecting chamber, leaving the solids retained inside the screen. As the drum rotates, a small jet of filtered effluent is used to continuously backwash the screen as it passes the highest point of rotation, with the filtered sediment being flushed into a small collecting trough inside the drum, which is then piped away to the inlet of the plant.

Microstrainers are only used for 20:30 effluents, with the loading rate depending on the grading of mesh used. For example, the range of mesh sizes normally used ranges from 90 to 390 apertures per mm^2, with the finest grades only used for potable water treatment, allowing loadings of secondary wastewaters of 300 to 700 $m^3 m^{-2} d^{-1}$. As a rough guide, a mesh aperture of 35 μm would give a 15:15 effluent, whereas a smaller mesh with a 23 μm diameter aperture would give a 10:10 effluent. Therefore, 30–80% removal of suspended solids and a 25–70% reduction in BOD_5 are possible, depending on the wastewater quality and mesh grade used (Table 2.6).

However, there is a very poor removal of enterobacteria by this method compared to either lagooning or grass plot irrigation. Between 2–5% of the filtrate is pumped from the collecting chamber and used for backwashing. Apart from blocking (blinding) due to the suspended solids in the wastewater, the mesh can also become clogged due to bacterial growth which can result in an excessive head loss. Such growths are difficult to remove manually or with chemicals and so are prevented by using a UV lamp. Microstrainers are self-contained and relatively small units that are normally kept under cover in specially constructed buildings. Although the meshes can last for up to 10 years, they require a high degree of maintenance and at least two full-scale units are required. This restricts their use to medium- and large-scale treatment plants, although they are increasingly rarely used (Institute of Water Pollution Control 1987a).

Sand filters

Gravity filtration through beds of sand have been used for many years in potable water treatment. In their simplest form, the filters are downflow units although developments on the original design have been made such as mixed media and upflow units. There are three types of sand filter used for the tertiary treatment of domestic wastewater, the slow sand filter, the rapid downward flow sand filter, and the upward flow sand filter.

Slow sand filters are comprised of a layer of fine sand approximately 450 mm in depth overlying a 200–300 mm layer of pea gravel with a nominal diameter between 20–30 mm. Settled secondary effluent can be filtered at a maximum rate of 2.5–3.5 $m^3m^{-2}d^{-1}$ (0.15 $m^3m^{-2}h^{-1}$). Because of the relatively low loading, slow sand filters require a large area and so often prove too expensive except for small treatment plants, although operating and maintenance costs are low. They are also particularly suitable for small treatment plants as they only require cleaning periodically. This is done by removing the top few millimetres of accumulated sludge and sand, which must be replaced occasionally with clean sand to maintain the depth of the filter. The contaminated sand is then washed and settled ready for reuse and the removed solids returned to the inlet of the plant. The surface layer of sand retains some of the suspended solids and quickly becomes covered with zoogloeal bacteria, which can significantly reduce the BOD_5 of the effluent. However, if the bacterial slime becomes too thick, then the flow through the filter is impeded. Lower down in the filter, nitrifying bacteria develop providing extra nitrification of the final effluent. Up to 60–80% of suspended solids are removed when a 20:30 effluent is passed through such

a filter, with a 30–50% reduction in BOD_5 and coliform bacteria possible (Table 2.6) (BSI 1972).

Rapid downward flow sand filters have a greater hydraulic head than slow sand filters (3–4 m), and when operated under pressure, give a hydraulic loading 40–50 times greater than slow sand filters at 200–250 $m^3m^{-2}d^{-1}$. Loaded at this high rate, the surface layer of sand rapidly binds under the pressure and so backwashing is required every 24–48 hours to clean the sand medium and to reduce compaction of the bed. Most rapid sand filters have an automatic backwashing facility which uses 2–3% of the total throughput for this purpose at a rate of 10 l $m^{-2}s^{-1}$. The wash-water can contain significant quantities of sand and so must be returned to the inlet so that the sand can be removed during grit extraction. The beds comprise of sand with a nominal particle size between 0.8 to 1.7 mm at a depth of 1.5 m. When operated at a hydraulic loading of 200 $m^3m^{-2}d^{-1}$, a rapid downward flow sand filter should remove 65–85% of the suspended solids and 20–35% of the BOD_5 from a 20:30 effluent (Table 2.6). Performance is not significantly affected by hydraulic loading or by the use of finer sand particles. Because of the regular backwashing, little biological activity occurs and so the BOD_5 removal is lower than in slow sand filters and there is little nitrification. Bacterial reductions are also poor and this type of tertiary treatment is not suitable if bacterial quality is important. However, rapid downward flow sand filters are widely used at large plants, although they do require a high level of maintenance and operational control (Sec. 6.1) (Institute of Water Pollution Control 1987a).

Upward flow sand filters achieve similar removal rates of suspended solids and BOD_5 to downward flow sand filters (Table 2.6), but at much higher hydraulic loadings (400 $m^3m^{-2}d^{-1}$) which makes them especially suitable for large treatment plants. The effluent is forced upwards through a bed of graded media under pressure or hydraulic head. The bed is made out of layers of graded media with the coarser particles at the base so that the effluent has to pass through layers of increasingly finer grade media as it progresses upwards. The entire bed of the filter is used to remove suspended solids which is why it is more efficient than the other filter systems, and does not require washing so frequently. A typical arrangement of media would be a 0.15 m layer of 40–50 mm gravel at the base with 0.25 m of 8–12 mm gravel followed by 0.25 m of 2–3 mm coarse sand, and finally a 1.5 m layer of 1–2 mm sand at the top. Such filters are washed by increasing the flow to 900 $m^3m^{-2}d^{-1}$ so that the interstices in the media are opened up and the accumulated solids flushed out in the washwater which is returned to the inlet.

Upward flow gravel bed clarifiers are either separate or more usually incorporated as part of the secondary sedimentation tank. Clarifiers act as a sedimentation tank with a coarse medium upward flow filter at the outlet. They were originally developed for use with small percolating filtration units to obtain better effluents than from just secondary settlement only. Upward flow clarifiers consist of a 150 mm layer of pea gravel (5–20 mm diameter) supported on a perforated metal platform near the top of a horizontal flow sedimentation tank with a surface overflow rate of between 15–25 $m^3m^{-2}d^{-1}$. Suspended solids are not physically strained from the effluent by the medium as the interstices are too large (1000–3000 μm diameter) compared to the solids (< 100 μm). However, the passage through the gravel promotes flocculation of the suspended particles which then settle on top of the gravel. The depth of water above the top of the gravel is therefore critical if flocculated solids are not to be carried over with the final effluent, thus the surface of the medium must be at least 150 mm, preferably 300 mm, below the surface. Other porous materials apart from gravel can also be used. These include plastic mesh and fine metal (wedge wire) that are easier to install and operate. The action of flocculation is the same as for the gravel with solids accumulating either in the interstices or on the surface, possibly dropping down through the tank to form a sludge on the tank floor.

Backwashing is required periodically to remove entrained solids, which is done by lowering the level of the water in the clarifier to below the level of the gravel bed or mesh and hosing it down. The exact frequency of backwashing will depend on the size of the gravel or media used and the suspended solids content of the effluent. No washwater is produced, because all the solids washed from the medium settle to the bottom of the tank. Desludging must be done frequently and at least once a week, and the flocculated solids should be drained off as necessary and returned to the inlet. A 30–50% removal of suspended solids is possible, although this depends largely on the size of the gravel, with up to 35% removal of BOD_5 (Table 2.6). Loading rates of up to 3 DWF are permitted, although the suspended solids from percolating filters flocculate far more readily than those from activated sludge processes. Therefore, higher loadings to clarifiers are permitted from percolating filters (42 $m^3m^{-2}d^{-1}$) than from activated sludge systems (30 $m^3m^{-2}d^{-1}$). The unit is capable of dealing with a wide fluctuation in suspended solids concentrations and hydraulic flows. Only a small hydraulic head is required (25 mm) and clarifiers are much smaller than other tertiary treatment units that are suitable for small treatment plants.

Mann (1979) has compared the tertiary treatment methods most commonly adopted for small treatment plants and has listed their potential advantages and disadvantages (Table 2.7). These methods are illustrated in Fig. 2.28.

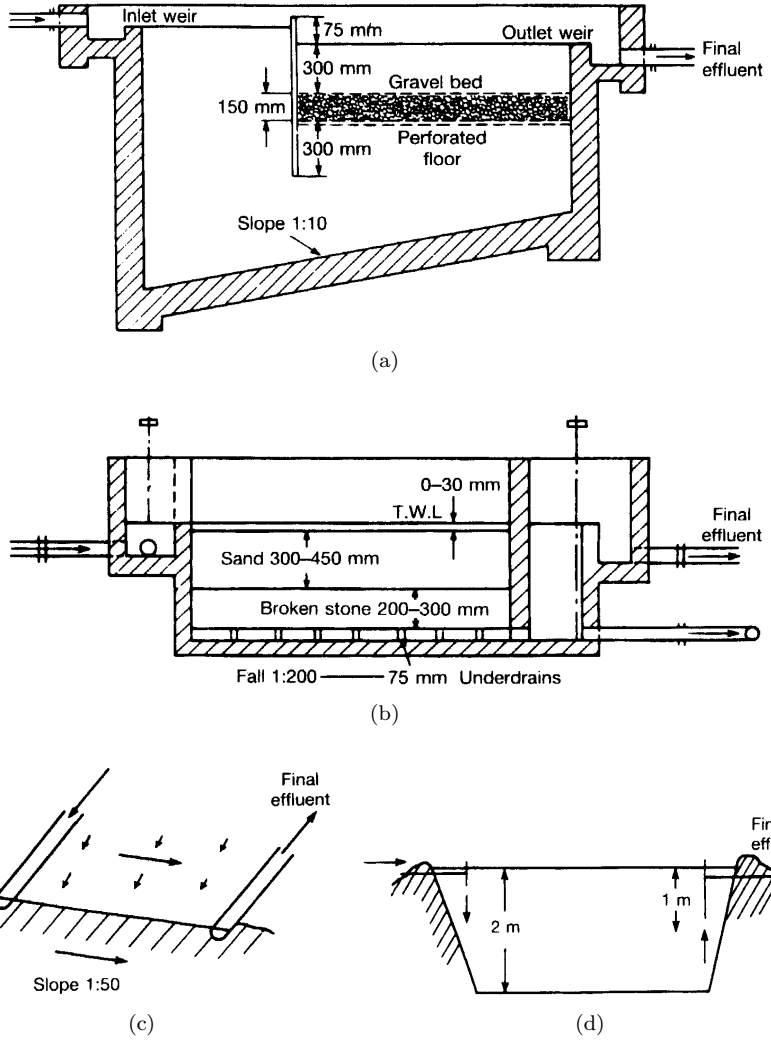


Fig. 2.28. Tertiary treatment methods widely used at small-scale treatment works. (a) Upward flow gravel bed clarifier which is installed in the final sedimentation tank, (b) slow sand filter of which at least two will be required for continuous operation, (c) grass plot or land treatment, (d) maturation or tertiary treatment lagoon (Mann 1979).

Table 2.7. Comparison of the performance and operation of tertiary treatment methods shown in Fig. 2.28 (Mann 1979).

System	Hydraulic loading rate ($m^3 m^{-2} d^{-1}$)	Removal (%) BOD	Removal (%) Suspended solids	Specific advantages	Potential disadvantages
Upward flow gravel clarifier	24	30	50	Can be fitted in the top of the humus tank	Requires regular backwashing; not less than weekly
Sand filter	3.0	40	60	Positive system. Little possibility of short-circuiting	Regular cleaning necessary. Two filters are needed in alternate use. Highest cost system
Grass plots	0.85	50	70	Very low cost, low maintenance, high efficiency	Can be unsightly, if maintenance neglected. Can encourage breeding of flies. A spare plot is needed to permit resting and maintenance
Lagoons	0.5	40	40	Efficiency can be increased by use of lower application rates. Very low maintenance requirements	Lagoons must be watertight. Can be unsightly if overloaded owing to formation of scum. May encourage breeding of insects

2.4.2. *Advanced wastewater treatment*

The term tertiary treatment is often used as a synonym for advanced water treatment (AWT) and while similarities exist, the two are not precisely the same. Whereas tertiary treatment is an additional step applied after secondary treatment to reduce the suspended solids and, to some extent the BOD_5, AWT is any process or system used after conventional treatment, or to modify or replace one or more conventional steps. AWT systems remove refractory and mainly soluble pollutants which are not readily removed by conventional biological treatment. Several of these pollutants can affect aquatic life. For example, unionised ammonia (NH_3) is highly toxic to fish and can cause deoxygenation as it is oxidised; nitrogen and phosphorous promote eutrophication in rivers and lakes respectively; while nitrogen compounds, trace organics and pathogens can hinder the reuse of surface water for supply. The main treatment methods include the removal of ammonia by nitrification; air-stripping or breakpoint chlorination; the removal of inorganic nitrogen by denitrification; phosphate removal by algal synthesis or chemical precipitation using iron or aluminium coagulants and lime; reduction of dissolved organics (residual organic matter) using activated carbon, chemical or ozone treatment; removal of inorganic salts, especially sulphates and chlorides, by ultrafiltration, reverse osmosis or electrodialysis; and finally disinfection of effluents to control pathogens, especially viruses. Some of these techniques are dealt with elsewhere in the text, where appropriate.

Further reading

General: Hammer 1977; Viessman and Hammer 1985; Eilbeck and Mattock 1987; Metcalf and Eddy Inc. 1991.

Tertiary treatment: Institute of Water Pollution Control 1987a.

Advanced wastewater treatment: Culp and Culp 1978; McCarty and Reinhard 1980; Jaap 2001.

3

The Role of Organisms

3.1. Stoichiometry and Kinetics

In biological wastewater treatment, the most widely occurring and abundant group of micro-organisms is the bacteria, and it is this group which is most important in terms of utilising the organic matter present in wastewater. The following section deals with the kinetics of biological treatment systems and concentrates mainly on the bacteria, although the basic principles are applicable to the other groups of micro-organisms.

The organic matter in wastewater is utilised by micro-organisms in a series of enzymatic reactions. Enzymes are proteins, or proteins combined with either an inorganic or low molecular weight organic molecule. They act as catalysts forming complexes with the organic substrate which they convert to a specific product, releasing the original enzyme to repeat the same reaction. The enzymes have a high degree of substrate specificity and a bacterial cell must produce different enzymes for each substrate utilised. Two types of enzymes are produced: *extracellular* enzymes, which convert substrate extracellularly into a form that can be taken into the cell for further breakdown by the *intracellular* enzymes, which are involved in synthesis and energy reactions within the cell. Normally, the product of an enzyme-catalysed reaction immediately combines with another enzyme until the final end-product required by the cell is reached after a sequence of enzyme-substrate reactions.

A portion of the absorbed material in the bacterial cell is oxidised to provide energy while the remainder is used as "building blocks" in cellular synthesis. In the oxidation of organic matter, for example the carbohydrate

glucose, oxygen only becomes involved at the end of a series of meticulously integrated enzyme chemical transformations:

$$C_6H_{12}O_6 \rightarrow 6CO_2 + 6H_2O + 678 \text{ cal energy}$$

The oxidation reaction that has occurred can be described in a number of ways such as, the use of oxygen, the loss of hydrogen from the substrate (normally the food source), which in this case is glucose, or the loss of electrons from the glucose substrate. These reductions are described in terms of hydrogen or electron donors (substrate) and hydrogen or electron acceptors (in this case, oxygen).

The electron donor gives up electrons that are transported via complicated biochemical pathways to the ultimate or terminal electron acceptor, which is oxygen, for an aerobic reaction. Organic electron donors are utilised in heterotrophic metabolism whereas autotrophic metabolism uses inorganic electron donors. It is the terminal electron acceptor that determines the amount of energy which is available from the substrate. The general relationship between energy yields of aerobic and anaerobic metabolism is summarised in Fig. 3.1. Energy stored in organic matter (AH_2) is released in the process of biological oxidation by dehydrogenation of substrate followed by transfer of an electron or electrons to an ultimate acceptor. The higher

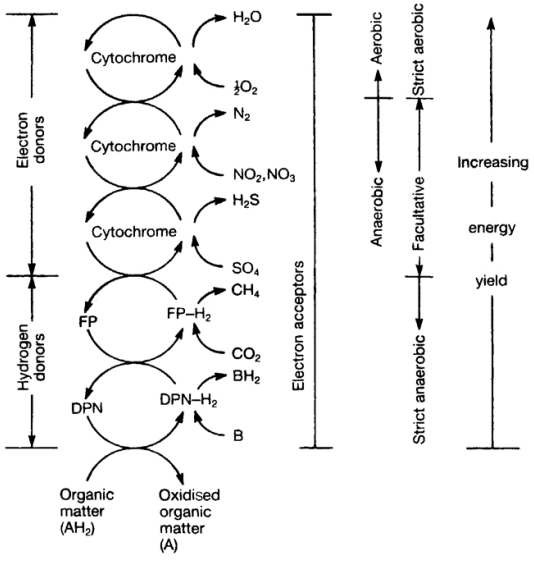


Fig. 3.1. Schematic representation of the dehydrogenation of wastewater, showing the relative energy yields for various ultimate electron acceptors (adapted Clark *et al.* 1977).

Table 3.1. The preferential selection of electron acceptors during microbial oxidation of organic matter.

Aerobic	$AH_2 + O_2 \rightarrow CO_2 + H_2O$ + energy	|
|	$AH_2 + NO_3^- \rightarrow N_2 + H_2O$ + energy	Decreasing
Facultative	$AH_2 + SO_4^{2-} \rightarrow H_2S + H_2O$ + energy	energy
↓	$AH_2 + CO_2 \rightarrow CH_4 + H_2O$ + energy	yield
Anaerobic	$AH_2 + B \rightarrow BH_2 + A$ + energy	↓

the ultimate electron acceptor is on the energy or electromotive scale, the greater the energy yield from the oxidation of 1 mole of substrate. Aerobic metabolism using oxygen as the ultimate electron acceptor yields the greatest amount of energy. Aerobic respiration can be traced in Fig. 3.1 from reduced organic matter (AH_2) at the base through the hydrogen and electron carriers to oxygen. Facultative respiration, using oxygen bound in nitrate or sulphate, yields less energy than aerobic metabolism. The least energy results from strict anaerobic respiration, where the oxidation of AH_2 is coupled with reduction of B (an oxidised organic compound) to BH_2 (a reduced organic compound).

The preferential use of electron acceptors based on energy yields in a mixed bacterial culture is shown in Table 3.1. Electron acceptors are used in the general descending order of dissolved oxygen, nitrate, sulphate, and oxidised organic compounds. Therefore, hydrogen sulphide formation follows nitrate reduction but precedes methane production (Clark *et al.* 1977).

During the oxidative reaction, energy is conserved by the cell in two ways. The micro-organisms function by capturing energy liberated by exergonic (energy-releasing) reactions (i.e. catabolism), using this energy to drive endergonic (energy-requiring) reactions (i.e. anabolism). The energy that is released in the cell by oxidising organic or inorganic matter or by photosynthesis is captured and stored within the cell in the form of a phosphate bond on a adenosine diphosphate molecule (ADP) forming adenosine triphosphate (ATP), which is known as substrate-level phosphorylation. When required, the stored energy can be released to drive other reactions, forming needed metabolites for cell synthesis or mobility by the hydrolysis of the ATP molecule to ADP:

$$ATP + H_2O \rightarrow ADP + H_3PO_4$$

Under standard conditions of concentration (0.1 M), temperature (30°C) and pH (7), 8.4 kcals of energy becomes available to the micro-organisms for biosynthesis for each mole of phosphate released (Fig. 3.2).

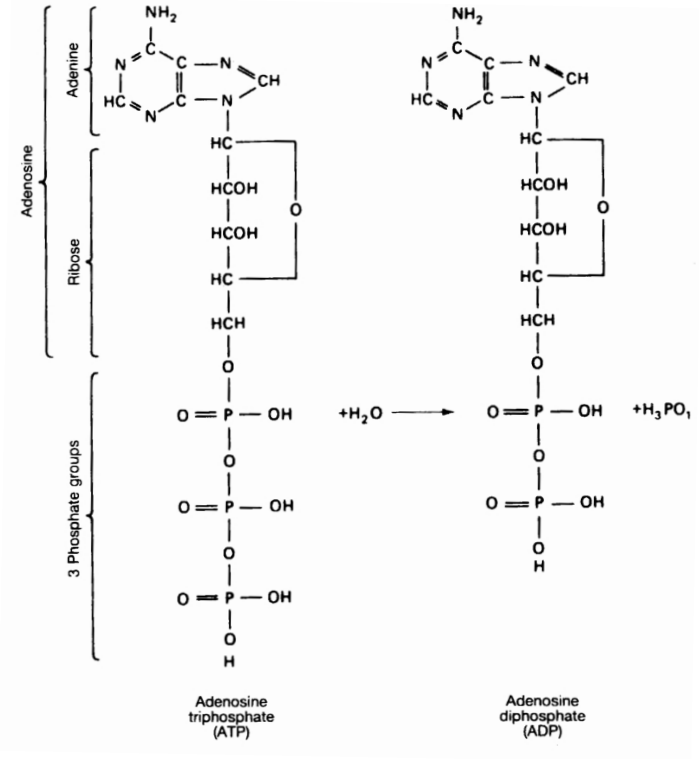


Fig. 3.2. The hydrolysis of adenosine triphosphate (Pelczar and Reid 1972).

The second method of recovering energy is by oxidative phosphorylation. Electrons, normally pairs, are produced by the oxidation of an electron donor (DH_2) and are passed through an electron-transport system to a terminal electron acceptor (A). The electron-transport system is a series of electron carriers arranged so that the large amount of energy produced by the oxidation of the electron donor is released in small packets, which are used to drive the endergonic phosphorylation reaction of ADP to ATP.

In Fig. 3.1, the oxidation-reduction reactions are carried out exclusively by enzymes. While some enzymes depend solely on their structure as proteins for activity, some require co-factors. Co-factors are either metal ions or complex organic molecules, generally known as co-enzymes, which function as carriers of electrons, specific atoms or functional groups which are transferred during the enzymatic reaction. The co-enzyme component of the enzyme determines what chemical reaction will occur. One of the most important co-enzymes is nicotinamide adenine dinucleotide, known as NAD

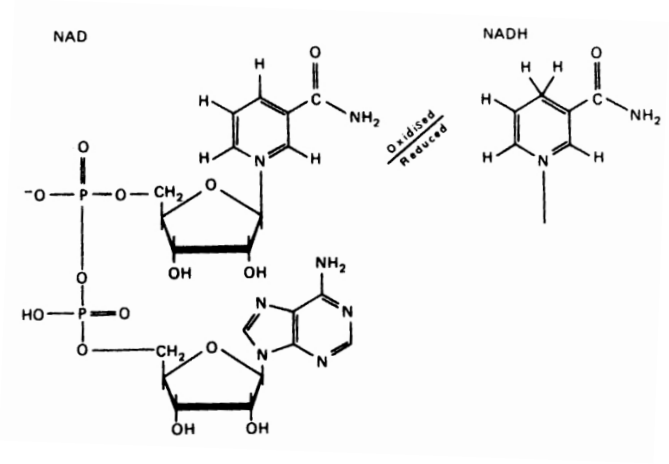


Fig. 3.3. Structure of oxidised and reduced forms of nicotinamide adenine dinucleotide (Brock 1971a).

in its oxidised form and NADH + H in its reduced form (Fig. 3.3). Flavoproteins (FP) which are responsible for hydrogen transfer are also important co-enzymes. Cytochromes are respiratory pigments that can undergo oxidation reduction and serve as hydrogen carriers. The various pathways of energy metabolism of organisms are considered in more detail in the following sections of this chapter.

3.1.1. *Stoichiometry*

It is essential in the design of biological treatment systems to be able to calculate the necessary inputs into the system such as oxygen and nutrients as well as the outputs such as carbon dioxide, sludge (i.e. biomass), and methane. This stoichiometric approach to energy production and cell synthesis has been developed by McCarty (1971). A table of half-reactions (Table 3.2) allows the organic substrate that has been utilised by the bacteria to be divided into two portions; first, that which has been oxidised to produce energy, and secondly that which is used for synthesis of new cellular material. The energy and synthesis reactions add together to become the overall reaction or total metabolism (Christensen and McCarty 1975).

Using Table 3.2, it is possible to calculate the energy yield for the metabolism of all the commonly encountered substrates under aerobic, anoxic or anaerobic conditions. For example, if we assume the composition of domestic sewage to be $C_{10}H_{19}O_3N$, the energy yields for different electron acceptors can easily be calculated by using Eq. 7 for electron donor

Table 3.2. Oxidation half reactions (Christensen and McCarty 1975).

Equation no.	Half-reaction		$\Delta G^\circ(W)^*$ kcal per electron equivalent
	Reactions for bacterial cell synthesis (R_c)		
	Ammonia as nitrogen source:		
1	$1/5CO_2 + 1/20HCO_3^- +$ $1/20NH_4^+ + H^+ + e^-$	$= 1/20C_5H_7O_2N + 9/20H_2O$	
	Nitrate as nitrogen source:		
2	$1/28NO_3^- + 5/28CO_2 +$ $29/28H^+ + e^-$	$= 1/28C_5H_7O_2N + 11/28H_2O$	
	Reactions for electron acceptors (R_a)		
	Oxygen		
3	$1/4O_2 + H^+ + e^-$	$= 1/2H_2O$	−18.675
	Nitrate:		
4	$1/5NO_3^- + 6/5H^+ + e^-$	$= 1/10N_2 + 3/5H_2O$	−17.128
	Reactions for bacterial cell synthesis (R_c)		
	Sulphate:		
5	$1/8SO_4^{2-} + 19/16H^+ + e^-$	$= 1/16H_2S + 1/16HS^- + 1/2H_2O$	5.085
	Carbon dioxide (methane fermentation):		
6	$1/8CO_2 + H^+ + e^-$	$= 1/8CH_4 + 1/4H_2O$	5.763

Table 3.2. (*Continued*)

Equation no.	Half-reaction		$\Delta G°(W)^*$ kcal per electron equivalent
	Reactions for electron donors (R_d)		
	Organic donors (*heterotrophic reactions*)		
	Domestic wastewater:		
7	$9/50CO_2 + 1/50NH_4^+ +$ $1/50HCO_3^- + H^+ + c^-$	$= 1/50C_{10}H_{19}O_3N + 9/25H_2O$	7.6
	Protein (amino acids, proteins, nitrogenous organics):		
8	$8/33CO_2 + 2/33NH_4^+ +$ $31/33H^+ + e^-$	$= 1/66C_{16}H_{24}C_5N_4 + 27/66H_2O$	7.7
	Carbohydrates (cellulose, starch, sugars):		
9	$1/4CO_2 + H^+ + e^-$	$= 1/4CH_2O + 1/4H_2O$	10.0
	Grease (fats and oils):		
10	$4/23CO_2 + H^+ + e^-$	$= 1/46C_8H_{16}O + 15/46H_2O$	6.6
	Acetate:		
11	$1/8CO_2 + 1/8HCO_3^- + H^+ + e^-$	$= 1/8CH_3COO^- + 3/8H_2O$	6.609
	Propionate:		
12	$1/7CO_2 + 1/14HCO_3^- + H^+ + e^-$	$= 1/14CH_3CH_2COO^- + 5/14H_2O$	6.664
	Benzoate:		
13	$1/5CO_2 + 1/30HCO_3^- + H^+ + e^-$	$= 1/30C_6H_5COO^- + 13/20H_2O$	6.892

Table 3.2. (*Continued*)

Equation no.	Half-reaction		$\Delta G^\circ(W)^*$ kcal per electron equivalent
	Ethanol:		
14	$1/6CO_2 + H^+ + e^-$	$= 1/2CH_3CH_2OH + 1/4H_2O$	7.592
	Lactate:		
15	$1/6CO_2 + 1/12HCO_3^- + H^+ + e^-$	$= 1/12CH_3CHOHCOO^- + 1/3H_2O$	7.873
	Pyruvate:		
16	$1/5CO_2 + 1/10HCO_3^- + H^+ + e^-$	$= 1/10CH_3COCCO^- + 2/5H_2O$	8.545
	Methanol:		
17	$1/6CO_2 + H^+ + e^-$	$= 1/6CH_3OH + 1/6H_2O$	8.965
	Inorganic donors (*autotrophic reactions*)		
18	$Fe^{3+} + e^-$	$= Fe^{2+}$	−17.780
19	$1/2NO_3^- + H^+ + e^-$	$= 1/2NO_2^- + 1/2H_2O$	−9.430
20	$1/8NO_3^- + 5/4H^+ + e^-$	$= 1/8NH_4^+ + 3/8H_2O$	−8.245
21	$1/6NO_2^- + 4/3H^+ + e^-$	$= 1/6NH_4^+ + 1/3H_2O$	−7.852
22	$1/6SO_4^{2-} + 4/3H^+ + e^-$	$= 1/6S + 2/3H_2O$	4.657
23	$1/8SO_4^{2-} + 11/16H^+ + e^-$	$= 1/16H_2S + 1/16HS^- + 1/2H_2O$	5.085
24	$1/4SO_4^{2-} + 5/4H^+ + e^-$	$= 1/8S_2O_3^{2-} + 5/8H_2O$	5.091
25	$H^+ + e^-$	$= 1/2H_2$	9.670
26	$1/2SO_4^{2-} + H^+ + e^-$	$= 1/2SO_3^{2-} + 1/2H_2O$	10.595

* Reactants and products at unit activity except $(H^+) = 10^{-7}$.

and Eq. 3 for aerobic conditions, Eq. 4 for anoxic conditions or Eq. 6 for anaerobic conditions.

Thus, the energy yield from the metabolism of domestic sewage under aerobic conditions is calculated by writing the half reactions:

Donor:

$$1/50C_{10}H_{19}O_3N + 9/50H_2O = 9/50CO_2 + 1/50NH_4^+ + 1/50HCO_3^- + H^+ + e^-$$

$$\Delta G^\circ = +7.6 \text{ kcal/e}^-$$

Acceptor:

$$1/4O_2 + H^+ + e^- = 1/2H_2O$$

$$\Delta G^\circ = -18.675 \text{ kcal/e}^-$$

Adding the half reactions yields:

$$1/50C_{10}H_{19}O_3N + 9/25H_2O + 1/4O_2 + H^+ + e^- = 9/50CO_2 + 1/50NH_4^+ + 1/50HCO_3^- + H^+ + e^- + 1/2H_2O$$

If the above equation is balanced by multiplying by 50, then:

$$C_{10}H_{19}O_3N + 18H_2O + 12.5O_2 = 9CO_2 + HN_4^+ + HCO_3^-$$

The energy yield by the reaction is:

$$\Delta G^\circ = \Delta G^\circ \text{ of products} - \Delta G^\circ \text{ of reactions}$$

As each mole of wastewater utilised result in the transfer of 50 electrons (Table 3.2). The energy resulting from the aerobic metabolism of wastewater is:

$$\Delta G^\circ = \frac{50 \text{ electrons}}{\text{mole wastewater}} \times [-18.675 \text{ kcal/e}^- - 7.6 \text{ kcal/e}^-]$$

$$= -1313.75 \text{ kcal per mole wastewater}$$

In a similar way, the energy yield from wastewater under anoxic conditions using NO_3^- as the electron acceptor or anaerobic conditions using CO_2 as the electron acceptor can be calculated as –1236.4 and –91.85 kcal per mole of wastewater respectively (Table 3.2). This demonstrates that the energy yields for specific substrate are essentially the same under aerobic and anoxic conditions but significantly less from anaerobic metabolism.

Synthesis is the biochemical process of substrate utilisation to form new protoplasm for growth and reproduction. The new cell material formed is a complex of organic compounds including proteins, carbohydrates, and lipids. Therefore, protoplasm is mainly composed of carbon, hydrogen, and oxygen, although on a dry weight basis it also contains 10–20% nitrogen, about 2.5% phosphorus, together with other essential elements present in trace amounts. The general formula used to characterise bacterial cells is $C_5H_7O_2N$, which is used in all stoichiometric calculations. The primary product of metabolism is energy and the major use of energy is for synthesis, and as energy release and synthesis are coupled then the maximum rate of synthesis occurs simultaneously with maximum rate of energy yield or metabolism. In wastewater treatment terms, the maximum rate of oxidation of organic matter for a given population of heterotrophic micro-organisms, occurs during maximum biological growth and the lowest oxidation rate occurs when growth ceases.

Calculating the proportion of substrate utilised as either energy or synthesis can be useful to the wastewater engineer, as both the amount of electron acceptor (oxygen) required and the end-products produced can be calculated by knowing what portion of the substrate is synthesised into new cellular material.

As part of the substrate goes into energy formation and the remainder goes to cell synthesis, all the reacting material can be expressed as:

$$f_e + f_s = 1$$

where f_e is the fraction of electron donor used for energy and f_s the fraction of electron donor used for synthesis. McCarty (1975) calculates the amount of substrate metabolised by bacteria to form energy and new cells by using a balanced half equation. The overall reaction is constructed from three half reactions (Table 3.2), one for the synthesis of bacterial cells, which are assumed to be $C_5H_7O_2N$ (R_c), one for the electron acceptor (R_e) and one for the electron donor (R_d), which combine to give the relationship where R is the overall reaction:

$$R = f_s R_c + f_e R_e - R_d$$

The ratio of f_e to f_s depends on the age of the cell culture as well as the substrate electron donor. An increase in the age of the cells in the system will reduce the net amount of substrate material converted to new cell mass. The age of cells is commonly referred to as sludge age or mean cell residence time (θ_c) and is defined as the average time in days an organism

Table 3.3. Maximum cell yield $(f_s)_{max}$ (i.e. the maximum fraction of the electron donor used for synthesis) for various electron donors and electron acceptors.

Electron donor	Electron acceptor	$(f_s)_{max}$
Heterotrophic reactions		
Carbohydrate	O_2	0.72
Carbohydrate	NO_3	0.60
Carbohydrate	SO_4	0.30
Carbohydrate	CO_2	0.28
Protein	O_2	0.64
Protein	CO_2	0.08
Fatty acid	O_2	0.59
Fatty acid	SO_4	0.06
Fatty acid	CO_2	0.05
Glucose	O_2	0.79
Lactose	O_2	0.74
Sucrose	O_2	0.75
Glycine	O_2	0.52
Alanine	O_2	0.52
Propionate	O_2	0.58
Acetate	O_2	0.58
Methanol	NO_3	0.36
Methanol	CO_2	0.15
Propionate	CO_2	0.07
Acetate	CO_2	0.06
Glucose	CO_2	0.27
Sewage sludge	CO_2	0.11
Autotrophic reactions		
S	O_2	0.22
S_2O_3	O_2	0.11
S_2O_3	NO_3	0.20
NH_4	O_2	0.10
H_2	O_2	0.24
H_2	CO_2	0.04
Fe	O_2	0.07

remains within the treatment system. Maximum values of f_s are given in Table 3.3 for various substrates. However, these values are for young, rapidly growing cultures and represent a maximum value of f_s. In older cultures the f_s value is lower and in oxidation ditches, for example, that have very

long cell residence times f_s can be as low as 20% of $(f_s)_{max}$. It is convenient to estimate the fraction f_s by using the relationship:

$$f_s = (f_s)_{max} 1 - [0.8b\theta_c/(1 + b\theta_c)]$$

where θ_c is the sludge age in days and the coefficient b represents the rate of cell death and decay which is assumed to be 0.03.

As an example, the oxygen required by the aerobic biological treatment of domestic sewage can be calculated by first estimating $(f_s)_{max}$. No value of $(f_s)_{max}$ is given for wastewater in Table 3.3, therefore it must be calculated from basic constituents. Domestic sewage consists of approximately 50% protein, 40% carbohydrate, and 10% fat (Sec. 1.2). Thus, by reference to Table 3.3, $(f_s)_{max}$ is calculated as:

$$\begin{aligned}(f_s)_{max,\,sewage} &= (\%\ \text{protein} \times (f_s)_{max\ \text{protein}}) \\ &\quad + (\%\ \text{carbohydrate} \times (f_s)_{max\ \text{carbohydrate}}) \\ &\quad + (\%\ \text{fat} \times (f_s)_{max,\,\text{fat}}) \\ (f_s)_{max,\,sewage} &= (0.50 \times 0.64) + (0.40 \times 0.72) + (0.10 \times 0.59) \\ (f_s)_{max,\,sewage} &= 0.67\end{aligned}$$

If the value of f_s is 20% of $(f_s)_{max}$ for an oxidation ditch in which the cells are undergoing endogenous respiration, with a long mean cell residence time, then the value of f_s is 0.13, under these conditions.

The approximate half-reactions are selected from Table 3.2.

$$\begin{aligned}R_c &: 1/5CO_2 + 1/20HCO_3^- + 1/20NH_4^+ + H^+ + e^- \\ &= 1/20C_5H_7O_2N + 9/20H_2O \\ R_e &: 1/4O_2 + H^+ + e^- = 1/2H_2O \\ R_d &: 9/50CO_2 + 1.50NH_4^+ + 1/50HCO_3^- + H^+ + e^- \\ &= 1/50C_{10}H_{19}O_3N + 9/25H_2O\end{aligned}$$

The overall reaction is constructed using $f_s = 0.13$. In accordance with $R = f_s R_c + f_e R_e - R_d$, the donor reaction (R_d) is not changed, the cell reaction (R_e) is multiplied through by $f_e = 1 - f_s = 0.87$ to obtain the following three equations:

$$\begin{aligned}R_c \times f_s &: 0.026CO_2 + 0.0065HCO_3^- + 0.0065NH_4^+ + 0.130H^+ + 0.130e^- \\ &= 0.0065C_5H_7O_2N + 0.059H_2O\end{aligned}$$

$$R_e \times f_e: 0.218O_2 + 0.870H^+ + 0.870e^- = 0.435H_2O$$

$$R_d: 0.18CO_2 + 0.02HN_4^+ + 0.02HCO_3^- + H^+ + e^-$$
$$= 0.02C_{10}H_{19}O_3N + 0.36H_2O$$

When added, the overall reaction R is:

$$0.02C_{10}H_{19}O_3N + 0.218O_2 = 0.0065C_5H_7O_2N + 0.0135HCO_3^-$$
$$+ 0.0135NH_4^+ + 0.154CO_2 + 0.134H_2O$$

Multiplying by $1/0.02 = 50$

$$C_{10}H_{19}O_3N + 10.9O_2 = 0.325C_5H_7O_2N + 0.675HCO_3^- + 0.675NH_4^+$$
$$+ 7.70CO_2 + 6.7H_2O$$

Thus, in the oxidation ditch system 1 mol of domestic sewage ($C_{10}H_{19}O_3N$) will yield 0.325 mol of micro-organisms ($C_5H_7O_2N$) and will require 10.90 moles of oxygen. It is also possible to express these values in terms of mg oxygen required per mg COD or mg volatile suspended solids per mg COD (Mandt and Bell 1982).

Another example of energy and synthesis calculations is given in Sec. 3.4.3, where the methane production of sewage sludge by heterotrophic fermentation is estimated.

In anaerobic decomposition, the low energy yield per unit of substrate due to a lack of electron acceptors is a limiting factor, which results in the incomplete breakdown of the substrate. Metabolism and synthesis cease when the supply of biologically available energy is exhausted. With aerobic metabolism, the biologically available carbon is the limiting factor, with no shortage of electron acceptors because of the abundance of oxygen.

However, the supply of substrate carbon is rapidly exhausted because of respiration of carbon dioxide and synthesis (Clark *et al.* 1977). The energy conversion efficiencies of aerobic and anaerobic metabolism are shown in Fig. 3.4. Incomplete metabolism, a small amount of biological growth, and the production of high-energy products, such as acetic acid and methane, characterises the anaerobic reaction. In contrast, the complete metabolism and synthesis of substrate, ending up with large amounts of biological growth, is characteristic of aerobic metabolism. The end-products of these metabolisms are compared in Table 1.28.

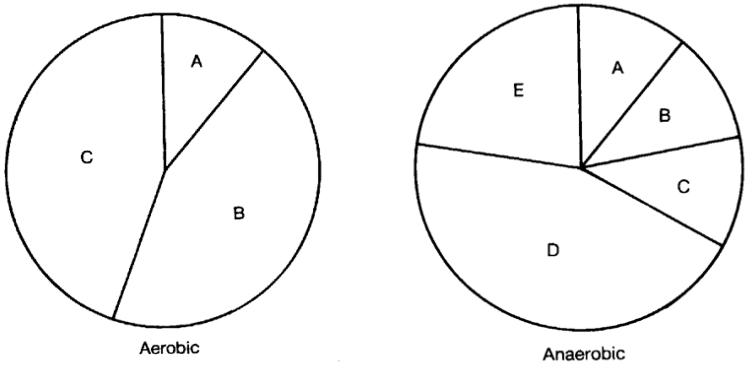


Fig. 3.4. Proportion of the total energy in wastewater utilised under aerobic and anaerobic metabolism as: (A) lost heat energy; (B) respiration; (C) synthesis; (D) energy bound in end-products; and (E) unused energy due to a lack of hydrogen acceptors.

3.1.2. *Bacterial kinetics*

In terms of substrate removal, the rate of carbonaceous oxidation, nitrification and denitrification depends on the rate of microbial growth, and in particular the rate of bacterial growth. This is best illustrated by observing the development of a microbial population in batch culture. When a small inoculum of viable bacterial cells are placed in a closed vessel with excess food and ideal environmental conditions, unrestricted growth occurs. Monod (1949) plotted the resultant microbial growth curve from which six discrete phases of bacterial development can be defined (Fig. 3.5). The microbial concentration is usually expressed as either the number of cells per unit volume, or the mass of cells per unit volume of reactor. However, it is not possible to directly translate one set of units from the other as the size of the cells and the creation of storage products by cells dramatically affects the mass but not the number of cells (Fig. 3.6).

The lag phase represents the acclimatisation of the organisms to the substrate with the bacterial cells having long generation times and zero growth rates. Nutrients are taken into the cells and both the size and the mass of bacteria increase as the amount of enzymes and nucleic acid increases. However, depending on the size and degree of adaptation of the inoculum to its new environment, the lag phase may be very short or even absent.

The bacterial inoculum normally comprises of cells in the stationary growth phase. However, if log-phase cells are used instead, then the lag phase can be shortened even further. Cells only begin to divide when a

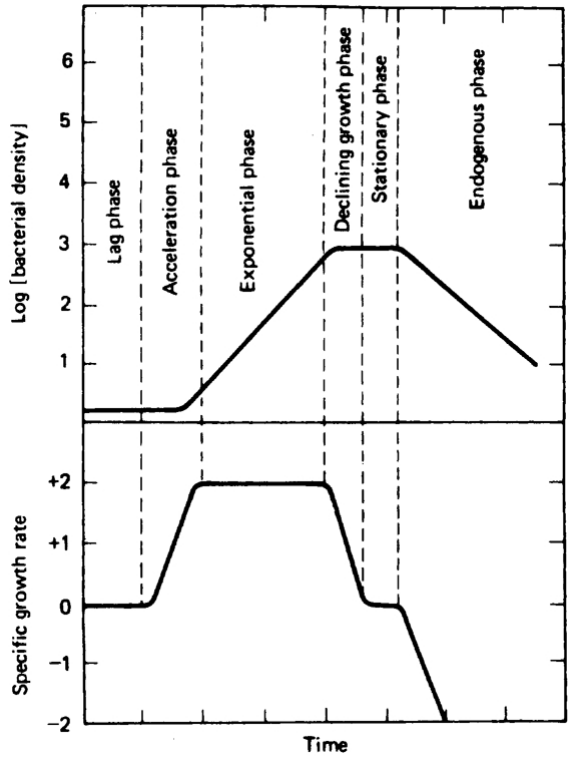


Fig. 3.5. The microbial growth curve showing bacterial density and specific growth rate at various microbial growth phases (Benefield and Randall 1980).

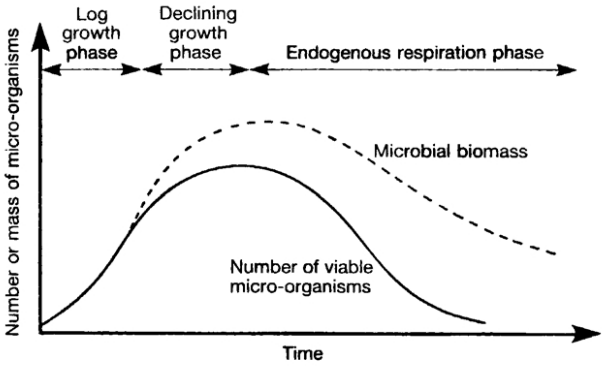


Fig. 3.6. Microbial growth curves comparing total biomass and viable biomass (Clark *et al.* 1977).

sufficient concentration of the appropriate enzymes have built up, but once division has started the population density of bacteria rapidly increases. In the acceleration phase, the generation time decreases and there is a discernable increase in the growth rate leading to the exponential or log phase. In this phase, the generation time is minimal, but constant, with a maximum and constant specific growth rate resulting in a rapid increase in the number and mass of micro-organisms. This is the period when the substrate conversion is at its maximum rate. The steady-state condition of growth is indicated by a near constant ratio of DNA/cell, RNA/cell, and protein/cell as well as constant cell density and minimum cell size. The rate of metabolism and, in particular, the growth rate is limited only by the microbial generation and its ability to process substrate. The exponential phase continues until the substrate becomes limiting. This produces the declining growth phase where the rate of microbial growth rapidly declines as the generation time increases and the specific growth rate decreases as the substrate concentration is gradually diminished. It is at this stage that the total mass of the microbial protoplasm begins to exceed the mass of viable cells as many of the micro-organisms have ceased reproducing due to substrate-limiting conditions (Fig. 3.6). In batch situations the accumulation of toxic metabolites or changes in the concentration of nutrients, or other environmental factors such as oxygen or pH can also be responsible for the onset of the declining growth phase. The microbial growth curve flattens out as the maximum microbial density is reached with the rate of reproduction apparently balanced by the death rate. This is the stationary phase where the substrate and nutrients are exhausted and there is a high concentration of toxic metabolites. It has been suggested that the majority of cells remain viable during this phase but in a state of suspended animation, without the necessary substrate or environmental conditions to continue to reproduce (Williams 1975). The final phase is the endogenous or log death phase. The substrate is now completely exhausted and the toxic metabolites have become unfavourable for cell survival. The microbial density decrease rapidly with a high micro-organism death rate resulting in the rate of metabolism and hence the rate of substrate removal also declining. The total mass of microbial protoplasm decreases as cells utilise their own protoplasm as an energy source (endogenous respiration) and as cells die and lyse they release nutrients back into solution, although there is a continued decrease in both the number and mass of micro-organisms.

The microbial growth curve is not a basic property of bacterial cells but is a response to their environmental conditions within a closed system. Because biological treatment processes are continuous flow systems

it is not directly applicable, although it is possible to maintain such systems at a particular growth phase by controlling the ratio of substrate to microbial biomass, commonly referred to as the food to micro-organism ratio (f/m). The f/m ratio maintained in the aeration tank in an activated sludge system controls the rate of biological oxidation as well as the volume of microbial biomass produced by maintaining microbial growth either in the log, declining or endogenous growth phase. The type of activated sludge process can be defined by the f/m ratio as being high-rate, conventional or extended (Fig. 5.11). For example, at a high f/m ratio micro-organisms are in the log-growth phase which is characterised by excess substrate and maximum rate metabolism. Whereas at low f/m ratios the overall metabolic action in the aeration tank is endogenous, with the substrate limiting microbial growth so that cell lysis and resynthesis occurs. The effect of the f/m ratio on the microbial dynamics of wastewater treatment systems are considered fully in Sec. 5.2.1.

Rates of reaction

The rate at which components of wastewater, such as organic matter, are removed and the rate at which biomass is produced within a reactor are important criteria in the design and calculation of the size of biological reactors. The most useful method of describing such chemical reactions within a biological reactor is by classifying the reaction on a kinetic basis by reaction order. Reaction orders can differ when there is variation in the micro-organisms, the substrate or environmental conditions and they must be measured experimentally.

The relationship between rate of reaction, concentration of reactant and reaction order (n) is given by the expression:

$$\text{Rate} = (\text{concentration})^{\text{n}}$$

or, more commonly, by taking the log of both sides of the equation:

$$\text{Log rate} = n \log (\text{concentration})$$

This equation is then used to establish the reaction order and rate of reaction. Thus, if the log of the instantaneous rate of change of the reactant concentration at any time is plotted as a function of the log of the reactant concentration at that instant, then a straight line will result for constant order reactions and the slope of the line will be the order of the reaction (Fig. 3.7). The rate of reaction is independent of the reactant concentration in zero-order reactions, which results in a horizontal line when plotted. The

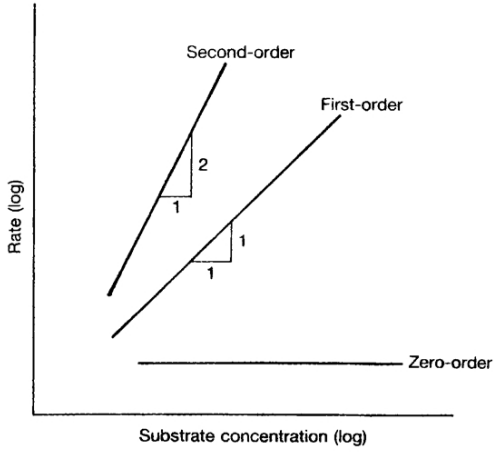


Fig. 3.7. Log plots of reaction rates.

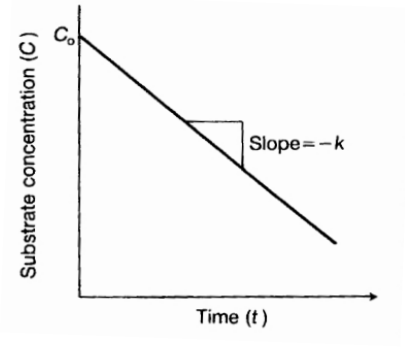


Fig. 3.8. Arithmetical plot of a zero-order reaction.

rate of reaction in first-order reactions is directly proportional to the reactant concentration, and with second-order reactions the rate is proportional to the concentration squared.

In practice, it is simplest to plot the concentration of reactant remaining against time in order to calculate the reaction rate. Zero-order reactions are linear when the plot is made on arithmetic paper (Fig. 3.8), following the equation:

$$C - C_0 = -kt$$

where C is the concentration of reactant (mg l^{-1}) at time t, C_0 the constant of integration (mg l^{-1}), which is calculated as $C = C_0$ at $t = 0$, and k the

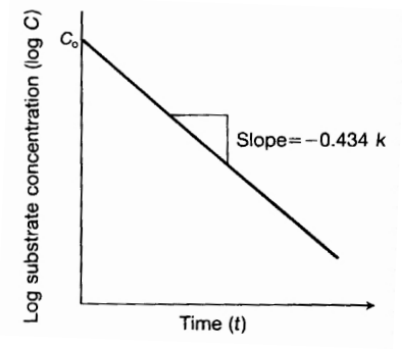


Fig. 3.9. Semi-log plot of a first-order reaction.

reaction rate constant (mg l^{-1} d^{-1}). As first-order reactions proceed at a rate directly proportional to the concentration of one reactant, the rate of reaction depends on the concentration remaining, which is decreasing with time. The plot of variation in concentration with time on arithmetic paper does not give a linear response but a curve. However, first-order reactions follow the equation:

$$\text{Log}\frac{[C_0]}{[C]} = \frac{kt}{2.3}$$

so that a plot of log C (the log of the concentration of the reactant remaining) against time will give a linear trace (Fig. 3.9). Second-order reactions proceed at a rate proportional to the second power of the concentration of a single reactant and obey the function:

$$\frac{1}{C} - \frac{1}{C_0} = kt$$

with the arithmetic plot of the reciprocal of the reactant concentration remaining ($1/C$) against time giving a linear trace, the slope of which gives the value of k (Fig. 3.10).

Thus, for any set of values C and t, such as the rate of removal of organic matter as measured by the BOD test at regular intervals, the reaction rate equations can be tested by making the appropriate concentration versus time plots and noting any deviation from linearity. It should be noted that although fractional reaction orders are possible it is normal for an integer value for reaction order to be assumed. A detailed and excellent account of reaction rate kinetics is given by Benefield and Randall (1980).

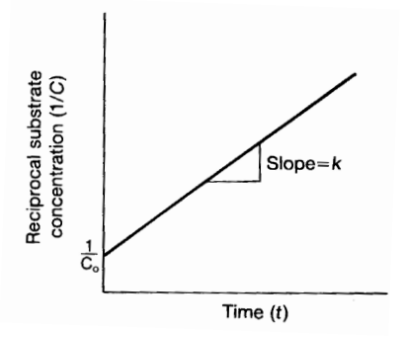


Fig. 3.10. Arithmetical plot of a second-order reaction.

Enzyme reactions

The overall rate of biological reaction within a reactor is dependent on the catalytic activity of the enzymes in the prominent reaction. If it is assumed that enzyme-catalysed reactions involve the reversible combination of an enzyme (E) and substrate (S) in the form of a complex (ES) with the irreversible decomposition of the complex to a product (P) and the free enzyme (E), then the overall reaction can be expressed as:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

where k_1, k_2, and k_3 represent the rate of the reactions. Under steady-state conditions the various rate constants can be expressed as:

$$\frac{(k_2 + k_3)}{k_1} = k_\mathrm{m}$$

where k_m is the saturation or Michaelis constant. The Michaelis-Menten equation allows the reaction rate of enzyme-catalysed reactions to be calculated:

$$r = \frac{R_\mathrm{max} - [S]}{k_\mathrm{m} + [S]}$$

where r is the reaction rate, R_max the maximum rate at which the product is formed (mg $\mathrm{l}^{-1}\mathrm{d}^{-1}$), and S the substrate concentration (mg l^{-1}). If this equation is plotted graphically (Fig. 3.11) it can be seen that the rate of an enzyme-catalysed reaction is proportional to the substrate concentration at low substrate concentrations (first-order). However, as the substrate concentration increases, the rate of reaction declines finally becoming constant and independent of the substrate concentration (zero-order). In practical

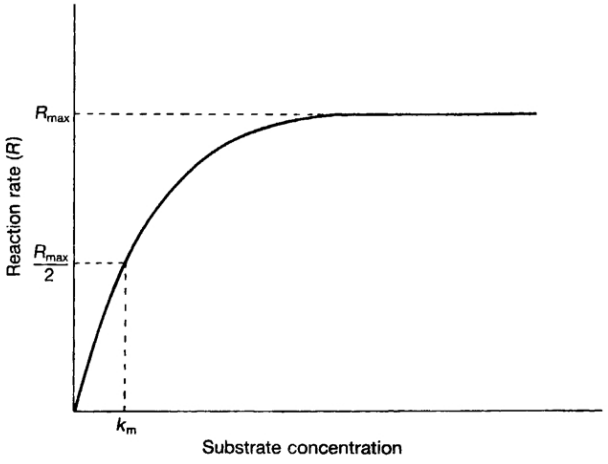


Fig. 3.11. The rate of enzyme-catalysed reactions as presented by the Michaelis-Menton equation.

terms, this is seen when a batch reactor is started and no further substrate is added.

Initially, the rate of reaction is only restricted by the ability of the enzymes to utilise the substrate which is in excess thus the reaction kinetics are zero-order. However, as the substrate is utilised the reaction begins to become substrate-limited resulting in fractional-order reactions until the substrate concentration is so low that the rate of reaction becomes totally limited by the substrate concentration and thus first-order kinetics result. First-order kinetics are assumed when $[S] \leq k_{\mathrm{m}}$. From Fig. 3.11 it can be seen that the saturation constant k_{m} is equal to the substrate concentration when the reaction rate is equal to $(R_{\max}/2)$.

Most biological wastewater treatment systems are designed to operate with high micro-organism concentrations which results in substrate-limiting conditions. Therefore, the majority of wastewater treatment processes can be described by first-order kinetics.

Environmental factors affecting growth

A number of environmental factors affect the activity of wastewater microbial populations and the rate of biochemical reactions generally. Of particular importance are temperature, pH, dissolved oxygen, nutrient concentration, and inhibition by toxic compounds. It is possible to control all these factors within a biological treatment system, except for

temperature, in order to ensure that microbial growth continues under optimum conditions. The majority of biological treatment systems operate in the mesophilic temperature range, growing best in the temperature range 20–40°C. Aeration tanks and percolating filters operate at the temperature of the wastewater, 12–25°C, although in percolating filters the air temperature and the rate of ventilation can have a profound effect on heat loss. The higher temperatures result in increased biological activity that in turn increases the rate of substrate removal. The increased metabolism at the higher temperatures can also lead to problems of oxygen limitations. Generally, activated sludge systems perform better than percolating filters below 5–10°C although heterotrophic growth continues at these temperatures. However, the practice in colder climates of covering filters and controlling the rate of ventilation, thus reducing heat loss within the filter bed, has largely overcome this problem. Van't Hoff's rule states that the rate of biological activity doubles with every 10°C rise in temperature within the range 5–35°C. The variation in reaction rate with temperature is represented by the modified Arrhenius expression:

$$k_{\mathrm{T}} = k_{20}\ \theta^{\mathrm{T}-20}$$

where T is the temperature (°C), k the reaction rate constant at temperature T (d^{-1}), k_{20} the reaction rate constant at 20°C (d^{-1}), and θ the temperature coefficient. There is a rapid decrease in growth rate as the temperature increases above 35°C which falls to zero as the temperature approaches 45°C (Benefield and Randall 1980; Barnes *et al.* 1983). Anaerobic digestion tanks are normally heated as near to the optimum temperature of the mesophilic range as possible (35–37°C). The pH and dissolved oxygen concentration in a biological reactor can be controlled by the operator. The optimum pH range for carbonaceous oxidation lies between 6.5–8.5. At $\mathrm{pH} > 9.0$ microbial activity is inhibited whereas at $\mathrm{pH} < 6.5$ fungi dominate over the bacteria in the competition for the substrate. Fluctuations in the influent pH are minimised by completely mixed aeration reactors that offer maximum buffering capacity. If the buffering capacity is not sufficient to maintain a pH within the acceptable range, then pH adjustment will be required. Anaerobic bacterium have a smaller pH tolerance ranging from pH 6.7–7.4 with optimum growth at pH 7.0–7.1. A dissolved oxygen concentration between 1–2 $\mathrm{mg\,l^{-1}}$ is sufficient for active aerobic heterotrophic microbial activity, although optimum growth is dependent on sufficient essential nutrients and trace elements being present. Apart from organic carbon, nitrogen, and phosphorus,

true elements such as sulphur, iron, calcium, magnesium, potassium, manganese, copper, zinc, and molybdenum must also be available for bacterial metabolism. Normally, all these nutrients and elements are present in excess in sewage although they may have to be supplemented in industrial wastewaters where nitrogen and phosphorus are usually only present in low concentrations. It is desirable to maintain a BOD_5:N:P ratio of 100:5:1 in order to ensure maximum microbial growth (Sec. 1.2). Toxic compounds such as phenol, cyanide, ammonia, sulphide, heavy metals, and trace organics can totally inhibit the microbial activity of a treatment plant if the concentration exceeds threshold limits for the micro-organisms. However, constant exposure to low concentrations of these substances results in the microbial community becoming acclimatised and increasing the concentration to which they can tolerate. Completely mixed aeration tanks are able to dilute shock toxic loads reducing the influent concentration to the final effluent concentration, whereas in filters the contact time between the micro-organisms and the toxic material is relatively short. The effect of environmental factors on both aerobic and anaerobic heterotrophic as well as autotrophic micro-organisms is discussed more fully in Secs. 3.2 and 3.3.

Kinetic equations of bacterial growth

The various growth phases on the microbial growth curve (Fig. 3.5) can be represented quantitatively. The common autocatalytic equation is used to describe microbial growth during the exponential growth phase:

$$\frac{dX}{dt} = \mu X$$

where X is the concentration of micro-organisms (mg l^{-1}), μ the specific growth rate (d^{-1}), and t the time in days. The integrated form of this equation when plotted on semi-log arithmetic graph paper results in a straight line, hence the term logarithmic growth phase. The equation assumes that all micro-organisms are viable and although this may be true for a test-tube culture, it cannot be the case for a wastewater treatment unit with long retention times. It is, however, assumed that a constant fraction of the organisms within the biological treatment unit will remain viable (Weddle and Jenkins 1971).

Exponential growth will continue so long as there is no change in the composition of the biomass and the environmental conditions remain constant (Pirt 1975). In a batch reactor, a change in environmental conditions will inevitably occur, usually substrate limitation, which will cause

a derivation from exponential growth into the declining growth phase. The most commonly used model, relating microbial growth to substrate utilisation, is that of Monod (1942, 1949, 1950). Monod observed that the growth rate dX/dt was not only a function of organism concentration but also of some limiting substrate or nutrient concentration. He described the relationship between the residual concentration of the growth limiting substrate or nutrient and the specific growth rate of biomass (μ) by the classical function that takes his name:

$$\mu = \mu_{\mathrm{m}} \frac{S}{k_{\mathrm{s}} + S}$$

where μ_{m} is the maximum specific growth rate at saturation concentration of growth limiting substrate (d^{-1}), S the substrate concentration (mg l^{-1}), and k_{s} the saturation constant (mg l^{-1}), which is the concentration of limiting substrate at which the specific growth rate equals one-half of the maximum specific growth rate ($\mu = \mu_{\mathrm{m}}/2$).

From this relationship, it can be seen that specific growth rate can have any value between zero to μ_{m} provided that the substrate can be held at a given constant value. This is the basis for all continuous flow treatment processes in biological wastewater treatment in which microorganisms are continuously cultivated but the overall rate of metabolism is controlled by the substrate concentration. When plotted, the Monod relationship between specific growth rate and the growth limiting substrate concentration (Fig. 3.12) has the same form as the Michaelis-Menton equation, which describes the rate of reaction of an enzyme with the substrate

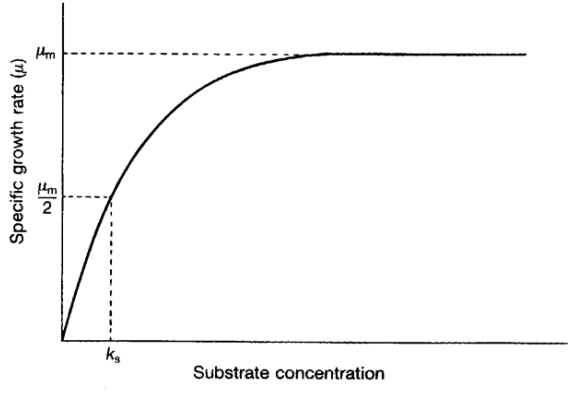


Fig. 3.12. The relationship between specific growth rate and growth-limiting nutrient concentration.

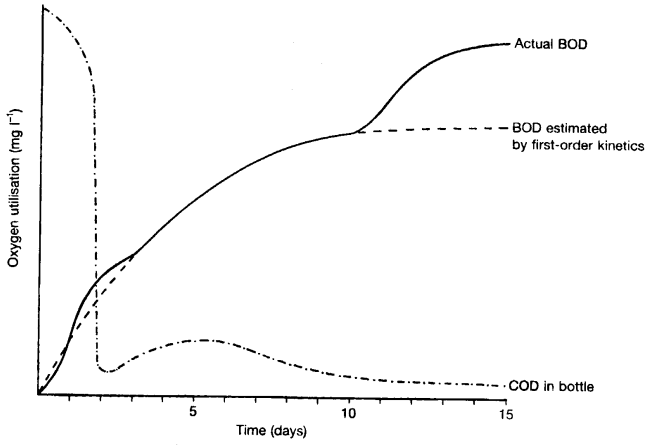


Fig. 3.13. BOD progression curve.

concentration (Benefield and Randall 1980). Figure 3.13 shows that the microbial growth rate increases as the availability of substrate increases until the maximum specific growth rate is achieved at which point a factor other than substrate, such as generation rate or a specific nutrient, becomes growth-limiting.

The specific growth rate of the equation of microbial growth under exponential growth conditions $dX/dt = \mu X$ can be replaced by the Monod function so that

$$\frac{dX}{dt} = \mu_{\mathrm{m}} \frac{SX}{k_{\mathrm{s}} + S}$$

where the growth rate (dX/dt) is directly proportional to substrate concentration (first-order). When the substrate concentration is much larger than k_{s} the expression for growth rate reduces to

$$\frac{dX}{dt} = \mu_{\mathrm{m}} x$$

where the growth rate is independent of substrate concentration (zero-order).

The organic carbon as measured by BOD or COD is usually considered to be the rate-limiting substrate in aerobic wastewater treatment systems, although the growth rate of the micro-organisms can also be controlled by other substances such as ammonia, phosphate, sulphate, iron, oxygen, carbon dioxide, and light (Andrews 1983). In low substrate concentrations, the rate of mass transfer into the cell may also control the rate of growth.

The thickness of microbial flocs or film may be orders of magnitude greater than the size of an individual micro-organism and the rate of mass transfer may limit growth under certain conditions (Powell, 1967; Baillod and Boyle 1970). However the Monod function can still be used if k_s is considered as a variable dependent upon the degree of mixing in the reactor.

In nearly all biological wastewater treatment systems the retention time of micro-organisms is such that the endogenous phase occurs. Some unit processes, such as oxidation ditches, are designed to operate specifically in this phase. The basic equation for microbial growth can be modified to incorporate endogenous decay:

$$\frac{dX}{dt} = (\mu - k_d)X$$

where k_d is the specific endogenous decay rate which includes endogenous respiration, death, and subsequent lysis. The specific endogenous decay rate k_d is of little significance when the retention time is short, being an order of magnitude less than μ. However, when the system is operated in the endogenous growth phase, k_d is important in the calculation of the net amount of micro-organisms produced and the oxygen utilisation rate.

The mass of organisms produced is related to the mass of substrate consumed, using the expression:

$$\frac{dX}{dt} = -Y\frac{[ds]}{[st]}$$

where X is the concentration of micro-organisms, s is the concentration of substrate, Y the yield coefficient, and t the time. The yield coefficient is a function of the species of micro-organisms present, the type of substrate, and environmental conditions. However, it is usually assumed to be constant for a given biological treatment process treating a specific wastewater. The yield coefficient is determined experimentally and such factors as formation of storage products (i.e. glycogen and poly-β-hydroxybutyrate), temperature, pH, and the variation in the fraction of viable cells, all of which can significantly affect the coefficient, must be taken into account. This is done by ensuring that the experimental conditions under which the coefficient is measured are the same as those encountered in practice, or by taking into account the factors used in the model of yield. The BOD or COD are used usually as a measure of substrate concentration and volatile suspended solids as an index of organism concentration. Yield coefficient is expressed as mass (or mole) of organism produced per mass (or mole) of substrate consumed. Similar relationships can be established for the utilisation of other

substances, such as oxygen or light energy, or the formation of products, such as methane or carbon dioxide.

The expression of yield can be combined with the constant growth rate equation ($dX/dt = \mu X$) to give the rate of substrate utilisation:

$$\frac{ds}{dt} = -\frac{\mu}{Y}X$$

When the amount of substrate utilisation for cell maintenance is expected to be significant, i.e. at high temperatures, long residence times and high cell concentrations, the above equation should be modified:

$$\frac{ds}{dt} = -\frac{\mu}{Y}X - k_{\mathrm{m}}X$$

where k_{m} is the specific maintenance coefficient (Benefield and Randall 1980).

Kinetic constants should be determined experimentally using bench or pilot scale methods (Giona *et al.* 1979). However, because of the biological and chemical nature of such experiments, the wastewater engineer and designer often prefer to rely on data presented in the literature. The variation in the kinetic constants quoted in the literature, due to the variation in wastewater quality and environmental conditions, is large. For example, $Y = 0.35$–0.45 mg VSS mg COD^{-1}, $k_{\mathrm{d}} = 0.05$–0.10 d^{-1}, $k_{\mathrm{s}} = 25$–100 mg l^{-1}, and $\mu_{\mathrm{m}} = 3.5$–10.0 d (Mandt and Bell 1982). Thus, clearly, kinetic analysis can only be performed with any degree of confidence on experimentally collected data.

3.1.3. *The BOD test*

The biochemical oxygen demand (BOD_5) test was introduced in Sec. 1.4.2 and is a quantitative measure of the oxygen uptake by aerobic microorganisms, mainly bacteria, as they oxidise organic matter. The test initiates the heterotrophic microbial activity that occurs both during the self-purification process in rivers and in the breakdown of organic wastewaters in biological treatment processes. The test provides a useful laboratory model describing what happens during the biodegradation of organic matter.

Microbial growth

Unlike the wastewater treatment plant or river, the BOD bottle is a closed microbial ecosystem which is devoid of external influences such as

light, turbulence, nutrient imbalance, and factors that affect the oxygen balance. The test is done under controlled conditions, in darkness at 20°C and by plotting the oxygen uptake against time the characteristic BOD curve is obtained (Fig. 1.18). Similar to the microbial growth curve (Fig. 3.5), it can be divided into the various growth phases: lag, log (acceleration), stationary, and endogenous respiration phases. There is a fifth phase in the BOD curve, that of nitrification (Fig. 1.19). Of course, the exact nature and extent of each growth phase is dependent on the composition of the sample being tested, thus in certain cases one or more phases may not be in evidence.

The lag phase is dependent upon the nature of the micro-organisms present in the sample. Its duration is a function of the number of bacteria present and whether or not they are acclimatised to the substrate. Normally, only small numbers are present and these have to acclimatise to their new environment in the BOD bottle, resulting in a delay before log growth and maximum oxidation occurs. Initially, the soluble substrate is rapidly assimilated and while cell numbers remain constant, cell size and mass increase. There is relatively little oxygen utilised during this phase as synthesis reactions require much less oxygen per unit of substrate utilised than does oxidation to carbon dioxide. Therefore, in order to avoid underestimating the BOD, the lag phase should be as short as possible, which is achieved by using laboratory grown micro-organisms to seed samples that are acclimatised both to the test temperature and to the substrate. Organisms transferred from a log growth phase will have a shorter lag phase than if transferred from any other growth stage (Young and Clark 1965). The effect on the BOD of prolonged lag phases because of insufficient bacteria when a sample has not been seeded, using unacclimatised seed or in the presence of inhibitory or toxic substances, is shown in Fig. 3.14 (HMSO 1983a).

During the log-growth phase the organic matter present in the sample is utilised by the heterotrophs for energy and growth. Once the mass of micro-organisms is large enough and is acclimatised to the substrate, the synthesis reaction rate declines and the oxidation reaction rate increases. During the log growth phase the available food supply (substrate) is in excess, resulting in a high food to micro-organism ratio (f/m), so that microbial growth is unrestricted. However, the micro-organisms are at their most vulnerable during this period because their generation time is reduced to a minimum, cell size is decreasing, and their resistance to toxic and inhibitory substances is low (Young and Clark 1965). This phase generally lasts between 24–36 hours during which time about 50% of

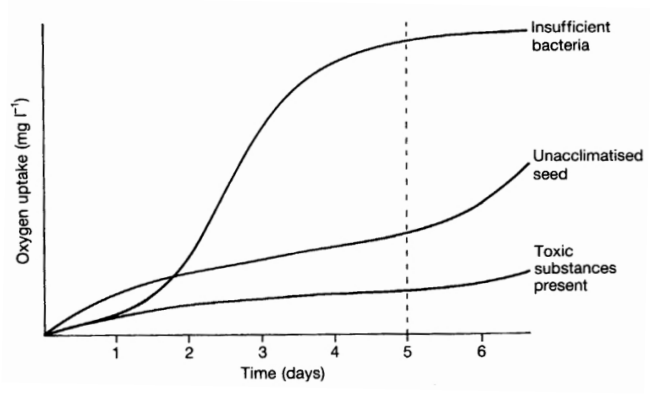


Fig. 3.14. Lag growth phases in the BOD test (adapted HMSO 1983a).

the organic matter will be oxidised. Eventually, with the micro-organisms having reached maximum concentration, the substrate becomes limiting (a low f/m ratio) and more difficult to assimilate. With a consequent decrease in growth rate, there is a decrease in the rate of oxygen uptake. The rate continues to decrease and levels out at near zero uptake rate over a period of up to 30 hours, resulting in a plateau called the stationary growth phase.

In the stationary phase all the available soluble substrate has been utilised. Continued oxygen uptake rate after this phase is due to endogenous respiration and the presence of predators, such as protozoans feeding on the bacterial biomass, produced during the log-growth phase (Busch 1958; Schroeder 1968). Bhatla and Gaudy (1965) found that the occurrence and duration of the plateau is usually linked to the population of predators, especially the protozoans, present at the end of the log-growth phase. For example, if protozoan growth lagged significantly behind bacterial growth, then a long plateau could be expected.

With a low f/m ratio, the declining bacterial population is forced into endogenous respiration. This normally occurs 3–5 days after incubation commences. Endogenous respiration is the oxidation of the portion of organic matter that was converted into cellular tissue during the log-growth phase. The first compounds to be oxidised are the expendable storage products such as glycogen and PHB (poly-β-hydroxybutyrate), leaving the more essential cellular constituents such as amino acids, proteins, and nucleic acids to degrade only when these have been utilised (Painter 1983). Oxidation occurs at a much slower rate than during the log-growth phase. By the fifth or sixth day, the reduced bacterial population begins to limit the growth of protozoans, which are also forced into

endogenous respiration. All the micro-organisms continue their endogenous respiration for about a further 15 days after which time biochemical oxidation should be over 90% complete.

The final stage in the BOD curve is only observed in samples containing a high concentration of ammonia and a significant population of nitrifying bacteria (Sec. 3.5). With the exception of partially nitrified effluents, nitrification proceeds very slowly during the initial stages of the BOD test, with no appreciable demand for oxygen. Thus, in practice, the effect of nitrification on the 5-day test (BOD_5) is usually negligible. This is due to the very slow reproduction rate of the nitrifying bacteria, which is between 2–6 days for most genera and results in a noticeable nitrification oxygen demand only after 10 days incubation. Nitrification can exert an appreciable oxygen demand in the BOD_5 when partially nitrified sewage effluents are tested. Such samples contain a high concentration of ammonia as well as an established population of nitrifying bacteria.

If the oxidation of organic matter is allowed to proceed in the BOD bottle, complete oxidation is achieved between 20–100 days depending on substrate. The total oxidation of all organic carbon, nitrogen, and hydrogen is referred to as the ultimate BOD (L). In practice, L is approximated mathematically from the rate of oxygen uptake during the initial incubation period, rather than actually incubating samples for such a lengthy period. (Fig. 1.22).

BOD kinetics

The kinetics of the BOD test have been fully explained in Sec. 1.4.2.1 and are based on first-order reaction principles. However, the concept of the oxygen uptake rate in the BOD test conforming to a first-order reaction has been widely disputed (Rivera *et al.* 1965; Young and Clark 1965; Landine 1971; Stones 1981, 1982). Stones (1982) applied both first- and second-order equations to the results of an experiment in which a 0.01 dilution of settled domestic sewage was incubated at 20°C and the residual dissolved oxygen concentrations determined at daily intervals over 15 days, from which k_1 values were determined (Table 3.4). His results clearly show that the values of $k_{1.1}$ and $k_{1.2}$, computed using first-order reaction kinetics, decrease rapidly as oxidation proceeds. This is in contrast to the $k_{1.3}$ values, computed using second-order reaction kinetics, which are virtually constant from the second to the tenth day of incubation, with the observed decline after 10 days attributable to the onset of nitrification. There is little doubt that the second-order reaction equation gives a better mathematical

Table 3.4. Values of the velocity coefficients $k_{1.1}$, $k_{1.2}$, and $k_{1.3}$ computed using first- and second-order reaction kinetics (Stones 1981, 1982, 1985).

Days	$k_{1.1}$	$k_{1.2}$	$k_{1.3}$
1	0.350	0.200	0.0397
2	0.318	0.170	0.0390
3	0.295	0.149	0.0386
4	0.277	0.132	0.0383
5	0.263	0.119	0.0381
6	0.252	0.109	0.0381
7	0.244	0.100	0.0381
8	0.236	0.092	0.0381
9	0.230	0.085	0.0382
10	0.224	0.080	0.0382
12	0.218	0.070	0.0391
15	0.212	0.059	0.0404

$k_{1.1}$, velocity coefficient calculated using a *first-order* reaction equation in which it is assumed that the rate of biochemical oxidation varies with the *unsatisfied BOD*.

$k_{1.2}$, velocity coefficient calculated using a *first-order* reaction equation in which it is assumed that the rate of biochemical oxidation varies with the *residual DO concentration*.

$k_{1.3}$, velocity coefficient calculated using a *second-order* reaction equation in which it is assumed that the rate of biochemical oxidation varies with *both* the *unsatisfied BOD* and the residual *DO concentration*.

description of the log-growth phase. However, the overall BOD curve is made up of a minimum of three growth phases each having an entirely different growth rate. Thus, BOD decay in natural environments represents a complex interaction between a diverse assemblage of bacteria and a heterogeneous organic substrate (Swamee and Ojha 1991). This is supported by other studies that show that no single fixed value, whether first-, second-, or half-order, can appropriately describe all wastes (Young and Clark 1965; Adrian and Saunders 1992, 1998; Adrian *et al.* 1999). Therefore, the use of a first-order reaction equation is to be preferred as it more accurately describes the summation of all the individual growth rates that make up the BOD curve. For this reason the first-order decay model is widely used in water quality modelling to describe the deoxygenation of organic wastes.

Borsuk and Stow (2000) have proposed an alternative to the simple fixed reaction order approach by allowing L to remain a free parameter. Thus, rather than assuming a first-order decay process *a priori*, they acknowledged that the BOD exertion is a mixture of decay processes and allow the data itself to determine the reaction order. BOD exertion is modelled as a first-order decay process in which the oxygen consumption is proportional to the concentration of BOD remaining (L_t):

$$\frac{dL_\mathrm{t}}{dt} = -k_1 L_\mathrm{t}$$

with L_t a free parameter as proposed by Borsuk and Stow, this is rewritten as:

$$\frac{dL_\mathrm{t}}{dt} = -k_\mathrm{n} L_\mathrm{t}^\mathrm{n}$$

This integrates to:

$$L_\mathrm{t} = \{L_0^{1-\mathrm{n}} - k_\mathrm{n}^\mathrm{t}(1-\mathrm{n})\}\frac{1}{1-\mathrm{n}}$$

where L_0 is the ultimate BOD (mg l^{-1}), L_t the BOD remaining after time t (mg l^{-1}), k_1 the first-order reaction rate constant (d^{-1}), t the time (d^{-1}), n a pseudo-order parameter, and k_n the mixed order reaction rate constant ((mg l^{-1})$^{(1-\mathrm{n})}\mathrm{d}^{-1}$).

Substituting $(L_0 - Y)$ for L_t:

$$Y = L_0 - \{L_0^{1-\mathrm{n}} - k_\mathrm{n}^\mathrm{t}(1-\mathrm{n})\}\frac{1}{1-\mathrm{n}}$$

where Y is the BOD exerted at time t $(L_0 - L_\mathrm{t})$ mg l^{-1}.

The authors used a Bayesian approach to estimate the parameters and found that the mixed order model described above resulted in a better fit to observed data and produced more realistic predictions of the ultimate BOD than first-order expressions.

Further reading

General: Benefield and Randall 1980; Andrews 1983; Jank and Bridle 1983; Atkinson and Mavituna 1983; Schugerl 1987; Bu'lock and Kristiansen 1987.

Stoichometry: Lawrence and McCarty 1970; McCarty 1972, 1975; Christensen and McCarty 1975; Schugerl 1987.

Kinetics: Giona *et al.* 1979; Schugerl 1987; Sinclair 1987.

3.2. Energy Metabolism

There are three general methods by which heterotrophs obtain energy: by fermentation; and by either aerobic or anaerobic respiration. Fermentation and respiration are the two fundamental types of metabolism, and differ in that respiration requires an external electron acceptor; oxygen in aerobic respiration and carbon dioxide, sulphate or nitrate in anaerobic repiration. Fermentation does not require an external electron acceptor as the substrate is the electron donor and the other participating organic molecule acts as the electron acceptor.

Heterotrophic micro-organisms, including bacteria, fungi, and, to a lesser extent, protozoans, are capable of utilising a wide range of organic compounds as a carbon source. These vary from simple sugars, alcohols or amino acids to complex carbohydrates, lipid polymers and proteins. However, these carbon sources are converted to a comparatively small number of intermediates such as pyruvate and acetyl-Co enzyme A (acetyl-CoA), which are common to all living tissue and are the "building blocks" for cell synthesis. The anabolic pathways of cell synthesis are essentially the same in all micro-organisms whether heterotrophs or autotrophs. As carbohydrates are broken down to glucose before entering more complicated biochemical routes and as the majority of bacteria can metabolise carbohydrates, by taking glucose as the basic substrate then it is possible to compare all the major steps in heterotrophic metabolism (Fig. 3.15). The steps from carbohydrate to glucose and then to pyruvic acid are common to all organisms metabolising carbohydrate. The substrate is broken down in a series of enzyme-catalysed reactions with small amounts of energy released at each oxidation stage and recovered by substrate-level phosphorylation (ADP $\rightarrow$ ATP). As this is achieved without the use of an electron acceptor, it is a fermentation reaction. The term fermentation describes any anaerobic metabolism of energy production that does not involve an electron transport chain. The glucose is broken down to pyruvate in three stages (Fig. 3.16). Stage 1 is an endergonic reaction using energy from ATP, whereas in stage 2 the 6-carbon sugar is cleaved to form two interconvertible 3-carbon compounds. The final stage is energy-yielding, with substrate-level phosphorylation occurring, which results in the formation of pyruvate. Pyruvic acid is the pivotal compound in metabolism, and from it many different products can be metabolised, depending on the organism and the environmental conditions (Fig. 3.17). If an external electron acceptor is present then the pyruvic acid can be converted to acetyl-CoA which enters the citric acid cycle (also known as the Krebs or

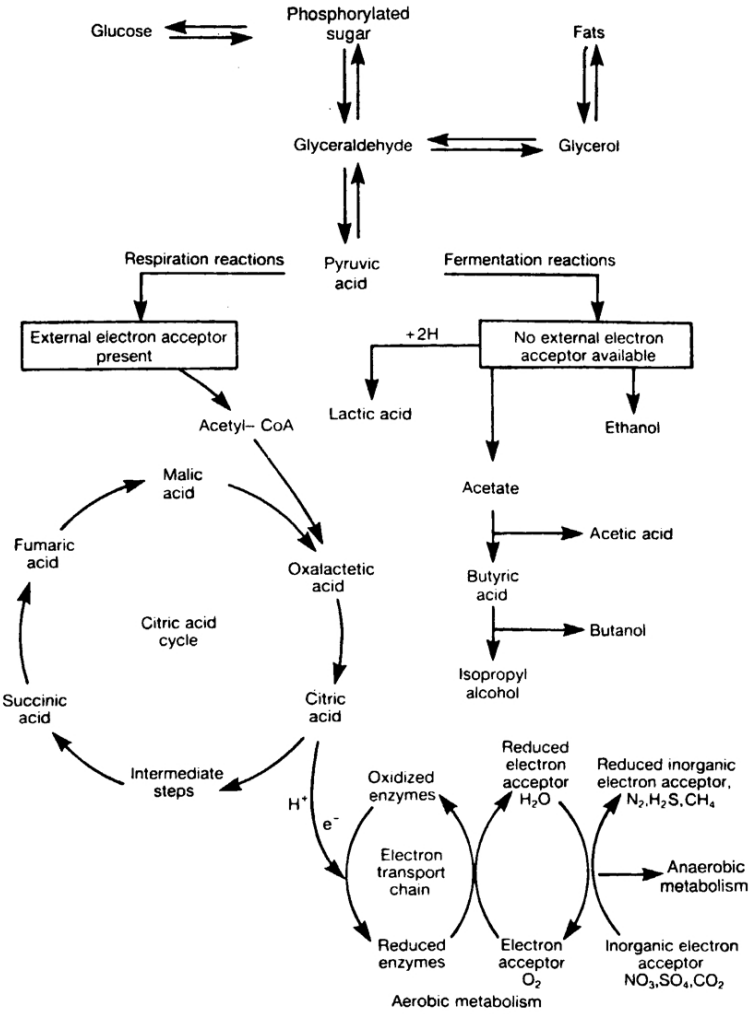


Fig. 3.15. Major metabolic steps in the fermentation of glucose. The diverse pathways after a pyruvic acid intermediate stage can be exploited to manufacture a variety of organic chemicals. Aerobic metabolism occurs by way of the citric acid cycle. Oxygen enters the reaction only at the last step as an electron acceptor.

tricarboxylic acid cycle) (Fig. 3.15). However, if there is no external electron acceptor available then the pyruvic acid may undergo a further stage in the fermentation pathway involving anyone of a series of alternative reactions, which not only serve to regenerate NAD and NADH, but also produce a wide range of fermentation products such as ethanol.

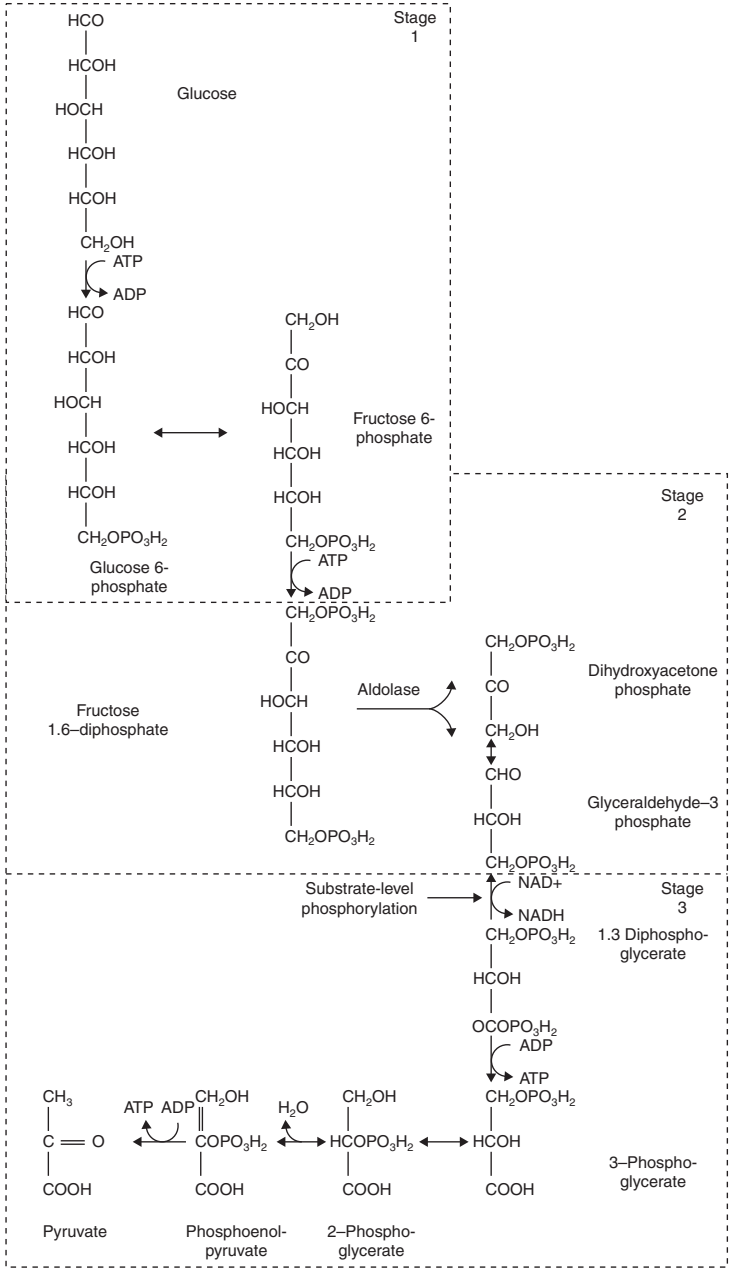


Fig. 3.16. Fermentation pathway of glucose to pyruvate (Brock 1970).

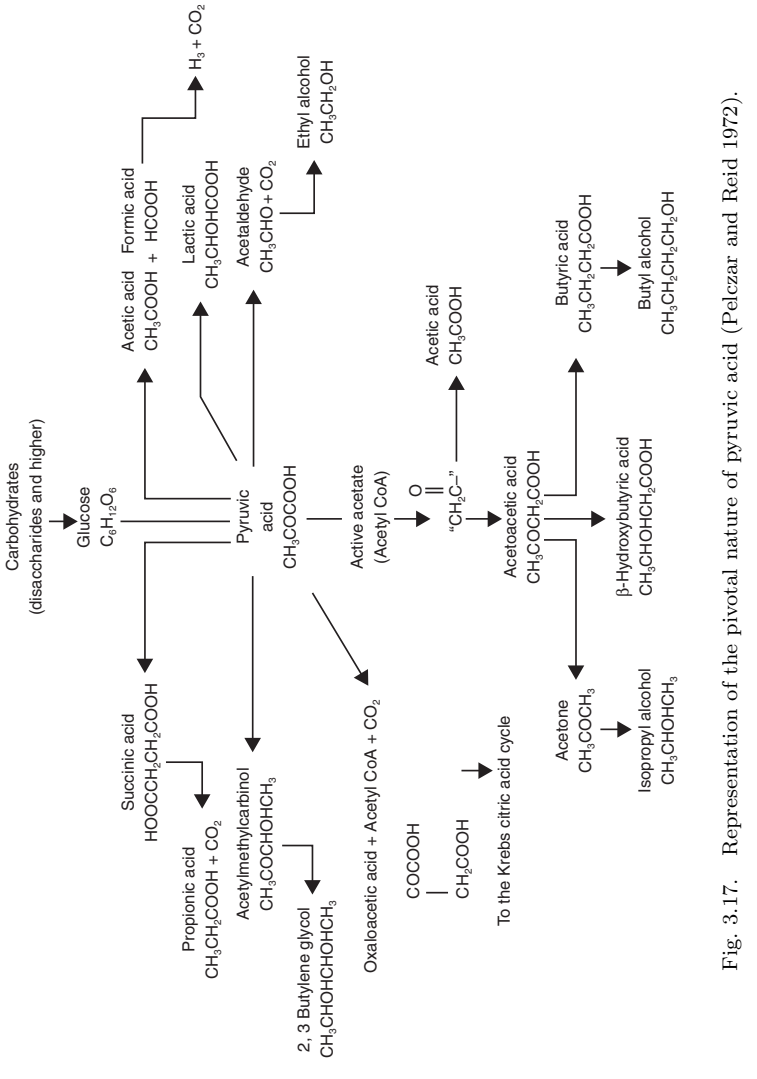
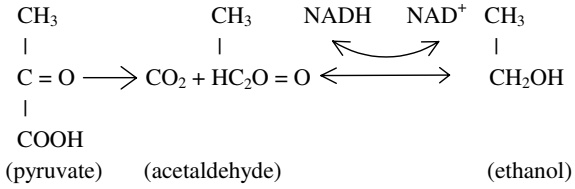


Fig. 3.17. Representation of the pivotal nature of pyruvic acid (Pelczar and Reid 1972).



The end-products of fermentation are still rich in energy even though the fermentation pathway is complete. For example, although the breakdown of glucose to lactic and acetic acids yields energy, the complex end-products are still potentially energy-rich.

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \rightarrow 2\underset{\text{(lactic acid)}}{CH_3CHOHCOOH} + \text{energy (22.5 kcal)}$$

$$\underset{\text{(glucose)}}{C_6H_{12}O_6} \rightarrow 3\underset{\text{(acetic acid)}}{CH_3COOH} + \text{energy (15 kcal)}$$

When external electron acceptors are present, pyruvic acid is converted to acetyl-CoA, which enters the citric acid cycle (Fig. 3.18). Metabolism now becomes respiration and not fermentation. However, in the cycle a hydrogen ion and electrons are released which enter the electron transport system where they react with either oxygen (aerobic respiration) or an inorganic electron acceptor (anaerobic respiration).

In aerobic respiration, most energy comes from oxidative phosphorylation as electrons are carried through the electron transport system, which reoxidises reduced co-enzymes that are formed as a result of oxidative reactions. In the electron transport system used by most wastewater bacteria there are three phosphorylation sites along the transport chain (Fig. 3.19). Thus, if the electrons enter at the NAD level each pair of electrons will result in the formation of three ATP molecules compared with only two ATP molecules if electrons enter the chain at the FAD level. Whereas the metabolic pathways followed in the breakdown of the carbon and the energy source is the same for both anaerobic and aerobic respiration. There are two important differences between these two processes. In anaerobic respiration, inorganic acceptors are utilised, such as carbon dioxide, sulphate or nitrate, which are reduced to methane, hydrogen sulphide, or ammonia, nitrous oxide or molecular nitrogen respectively (Fig. 3.20). The amount of ATP formed by oxidative phosphorylation is also different, being much less during anaerobic as opposed to aerobic respiration. The reason is that the amount of ATP formed by the passage of pairs of electrons through the electron transport system depends on the difference in redox potential between the electron donor and acceptor (Fig. 3.21).

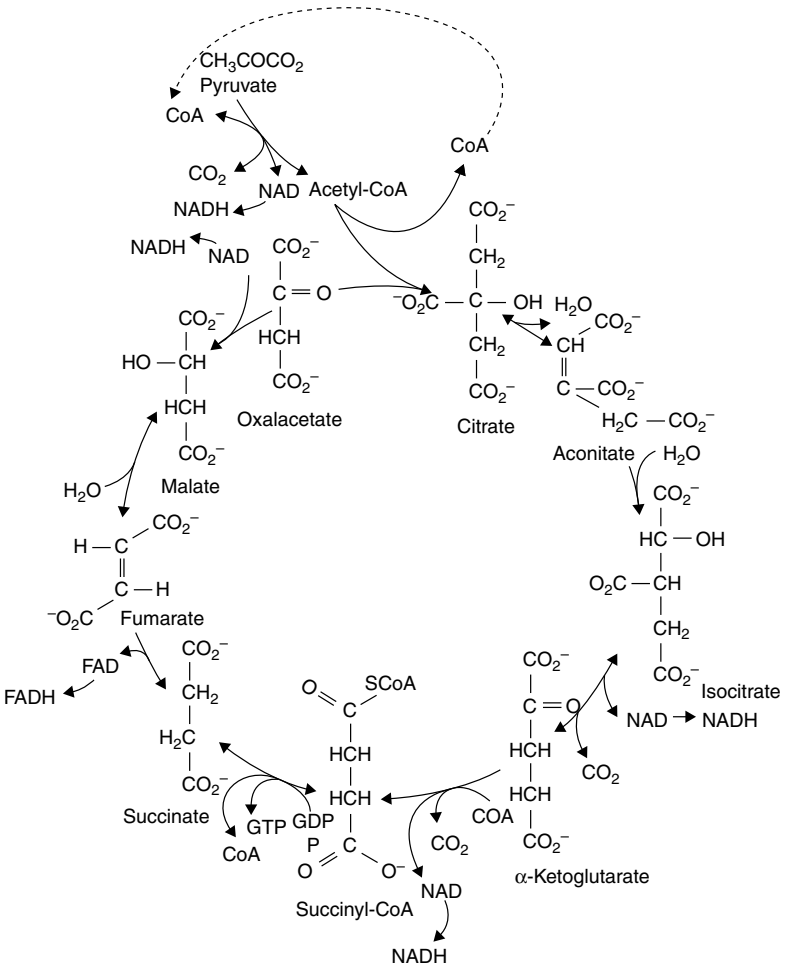


Fig. 3.18. The Krebs cycle (Brock 1970).

As oxygen has a lower redox potential than the inorganic electron acceptors, more ATP is normally released during aerobic respiration. In terms of energy yield, the fermentation of glucose realises two ATP molecules per hexose unit compared with 38–39 ATP molecules per hexose unit during aerobic respiration. The greater energy utilisation during aerobic respiration means that a much greater proportion of the glucose will be assimilated as cell material compared with anaerobic respiration (Table 3.2) (Benefield and Randall 1980). The energy-generating mechanisms have been excellently reviewed by Anderson (1980) and Bitton (1999).

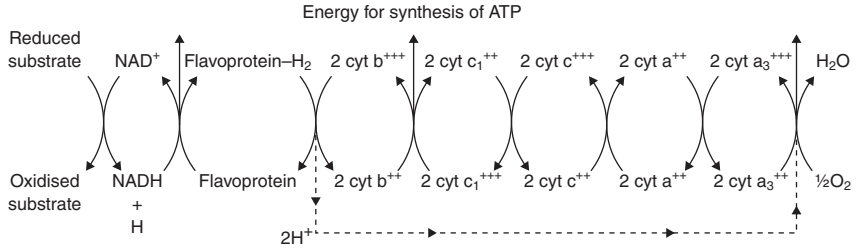


Fig. 3.19. Electron transport system common to most bacteria (Pelczar and Reid 1972).

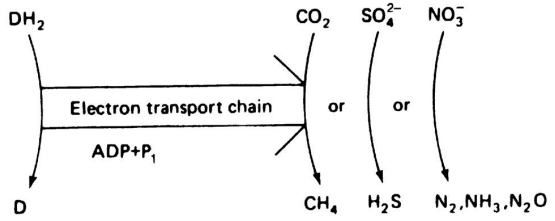


Fig. 3.20. Electron transport chain for anaerobic respiration (Benefield and Randall 1980).

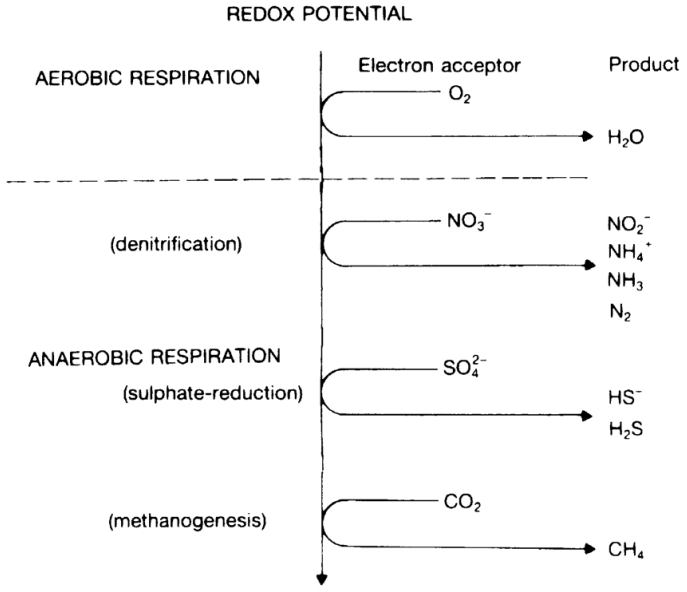


Fig. 3.21. The redox potential of water and sediment indicates which inorganic electron acceptor is being used by the microbial community (Lynch and Poole 1979).

Further reading

General: Mandelstam and McQuillen 1973; Haddock and Hamilton 1977; Lynch and Poole 1979; Anderson 1980; Bitton 1999.

3.3. Aerobic Heterotrophic Micro-organisms

3.3.1. *The organisms*

Bacteria

Bacteria are the most versatile of all the organisms associated with wastewater treatment in terms of the conditions under which they can thrive and the substrates they can metabolise. Undoubtedly, it is the bacteria that are the most important group of organisms in biological treatment systems, although fungi, protozoans, and a range of other invertebrate organisms also play important but comparatively minor roles.

The bacteria form the basic trophic level in all the biological treatment systems and so form the major proportion of the biomass, with the exception of waste stabilisation ponds in which algae may predominate (Sec. 6.3). The dominant bacteria are the aerobic heterotrophs that degrade and eventually mineralise organic compounds present in wastewater to carbon dioxide and water. It is the small size of bacteria and their resultant large surface area to volume ratio which makes them so efficient, in terms of nutrient and catabolic exchange, with the liquid medium in which they are either suspended or are in contact. Their short doubling times, which can be as little as 20 minutes in pure culture, enable bacteria to rapidly take advantage of increased substrate availability compared with other organisms. Protozoans, for example, have an average doubling time in the order of 10 hours, although some species do have doubling times of less than 2 hours, such as *Tetrahymena pyriformis* (Curds and Cockburn 1971).

Estimation of the numbers of bacteria in sewage and treatment processes can be made by direct microscopic observation using a special counting chamber such as a Petroff-Hauser chamber or by culturing bacteria on various nutrient media at 22°C or 30°C (Pike 1975; APHA 1998). Serious discrepancies exist between total counts of bacteria in treatment processes made by microscopy, which cannot distinguish between viable and non-viable cells, and by various culturing techniques that measure only the viable bacteria (Table 3.5). The latter technique gives results that are often two orders of magnitude less due to the proportion of the microbial biomass, which is either dead or non-viable, because of most processes being operated

Table 3.5. Numbers of total and viable bacteria (geometric means) at different stages of treatment (Pike and Carrington 1972).

Source (and number) of samples[b]	Bacterial counts[a]				Viability (%)	Total suspended solids (mg l^{-1})
	In samples (No./ml)		In suspended solids (No./g)			
	Total	Viable	Total	Viable		
Settled sewage (46)	5.6×10^8	6.3×10^6	3.0×10^{12}	3.4×10^{10}	1.1	190
Activated sludge mixed liquor, conventional rate (18)	5.9×10^9	4.9×10^7	1.3×10^{12}	1.1×10^{10}	0.83	4600
Activated sludge mixed liquor, high rate (24)	1.4×10^{10}	2.4×10^8	3.0×10^{12}	5.0×10^{10}	1.7	4800
Filter slimes (18)	6.2×10^{10}	1.5×10^9	1.3×10^{12}	3.2×10^{10}	2.5	54 000
Secondary effluents (16)	5.4×10^7	1.1×10^6	1.9×10^{12}	4.1×10^{10}	2.1	28
Effluents, high-rate activated sludge plants (24)	4.8×10^7	1.4×10^6	3.3×10^{12}	1.0×10^{11}	3.0	14
Tertiary effluents (11)	2.9×10^7	6.6×10^4	3.0×10^{12}	6.8×10^9	0.23	9.7

[a]Samples from sewage works and laboratory pilot plants; high-rate plants are pilot scale, working at loading of 0.46–2.5 kg BOD removed/kg MLSS day, secondary effluents from nine filters and seven activated sludge plants, tertiary effluents from ten lagoons, one grass plot.

[b]Total counts obtained with Helber counting chamber, viable counts by plate-dilution frequency method on CGY agar, incubation for 6 days at 22°C; viability expressed as percentage ratio of viable count to total count.

in the stationary or declining phase of the microbial growth curve (Fig. 3.5). Culturing techniques of aerobic heterotrophic bacteria are reviewed by Pike (1975) and Pickup (1991) who conclude that cultural methods cannot be used as estimates of biomass or microbial activity, as the enzymes of the non-viable bacteria remain active within the treatment system. However, this does not prevent culturing techniques being used for measuring the rates of increase or decline in the number of viable bacteria, for which they are selective. A major problem in culturing is that none of normal growth media supports the growth of all nutritional types of aerobic heterotrophic bacteria present in wastewater and treatment systems, although optimal counts are obtained using a casitone-glycerol-yeast extract agar (CGY) (Pike *et al.* 1972). Plate counts are determined by either spread plating, i.e. 0.1 ml of microbial suspension is spread onto the surface of an agar plate, or pour plate method, i.e. 0.1–1.0 ml of microbial suspension is mixed with the molten agar and poured into a petri dish. Each viable cell is assumed to produce a colony, with the number of counts expressed as colony-forming units (cfu). Membrane filters can also be used with dilute samples filtered through sterile cellulose nitrate filters and placed directly onto suitable growth medium (Sec. 9.3). To determine the MPN for total bacteria, samples are serially diluted in YP medium (2.5 g l^{-1} yeast extract, 2.5 g l^{-1} peptone) and incubated for 7 days at 28°C (APHA 1998). Culturing techniques are constantly being revised, thus, the latest edition of Standard Methods should always be checked (APHA 1998). Bacteria from treatment plants are usually aggregated as either sludge flocs or filter film, which must be completely broken up into individual cells randomly dispersed, before plating on to medium and incubating. Ultrasonics are used in preference to standard homogenisation procedures, although many cells are inevitably damaged or destroyed. Thick filter film can only be broken down to individual cells by extreme homogenisation methods which result in massive cell mortalities and produce counts which are misleadingly low.

Bacterial counts using counting chambers are done under phase contrast microscopy. Fluorochromes (e.g. acridine orange, DAPI) have largely replaced traditional counting chambers. Bacterial cells are retained on a membrane filter that has been treated to suppress auto-fluorescence (e.g. polycarbonate filters treated with Irgalan Black), then stained with the fluorochrome and counted using an epifluorescent microscope (Kepner and Pratt 1994). Active cells can be determined when epifluorescence microscopy is used in combination with oxidoreduction dyes e.g. INT (2(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride) or CTC (cyanoditolyl tetrazolium chloride) (Pyle *et al.* 1995; Posch *et al.* 1997).

Metabolic methods are used to estimate biomass and microbial activity. Substrate utilisation can be measured by determining the uptake of substrate itself, by oxygen consumption or dehydrogenase activity (Brock 1971b). As adenosine triphosphate (ATP) is only found in living cells and its concentration is linked to the organic content of cells, attempts have been made to use it to estimate biomass as well as the viability of sludges and films. However, the presence of large organisms, especially protozoans and rotifers, make it less useful as a specific measure of bacterial biomass or bacterial viability (Pike 1975). Other specific cell chemicals such as DNA, RNA, or proteins have also been used.

Bacterial identification is essentially a task for a specialist microbiologist, primarily because few bacterial species have been adequately described. Also, the biochemical tests used for positive identification are complex and time consuming. Bergey's manual (Holt 1994) is the definitive work on identification because it provides an extremely comprehensive list of bacteria, which are fully described and characterised in the literature. This manual is being slowly updated as a series of volumes. However, it is possible to identify the more common bacterial components of treatment systems and those species producing gelatinous matrices or filaments of cells, in particular, by using simple microscopic and histological tests (Farquhar and Boyle 1971a,b; Eikelboom 1975). The key by Eikelboom and Van Buijsen (1981) contains many excellent photomicrographs of all the major filamentous bacteria found in wastewater. The major isolation methods for filamentous micro-organisms have been compared by Strom and Jenkins (1984) and are discussed further in Sec. 5.4, when bulking of activated sludge is examined.

A healthy Western adult excretes approximately 135 g dry weight of faeces and 1400 ml of urine each day, with 30% of biomass being bacteria. As these bacteria form part of the gut flora they are predominantly obligate anaerobes, out-numbering aerobes by a factor of 40 to 1. Urine contains few bacteria and only exceed $> 10^5$ ml^{-1} when the urinary tract is infected. The major aerobic species in urine are Gram-negative, including *Escherichia coli*; *Proteus spp.*, and *Pseudomonas aeruginosa*. The bacterial flora of excreta is dealt with in more detail in the section on anaerobic heterotrophs (Sec. 3.4).

The bacterial count of sewage shows a marked diurnal variation which is linked to the water usage and excretion pattern of the population, following a similar curve to the BOD of domestic sewage (Fig. 1.3), but with a more differentiated peak in the evenings. Seasonal patterns have also been identified (Tomlinson *et al.* 1962; Gameson *et al.* 1967; Harkness 1966),

with maximum numbers found during August and September. However, it is likely that this has been due to other factors, such as the temporary removal of toxic and inhibitory substances in the waste, or an increase in population size because of tourists. Pathogenic bacteria are also present in sewage, and these are discussed fully in Chapter 9.

The bacterial communities found in wastewater treatment plants are complex, with a variety of genera present, in addition to the dominant genera that are usually aerobic, heterotrophic, Gram-negative, rod-shaped bacteria with polar flagella. The bacterial flora of all aerobic treatment systems are basically the same, with *Zoogloea*, *Pseudomonas*, *Chromobacter*, *Achromobacter*, *Alcaligens*, and *Flavobacterium* the major genera present. All are able to oxidise organic compounds to carbon dioxide and water. *Escherichia coli* and the other faecal indicator bacteria (Sec. 9.3) are universally present in treatment systems but are not indigenous members of the microbial community (Table 3.6).

The bacterial communities of activated sludge systems are far more specialised than those associated with fixed-film reactors and also have a lower diversity. The bacteria in activated sludge comprise of two major types. Dispersed species are constantly removed by protozoan grazing and by being discharged with the effluent. The dispersed species grow faster than the second major type, the flocculating bacteria, which form flocs and, due to the design of the process, are retained in the system. Although the activated sludge process selects the flocculating bacteria in preference to the dispersed species, the latter play a major part in substrate utilisation. Fixed-film reactors have a greater range of micro-habitats compared with activated sludge and so support a less specialised flora of bacteria comprising a much greater diversity. There is a noticeable variation in the numbers and abundance of bacteria with filter depth, whereas the community structure in both treatment processes are affected by seasonal and operational factors. The individual bacterial communities associated with each treatment process are discussed in the relevant chapters below. However, two bacteria, *Zoogloea* and *Sphaerotilus natans*, which are important components of wastewater treatment systems, can be considered as typical examples of aerobic heterotrophic bacteria and are considered in detail here. Much research has been done on these species as they are easily isolated and subsequently cultured. They are extremely common in wastewater treatment systems and are also found in natural watercourses receiving organic enrichment. Another important feature is that they are among the easiest species of bacteria to identify.

Table 3.6. Outline of useful organisms and reactions (Berthoux and Rudd 1977).

	Organism	Reaction	Oxygen requirement	pH	Temp. (°C)	Comments
1.	Organic degraders	Organics to CO_2				
	Escherichia coli	and H_2O	Facultative	6–8	10–30	Indicator organism
	Zooglea ramigera	”	”	”	”	Active in sewage treatment — utilises polysacharides and carbohydrates
	Sphaerotilus natans	”	”	”	”	
	Pseudomonas sp.	”	”	”	”	
	Chromobacteria sp.	”	”	”	”	
	Achromobacter sp.	”	”	”	”	
	Corynebacteria	”	”	”	”	
	Clostridia sp.	”	Anaerobic	7–8	20+	Proteolytic
	Bacillus sp.	”	Facultative			
2.	Nitrogen users					
	Nitrosomonas	$NH_3 \rightarrow NO_2^-$	Aerobic	7–8	10–25	Nitrification — common in soils and streams
	Nitrobacter	$NO_2^- \rightarrow NO_3^=$	Aerobic	”	”	
	Rhizobium	$N_2 \rightarrow NO_3^=$				Symbiotic with legumes
	Azobacter sp.	”				Non-symbiotic
	Clostridium pasteurianum	”				Non-symbiotic
	Thiobacillus denitrificans	$NO_3^{2-} + 2S + H_2O \rightarrow HSO_4^- + SO_4^{2-} + N_2$	Anaerobic			Autotrophic
	Micrococcus denitrificans	$NO_3^{2-} \rightarrow N_2$	Anaerobic			Heterotrophic
	Pseudomonas	”	”			Heterotrophic

Table 3.6. (*Continued*)

	Organism	Reaction	Oxygen requirement	pH	Temp. (°C)	Comments
3.	Sulphur bacteria					
		Oxidation of S				
	Beggiatoa	$2H_2S + O_2 \rightarrow 2S + H_2O$	Aerobic	Low		Autotrophic
	Thiobaccillus thiooxidans	$2S + 2H_2O + 3O_2 \rightarrow 2SO_4^{2-} + 4H^+$	”	”		”
		Reduction of S				
	Desulfovibrio	$CH_3COOH + SO_4^{2-} \rightarrow 2CO_2 + H_2S + 2OH^-$	Anaerobic	Low		Organic consumed in reaction
		$4H_2 + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$				Autotrophic reaction
4.	Methane bacteria					
	Methanobacillus omelianskii	Alcohols $\rightarrow CH_4 + CO_2$	Anaerobic	6.4–7.2	35	Methane fermentation exploited in sludge
	Methanococcus vanieli	Formate $+ H_2 \rightarrow CH_4$	”	”	”	Digestion and other anaerobic sewage treatment processes
	Methanobacterium formicicum	$CO_2 + H_2 \rightarrow CH_4$				Carbon dioxide reduction
	Methanobacterium sohngeni	Acetate butyrate $\rightarrow CH_4$				
	Methanomonas	$CH_4 + 2O_2 \rightarrow CO_2 + H_2O$	Aerobic			Methane oxidation

Table 3.6. (*Continued*)

	Organism	Reaction	Oxygen requirement	pH	Temp. (°C)	Comments
5.	Iron bacteria					
	Leptothrix sp.	$Fe^{2+} \rightarrow Fe^{3+}$	Aerobic			Autotrophic — causes rust and corrosion
	Gallionella sp.	”	”			
6.	Others					
	Pseudomonad	$CO + 1/2O_2 \rightarrow CO_2$	Aerobic			
	Thiocyanoxidans	oxidise cyanide		7		
	Cellulomonas sp.	cellulose-protein			35	

Sphaerotilus natans is a Gram-negative non-sporing bacterium made up of individual rod-shaped cells with rounded ends, each 1–4 × 4–10 μm in size, enclosed within a sheath of varying thickness (Stokes 1954; Mulder and Van Veen 1963; Phaup 1968). It is a variable species, the appearance of the cells and the amount of sheath material varying with nutritional regime. The sheath is a protein-polysaccharide-lipid complex which is itself enclosed in a capsule composed of a simple polysaccharide material whose composition varies with the nutrient regime (Fig. 1.15). The cells often contain globular inclusions of the food reserve material poly-β-hydroxybutyrate. These inclusions are visible even in the early phases of growth and has been shown to be utilised under nutrient-limited conditions. A high C:N ratio favours the formation and storage of poly-β-hydroxybutyrate which can comprise up to 40% of the dry weight of the cells (Rouf and Stokes 1962). However, as the filaments age so the individual bacterial rods become smaller in size and the inclusions disappear (Mulder and Van Veen 1963; Sanders 1982). In young cultures, the filaments sometimes appear to be non-septate and as they age the cells become more distinct. Occasionally, a single sheath contains two or even three rows of cells with false branching occurring by single rods slipping sideways and growing into a new filament (Phaup 1968). Two varieties of *S. natans* are found in treatment plants and polluted waters (Gray 1982). When the organic carbon concentration is low the form *S. natans* var. *diochotoma* is common, the filaments appearing highly branched and enclosed in a thin sheath. Whereas, if the organic carbon concentration is high, especially in the form of low polymer carbohydrate and organic acids, then *S. natans* var. *typica* is found. This latter variety is normally associated with treatment plants and branching is weakly developed and often absent. The sheath is also thicker and in polluted rivers has a more slimey consistency (Gray 1985a). Reproduction occurs by fragmentation of the filament or by the production of motile flagellated cells. The flagellated cells, commonly referred to as swarm cells, become attached to new suitable substrates, by a gelatinous holdfast formed at the non-flagellated end, which then lose their flagella and grow into new filaments (Phaup 1968). Isolation of *S. natans* into pure culture is relatively simple, colonies on agar medium being dull white and of variable form (Gaudy and Wolfe 1962; Marcus *et al.* 1978). Identification should be made using the characters described by Gaudy and Wolfe (1962) or Buchanan and Gibbons (1975). *Sphaerotilus natans* was originally described by Kutzing in 1833, who considered it to be an alga, and since then much confusion has developed over the taxonomy of this genus which is now firmly placed in the family *Chlamydobacteriaceae*. Many synonymous genera and species have

been described in the past including *Cladothrix*, *Chlamydothrix*, *Clonothrix*, and *Streptothrix*. However, *S. natans* and *S. discophorous* are now considered to be the only valid species (Pringsheim 1949). The genus *Leptothrix* is distinguished from *Sphaerotilus* on the basis of its differing ability to oxidise manganese and iron (Van Veen *et al.* 1978).

Studies on wastewater microbiology have generally not tried to characterise zoogloeal-forming organisms as they can be difficult to isolate and are not well defined taxonomically. The category includes *Zoogloea ramigera*, *Pseudomonas* spp., and zoogloeal forms of *Sphaerotilus*. They are extremely common in aerobic wastewater treatment systems (Rossello-mora *et al.* 1995), as well as being important components in slimes found in both natural and man-made environments (Williams and Unz 1983). The organism comprises small Gram-negative non-sporing cells (0.5–10 × 1.0–3.0 μm) not arranged in filaments but embedded in a gelatinous (polysaccharide) matrix, known as glycocalyx, forming lobed and unlobed spherical masses (Curtis 1969; Buchanan and Gibbons 1975; Gray 1982a). The glycocalyx anchors the bacteria and helps in the removal of complex organic and inorganic materials (Bitton 1994). Since the proposal of *Z. ramigera* in 1868 (Itzigsohn 1868), there has been much confusion regarding both the genus and its species as all the original type cultures have been lost, although new strains have been proposed as neotypes (Crabtree and McCoy 1967; Zvirbulis and Hatt 1967). These strains are non-proteolytic, oxidise carbohydrates but are unable to ferment them. Ammonia is utilised as a sole source of nitrogen in the presence of vitamin B_{12}. However, zoogloeal forms are so widespread it would appear that they have an ability to utilise other nitrogen sources. Friedman and Dugan (1968) have isolated several *Zoogloea* strains, which although morphologically distinct from the neotype proposed by Crabtree and McCoy (1967), are biochemically similar. Unz and Dondero (1967a,b) isolated 65 *Zoogloea* strains using single cell isolations taken from naturally branching wastewater zoogloeal masses, all of which were able to reduce nitrate to nitrogen, possessed urease, catalase and an oxidative reaction, but could not produce acid from carbohydrates. It would appear, therefore, that zoogloeal bacteria in terms of wastewater treatment describes all non-fermentative bacteria forming zoogloeal masses or flocs (Gray 1985a). One of the *Z. ramigera* strains isolated by Unz and Dondero (1967a) has been accepted as the neotype strain (106, ATCC 19544) (Unz 1984). However, while the neotype strain is related to the bacteria within zoogloeal projections, it is not related to other bacteria previously termed *Z. ramigera*. (Shin *et al.* 1993; Rossell-mora *et al.* 1995). Lu *et al.* (2001) have evaluated a number of immunological detection methods for *Z. ramigera*.

These were developed using polyclonal antibodies against the cells or the isolated exocellular polymer of the neotype strain 106(ATCC 19544) allowing detection of *Z. ramigera* in environmental samples. They found the neotype strain in all wastewater treatment systems as well as in all eutrophic and mesotrophic lakes. Rossello-mora *et al.* (1995), examining activated sludge flocs, found that up to 10% of the bacteria reacted to a fluorescein-labelled oligonucleotide probe complementary to 16S rRNA of *Z. ramigera* 106(ATCC 19544), showing the importance of this bacterium to wastewater treatment.

Fungi

Heterotrophic fungi can be as efficient as heterotrophic bacteria in the removal of organic matter from wastewater (Tomlinson and Williams 1975). But fungi produce a greater biomass per unit weight of substrate utilised than many of the important bacteria in wastewater treatment units, which results in more film and sludge production per kg BOD removed. Although this is disadvantageous in conventional treatment systems, this higher biomass production from fungi is potentially extremely useful in the recovery and reuse of energy-rich substrates discharged from the food-processing industries (Sec. 10.3).

A range of recovery and isolation techniques have been used which are fully reviewed by Cooke (1963) and Tomlinson and Williams (1975). Fungi are allowed to colonise a new substrate placed in the treatment units, such as glass slides (Tomlinson 1941) or a bait, such as hemp seeds. They are subsequently isolated from this substrate using special agar medium such as rose-bengal agar which contains aureomycin to reduce bacterial contamination on plates. However, a number of fungi, in particular *Subbaromyces splendens* and *Sepedonium* spp., are very sensitive to antibiotics and are not recovered by this method. A more recent technique is to plate out the actual film or sludge floc directly on to the medium using the ring-plate method. The material is collected from the treatment plant and scanned under the microscope so that any macro-invertebrates can be removed. It is then placed within the glass ring which stands on three glass bead feet which is set into modified Czapek-Dox agar, which does not contain any antibiotics. The bacteria are physically retained within the ring, whereas the fungi are able to grow through the medium between the feet of the glass ring and colonise the outer areas of the plate (Tomlinson and Williams 1975). Estimation of fungal abundance is difficult. Direct counts have been used but problems in the interpretation as to what constitutes a viable growth

unit is difficult (Gray 1983b). As each intact cell could potentially give rise to a new colony, direct counts are affected by the degree that film or sludge samples have been broken down during collection and microscopic preparation. A more accurate method is to measure the concentration of muramic acid that will give an accurate estimation of fungal activity.

Identification of fungi is difficult as most species produce extensive vegetative growth in the presence of wastewater but rarely produce spores. Some common wastewater fungi rarely produce spores even in pure culture. Identification is made mainly on morphological characters using the laboratory guide to fungi in polluted water by Cooke (1963) and the excellent taxonomic summaries and photographs produced by Tomlinson and Williams (1975), and the drawings by Dr Irene Williams of Aston University (Fig. 4.15). Examination of species in pure culture may also be useful, especially to distinguish between superficially similar species, such as *Subbaromyces splendens* and *Ascoidea rubescens*. Some species are morphologically very distinct, such as *Leptomitus*, which is aseptate and has intermittent constrictions in the hyphae and contains spherical cellulin plugs, whereas other species, such as *Fusarium aquaeductuum* and *S. splendens*, often produce conidia within the treatment plant so aiding identification. As a general guide, *Geotrichium candidum*, *Sepedonium* sp., and *F. aquaeductuum* all form visable mats over the surface of the medium in percolating filters. *Geotrichium candidum* forms a soft textured mat, grey to brown in colour, which loosely follows the contours of the medium, whereas *Sepedonum* sp. forms much thicker and tougher mats which often have a leathery appearance and are olive-green to yellow-grey in colour. *Fusarium aquaeductuum* also form mats that have a distinctive orange to pink colouration, but can also form discrete lumps of growth on the sides of the medium or as fluffy growths in surface pools when the interstices are blocked. This discrete growth pattern is the normal growth form for *S. splendens* and *A. rubescens. Leptomitus lacteus* and *Trichosporon cutaneum* have never been recorded as forming visible growths in percolating filters and thus, can only be seen under the microscope. A simple key to these common fungi, which regularly occur in percolating filters, is given in Table 3.7. Well over 100 fungi have been isolated from wastewater treatment plants (Cooke 1958), and although the majority of these species will have entered the plant with the influent, few can survive or flourish within the treatment units. For example, of the 112 species Cooke isolated from percolating filters, only 29 were isolated frequently enough or in sufficient numbers to be considered as permanent members of the microbial community. Furthermore, few appeared to be important components

Table 3.7. A simple guide to the major fungi occurring in percolating filters.

1.	Hyphae:	Non-septate ... 2
		Septate ... 3
2.	Non-septate hyphae with constrictions	*Leptomitus lacteus*
3.	Septate hyphae with conidia or arthrospores:	Absent ... 4
		Present ... 5
4.	No conidia or arthrospores formed but chlamydospores present	*Sepedonium* sp.
5.	Conidia formed:	Conidia boat-shaped and septate (25–35 × 4–5 μm) ... *Fusarium aquaeductuum*
		... Conidia formed singly, oval shaped (35–40 × 10–15 μm), non-sepatate, produced on sparingly branched conidiophores ... *Subbaromyces splendens*
		... Conidia formed successively at conidiophore apex to form tight clusters, conidiophore unbranched, conidia less oval than *S. splendens* but more spherical (20–25 × 12–15 μm), endoconidia formed ... *Ascoidea rubsescens*
	Arthrospores formed: ...	Blastospores present ... *Trichosporon cutaneum*
		Blastospores absent ... *Geotrichium candidum*

of the film. Only two species were considered to be primarily associated with wastewater treatment plants, *Ascodesmis microscopia*, which is only known from mammalian dung and *Subbaromyces splendens*, which has only been isolated from percolating filters and its natural habitat remaining unknown (Hesseltine 1953), the other species are equally abundant in natural habitats.

The origin of sewage and other wastewaters is such that viable units, either as hyphal fragments or spores, of a wide range of fungal species will be present. The conditions in the aerobic treatment units of excess substrate and nutrients plus sufficient oxygen ensures that some of these species will colonise and successfully develop. Fungi are commonly associated with all wastewater units, including sludge tanks and anaerobic digesters, although most of the records relate to their occurrence in the film of percolating filters. Large amounts of fungi are considered to be undesirable in all types of fixed-film reactors and percolating filters because they cause blockage of the interstices between the stone medium (Fig. 4.4), which impedes drainage and aeration and eventually leads to ponding (Sec. 4.1). Fungal films are tougher than predominantly bacterial films and are less readily sloughed off. All the fungi have seasonal patterns of growth reaching a peak in winter or early spring and disappearing or becoming scarce by mid-summer. Most of the fungi that flourish in the fixed-film reactors show largely or entirely vegetative growth with spores being rare. At the filters in Minworth (Birmingham), six fungi accounted for the majority of the mycelium in filter film, these were *Ascoidea rubescens*, *Fusarium aquaeductuum*, *Geotrichium candidum*, *Sepedonium* sp., *Subbaromyces splendens*, and *Trichosporon cutaneum*. Other fungi that were also present, but much less common, were *Phoma* and the phycomycetes *Leptomitus lacteus* and *Pythium gracile*. Fungal filaments are often observed in activated sludge flocs, although fungi are rarely dominant. However, fungi are especially associated with industrial effluent and will grow profusely if the bacterial component is inhibited, especially by low pH conditions. When fungi dominate, the reduced density of flocs reduces their settling velocity and may eventually lead to bulking. In a survey of activated sludge fungi, Cooke and Pipes (1968) found that 90% of the species isolated belonged to four common genera. *Geotrichium candidum* was the most abundant fungal species recorded being present in nearly all the activated sludge units examined together with a species of *Trichosporon* (Table 3.8). Predacious fungi are common in treatment units. They either capture prey by modified hyphal traps or parasitise hosts via a range of conidial adaptations, attacking a wide range of mesofauna in particular, nematodes, protozoans, and rotifers (Gray 1984a).

Table 3.8. Occurrence of the major wastewater fungal species in the activated sludge and percolating filter processes (+, presence; − absence).

Fungi	Activated sludge	Percolating filtration
Ascoidea rubescens	−	+
Fusarium aquaeductuum	−	+
Geotrichium candidum	+	+
Leptomitus lacteus	+	+
Sepedonium sp.	−	+
Subbaromyces splendens	−	+
Trichosporon cutaneum	+	+

Table 3.9. Maximum theoretical growth rates (μ_{max}), doubling times (t_d), and saturation constants (k_s) of six filter fungi (Tomlinson and Williams 1975).

Fungus	Medium	μ_{max} (h^{-1})	t_d (h)	k_s (g l^{-1})	k_s (BOD mg l^{-1})
Sepedonium sp.	CYG	0.124	5.6	2.5	1850
	CYGS	0.167	4.2	1.25	900
Subbaromyces splendens	CYG	0.0628	11.0	3.84	2460
	CYG	0.077	9.0	1.54	990
Ascoidea rubescens	CYG	0.0887	7.8	1.16	1000
	CYG	0.053	13.1	1.54	1375
Fusarium aquaeductuum	CYG	0.188	3.7	0.435	278
Geotrichum candidum	CYG	0.30	2.3	0.875	560
Trichosporon cutaneum	CYG	0.22	3.2	1.0	640
	'Complan'	0.178	3.9	0.182	100

Note: All fungi grown in batch culture in liquid media at 25°C, the flasks continuously shaken.

Fungal growth is similar to that of bacteria, with logarithmic growth occurring in the early stages of colonisation following the Michaelis-Menten equation as expressed by Monod, where the maximum rate of growth occurs when the substrate concentration is not limiting. Maximum theoretical growth rates (μ_{max}), doubling times (t_d), and saturation constants (k_s) for the major fungi are given by Tomlinson and Williams (1975) who were able to express k_s in terms of BOD_5 (mg l^{-1}), (Table 3.9). They estimated expected growth rates of fungi, expressed as doubling times (t_d) using the expression:

$$t_d = t_{dmin}1 + \frac{k_s}{s}(h)$$

Table 3.10. Change in weight of *Sepedonium* and *Subbaromyces* after 3 days at 25°C in sewage diluted with water (Tomlinson and Williams 1975).

BOD mg l^{-1}	*Sepedonium*	*Subbaromyces*	BOD mg l^{-1}	*Sepedonium*	*Subbaromyces*
	Initial wt. (mg)			Initial wt. (mg)	
	3.54	2.46		0.62	0.67
200	4.40	2.46	220	2.93	2.60
100	3.85	2.10	110	1.71	2.46
50	3.15	2.24	55	1.02	1.35
25	3.30	1.88	27.5	0.45	1.00
12.5	2.60	1.80	14	0.32	0.66
			0	0.39	0.55

where t_{dmin} is the minimum doubling time shown in Table 3.9 and s is 250 mg l^{-1}, where *Sepedonium* 33 h (79 h), *Subbaromyces* 80 h (216 or 560 h), *Ascoidea* 58 h (135 h), *Fusarium* 8 h (13 h), *Geotrichium* 7.5 h (9 h), and *Trichosporon* 7.8 h (22 h) at the summer temperature of 25°C (and winter temperature of 12°C). If these values of t_{d} are compared with similar values for zoogloeal bacterial grown at 25°C which ranged from 10–25 h, then clearly only *Fusarium*, *Geotrichium*, and *Trichosporon* are able to compete with bacteria at summer temperatures. Tomlinson (1941) measured the maintenance energy requirements of two fungi by measuring the increase in biomass at different substrate loadings and estimating the minimum loading at which zero growth occurred (Table 3.10). As bacteria have lower k_{s} values than fungi, the growth of bacteria is not significantly affected by reducing the substrate loading below that required for maintenance of fungi. Therefore, in percolating filters weak sewages (BOD $<$ 280 mg l^{-1}) limit the growth of fungi especially at summer temperatures, whereas stronger sewages and lower winter temperatures favour fungi. The fact that excessive fungal growths are rarely reported in American filters is due to the weaker wastewater treated (Sec. 1.2).

3.3.2. *Nutrition*

Domestic sewage, and to a lesser extent wastewater from slaughter houses, breweries, and food-processing industries, contain a rich variety of organic and inorganic compounds including important trace elements and organic growth factors (Sec. 1.2). All the nutritional requirements for bacterial and fungal growth are present in the wastewater. Thus, provided that

the environmental factors are favourable, a wide and diverse range of heterotrophic micro-organisms will develop within the biological reactor of a wastewater treatment plant. The micro-organisms present are not only those species which can metabolise the raw constituents of the wastewater, but also those that utilise the breakdown products of other micro-organisms and species which prey upon the micro-organisms. As already discussed in Sec. 1.3, heterotrophs need two categories of nutrients: those specifically required to produce energy for growth and metabolism; and those chemical elements required for biosynthesis. Although phototrophs utilise light energy in wastewater treatment processes, the chemotrophs are most important and they require a chemical source of energy.

Plant and animal tissue is primarily composed of carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulphur, and bacterial and fungal metabolism will require these specific elements in the greatest quantities. Other chemical constituents are also necessary for successful heterotrophic growth including potassium, magnesium, manganese, and calcium (which are used for the production of enzyme co-factors and in protein construction), trace amounts of iron, cobalt, copper, zinc, and molybdenum (which are important in the production of co-factors for specific enzymes), and a range of other growth factors (Jefferson *et al.* 2001) (Table 3.11). It is interesting that sodium has rarely been shown as an essential element in microbial metabolism, although it is always present in excess in wastewaters. The specific chemical nature of the wastewater will determine the range of heterotrophic species present. For example, the nature of nitrogen will limit the species present as will the C:N:P ratio. Certain industrial wastes are deficient in one or more important elements, e.g. citrus wastes lack nitrogen, whereas coke oven liquors lack phosphorus. Thus, these elements will have to be added as suitable soluble salts if biological treatment of wastewater is to be successful (Painter 1983; Beardsley and Coffey 1985). In their extensive study on nutrient and micronutrient balancing of influent wastewaters to aid biodegradability, Jefferson *et al.* (2001) recommended that biological treatment can normally be maximised by nitrogen and phosphorus balancing followed by supplements of zinc and copper. However, nutrient balancing and micronutrient addition must be tailored to each individual wastewater.

Carbon

Most aerobic heterotrophs utilise the same compound or compounds for both the energy and carbon source. A wide variety of organic compounds

Table 3.11. Microbial nutrient requirements and concentrations present in domestic wastewaters (Jefferson *et al.* 2001).

Nutrient	Reported requirements (mg l^{-1})[a]	Role of trace metal[a]	Concentration detected (mg l^{-1})	
			Real greywater	Synthetic greywater
N, P, S	15, 3, 1 min.[b]	—	5.00, 1.37, 16.3	5.00, 0.047, 17.5
Ca	0.4–1.4	Cell transport systems and osmotic balance in all bacteria. Bridging anionic ECP and aiding flocculation. Increase growth rates. Requirements and effects vary. Interacts with other metals.	47.9	47.0
K	0.8–> 3.0	Cell transport systems and osmotic balance in bacteria.	5.79	3.96
Fe	0.1–0.4	Growth factor in bacteria, fungi and algae. Adsorbed in proportion to the concentration available. Electron transport in cytochromes. Synthesis of catalase, peroxidase and aconitase. Ion reduction for floc formation.	0.017	0.009
Mg	0.4–5.0	Enzyme activator for a number of kinases and phosphotransferase in heterotrophic bacteria.	5.29	5.02
Mn	0.01–0.5	Activates bacterial enzymes. Often interchangeable with magnesium in kinase reactions. Lower affinity for binding sites than other metals but still can inhibit metabolism at 1 mg l^{-1}.	0.04	0.02
Cu	0.01–0.5	Bacterial enzyme activator required in trace quantities. Can inhibit metabolism. Chelates other substances, reducing their toxicity.	0.006	0
Al	0.01–0.5	Not known. Affects the species found in sludge.	0.003	0

Table 3.11. (*Continued*)

Nutrient	Reported requirements (mg l^{-1})[a]	Role of trace metal[a]	Concentration detected (mg l^{-1})	
			Real greywater	Synthetic greywater
Zn	0.1–0.5	Bacterial metallic enzyme activator of carbonic anhydrase and carboxypeptidase A. Dissociable on active site of enzymes. Stimulates cell growth. Toxic at 1 mg l^{-1}, especially to protozoa. Can exacerbate toxic effects of other metals and inhibit metabolism.	0.03	0
Mo	0.2–0.5	Molybdenum is a common limiting nutrient.	0	0
Co	0.1–5.0[c]	Bacterial metallic enzyme activator. Dissociable on active site of enzymes. Activates carboxypeptidase for synthesis of vitamin B_{12} (cyanocobalamin) but otherwise toxic. Can inhibit metabolism.	0	0

[a]Burgess *et al.* (1999a).
[b]From COD:N:P ratio, Beardsley and Coffey (1985).
[c]Sathyanarayana and Srinath (1961).

can be utilised, although the majority of species can readily utilise the soluble compounds, such as sugars, organic acids, and amino acids. Most of the naturally occurring sugars, and in particular glucose, are assimilated by bacteria and fungi. However, fats and proteins and their breakdown products are less acceptable, with a varying ability in utilisation between species. Although insoluble and relatively insoluble substances, such as cellulose, lignin, and higher fatty acids, are utilised by specialist micro-organisms, many of these species only utilise them as secondary substrates.

Work on the nutritional requirements of wastewater micro-organisms is limited (Painter 1983), and has been largely restricted to a number of key species that are also important in aquatic ecosystems (Gray 1985a). However, these key species do provide a useful indication of the requirements of aerobic heterotrophic micro-organisms found in wastewater treatment plants. Considerable work done on the utilisation of carbon sources by *Sphaerotilus natans* has been done in laboratory pure culture studies (Mulder and van Veen 1963; Phaup 1968). The carbon sources providing favourable growth are glucose, mannose, galactose, fructose, maltose, lactose, sucrose, mannitol, succinate, lactate, fumarate, pyruvate, acetate, and glycerol. Other compounds, such as amino acids, alcohols, and organic acids, are able to substitute for the carbon requirements if glucose is absent (Scheuring and Hohnl 1956). When *S. natans* and *S. discophorous* are grown in media containing glucose, they only have a limited capacity to oxidise sugars, amino acids, and organic acids (Stokes and Powers 1967), but when grown in the absence of glucose these compounds are readily utilised. These results suggest that represssion of enzyme synthesis is involved. Starch and peptone were used to culture *Sphaerotilus* by Bisset and Brown (1969), and Roberts (1977) observed that both of his strains of *S. natans* could utilise soluble oxidised starch from a paper mill effluent. Roberts also found that high molecular weight polysaccharides (molecular weight $> 10{,}000$) present in the effluent were also utilised by *S. natans* in pure culture. This is clearly due to the mechanism by which the bacterium utilised these large polymeric molecules. Extracellular enzymes are produced to degrade the polymer to a molecular size that is able to penetrate the bacterial cell wall membrane. In batch culture, carried out at comparatively high concentrations, the activity of extracellular amylase is able to build up at a level at which starch can be degraded at an acceptable rate. This is because amylase production is a function of substrate concentration and in batch culture the enzyme remains undiluted. However, at the lower concentrations present in the biological reactor of a wastewater treatment plant, extracellular enzyme production is reduced and may be unable to

build up to effective concentrations of amylase, resulting in very slow starch degradation (Roberts 1978; Gray 1985a). The fungi *Leptomitus lacteus* and *Fusarium aquaeductuum* both utilise fatty acids up to C_8. Glucose and maltose both produce excellent growths of both fungi in pure culture, and xylose and arabinose to a lesser degree. Ethanol supports sparse growth of *L. lacteus*, whereas glycerol is utilised by *F. aquaeductuum*. Pyruvic acid is utilised by both organisms, whereas only *F. aquaeductuum* uses succinic and citric acids. Malic and fumaric acids both inhibit the growth of these fungi. *Ascoidea rubescens*, *Geotrichum candidum*, and *Trichosporon cutaneum*, like *Fusarium*, are able to utilise glucose, sucrose, arabinose (except *G. candidum*), and glycerol. Growth of *Subbaromyces splendens* is not stimulated by glucose but, together with *Sepedonium*, grows extremely well on complex organic compounds, with high molecular weights, such as casein. *Sepedonium* grows well on starch as it has levels of strong amylase activity (Tomlinson and Williams 1975). Full details of the carbon substrates utilised by wastewater fungi are given by Tomlinson and Williams (1975).

Nitrogen, basal salts, and other growth factors

Species vary in their ability to utilise forms of nitrogen. Although most bacteria and fungi are able to utilise ammonia as the sole nitrogen source, better growth is usually obtained when an organic nitrogen source is available for use. Growth of *S. natans* is certainly less luxuriant on inorganic nitrogen. Most amino acids support growth (Wilson 1960; Phaup 1968), and in pure culture the nitrogen requirements of *S. natans* can be met using organic nitrogen in the form of amino acids or short-chain peptides (Cawley 1958). However, those that are not suitable or toxic, are serine, histidine, lysine, ornithine, threonine, tryptophane, tyrosine, and valine (Phaup 1968) and, although necessary, most of the amino acids can be toxic in high concentrations. Asparagine, glutamine, aspartic, and glutamic acids are used as both carbon and nitrogen sources, whereas methionine and glycine are only used as a nitrogen source (Mulder and van Veen 1963). The growth of *S. natans* is also promoted when inorganic forms of nitrogen are available, and ammonia, nitrite, and nitrate ions can all be utilised, although vitamin B_{12} is also required under these conditions (Okrend and Dondero 1964; Dias and Huekelekian 1967). The fungi *Geotrichium candidum* and *Trichosporon cutaneum* are both able to utilise ammonia and organic nitrogen, although *Sependonium* sp. and *L. lacteus* are supported only by organic sources of

nitrogen (Schade 1940; Painter 1954; Cantino 1966). *Ascoidea rubescens* and *Subbaromyces splendens* both require an organic source of nitrogen for growth, whereas *F. aquaeductuum* was the most versatile of the filter fungi being able to utilise nitrate, ammonia, and organic nitrogen (Tomlinson and Williams 1975). Comparing the nutritional requirements of *L. lacteus* and *F. aquaeductuum*, Williams (1983) tested a wide variety of possible nitrogen sources. *Leptomitus lacteus* could not utilise methylamine, acetamide or urea, although *F. aquaeductuum* did use them as sole nitrogen source. Leucine, asparagine, and glutamine were utilised as nitrogen sources by *L. lacteus*, whereas glycine could not be used. *Fusarium aquaeductuum* appears to utilise glutamine and leucine as both nitrogen and carbon sources. The ratio of BOD_5 to N (C:N) should be equal to, or less than 18 if the nitrogen requirements of micro-organisms are to be fully satisfied and optimum microbial growth is to occur. As the ratio increases above 22 then BOD_5 removal efficiency will begin to fall off as microbial activity becomes nitrogen-limited. Under high C:N conditions, there will be a tendency for increased filamentous growth in activated sludge tanks, possibly resulting in bulking (Hattingh 1963a,b).

A large number of other elements are also required, although the concentrations required for optimum growth depends on species and other factors, such as growth rate. Phosphorus is required in comparatively large quantities, although at much lower levels than nitrogen. Phosphates are common constituents of sewage and satisfy all the requirements of the micro-organisms provided that the BOD:P (C:P) ratio is less than 90–150. If the C:P ratio rises above 165–170 then microbial treatment efficiency in terms of BOD_5 removal falls, resulting in enhanced filamentous growth and possible bulking in an activated sludge plant (Greenberg *et al.* 1955; Hattingh 1963a,b). Laboratory growth studies of *S. natans* have shown that the bacterium requires a basal salt supplement of sodium, potassium, calcium, magnesium, phosphate, sulphate, and chloride (Gray 1985a). Iron is required in trace amounts but is toxic to *S. natans* in low concentrations (Waitz and Lackey 1959), and Razumor (1961) used this relationship with iron to separate *Sphaerotilus* from *Leptothrix* and *Cladothrix*. Dias and Dondero (1967) found that both *S. natans* and *S. discophorous* required calcium. Using a continuous-flow apparatus, they found that calcium is required for the development of the sheath (Dias *et al.* 1968). The percolating filter fungus *Sepedonium* sp. requires calcium (5.0–12.5 mg l^{-1}), zinc (0.5–1.0 mg l^{-1}) and trace amounts of iron, copper, and manganese for optimum growth (Painter 1954). Imbalances of trace metals in particular,

that often results when metal wastes are present, can reduce the diversity of micro-organisms present in sludges and filter film and can result in excessive growths of tolerant species that can cause bulking or ponding (Pfeffer *et al.* 1965).

Some micro-organisms require organic growth factors such as B-group vitamins, which act as co-enzymes or enzyme precursors, although many species are able to synthesise their own growth factors. Agricultural wastes, domestic sewage, and some industrial wastes contain adequate concentrations of required growth factors. Sewage, in particular, is rich in B-group vitamins, and riboflavin, nicotinamide, pantothenic acid, thiamine, and biotin have all been isolated (Painter 1983). Painter (1954) demonstrated that sewage also contained other growth factors by showing that enhanced growth by *Sepedonium* sp. was achieved by the addition of small amounts of sewage to growth medium containing all the B vitamins and a number of other known growth factors. *Zoogloea* spp. and *Comamonas* spp. isolated from activated sludge, required either amino acids or vitamins, or both, and that biotin, thiamine, and cobalamine were the most frequently required growth factors (Painter 1983). Laboratory growth studies indicate that *S. natans* does not require a supply of accessory growth factors apart from trace amounts of vitamin B_{12} (cyanocobalamin) or methionine, from which B_{12} can be synthesised (Okrend and Dondero 1964). Vitamin B_{12} was shown to be necessary for the growth of 34 *Sphaerotilus* and *Leptothrix* strains, although it could be replaced by much higher concentrations of methionine. The presence of vitamin B_{12} was shown to be necessary for the utilisation of inorganic nitrogen by *S. natans*, whereas *S. discophorous* had a growth requirement for adenine, guanine, thiamine, and biotin as well as vitamin B_{12}. *Sphaerotilus natans* does not require any growth factor except vitamin B_{12}. Among those tested and found not to be required were thiamine, riboflavin, nicotinic acid, pryridomine, pantothenic acid, biotin, folic acid, *p*.-amino benzoic acid, adenine, xanthine, guanine, and urea (Mulder and van Veen 1963). *Fusarium aquaeductuum* does not require thiamine and, like *Geotrichium candidum*, is independent of an external supply of B-group vitamins, although both *T. cutaneum* and *Sepedonium* sp. require thiamine, with the latter also requiring biotin (Painter 1954). Although a number of the more common wastewater micro-organisms have a requirement for external sources of vitamins and other growth factors, the majority do not. Therefore, any deficiency would only result in a slight reduction in species diversity, and treatment efficiency would remain unimpaired (Painter 1983).

3.3.3. *Environmental factors*

Dissolved oxygen

Dissolved oxygen serves only as an electron acceptor for heterotrophic aerobes in wastewater. In terms of substrate utilisation and energy production, aerobic heterotrophs are far more efficient than anaerobic micro-organisms (Fig. 3.4). The facultative micro-organisms also grow better and more efficiently in the presence of oxygen. The growth rate of an aerobe increases with the concentration of oxygen until a critical dissolved oxygen concentration is reached, at which time maximum growth rate occurs under prevailing conditions and no further increase is possible. The critical concentration is normally below 1 mg l^{-1} for dispersed bacteria, but as the critical concentration is governed by the rate of diffusion and the diameter of the organism, the critical concentration in the activated sludge process is somewhat higher due to the aggregation of bacteria into flocs. Filamentous fungi also have a critical concentration of > 1 mg l^{-1}.

Whereas percolating filters obtain oxygen from natural ventilation, dissolved oxygen has to be supplied by mechanical means in the activated sludge process. The higher the concentration at which the dissolved oxygen is maintained in the aeration tank, the greater the energy input required. Also, as the oxygen deficit of the wastewater is reduced then the aeration efficiency also declines. The cost of aerating wastewater is extremely expensive and it is important to maintain the wastewater in the activated sludge tank as near as possible to the critical dissolved oxygen concentration in order that maximum micro-organism efficiency can be achieved at minimum cost (Fig. 3.22). Turbulence is an important factor in the activated sludge process as it reduces localised dissolved oxygen and substrate deficiencies. Efficient mixing ensures that the dissolved oxygen and substrate concentration are maintained at maximum concentration, which maintains a maximal diffusion rate through the cell-liquid interface. However, diffusion through flocs of activated sludge require higher critical concentrations of dissolved oxygen to give maximum oxygen uptake, compared with dispersed bacterial cultures where diffusion is simply across the cell wall. For example, in zoogloeal flocs, maximum uptake occurs at dissolved oxygen concentrations of between 0.6 to 2.5 mg l^{-1}, depending on floc size, compared with < 0.1 mg l^{-1} for dispersed zoogloeal cells. However, the normal floc size in activated sludge is such that maximum oxygen uptake occurs at concentrations of 2 mg l^{-1} or less, although a survey of activated sludge plants showed that 75% contained flocs with nominal diameters of < 43 μm, indicating maximum uptake rates to be < 0.6 mg l^{-1} (Muller *et al.* 1968). The dissolved

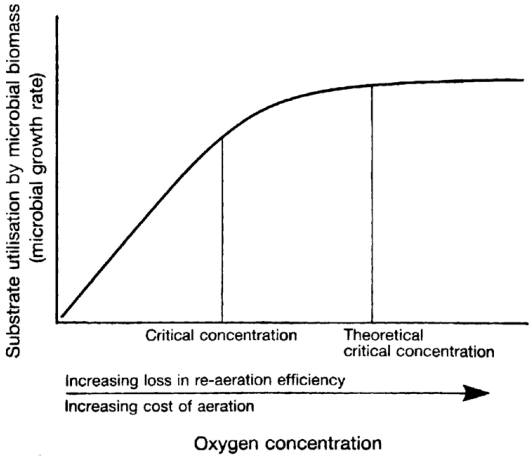


Fig. 3.22. The effect of oxygen concentration on activated sludge activity showing critical and theoretical critical oxygen concentrations.

oxygen concentration in aeration tanks is controlled by oxygen electrodes placed in the tank and linked to the aeration equipment. The desired oxygen concentration in aeration tanks is normally between 0.6–2.0 mg l^{-1}, with 1.0 and 1.2 mg l^{-1} being generally used. Selection is dependent on such factors as tank design, wastewater characteristics, and microbial characteristics, and a precise critical concentration for a specific treatment unit can only be derived from practical operational experience (Sec. 5.2.3). It has been suggested that enhanced agitation encourages the growth of dispersed bacteria and inhibits filamentous forms such as *S. natans*.

In fixed-film reactors oxygen only diffuses to a maximum depth of 0.1–0.2 mm, below which the film is either anoxic or anaerobic. When filamentous fungi are present, the critical depth increases to 2 mm as the diffusion of oxygen is assisted by protoplasmic streaming (Tomlinson and Snaddon 1966). Where the film is well developed and the interstices are small, thus restricting the rate of ventilation, the oxygen concentration can become a limiting factor (Sec. 4.1.4).

Temperature

Temperature has a profound effect on wastewater micro-organisms, not only in governing the rate of reaction (Sec. 3.1.2), but changes in temperature also give rise to significant alterations in the community structure. In terms of removal efficiency, higher temperatures give rise to higher rates of

BOD removal up to 35–40°C, after which bacterial cells become reduced in size and number, thus growing in a dispersed phase and resulting in turbid effluents (Rogovskaya *et al.* 1969). In pure culture, *S. natans* has a wide temperature tolerance ranging from 4–40°C with optimal growth between 25–30°C (Sanders 1982). The rate of metabolism is very much reduced at lower temperatures which is illustrated when the filamentous bacteria form microscopic slime growths (sewage fungus) in polluted rivers. For example, the slime growth in River Altamaha (Georgia, USA) in summer (30°C) was limited to 150–250 m below the effluent source, whereas in winter (10°C) it extended for 24–64 km (Phaup and Gannon 1967). *Sphaerotilus natans* has the ability to out-compete other bacteria for available nutrients at lower temperatures, although fungal components are known to have more rapid growth rates than the bacterial component at reduced temperatures, provided suitable nutrients are available. This is clearly demonstrated by experimental channel experiments where the standing crop of zoogloeal/*Fusarium* slime is closely related to temperature. Growth was initially more rapid at 20°C than at 10°C. However, a much more luxuriant and heavier *Fusarium* growth was eventually established at the lower temperature with the biomass six times greater at 10°C compared with 20°C after 30 days (Ministry of Technology 1970).

The rates of growth of all the major wastewater fungi increase linearly with temperature. At 25°C, which is the maximum summer temperature of wastewater, the fungal growth rate is approximately double the growth rate at 12°C, the average winter temperature. *Fusarium aquaeductuum* and *L. lacteus* both grew well over the range 10–20°C with optimum growth occurring between 15–25°C and 20–25°C respectively (Williams 1983). *Leptomitus lacteus* has been recorded growing in the heated effluent from an alcohol factory at 27°C (Gray 1985a), and Zehlender and Boek (1964) recorded optimum growth at 8°C at pH 6. *Sepedonium* sp. and *G. candidum* have similar optimum temperatures at 20°C and 20–25°C respectively (Tomlinson 1942; Pipes and Jones 1963). Clearly, the fungi in percolating filters do not all have the same temperature range. For example, in contrast to *Sepedonium* sp., *S. splendens* predominates in filters during the summer and autumn because of its high optimum temperature of 25–27°C. Conidia fail to germinate below 5°C and are restricted up to 15°C, limiting the dominance of this fungus to the warmer months, although it is present in filters throughout the year (Williams 1971; Gray 1983b).

Activated sludge liquors are reported as being less filamentous at higher temperatures (Hunter *et al.* 1966) and have been shown to take longer to acclimatise to changes in temperature than to toxic compounds. Complete

acclimatisation takes at least 2–3 weeks and takes up to five times longer at 5°C compared with 30°C (Rogovskaya *et al.* 1969). In percolating filters, maximum microbial activity occurs between 20–35°C, although some yeasts are active up to 40°C. However, the activity of most of the important micro-organisms is inhibited at temperatures < 7°C and > 36°C, although viability of most of the organisms are not lost if the temperature only exceeds these limits marginally for a few days.

pH

The importance of pH on microbial growth has been demonstrated by studies on polluted rivers in Norway. The community structure of heterotrophic slimes was different in rivers with different pH values. For example, the River Dams supported a *S. natans*-dominated slime (pH 7.0), whereas the River Otta (pH 5.8) supported a *Fusarium*-dominated slime (Ormerod *et al.* 1966; Baalsrad 1967). The same affect is found in fixed and mixed biological reactors, with fungi growing over a wider pH range than bacteria dominating at acidic pH values (pH < 6.5), with the bacteria preferring neutral pH values and generally dominating at pH values > 7.0. Domestic wastewaters have a pH range of between 6–8, and are amenable to biological oxidation. However, some industrial wastewaters can be quite acidic or alkaline and need to be neutralised before biological treatment. The optimum pH range for carbonaceous oxidation lies between 6.5 and 8.5.

In pure culture studies, *S. natans* can tolerate a wide pH range (pH 5–10) although most rapid growth occurs between 6.5–8.1, with optimum growth at pH 7.5 and growth inhibited at pH < 6.2. *Zoogloea* spp. are found over a similar range although the pH has been shown to affect the growth form, being dispersed at pH 7 but forming aggregates at pH 6 (Angelbeck and Kirsch 1969). All the fungi can grow over a wide range of pH values with *F. aquaeductuum* (pH 4–9), *G. candidum* (pH 3–9), *L. lacteus* (pH 2.5–7.5), and *T. cutaneum* (pH 4–9) all having wide pH tolerances (Painter 1954; Pipes and Jones 1964; Zehlender and Boek 1964). Although *Sepedonium* sp., which is found over a wider pH range (pH 4–10), has a restricted optimum growth range 7.0–8.5 (Tomlinson and Williams 1975), Tomlinson and Williams (1975) suggest that the effect of pH on *F. aquaeductuum*, *G. candidum*, and *T. cutaneum* depends largely on the composition of the growth medium. They concluded that the common filter fungi can be classified as "colonial" species (*F. aquaeductuum*, *G. candidum*, *T. cutaneum*), which can tolerate low pH values but are susceptible to inhibition by undissociated organic acids and the "spreading" species (*Sepedonium* sp., *S. splendens*, *A. rubescens*), which have a pH optimum in the neutral range.

Light

Heterotrophic micro-organisms are independent of light, often growing in complete darkness within the biological reactor. However, in facultative waste stabilisation ponds (Sec. 6.3.2) light is vital in order for the algae to produce enough oxygen to maintain the aerobic heterotrophic demand. The organic loading controls the ratio of phototrophic to chemotrophic organisms. Reducing the loading will increase the ratio of sunlight energy to chemical energy into the system, increasing the ratio of phototrophic to heterotrophic organisms. With the exception of macrophyte-based systems (Sec. 6.2), plants play only a minor role in biological treatment systems. However, algae and diatoms are commonly recorded on the surface of percolating filters, although excessive growths of filamentous algae and mosses can result in a reduction in performance by causing filters to pond (Benson-Evans and Williams 1975; Hussey 1982; Gray 1984b).

Fungi are generally independent of light with the exception of *F. aquaeductuum*. Both conidia formation and pigmentation in this species are light-dependent, the pigment being a photo-induced carotenoid (Rau 1967), which is why this species is restricted to the surface of percolating filters.

3.3.4. *Inhibition*

Many heavy metals and organic compounds are toxic to aerobic heterotrophs, both in pure culture and in the treatment plant. However, in the mixed culture of micro-organisms that makes up activated sludge, these toxic compounds need to be at far greater concentrations compared with pure cultures before any inhibitory effect is noticed. This is due to physical adsorption of the compounds onto organic matter and flocs, and also due to chemical reactions such as precipitation or chelation with other constituents of wastewater including other toxic substances. However, as the concentration of the toxin increases, the inhibition or life processes becomes increasing severe until the cells eventually die and lyse. When treating wastewater containing inhibitory substances, some degree of acclimatisation and selection of tolerant species will occur. However, the performance of such systems will rarely be as good as toxic-free systems (Moulton and Shumate 1963). Metals such as copper and mercury are particularly toxic, complexing with enzymes and other metabolic agents connected with respiration and rendering them inactive. The common filter fungus *Sepedonium* sp. is surprisingly sensitive to zinc, being inhibited at concentrations of between 4–10 mg l^{-1}, when other trace metals are also deficient in the medium.

Other substances, especially organic complexes containing nitrogen, and occasionally sulphur, compete with enzymes for essential metals which act as co-enzymes and catalysts, whereas phenols and detergents act by transforming the cell or causing it to disintegrate (Painter 1983). Wastewaters with high concentrations of salts or nitrogen compounds also inhibit biological treatment processes and affect the aerobic heterotrophs, in particular. Salt concentrations of 30,000 mg l^{-1} severely inhibit the activated sludge processes due to osmotic stress (Kincannon and Gaudy 1966), although shock loads of sodium chloride have little effect on the micro-organisms forming the film in percolating filters (Gray 1981). *Fusarium aquaeductuum*, *G. candidum*, and *T. cutaneum*, which are able to use ammonia as a sole nitrogen source, are not inhibited by ammonia at concentrations up to 2,000 mg l^{-1}. However, *A. rubescens*, *Sepedonium* sp., and *S. splendens*, which all require an organic nitrogen source, are inhibited by ammonia. Many bacterial species cannot tolerate high ammonia concentrations and the activated sludge process is severely inhibited if ammonia is present in excess. Anionic detergents, which are normally present in concentrations of 35 mg l^{-1} as Manoxol OT in domestic sewage, inhibit a wide range of heterotrophs. At concentrations of 5 mg l^{-1} there is little inhibitory effect on fungi, but at concentrations in excess of 20 mg l^{-1} many fungal species are inhibited or eliminated. *Sepedonium* sp. is very susceptible to concentrations of detergents above 10 mg l^{-1} and *S. splendens* is affected at concentrations in excess of 30 mg l^{-1}, when conidia formation is also inhibited, which is probably why conidia are so rarely seen in filters treating domestic sewage. *Ascoidea rubescens* is unaffected by anionic detergents up to 50 mg l^{-1} and *T. cutaneum* is also very tolerant (Tomlinson and Williams 1975). *Geotrichum candidum* and *F. aquaeductuum* are able to inhibit the growth of certain other fungi by the production of antibiotics. *Geotrichum candidum* inhibits the growth of *F. aquaeductuum* and *T. cutaneum*, and *F. aquaeductuum* inhibits *Sepedonium* sp., *S. splendens*, and *T. cutaneum* (Table 3.12).

Further reading

General: Haddock and Hamilton 1977; Painter 1983; Bitton 1999.
Bacteria: Phaup 1968; Pike 1975; Painter 1983.
Fungi: Tomlinson and Williams 1975.
Nutrition and environmental factors: Tomlinson and Williams 1975; Painter 1983; Bitton 1999; Jefferson 2001.

Table 3.12. Interactions between five species of fungi on nutrient agar plates (Tomlinson and Williams 1975).

	Se	*Su*	*G*	*F*	*T*
Sepedonium (*Se*)		=	=	>	=
Subbaromyces (*Su*)	=		=	>	=
Geotrichum (*G*)	=	=		<	<
Fusarium (*F*)	<	<	>		<
Trichosporon (*T*)	=	=	>	>	

=, indicates no interaction; >, indicates that organism in vertical column was inhibited by organism in horizontal column; <, indicates that organism in horizontal column was inhibited by organism in vertical column.

3.4. Anaerobic Heterotrophic Micro-organisms

3.4.1. *Introduction*

Anaerobic heterotrophic bacteria are either obligate and unable to grow in the presence of oxygen, or facultative and can adapt to environments either with or without oxygen. The latter form a bridge between obligate aerobes and obligate anaerobic species. Growth and metabolism of obligate anaerobes are inhibited by oxygen, with oxygen toxicity depending on the redox potential (E_{h}), partial pressure, composition of the substrate, growth rate, and cell density (Hughes 1980).

The major role of anaerobic heterotrophs is in sludge digestion converting unstable sewage into a more stabilised form. Digestion normally takes place in specially constructed reactors, although anaerobic digestion also occurs in the sludge blanket of waste stabilisation ponds. The pressure of anaerobic activity in aerobic treatment processes is generally undesirable with the commonest effects of anaerobosis being the production of foul odours and interference of floc settling due to denitrification occurring in the sedimentation tank after the aeration basin in the activated sludge process.

Anaerobic treatment is an effective method for the complete treatment of many organic wastes, especially animal wastes and organic effluents from the food processing industries (Stafford *et al.* 1980). The organic substrate is degraded in the absence of oxygen to carbon dioxide and methane with only a small amount of bacterial growth. Approximately 90% of the available chemical energy, in the form of organic material, is retained as

methane production (McInnery *et al.* 1980). Apart from the economic value of the methane gas produced, anaerobic treatment has many advantages over aerobic treatment processes, such as less biomass produced per unit of substrate utilised (the lower biomass production means a lower requirement for nitrogen, phosphorus, and other nutrient and growth factors), higher organic loadings are possible as anaerobic processes are not limited by oxygen transfer rates, and the lower constructional and operational costs compared with aerobic processes. However, the major disadvantage in temperate and colder climates is the elevated temperatures required to maintain microbial activity at a reasonable level.

3.4.2. *Presence in the treatment plant*

Wastewater

Anaerobic bacteria are common in sewage and other wastewaters. They originate from the human intestine, although other sources include land drainage, stormwater, and biological processes in industry. In sewage, the faecal bacteria outnumber the other micro-organisms present, although no really satisfactory methods of counting anaerobic bacteria have been developed. But as coliform counts are in excess of 10^6–10^7 ml^{-1} in sewage, and coliforms only represent a small portion of total anaerobic bacteria, the total count must be very high.

Treatment plant operators try to prevent incoming wastewater from becoming anaerobic. Not only because this makes it more difficult to treat but to prevent anaerobic activity within the sewers. Anaerobic activity is usually indicated by offensive smells such as amines, skatole, and, more commonly, hydrogen sulphide (Table 1.18). These gases have to be vented from the sewer as they are extremely toxic to men working in the sewer environment and can also cause corrosion of concrete pipework.

Primary sedimentation tanks

The wastewater in primary sedimentation tanks is normally aerobic and inhibits the anaerobic bacteria present. The retention times in the tanks are normally < 8 h, which is not long enough for the sulphide bacteria to build up. Therefore, odours are rarely produced unless the wastewater is already anaerobic when entering the treatment works. When desludging is irregular or the radial scrapers are not effective, anaerobic fermentation can occur in the compacted sludge layer causing the sludge to rise.

Aerobic treatment units

Although present, obligate anaerobes are not active in aerobic treatment systems and only dominate when anaerobic conditions exist. In percolating filters anaerobic conditions only occur as a result of gross overloading when the interstices of the medium become blocked by excessive film accumulation; or after a toxic shock which kills and subsequently inhibits the aerobic micro-organisms. In the activated sludge process, anaerobic conditions occur because of factors such as poor design, insufficient aeration, and gross overloading. It is less likely for a percolating filter to become completely anaerobic; therefore some treatment capacity will normally always remain. However, once an activated sludge aeration unit becomes anaerobic the entire treatment capacity of the system is lost, which results in foul odours and sludges with extremely poor settleabilities. The role of facultative bacteria in aerobic systems is unclear, and although they are capable of utilising oxygen they have not been recorded as dominant in the activated sludge processes (Lighthart and Oglesby 1969).

Percolating filters and, to a lesser extent sludge aeration basins, provide a wide diversity of micro-habitats for micro-organisms and therefore there are microniches where anaerobic bacteria can flourish, albeit in small numbers. Oxygen can only diffuse to a maximum depth of 0.2 mm into filter film and the inner layers are normally anaerobic (Sec. 4.1.1). The centre of activated sludge flocs can also be anaerobic because of the problem of oxygen diffusion, which is reduced considerably as the oxygen concentration in the aeration basin falls below 1 mg l^{-1} (Sec. 5.5).

Final settlement tanks

It is in the final settlement process that anaerobic bacteria can most frequently cause problems. In the final settlement tank of the activated sludge process, solids are retained for only a short time in order to maintain the aerobic character of the sludge, which is returned to maintain the microbial density in the aeration basin. Two problems may arise. If the sludge is not removed continuously or very frequently, but instead allowed to remain in the tank, then facultative anaerobes begin to multiply and can become incorporated into the activated sludge thus reducing efficiency. If anaerobic conditions are allowed to develop, gas will be produced and floc particles will rise to the surface because of bubbles becoming entrapped or attached to particles. This is nearly always due to the conversion of nitrate, formed by nitrification in the aeration tank, to nitrogen gas, a process known as denitrification (Sec. 3.4.5).

Anaerobic processes

It is in the digestion of sludge, in specially designed anaerobic reactors (fermentors or digesters), that the activities of anaerobic micro-organisms are used to advantage in wastewater treatment (Sec. 7.2). The settled sludge, from both primary and final sedimentation tanks, is converted from its highly putrid state to a stable and disposable product that neither smells nor undergoes further decomposition on storage. The main advantages of treating sludges anaerobically include: the high degree of stabilisation; the low production of extra biological sludge; the low nutrient requirements; no oxygen requirement; and the production of useful end-products, in the form of methane and a useful biomass.

The main constituents of raw sludge are protein, fats, and polysaccharides. Typical chemical analysis of a sewage sludge is protein (25%), cellulose and lignin (25%), and fats (20%) (Heukelekian 1957). If anaerobic breakdown is complete then the end-products will be methane, carbon dioxide, water, and new bacterial cells. However, incomplete breakdown due to environmental conditions or inhibitors could result in intermediate products being formed, such as volatile fatty acids, alcohols, and ammonia.

3.4.3. *Anaerobic digestion*

The process of sludge digestion is generally considered as a two-phase process — the non-methanogenic followed by a methanogenic phase. It is more

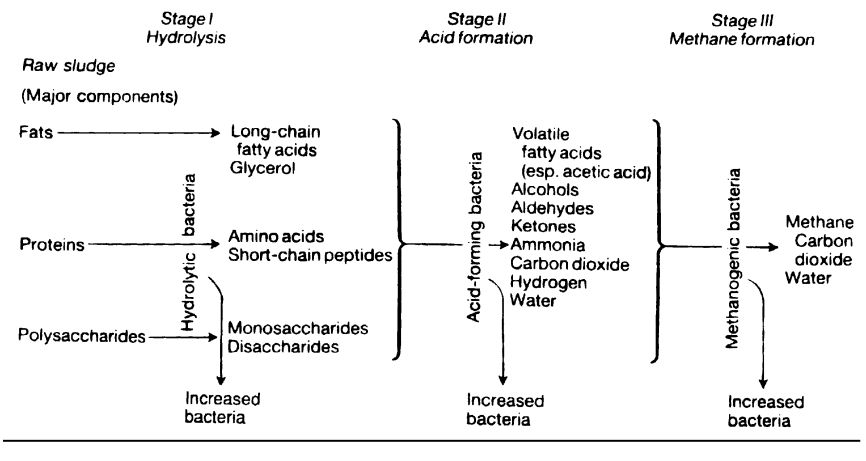


Fig. 3.23. Three stages of sludge digestion. Some hydrolytic bacteria are known to be able to carry out both stages I and II.

accurate to describe the process of anaerobic digestion as being comprised of three discrete stages; hydrolysis and acid formation (the non-methanogenic phase), followed by methane formation (methanogenic phase) (Fig. 3.23).

$$\text{Insoluble organics} \xrightarrow[\text{bacteria}]{\text{Hydrolytic}} \text{Soluble organics} \xrightarrow[\text{bacteria}]{\text{Acid forming}}$$

$$\text{Volatile acids} \xrightarrow[\text{bacteria}]{\text{Methanogenic}} \text{Gases}$$

It is convenient to think of these stages as different trophic levels, and although all three stages are normally occurring simultaneously within a digester, the micro-organisms involved at each stage are metabolically dependent on each other for survival. For example, the methanogenic bacteria require the catabolised end-products of the acid-forming bacteria. However, the latter species would eventually become inhibited by their own end-products if these were not degraded by the methanogenic bacteria. Although bacteria are the major group of micro-organisms involved in anaerobic digestion, fermentative ciliate and flagellate protozoa and some anaerobic fungi also occur (Hobson *et al.* 1974). The process does not readily occur in the presence of electron acceptors such as oxygen, sulphate, or nitrate. Energy transformation is by the ATP system with energy being stored by the reaction of ADP and inorganic phosphate (P_i) to form ATP. The energy conserved in the pyrophosphate bond is used by splitting ATP into either ADP + P_i or adenosine-5-monophosphate (AMP) and pyrophosphate (PP_i).

In the first stage, the major substrates in the sludge are hydrolysed to basic components; proteins to amino acids, fats to glycerol and long-chain fatty acids, and polysaccharides to mono- or disaccharides (Fig. 3.23).

Proteins are hydrolysed to smaller units such as polypeptides, oligopeptides, or amino acids by extracellular enzymes called proteases, which are produced by only a small proportion of the bacteria. The majority of bacteria are able to utilise these smaller peptides or the amino acids, which pass through the cell wall and are broken down intracellularly. The production of protease is far in excess of that required, even though protease-producing bacteria represent such a small percentage of the total bacteria present. Estimates indicate that this over-production could be in the order of 50 times more than is required (Hattingh *et al.* 1967). The most active proteolytic bacteria are the spore-forming *Clostridium* sp. In anaerobic sludge, proteolytic bacteria can reach concentrations of 6.5×10^7 ml^{-1}, of which 65% of the isolates examined by Toerien (1970) were spore formers, 21% cocci, and the remainder non-sporing rods and bifid-like bacteria. Little

Table 3.13. Enumeration and identification of anaerobic bacterial populations in sewage sludge digesters (Zeikus 1980).

Group	Numbers (per ml)	Generic identity
Hydrolytic bacteria		Majority unidentified
Total	10^8–10^9	Gram-negative rods
Proteolytic	10^7	*Eubacterium*
Cellulolytic	10^5	*Clostridium*
Hydrogen-producing acetogenic bacteria	10^6	Unidentified Gram-negative rods
Homoacetogenic bacteria	10^5–10^6	*Clostridium* *Acetobacterium*
Methanogens	10^6–10^8	*Methanobacterium* *Methanospirillum* *Methanococcus* *Methanosarcina* '*Methanothrix*'
Sulphate reducers	10^4	*Desulfovibrio* *Desulfotomaculum*

is known about the lipolytic bacteria even though they have been shown to be highly effective in anaerobic digesters (Crowther and Harkness 1975). They are present in densities of up to 7×10^4 ml^{-1} and the addition of vegetable oil to digesters to enhance gas production is commonly practised in some countries. Anaerobic cellulolytic bacteria are present in anaerobic sludge at concentrations of between 10^4–10^5 ml^{-1} and are predominantly Gram-negative coccobacci, with *Bacteroides ruminicola* being a particularly common species. Other species, including a Gram-positive species forming curved rods which formed short chains, have been isolated by Hobson and Shaw (1971). In sewage sludge, the ability to hydrolyse starch is the most common activity of these bacteria (Table 3.13).

The heterogeneous group of facultative and anaerobic bacteria, which are responsible for hydrolysis, are also responsible for acid formation. In this second stage, the hydrolysed substrate is converted to organic acids and alcohols, with new cells also being produced. Various biochemical pathways are utilised, including fermentation and β-oxidation. There is very little stabilisation of the substrate in terms of BOD_5 or COD removal, with the products of acid fermentation being large organic molecules. Obligate anaerobic bacteria occur in much larger numbers than the facultative or aerobic bacteria, with ratios of 1:100 and numbers in the order of 10^7–10^8 ml^{-1} are not uncommon (Kirsch 1968). The major acid-forming

organisms are *Bacillus* sp., *Micrococcus* sp., and *Pseudomonas* sp., although little taxonomic work has been done (Crowther and Harkness 1975). Mono- and disaccharides, long-chain fatty acids, glycerol, amino acids, and short-chain peptides provide the main carbon source for growth, with saturated fatty acids, carbon dioxide, and ammonia being the main end-products. Alcohols, aldehydes, and ketones are also produced but only in minute quantities (Fig. 3.23). The concept of pyruvate as the pivotal compound in metabolism was discussed earlier. When no external electron acceptor is present, as is the case in acid fermentation, pyruvate can undergo several alternative reactions that regenerate NAD from NADH (Fig. 3.17). Acetic, propionic, butyric, and lactic acids are the most frequently produced end-products during stage II and Toerien (1970) found that these acids were produced by 87%, 67%, 10%, and 70% respectively of the 92 acid-forming bacterial isolates he examined. Propionic and longer chain fatty acids are degraded by an intermediate microbial group called the obligate hydrogen-producing acetogenic bacteria, whereas other acid-producing bacteria, referred to as the homacetogenic bacteria, produce acetic and sometimes other acids (McInerney *et al.* 1980).

The third and final stage in anaerobic digestion is methane fermentation where the end-products of acid fermentation are converted to gases, mainly methane and carbon dioxide, by several different species of obligate anaerobic bacteria. In this stage, complete stabilisation of the substrate occurs and as the end-products are only gases it is more efficient than complete aerobic stabilisation. Methane is an ideal end-product as it is non-toxic, easily escapes from the site of production with the use of a separation process, is not very soluble, inert under anaerobic conditions, and can be readily collected and used as an energy source. In the overall anaerobic fermentation of carbohydrates to carbon dioxide and methane, equal volumes of each gas are produced.

$$(C_6H_{12}O_6)_x + xH_2O \rightarrow xC_6H_{12}O_6 \rightarrow 3xCH_4 + 3xCO_2$$

The carbon dioxide evolved only partially escapes as gas, because unlike methane, it is relatively soluble in water. It also reacts with any hydroxide ions (OH^-) in the system to produce bicarbonate ions (HCO_3^-). The evolution of carbon dioxide gas is therefore a function of factors such as pH, bicarbonate concentration, temperature, and substrate composition. The biodegradable protein is deaminated to produce ammonia which reacts with water:

$$NH_3 + HOH \rightleftharpoons NH_4^+ + OH^-$$

This is the major source of hydroxide ions which react with the carbon dioxide evolved during methanogenesis to form bicarbonate ions:

$$CO_2 + HOH \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$H_2CO_3 + OH^- \rightleftharpoons HCO_3^- + HOH$$

Therefore, the protein content of the wastewater substrate will significantly affect the quantity of carbon dioxide actually released from solution as well as the buffering capacity of the system in terms of bicarbonate. The portion of carbon dioxide incorporated in the bicarbonate ion is eventually removed from the reactor in the liquid rather than in the gas phase (Pfeffer 1980).

Anaerobic digestion and methane production are not unique to anaerobic digesters, they occur in natural environments including the digestive tract of most animals, in the sediments of lakes and rivers, and in estuaries, swamps, marshes, and peat bogs. The bacteria responsible for methanogenesis in digesters are similar to those found in other environments, although little is known about them because of the problems of isolating and maintaining cultures of bacteria under anaerobic conditions. Most methanogenic bacteria belong to the genera *Methanobacterium*, *Methanosarcina*, *Methanospirillum*, and *Methanococcus*. The methanogenic genera isolated so far, are limited to the catabolism of either one carbon (e.g. H_2/CO_2, CH_3OH, CO, HCOOH, CH_3NH_2) or two carbon compounds (e.g. CH_3COOH). Methanogens are unusual in that they are composed of many species with very different cell morphology. They require a strict anaerobic environment for growth with a redox potential below −300 mV. They have simple nutritional requirements; carbon dioxide, ammonia, and sulphide. Ammonia is the essential nitrogen source for growth and no methanogenic species are known to utilise amino acids or peptides, sulphide is the most common sulphur source, although some species can use cysteine instead. Methanogens contain a number of unique co-enzymes, for example, co-enzyme 420 which is involved in electron transfer instead of the usual ferredoxin, co-enzyme M which is used in methyl transfer reactions, and factor B which is required for the enzyme formation of methane from methyl co-enzyme M. The synthesis of ATP appears to be via electron transport linked to phosphorylation. The ultrastructure and cellular composition of methanogens make them very different from typical bacteria and some workers have suggested that they are in fact a primitive group of bacteria. The group does not contain muramic acid in their cell walls and this, linked with their unique co-enzymes and the unique oligo-nucleolide

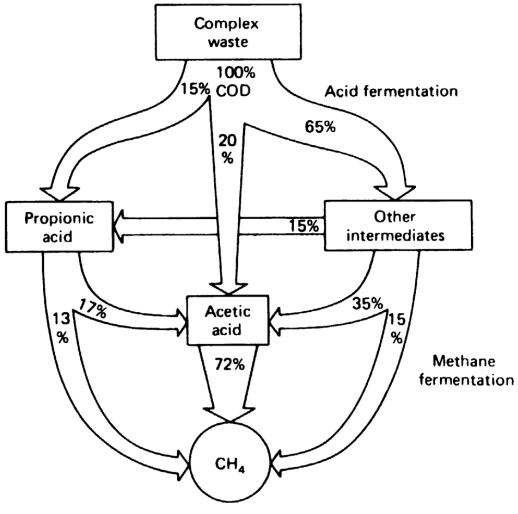


Fig. 3.24. Pathways in methane fermentation of complex wastes. Percentages represent conversion of waste COD by various routes (McCarty 1964).

sequences of the 16S ribosomal RNA molecule, has been used to re-classify the micro-organisms of the group (Table 3.14). Further taxonomic and physiological details are given by Wolfe (1971), Crowther and Harkness (1975), Morris (1975), Thauer *et al.* (1977), and Zeikus (1977, 1980).

There is a close relationship between gas production and the numbers of methanogenic bacteria present in a digester, generally being present in numbers between 10^6–10^8 ml^{-1} (Table 3.13). It is from acetic and, to a lesser extent, propionic acid that the greatest percentage of methane is derived during methane fermentation, whereas formic acid fermentation and methane fermentation associated with β-oxidation of long-chain fatty acids probably accounts for most of the small percentage of methane not derived from acetic and propionic acids (Fig. 3.24).

Methane formation from acetic acid is a single-step process carried out by one group of methanogenic bacteria:

$$\underset{\text{(acetic acid)}}{CH_3COOH} \rightarrow \underset{\text{(methane)}}{CH_4} + CO_2$$

Methane fermentation of propionic acid is a two-step process involving two groups of methanogenic bacteria, with acetic acid as the intermediate

Table 3.14. Proposed taxonomic scheme based on comparative cataloguing of the 16S ribosomal RNA and substrates used for growth and methanogenesis (McInerney *et al.* 1980).

	Type strain	Former designation	Substrates for growth and CH_4 production
Order I. *Methanobacteriales* (type order)			
Family I. *Methanobacteriaceae*			
Genus I. Methanobacterium (type genus)			
1. *Methanobacterium formicicum* (neotype species)	MF	*Methanobacterium formicicum*	H_2, formate
2. *Methanobacterium bryantii*	M.o.H.	*Methanobacterium* sp. strain M.o.H.	H_2
Methanobacterium bryantii strain M.o.H.G.		*Methanobacterium* sp. strain M.o.H.G.	H_2
3. *Methanobacterium thermoautotrophicum*	ΔH	*Methanobacterium thermoautotrophicum*	H_2
Genus II. *Methanobrevibacter*			
1. *Methanobrevibacter ruminantium* (type species)	MI	*Methanobacterium ruminantium* strain MI	H_2, formate
2. *Methanobrevibacter arboriphilus*	DHI	*Methanobacterium arboriphilicum*	H_2
Methanobrevibacter arboriphilus strain AZ		*Methanobacterium* sp. strain AZ	H_2
Methanobrevibacter arboriphilus strain DC		*Methanobacterium* strain DC	H_2
3. *Methanobrevibacter smithii*	PS	*Methanobacterium ruminantium* strain PS	H_2, formate
Order II. *Methanococcales*			
Family I. *Methanococcaceae*			
Genus I. *Methanococcus*			
1. *Methanococcus rannielii* (neotype species)	SB	*Methanococcus rannielii*	H_2, formate
2. *Methanococcus roltae*	PS	*Methanococcus* sp. strain PS	H_2, formate

Table 3.14. (*Continued*)

	Type strain	Former designation	Substrates for growth and CH_4 production
Order III. *Methanomicrobiales*			
Family I. *Methanomicrobiaceae* (type family)			
Genus I. *Methanomicrobium* (type genus)			
1. *Methanomicrobium mobile* (type species)	BP	*Methanobacterium mobile*	H_2, formate
Genus II. *Methanogenium*			
1. *Methanogenium carioci* (type species)	JR1	Cariaco isolate JR1	H_2, formate
2. *Methanogenium marisnigri*	JR1	Black Sea isolate JR1	H_2, formate
Genus III. *Methanospirillum*			
1. *Methanospirillum hungatii*	JF1	*Methanospirillum hungatii*	H_2, formate
Family II. *Methanosarcinaceae*			
Genus II. *Methanosarcina* (type genus)			
1. *Methanosarcina barkeri* (type species)	MS	*Methanosarcina barkeri*	H_2, CH_3OH, CH_3NH_2, acetate
Methanosarcina barkeri strain 227		*Methanosarcina barkeri* strain 227	H_2, CH_3OH, CH_3NH_2, acetate
Methanosarcina barkeri strain W		*Methanosarcina barkeri* strain W	H_2, CH_3OH, CH_3NH_2, acetate

step.

$$\text{Step 1:}\quad \underset{\text{(propionic acid)}}{CH_3CH_2COOH} + 0.5H_2O \rightarrow \underset{\text{(acetic acid)}}{CH_3COOH} + 0.25CO_2 + \underset{\text{(methane)}}{0.75CH_4}$$

$$\text{Step 2:}\quad \underset{\text{(acetic acid)}}{CH_3COOH} \rightarrow CO_2 + \underset{\text{(methane)}}{CH_4}$$

$$\text{Overall:}\quad \underset{\text{(propionic acid)}}{CH_3CH_2COOH} + 0.5H_2O \rightarrow 1.25CO_2 + \underset{\text{(methane)}}{1.75CH_4}$$

The acid-fermenting bacteria are tolerant to changes in pH and temperature and have a much higher rate of growth than the methane fermenting bacteria. This difference in growth rate, linked with a greater sensitivity to environmental factors, results in the methanogenic bacteria becoming the major factor controlling the overall rate of anaerobic digestion (Benefield and Randall 1980). However, in the digestion of other wastes, the first stage of anaerobic digestion, the enzymic hydrolysis of polymers to monomers and cellulose hydrolysis in particular, has been reported as the rate-limiting step in the conversion of cellulose to methane (Chan and Pearson 1970) and in the digestion of household refuse (Pfeffer 1974). The effect of environmental factors and the inhibition of toxic compounds in the anaerobic process are considered in Chapter 7.

The utilisation of the organic fraction of wastewater is directly related to methane production and *vice versa*. Where the exact chemical nature of the sludge is known the quantity of methane produced can be estimated by the following equation (Buswell and Mueller 1952):

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4$$

However, McCarty (1964) estimated the theoretical methane production from the complete stabilisation of 1 kg of COD as 0.348 m^3 at standard temperature and pressure.

The volume of bacteria biomass and methane produced in an anaerobic sewage sludge digester can be calculated using stoichiometry (Sec. 3.1). Using the tables devised by McCarty (1975) (Tables 3.2 and 3.3), the appropriate half reactions can be selected with carbon dioxide as the electron acceptor:

$$\text{Rc: } 1/5CO_2 + 1/20HCO_3^- + 1/20NH_4^+ + H^+ + e^-$$
$$= 1/20C_5H_7O_2N + 9/20H_2O$$

$$\text{Re: } 1/8CH_4 + 1/4H_2O = 1/8CO_2 + H^+ + e^-$$

$$\text{Rd: } 1/50C_{10}H_{19}O_3N + 9/25H_2O$$
$$= 9/50CO_2 + 1/50NH_4^+ + 1/50HCO_3^- + H^+ + e^-$$

Table 3.3 gives a $(f_s)_{max}$ value of 0.11 for sewage sludge and as the digester has a mean cell residence time of 20 days, f_s is calculated as:

$$f_s = 0.11\left(1 - \frac{0.8(0.03)(20)}{1 + 0.03(20)}\right) = 0.077$$

$$\text{As} \quad f_s = 1 - f_e\,, \quad f_e = 0.923$$

Therefore, the revised reactions using the relationship R = f_s Rc + f_e Re – Rd are

Rc xf_s:

$$0.0154CO_2 + 0.0039HCO_3^- + 0.0039NH_4^+ + 0.007H^+ + 0.007e^-$$
$$= 0.0039C_5H_7O_2N + 0.035H_2O$$

Re xf_e:

$$0.115CO_2 + 0.923H^+ + 0.923e^- = 0.115CH_4 + 0.231H_2O$$

Rd:

$$0.02C_{10}H_{19}O_3N + 0.36H_2O = 0.18CO_2 + 0.02NH_4^+ + 0.02HCO_3^- + H^+ + e^-$$

When added and normalised by dividing by 0.02, the overall reaction R is:

$$C_{10}H_{19}O_3N + 4.7H_2O = 0.195C_5H_7O_2N + 5.75CH_4 + 2.48CO_2$$
$$+ 0.81NH_4^+ + 0.81HCO_3^-$$

Therefore, for each mole of sewage sludge, 0.195 mole of new cells are produced and 5.75 moles of methane is released.

3.4.4. *Sulphide production*

The production of hydrogen sulphide is the most common and well-known manifestation of anaerobiosis. Sulphide can be produced by anaerobic micro-organisms in two ways.

Protein is broken down to amino acids, and those containing sulphur, e.g. cysteine, cystine, and methionine, are degraded further with sulphide being produced. Most anaerobic bacteria are able to produce sulphide from protein, e.g. *Proteus*, *Bacteroides* spp., and some *Clostridium* spp. Although all can grow anaerobically, only *Bacteroides* spp., which can be present in

faeces at concentrations up to 10^{10} g^{-1}, are obligate species (Houte and Gibbons 1966). In wastewater systems, most sulphide is produced from sulphate reduction by the anaerobic sulphate-splitting bacterium *Desulfovibrio desulfuricans*, although species of the genus *Desulfotomaculum* are also routinely isolated from digesters (Zeikus 1980). The former species is present in low numbers in sewage (60–600 ml^{-1}) but rapidly increases on storage up to concentrations in excess of 100,000 ml^{-1} over 14 days.

The sulphate-reducing bacteria only utilise a restricted range of carbon compounds, such as lactate and malate, and rely on the metabolic products of other anaerobic bacteria that are able to utilise more complex organic compounds. Sulphate-reducing bacteria are found in a wide range of anaerobic environments where there is a supply of sulphate, which they utilise instead of oxygen for respiration, organic matter, and a suitable bacterial population able to utilise the organic matter to produce compounds such as lactate (Lynch and Poole 1979). Sulphate is a major ion in seawater and bacterial sulphate reduction is an important reaction in anoxic estuarine and marine environments. When heavy metals are present in the sediment where sulphate-reducing bacteria are active then the sulphide produced reacts to form insoluble salts; the black discolouration so often associated with anaerobic sediments is due largely to the formation of ferrous sulphides. If no metals are present, the sulphide escapes into the water column or the atmosphere as hydrogen sulphide. The metabolism of the sulphate-reducing bacteria has been extensively studied and reviewed (Postgate 1965; Dart and Stretton 1977). The release of hydrogen sulphide depends on the pressure, temperature, and pH. Hydrogen sulphide is the compound responsible for the unpleasant smell associated with anoxic estuaries, such as Dublin Bay and many of the estuaries receiving high organic loads. When produced in sewers, sulphide can cause severe corrosion of the concrete pipework (Sec. 1.3.1) and is extremely toxic, especially in enclosed environments where it is fatal to air breathing organisms including man. The sulphide can be used as a source of energy by sulphur oxidising bacteria or as an electron donor by some of the photosynthetic bacteria. More commonly, it is chemically oxidised, thus maintaining anoxic conditions (Fig. 3.25).

3.4.5. *Denitrification*

Nitrate can be converted via nitrite to gaseous nitrogen under low dissolved oxygen conditions by the process known as denitrification. The process occurs in any nitrified effluent when deprived of oxygen i.e. nitrate serves

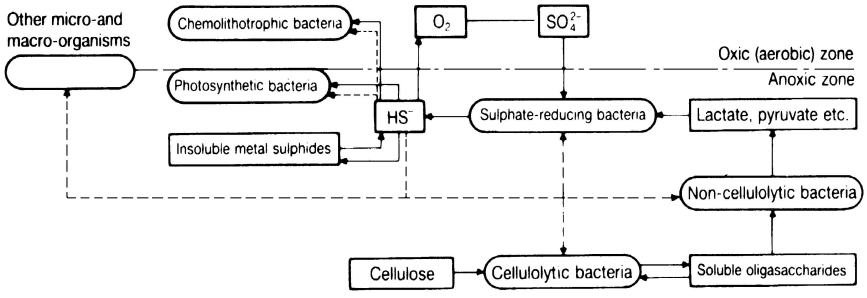


Fig. 3.25. How sulphate-reducing bacteria interact with the anoxic sediment zone (Lynch and Poole 1979).

as the terminal electron acceptor. Therefore, unless a specific denitrifying reactor is constructed (Secs. 5.6 and 7.3.3), nitrification is only likely to occur in the sludge layer of the sedimentation tank after a biological reactor where nitrification occurs. Denitrification occurs in the following sequence:

$$NO_3 \xrightarrow[\text{reductase}]{\text{Nitrate}} NO_2 \xrightarrow[\text{reductase}]{\text{Nitrite}} NO \xrightarrow[\text{reductase}]{\text{Nitric oxide}} N_2O \xrightarrow[\text{reductase}]{\text{Nitrous oxide}} N_2$$

Nitrous oxide can, under certain circumstances (e.g. low COD:NO_3 ratios, short sludge residence time and low pH), be produced during denitrification with up to 8% of the NO_3 converted to N_2O. Nitrous oxide is a major air pollutant thus operational care must be taken to prevent its formation (Hanaki *et al.* 1992).

Denitrification can only proceed under anoxic conditions (when the dissolved oxygen conditions are very low but not necessarily zero), with the dissolved oxygen concentration $< 2\%$ saturation (Kiff 1972), and when a suitable carbon source is available to act as electron donor. There is enough organic carbon remaining in the treated effluent and sludge within secondary settlement units to allow denitrification to proceed, although in specifically constructed denitrifying reactors the carbon is supplied as either methanol or settled sewage. The process is carried out by a wide range of facultative anaerobes, the most common genera being *Pseudomonas* (*P. fluorescens*, *P. aeruginosa*, *P. denitrificans*) and *Alcaligenes*, with *Achromobacterium*, *Denitrobacillus*, *Spirillum*, *Micrococcus*, and *Xanthomonas* frequently present (Painter 1970; Tiedje 1988). The overall stoichiometric equations for denitrification using methanol as the carbon source has

been calculated by McCarty *et al.* (1969) as:

Overall nitrate removal:

$$NO_3^- + 1.08CH_3OH + H^+ \rightarrow 0.065C_5H_7O_2N + 0.47N_2 + 0.76CO_2 + 2.44H_2O$$

Overall nitrite removal:

$$NO_2^- + 0.67CH_3OH + H^+ \rightarrow 0.04C_5H_7O_2N + 0.48N_2 + 0.47CO_2 + 1.7H_2O$$

Overall de-oxygenation:

$$O_2 + 0.93CH_3OH + 0.056NO_3^- + 0.056H^+ \rightarrow 0.056C_5H_7O_2N + 0.65CO_2 + 1.69H_2O$$

The maximum growth rate of denitrifiers (μ_{max}) is affected by nitrate and methanol concentrations, temperature, and pH. The growth rate of denitrifiers (μ_D) is expressed as:

$$\mu_D = \mu_{max} = \left(\frac{D}{k_d + D}\right)\left(\frac{M}{k_m + D}\right)$$

where D is the nitrate concentration (mg l^{-1}); k_d the half-saturation constant for nitrate (mg l^{-1}); M the methanol concentration (mg l^{-1}), and k_m the half-saturation constant for methanol (mg l^{-1}). Denitrification rate is related to growth rate as:

$$q_d = \frac{\mu_D}{Y_d}$$

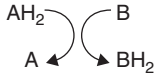
where q_d is the nitrate removal rate (mg NO_3-N mg$VSS^{-1}d^{-1}$) and Y_d is the growth yield (mg VSS per mg NO_3 removed).

Denitrifying micro-organisms are far less sensitive to toxic chemicals than nitrifying bacteria. Some trace metals, such as molybdenum, which is required for the synthesis of nitrate reductase, stimulates denitrification. The optimum pH is 7.0 with both the pH and alkalinity increasing as denitrification proceeds. A theoretical maximum of 3.6 mg of alkalinity as $CaCO_3$ is produced per mg of nitrate reduced to nitrogen gas, although 3.0 mg is used for design purposes. Thus, in practice, about half of the alkalinity consumed during nitrification is replaced by denitrification. Optimum temperature is 25–30°C although denitrification occurs over a wide range (5–50°C)(USEPA 1975).

Denitrification is considered further in Secs. 5.6 and 7.3.

3.4.6. *Redox potential*

Oxidation-reduction (or redox) reactions in biological reactions are normally defined in terms of loss and gain of hydrogen or electrons. Each oxidation is accompanied by a reduction and can be summarised as:



where AH_2 is the hydrogen donor and B the hydrogen acceptor. Each couple (AH_2/A or B/BH_2) has a tendency to either donate reducing equivalents and be oxidised ($AH_2 \rightarrow A$) or accept them and be reduced ($B \rightarrow BH_2$). When both couples combine in a complete redox reaction, the net flow of the reaction can be determined by the relative tendency of each couple to donate or accept reducing equivalents, which is the redox potential. This can be measured using a galvanic cell consisting of two electrodes connected by a conducting solution: oxidation occurs at the negative electrode (anode) and electrons are produced, whereas electrons are consumed and reduction takes place at the positive electrode (cathode). The redox potential is quantified by comparison with a standard redox couple. By convention, the standard redox couple is that present at a hydrogen electrode consisting of a platinum electrode, with hydrogen ions in solution. In the presence of platinum as the catalyst, the reaction is:

$$H_2 \rightleftharpoons 2H^+ + 2e^-$$

and the tendency to donate reducing equivalents, as electrons in this case, is measured as the voltage (potential) of the electrical current generated, when the electrode is coupled in series with another redox couple electrode. Under standard conditions, 25°C, 1 atm of H_2 and at pH 0, the redox potential of the hydrogen electrode is zero. At pH 7, the potential of the redox couple $H_2/2H^+ + 2e^-$ is -420 mV. The symbol E_h is used for the redox potential under standard conditions. The theory of redox measurement is fully discussed by Kokholm (1981).

A couple of lower redox potential will always donate reducing equivalents to a couple of higher potential and, during the oxidation of a substrate, reducing equivalents are transferred in the direction of increasing potential. This transfer is accompanied by the release of free energy, the magnitude of which is given by the standard free energy change,

$$\Delta G^{o\prime} = -nF\Delta E_h$$

where n is the number of electrons transferred in the reaction, F the charge on one mole of electrons, which is Faraday's constant (96.649 kJV^{-1} mol^{-1}),

and ΔE_h the standard electrode potential. Biological systems have evolved to conserve this energy and to convert it into biologically useful forms (i.e. oxidative phosphorylation).

In practical terms, the redox potential can be used to indicate which oxidative-reduction reactions will occur within a wastewater system and is particularly useful in the management of anaerobic systems (Fig. 3.21) (Dirasian 1968a,b). The redox potential gives a measure of the general condition of the liquid. Anaerobic processes similar to those found in sludge lagoons and digesters will have low values of E_h (< -200 mV), whereas aerobic processes will have higher values ($> +50$ mV). More precisely, values of E_h -150 mV to -420 mV are found in anaerobic environments, whereas aerobic environments vary between -200 mV and $+420$ mV. Facultative environments change from aerobic to anaerobic systems at about $+100$ mV (Hughes 1980). Redox potential is a more reliable measure of aerobic conditions than a dissolved oxygen concentration measurement as is often used in the control of environmental conditions in activated sludge tanks. Because odours are produced from anaerobic rather than aerobic processes, redox potential appears to be a convenient measure to determine the onset of odour production during the treatment of high strength wastes, such as animal wastes. Odours at redox potentials that are far more negative than that for zero-dissolved oxygen. Thus, the control of an aeration system to prevent odour production will be far more effective if based on redox potential rather than on dissolved oxygen concentration. This is because of the closer correlation between redox potential and odour breakthrough, compared with dissolved oxygen concentration and odour breakthrough. In practice, a minimum redox potential of $E_h = +40$ mV has been shown to be most efficient, although minimum thresholds of -100 mV have been used. Therefore, by automatically aerating the surface wastewater when E_h falls below $+40$ mV, no odours are produced and, by using redox potential instead of dissolved oxygen concentration, savings in aeration costs of up to 75% have been achieved (Barnes *et al.* 1985).

Most aquatic sediments are prone to low oxygen conditions due to the problems of oxygen diffusion from the water column into the sediment. This depends on the oxygen concentration of the water, the physical and chemical nature of the sediment, and the burrowing activities of bottom-dwelling fauna. Once the sediment becomes anoxic the fauna population will become progressively excluded. Under these conditions, the redox potential is a useful indicator of which electron acceptor is being used by the anaerobic bacteria present in the sediment (Fig. 3.21).

Further reading

General: Lynch and Poole 1979; Benefield and Randall 1980; Stafford *et al.* 1980; Schugerl 1987; Bitton 1999.
Organisms and physiology: Wolfe 1971; Morris 1975; Zeikus 1977, 1980.
Biochemistry: Thauer *et al.* 1977.
Denitrification: Barnes and Bliss 1983; Winkler 1984.
Redox: Dirasian 1968a,b; Kokholm, 1981; Barnes *et al.* 1985.

3.5. Autotrophic Micro-organisms

3.5.1. *Introduction*

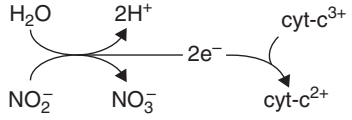
Unlike heterotrophic micro-organisms, autotrophs are unable to utilise organic matter. They use carbon dioxide, bicarbonate, or carbonate as their sole carbon source for the synthesis of cellular material, and obtain energy for metabolism by the oxidation of reduced inorganic compounds (chemo-autotrophs) or by photosynthesis (photo-autotrophs).

Chemo-autotrophic bacteria are able to oxidise a range of elements that are present in a reduced form (i.e. low oxidation state) (Table 3.15). However, the conditions required for the active growth of these bacteria are critical, and in some ways conflicting. The group requires a plentiful supply of the reduced inorganic compound (e.g. NH_3, NO_2^-, H_2S), oxidising agent (e.g. O_2, or for some species NO_3^-), which must have a more positive redox potential than the reducing agent, and carbon dioxide as the carbon source. Chemo-autotrophs are aerobic organisms, although they can grow at low partial pressures of oxygen and some species are able to utilise other oxidising agents. Unlike the photo-autotrophs, they do not require light and do not contain pigments. However, they are restricted in wastewater treatment units to the areas where there are plentiful supplies of reduced inorganic compounds, carbon dioxide, and some oxygen. As a group, they can oxidise a wide range of reduced inorganic compounds, although individual species are highly specific in the reactions they catalyse and in the environmental conditions they require. The energy metabolism of autotrophs is essentially the same as heterotrophs, with ATP formation by oxidative phosphorylation. The electrons produced from the oxidation of reduced inorganic molecules enter the electron transport system at the level of cytochrome-c (Fig. 3.19). In all chemo-autotrophic reactions, oxygen is the terminal electron acceptor, and the subsequent electron flow through the electron transport chain, via a series of biological redox carriers in

Table 3.15. Primary energy yielding reactions of chemo-autotrophic bacteria.

	Electron donor	Product	ΔG° (kJ mol^{-1})	Major genus
Sulphur bacteria	$S^2 + 2O_4^{2-} \rightarrow$	SO_4^{2-}	–715	*Thiobacillus*
	$S^\circ + 1.5O_2 + H_2O \rightarrow$	$SO_4^{2-} + 2H^+$	–502	*Thiobacillus*
Nitrifying bacteria	$NH_3 + 1.5O_2 \rightarrow$	$NO_2^- + H_2O + H^+$	–274	*Nitrosomonas*
	$NO_2^- + 0.5O_2 \rightarrow$	NO_3^-	–73	*Nitrobacter*
Hydrogen bacteria	$H_2 + 0.5O_2 \rightarrow$	H_2O	–234	*Hydrogenomonas*

exergonic reactions to oxygen, will produce a negative free energy change which is used for the synthesis of ATP (Anderson 1980). For example, in the case of the oxidation of NO_2^- to NO_3^- only one ATP molecule per NO_2^- unit oxidised is produced:



Because ADP and NAD have similar redox potentials, $NADH_2$ can be synthesised instead of ATP as cytochrome-c is reduced (Fig. 3.26).

Photo-autotrophs use free carbon dioxide as a carbon source and derive energy from sunlight. There are many photo-autotrophic bacteria present in small numbers in wastewater treatment systems, but the most important group is the eukaryotic photo-autotrophs which include the true algae, bryophytes, and the higher plants. With the exception of bryophytes, algae are the only eukaryotic phototrophs found in conventional wastewater treatment systems, although macrophytes are used in warmer climates (Sec. 6.2). Although algae are found in small numbers in most aerobic treatment systems they are particularly important in waste stabilisation ponds where they provide oxygen for heterotrophic and chemo-autotrophic bacteria (Sec. 6.3.2). Like other plants, algae use free carbon dioxide as a

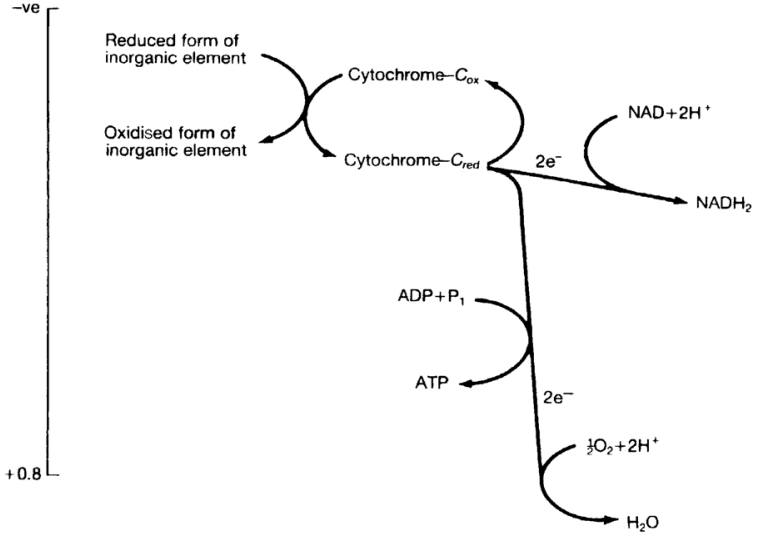


Fig. 3.26. Reduction of cytochrome by NAD (Anderson 1980).

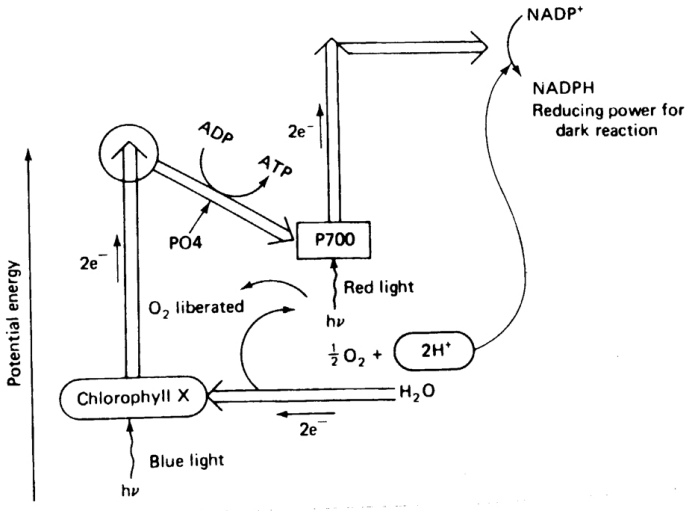


Fig. 3.27. Electron flow during photosynthesis (Benefield and Randall 1980).

carbon source and derive all their energy requirements from sunlight by the process of photosynthesis (photophosphorylation). Essentially, water is oxidised by releasing electrons which are then used to reduce carbon dioxide to a carbohydrate. Two basic reactions occur, a light and a dark reaction, both of which occur during the hours of daylight, although the dark reaction utilises the product of the light reaction which is dependent on light energy. Short-wave light energy is absorbed in the light reaction by chlorophyll, which supplies the energy to oxidise water, releasing oxygen and electrons. The energy level of the electrons is reduced by passing through an electron transport system, which releases energy in the form of ATP. The electrons are re-energised by the absorption of long-wave light energy by pigment P700 in the plant tissue. This is used to reduce nicotinamide adenine dinucleotide phosphate (NADP) to NADPH, which provides the energy to drive the dark reaction (Fig. 3.27). The ATP and NADPH produced by the light reaction are utilised to reduce six molecules of carbon dioxide to a hexose molecule in the dark reaction with the enzyme 6-ribulose-l,5-diphosphate required to catalyse the reaction that is regenerated at the end of the cycle:

$$\begin{aligned}&\text{6-Ribulose-l, 5-diphosphate} + CO_2 + 18\text{ATP} + 12\text{NADPH} + 12H^+\\ &\quad\rightarrow \text{6-Ribulose-l, 5-diphosphate} + \text{hexose} + 18P_i + 18\text{ADP} + 12\text{NADP}\end{aligned}$$

There are important differences in the pigments between various groups of eukaryotic photo-autotrophs, which reflect their adaptation to the

environment in which each grows. For example, green algae are found close to the surface of the water in waste stabilisation ponds where they absorb red light, whereas red algae grow at lower depths in ponds where there is poor penetration of red light and they use the pigment phycoerythrin to absorb light of shorter wavelengths.

Two major groups of photo-autotrophic bacteria are associated with wastewater treatment. The green photosynthetic bacteria, the most important family being the *Chlorobacteriaceae*, are the simplest of the photo-autotrophs. They contain the pigment bacteriochlorophyll and require carbon dioxide, light, anaerobic conditions, and either hydrogen or a reduced form of sulphur (e.g. H_2S) for growth. They are commonly isolated from anaerobic environments rich in hydrogen sulphide, such as sludge digesters or the sludge layer in waste stabilisation ponds, there they are referred to as green photosynthetic sulphur bacteria. In the oxidation of hydrogen sulphide by *Chlorobium* sp., elemental sulphur (S°) is produced, which is released into the environment:

$$2H_2S + CO_2 \xrightarrow{\text{Light } (h_\nu)} \underset{\text{(new cells)}}{[CH_2O]} + 2S^\circ + H_2O$$

Hydrogen sulphide is not a sufficiently strong reducing agent (i.e. the redox potential of hydrogen sulphide is insufficiently negative) to reduce carbon dioxide. Oxidation is, in fact, light-dependent, with visible light absorbed by the bacteriochlorophyll, which oxidises the hydrogen sulphide, releasing electrons that are subsequently trapped by a compound with a more negative redox potential (Anderson 1980).

Purple photosynthetic bacteria also contain the green pigment bacteriochlorophyll but appear red or purple due to the presence of carotenoid pigments. There are two main groups. The purple sulphur bacteria or the *Thiorhodaceae* (e.g. *Chromatium* sp.) are motile and require light, anaerobic conditions, and either hydrogen sulphide or hydrogen for the assimilation of carbon dioxide. Species of this group occur in the sludge layers of waste stabilisation ponds and produce elemental sulphur from hydrogen sulphide, which is deposited in bacterial cells where it accumulates. The purple non-sulphur bacteria, the *Athiorhodaceae* (e.g. *Rhodopseudomonas* sp. and *Rhodospirillum* sp.) are unable to use reduced forms of sulphur but utilise hydrogen instead. They are also restricted to anaerobic environments and require light and carbon dioxide. This group is also capable of utilising simple organic compounds such as acetate instead of carbon dioxide, although the assimilation of the organic carbon is still enhanced by light. This ability shows that all photosynthetic organisms are autotrophic and

that this group is more accurately described as being photoheterotrophic.

The blue-green algae, *Cyanophyceae*, are not an important group in wastewater treatment, although they are occasionally isolated from aerobic and anaerobic systems. Under anaerobic conditions, they use light to reduce gaseous nitrogen to ammonia, a process known as nitrogen fixation, whereas under aerobic conditions they carry out light-dependent carbon dioxide assimilation without the use of any strong reducing agent, such as hydrogen sulphide or hydrogen. They produce oxygen at the same rate as carbon dioxide is assimilated.

3.5.2. *Nitrification*

The principal sources of organic nitrogen are domestic wastes, animal slurry from intensive farming, and high-protein wastes from certain processing industries, especially the meat trade. In the sewerage system, organic nitrogen is rapidly deaminated and urea is hydrolysed by the enzyme urease to release ammonia:

$$\underset{\text{(urea)}}{\begin{array}{l}\mathrm{NH_2}\\ |\\ \mathrm{C=O}\\ |\\ \mathrm{NH_2}\end{array}} + 2\mathrm{H_2O} \xrightarrow{\text{urease}} \underset{\text{(ammonium carbonate)}}{(\mathrm{NH_4^+}):\mathrm{CO_3^{2-}}}$$

Apart from urea, proteins are a major source of nitrogen in domestic wastewater and are converted to peptides and amino acids by extracelular proteolytic enzymes. The amino acids are deaminated in either oxidative or reductive states to produce ammonia:

Oxidative deamination

$$\underset{\text{amino acid}}{\begin{array}{l}\mathrm{R{-}CH{-}COOH}\\ |\\ \mathrm{NH_2}\end{array}} + 0.5\mathrm{O_2} \rightarrow \underset{\text{keto acid}}{\begin{array}{l}\mathrm{R{-}C{-}COOH}\\ |\\ \mathrm{O}\end{array}} + \mathrm{NH_4}$$

Reductive deamination

$$\underset{\text{amino acid}}{\begin{array}{l}\mathrm{R{-}CH{-}COOH}\\ |\\ \mathrm{NH_2}\end{array}} + 2\mathrm{H} \rightarrow \underset{\text{keto acid}}{\mathrm{R{-}CH_2{-}COOH}} + \mathrm{NH_4}$$

By the time raw domestic sewage enters the treatment plant, 90% of the nitrogen is either present as ammonia or unstable organic compounds,

which are readily transformed to ammonia (Culp *et al.* 1978). Domestic sewage, with an ammonia-nitrogen concentration of 35 g m^{-3}, is extremely weak in terms of ammonia-nitrogen concentration compared with other nitrogen-rich wastewaters, due to the large dilution it receives.

Domestic sewage has nitrogen in excess of the microbial requirement to oxidise the amount of carbon present (Sec. 1.2), therefore only part of the nitrogen is removed by conventional heterotrophic activity, being incorporated into the microbial biomass. This is also true for phosphorus. The residual nitrogen stimulates autotrophic activity, which, if discharged into a watercourse, will be in the form of photo-autotrophic activity, i.e. eutrophication. The utilisation of nitrogen by photo-autotrophs produces a large quantity of biomass in the form of algae, because the proportion of nitrogen in the biomass is small. The problem is that the nutrient is assimilated into the cells for the synthesis of amino acids, enzymes, and nucleic acids, thus a direct relationship between nutrient removal and biomass production exists. Only a small proportion of the ammonia-nitrogen is assimilated into the heterotrophic biomass during wastewater treatment, and under low loading conditions in most fixed-film and mixed biological treatment units the remainder is oxidised by chemo-autotrophic bacteria. Autotrophic bacteria are able to use the nitrogen in a non-assimilative way, as an energy source, so only small amounts of biomass are produced. Ammonia, the reduced form of nitrogen, is oxidised by autotrophic nitrifying bacteria to nitrate via nitrite, a process known as nitrification.

The microbial oxidation of ammonia and ammonium ions occurs in two distinct stages, each involving different species of chemo-autotrophic nitrifying bacteria. The chemo-autotrophs utilise ammonia or nitrite as an energy source, oxygen as the terminal electron acceptor, ammonia as the nitrogen source, and carbon dioxide or carbonate as a carbon source.

The first stage is the oxidation of ammonium ions to nitrite (nitrosification):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2 + 2H^+ + H_2O \qquad -58 \text{ to } 84 \text{ kcal}$$

This reaction is generally considered to be catalysed by the genus *Nitrosomonas*, and indeed three species of this genus *N. europa*, *N. oligocarbogenes*, and *N. monocella* are frequently isolated. However, other genera have also been identified as being able to carry out the first stage of nitrification, these include *Nitrosococcus*, *Nitrosospira*, *Nitrosocystis*, and *Nitrosogloea* (Belser 1979). These bacteria belong to the β- and γ-subdivisions of the proteobacteria (Head *et al.* 1993, 1998). The hydrogen ions released in the

oxidation of ammonia to nitrite lowers the pH, and during nitrification a discernible fall in the pH of the wastewater can be recorded. This can be a problem in enclosed systems, or systems with a long retention time, where there is sufficient ammonia present for nitrification to occur as the pH will be reduced until nitrification is inhibited and eventually stopped.

In the second stage, nitrite is oxidised to nitrate:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad -15.4 \text{ to } 20.9 \text{ kcal}$$

The genus *Nitrobacter* is considered to be responsible for the second nitrifying reaction, with *N. winogradskyi* and *N. agilis* commonly isolated. *Nitrocystis*, *Nitrococcus*, and *Nitrospira* have also been cited as being able to oxidise nitrite to nitrate (Focht and Verstraete 1977; Belser 1979; Winkler 1981).

The overall nitrification reaction shows that the oxidation of ammonia to nitrate requires a high input of oxygen, about 4.5 kg for each kg of ammonia-nitrogen (NH_4-N) oxidised:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad -73.4 \text{ to } 104.9 \text{ kcal}$$

There is some evidence that the nitrifying bacteria may not be obligate autotrophs, but have the ability to utilise certain organic compounds, as do the heterotrophic nitrifying bacteria found in soil (e.g. *Arthrobacter* sp., *Aspergillus* sp.).

Kinetics and environmental factors

Growth of nitrifying bacteria is represented by Monod kinetics (Sec. 3.1.2):

$$\mu = \frac{\mu_m \cdot S}{k_s \cdot S}$$

where μ is the specific growth rate of the nitrifying bacteria, μ_m the maximum specific growth rate, k_s the saturation constant, and S the residual concentration of the growth-limiting nutrient.

As nitrite is not accumulated under steady-state conditions the rate-limiting step in nitrification is assumed to be the oxidation of ammonia to nitrite. Therefore, it is more convenient to model nitrification on the specific growth rate of *Nitrosomonas* (μ_{NS}):

$$\mu_{NS} = \mu_{m \cdot NS} \frac{[NH_4\text{-N}]}{k_N + [NH_4\text{-N}]}$$

where $\mu_{m \cdot NS}$ is the maximum specific growth rate of *Nitrosomonas* (d^{-1}), NH_4-N the ammonia-nitrogen concentration of the wastewater in the

reactor (g m^{-3}), and k_N the saturation constant (g m^{-3}) (Table 3.16).

However, the specific growth rate, thus the rate of nitrification, is affected by a number of environmental factors. In particular, nitrification is inhibited by short retention times, low dissolved oxygen concentrations, low temperatures, a wide range of inorganic and organic compounds, extreme pH, and deficiencies of key nutrients.

Nitrifying bacteria, when compared with the heterotrophic organisms, are very much slower growing. Nitrification, therefore, proceeds at a much slower rate, 3–4 times slower in fact, than carbonaceous oxidation (Sec. 1.4). In order to maintain an effective population of nitrifying bacteria within a biological reactor, a long retention time is required. This provides sufficient contact between the wastewater and the bacteria to ensure maximum nitrification. Also, a long retention time or sludge residence time (SRT) prevents the rate of loss of nitrifying organisms exceeding the rate of production of new organisms, thus maintaining the nitrifying population.

Nitrification is generally only associated with low-rate loadings, with the degree of nitrification being progressively lost as loading increases. In the activated sludge process the shortest possible SRT to avoid wash-out of the nitrifiers and to maintain nitrification is determined by the specific growth rate of the organism, which is dependent on the other environmental or operating factors, especially temperature and pH. A generalised expression of the estimation of the minimum SRT ($t_{s(min)}$) at temperature (T°C) has been devised by Marais (Jones and Sabra 1980):

$$t_{s(min)} = 3.05 \times (1.127)^{(T-20)} \text{ d}^{-1}$$

Dissolved oxygen is used as the terminal electron acceptor by nitrifying organisms and, in general terms, nitrification is inhibited at low dissolved oxygen concentrations. In the activated sludge process, it is generally accepted that nitrification does not occur below 0.2–0.5 mg O_2 l^{-1}. However, no inhibition is found at oxygen concentrations > 1.0 mg l^{-1}

Table 3.16. Normal values for the various bio-kinetic constants applicable to the nitrification process (Hultman 1973).

Constant	Value
$(\mu_{max})_{NS[20°C,(pH)_{opt}]}$	0.3–0.5 d^{-1}
k_N	0.5–2.0 mg/l
Y_N	≈ 0.05 mg VSS/mg [NH_4^+-N]
$(pH)_{opt}$	8.0–8.4

(Wild *et al.* 1971), and as long as the aeration system is adjusted to maintain a minimum dissolved oxygen concentration of 2 mg l^{-1}, then the effects of dissolved oxygen on nitrification can be ignored. In high purity oxygen systems, high dissolved oxygen concentrations can be achieved. Nitrification does not appear inhibited up to oxygen concentrations of 20 mg l^{-1} (Winkler 1981), although there is some evidence to suggest that above this concentration some inhibition may occur. Other workers, however, have reported nitrification at even higher dissolved oxygen concentrations, up to a maximum of 60 mg l^{-1} (Haug and McCarty 1972). Little or no loss in nitrifying ability occurs if activated sludge is stored anaerobically for short periods < 4 h, although over longer periods > 24 h the nitrifying bacteria are killed (Department of Scientific and Industrial Research 1963a). Clearly, oxygen is a growth-limiting substrate in terms of nitrifying bacteria, and this can be expressed using a Monod-type relationship:

$$\mu_{\mathrm{NS}} = \mu_{\mathrm{m\cdot NS}} \frac{[DO]}{k_{\mathrm{O2}} + [DO]}$$

where DO is the dissolved oxygen concentration and k_{O2} the oxygen saturation coefficient. Reported values of k_{O2} are in the range of 0.15–2.0 g m^{-3} for nitrification in the activated sludge process. A mid-value of 1.3 g m^{-3} is usually used, although Eckenfelder and Argaman (1978) have reported a K_{O2} value of approx. 1.0 g m^{-3}. The effect of dissolved oxygen on nitrification has been excellently discussed elsewhere (de Renzo 1978; Benefield and Randall 1980; Stenstrom and Poduska 1980; Winkler 1981).

The overall rate of nitrification has been shown to decrease with a decrease in temperature (Downing and Hopwood 1964). *Nitrosomonas* isolated from activated sludge has an optimum growth rate at 30°C (Loveless and Painter 1969), although a slightly higher range has also been reported 30–35°C (Buswell *et al.* 1954). There is little growth below 5°C and no growth of *Nitrobacter* below 4°C. *Nitrobacter* has a slightly higher optimum temperature for growth at 35°C, although maximum growth has been reported up to 42°C (Deppe and Engel 1960; Laudelout and Tichelen 1960). Thus, the specific growth rate of *Nitrosomonas* ($\mu_{\mathrm{M\cdot NS}}$) and the saturation constant k_{N} are affected by the temperature according to the relationship:

$$\mu_{\mathrm{m\cdot NS}} = \mu_{\mathrm{m\cdot NS\ (15^{\circ}C)}} \mathrm{e}^{0.95(T-15)}$$

and

$$k_{\mathrm{N}} = 10^{0.051(T)-1.158}$$

where $\mu_{m \cdot NS}$ is the maximum specific growth rate of *Nitrosomonas* at operating temperature T°C (d^{-1}) and $\mu_{m \cdot NS\ (15°C)}$ is the maximum specific growth rate of *Nitrosomonas* at 15°C (d^{-1}), and k_N is the saturation constant in g m^{-3} as N. Therefore, the specific growth rate of *Nitrosomonas* should always be adjusted for temperature before use in the calculation of nitrification rates or yields. The Q_{10} value (20–30°C) for *Nitrosomonas* in pure culture is ∼1.8 (Buswel *et al.* 1954) but 3.3 in activated sludge (Downing and Hopwood 1964).

Organic matter is known to inhibit nitrification and increases in organic loadings result in rapid decreases in the rate of nitrification. This is probably due to the increased activity of heterotrophs, which because of their more rapid growth rates, successfully compete with the nitrifying bacteria for dissolved oxygen and nutrients. This direct competition from heterotrophs, or photo-autotrophs if light is available (Winkler and Cox 1980), is a major cause of nitrification failure in biological treatment systems. Most organic and inorganic compounds, especially metals, inhibit nitrification (Painter 1977; Stankewich and Gyer 1978).

Nitrification is favoured by mildly alkaline conditions, pH 7.2–9.0, with an optimum pH of between 8.0 and 8.4. Below pH 8 the rate of nitrification decreases becoming completely inhibited at pH < 5 (Wild *et al.* 1971), even though nitrifying bacteria can be acclimatised to slightly more acidic pH values. Acclimatisation to a different pH may take several weeks. For example, a pH shift from 7 to 6 required 10 days of acclimatisation before nitrification returned to its former rate (Haug and McCarty 1972). Nitrosification produces hydrogen ions and if there is insufficient alkalinity present to buffer the wastewater, as would be the case in a soft water catchment area and when there was a high concentration of ammonium salts in the wastewater, then the pH will gradually decrease. However, this phenomenon is not restricted to soft waters. Painter (1983), cites an example of a domestic sewage in a hard water area with a hardness of 300–400 mg $CaCO^3$ l^{-1} in which 60 mg NH_4-N l^{-1} was being oxidised, resulting in pH values as low as 5.0–5.5. This decrease in pH will not only inhibit nitrification but also heterotrophic activity, reducing BOD_5 removal. Thus, when this occurs a characteristic cycle in effluent quality can be identified, with high nitrate and low BOD_5 concentrations alternating with low nitrate and high BOD_5 concentrations. There is very little carbon dioxide in the atmosphere. About 0.05% by weight, and instead the carbon dioxide produced from heterotrophic activity is utilised by the nitrifying organisms. However, due to the acidity of nitrification, the carbon dioxide from heterotrophic activity is often in the form of carbonate or bicarbonate ions. In purely nitrifying

systems (e.g. nitrifying filters) where there is no associated heterotrophic activity, a supplementary source of carbon is required, normally in the form of a carbonate or bicarbonate supplement. Therefore, if nitrifying bacteria consume carbonate ions, then the reduction in alkalinity may seriously affect the buffering capacity of the system. The maximum specific growth rate of *Nitrosomonas* ($\mu_{\mathrm{m\cdot NS}}$) can be adjusted for pH variation by using the equation devised by Hultman (1971):

$$\mu_{\mathrm{m\cdot NS}} = \frac{(\mu_{\mathrm{m\cdot NS}} \text{ at optimum pH})}{1 + 0.04(10(\mathrm{pH_x} - \mathrm{pH}) - 1)}$$

where $\mathrm{pH_x}$ is the optimum pH for growth of *Nitrosomonas* (usually taken as 8.2) and pH is the operating pH.

The process of nitrification does not remove nitrogen from the wastewater, it transforms it from ammonia to nitrate, with the latter being discharged in soluble form in the final effluent. During the process of nitrification the ammonia and nitrite are used as an energy source and little biomass is produced in terms of either NH_4-N or NO_2-N removed (Water Research Centre 1971):

Stage 1:

$$55\mathrm{NH_4^+} + 5\mathrm{CO_2} + 76\mathrm{O_2} \rightarrow \underset{\text{(bacterial biomass)}}{\mathrm{C_5H_7O_2N}} + 54\mathrm{NO_2^-} + 52\mathrm{H_2O} + 109\mathrm{H^+}$$

Stage 2:

$$400\mathrm{NO_2^+} + 5\mathrm{CO_2} + \mathrm{NH_4^+} + 195\mathrm{O_2} + 2\mathrm{H_2O} \rightarrow \underset{\text{(bacterial biomass)}}{\mathrm{C_5H_7O_2N}} + 400\mathrm{NO_3^-} + \mathrm{H^+}$$

Thus, in the oxidation of 1 kg of NH_4-N only 150 g (dry weight) of bacterial biomass in the form of *Nitrosomonas* is produced in the first stage and 20 g (dry weight) of *Nitrobacter* in the second stage. Only approximately 2% of the original NH_4-N is assimilated into the biomass, this being used to form new cellular material, therefore all the nitrification reactions can be considered as stoichiometric.

The biomass of *Nitrosomonas* produced (Y_{N}) and the rate of ammonium oxidation can be expressed as:

$$\mu_{\mathrm{NS}} = Y_{\mathrm{N}}(q)_{\mathrm{NS}}$$

where μ_{NS} is the specific growth rate of *Nitrosomonas* ($\mathrm{g\ m^{-3}}$), Y_{N} the yield coefficient, or the biomass of *Nitrosomonas* produced per unit of ammonium oxidised, and $(q)_{\mathrm{NS}}$ the specific ammonium oxidation rate ($\mathrm{d^{-1}}$).

Table 3.17. Typical kinetic coefficients for nitrification in the activated sludge process obtained from pure culture experiments[a] (Metcalf and Eddy 1984).

Coefficient	Basis	Value	
		Range	Typical[b]
Nitrosomonas			
μ_m	d^{-1}	0.3–2.0	0.7
k_s	NH_4^+–N, mg l^{-1}	0.2–2.0	0.6
Nitrobacter			
μ_m	d^{-1}	0.4–3.0	1.0
k_s	NO_2^-–N, mg l^{-1}	0.2–5.0	1.4
Overall			
μ_m	d^{-1}	0.3–3.0	1.0
k_s	NH_4^+–N, mg l^{-1}	0.2–0.5	1.4
Y	NH_4^+–N, mg VSS[c] mg^{-1} d^{-1}	0.1–0.3	0.2
k_d		0.03–0.06	0.05

[a]Values for nitrifying organisms in activated sludge will be considerably lower than the values reported in this table.
[b]Values reported are for 20°C.
[c]VSS = volatile suspended solids.

Typical kinetic coefficients for stage 1 (*Nitrosomonas*), stage 2 (*Nitrobacter*), and overall nitrification reactions in the activated sludge process are explained by Metcalf and Eddy (1984) (Table 3.17). The kinetic models in this section can be used for any stage in the nitrification process.

Similar to other micro-organisms, the nitrifying bacteria require trace elements; *Nitrosomonas* require trace quantities of calcium, magnesium, and copper (Loveless and Painter 1968); and *Nitrobacter* trace quantities of molybdenum (Finstein and Delwiche 1965). *Nitrosomonas* and *Nitrobacter* are more susceptible to inhibition than heterotrophic micro-organisms and nitrification in percolating filters is less susceptible to inhibition compared with the activated sludge process (Department of Scientific and Industrial Research 1963a). However, details on inhibition are difficult to obtain because in mixed cultures (i.e. within the biological treatment unit) the concentration of a toxic compound causing inhibition is much higher than is found in pure culture studies. Also, nitrifying bacteria are able to acclimatise to much higher concentrations of the inhibitory compound if they are allowed to become accustomed slowly to increasing concentrations. This is discussed fully by Painter (1983) who cites the example of thiourea toxicity to *Nitrosomonas*. Thiourea is thought to utilise any

available copper, which is an essential requirement for an enzyme system in *Nitrosomonas*. The inhibition of *Nitrosomonas* will also reduce the *Nitrobacter* population, which grows on the product of the former genus, thus thiourea (or allylthiourea) is used as an inhibitor of nitrification in the BOD_5 test to differentiate between carbonaceous oxidation and ammonia oxidation (Sec. 1.4.2.3). In pure culture, the genus is completely inhibited at 0.5 mg l^{-1} thiourea, but in activated sludge 10 times this concentration can be tolerated. In time, *Nitrosomonas* can build up a tolerance to thiourea up to 92 mg l^{-1}. Although pure culture studies have shown nitrifying bacteria to be slightly sensitive to a wide range of inorganic and organic compounds, their ability to acclimatise to relatively high concentrations means that, in practice, they are rarely inhibited by the range of ions found under normal wastewater conditions. A comprehensive list of chemicals, that cause nitrification inhibition during sewage treatment, has been prepared by Richardson (1985).

3.6. Assessing Treatability, Toxicity, and Biodegradability

3.6.1. *Introduction*

Treatability of a substance is defined as the amenability of the substance to removal during biological wastewater treatment without adversely affecting the performance of the treatment plant. Methods that simulate treatment systems such as pilot plants are costly and time-consuming to perform, so quicker and less-expensive methods are normally used, which will give an indication of the treatability and toxicity of industrial wastewaters and individual compounds to the treatment process. Such methods, called screening or indicative methods, are either biochemical or microbial tests, that can be carried out relatively quickly in a wastewater treatment laboratory using standard equipment. As treatability implies that substances present in a wastewater are biologically degraded within the retention period of the inoculum (biomass) in the treatment plant, conditions under which indicative tests are carried out must be similar to full-scale wastewater treatment. Clearly conditions under which indicative tests are carried out cannot be similar in every way to full-scale treatment. Tests tend to be more stringent in that the substance under examination has to be at a much higher concentration than found in the environment; and as no other organic compounds are present, there is no chance of cometabolism or secondary oxidation. Also some wastewaters or chemical substances will require a period of acclimation (adaptation), before yielding to oxidation,

which cannot be accommodated in the indicative tests unless pre-acclimated micro-organisms are used.

Toxicity, or the extent to which a substance adversely affects micro-organisms, is most likely dependent on the concentration of micro-organisms present, that is on the food:micro-organism ratio (f/m). It is therefore useful to conduct tests at various concentrations of inoculum, and to quantify the toxic effects of substances as EC_{50} i.e. the concentration of substance giving 50% inhibition of the control.

Biodegradation can be defined as the breakdown of a substance by micro-organisms. This may be either primary biodegradation, the alteration of the chemical structure of a substance, resulting in loss of a specific property of a substance, or ultimate biodegradation, the complete breakdown of a substance to either fully oxidised or reduced simple molecules and the formation of new cells. Current legislation in the European Community requires that biodegradation testing is carried out on new chemicals as part of the seventh amendment on the Classification, Packaging and Labelling of Dangerous Substances Regulations (E.C. 1992). The test methods are based primarily on the OECD (Organisation for Economic Cooperation and Development) test guidelines (OECD 1981), some which have been revised (OECD 1992). These methods are discussed below.

Many of the biodegradability assessment techniques were originally designed for measuring the biodegradability of surfactants but have proved to be just as effective for other substances. These and other methods for determining the treatability, toxicity, and biodegradability of chemicals are discussed below.

3.6.2. *Biochemical tests*

A biochemical test is one that is based on the measurement of the activity of specific metabolic products of micro-organisms. These have traditionally been used for the determination of microbial biomass and activity in soils and water, but more recently have found application as toxicity screening techniques.

Enzymatic assays

The impact of toxicants on enzymes can be determined by convenient, sensitive, and relatively rapid assays. The advantages of such enzyme testing systems *in vitro* are the use of commercial products of standardised purity that are relatively inexpensive. Enzymatic assays consist of measuring the rate of depletion of substrates or the rate of formation of new products. This

is achieved with the aid of spectrophotometric, fluorometric, or automatic titration methods or with the use of radioactively labelled compounds.

Dehydrogenases. Dehydrogenases, being intracellular enzymes, the activity of which is linked to cell respiration, are good indicators of cellular viability (Bitton and Koopman 1986). In wastewater treatment plants, the biologically active solids under aeration are commonly approximated as the volatile fraction of the mixed liquor suspended solid concentration (MLVSS) (Sec. 5.2.1). However, this fraction also contains inert organic solids and inactive microbial cells. Ford *et al.* (1966) proposed that dehydrogenase activity measurement could be a useful additional indicator of activated sludge activity.

Tetrazolium salts have been widely used to demonstrate reduction reactions in a wide range of applications. With regard to their use in toxicity testing, four compounds have been suggested (TTC, INT, NBT, and MTT). The characteristics of these are outlined in Table 3.18. It is important to know the site of action of tetrazolium salts along the electron transport chain that has been achieved through study of the mitochondrial succinoxidase system. Investigators have used specific inhibitors (malonate; 4,5-dichloro-2-trifluoro methyl benzimidazole; actimycin; and cyanide) to block the electron transport system at specific sites and assess their effect on formazan production. Thus for the purpose of toxicity testing, formazan production is inhibited when the site of action of the tetrazolium salt is beyond the site of action of the toxicant. However, tetrazolium salts may act at more than one site along the electron transport chain, and it is possible to use a range of tetrazolium salts that cover the electron transport chain in order to determine the site of action of the inhibitor.

2,3,5-triphenyl tetrazolium chloride (TTC) is reduced through the action of dehydrogenases to triphenyl formazan, a red insoluble precipitate. Following incubation of the test sample in the presence of TTC, the resulting red formazan is dissolved in an organic solvent and the absorbance is read at 485 nm. The TTC reduction assay is based on the inhibition of dehydrogenase activity by chemical toxicants, and is controlled by many factors, including incubation time, temperature, light, substrate, TTC concentration, pH, and oxygen (Jones and Prasad 1969). A modification of this method, using lysozyme in the incubation medium, improved its reproducibility (Ryssov-Nielsen 1975). A further modification was the addition of peptone to accelerate the test, and this method has been used as a rapid toxicity test (0.5 to 1 hour) to determine the treatability of both industrial

Table 3.18. Properties of four tetrazolium salts (Bitton and Koopman 1986).

Name	Formula	Molecular weight	Lipid solubility of formazan	Colour of formazan	Competitive with oxygen	Absorbance peak (nm)
2,3,5-Triphenyl tetrazolium chloride (TTC)	$C_{19}H_{15}ClN_4$	334.8	Yes	Red	Yes	485
2-*p*-Iodophenyl-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride (INT)	$C_{19}H_{13}ClIN_5O_2$	505.7	Yes	Orange-red	No	490
3,3′-(3,3′-Dimethoxy-4,4′-biphenylylene)-bis-(2-*p*-nitrophenyl-5-phenyl tetrazolium chloride (NBT)	$C_{40}H_{30}Cl_2N_{10}O_6$	817.6	No	Blue	No	572
3-(4,5-Dimenthyl-2-thiazolyl-2,5-diphenyl-tetrazolium bromide (MTT)	$C_{18}H_{16}BrN_5S$	414.3	Yes	Purple, blue and grey	–	550

wastewaters and municipal effluents in activated sludge plants (Gorban *et al.* 1987). Its main disadvantage is that the assay must be carried out in the absence of oxygen.

2-*p*-Iodophenyl-3-*p*-nitrophenyl-5-phenyl tetrazolium chloride (INT) is reduced by the electron transport system of active micro-organisms to water insoluble red INT-formazan crystals (INTF). These can be viewed microscopically or can be extracted with an organic solvent such as dimethylsulphoxide. Dehydrogenase activity is proportional to the fraction of cells containing INTF crystals or to the optical density of INTF extract (Koopman and Bitton 1987). Preliminary results indicate the INT assay is sensitive to both heavy metals and toxic organic compounds (Dutton *et al.* 1986). Inhibition values were obtained for nickel, zinc, copper, mercury, phenol, 3,4-dichlorophenol, sodium dodecyl sulphate (SDS), tetrachloroethylene, and formaldehyde. Values obtained with inocula from different treatment systems or with inocula from the same system sampled on different days were in good agreement. The test was particularly sensitive to heavy metals, as indicated when a comparison of inhibition values with those obtained from such bioassays as the *Spirillum volutans* assay, *Pseudomonas fluorescens* inhibition, and *Photobacterium phosphoreum* (Microtox) bioassay, and the dehydrogenase TTC test. The INT test was less sensitive to selected organic compounds, i.e. phenol and SDS, than the Microtox® assay or *S. volutans* inhibition, but was similar in sensitivity to growth inhibition assays and the TTC reduction test.

Two *in vitro* dehydrogenase activity assays kits for glucose-6-phosphate dehydrogenase, marketed by Sigma Chemicals, are based on the reduction of NADP to NADPH. NADPH can be measured colorimetrically (NADPH, in the presence of phenazine methosulphate, reduces a blue dye to a colourless state and the rate of disappearance of the dye is proportional to the dehydrogenase activity) or spectrophotometrically (based on the rate of increase of absorbance at 340 nm accompanying the formation of NADPH). Ruthford *et al.* (1979) applied this assay to the measurement of petroleum effluents. They found similar inhibition curves for both colorimetry and spectrophotometry results, and obtained similar results from invertebrate and fish bioassays. More recent work investigating the toxicity of metal-working fluid preservative indicated that the *in vitro* dehydrogenase assay was less sensitive than the Microtox® or a fish bioassay (Mallak and Brunker 1984).

Resazurin, an oxido-reduction inhibitor, is reduced in two stages. The first is irreversible and involves the reduction of blue resazurin to pink resorufin. In the second stage, resorufin is reversibly reduced to colourless

dihydroresorufin. The irreversible character of the first stage of the reduction process makes resazurin suitable for toxicity testing, these tests being based on the reduction of resazurin to resorufin by microbial dehydrogenases. The dehydrogenase assay consists of measuring the absorbance at 610 nm, the maximum absorbance of unreduced resazurin. The high fluorescence of resorufin could enable the test to become more sensitive and rapid as fluormetric measurement techniques could be used.

Liu (1983) found a good correlation between the rate of resazurin reduction and the concentration of mixed liquor suspended solids (MLSS) of activated sludge, and with other tests of biological activity such as viable plate counts and oxygen uptake. A rapid assay involves incubation of the test chemical with activated sludge and resazurin for 15 minutes at 21°C and extraction with n-amyl alcohol and phthalate-HCl buffer. The absorbance of the supernatant is measured at 610 nm. The extent of inhibition of dehydrogenase activity, as indicated by the retardation of resazurin reduction, can be used to estimate the toxicity of the test chemical. This assay demonstrated mercury toxicity in activated sludge as well as the inhibitory and stimulatory effects of chlorobenzenes on bacteria. A similar assay uses a pure culture of *Bacillus cereus* as the test micro-organism (Liu 1986).

Beta-galactosidase. A simple rapid assay developed by Reinhartz *et al.* (1987) is based on the ability of toxicants to inhibit the biosynthesis of an inducible enzyme, e.g. beta-galactosidase, by a rough mutant of *Escherichia coli*, which is highly sensitive to a wide spectrum of toxic substances such as pesticides, mycotoxins, and heavy metals. The test is performed under stress conditions for the bacterium, since an improved sensitivity when using a low concentration of the test substance is usually obtained. Serial dilutions of the test sample are mixed with the stressed bacteria and a mixture containing the specific inducer for the chromogenic enzyme and essential factors required for the recovery of the bacteria from their stressed condition. The ability of cells to synthesise beta-galactosidase under these conditions depends on their ability to recover from the stress. Toxic materials interfere and/or inhibit the recovery process and with it the synthesis of beta-galactosidase. The amount of the synthesised enzyme is determined by a colorimetric reaction. A commercial kit has been developed based on this assay. The Toxi-Chromotest® contains bacteria in a lyophilised form and all of the reagents have been stabilised so that the kit may be stored for long periods. Various pesticides and mycotoxins have been tested using this bioassay, and it has been found to be similar in sensitivity to the Microtox® test and mammalian cell cultures. It was found that the sensitivity of this

bioassay was increased by altering the outer membrane permeability of the cell wall by pretreating the mutant *E. coli* cells with a polycationic antibiotic, Polymyxin B (Koopman *et al.* 1988).

The MetPAD® bioassay was developed for the specific determination of the toxicity of heavy metals, and was evaluated for its use as a toxicity screening assay for industrial effluents (Bitton *et al.* 1992). This commercial kit, for use in the field, includes lyophilised *E. coli*, deionised water as a diluent, phosphate buffer, and petri dishes containing filter pads saturated with beta-galactosidase substrate. The test substance is incubated with the bacterial reagent at 35°C for 90 minutes and is dispensed onto the assay pad. The intensity of the purple colour gives an indication of the enzyme activity. The minimum inhibitory concentration can be determined by assaying several dilutions of the test substance. Assays of nine industrial effluents were carried out and the results indicated that the MetPAD® did not respond well to organic chemicals in comparison to the Microtox® assay (a bioluminescence assay), but appeared to be sensitive to heavy metals. It was concluded that this assay could have application as a complimentary test to another bacterial assay in order to specifically test for the presence of heavy metals in industrial effluents, sludges, soils and sediments.

In-vitro urease activity. This simple test system is based on the enzymatic cleavage of urea to ammonia and carbon dioxide by urease (Obst *et al.* 1988). This enzyme reaction was selected because the microbial degradation of urea is a typical reaction in wastewater. In addition, urease is very sensitive to heavy metals. The influences on the enzymatic cleavage of urea are determined either electrochemically or titrimetrically, as the pH value increases due to the release of ammonia.

ATP assays

Adenosine triphosphate (ATP) is the universal energy currency shared by animal, plant, and microbial cells. It is generated during oxidative phosphorylation in heterotrophs, photophosphorylation in photoautotrophs, or as a result of oxidation of inorganic chemicals by chemoautotrophs. Since ATP is rapidly inactivated upon cell death, it can be conveniently used as a measure of live biomass. When rapidly growing bacterial cells are exposed to low concentrations of toxicants, growth inhibition usually occurs (Xu and Dutka 1987a,b). After several life cycles, the toxic effect can be estimated by comparing sample cell growth to the control via ATP content. Some toxicants inhibit not only bacterial growth, but also affect the

luciferase activity during ATP determinations. This assay proposes that the observed light output reduction of the test system is a net result of both bacterial growth and luciferase inhibition. Luciferase activity inhibition can be determined by adding a standard ATP solution, as enzyme substrate to the sample, and distilled water for the control, and monitoring the light emission of the enzyme (ATP-Tox system). It is believed that any bacterium with a short duplication period may be used as a testing organism, although *E. coli* is the preferred organism as it appears to be very sensitive to toxicants. Data available in the literature are contradictory for this assay. Brezonik and Patterson (1971) found that ATP assays were relatively insensitive to the action of nickel and copper. Kennicutt (1980) found acrolein and mercury to be the most toxic chemicals investigated, mercuric chloride at a concentration of 1 ppm caused nearly 100% loss of ATP in 1 hour, and concluded that this method was sensitive to a variety of chemicals with varying toxicities. However, more research is necessary in order to consider this biochemical process as a reliable toxicity test parameter.

3.6.3. *Bacterial tests*

Growth inhibition and viability of bacterial cells

In many bioassays, inhibition of growth or viability is used to assess the effect of a toxicant. Since growth is a summation of cellular processes, it reflects toxic effects on numerous biological functions (Trevors 1986).

Spirillum volutans bioassay. Spirillum volutans is a relatively large aquatic bacterium with a rotating fascicle of flagella at each pole. Under normal conditions, the polar fascicles form oriented revolving cones allowing the bacterium to move and reverse directions. During the reversing process, the polar fascicles reorientate simultaneously. This bioassay is based on observing a decrease in reversing motility of 90% of the test cells, which is considered a positive toxic effect. A defined test medium, the test chemical and log-phase *S. volutans* cells (18-hour culture) are incubated for 2 hours and are then examined under the phase contrast microscope. Mixtures of metals tested at concentrations that are only slightly toxic when tested alone became more toxic, indicating a synergistic response (Dutka 1986).

Bacterial growth inhibition test. In the method described by Alsop *et al.* (1980) the test material was evaluated at several concentrations in a mixture containing buffer, nutrients, growth substrate and microbial seed. The mixture was incubated for 16 hours at 20°C while agitating, and turbidity

values were read at 530 nm. The toxicant concentration inhibiting growth by 50% could be determined. A similar method was carried out using a pure culture of *Pseudomonas putida*, and turbidity was measured after 6 hours incubation at 27°C at 600 nm (Slabbert and Grabow 1986). The original method has been adopted by the UK Standing Committee of Analysts (1988).

Another bioassay determined the effect of toxic substances on a pure culture of *Pseudomonas fluorescens*, (Netherlands Standard NEN 6509) (Dutka and Kwan 1982). Log growth phase cells were inoculated into nutrient broth containing different concentrations of test compound. After incubating on a rotary shaker for 18 hours at 37°C, absorbance measurements were carried out at 650 nm using a spectrophotometer. It was observed that synergistic effects were easily detected using this assay.

Another growth inhibition microassay was based on the effect of toxicants on the growth of the bacterium *Aeromonas punctata* (Slabbert 1988). Cultures grown overnight at 35°C in minimal medium or diluted nutrient broth were diluted with fresh media to optical densities of approximately 0.09 and 0.08, respectively, 30 minutes before inoculation of test samples. Plates were incubated at 35°C for 6 hours. Growth was measured photometrically at 620 nm and the percentage inhibition of various concentrations of test substances was calculated.

The agar plate assay was another growth inhibition assay where agar plates thinly coated with fresh bacterial culture (pure or unmixed) were spotted with test chemicals, and the plates were incubated at room temperature for 18 hours. The degree of toxicity of the test chemical to the bacterial culture was determined by the minimal concentration of the toxicant required to produce a clean spot on the seeded agar plate, indicating the inhibition of growth of the culture (Liu 1987). *Bacillus cereus*, which was originally isolated from activated sludge, was routinely used as the testing bacterium.

A similar assay has been proposed using *Pseudomonas putida* as the test organism (Slabbert and Grabow 1986). Membrane filter pads with filter-sterilised test samples were incubated on top of membranes saturated with inoculum. Pads saturated with deionised water were used as the control. Incubation was carried out on half-strength agar at 35°C for 24 hours. Toxicity was again determined according to the inhibition of growth of the micro-organism. The toxicity data generated by the agar plate method were mostly qualitative in nature. However, this could be used as a convenient screening test for determining the toxicity potential of test chemicals or industrial wastes.

Bioluminescence. The Microtox® bioassay has been developed by Beckman Instruments Incorporated for assessing acute toxicity in aquatic samples. The assay is based on the measurement of the activity of a luminescent bacterium *Photobacterium phosphoreum*, which emits light under normal metabolic conditions. On exposure to toxicants, the light output is reduced, and this reduction is proportional to the toxicity of the test substance. Intensity of the light output depends on several external factors including temperature, pH, salinity, nature and concentration of the toxicant (Ribo and Kaiser 1987). In order to minimise the variability of the measured toxic concentrations, rigorous control of such external factors is necessary. The test involves the addition of luminescent bacteria into a vial of precooled (15°C) diluent solution, followed by a 15-minute stabilisation period. Immediately, a test compound is added to the vial and is placed adjacent to a photomultiplier tube in the dark. Total light emissions are recorded over a 15-minute period and displayed on a digital meter. The Microtox® bioassay has been applied widely to the toxicity testing of chemicals and industrial effluents. Green *et al.* (1985), used the Microtox® test to evaluate the toxicity of copper, acetone and methanol and Tarkpea *et al.* (1986), determined the EC_{50} values for alcohols and aromatic compounds. The reduction in toxicity of biologically treated landfill leachate containing phenoxyacetic acid herbicides and chlorophenols was determined using the Microtox® assay (McAllister *et al.* 1991). The Microtox® test has also been evaluated for use as a toxicity test to establish optimum loading rates of hazardous organic wastes for land treatment in order to ensure protection of soils and groundwater (Casarini *et al.* 1991). Gray and O'Neill (1997) compared the Microtox® test to the BOD inhibition and activated sludge respiration inhibition tests in the assessment of acid mine drainage toxicity. They found that Microtox®, although expensive, was rapid and simple to use. It was the most sensitive of the three tests compared and did not suffer the problems of repeatability and reproducibility encountered in the other two tests. The sensitivity of the Microtox® test is comparable to those of many other acute toxicity aquatic bioassays, but it does not provide real threshold values. Any relationships with sublethal effects have largely been unexplored.

The Mutatox® test, based on the use of a dark mutant strain of *P. phosphoreum*, was developed for investigating the mutagenicity of chemical substances by the Microbics Corporation (1988). The genotoxic chemicals restore the light-emitting properties of the bacterium and can be measured using a modified Beckman Microtox® Analyser. The test was evaluated

and proved to be a valuable addition to the available toxicity test methods for the environmental screening of mutagenic chemicals (Kwan *et al.* 1990).

Inhibition of growth of bacteria involved in nutrient and mineral cycles. Williamson and Johnson (1981), have described a bioassay with *Nitrobacter* as the test organism, which has shown to successfully detect various toxicants in municipal and industrial wastewaters. An enriched culture of *Nitrobacter* is prepared in a flow column packed with polyethylene beads and a nutrient solution. When a bacterial mass is established, the cells are removed from the column and are used immediately in toxicity measurements or freeze-dried and used at a later date. Each flask is sampled every 30 minutes for 4 hours for a decrease in nitrate, using an ion-electrode or the standard spectrophotometric assay. Inhibition of *Nitrobacter* metabolic activity can be estimated for different concentrations of the test compound. A similar method tests for the inhibition of nitrification of activated sludge (International Standards Organisation 1986). A mixture of washed sludge, 3000 mg l^{-1} MLSS, from a nitrifying activated sludge treatment plant is aerated with synthetic sewage (Table 3.19), nutrient medium and test material. Rates of production of oxidised nitrogen or removal of ammoniacal nitrogen are compared with those of a control containing no test material (Robinson 1988).

The toxicities of 43 chemicals, including chlorinated phenols, chlorinated benzenes, chlorinated aliphatics and alcohols were determined using *Nitrobacter* as the test organism (Tang *et al.* 1992). The *Nitrobacter*-enriched culture was maintained in a 20-litre reactor, and the seed, substrate, and test chemical were added to a 150 ml serum bottle. Oxygen was added by injecting 30 ml of pure oxygen into the bottle, and the sample was agitated on a shaker table for 24 hours. After centrifugation, the nitrite concentration was determined by colorimetry. The EC_{50} value of each chemical was obtained by interpolation of a plot of percentage inhibition versus concentration. The method was found to be both effective and simple to perform.

Tam and Trevors (1981) have reported using *Azotobacter vinelandii* as the test micro-organism in a toxicity bioassay. *Azotobacter vinelandii* was inoculated into a nitrogen-free medium containing the test chemical, and pure acetylene was added to a certain concentration after an equivalent volume of the gas phase was removed. The mixtures were incubated at 30°C in the dark for 12 hours. At appropriate intervals, 0.2 ml gas samples were removed and analysed by gas chromatography for ethylene as a measure of nitrogen-fixation and CO_2 and O_2 as a measurement of respiration. The O_2 concentration in each flask was adjusted every hour

Table 3.19. To produce synthetic sewage dissolve the following in 1 litre of distilled water (OECD 1984). This gives a stock solution of synthetic sewage that should be diluted 100 fold. Meat extract can be replaced by 11 g of Bovril® which, when diluted 40 fold (i.e. 25 ml of stock solution to 1 litre of water), gives a synthetic sewage with a BOD of 300 mg l^{-1} and COD of 600 mg l^{-1}. However, meat extract is less odourous in aerated laboratory-scale treatment units. The stock solution of both recipes should be stored at 4°C and discarded after 7 days.

16 g	Peptone
11 g	Meat extract
3.0 g	Urea
2.8 g	Dipotassium hydrogen phosphate
0.7 g	Sodium chloride
0.4 g	Calcium chloride dihydrate
0.2 g	Magnesium sulphate heptahydrate

The pH of the solution must be adjusted to 7.5.

by a known concentration of pure O_2 to replace the amount consumed in respiration. The CO_2 evolution, the O_2 consumption, and the reduction of ethylene were inhibited by sodium pentachlorophenol at concentrations of 50 μg l^{-1}.

Bacterial cell permeability to toxicants is an important parameter in toxicity tests using bacteria (Bitton *et al.* 1988). Gram-negative bacteria are generally more resistant to toxicants than Gram-positive bacteria because of their more complex cell wall, and their use as a tool in toxicity assays requires alteration of their cell wall to increase their permeability to toxicants. To date only some mutational and physical (freeze-drying) alterations of bacteria have been evaluated for use in toxicity assays. Further research into techniques such as chemical treatments (EDTA, tris buffer, calcium chloride, antibiotics) and physical treatments (heating, freeze-thawing, drying) to increase the permeability of the outer wall membrane may lead to the development of more sensitive toxicity analytical techniques.

Assays based on the inhibition of oxygen uptake by bacterial cells.

Aerobic bacteria require O_2 for cell maintenance, growth and division. Thus the rate of O_2 uptake, or the respiration rate, is a useful parameter for

assessing whether a bacterium is in a normal, healthy, and active state. The respiration rate of a culture responds rapidly to the presence of inhibitors, and measurements have the advantage of speed and simplicity. However, not all inhibitors affect respiratory processes of bacteria, a cell may still respire after it has lost the ability to grow and divide, i.e. it is active, but not viable. Loss of respiration signifies death of a cell and is therefore a measure of acute toxicity. In short-term tests, which do not span more than one generation of bacteria, effects on growth and cell division are not manifested and therefore short-term respiration studies will not identify effects which become obvious only after a number of generations have been produced (King and Dutka 1986). Respiration rate measurements can be made in two distinct ways. Electrolytic respirometry involves the electrolytic production of sufficient oxygen to replace that absorbed by microbial action; in manometric repirometry the change in volume (or pressure) is measured by means of a calibrated scale. In the second method, the decrease in dissolved oxygen concentration is measured, the sample is confined so that no further replenishment of the dissolved oxygen can take place. Oxygen uptake due to nitrification and carbonaceous oxidation may be distinguished by means of a specific inhibitor of nitrification (Painter and Jones 1963).

Manometry. The earliest measurements of respiration rates with wastewater were carried out using manometric methods (Sierp 1928). Many similar manometric respirometers have been developed, the Warburg manometric technique being probably the most widely used (Umbreit *et al.* 1964; McKinney and Jeris 1955; Gerhold and Malaney 1966). A simplified manometric apparatus, marketed by the Hach Chemical Company, Ames, Iowa, U.S.A., is commercially available. A measured volume of test substance is stirred in a partially filled bottle which is connected to a closed-end mercury manometer. Oxygen consumption is measured by observing the change in level of the mercury column in the manometer. Carbon dioxide that evolves into the bottle atmosphere is absorbed by alkali, which is held in a small cup within the bottle cap (Sec. 1.4.2). Temperature control is usually achieved by placing the equipment in an incubator (U.K. Standing Committee of Analysts 1982). This method offers some advantages over others in that it requires little attention and also simulates quite closely the conditions found in a wastewater treatment plant. A comparison is made of the rate and amount of oxygen taken up in closed test vessels containing inoculated reference sewage with that taken up in vessels containing the test material either alone or in a mixture with the reference sewage (OECD 1992).

The biodegradation rates of 25 organic chemicals were determined manometrically with an 18-place Warburg apparatus. The toxicity values

determined were found to correlate well with those from aquatic toxicity tests, although it appeared that micro-organisms were less sensitive to the test chemicals than the aquatic organisms used (Vaishnav and Korthals 1990).

Electrolytic respirometry. A more sophisticated type of respirometer overcomes the problem of having to estimate the oxygen consumption of the samples before starting by including provision for reoxygenation of the culture as the oxygen is depleted. The respiring mixture is sealed in a flask with a CO_2 absorber dish suspended in an airspace. When the pressure in the airspace falls, as O_2 is utilised in the culture, a pressure sensing device causes a current to pass through the electrodes of an electrolytic cell mounted on the flask, thus producing an increment of O_2 to make up the deficit. The current passes through the electrodes and the length of time can be controlled so that a known increment of O_2 is produced at each operation of the electrolytic cell. Electrolytic repirometry ensures that the concentration of oxygen stays fairly constant, while in the classic manometric methods the oxygen concentration gradually falls. In full-scale wastewater treatment units, such as the activated sludge process, the dissolved oxygen concentration remains fairly constant at any given point within the reactor. In this way electrolytic methods simulate more closely full scale treatment.

The pHox® system, developed by the former Water Pollution Research Laboratory, U.K. (Montgomery *et al.* 1971), the Sapromat® respirometer marketed by Voight-Sapromat (1980), and the B1-1000® electrolytic respirometer, marketed by Bioscience Incorporated (1992) are electrolytic respirometers that are widely used. The Sapromat® is available in a completely automated version that has a compensating vessel for atmospheric pressure changes, and automatically plots the O_2 uptake curves in several samples simultaneously. The replenishment of O_2 allows the tests to be extended for longer periods than batch tests in sealed systems so that longer term effects, such as acclimation to overcome inhibition, may be investigated. The Micro-Oxymax® is a closed-circuit respirator that can measure both the O_2 consumption and the CO_2 production (Columbus Instruments International Corporation 1991). This instrument uses gas sensors to measure the changes in the O_2 and the CO_2 concentrations in the head space of a measuring vessel, and has the ability to measure up to 20 samples simultaneously. The operation of this system is automatically controlled by a PC and experimental data are maintained by computer software. As the measurements of O_2 and CO_2 are by external sensors,

fluctuations in temperatures have no influence on readings, resulting in accurate measurements.

Direct measurement of dissolved oxygen. Possibly the simplest technique for the determination of the activated sludge respiration rate is the use of a galvanic cell O_2 probe (Suschka and Ferreira 1986). Suitably aerated samples are introduced into a vessel containing an electrode which detects the decrease in dissolved oxygen concentrations, which is recorded as a function of time. The slope of the resulting line is the respiration rate in mg $O_2 l^{-1}$ per unit time. By measuring the MLSS or MLVSS it is possible to express respiration or specific oxygen uptake rate, mg $O_2 g^{-1}$ MLVSS per hour. The percentage inhibition at a particular concentration of test substance or the concentration giving 50% inhibition can be estimated (EC_{50}). Oxygen electrodes have been incorporated into a number of devices so that the sample may be flushed through the system and, when required, a portion can be sealed off for measurement of the respiration rate. Examples of such systems were developed by Blok (1974), and also the Rank Cell® (Green *et al.* 1975), the submersible electrode (U.K. Standing Committee of Analysts 1982), and van Kessel (1965). The Toximeter® was developed at the BASF wastewater treatment plant in Ludwigshafen, Germany, in order that toxic shock loading on the plant could be avoided. The Toximeter® consists of a pilot plant unit, with a continuous measurement of the respiration rate of the biomass. A prolonged decrease in the respiration rate results in the activation of an alarm and the toxic wastewater can be re-routed to a containment tank for further treatment (Pagga and Gunthner 1981).

Similar biosensors for on-line control in wastewater treatment plants are the rapid oxygen demand and toxicity tester (RODTOX®) described by Herricks *et al.* 1991, and the RA-1000® respiration analyser marketed by Manotherm BV (Spanjers and Klapwijk 1990). The RA-1000® continuous on-line respirometer consists of a 1-litre closed, completely mixed respiration chamber. The activated sludge is continuously pumped through the chamber, and the dissolved oxygen is periodically monitored at the inlet and the outlet. The respirometer is connected to a microprocessor, which records the dissolved oxygen concentration data, calculates the respiration rates, and as a result controls the process flows.

The following are methods for determining the EC_{50} of a chemical or wastewater using mixed cultures of micro-organisms, i.e. activated sludge:

(*i*) *Determination of immediate toxicity*, EC_{50} (*WRC*). Developed by the Water Research Centre (WRC), this is a rapid screening method. Activated sludge in the presence of sewage will respire rapidly and the addition of a

toxic concentration of a chemical will result in a decrease in the respiration rate proportional to the toxicity of the chemical (U.K. Standing Committee of Analysts 1982; OECD 1984). The Activated Sludge Respiration Inhibition Test (OECD 1984) involves incubating unacclimated samples of activated sludge mixed liquor from a municipal treatment plant. Activated sludge is continuously aerated with a feed substrate in the presence of varying concentrations of the test chemical for 30 minutes. The EC_{50} is the concentration of test chemical (as a percentage dilution) required to reduce the respiration rate (mg O_2 h^{-1}) by 50%, and it is calculated by comparison with an uncontaminated control. The mixed liquor suspended solids concentration should be maintained at 3000 mg l^{-1} and the respiration rate expressed as the specific oxygen uptake rate in mg O_2 g^{-1} MLSS h^{-1}. Diluted synthetic sewage is added to aerated activated sludge and the oxygen utilisation measured using an oxygen probe placed in the sample which is continuously stirred in a standard BOD bottle. The oxygen concentration is recorded every 15 seconds or continuously using a chart recorder over a total period of 30 minutes.

The concentration of dissolved oxygen is plotted against time and a regression line fitted. The respiration rate of the test samples and controls are calculated from the slopes (concentration of oxygen over time). Percentage inhibition is plotted for each dilution using the equation:

$$\% \text{ Inhibition} = \frac{R_c - (R_t - R_{pc})}{R_c} \times 100$$

where R_c is the respiration rate of the control, R_t the respiration rate of the test sample, and R_{pc} the respiration rate of the physico-chemical uptake at the test dilution (i.e. no activated sludge added). The percentage inhibition values (x axis) are then plotted against the various dilutions of the test chemical (y axis) and the 50% inhibition point calculated from the slope of the regression line. Poor reproducibility due to the varying nature of the activated sludge inoculum used should be taken into consideration when using this test. To some extent this is a treatment plant specific assessment procedure. The new 30-minute Polytox® toxicity procedure overcomes this problem by using a standard bacterial preparation in place of the activated sludge, significantly improving reproducibility (Elnabarawy *et al.* 1988).

(*ii*) *Modified "ETAD" method.* This method is based on that described by ETAD, (Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry), in which activated sludge obtained from a sewage treatment plant at a concentration of 1500 mg l^{-1} MLSS (approximately 10^7–10^8 organisms per ml) is used as a seed. The initial respiration rates

and the respiration rates after 3 hours of a standard amount of synthetic sewage and a suitable range of concentrations of test substances, including a control and a physico-chemical control, are measured (OECD 1971; OECD 1984; E.C. 1981).

(*iii*) *BOD inhibition test.* One of the simplest ways of assessing the toxicity of a test substance is to determine its effect on the uptake of oxygen by micro-organisms grown on a known, readily degraded substrate as measured by the standard 5-day biochemical oxygen demand (BOD) test. A number of standard substrates have been suggested, one being a mixture of glutamic acid and glucose (U.K. Standing Committee of Analysts 1982; Fitzmaurice and Gray 1987a,b). The test substances and the standard substances are dissolved in BOD dilution water. The mixture is saturated with air, seeded with a microbial inoculum (usually 5 ml of sewage effluent per litre or approximately 10^1–10^3 cells per ml), and incubated in the dark at 20°C for 5 days. The amount of O_2 consumed is measured with a dissolved oxygen probe and is compared to the O_2 uptake by the standard substances. A range of concentrations of test substances gives a set of BOD_5 values from which the EC_{50} of the test substance can be determined. The test substances may be inhibitory at higher concentrations but biodegradable at lower ones, while mixtures of industrial wastes may contain biodegradable and toxic components. It is then necessary to determine the BOD of the test material at various concentrations in the absence of the standard solution of glucose-glutamic acid (Sec. 1.4.2). The standard substrate is made up of 0.15 g l^{-1} glucose and 0.15 g l^{-1} glutamic acid which has a calculated BOD of 220 mg l^{-1}. A standard bacterial seed (e.g. Bioseed®) should be used instead of settled sewage. Percentage inhibition is calculated using the equation below:

$$\% \text{ Inhibition} = \frac{BOD_s - BOD_t}{BOD_s} \times 100$$

where BOD_s is the BOD of the control solution and BOD_t the BOD of the test solution. Percentage inhibition is plotted against the dilution of the test chemical with the EC_{50} calculated from the slope of the plot.

The OECD closed-bottle test is a measure of the biodegradability of organic compounds in an aerobic medium at a concentration of 2–5 mg l^{-1} of test substance, with an inoculum concentration of 0.5 ml l^{-1} of secondary effluent (OECD 1992). Degradation is followed by analysis of the dissolved oxygen concentration over a 28-day period. The BOD is determined and the biodegradation is defined as a ratio of the BOD to either the theoretical oxygen demand or the COD. In practice the theoretical oxygen demand

should be used as many chemicals are not fully oxidised, or are lost by evaporation, by the dichromate reagent. For example, some hydrocarbons will give 200% COD degradation compared to only a 40% theoretical oxygen demand.

A modification of the Japanese MITI (Ministry of International Trade and Industry) test is used to determine the degradation of a test substance by measuring the dissolved oxygen concentration continuously using an enclosed respirometer (OECD 1992). The test temperature range of $25 \pm 1°C$, an activated sludge concentration of 30 mg l^{-1}, and a 28-day test period are the recommended test conditions. Additional analyses are carried out by measuring the dissolved organic carbon (DOC) or the concentration of residual chemicals.

The toxicity of metals has been investigated using the BOD test (Mowat 1976; Ademoroti 1988). Mowat determined the toxicities of a range of metals by using the standard BOD test with an inoculum of 5% raw wastewater composite. Metals were found to decrease in toxicity in the following order: mercury, silver, copper, chromium, iron, aluminium, cadmium, cobalt and nickel, tin, and zinc. Limitations of the method were found to be the relationship between the toxic concentration and the quantity of suspended solids, and the affect of the nutrients in the dilution water on the solubility of certain metals, as some precipitated out of solution. BOD measurements of poultry farm wastewater showed that various concentrations of copper (II) and zinc ions had suppressing effects on the BOD values, inhibition occurring at concentrations as low as 1 mg l^{-1} (Ademoroti 1988) (Sec. 1.4.2.3).

The following are methods used to determine the EC_{50} of test substances using pure cultures of micro-organisms:

(*i*) *Synthetic activated sludge procedure.* Toxicity screening procedures based on the BOD method using pure cultures have been proposed for screening biological wastes (Busch 1982). Busch indicated that the bacterial seed represented the major variable in the BOD test and felt that it could be controlled both quantitatively and qualitatively by the use of pure cultures. A synthetic activated sludge procedure using a mixture of six micro-organisms and a medium of constant composition has been developed in order to improve the reproducibility of toxicity screening tests using activated sludge (Kwan 1988). Such factors as bacterial species composition, bacterial concentrations, sludge composition, and toxicant presence, make interlaboratory toxicity screening comparison studies difficult when activated sludge is used and the data contains

large variations. Six bacteria were selected to form the synthetic activated sludge from two to four different domestic and industrial effluents. Appropriate dilutions of effluents were spread onto the synthetic sewage agar (synthetic sewage and 15 grams of agar per litre) and incubated for 7 days at 20°C. At least six of the most predominant colony types were isolated and purified by subculturing 2 to 3 times onto fresh synthetic agar plates, and could be frozen for stock cultures. Each of the purified isolates were then inoculated into sewage broth and maintained on a rotary shaker for 7 days at 20°C. After 7 days, all flasks were pooled, the composite resulting in the synthetic activated sludge. Synthetic sewage, different concentrations of test substances, and synthetic activated sludge as seed, were added to a series of BOD bottles. Three controls were run with each test, a negative control, a physicochemical control, and a positive control (containing either mercury or 3,5-dichlorophenol). All bottles were stoppered and stirred at 20°C. The initial dissolved oxygen concentration was measured as was the concentrations after 30-minute intervals for 180 minutes. The EC_{50} could be determined from a plot of percentage inhibition versus concentration of test substance. The advantage of the synthetic activated sludge is the consistency of seed inoculation and reproducibility of data, while the fact that the effect of acclimation to specific inputs (toxicants or recalcitrant molecules) is not accounted for is a disadvantage. The synthetic activated sludge procedure can be standardised further by first identifying the organisms used as the bacterial seed, and then purchasing similar organisms from the ATCC collection of numbered strains. This would enable researchers anywhere to purchase the same strains and obtain comparable pure chemical data. Another variation would be to select 5 or 6 heterotrophic organisms commonly found in sewage, purchase those organisms from the ATCC and use them in the synthetic activated sludge technique. Again this would allow other laboratories to compare results and efficiencies of sewage treatment systems in reducing toxicant concentrations (King and Dutka 1986) (Sec. 10.4.3).

A commercially prepared seed produced by the Polybac Corporation is composed of a blend of bacterial cultures. Elnabarawy *et al.* (1988), compared the sensitivity of activated sludge respiration inhibition using this seed with the two published assay procedures, the Microtox® *Photobacterium phosphoreum* assay, and the OECD activated sludge respiration inhibition test. The following commonly occurring trace contaminants were tested: mercury, zinc, copper, cadmium, chromium, cobalt, cyanide, pentachlorophenol, 3,5-dichlorophenol, sodium lauryl sulphate, toluene, phenol,

formaldehyde, and chloroform. The Microtox® system was thought to be the most sensitive, followed by the OECD test using sewage micro-organisms as seed. The least sensitive was the commercially prepared bacterial seed. The major advantage of using a standard bacterial population in place of activated sludge is that a common reagent (inoculum) is available for inter-laboratory comparative studies.

(*ii*) *Oxygen uptake by micro-organisms.* A rapid screening assay for toxicity testing has been developed using the aquatic bacterium *Pseudomonas putida* (Slabbert and Grabow 1986). This bacterium requires oxygen to carry out basic physiological functions, and any effect on cellular metabolism and activity will thus be reflected by the respiratory activity. The test is carried out by adding a test sample to a suspension of *Ps. putida*, and the dissolved oxygen uptake is measured using a dissolved oxygen electrode before, during, and after exposure. If sublethal levels of toxicants are present in the test sample, oxygen uptake is inhibited or stimulated. If lethal concentrations of toxicants are present, oxygen uptake will be reduced. Using this method the toxicities of copper, cadmium, zinc, mercury, cyanide, phenol, and acetone were measured. It was concluded that this procedure was relatively sensitive, simple, economical, and exceptionally fast, and would prove useful for the rapid testing of many wastewaters.

A broader base for assessing the damaging action of pollutants can be obtained by using different organisms in analogous test procedures. The protozoan *Tetrahymena pyriformis* has been applied in comparable tests (Slabbert 1988). The toxicity of copper found in sediment samples was determined by measuring the inhibition of oxygen consumption of a pure culture of *Aeromonas hydrophila*. The microbial strain was isolated from the sediment under study (Flemming and Trevors 1989).

Microbial oxygen electrode screening tests. Modifications of oxygen electrodes have been reported recently in which a pure culture of bacteria is incorporated in or under the membrane of the probe to detect the presence of toxic materials. Immobilised *Clostridium butyricum* embedded in a collagen membrane over a dissolved oxygen probe has been investigated, as have the suitability of a number of bacteria, moulds, yeasts, and actinomycetes (Hikuma *et al.* 1980).

King and Dutka (1986) incorporated 18-hour cultures of *Bacillus subtilis* onto a membrane filter, mounted the filter onto the teflon membrane of a Clark-type oxygen electrode, and monitored the rate of inhibition of respiration at different concentrations of test compound in a glucose buffer solution.

A microbial sensor for the rapid measurement of BOD was developed using the yeast *Trichosporan cutaneum* immobilised in polyvinylalcohol and mounted on the polyethylene membrane of a modified oxygen electrode (Riedel *et al.* 1990). Inhibition of the biosensor by heavy metals and an increase in sensitivity of the sensor by pre-incubation with the test substance indicated that this microbial sensor could have wide application in wastewater treatment process control and as a toxicity assessment method.

Assays based on substrate utilisation

Modified OECD screening test (*MOST*). The modified OECD screening test is designed to pick out substances that are readily biodegradable (OECD 1992). The test substance is dissolved in an inorganic medium at a concentration of 10–40 mg carbon per litre, the medium is inoculated with a relatively low concentration (10^2 cells per ml) of micro-organisms from a mixed population and aerated at $22 \pm 2°C$ in the dark for a maximum of 28 days. Biodegradation is followed by dissolved organic carbon (DOC) analysis (U.K. Standing Committee of Analysts 1979). Control flasks are run in parallel to determine the DOC blank together with reference material of known biodegradability. Any substance which requires a period of greater than 18 days for acclimation of the relevant bacteria is found to be poorly or non-biodegradable. Substances passing this test, i.e. that exhibit greater than 70% DOC removal within 10 days, can be assumed to be very biodegradable and will be rapidly removed from a wastewater treatment plant.

DOC die-away test. This test was originally based on the method of the U.K. Standing Technical Committee on Synthetic Detergents (1966). It is similar to the MOST test but employs a much higher concentration of micro-organisms. This allows compounds to be degraded which would not pass the MOST test yet which degrade at a sufficiently high specific rate to be removed in sewage treatment plants under normal conditions (sludge retention time of 3 to 10 days). The behaviour of the test compound is compared with that of two surfactants, one is removed to a low degree in sewage treatment, the other, although well removed, is not readily degraded in tests with smaller inocula. The test compound is dissolved in an inorganic medium at a concentration of 10–40 mg carbon per litre, the medium is inoculated with a relatively large number of micro-organisms (10^5–10^6 per ml) from a mixed population (30 mg activated sludge per litre) and aerated at constant temperature for 28 days in the dark or diffuse light. If primary degradation is to be determined, specific analysis is

carried out; for determination of ultimate biodegradation DOC analysis is necessary (ISO 1984; OECD 1992).

Static-culture flask screening procedure. Some compounds are amenable to biodegradation only if other organic compounds (co-metabolism) or specific compounds (analogue metabolism) are being simultaneously biodegraded (Bunch and Chambers 1967). This test method is intended as a simple screening procedure for comparing the microbiological degradation of all organic compounds under static aerobic conditions. It also permits a moderate degree of acclimation. Since other organic compounds are present, primary, but not ultimate, biodegradability is measured. The test substance (up to 20 mg l^{-1}) is dissolved in BOD dilution water containing yeast extract as an additional carbon source, and an inoculum of raw sewage (10% v/v). Incubation is at room temperature and weekly subcultures are made for 3 consecutive weeks. After incubation for 7 days each subculture is analysed for the test compound to determine the extent of biodegradation. A parallel control of known biodegradability and preferably of similar chemical structure is included. This method utilises a culture-enrichment technique. Under normal wastewater treatment conditions, such culture-enrichment processes are different and the feed and sludge retention times may not always be optimum for the establishment of an acclimated microbial population to efficiently treat the test compound. Furthermore, the concentration of the organic test compounds in wastewater are probably significantly smaller than those used in the biodegradation studies, and as a result, may not elicit an induction enzyme formation response by the activated sludge biomass. This method is, however, useful in predicting the treatment of test compound by normal wastewater treatment processes, provided the methodology is modified to allow for adaptation of these compounds to occur.

A comprehensive screening study of priority pollutants was carried out using this procedure by Tabak *et al.* (1981). The test was modified to include the capability of studying the biodegradation of water insoluble and/or volatile compounds comprising the priority pollutant list and to facilitate the use of both the gas chromatographic as well as the DOC and total organic carbon (TOC) analytical procedures for assessing the extent of biodegradation of the test compound.

Modified semi-continuous activated sludge (SCAS) test. This method is an adaptation of the American Soap and Detergent Association (1965) semi-continuous activated sludge procedure that has been recommended by the OECD Chemicals Group (OECD 1976, 1981) and the European

Community (E.C. 1981). Only substances that are not readily biodegradable are subjected to this test, which, because of the high mean retention time of the sludge (> 100 days) and intermittent addition of the inoculum, does not simulate conditions experienced in conventional sewage treatment. The semi-continuous activated sludge (SCAS) unit is operated on a fill and draw cycle, where fresh sewage and the test compound is added once a day after the settling of the sludge and the removal of the supernatant. The MLSS concentration is initially in the range of 1000 to 4000 mg l^{-1}, and the sewage is usually degraded within 8 hours, leaving a 16-hour period of degradation of the test compound when the biomass is respiring endogenously. The conditions provided by the test are highly favourable for the selection and/or adaptation of micro-organisms capable of degrading a new compound and for co-metabolism. However, the procedure is most useful as a test for inherent biodegradability and may also be used to produce acclimated inocula for other procedures. The DOC concentration in the effluent of a SCAS unit which is being dosed with sewage and a known concentration of test substance is compared with the DOC of the effluent from a control unit dosed with sewage alone. Any difference in the concentration of DOC in the two units is assumed to be due to the residual test substance.

King and Painter (1985) investigated acclimation using this procedure by dosing activated sludge with a low concentration of one of six inhibitory organic chemicals in domestic sewage over a period of 3 months. The EC_{50} was assessed at the start and after various periods of exposure by inhibition of respiration (OCED 1984), nitrification (U.K. Standing Committee of Analysts 1981), and growth. An attempt was also made to isolate specially tolerant bacteria from the acclimated sludges and to compare the EC_{50} of these with the EC_{50} of exposed, but unacclimated, and of unexposed, presumably less tolerant bacteria. Results of this study were that selection in the SCAS test of specially tolerant or inhibitor degrading species had not occurred, except to some extent for pentachlorophenol.

Zahn-Wellens/EMPA test. The Zahn-Wellens test is another standard test for inherent biodegradability currently in use and has been widely used in Germany (Zahn and Wellens 1974; OECD 1981). Proposals made by Germany and Switzerland were to modify this test by combining it with a test developed by the Swiss Federal Laboratories for Materials Testing and Research, EMPA (OECD 1992). A mixture of the test substance, mineral nutrients and a relatively large amount of activated sludge is agitated and aerated at 20–25°C in the dark or diffuse light for 28 days. A relatively high

concentration of test substance (50–400 mg l^{-1} DOC) gives the advantage of greater analytical reliability. Biodegradation is measured by either DOC or COD analysis. This test, although useful, does not take into account the acclimation of the biomass to the test compound, unlike the SCAS test method (Nyholm 1991).

COD removal test. The determination of COD in the evaluation of degradability was first used by Konecky *et al.* (1963), and later by Pitter (1968). On the basis of experience obtained, the Department of Water Technology and Environmental Engineering (Prague) has developed a standard method for the comparison of biological degradability of organic substances (Pitter 1976). The evaluation of degradability is dependent on the decrease of organic carbon from the biological medium, with the rate of removal of COD indicating the rate of degradation. The degree and rate of degradation can be reliably quantitatively expressed and compared. The test substance is dissolved in a beaker containing biological medium at a concentration corresponding to 200 mg l^{-1} COD. The activated sludge, originally obtained from a sewage treatment plant, is adapted by vigorous aeration, driving off 20% of the sludge volume per day. After sedimentation of a further 60% of the sludge volume, the residue is diluted with water, starch or glucose, peptone, phosphate buffer and the test compound, to the original volume. Further aeration is carried out until the test compound is gradually increased so that after 20 days adaptation it is at a concentration of 200 mg l^{-1} COD. Thickened, adapted activated sludge is added to the biological medium giving a dry matter content of 100 mg l^{-1} of inoculum. The mixture is stirred in the dark at 20°C, the initial COD of the liquid phase is determined as are filtered samples at suitable intervals. The degree of degradation and the average specific rate of degradation are expressed in mg COD (or organic carbon) removed by a gram of dry matter of activated sludge per hour. Pitter (1976), using this method, evaluated the biodegradability of a variety of aliphatic and aromatic compounds. Results were expressed in percentage COD removal and rate of biodegradation.

Dextrose removal. In order to develop a rapid, easy methodology for evaluating the toxicity of chemicals to activated sludge, Hickman and Novak (1984) investigated the inhibition of dextrose uptake by activated sludge using pentachlorophenol (PCP) as a test chemical. An additional objective of the study was to determine if activated sludge could acclimate to low levels of PCP and if acclimation would protect the biomass against detrimental effects of shock loads of either PCP or related priority pollutants. The degree of metabolic activity of the activated sludge was measured in

terms of its specific rate of dextrose uptake. This was measured by a batch method. Activated sludge, at 20°C, was mixed with dextrose (to a concentration of 500 mg l^{-1}) plus the desired toxicant dose. After 5 and 15 minutes, the COD was determined by the dichromate reflux method. The MLSS concentration of the sludge was determined and the specific uptake rate was calculated as COD mg l^{-1} per MLSS mg l^{-1} $hour^{-1}$. Bench-scale reactors containing activated sludge become acclimated to low levels of PCP after 1 month. Low levels of PCP were found to give protection from shock loads of PCP, the amount of protection afforded was directly related to the acclimation concentration. Acclimation of activated sludge to PCP also provided protection from shock loads of related priority pollutants.

Thymidine uptake. The thymidine uptake procedure was developed as a microbial toxicity and degradation test method (Fuhrman and Azam 1980). Effluent from a laboratory activated sludge unit was mixed with an equal volume of a mineral salts medium and incubated at room temperature on a shaker for 48 hours (Sugatt *et al.* 1984). The medium, after filtration through glass wool, was very slightly turbid, and contained approximately 10^7 cells ml^{-1}. The bioassay procedure involved the addition of mineral salts medium to scintillation vials. Replicate vials were either unsupplemented with a carbon source or received either glucose or a commercial C_{12} linear alkylbenzene sulphonate (LAS) and methyl-^{3}H thymidine. Sterile controls containing 1% formaldehyde were also included. The assay was initiated with the addition of 0.1 ml of the inoculum. Periodically, for assessment of thymidine incorporation, replicate vials received cold trichloroacetic acid, and were placed on ice for 45 minutes. The contents were then filtered, washed with deionised water, and the filters were dissolved by the addition of ethylamine and Instagel. Radioactivity was assayed on a liquid scintillation counter. Results depicted thymidine incorporation into cells over time as a function of substrate concentration, i.e. glucose and LAS. The assay procedure as studied was highly artificial, as tests were conducted with relatively low levels of cells (10^3–10^4 organisms ml^{-1}) in a synthetic medium so that responses to low levels of substrates could be observed. Future studies are to address the applicability of assay conditions that are more readily found in the natural environment (Gledhill 1987).

Glucose uptake. Inhibition of glucose uptake can be used as a rapid, specific, and accurate method to determine the toxicity of chemicals to activated sludge (Olah and Princz 1986). The specific rate of glucose assimilation by activated sludge can be determined by adding a known quantity of glucose to the sludge while aerating and measuring the change of glucose

concentration with time by using a glucose-selective membrane electrode. This is an amperometric dissolved oxygen sensor, the teflon membrane of which is coated with immobilised glucose-oxidase enzyme. The oxygen necessary for the reaction is removed from the sludge by the enzyme, so the dissolved oxygen of the sludge is also measured. Toxicity of a chemical can be determined by measuring the inhibition of glucose uptake by the sludge.

A more complex method follows the changes of glucose content in activated sludge by using ^{14}C-labelled glucose (Larson and Schaeffer 1981). Activated sludge (2000–2500 mg l^{-1} MLVSS) and ^{14}C glucose were exposed to various concentrations of test chemicals for 15 minutes. The reactions were terminated with the addition of hydrochloric acid. Inhibition of ^{14}C glucose uptake was measured using a liquid scintillation counter. Data for the decrease in glucose uptake as a function of the log of the test chemical concentration were analysed by a nonlinear regression model to determine the EC_{50} values. The EC_{50} values of mercuric chloride and 3,5-dichlorophenol agreed well with values obtained by inhibition of respiration and microbial toxicity tests cited in the literature.

Assays based on CO_2 production.

The Sturm test, based on the evolution of CO_2 (Larson 1979; Sturm 1973), has been used extensively for the study of biodegradation and has been adopted as an OECD Guideline method (OECD 1992). Since it does not employ analysis of the test compound, it is useful for assessing the biodegradability of insoluble as well as soluble organic chemicals; although the amount of biodegradation of insoluble materials depends on the effectiveness of the agitation to keep the material in suspension and available for bacterial attack. The production of CO_2 from an organic compound means that it has been degraded, so that the comparison of the calculated CO_2 production with that observed gives a measure of the extent of biodegradation. However, complete biodegradation will never be reached even with readily biodegradable substances, since only a proportion of the test substance is used in respiration for energy, while some breakdown products are incorporated as new cells, which, in the duration of the test (28 days) will not be broken down (Weytjens *et al.* 1994).

A chemically defined liquid medium, essentially free of other organic carbon sources, is spiked with the test material and inoculated with a relatively large number of micro-organisms (10^5–10^6 cells per ml) from a mixed population and aerated at constant temperature. The CO_2 released is trapped as barium carbonate. After reference to suitable blank controls,

the total amount of CO_2 produced by the test compound is determined for the test period and calculated as the percentage of total CO_2 that the test material could have produced based on carbon composition. The main disadvantage of the Sturm test is that it is labour intensive, time consuming and as a consequence, replications of the test are difficult (Boatman *et al.* 1986). The minimum amount of test compound used for this test is 5 mg l^{-1}, but when this method is adapted to handle ^{14}C-labelled compounds the test compound concentration can be much lower. Other disadvantages of CO_2 evolution systems are that biodegradation is measured in the absence of alternate carbon and energy sources, and the level of micro-organisms used for the inoculum can be susceptible to toxic properties of the test material. Where the test substances are volatile, stripping may occur through the continuous purging of the CO_2 from the test vessels. Volatile chemicals can be tested by the oxygen uptake methods and, by using special vessels, in the DOC die-away method. The degree of biodegradation can also be calculated from additional DOC analyses made at the beginning and end of the test period (OECD 1992).

A study of the aerobic degradation of natural and xenobiotic organic compounds by subsurface microbial communities was carried out using aquifer solids samples (Swindoll *et al.* 1988). Biodegradation was measured as both the conversion of radiolabelled substrate to $^{14}CO_2$ and the incorporation of ^{14}C into cellular biomass. Data showed that the microbial community degraded many of the organic chemicals and that uptake into cell biomass represented a large fraction of total metabolism for many of the xenobiotic compounds.

Degradation studies were carried out by measuring the respiratory release of $^{14}CO_2$ using naturally occurring micro-organisms from a brown-water lake with a high content of humic compounds (Larsson *et al.* 1988). The ^{14}C-labelled recalcitrant aromatic pollutants were degraded more readily by the brown-lake microbes than microbial communities from a clear-water lake, and this was explained by the structural similarities between the test compounds and natural humic substances.

A method described by Larson (1979) to estimate the biodegradation potential of soluble, insoluble and unknown organic chemicals has two stages. Firstly, a microbial inoculum is generated in a bench scale SCAS system during which micro-organisms are acclimated to test material and the removal of DOC is monitored, followed by biodegradability testing (CO_2 evolution) in a defined minimal medium containing the test material as the sole carbon and energy source and a dilute bacterial inoculum from the supernatant of homogenised activated sludge generated in a

SCAS. Biodegradability data, accurately described by a non-linear model, allows the rate and extent of biodegradation for different compounds to be compared and statistically examined. The combined CO_2-SCAS system can be applied to a variety of compounds since non-specific methods are used to follow carbon metabolism. The combined system using high and low population levels tends to compensate for limitations of either system used singly. Removal and biodegradation can be measured in the SCAS and CO_2 system, respectively, for chemicals of known structure if the amount of organic carbon in the chemical can be measured.

The biodegradability of 18 test chemicals was determined using a carbon analyser to monitor the evolution of CO_2 (Struijs and Stoltenkamp 1990). Solutions of the test substance and activated sludge were prepared in mineral nutrient medium, and were incubated at 20°C on a rotary shaker. The CO_2 produced was measured weekly by headspace analysis and dissolved organic carbon analysis was carried out where possible. This test method was shown to have advantages over the Sturm test in that the biodegradability of poorly soluble compounds could be determined.

A short-term toxicity assay developed by Jardim *et al.* (1990), was the inhibition of respiration of *E. coli* by measuring the final CO_2 concentration in the culture medium using flow injection analysis and an electrochemical sensor. The method proved to be very rapid, in particular for mercury and an antibiotic where an inhibition in the respiration of the *E. coli* cells was detected in less than 20 minutes. However, it was noted that interference could be caused by some inorganic ions such as sulphide, cyanide, and acetate.

An improvement in the Sturm test has been developed by the use of infra-red analysers for the measurement of CO_2 (Birch and Fletcher 1991). This modification has improved the test in terms of speed, simplicity, and precision. Both the methods developed by Struijs and Stoltenkamp (1990) and Birch and Fletcher (1991) use enclosed serum bottles, with the headspace CO_2 determined after acidification in the former and without acidification in the later method. In contrast, the Sturm test is a flow-through system with CO_2 free air being bubbled through and the CO_2 collected in solution. So while volatile and insoluble chemicals may be tested in the enclosed methods, volatiles cannot be tested by the Sturm procedure.

3.6.4. *Other approaches*

Microcalorimetry

The use of microcalorimetry to study the effects of potential toxicants on micro-organisms is a developing concept. There are two main responses in

heterotrophic micro-organisms when they are subject to stress, one is to effect changes in biomass or community structure and the other response is based on changes in total or specific activities, e.g. motility and heat production. Heat changes which accompany all biological activity reflect the total activity in a community and could be a useful parameter for studies on the integrated effect of ecocontaminants under aerobic as well as anaerobic conditions (Bitton and Dutka 1986).

Calorimetric instrumentation can be broadly divided according to the operation mode; batch or flow. In the former, the reactive mixture is contained within a closed cell and experiments are carried out by fixing mixed amounts of reactants following thermal equilibration of the system. In flow calorimeters, the reactive mixture is circulated through a flow cell, the reacting components can be mixed either directly in the flow cell or before their introduction. Flow systems have gained widest acceptance in analytical-type biological studies because of the advantages they offer in ease of sample handling, the possibility of continuous on-line monitoring of processes, and the possibility for automation and computer control in routine experiments. Several types of flow reaction microcalorimeters are commercially available, such as those developed by the LKB Company and SODEV Incorporated (Jolicoeur and Beaubien 1986). Two wastewater treatment systems, an activated sludge plant treating wastewater from a textile factory and a methane fermentation plant treating a cheese factory effluent, were using a prototype flow microcalorimeter designed by SODEV. In both systems, the heat flux resulting from the metabolic activity during biodegradation of organic substances in the wastewaters could be measured accurately and rapidly, and could be operated continuously (Jolicoeur *et al.* 1988).

The development of toxicity bioassays using flow microcalorimetry must satisfy a number of primary physical and biological requirements. Firstly, the solutions or suspensions investigated must circulate in tubing and cells having internal diameters of 1 mm, the sample should show no evidence of sedimentation during the measuring period, nor should the micro-organisms exhibit appreciable adhesion to the cell wall. Care must be exercised that the oxygen and nutrients do not become limiting during the residence time of the biological mixture within the calorimetric cell. If the travel time of the sample to the calorimetric detector is too long, or if nutrient concentrations are too low, the observed heat effect may be artificially diminished. However, calorimetric measurement can tolerate a number of conditions which may create problems in other types of measurements, for instance, the presence of air bubbles, suspended materials and high concentrations of numerating species are generally well tolerated in calorimetric experiments.

Due to the recent application of microcalorimetry to chemostat-grown cultures, only a few studies dealing with the influence of toxicants on continuous bioprocesses have been carried out. In one particular study, it was reported that the addition of sodium azide to a glucose-limited culture of *Klebsiella aerogenes*, simultaneously increased the heat output, the respiration rate, and the CO_2 production (James and Djauan 1982). A significant decrease in biomass was also observed, approximately 30% in 30 minutes, indicating a less efficient utilisation of the energy source.

Fortier *et al.* (1980) investigated the influence of various toxicants, (cyanide, phenol, copper, chromium, and cadmium), on a mixed aerobic continuous culture used as a model for activated sludge processes. Addition of the heavy metals significantly reduced the heat production of the culture, indicating major inhibitory effects. In a more recent study, the toxicity effects induced by aliphatic alcohols in a heterogeneous aerobic culture were investigated. An increase in heat production at low alcohol concentrations and a marked decrease at high concentrations suggested metabolism occurred at low concentrations (Beaubien *et al.* 1985). A comparative study undertaken by Beaubien *et al.* (1986) used fast flow microcalorimetry to determine the toxicity of copper, mercury, zinc, phenol, sodium dodecyl sulphate and cetyl trimethyl bromide to mixed bacterial cultures and EC_{50}'s were compared with those obtained with the Microtox® test and with acute toxicity tests conducted with the aquatic invertebrate *Daphnia magna* and the freshwater alga, *Selenastrum capricornutum*. Despite differences between inhibition values obtained, results suggested that under standard conditions a mixed aerobic culture could yield toxicity estimates comparable to those obtained from multispecies testing. Considerably more work along these lines would be required for a standard toxicity assay, but the initial results appeared to be highly encouraging.

Microcosm toxicity tests

Microbial degradation of a potential toxicant or pollutant in the natural environment depends on the concentration and availability to the indigenous microbial population. Microcosm approaches using natural waters, solids, or sediments as microbial seed have now been used to determine the fate and effect of a variety of chemicals in aquatic environments. Microcosm tests have the advantage of providing data on the responses of many species simultaneously, and of including the effects of toxicants on interactions between species or species and their environments.

Gasoline leaking from underground storage tanks or from spillages poses a serious threat to ground-water quality due mainly to the water-soluble components of benzene, toluene, and xylenes. Major *et al.* (1988) investigated the biodegradation of these aromatic hydrocarbons in anaerobic batch microcosms containing shallow aquifer material. The study assessed the potential to remediate contaminated aquifers by nutrient addition or by addition of an electron acceptor, and the conclusion was that the addition of nitrate to gasoline-contaminated aquifers could prove to be an inexpensive *in situ* remedial technique.

Microcosm toxicity tests using naturally derived protozoan communities to estimate permissible concentrations of copper have been carried out (Pratt *et al.* 1987). Polyurethane foam substrates were placed in the selected aquatic environment for 14 days. These artificial substrates termed epicenters were colonised by protozoans as well as bacteria, diatoms, fungi, rotifers, annelids, and crustaceans, and have been used to study the dynamics of protozoan communities (Cairns 1982). The most consistent and sensitive response monitored has been the colonisation of barren substrates as an indicator of toxicant effects. Eighteen of these naturally colonised epicenter substrates were introduced into an illuminated headbox with dechlorinated tapwater. The diluent, along with any immigrating organisms, then entered a flow-splitter and was partitioned into 18 mixing chambers where stock solutions of copper sulphate were introduced. From the mixing chambers, media was delivered to test chambers containing barren polyurethane foam substrates. Taxonomic richness of communities were adversely affected at concentrations > 12.7 μg l^{-1} after 21 days. Colonisation was also affected at > 12.7 μg copper l^{-1}.

Quantitative estimates for environmental fate can still only be achieved by the extrapolation of laboratory estimates to an *in situ* ecosystem, and it is suspected that laboratory conditions may overestimate degradation rates or toxicity effects (Bitton and Dutka 1986).

3.6.5. *Continuous simulation tests*

Simulation tests are applied to substances and industrial wastewaters which are expected to be discharged to a sewer, in order to ensure that at the concentration used, they do not exert any adverse effects on the wastewater treatment process. Since they are relatively expensive tests, they are usually applied only to substances that are used in large quantities or which are of economic importance. Two simulation tests for the activated sludge process are the Husmann apparatus for testing the biodegradability

of synthetic surfactants (OECD 1971; E.C. 1981) and the Porous Pot apparatus, developed by the Water Research Centre (WRC) (Painter and King 1978). The Husmann apparatus is made from acrylic polymer or glass and consists of a cylindrical aeration chamber of 3-litre capacity with a conical base. The aeration vessel has an outlet at one side which passes into the conical base of a settlement chamber, which is also cylindrical and 60% the diameter of the aeration chamber. The Porous Pot is constructed from sheets of porous polythene which are made into cylinders. The Porous Pot is contained in a PVC vessel 15 cm in diameter with an outlet for the effluent. Comparison is made of the performance of laboratory-scale activated sludge units receiving the industrial wastewater alone or in a mixture of waste and sewage. Performance is assessed by the removal of polluting matter, the degree of nitrification, sludge growth and settleability. Further indications of toxic effects of the individual wastewaters may be given by microbial examination of the sludge. However, both these units fail to simulate true conditions of activated sludge treatment; the Husmann apparatus has an air-lift pump which returns settled sludge at an abnormally high rate and which does not allow anaerobic conditions to develop during settlement which can happen in practice, and the Porous Pot has no settlement phase at all. Although not widely used, simulation methods for fixed film wastewater treatment processes are also available (U.K. Standing Committee of Analysts 1981, 1982).

The treatability of wastewaters containing copper, zinc, and nickel and various industrial wastewaters was investigated using porous pot activated sludge units (Green *et al.* 1975). Useful information was obtained in these studies as acclimation to the biomass was taken into account. The treatability of *para*-dichlorobenzene in sewage treatment plants was studied using a modified porous pot activated sludge apparatus (Topping 1987). The porous pots were operated at a lower aeration rate, and various temperatures and sludge retention times were investigated. The plants were acclimated for 41 days and a second plant was established from waste sludge from the original and the control. Plant performance was determined by the level of TOC and ammoniacal nitrogen in the final effluent.

Biological treatability studies were carried out by the WRC for Sterling Organics, England, manufacturers of the drug paracetamol using the porous pot units (Dumbleton 1991). The aqueous wastes containing p-amino-phenol (PAP), sulphites, and inorganic salts were shown to be biodegradable resulting in a BOD of less than 35 mg l^{-1} at a 2% PAP dilution. The treatability of 12 organic compounds was investigated using continuous flow activated sludge units with an aeration volume of 3-litre and a settling compartment of 3.23 l (Stover and Kincannon 1982). The test

compounds were added individually to a synthetic wastewater composed of known biodegradable substances and the units were operated at different mean cell residence times. The treatment performance was monitored using BOD, COD, and TOC analyses.

Continuously fed laboratory-scale activated sludge units, with an aeration basin of 6 l capacity, and a final clarifier of 2 l capacity, were operated in order to determine the effect of waste from a detergent manufacturing plant on a municipal wastewater treatment plant (Versteeg and Woltering 1990). The units were operated for 14 days so that they could reach steady state conditions, and were then fed with different concentrations of the waste. Toxicity testing of the effluent was then carried out using aquatic organisms.

Kilroy and Gray (1992a) operated completely mixed activated sludge pilot units, each with a capacity of 80 l, in parallel to investigate the treatability of ethylene glycol, an organic chemical commonly used as a coolant in the pharmaceutical industry. While a concentration of the test chemical of 0.25% in the raw wastewater was treatable, there was a slight deterioration of floc structure in the biomass. A higher concentration of 0.5% ethylene glycol promoted extensive *Nocardia* scum formation, with a resultant loss in performance. From these simulation tests a safe discharge concentration of 0.1% was determined.

The treatability of several xenobiotic compounds by activated sludge micro-organisms was investigated using fill and draw laboratory-scale units with a capacity of 2.5 l, which were operated in series (Jacobsen *et al.* 1991). The test chemicals were investigated at concentrations ranging from 1–200 μl, and synthetic sewage was used as the primary substrate. It was observed that the biodegradation of pentachlorophenol (PCP) increased with an increased sludge retention time, indicating that biodegradation occurred by catabolic growth of a specific slow growing fraction of the biomass. However, the degradation of the substance lindane was increased with the increased degradation of the synthetic sewage, indicating that biodegradation occurred by co-metabolism.

Investigations of the treatability of ammonia-rich wastewaters in activated sludge systems was carried out using four laboratory, completely mixed and continuous flow single-stage units with a capacity of 14 l treating a highly nitrogenous synthetic wastewater with a low BOD (Al-Sa'ed 1988). At sludge retention times greater than 10 days, almost complete nitrification took place, while the efficiency of nitrification decreased at low sludge retention times. At sludge ages less than 10 days and high BOD:N ratios, it was observed that sludge bulking occurred.

The treatability of PCP in municipal activated sludge plants was assessed using three 20-litre continuous flow activated sludge reactors with clarifiers (Melcer and Bedford 1988). Data indicated that a relatively long sludge retention time, between 10 and 20 days, was required for maximum removal of PCP, and that a background level of PCP in the influent was required to maintain a PCP degrading population. Further studies using laboratory-scale units with an aeration basin of 5 l capacity and a clarifier of 4.7 l capacity were carried out to determine the treatability of variable amounts of volatile organic chemicals including chloroform, tetrachlorethylene, trichlorethane, toluene, xylene, and five heavy metals (Melcer *et al.* 1991). It was concluded that different mechanisms were responsible for the removal of short-term variable inputs of volatile organic compounds.

Simulation studies have been used to investigate the growth of filamentous organisms in activated sludge systems and for studying effective methods of sludge bulking control. Four 80 l pilot plants were fed with 100 litres per day of settled sewage, and were operated having an anaerobic, anoxic and aerobic zone, with retention times of 5, 3 and 9 hours respectively (van Leeuwen 1988). Effluent from each pilot plant flowed into a settler from where sludge was recycled to the anaerobic zone. Continuous dosing of ozone resulted in a vastly improved sludge settleability, and improved COD and colour removal. Nitrification-denitrification and phosphate removal appeared to be unaffected and it was concluded that ozone was an effective method for the control of sludge bulking.

A study to investigate the treatability of airport wastewater containing aircraft de-icing fluids was carried out using a bench-scale reactor, consisting of a 51 l aeration tank and a 8.5 l clarifier, to determine the optimum organic loading conditions for treatment (Jank *et al.* 1974). Further pilot plant studies using a large extended aeration plant with a 132 m^3 aeration basin, a 26 m^3 clarifier and a 24-hour retention time were conducted in order to investigate any possible operational problems during treatment of this wastewater. Problems associated with the growth of filamentous micro-organisms at higher organic loadings were highlighted during these simulation studies.

Simulation test methods have applications in determining the most suitable form of secondary treatment. Hannah *et al.* (1986) operated four pilot plants that were models of a trickling filter, an activated sludge plant, an aerated lagoon, and a facultative lagoon, to evaluate the most successful plant design for the removal of toxicants and to determine how the toxics partition and interact with the processes. Bench-scale studies were conducted at the Merck & Company Incorporated, Virginia, in order to

investigate the treatability of the factory's raw pharmaceutical wastewater. The results were used for the design of the full-scale plant (Donahue 1983).

3.6.6. *Conclusion*

Although useful, short-term tests are limiting as experimental conditions differ considerably from those in full-scale plants. The tests are batch processes in which the progress of a biochemical reaction is investigated over a relatively short time period, from 30 min in respiration inhibition studies to 5 d in BOD toxicity testing. The mixing characteristics therefore exhibit completely plug-flow dispersion. In contrast, the dispersion in full-scale continuous-flow plants approaches either completely mixed or dispersed plug-flow character. Substances described as non-toxic and degradable in short-term tests can exhibit other deleterious effects on full-scale operation. In short-term tests the biodegradation of a substance does not require that micro-organisms should be in any particular state, whereas the efficient operation of full-scale activated sludge plants requires that the flocculation of biomass occurs in order that separation of solids from the final effluent is achieved. Some substances can cause deflocculation without inhibiting respiratory activity. Other substances are responsible for the proliferation of filamentous micro-organisms resulting in sludge bulking. For these reasons simulation tests should be carried out in order to determine the treatability of chemical substances in activated sludge plants.

Further reading

General: Anderson 1980; Painter 1983; Bitton 1999.
Nitrification: Wild *et al.* 1971; Hultman 1973; Poduska and Andrews 1975; Sharma and Ahler 1977; Hall and Murphy 1980.
Treatability: APHA 1999.

4

Fixed-Film Reactors

It is clear, when examining stones or other submerged objects in an enriched river or the wall of a sewer, that micro-organisms will readily colonise any suitable surface provided that sufficient nutrients are present. This principle has been utilised in fixed-film or attached growth systems where the microbial biomass is present as a film which grows on the surface of an inert and solid medium. Purification is achieved when the wastewater is brought into contact with this microbial film. Because the active biomass is largely retained within the reactor there is no need to recirculate any displaced biomass back to the reactor in order to maintain a sufficient density of micro-organisms, as is the case in the activated sludge process (Chap. 5). The required contact between the film and the wastewater is achieved in most fixed-film reactors by allowing the wastewater to pass over the stationary medium in which the film has developed. However, it is not essential for the medium to be stationary, and in some reactor designs the medium itself moves through the wastewater (Sec. 4.2). Fixed-film reactors are designed as secondary treatment processes to partially treat (high-rate filter, anaerobic filter) or fully treat settled wastewater (percolating filter, rotating biological contractor, submerged filters), and for tertiary treatment to provide nitrification or denitrification. The most widely used fixed-film reactor is the percolating filter, which is considered in detail below. Rotating biological contactors, biological aerated flooded filters, and submerged aerated filters are considered in Sec. 4.2. Reed beds, another type of fixed film reactor, are discussed in Chap. 6. These processes are all aerobic. However, anaerobic filters, which involve the development of an anaerobic film on a suitable support medium, are also widely used

(Sec. 7.3). Anaerobic filters are used for treating wastewater with a high concentration of soluble BOD (> 500 mg l^{-1}) and which contain little suspended solids. Under anaerobic conditions, the organic matter is converted to gaseous end-products with little sludge being produced. The most widespread use of anaerobic filters is for the removal of oxidised nitrogen from purified effluents by denitrification, with the nitrogen present reduced to elemental nitrogen and released from the filter as a gas. Denitrification requires a carbon source, and in the removal of oxidised nitrogen (nitrite, nitrate) from purified effluents this is usually supplied as methanol (Anderson *et al.* 1984; Bailey and Thomas 1975). Anaerobic fixed-film reactors are also known as anoxic filters. The use of anaerobic fixed-film reactors is fully reviewed in Sec. 7.3.

4.1. Percolating Filters

The design and function of biological filters has been described by numerous workers (Bruce 1969; Warren 1971; Pike 1978; Bruce and Hawkes 1983). In its simplest form, the filter consists of a bed of graded hard material, the filter medium, about 2 m deep. The medium has interstices or voids that allow air and applied wastewater to reach all parts of the bed. The filter has a ventilating system to ensure free access of air to the bed and a distributor to regulate the volume and frequency of application of the sewage (influent) over the surface (Fig. 4.1). The medium provides the necessary base for the attachment of non-motile micro-organisms, principally bacteria and fungi, which form a film. Motile organisms, both micro- and macroscopic, live in the shelter of the interstices, feeding on and controlling the accumulated film. The action of this grazing fauna prevents heavy film growths blocking the interstices (Hawkes 1963), which would cause ponding and anaerobic conditions within the filter bed. The accumulation of the film follows a seasonal pattern, becoming thicker during the winter months. The action of the grazing fauna loosens and breaks down the film, resulting in a large removal of film each spring which is known as sloughing. The nutrients in the wastewater promote the growth of the micro-organisms that comprises the film, thus, as the wastewater percolates downwards over the surface of the film-covered medium, biological oxidation and conversion takes place.

Percolating filters are the most widely used secondary treatment process in Europe. They are equally common in other temperate regions including the USA, where they are employed at over 3700 separate municipal sewage treatment plants. Although biofiltration was historically the first process

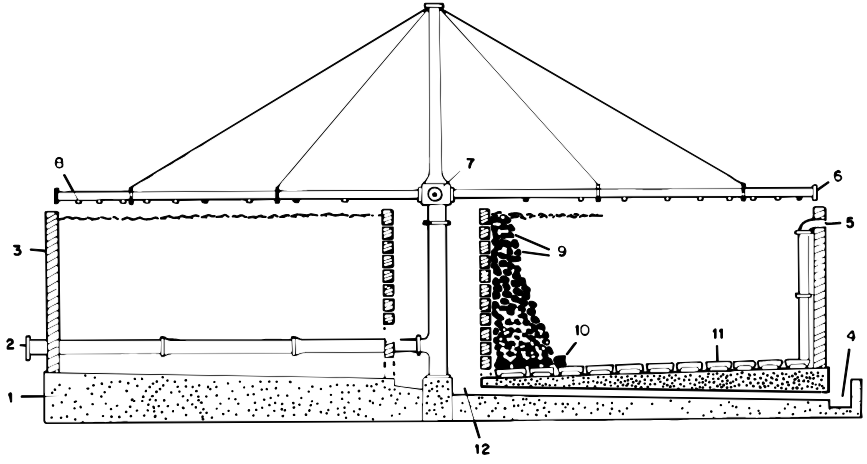


Fig. 4.1. Basic constructional features of a conventional percolating filter. 1, foundation floor; 2, feed pipe; 3, retaining wall; 4, effluent channel; 5, ventilation pipe; 6, distributor arm; 7, rotary seal; 8, jets; 9, main bed of medium; 10, base layer of larger medium; 11, drainage tiles; 12, central well for effluent collection (Bruce and Hawkes 1983).

used, it still has certain advantages over the activated sludge process. Filters require virtually no skilled maintenance or close control. In energy terms, percolating filters are more economical than the activated sludge process, and are more versatile in responding to changes in flow and character of wastewater (Hawkes 1963). Filters are more tolerant of continual or shock discharges of certain pollutants compared with activated sludge (Cook and Herning 1978), including toxic industrial wastes containing heavy metal ions, phenols, cyanides, sulphides, and formaldehyde. They are widely used for both total and partial treatment of a wide range of industrial wastewaters (Bruce 1969; Calley *et al.* 1987; Pike 1978). Their major disadvantage is capital cost, being normally uneconomic in serving populations greater than 50000. This is due not only to higher capital costs compared to activated sludge systems but also to the larger area of land they occupy, which is often at a premium in urban areas (Jeger 1970) (Table 4.1). For this reason, the activated sludge process predominates at very large sewage treatment works. Although the proportions of the population of England and Wales served by these two bio-oxidation processes are about the same, many more of the 5000 or so sewage treatment plants use percolating filters rather than the activated sludge process (Institute of Water Pollution Control 1972). Bruce (1969) concluded, in his review on percolating filtration, that there was no indication that the use of percolating filtration was likely

Table 4.1. Comparison between the activated sludge and percolating filter processes.

	Activated sludge	Percolating filtration
Capital cost	Low	High
Area of land	Low: advantageous where land availability is restricted or expensive	Large: 10 times more area required
Operating cost	High	Low
Influence of weather	Works well in wet weather, slightly worse in dry weather, less affected by low winter temperatures	Works well in summer but possible ponding in winter
Technical control	High: the microbial activity can be closely controlled; requires skilled and continuous operation	Little possible except process modifications. Does not require continuous or skilled operation
Nature of wastewater	Sensitive to toxic shocks, changes in loading, and trade wastewaters; leads to bulking problems	Strong wastewaters satisfactory, able to withstand changes in loading and toxic dischanges
Hydrostatic head	Small: low pumping requirement, suitable for site where available hydraulic head is limited	Large: site must provide natural hydraulic head otherwise pumping is required
Nuisance	Low odour and no fly problems. Noise may be a problem both in urban and rural areas	Moderate odour and severe fly problem in summer possible. Quiet in operation
Final effluent quality	Poor nitrification but low in suspended solids except when bulking	Highly nitrified, relatively high suspended solids
Secondary sludge	Large volume, high water content, difficult to dewater, less stabilised	Small volume, less water, highly stabilised
Energy requirement	High: required for aeration, mixing, and maintaining sludge flocs in suspension and for recycling sludge	Low: natural ventilation, gravitational flow
Synthetic detergents	Possible foaming, especially with diffusers	Little foam
Robustness	Not very robust, high degree of maintenance on motors, not possible to operate without power supply	Very sturdy, low degree of maintenance, possible to operate without power

to be outmoded and this remains true even with the introduction of new processes and the development of packaged plants. However, the majority of new domestic and municipal sewage treatment plants built since 1970 have been of the activated sludge type. Small domestic plants serving populations of < 2000 are often of the rotating biological contactor or submerged aerated filter design. Reed beds are increasingly being used for secondary and tertiary treatment for small rural plants serving < 1000 PE. Parker (1999) examines the future role of percolating filters.

The term percolating filter in Ireland and the UK is still used, although there are many derivations of the name such as biological filter, bacteria bed, percolater, and, in the USA, trickling filter. However, the term filter often causes confusion as the process is essentially biological, even though there is some physical removal of fine solids. The confusion arises because when the process was first developed it was thought that purification was brought about by physical filtration, as in a sand filter, and percolating filtration was developed from such physical filter processes. Experiments were carried out at the Lawrence Experimental Station of the Massachusetts State Board of Health (USA) using small filter beds to test the efficiency of various soils and aggregates, e.g. different grades of sand and gravel, to physically remove solids from wastewater. They found that effective purification of settled sewage could be achieved using quite coarse gravel (19–25 mm grading) and at very high rates of application (0.2–0.5 $m^3m^{-2}d^{-1}$), so that it was not always necessary to use sand filtration or land treatment, both of which required much lower hydraulic loading rates to be as effective (Mills 1890). Although it was quickly realised that this new type of treatment must involve a degree of biological oxidation, it was not until the process was well established that the physical role of filtration was found to be minor in comparison to the biological function, by which time the terms, trickling and percolating filtration had become established. The impact on sewage treatment practice was immediate, because this was a process that required only one-tenth of the area that existing land treatment required and, as the beds were specially constructed, treatment became independent of the suitability of the soil for the first time. The first percolating filters were constructed in Salford (England) in 1892. This first installation was designed by Corbett (1902) and it was here that many of the practical problems of design and construction were solved. The first municipal trickling filter installed in the USA was in Atlanta, Georgia, and was commissioned in 1903 (Stanbridge 1976; Bruce and Hawkes 1983).

4.1.1. *Design and operation*

Design

The design of percolating filters has changed very little since they were first introduced and, with a working life of 80 years, many of the original filters are only now reaching the end of their useful lives. However, replacement is more often due to an increase in the loading in excess of their original design, rather than structural or mechanical failure. The major factors that need to be taken into account in the design and operation of percolating filters are: medium type, and in particular, specific surface area and voidage; depth; area of the bed; organic, and hydraulic loading rates.

The depth of filters is arbitrary, and when all types of percolating filtration are taken into consideration there is a total range of between 0.9 and 15.0 m. The standard (low-rate) percolating filter in the UK is usually about 1.8 m deep and rarely less than 1.5 m or in excess of 2.5 m. Filter beds in the rest of Europe tend to be deeper than this and in Germany most filters are between 3–4 m in depth. Low-rate filters in the USA are a similar depth to those in the UK, at between 1.5–2.0 m, although shallow beds (< 1 m) are used for high-rate filtration. High-rate filters using modular plastic medium are used to treat industrial wastewaters. The medium is very light and can be stocked, like building blocks, into a tall tower which is essentially free-standing. These filter towers are housed in lightweight prefabricated material or are built out of breeze blocks and can be constructed as high as 12–15 m.

In terms of treatment efficiency, the majority of BOD and suspended solids removal occurs within the top 750 mm of the filter bed, which is also about the minimum depth when using conventional mineral medium in order to avoid short-circuiting of wastewater through the bed. Apart from constructional costs, the major limiting factor in the selection of the depth of filters is the loss of hydraulic head, with the greater the depth the greater the loss of head occurring. Unless the plant is built on a slope, pumping will be required. From experience, it would appear that a depth of 1.8 m, when using conventional medium, is a good compromise between treatment efficiency, loss of hydraulic head, and cost. The influence of depth on performance is considered in Sec. 4.1.4.

There appears to be a wide variety in the configuration, or plan, of percolating filters (Learner 1975a), although it is dependent to a large extent on the site and the total loading. They are usually either rectangular or circular in plan. Rectangular filters are normally used for large populations because they are more compact and are cheaper to construct because they

share retaining walls. Maximum size of such filters rarely exceeds 10 m in width and 100 m in length. They can also be used for very small populations (< 40 people) when the rectangular shape is more compatible with the mechanical distribution system used. This comprises of a tipping trough that discharges the wastewater intermittently into a fixed set of distribution channels that are laid across the filter. Circular plan filters are generally preferred as they allow greater control over the frequency of wastewater application. The maximum size of these filters is limited by the maximum diameter of rotating distributor available, which is 40 m.

Percolating filters are built on a concrete base covered with drainage tiles that provides a raised floor on which the medium rests (Fig. 4.1). This provides an unrestricted area of underdrainage, which takes the final effluent away. It is important to ensure that the underdrainage area is deep enough to allow the passage of air from ventilation pipes lining the base of the filter bed. The ventilation pipes are connected to the atmosphere so that there is a free flow of air through the whole filter, providing the necessary aeration. Natural convection causes aeration because of the difference between the air temperature and the temperature within the bed. The concrete base is sloped to at least 1:100, normally 1:50, to ensure a minimum flow of 0.6 m s^{-1}, which prevents settlement and the accumulation of solids washed from the medium. Drainage tiles can be replaced by a series of half-round field drains laid out in a herringbone pattern in rectangular filters, or arranged radiating out from the centre of circular shaped filters to a central drainage channel or well (Fig. 4.2) (IWEM 2000). As is the case with the tiles, the drains must be of sufficient size to allow for adequate ventilation.

Filter bed walls have to be able to support a considerable weight of medium, attached film, and water (Table 4.2). The retained material exerts lateral pressure on the walls, which increases towards the base and it is thus

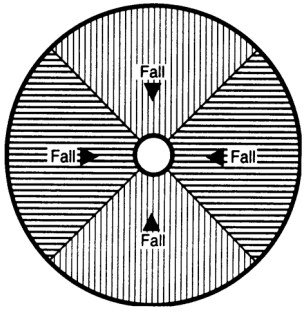


Fig. 4.2. Typical arrangement of filter floor tiles draining to a central wall.

Table 4.2. Comparison of the bulk density of a mineral medium and a random plastic medium of the same nominal grade (50 mm).

	Dry weight (kg m^{-3})	Total bulk density due to film accumulation (kg^3m^{-3})		Weight when totally saturated with water (kg^3m^{-3})
		minimum film	maximum film	
Flocor R. C.	97.84	133.73[a] 140.68[b]	290.59[a] 354.77[b]	1004.84
50 mm slag	886.31	1014.55[a] 1050.01[b]	1277.32[a] 1180.33[b]	1401.31

[a]Low-rate loading, [b]high-rate loading.

cheaper and safer to construct filter beds partially or completely buried in the ground. This allows breeze blocks or a single layer of bricks to be used for the retaining wall and also ensures maximum thermal insulation provided by the surrounding earth. Filter walls are normally under ring tension with the forces involved increasing with diameter of the filter (Haywood and Cross 1976). When constructed entirely above ground the retaining walls must be able to take the maximum bulk density of the filter medium contained within the bed. In this case, the walls are constructed out of reinforced concrete, thick brickwork, or prefabricated concrete sections bolted together. When precast sections are used the joints do not need to be water tight as the lateral spread of effluent in the bed is limited under low to moderate hydraulic loadings. At higher loadings some seepage of wastewater may occur at the joints. In the early years, some small filter beds were built free-standing. This was done by inclining the sides sufficiently so that the media formed a stabilised bank which was then covered using a larger grade of aggregate. Drystone walling was also popular, although both systems were liable to the walls collapsing and surface water overflowing down the sides. Modular plastic medium exerts a negligible amount of lateral pressure on the retaining walls and therefore lightweight cladding, usually coated steel panels, or single thickness breeze block housing are adequate. This is not the case with random plastic filter medium, as is seen when the maximum bulk density exerted on the retaining walls can be almost equal to that of mineral medium if ponding occurs (Table 4.2) (Gray 1983b).

The main function of the distribution system is to apply the wastewater to the top of the filter bed as evenly as possible. Distribution systems can be categorised as either static or moving. Static distributions are a network of pipes or open troughs which are permanently fixed in position above the surface of the medium. The wastewater is discharged through fixed outlets

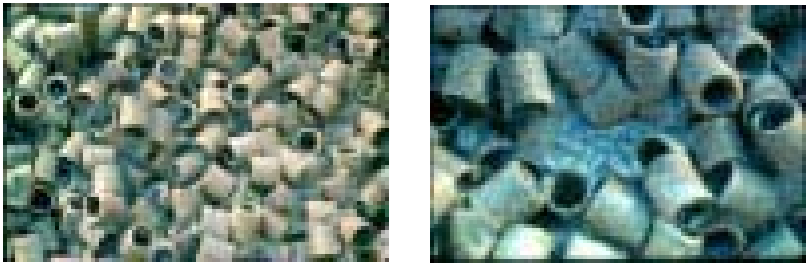
that are usually spray nozzles (where pipes are used) or via V-notches from open channels. However, the uniformity of dosing and the completeness of wetting the surface of the medium is not as good as obtained when using moving distributors, and static distribution systems are restricted to very small percolating filters. High-rate filters using modular media are often square or rectangular in plan (Sec. 4.1.2) and because it is important to have even distribution in order to utilise all the potential surface area of the medium, static spray nozzles are frequently used. There are two types of moving distributors, rotary distributors that are used for circular filters and reciprocating distributors that are employed on rectangular beds. Rotary distributors are either 2 or 4 radial sparge pipes which are pivoted in the centre of the filter on a rotating column (Fig. 4.1). The outlet holes are spaced along the arm to ensure a uniform loading, with the holes becoming more closely spaced further away from the centre. The outlets must be large enough to ensure that blockages from solids carried over from the settlement tank or accumulated grease rarely occur. Once blocked, that area of the bed fed by that outlet hole will be without any influent until the blockage is cleared. For this reason, simple holes, with splash plates situated below to ensure maximum dispersion of the wastewater, are preferred to jets or spray nozzles. The distributor arms can be driven by water wheels, electrically powered wheels that drive the distributor arm around or, more commonly, by jet reaction as the water leaves the outlet. Whereas jet reaction does not require a power supply and is mechanically simple, it does require a liquid head of 0.5 m as well as an extremely low friction bearing to support very well-balanced distributor arms. The speed of rotation varies according to the hydraulic loading and such systems are affected by changes in wind direction, which can result in the distributor stopping altogether and causes local overloading of the bed and a subsequent reduction in effluent quality (IWEM 1988, 2000).

Reciprocating distributors are associated only with filters that are rectangular in plan and are only common in the UK. The walls of the bed serve as a track to convey as well as support the distributor as it moves back and forth along the bed. Wastewater is fed into the moving sparge pipe from a longitudinal supply channel by syphonic action (IWEM 2000). The moving distributor is propelled either by a water wheel mechanism or an electric motor using cables. Because the distributor cannot travel in excess of 10 m min^{-1} it takes a considerable time to travel from one end to the other. This can result in long intervals between successive dosing of wastewater, especially at the far ends of the bed, thus particularly long beds may employ several distributors each operating over a particular area (Stanbridge 1972).

Loading

For conventional single-pass filtration, where settled sewage passes through a single percolating filter bed before having the solids (humus) removed in a secondary sedimentation (humus) tank, loading can be categorised as either low- or high-rate. Hydraulic loading is expressed in cubic meters of settled wastewater per cubic metre of filter medium per day ($m^3m^{-3}d^{-1}$) so that loadings $< 3m^3m^{-3}d^{-1}$ are classed as low-rate and $> 3m^3m^{-3}d^{-1}$ as high-rate filtration. However, there is no precise demarcation between low and high rate filtration and these figures are only guidelines. Hydraulic loading is not always expressed as a volumetric loading. It is also expressed as a surface loading, which is often referred to as the irrigation rate, in cubic metres of wastewater per square metre of superficial bed area per day ($m^3m^{-2}d^{-1}$). The hydraulic loading rate depends solely on the physical characteristics of the medium and the degree of film accumulation within the bed. The upper hydraulic limit is determined by the onset of flooding because the wastewater is being applied at a faster rate than it can percolate through the bed. Where this occurs then the interstices of the medium become flooded and the access of air to the interior of the bed is prevented. The lower limit is known as the minimum wetting rate, which is the loading rate at which the medium is kept sufficiently moist to prevent drying and the microbial film to remain active (Winkler 1981). Organic loading is measured as the BOD per cubic metre of filter medium per day (kg BOD $m^{-3}d^{-1}$) with low-rate filtration classed as filters receiving loads of < 0.6 kg BOD $m^{-3}d^{-1}$ and high rate > 0.6 kg BOD $m^{-3}d^{-1}$. In practice, in order to produce a Royal Commission standard effluent (20 mg l^{-1} BOD, 30 mg l^{-1} suspended solids) after settlement with a high degree of nitrification, filters treating domestic sewage should receive an organic loading of between 0.07–0.10 kg BOD $m^{-3}d^{-1}$ and a hydraulic loading between 0.12–0.60 $m^3m^{-3}d^{-1}$. Generally, increases in organic loading in excess of 0.10 kg BOD $m^{-3}d^{-1}$ will result in heavier film growths, which may result in ponding. In an attempt to produce more efficient percolating filters, which would operate at much higher loadings, a number of modifications of the basic process have been adopted. By using larger mineral medium, for example, greater loads can be applied to filters without the risk of ponding. Such high rate filtration will produce a 20:30 effluent with an increased hydraulic loading of up to 1.8 $m^3m^{-3}d^{-1}$, but with little or no nitrification (Institution of Public Health Engineers 1978). If a less stabilised effluent is required, such as roughing treatment for strong industrial wastes, then loadings of up to 12 $m^3m^{-3}d^{-1}$, with organic loads up to 1.8 kg BOD $m^{-3}d^{-1}$ will give 60–70% removal. Treatment at such high rates is facilitated by

Fig. 4.3. Surface of a biotower housing modular plastic medium (Flocor E®). Splash plates are used to ensure even distribution of influent wastewater over the surface of the medium ensuring maximum utilization of the available surface area.

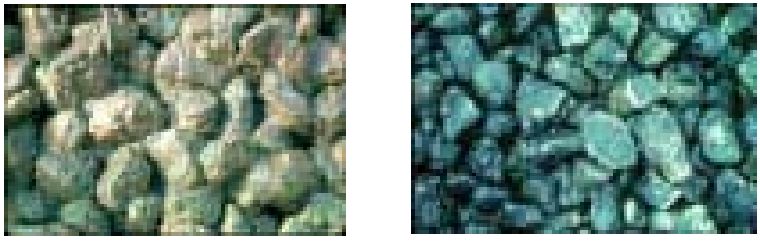


(a) (b)

Fig. 4.4. (a) Bacterial film development on the random plastic medium Flocor RC®. (b) Even with high voidage, random plastic media can suffer from ponding due to the fungus *Subbaromyces splendens*, seen here forming thick greyish-white colonies.

using modular (Fig. 4.3) or random plastic medium (90% voids) (Fig. 4.4) in tall towers, in place of the usual stone medium (40% voids) (Fig. 4.5), thus reducing the risk of ponding. Modifications of the percolating filter process are dealt with in Sec. 4.1.2.

If the amount of biological film growing per unit area of medium remains more or less constant then the organic loading corresponds to the sludge loading or the food to micro-organism ratio (f/m ratio) as used in the activated sludge process (Sec. 5.2). This is difficult to measure directly as film accumulation is rarely even throughout the filter bed and is certainly more abundant in the upper areas of the bed where food is a non-limiting factor. Also, a considerable proportion of the biomass will be non-active being comprised of inert macro-invertebrate fragments, and the proportion of non-active material will increase towards the base of the filter. Thus, in theory, using sludge loading in a percolating filter offers a degree of control on par with that of the activated sludge process. However, in practice,



(a) (b)

Fig. 4.5. The surface film development on blast-furnace slag varies according to temperature and distribution system. (a) Winter bacterial dominated film on a filter employing splash plates; (b) summer algal dominated film. The influent wastewater flows directly from the nozzles, without splash plates, scouring the medium directly below the outlets of the distributor arm. Thick algal mats are formed in the splash zones between the outlets.

accurate estimations of film accumulation can only be made for high-rate filters, especially those using modular plastic medium, where seasonal variation in film development and the problem of inert solids being retained in small interstices have been largely eliminated. Another important factor is that none of the medium in a high-rate filter should be either food or oxygen-limited, thus ensuring an even growth of film throughout the bed. Estimates of film accumulation have been made. For example, the active depth of film is known to be about 0.2 mm, which gives an average weight of active film per m^2 of medium surface of around 0.2 kg. If it is assumed that the water content of film is about 97%, then each square metre of medium surface is supporting 6 g (DW) of active microbial biomass. The exact amount in the filter will depend on the surface area of the medium, but if this is assumed to be 100 m^2m^{-3} then the active microbial biomass will be 0.6 kg (DW) m^{-3}. Thus, at an organic loading of 0.1 kg BOD $m^{-3}d^{-1}$ the sludge loading will be about 0.2 kg BOD $kg^{-1}d^{-1}$. For high-rate systems, assuming the same surface area for the medium, the sludge loading will be approximately 1 kg BOD $kg^{-1}d^{-1}$ at an organic loading of 0.6 kg BOD $m^{-3}d^{-1}$. Therefore, there would appear to be agreement between the term low- and high-rate as used in both the percolating filtration and the activated sludge processes (Winkler 1981).

Filter media

The function of the medium in a percolating filter is to provide an extensive surface to support the biological film and the associated fauna necessary for the purification of settled wastewaters. At the same time it must allow

sufficient ventilation for the process to operate aerobically and ensure maximum contact between the active film and the waste liquid.

Mineral media. The important features of mineral media were summarised in the original British Standard in 1948 (BSI 1948). It stated that a mineral filter medium should be selected for "high surface area consistent with adequate voidage, and for satisfactory grading (within specified size limits), durability, roughness of texture, satisfactory shape characteristics and low cost". Thompson (1925) had shown that the material selected had to be mechanically and chemically stable if the medium was not to degrade filling the voids with smaller pieces of aggregate broken off the parent medium. Whereas Levine *et al.* (1936), comparing a number of different filter media, found that the performance was directly related to the surface area of the medium. After the publication of the 1948 British Standard, it became the practice for engineers to choose a medium which was predominantly cubic in shape. This view was based on the supposition that a filter medium containing a high proportion of flattish pieces would have an undesirably low voidage, and many textbooks still retain this view. The more irregular a material is for a given nominal size, the greater the surface area. Therefore, although more costly, blast furnace slag or clinker is to be preferred to gravel or pebbles. For material of a given shape or uniform grade, the voidage is independent of size. However, the important factor is the actual dimensions of the void spaces (the interstices), as these will determine whether or not a given medium will clog during operation or will allow adequate ventilation. Excessive accumulation of film also reduces the effectiveness of the surface area. The rejection of flaky material (i.e. particles with one excessively thin dimension) by British Standard 1438:1948 was proved to be unjustified by Schroepfer (1951). When examining the effect of particle shape on voidage and surface area, he found that the more cubical material possessed a lower voidage than the flaky material and concluded that an increase in angularity of particles, of which flaky particles are an extreme example, resulted in an increase in both voidage and surface area. Compaction will reduce the available voidage by between 5–7% (Moncrieff 1953). In a review of the relationship of particle shape and voidage, Bruce (1968) supported the earlier findings of Schropfer, that flaky media possess a higher voidage and surface area than regular media of the same sieve size. However, the average volume of flaky particles is smaller and this controls the actual size of the voids, although the use of flaky material of a large grading would compensate for this. These findings led to a relaxation of the "index of flakiness" and the withdrawal of the "index of elongation" in the revised British Standard on Percolating Filter Medium (BSI 1971, 1980).

Numerous comparative investigations on the ideal characteristics of filter media followed the publication of the British Standard in 1948, in particular, those carried out at Minworth near Birmingham (Hawkes and Jenkins 1955, 1958) and at Stevenage (Wilkinson 1958; Truesdale *et al.* 1962). These investigations showed that the smaller media consistently produced better quality effluents, and that medium with a rough surface gave marginally better performance than the smooth surface materials such as gravel. Truesdale *et al.* (1962) found that although small grade, rough-textured medium was extremely efficient in treating large organic loads of settled sewage during the summer (0.18 kg BOD $m^{-3}d^{-1}$), it suffered from excessive film accumulation during the colder winter months. This resulted in ponding and the eventual clogging of the filter. Experience has shown that in order to achieve maximum efficiency throughout the year, a 50 mm mineral medium with a rough surface provides the best compromise between large surface area and the provision of large voidage, and that this will produce a high quality effluent in a conventionally operated British plant (Hawkes 1963).

Learner (1975a), in a survey of percolating filters, found that granite, clinker, and blast furnace slag were the most frequently used media (Table 4.3). While the pitted nature of the clinker and blast-furnace slag makes them superior to other mineral media, the choice of filter medium does not only depend on its suitability but also on availability and cost. As Learner points out, the majority of filters using clinker as medium were constructed prior to 1956, and since then clinker has become more expensive

Table 4.3. Types of media most commonly used in percolating filters in the UK (Learner 1975a).

Type of medium	%
Granite	26
Clinker	24
Blast-furnace slag	23
Rounded gravel	6
Limestone and clinker	6
Limestone	4
Coke	4
Clinker and gravel	3
Slag and coke	1
Saggar chippings	1

Table 4.4. Percentage occurrence of grades of filter media used in UK percolating filters (Learner 1975a).

Grades of medium (mm)	0–13	13–25	25–38	38–51	51–64	64–76	> 76
%	2	21	39	29	6	3	0

and extremely difficult to obtain. Another interesting aspect of the survey was that the majority of the filters sampled in Scotland used granite as a filter medium; although this may not be the most suitable medium it was the most readily available in the area. A similar situation exists in Ireland where neither blast furnace slag or clinker are available (Earle and Gray 1987). For this reason, the majority of percolating filters use the locally available stone which is normally basalt or granite. Learner (1975a) found that the commonest range of media grades used was 13 to 51 mm (Table 4.4), based on particle size analysis. Bruce (1969) states, in his review of percolating filters, that the commonest grades of media used in the UK are the nominal sizes of 38–51 mm. As previously stated, 50 mm mineral media provides a good compromise between surface area and voidage and has been shown to produce good quality effluents under low-rate conditions (Hawkes and Jenkins 1955, 1958).

Physical nature of media. The shape of media is defined by using a descriptive classification based on visual inspection. They can be classed as being flat, angular, irregular, rounded, or spherical. The different shapes are beautifully compared in a series of photographs in British Standard 1438 (1971). The voidage increases with an increase in the proportion of flaky media, whereas angular shaped media have a higher voidage than rounded media. Typically, basalt and granite media are either flat or angular (50–54% voidage), blast-furnace slag is irregular (50%), and gravel is rounded or spherical (38–40%). The voidage can be determined directly using a cylindrical metal vessel with spouted outlets at two levels and having a volume of at least 25 litres between the two outlets. The voidage is calculated by first filling the vessel full of media and then filling it with water. It may be necessary to leave the medium submerged in water for 24 hours in order to ensure that absorption by the aggregate is complete before topping up the vessel with water. The highest outlet is opened and allowed to drain fully and the volume of water filling the interstices of the known volume of medium between the two spouts is determined by measuring the volume of liquid that drains from the lower outlet. The results are expressed as a

percentage. The volume and overall diameter of the cylinder must be as large as practicable in order to minimise the wall effects (Bruce 1968).

Grading is normally done by the supplier of the medium who issues a certificate showing the nominal grade. It is important to obtain a medium which has a fairly uniform particle size, otherwise if there is a wide discrepancy between the sizes, the voidage and more importantly the size of the voids will be seriously reduced. When the aggregate is crushed, considerable dust and small fines are produced which must be removed by washing with high pressure hoses before the medium is placed in the filter bed. If this is not done, localised blockages of the voids deep within the filter will eventually occur. It is impossible, unless the media is manufactured, e.g. plastic media, for the medium to be comprised of particles of a single size. For this reason, the medium is graded by determining the percentage by weight passing each sieve and the nominal size of the medium is then calculated using the limits set by British Standard 1438 (Table 4.5). Small quantities of over- or under-sized medium do not affect the voidage significantly, thus for a nominal size of 50 mm, for example, up to 15% of the medium by weight can be > 50 mm and up to 30% < 37.5 mm. Particle size analysis is done using square hole sieves, according to British Standard 410, using a minimum sample of 100 kg of medium. In practice, grading proves more difficult as often the percentage passing each sieve does not conform exactly to the limit set in Table 4.5. Earle (1986) gives an example where

Table 4.5. Grading limits for media in British Standards 1438 (1971).

BS410 square hole perforated plate test sieves (mm)	Nominal sized (mm)					
	63	50	40	38	20	14
	% by weight passing					
75	100	—	—	—	—	—
63	85–100	100	—	—	—	—
50	0–35	85–100	100	—	—	—
37.5	0–5	0–30	85–100	100	—	—
28	—	0–5	0–40	85–100	100	—
20	—	—	0–5	0–40	85–100	100
14	—	—	—	7	0–40	85–100
10	—	—	—	—	0–7	0–40
6.3	—	—	—	—	—	0–7

the slag medium was crushed to a nominal size of 50 mm but was found on sieve analysis to have 100% (by weight) passing the 63.0 mm sieve; 70.5%, 50 mm; 15.1%, 37.5 mm, and 0%, the 28.0 mm sieve. This medium does not conform to the limits set for 50 mm as 29.5% was retained in the 50 mm sieve, so crushing was not done adequately. However, it would not appear serious enough to abandon the media, especially after it has been delivered, so what nominal grade is it? It clearly does not fit the limits set for 63 mm either, so the nominal grade of the medium lies somewhere between these two values. Table 4.5 does make it clear that the bulk of the medium of the 50 mm nominal size should be between 37.5–50.0 mm in size, and in this case 55.4% was within this particular range. As up to 30% of the medium is allowed to pass the 37.5 mm sieve then Table 4.5 indicates that 55% within the 50–37.5 mm is legitimate for a medium of 50 mm nominal grade even though it specifies limits of 85–100. Therefore, the sample tested appears to fit best within this category rather than the 63 mm nominal size grade. Interestingly, as filter media ages its particle-size distribution expands as pieces of medium are weathered and fractured (Table 4.6). For this reason, the use of an average size or median size for filter media has been proposed (Earle 1986).

In selecting the medium, a compromise must be reached between the conflicting requirements of a high specific area, which requires a fine grade, and large voids, which are obtained by using a coarse grade. Surface area (m^2m^{-3}) is affected by surface texture and particle shape, although the nominal size of the medium is the major influence (Table 4.7). A rough porous medium, such as clinker or blast-furnace slag can have a surface area up to 17% greater than smooth-surface media, such as gravel of the same nominal size. However, as the film develops, many of the small holes in rough-textured medium become filled with organic debris, fauna, or film itself. Thus, in practice, the surface area will tend to approach that for smooth-medium types. The numerous holes of various sizes and the irregular areas on rough-textured medium do provide a much greater variety of niches for both the micro-organisms and the larger grazers than on smooth-medium types. The rougher texture also prevents the film being so readily sloughed off and allows increased weights of biomass to accumulate within the filter.

It is not normally necessary to measure the surface area of medium directly, as the type and the grade being used will most likely conform to one of the three media in Table 4.7. Where necessary, surface area can be determined by the paint dipping technique. Using a known bulk volume of medium, each piece is coated with a free-flowing paint using a standard

Table 4.6. Problems of calculating nominal size of mineral filter medium using Table 4.5 when the medium has undergone weathering within the filter. Examples are given for Irish percolating filters and the nominal size compared with the mean size (mm) (Earle 1986).

Site	Bulk percentages passing the sieve grading (mm)					
	16	20	28	37.5	50	63
Ballygar	5	28	26	8	25	8
Enniskerry	0	0	1	13	55	31
Gort (i)	5	10	40	40	5	0
Gort (ii)	10	10	34	30	3	14
Kildare (i)	9	19	59	13	0	0
Kildare (ii)	9	18	59	14	0	0
Killincarring	41	36	23	0	0	0
Leighlinbridge (i)	2	4	54	38	3	0
Leighlinbridge (ii)	8	9	45	34	3	0
Loughrea	11	14	24	29	22	0
Mountbellew	0	0	2	41	56	0
Newcastle	1	2	11	41	46	0
Rathmore	8	19	45	23	6	0
Roundwood	1	2	8	24	46	19
Saggart (i)	3	3	9	17	45	22
Saggart (ii)	0	2	2	38	45	13

Site	Voidage (%)	Nominal size (mm)	Mean size (mm)
Ballygar	42	37.5	25
Enniskerry	49	50	44
Gort	44–45	37.5	27
Kildare	42–43	28	23
Kilincarring	44	20	16
Leighlinbridge	46–47	37.5	28
Loughrea	44	37.5	29
Mountbellow	45	37.5	40
Newcastle	49	37.5	37
Rathmore	44	37.5	24
Roundwood	43	50	42
Saggart	45–48	50	40

Table 4.7. Values for specific surface area (m^2m^{-3}) of three different types of filter medium in relation to nominal size and grading. The range for graded material represents maximum oversize or undersize material permitted by British Standard 2438 (1971).

Nominal maximum size of medium (mm)	Type of medium: Granite		Blast-furnace slag		Crushed gravel	
	Single-size	Range of graded material	Single-size	Range of graded material	Single-size	Range of graded material
25	194	185–237	208	200–246	176	169–208
37.5	135	129–149	146	140–163	125	120–140
50	97	94–111	104	101–118	89	86–101
63	75.5	73–85	81	79–90	69	67–77

dipping procedure (Schroepfer 1951). The weight of paint adhering to the medium is calculated by weighing the paint container before and after dipping. The increase in weight per unit area is calculated by dipping test blocks of known surface area into the same paint. These test blocks can be of wood or aggregate provided that the paint adheres to them and the surface area can be accurately calculated. Problems are encountered in obtaining an even distribution of paint over all the pieces of medium even after several dippings. Truesdale *et al.* (1962) noted that filter media has different absorptive capacities and, in practice, absorption of paint varies between individual pieces of the same medium. The problem of uneven coating, and of paint collecting and thickening at the base is overcome by painting individual pieces by hand and then calculating the increase of weight for each piece of medium. Using red lead paint (British Standard 2523, type B), which is thick enough to give good coverage and heavy to ensure a good increase in weight after application, the medium is coated individually using a 10 mm thick paint brush. This procedure is more time consuming than paint dipping, but does ensure that the coat of paint is even and that all the pores are covered. Surface area is then calculated by comparing the increase in weight of the media with the mean increase in weight obtained with the test blocks of directly measurable surface area. To overcome the varying absorptive capacity of the media the pieces of medium will have to be painted a number of times. Experiments have shown that the paint retained on test blocks varied considerably on the first painting but this variability became less with each subsequent coating of paint. Three coats of paint appear sufficient for the most porous media (Gray 1980).

Media must be strong enough to take the weight of the medium and accumulated film above it, be chemically non-reactive, and be durable so that the media does not need to be replaced for many years. The media will undergo weathering within the bed because of constant irrigation with wastewater, and surface microbial activity. On the surface of the bed the media will also be subject to normal weathering processes, such as freezing and heating. Once the media begins to disintegrate, the voids will become gradually clogged with fragmented particles. Some media are totally unsuitable for use and with time they will be broken down until the bed is full with a mush of small particles, e.g. peat briquettes. In general, most sedimentary rocks are unsuitable for use as percolating filter media. The media should remain completely stable for at least 50 years. Its suitability can be assessed by the sodium sulphate "soundness" test. Test pieces of medium are immersed in saturated solutions of sodium sulphate for a number of days before being dried at 105°C. This cycle is repeated 20 times

and the material is then examined for signs of possible defects or loss of weight (BSI 1971). Any medium that passes such a severe test will certainly survive 50 years in a percolating filter. However, some media which fail this test may also be suitable. The media should be chemically non-reactive with all types of wastewater that are normally treated in percolating filters. Problems of leaching from media do occur, especially metals, and it has been suggested that this may interfere with some sensitive species, successfully colonising the media, such as the nitrifying bacteria. Sulphur compounds can be leached from certain slag media and may lead to damage of the concrete base of the filter under certain circumstances (Lavender 1970). Although under normal operating conditions, excess sulphate will be diluted by the wastewater. Limestone is widely used both in Ireland and the UK, and can be used successfully, provided that the wastewater is either neutral or alkaline. Soft limestones and chalk are eventually broken down even in neutral wastewater, and where the wastewater includes the drinking water supplied from a catchment with acidic geology, dissolution will occur and the limestone medium will literally be dissolved away. Limestone should not be used to treat industrial wastewaters for this reason.

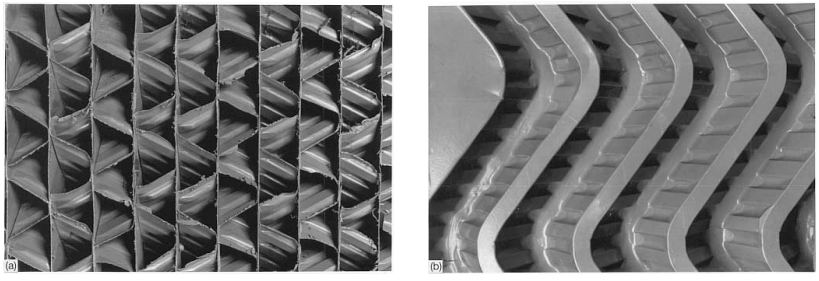
Plastic media. This medium has been freely available in Europe since the end of the 1950s and is now usually made out of PVC. It has a very high voidage (normally $> 90\%$), wide interstitial spaces, high specific surface area, and is only about 10% of the weight of the mineral medium when in operation. This allows tall filters to be built using prefabricated and light-weight structures, which is significantly cheaper than building conventional mineral filters. The plastic filter media must be robust and strong enough to withstand the weight of 3–12 m of media and the accumulated film. This strength is supplied not by the material but in the way it has been prefabricated. The other major advantage over mineral medium is that blockages because of accumulated film are very unlikely due to the high voidage and so high organic loads can be applied. The wide interstices allow sufficient air to reach all areas of the filter, whereas oxygen availability can be limiting in mineral filters. The specific surface area of plastic medium varies between 83–330 m^2m^{-3}, although it appears that there is very little practical difference in surface area between plastic and mineral media (Tables 4.7 and 4.8). Apart from the requirements already mentioned, the main requirements for plastic media are similar although not exactly the same as for mineral media. The advantage of manufacturing a filter medium is that no compromise is required. Generally, the main features of the ideal plastic media are: an ability to treat high hydraulic and organic loads; an open structure in order to avoid restricting film development or subsequent

Table 4.8. Comparison of the physical properties of widely used random and modular plastic filter medium with the mineral medium 50 mm blast furnace slag.

Medium	Composition	Specific surface area (m^2m^{-3})	Voidage (%)
Random			
Norton			
Actifil 90E	Polypropylene	101	95
Actifil 50E	Polypropylene	124	92
Actifil 75	Polypropylene	160	92
MT			
Filterpak 1127	Polypropylene	120	93
Filterpak 1130	Polypropylene	190	93
Mini ring (2)	Polypropylene	118	93
Mini ring (3)	Polypropylene	79	94
ICI			
Floor RC	PVC	330	91
Modular			
Cloisonyle	PVC	220	94
ICI			
Flocor E	PVC	90	95
Flocor M	PVC	135	95
Munsters			
Plasdek B2760	PVC	100	95
Plasdek B19060	PVC	140	95
Plasdek B12060	PVC	230	95
Mineral			
Blast furnace slag (50 mm)		143	51

blockages due to excessive film growth; wide interstices to permit thorough aeration; chemical inertness; durability; physical strength to support sufficient media and associated film to ensure adequate treatment; light bulk density so that structural costs can be reduced; configuration of the media which ensures uniform distribution and minimises channelling; and, finally, it must be cost-effective (Chipperfield 1968; Hemmings 1979).

There are two main categories of plastic media, modular (ordered) and random, with a wide variety of commercially available designs within each category. These have been described and their performances compared by various workers (Bruce and Merkens 1973; Forster 1977; Besselievre



(a) (b)

Fig. 4.6. Configuration of a modular plastic medium (Flocor E®). (a) The top surface over which wastewater is applied via spray nozzles and (b) the high surface area of the medium on which the biological film develops.

and Schwartz 1976; Winkler and Thomas 1978; Porter and Smith 1979; Rowlands 1979).

Two main designs of modular medium are generally used, vertical sheets, ribbed or corrugated, and vertical corrugated tube media. The former is the most widely used design of modular media and is made up of sheets of corrugated ribbed plastic assembled to form a rectangular module with a large number of open zigzag channels (Fig. 4.6). The modules can be prefabricated on site or delivered already made up into blocks. The media is simply stacked into the filter bed and the modules can be cut to size to exactly fit any configuration of bed. The individual sheets of plastic which comprise the modules are orientated vertically so that both surfaces of the channel are exposed to the wastewater. The design of the zigzag channel is such that wastewater is unable to free fall through the medium without coming into contact with the film. The media is available in a range of spacings that allow for extra accumulation of film. The smooth texture and vertical placement of the media discourages excessive build-up of film, and the film regularly sloughs because of its own weight (Pearson 1965: Eden *et al.* 1966). The most widely used media of this type is Flocor E, which has a dry bulk density of 40 kg m^{-3} and increases to up to 300 kg m^{-3} as the film develops. Because of such a large increase in weight when in operation, beds in excess of 3 m depth will need intermediate support (Hemmings 1979; Porter and Smith 1979). It has a specific surface area of 90 m^2m^{-3}, comparable to blast-furnace slag of a nominal size of 63 mm, which is also used for high-rate treatment (Tables 4.7 and 4.8). Cloisonyle® is the most frequently used vertical tube medium. It consists of a block of closely assembled vertical tubes between 4 to 6 m in length, which extends the whole depth of the filter bed. Each 80 mm diameter tube is subdivided into

14 smaller concentric tubules each about 15 mm in diameter. The medium has a high voidage (94%) and specific surface area (220 m^2m^{-3}) (Table 4.8). However, successful operation is due entirely to adequate distribution of wastewater, because as the tubes are not interconnected, any tubule not receiving a wastewater supply will remain dry throughout. Also, as the surfaces are entirely vertical there is a chance that wastewater can fall for some distance within a tubule without coming into contact with the attached film. It was quickly realised that modular media were of limited value in the complete treatment of domestic wastewater. However, modular media are extremely effective as roughing filters, removing large weights of BOD per unit volume of media at relatively low levels of efficiency in terms of final BOD concentration, i.e. 50–80% (Ministry of Technology 1968).

Even with careful design, the short contact time between the influent wastewater and the film, and the possibility of the free fall of wastewater, prevents the modular forms of plastic media from producing well-purified effluents. In order to increase the contact time but retain the high voidage and high surface area per unit volume of modular media, various new random plastic media were designed. These were shaped in such a way that free fall of wastewater experienced in some modular designs was prevented. Its random nature increased contact time with the film while maintaining the high voidage, thus reducing the problem of film accumulation and lack of ventilation experienced with mineral media (Ramsden 1972). Random packed media are made up of individual pieces of PVC or polypropylene moulded into specific shapes in the same nominal sizes used for mineral media such as 37.5, 50, and 65 mm, although some designs can be obtained in sizes up to 90 mm in diameter. The media is generally rings or short tubes with the surface area increased by external or internal fins and ribs (Fig. 4.7). The media is packed randomly into the filter bed in the same

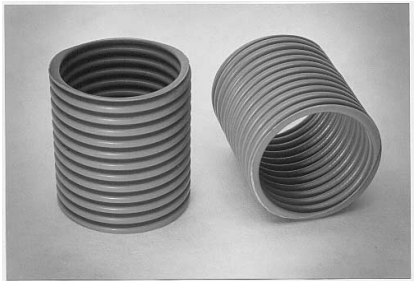


Fig. 4.7. Configuration of a random plastic medium (Flocor RC®).

way as mineral media. The advantages of random plastic over mineral media include large voidage, higher specific surface areas (Table 4.8), and extremely low bulk density (Table 4.2), which permits easy handling. Its lightweight nature allows it to be housed in prefabricated beds such as PVC sheeting attached to a mild steel framework, glass reinforced plastic (GRP), or brick. The higher voidage allows the influent to be continuously loaded using fixed sprays, although conventional distribution systems can also be used. The types of random plastic media available have been reviewed by Porter and Smith (1979).

Plastic media has been successfully used to treat effluent wastewater from a wide range of industries (Besselievre and Schwartz 1976). However, whereas modular media is used for high rate filtration, random plastic media is essentially used for low-rate applications. On the other hand, random plastic does not appear any more efficient than comparable mineral media at conventional low-rate loadings (< 0.1 kg BOD $m^{-3}d^{-1}$) and may even be less efficient in terms of nitrification (Eden *et al.* 1966). Also, a comparable plastic low-rate filter will be more expensive than a mineral system. Therefore, random plastic media is generally used for the primary biological treatment of strong wastewaters prior to their treatment by established methods (Anon 1979b; Hemming 1979). The performance of different media is compared in Sec. 4.1.4.

Purification and film development

The microbial film takes 3–4 weeks to become established during the summer and up to 2 months in the winter, and only then has the filter reached its maximum purification capacity, including nitrification. Unlike many industrial wastewaters, it is not necessary to seed domestic wastewaters treated by percolating filters because all the necessary micro-organisms are present in the sewage itself, with the dipteran grazers flying on to the filter and colonising it. The micro-organisms quickly form a film over the available medium. However, the film only develops on the surfaces that receive a constant supply of nutrients. Therefore, the effectiveness of media to redistribute the wastewater within the filter, in order to prevent channelling and to promote maximum wetting of the medium is an important factor affecting performance. The film is a complex community of bacteria, fungi, Protozoa, and other mesofauna, plus a wide diversity of macro-invertebrates, such as enchytraeids and lumbricid worms, dipteran fly larvae, and a host of other groups which all actively graze the film (Curds and Hawkes 1975) (Sec. 4.1.3).

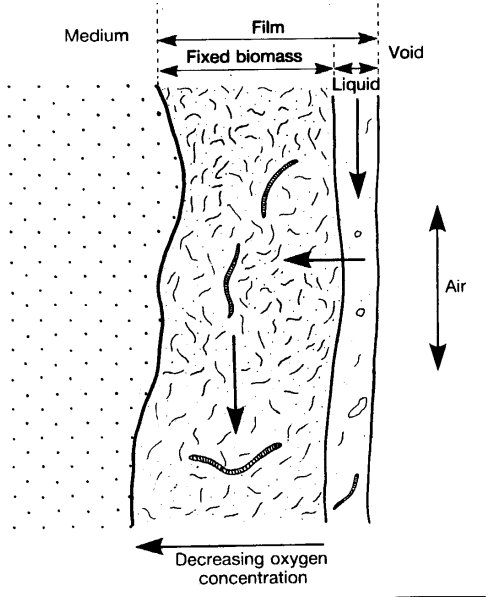


Fig. 4.8. Diagrammatic representation of the structure of film.

The organic matter in the wastewater is degraded aerobically by the heterotrophic micro-organisms that dominate the film. The film has a spongy structure, rather similar to activated sludge flocs, which is made more porous by the feeding activities of the grazing fauna which are continually burrowing through the film. The wastewater passes over the surface of the film and, to some extent, through it, although this depends on film thickness and the hydraulic loading (Fig. 4.8). In low-rate filters a large proportion of the wastewater may be flowing through the film matrix at any one time, and it is the physical straining action of this matrix that allows such systems to produce extremely clear effluents. Another advantage is that the greater the proportion of wastewater that flows through the film the greater the micro-organism-wastewater contact time, i.e. the retention time or hydraulic retention time (HRT). The higher the hydraulic loading the greater the proportion of the wastewater passing over the surface of the film, which results in a lower HRT and a slightly inferior final effluent. Whether the liquid forms a separate layer passing over the surface of the film or is actually making its way slowly through the film matrix, the first stage of purification is the adsorption of organic nutrients onto the film. Fine particles are flocculated by extracellular polymers secreted by the micro-organisms and adsorbed on to the surface of the film, where together

with organic nutrients, which have been physically trapped, they are broken down by extracellular enzymes secreted by heterotrophic bacteria and fungi. The soluble nutrients in the wastewater, and those produced from this extracellular enzymatic activity, are directly absorbed by the micro-organisms comprising the film and synthesised. Oxygen diffuses from the air in the interstices, first into the liquid and then into the film. Conversely, carbon dioxide and the end-products of aerobic metabolism diffuse in the other direction. The thickness of the film is critical, as the oxygen can only diffuse for a certain distance through the film before being utilised, leaving the deeper areas of the film either anoxic or anaerobic. The depth to which oxygen will penetrate depends on a number of factors, such as composition of the film and its density and the rate of respiration within the film itself. It has been estimated as being between 0.06–2.00 mm thick (Schulze 1957; Ekenfelder 1961). Tomlinson and Snaddon (1966) calculated this critical depth experimentally to be 0.2 mm for a predominately bacterial film. However, where fungal mycelium is present, oxygen diffusion is enhanced by movement within the hyphae, increasing the critical depth to 4 mm. Only the surface layer of the film is efficient in terms of oxidation, and only a thin layer of film is required for efficient purification. Tomlinson and Snaddon estimated that the optimum thickness in terms of performance efficiency was only 0.15 mm. This means that it is the total surface area of active film that is important and not the total biomass of the film.

The film accumulates and subsequently thickens within the filter during operation. This is due to an increase in microbial biomass from synthesis of the waste and to accumulation of particulate material by flocculation and physical entrainment where the accumulation rate exceeds the rate of solubilization and assimilation by the micro-organisms. Where the wastewater is largely soluble, such as food processing wastewaters, most of the film increase will be due to microbial growth. Whereas with domestic wastewater, that has been poorly settled, the accumulation of solids may account for the major portion of the film accumulation. Once the film exceeds the critical thickness an anoxic, and subsequently an anaerobic, environment is established below the aerobic zone. As the thickness continues to increase, most of the soluble nutrients will have been utilised before they can reach the lower micro-organisms thus forcing them into an endogenous phase of growth. This has the effect of destabilising the film because the lower micro-organisms lose their ability to hold on to the surface of the medium, which results in portions of the film becoming detached and washed away in the wastewater flow, a process known as sloughing. Although thick film growths do not reduce the efficiency of the filter, excessive growths can

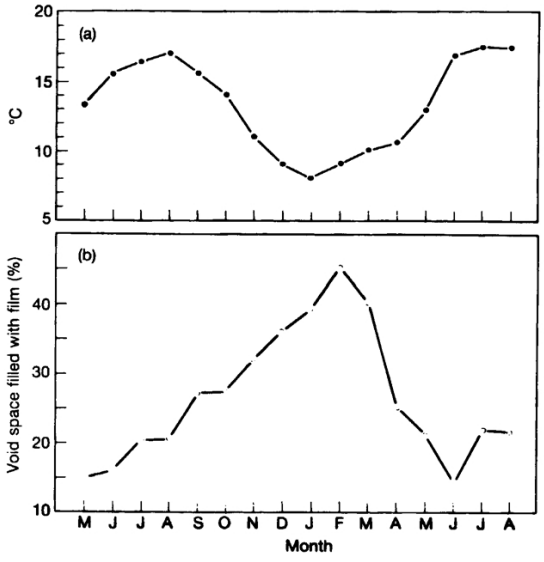


Fig. 4.9. Seasonal variation in the temperature of the sewage applied to a percolating filter and in the percentage of void space in the filter filled with film and water. (a) Monthly average temperature of applied sewage; (b) monthly average proportion of voids filled with film. Quantity of film and water measured by neutron scattering technique (Bruce *et al.* 1967).

reduce the volume of the interstices, reducing ventilation and even blocking them completely, preventing the movement of wastewater. Severe clogging of the interstices is known as ponding and is normally associated with the surface of the filter when whole areas of the surface may become flooded.

The accumulation of film within a filter bed follows a seasonal pattern; being low in summer due to high metabolic and grazing rates but high in the winter when the growth rate of the micro-organisms is reduced, as is the activity of the grazing fauna. As the temperature increases in the spring there is a discernible sloughing of the film that has accumulated over the winter months (Fig. 4.9). Wheatley and Williams (1976) found that no single factor directly influenced film accumulation within their experimental filters, but suggested that it was controlled by the interaction of a number of factors, namely ambient temperature, organic load, the distribution system, the microbial characteristics of the film, and the activity of grazers. Temperature has been shown to be an important factor in film accumulation by Hawkes and Shephard (1971), who demonstrated that at below 10°C, the rate of film accumulation increased rapidly. In a comparative experiment using laboratory filters with and without macro-invertebrate

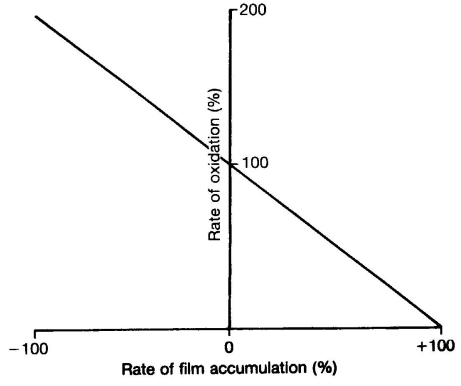


Fig. 4.10. Rate of film accumulation in percolating filters showing that as the rate of adsorption is constant, the rate of oxidation is proportional to the temperature of the film.

grazing fauna at 5 and 20°C, they examined the effects of grazers and temperature in film accumulation (Shephard and Hawkes 1976). They found that at higher temperatures, a greater proportion of the BOD removed by adsorption was oxidised, and therefore fewer solids accumulated. The rate of oxidation decreased as the temperature fell, although the rate of adsorption remained unaltered. Therefore, at the lower temperatures there was a gradual increase in solids accumulation which eventually resulted in the filters becoming clogged. This relationship is shown in Fig. 4.10, where it can be seen that in the warmest months the high microbial activity may exceed the rate of adsorption, thus reducing the overall film biomass. Seasonal variation in film accumulation in the USA in percolating filters is thought to be due to this differential microbial activity at different temperatures (Holtje 1943; Heukelekian 1945; Cooke and Hirsch 1958). However, such variations in the UK have been primarily attributed to the activity of the grazing fauna, with the film accumulating during the winter months when both population densities and the activity of grazers were suppressed (Reynoldson 1939; Lloyd 1945; Tomlinson 1946b; Hawkes 1957). It appears that the grazing fauna do play a significant role in reducing the overall film biomass by directly feeding on the film, converting it into biomass which is flushed from the filter, in the effluent (Hawkes and Shephard 1972). There can be little doubt that the spring sloughing of the accumulated film is pre-empted by the increased activity of the grazers, which loosen the thick film from the medium. Once the film has become detached it strips accumulated film from other regions of the bed as it is washed out. Although grazers suppress maximum film accumulation and maintain minimum film

accumulation for a long period after sloughing, it is temperature which primarily controls the accumulation of film. Whereas hydraulic loading in low-rate filters is of little significance compared to the action of macro-invertebrate grazers in controlling film. As the hydraulic loading increases, as is the case after modifications to the process such as recirculation or double filtration, physical scouring of the film by the wastewater becomes increasingly important. In high-rate filters, especially those employing modular plastic media, the high hydraulic loading controls the film development by scouring the film from the smooth surfaced media as it reaches critical thickness. In such filters sloughing tends to occur on a more regular basis rather than seasonally.

The organic matter in the settled sewage applied to a percolating filter is present as fine or colloidal matter, or in solution. The film transforms this material by biophysical processes (flocculation and adsorption) and bio-chemical processes (bio-oxidation and synthesis) to produce a variety of end-products. Some of these are soluble or gaseous end-products, such as nutrient salts or carbon dioxide from oxidation processes, or soluble products from the lysis of the micro-organisms comprising the film. The remainder is present as solids that require separation from the final effluent. These solids are of three types: flocculated solids; detached fragments of the accumulated film and the grazing fauna; and fragments of their bodies and faeces. These solids are collectively known as humus and like all secondary solids they require further treatment after settlement (Sec. 8.1). The mode of operation will influence the nature of the humus sludge. High-rate operation will produce a humus sludge mainly comprised of flocculated solids and detached fragments of film, whereas a low-rate filter sludge contains a large proportion of grazing fauna and animal fragments. Sludges containing animal fragments and grazing fauna will be more stable than sludges from high-rate systems where the grazing fauna is absent or reduced. The production of humus varies seasonally with mean production rates for low-rate filters between 0.20–0.25 kg humus per kg BOD removed. It varies from 0.1 kg kg^{-1} in the summer to a maximum of 0.5 kg kg^{-1} during the spring sloughing period. In high-rate systems a greater volume of sludge is produced due to the shorter HRT, which results in less mineralisation. The humus production does not vary seasonally to the same extent having a mean production rate of 0.35 kg kg^{-1}, although this is dependent on the nature of the influent wastewater. Sludge production is much less compared with activated sludge, being more stabilised and containing less water (Table 4.9), although as loading increases the mode of purification in percolating filters approaches that of the activated sludge process and the sludge

Table 4.9. Typical sludge volumes and characteristics from biological unit processes (Open University 1975).

Source	Volume (l per head d^{-1})	Dry solids (kg per head d^{-1})	Moisture content (%)
Primary sedimentation	1.1	0.05	95.5
Low-rate percolating filtration	0.23	0.014	93.9
High-rate percolating filtration	0.30	0.018	94.0
Activated sludge (wasted)	2.4	0.036	98.5

alters accordingly both in quality and quantity. Separation takes place in secondary settlement tanks traditionally called humus tanks. They are similar in principle to primary settlement tanks (Sec. 2.2.2), although often of different design. For example, deep square tanks of small cross-sectional areas and inverted pyramid bottoms with maximum upflow rates of < 1 m h^{-1} are common (IWEM 2000). Percolating filters usually achieve high levels of nitrification, and a long sludge retention time within the humus tank may give rise to anoxic conditions and problems of denitrification, which results in the carry over of sludge in the final effluent. High-rate sludges are more susceptible to denitrification than low-rate sludges that are more stabilised and so exert less of an oxygen demand within the settlement tank. Temperature is also important with gas production being much heavier during the summer. Whereas the bulk of the solids settle easily, there is a fraction of fine solids that do not, and are carried out of the humus tank in the final effluent. These fine solids are responsible for a significant portion of the residual BOD in the final effluent, and if a high quality effluent is required some form of tertiary treatment may be necessary. The residual BOD can be successfully removed by any tertiary treatment process, such as micro-straining, sand filtration, upflow clarification, or land treatment (Sec. 2.4.1). There are a number of simple models available to estimate final effluent quality from both low-rate and high rate filters (Pike 1978; Hoyland and Horwood 1980). More dynamic mathematical models are based on the Michaelis-Menton equation to measure the utilisation of substrate within the film, the Monod equation to estimate film growth and detachment, and Fick's law for substrate diffusion through the film. Raj and Murthy (1999) have compared a number of different models using experimental data from the treatment of synthetic dairy wastewater and have evaluated the kinetic parameters used in such models.

4.1.2. *Process modifications*

Percolating filters originally were designed to treat domestic wastewater on a single pass through one filter bed containing mineral media of nominal size between 25–50 mm. At a hydraulic loading of < 0.4 $m^3m^{-3}d^{-1}$ and an organic loading of 0.12 kg BOD $m^{-3}d^{-1}$ a final effluent with a BOD and suspended solids concentration not exceeding 20 and 30 mg l^{-1} respectively can be obtained. Assuming a third of the organic load is removed during primary sedimentation, the per capita loading rate of a single-pass low-rate filter is equivalent to approximately 2.6 people per cubic metre of filter medium. Over time, the general trend of increasing loads to treatment plants plus more stringent disposal standards has resulted in the necessity of expanding low-rate filters. However, because of cost and of land availability restrictions, ways of uprating the efficiency of this original system have been examined. Currently, there are a number of modifications in use that have effectively increased the per capita loading per unit volume of medium.

Filtration can be separated into single- and multi-stage processes. Wastewater treated by a single filter or by filters operated in parallel is known as single-stage filtration. Examples include low- and high-rate single filtration, and recirculation. In multi-stage processes the wastewater is treated progressively by filters operated in series and includes double filtration, alternating double filtration, and two-stage systems (Fig. 4.11).

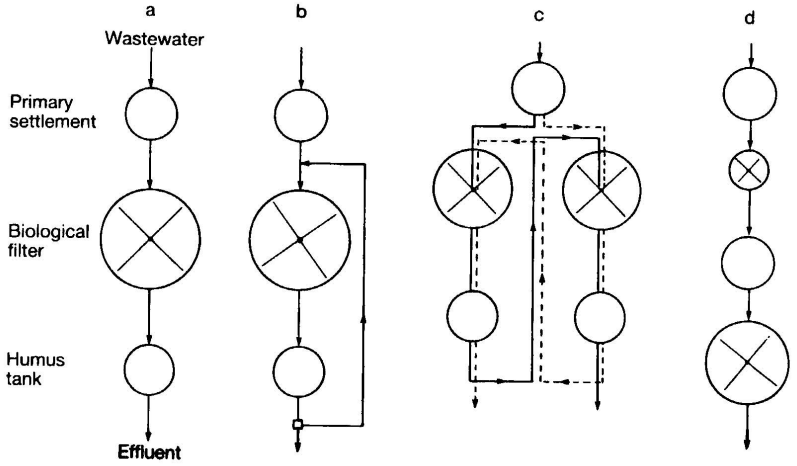


Fig. 4.11. Main systems of operation of percolating filters. (a) Single filtration; (b) recirculation; (c) alternating double filtration (ADF); (d) two-stage filtration with high-rate primary filter.

Single-stage systems

Conventional single-pass low-rate filtration (Fig. 4.11) aims to produce a final effluent of a very high standard. With good operational conditions, almost complete purification including nitrification, is possible. To achieve this, both the hydraulic and organic loadings must be kept low in order to obtain a low growth rate of film. Humus production is low and the sludge is highly mineralised and easy to dewater. Using a medium of a nominal size of 37.5–50 mm, the maximum loading rate, in the UK and Ireland, does not generally exceed 0.10–0.12 kg BOD $m^{-3}d^{-1}$, if a Royal Commission standard final effluent is required. In practice, the effluent BOD and suspended solids concentration fluctuates seasonally, being highest in the spring. Such systems are employed at small sewage treatment plants, and where an appropriate hydraulic head is available they can be operated without a power supply. Where the flow is $> 1,000\ m^3d^{-1}$ modifications to conventional single-pass filters are usually used.

The earliest modification to the percolating filter process was recirculation and is now built into most single filtration systems (Fig. 4.11). Recirculation has generally been used to dilute strong wastewaters or wastewater containing a large proportion of industrial waste, particularly to reduce toxic components to below inhibitory concentrations, or occasionally to "even-out" the diurnal fluctuations in flow to the filters, which ensures a uniform hydraulic loading. This modification results in an increase in the hydraulic loading to the filter, which has a number of advantages. The stronger flushing action prevents surface accumulation of film and reduces the film accumulation within the bed encouraging a thinner, faster-growing film that is more efficient in removing organic matter. The greater hydraulic loading increases the area of the media utilised. The diluted influent also restricts film accumulation at the surface of the filter because of a reduction in the available nutrients in the wastewater, whereas the recirculated fraction contains dissolved oxygen, nitrate, and micro-organisms that may be beneficial. Thus, by diluting the influent with recirculated treated effluent, higher organic loadings can be applied than to single-pass low-rate filters because excessive film accumulation is controlled. These factors allow the loading to be safely increased to between 0.15 and 0.20 kg BOD $m^{-3}d^{-1}$ with little effect on the final effluent quality (90–95% BOD removal), although nitrification will be slightly suppressed. When the nutrients in wastewater are soluble and readily oxidised, such as in food-processing wastes, the organic loading may be as high as 0.5–0.6 kg BOD $m^{-3}d^{-1}$ (Oliver and Walker 1961; Jackson and Lines 1972; Peacock 1977). At higher

hydraulic loadings, more of the filter depth is utilised by heterotrophic micro-organisms because the food is more evenly distributed. This results in the zone of carbonaceous oxidation extending much deeper and restricts the area available for nitrification to the very base of the filter and occasionally excludes it altogether. The ratio of recirculated effluent to influent wastewater is the recirculation ratio and for domestic wastewater it is generally either 1:1 or 2:1. The recirculation ratio can rise to as high as 25:1 for very strong industrial wastewaters. The ideal loading rate after dilution should be between 0.1 and 0.15 kg BOD $m^{-3}d^{-1}$, although the actual effluent BOD concentration is normally used to determine the ratio and therefore the organic loading is normally higher. The recirculation ratio can be calculated by estimating the BOD of the diluted influent fed to the filter (S_o):

$$S_o = (Q_i S_i + Q_r S_r)/(Q_i + Q_r)$$

where the flow rate (Q) and the BOD (S) are known for the influent (i) and the recycled or treated effluent (r). The recycle ratio R_r is $Q_r : Q_i$, and the equation can be rearranged as:

$$S_o = (S_i + R_r S_r)/(1 + R_r)\,.$$

Because the effluent BOD in low-rate systems is usually very low, S_o can be estimated as:

$$S_o = S_i/(1 + R_r)\,.$$

Recirculation can be considered as medium-rate filtration, whereas recirculation in high-rate systems has been found to have little effect on BOD removal efficiency. This is probably because the flushing action of the wastewater in plastic media filters is of less importance than in mineral beds. Recirculation has been studied, in detail, by Lumb and Eastwood (1958).

Conventional single-pass filtration is also used for partial treatment of wastewater (Fig. 4.11). Settled or raw wastewater that has been finely screened can be applied to filters containing mineral media with a large nominal size > 63 mm, random plastic medium, or more commonly modular plastic medium at high organic and hydraulic loadings. High-rate filtration can be used as preliminary or roughing treatment of high strength industrial effluents; for partial secondary treatment before discharge to a sewer or to surface water, especially estuaries and coastal waters, where the effluent

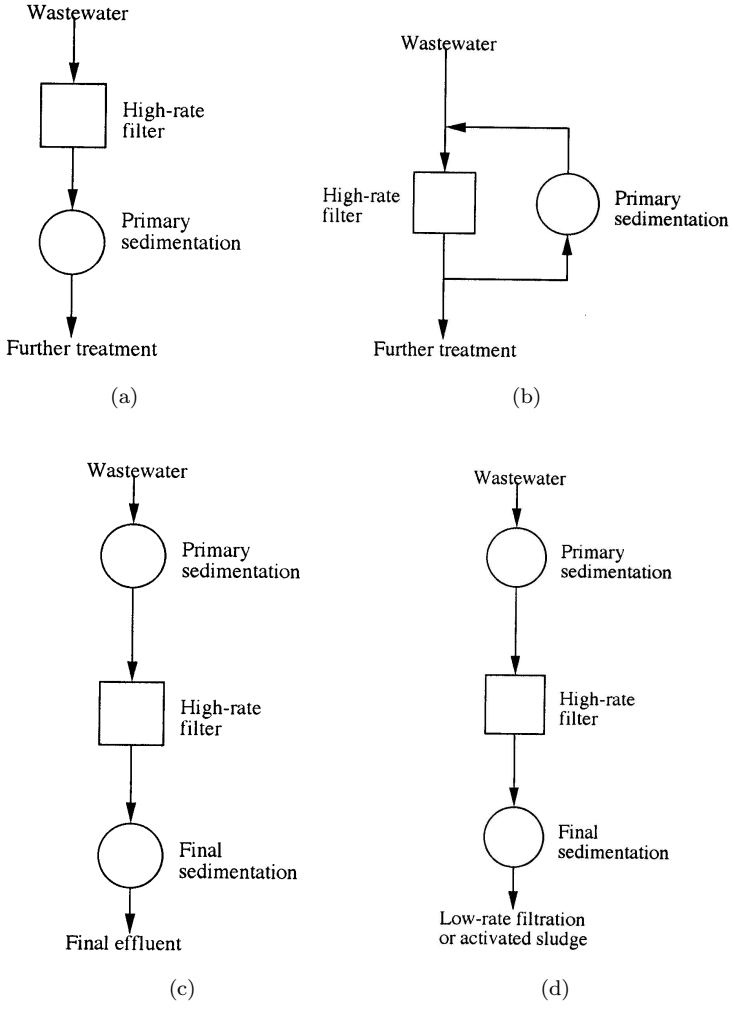


Fig. 4.12. Common applications of high-rate filtration: (a) Preliminary or roughing treatment; (b) preliminary or roughing treatment; (c) partial secondary treatment; (d) first stage of multi-stage secondary treatment.

discharge standards permit; for the first stage of secondary treatment followed by low-rate filtration or activated sludge (Fig. 4.12). The high voidage prevents ponding and heavy accumulations of film, so that film growth is rapid with a constant and high removal of film from the filter. The humus from such filters is poorly oxidised making it less stable than conventional low-rate humus and more difficult to dewater (Bruce and Merkens 1970). Almost any degree of partial treatment can be obtained (50–70% BOD

reduction), depending on the loading rate. High-rate filters are operated at hydraulic loading > 3 $m^3m^{-3}d^{-1}$ and at organic loadings > 0.6 kg BOD m^3d^{-1}. In general a loading of 1 kg BOD $m^{-3}d^{-1}$ will give a BOD removal rate of between 80–90%, whereas this falls to 50% at loadings of between 3–6 kg BOD $m^{-3}d^{-1}$. The specific removal rate depends on the nature of the wastewater and generalisations are difficult. Performance data for a range of wastewaters is given elsewhere (Chipperfield 1967; Jackson and Lines 1972; Anderson 1977). Coarse mineral media (63–150 mm) must be loaded at significantly lower organic loading rates than modular media. Modular and random plastic media have been successfully used to treat macerated and finely screened raw sewage. This eliminates the use of primary sedimentation which results in a considerable saving in primary treatment and subsequent sludge handling (Hemming 1979; Hoyland and Roland 1984). The efficiency of high-rate filters is generally measured in terms of weight of BOD removed per unit volume of medium per day, rather than percentage removal as is the case with low-rate filters. Low-rate filters remove a high percentage of the influent organic matter but the rate of removal is low in terms of mass of nutrients removed per unit volume. Conversely, high-rate systems remove a smaller proportion of the influent organic matter but at a much higher rate. This has given rise to the general terms of polishing and roughing filters for low- and high-rate systems respectively. The percentage removal of BOD decreases as the loading increases, whereas the mass of BOD removed increases with loading up to a critical limiting value above which the amount removed remains constant (Winkler 1981). The advantages and disadvantages of low-rate and high-rate filtration are compared in Table 4.10.

Multi-stage systems

The most widely used multi-stage system is double filtration (DF). In this modification two sets of similar beds are used in series. The media is essentially of the same grade (63–75 mm) and normally sedimentation is employed between each stage to minimise the risk of the humus solids clogging the interstices of the medium in the second filter. Intermediate sedimentation is not absolutely necessary, but if not used it will reduce the performance slightly and increase the amount of solids discharged from the second filter (Tomlinson and Hall 1953). The major problem with using identical filters was that the primary filter often became ponded during the winter (Tomlinson and Hall 1951; Barraclough 1954), which severely limited the overall loading rate. This has been overcome by a number of

Table 4.10. Summary of the advantages and disadvantages of low- and high-rate filtration.

Advantages	Disadvantage
Low-rate (L-R) filtration	
Well nitrified effluent	High land requirement
Natural ventilation adequate	Effluent sometimes turbid
No aerosols or foams	Fly nuisance may occur
Low sludge yield	No biological removal of N or P
No sludge recycling	Seasonal sloughing of film
Low operating costs	High capital cost
Resistant to shock loads	Final effluent BOD > 10 mg l^{-1}
No or low power requirement	Final effluent suspended solids > 10 mg l^{-1}
High-rate filtration	
Lower capital cost than L-R filtration	High BOD and suspended solids in effluent
High BOD removal per unit volume	No nitrification
Less prone to fly nuisance	Main source of odour at treatment plant
Lower area required than L-R filters	Sludge more difficult to dewater
	Higher sludge yield than L-R filters
	Often visibly obtrusive
	Power required

modifications. Some degree of control of film accumulation in the primary stage was achieved by using low frequency dosing, although the system was vastly improved by using high-rate medium in the first stage (two-stage filtration) and by alternating double filtration.

Two-stage filtration is a direct development of DF, although some textbooks fail to distinguish between the two. Here, different media are used in each filter with the first stage operated as a roughing filter and the second stage for polishing the partially treated wastewater. The first stage is operated as a high-rate filter at high loading rates using either a coarse mineral medium (75–130 mm) or plastic media with a high voidage. This first stage reduces the organic loading by 70% allowing the second stage, after intermediate sedimentation, to be operated as a conventional low-rate filter. At loading rates of between 1.6–2.3 kg BOD $m^{-3}d^{-1}$ for the first filter, and 0.04–0.12 kg BOD $m^{-3}d^{-1}$ for the second stage, a 20:30 effluent with a high degree of nitrification is possible (Forster 1977). The system can be further enhanced by using recirculation. Where very strong wastewaters are being treated it may be necessary to have several roughing filters in series to reduce the organic loading sufficiently to allow complete

treatment by the final low-rate stage. This multiple unit system is known as multi-stage filtration (Fig. 4.11).

A further development of the DF system is alternating double filtration (ADF). Identical filters are used, each containing the same medium, with the film accumulation in the first stage controlled by periodically reversing the sequence the filters are used, so that each filter is subject to successive periods of feeding and starvation (Fig. 4.11). The basic research was carried out at Minworth Sewage Treatment Works in Birmingham, where so much pioneering work on percolating filtration has been done (Wishart *et al.* 1941; Mills 1945; Tomlinson 1946b). The filters are filled with a coarser medium (63–75 mm) than normally used for single filtration, which allows a relatively high loading to the filter. The film is controlled to a certain extent by the high flow, distributing the growth of film more evenly through the filter and providing some restriction of film accumulation by washing out film. The rate of film growth at the high organic loadings used is very rapid in the first filter of the sequence. Loading in this order continues for 1–2 weeks until the filter has accumulated a heavy growth of film and is close to becoming clogged. At this point, the flow is reversed so that the second filter is being fed with dilute partially purified wastewater. This puts the film in the second filter under nutrient-limited conditions and forces the micro-organisms to enter an endogenous growth phase, which reduces the overall film biomass but at the same time retains a healthy and active film. Humus is removed after every stage and the ADF process allows the organic loading to be increased by at least a factor of two. For domestic wastewater, a 20:30 effluent can be produced at a loading of 0.24 kg BOD $m^{-3}d^{-1}$ (1.5 $m^3m^{-3}d^{-1}$) (Calley *et al.* 1977), although loadings of 0.30–0.47 kg BOD $m^{-3}d^{-1}$ are normal (Forster 1977). The starvation effect on the second filter is important to ensure film reduction, and with stronger wastes, such as meat-processing wastewaters, recirculation may also be necessary to achieve a wastewater dilute enough for the second stage. A major disadvantage with the ADF process is that nitrification is suppressed. Nitrifying bacteria are normally found at the base of conventional filters where all the carbonaceous material has been utilised. The nitrifying bacteria are slower growing than heterotrophs and are unable to compete for available space. Thus, by alternating the filters in this way they never become established, which results in poor nitrification (Bruce *et al.* 1975).

Modern percolating filter plants are designed with enough flexibility to allow the filters to be operated at single or double, and even ADF filtration, depending on the strength of the incoming wastewater and the degree of treatment required. Basic operating parameters are summarised in Table 4.11.

Table 4.11. Summary of the percolating filter operating variables where (I) is the first stage and (II) the second stage.

Key operating variables	Low-rate	Recirculation	Double filtration
Medium type	Blast furnace slag	Blast furnace slag	Blast furnace slag
Medium grade (mm)	50	28–40	(I) 100–150, (II) 28–40
Medium depth (m)	1.8	1.8	(I) 1.8–7.0, (II) 1.8
Organic loading rate (kg BOD m^3d^{-1})	0.07–0.15	0.10–0.20	0.10–0.20
Maximum influent BOD (mg l^{-1})	< 350	< 2000	< 2000
Recirculation ratio	0	1:1 to 5:1	0

4.1.3. *The organisms and their ecology*

While the activated sludge process is a truly aquatic habitat, the percolating filter provides a variety of habitats, ranging from aquatic to terrestrial. In this respect the filter bed is similar to the sea-shore, with the aquatic micro-organisms forming the film that supports a wide range of air breathing macro-invertebrate grazers. The natural habitat of these micro-organisms is the surface of stones and other submerged objects in rivers and streams, especially those receiving large nutrient inputs. The micro-organisms are also associated with similar aquatic habitats such as lakes and ponds, wetlands, and other damp environments.

The food-chain in a percolating filter contains several extra-trophic levels compared with the activated sludge process. The meiofauna provides the top trophic level in activated sludge, whereas in fixed-film reactors a diverse range of macro-invertebrates (Fig. 2.23) is also present. The basic unit of purification is the heterotrophic film, which converts the soluble and suspended organic matter in the influent wastewater into the bacterial and fungal biomass on which the rest of the community depends. Protozoa graze mainly on the film or on free-living bacteria, although a few are predators of other protozoans or saprophytes. The remaining meiofauna, mainly nematodes and rotifers, also feed on the film, although several are predatory. The grazing fauna, which are dominated by dipteran larvae and oligochaete worms, feed mainly on the film. The main energy transformations are given in Fig. 4.13. A typical list of species from a percolating filter is given in Table 4.12 and contains species of: *Bacteria*, *Fungi*, *Algae*, *Protozoa* (*Sarcomastigophora* and *Ciliophora*), *Nematoda*, *Rotifera*, *Annelida*, *Insecta*, *Crustacea*, *Arachnida* (*Acari* and *Araneida*), and *Mollusca*. This is by no means a definitive list and a vast diversity of species from all phyla have been recorded (Clay 1964; Learner 1975a; Curds and Hawkes 1975). Larger species also regularly visit the surface of the filter beds to feed, especially insectivorous birds such as Wagtails and various small mammals such as rodents and hedgehogs, although access to the mammals is often restricted by the filter bed wall. The ecology of percolating filters has been fully reviewed by Hawkes (1959, 1963, 1983b), with each major group examined in detail by Curds and Hawkes (1975).

Heterotrophs and phototrophs

Bacteria. Bacteria form the basic trophic level in filter beds and are predominant both in terms of numbers and biomass, with aerobic, anaerobic, and facultative species all present. The bacterial flora in percolating filters

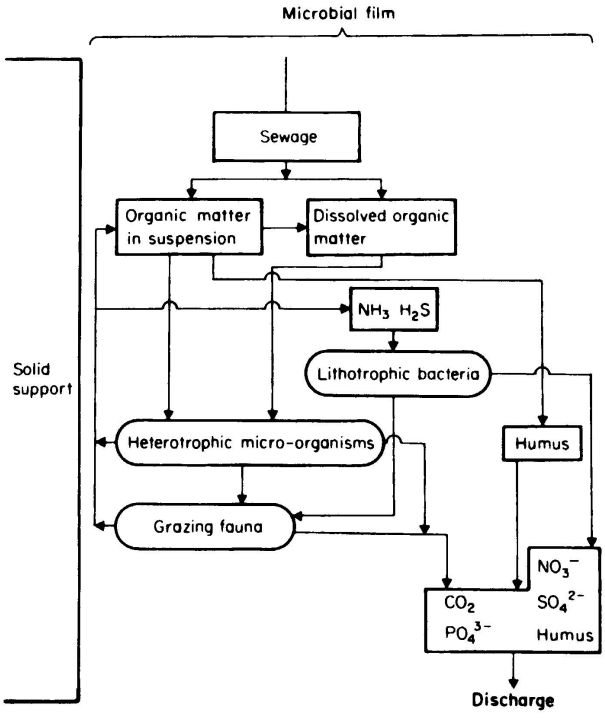


Fig. 4.13. Food web in the microbial film of percolating filters (Lynch and Poole 1979).

are similar to those found in activated sludge. These species are able to grow over an inert medium dominating filamentous forms. The dominant aerobic genera are the Gram-negative rods *Zoogloea*, *Pseudomonas*, *Achromobacter*, *Alcaligens*, and *Flavobacterium* (James 1964; Harkness 1966; Pike and Carrington 1972; Pike 1975). The role of bacteria in aerobic wastewater treatment has already been discussed in Sec. 3.3.1. The removal of pathogens is considered in Sec. 9.3.2. Filamentous bacteria such as *Sphaerotilus natans* and *Beggiatoa* sp. are frequently associated with percolating filters (Cooke 1959; Bruce *et al.* 1970; Wheatley 1976), but are rarely dominant. Their abundance is directly associated with organic loading in filters, although they do not cause operational problems as they do in activated sludge (Sec. 5.4). In a conventional low-rate single-pass filter, the heterotrophic bacteria and fungi are responsible for the primary oxidation of the organic matter in the wastewater. The actual microbial composition of the film varies seasonally with filter depth and the composition of the wastewater, and it is difficult to characterise the microbial community structure.

Table 4.12. Typical list of species recorded from a single percolating filter.

Bacteria
Zoogloeal forms, mainly *Zoogloeal ramigera*
Sphaerotilus sp.
Leptothrix sp.
Beggiatoa sp.
Fungi
Subbaromyces splendens Hesseltine
Sepedonium sp.
Fusarium aquaeductuum (Radimacher and Rabenhorst) Saccardo
Geotrichum candidum Link
Algae
Chlorella sp.
Scenedesmus sp.
Stigeoclonium sp.
Protozoa: Sarcomastigophora
Bodo sp.
Amoeba sp. mainly *Amoeba radiosa* Ehrenberg
Euglena sp.
Protozoa: Ciliophora
(Holotrichia)
Trachelophyllum pusillum Perty-Claparède and Lachmann
Hemiophrys fusidens Kahl
Hemiophrys pleurosigma Stokes
Chilodonella cucullulus (Müller)
Chilodonella uncinata Ehrenberg
Colpoda cucullus Müller
Uronema nigricans (Müller) Florentin
Glaucoma scintillans Ehrenberg
Colpidium colpoda Stein
Colpidium campylum (Stokes)
Paramecium aurelia Ehrenberg
Paramecium caudatum Ehrenberg
(Peritrichia)
Vorticella microstoma Ehrenberg

Table 4.12. (*Continued*)

Vorticella convallaria Linnaeus
Vorticella vernalis Stokes
Opercularia minima Kahl
Opercularia microdiscum Faure-Fremiet
Opercularia coarctata Claparède and Lachmann
Epistylis rotans Svec
(Spirotrichia)
Stentor roeseli Ehrenberg
Aspidisca costata (Dujardin) = *cicada*
Tachysoma pellionella (Müller-Stein)
Oxytricha ludibunda Stokes
(Suctoria)
Acineta cuspidata Stokes
Acineta foetida Maupas
Podophrya maupasi Bütschli
Podophrya carchesii Claparède and Lachmann
Podophrya mollis Bütschli
Sphaerophrya magna Maupas
Nematoda
Rotifera
(Bdelloidea)
Philodina roseola Ehrenberg
(Monogonota)
Lecane sp.
Dicranophorus sp.
Annelida
(Oligochaeta)
Enchytraeidae
Enchytraeus buchholzi Vejdovsky
Lumbricillus rivalis Levinsen
Lumbricidae
Dendrobaena rubida (Sav.) *f. subrubicunda* (Eisen)
Eiseniella tetraedra (Savigny)
Insecta
(Collembola)

Table 4.12. (*Continued*)

Isotomidae
Isotoma olivacea-violacea gp.
(Coleoptera)
Hydrophilidae
Cercyon ustulatus (Prey.)
Staphylinidae
Unidentified sp.
(Diptera)
Anisopodidae
Sylvicola fenestralis (Scop.)
Psychodidae
Psychoda alternata Say.
Psychoda severini Tonn. (= *P. albipennis*)
Chironomidae
Hydrobaenus minimus Mg. (= *Limnophyes minimus*)
Hydrobaenus perennis Mg. (= *Chaetocladius perennis*)
Metriocnemus hygropetricus Kieff. (= *M. eurynotus*)
Ephydridae
Scatella silacae Lw
Sphaeroceridae
Leptocera sp.
Cordyluridae
Spathiophora hydromyzina Fall.
(Chilopoda)
Lithobius forficatus Linn.
Crustacea
(Cyclopoida)
Cyclopidae
Paracyclops fimbriatus-chiltoni (Thomson)
Arachnida
(Acari)
Acaridae
Histiostoma carpio (Kramer)
Rhizoglyphus echinopus (Fumouze and Robin)

Table 4.12. (*Continued*)

Anoelidae
Histiostoma feroniarum (Dufour)
Ascidae
Platyseius italicus (Bertese)
(Araneida)
Linyphiidae
Unidentified sp.
Mollusca
(Gastropoda)
Limacidae
Agriolimax reticulatus (Müll.)

An example of seasonal and depth variation in a pilot filter containing 50 mm blast-furnace slag medium treating domestic wastewater is given in Fig. 4.14. As the wastewater passes through the filter bed it is purified by the film, and changes occur as it percolates over the medium resulting in the wastewater having a different composition at various depths. As a consequence, the microbial community structure varies in composition at different depths, having a greater number of species nearer the surface than at the base. This is why there is a greater diversity of species in percolating filters than in completely mixed systems such as activated sludge. The stratified distribution of species within the filter bed allows it to withstand shock loadings.

Fungi are more common in percolating filters than in activated sludge, as they are among the first species to colonise the media. Although many fungi can be isolated from percolating filters, only a few manage to take advantage of the habitat and flourish (Cooke 1959). Among those that do flourish are *Geotrichium candidum*, *Fusarium aquaeductuum*, *Sepedonium* sp., *Subbaromyces splendens*, *Ascoidea rubescens*, and *Trichosporon cutaneum* (Fig. 4.15; Table 3.7). Fungi dominate over heterotrophic bacteria under specific conditions. They outgrow bacteria at colder temperatures, under conditions of low pH such as wastewater from fruit and vegetable processing or effluents containing mineral acids, and also dominate filters treating strong wastewaters with a high organic content. For this reason, fungi are associated mainly with industrial effluents (Hesseltine 1953; Watson *et al.* 1955; Hawkes 1957, 1965; Tomlinson 1946b; Sladka and Ottova 1968). Fungi have a lower surface area to volume ratio compared

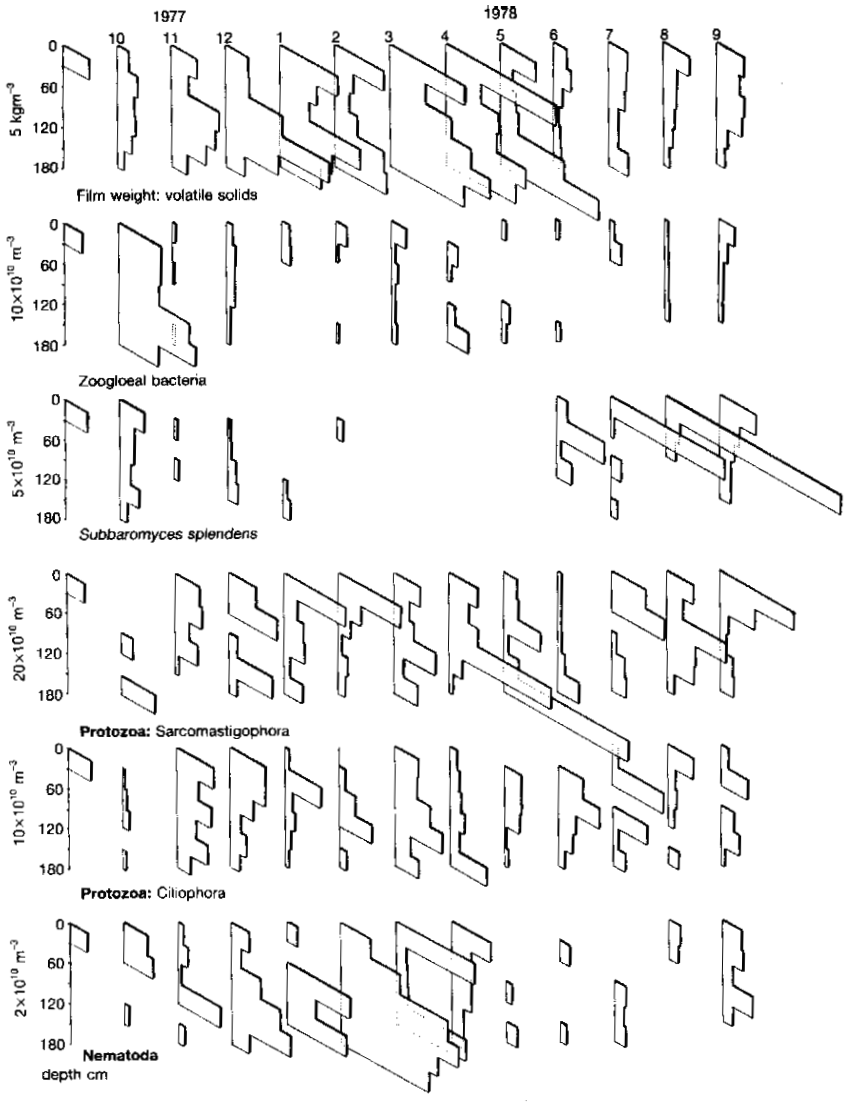


Fig. 4.14. Vertical distribution of film and microfauna in a low-rate filter containing 50 mm blast-furnace slag medium.

to bacteria and thus have a lower affinity for available substrate than heterotrophic bacteria. Therefore, bacteria will predominate when the substrate concentration is low, i.e. when the wastewater is weak, or at lower depths in the filter bed after a proportion of the substrate has been utilised

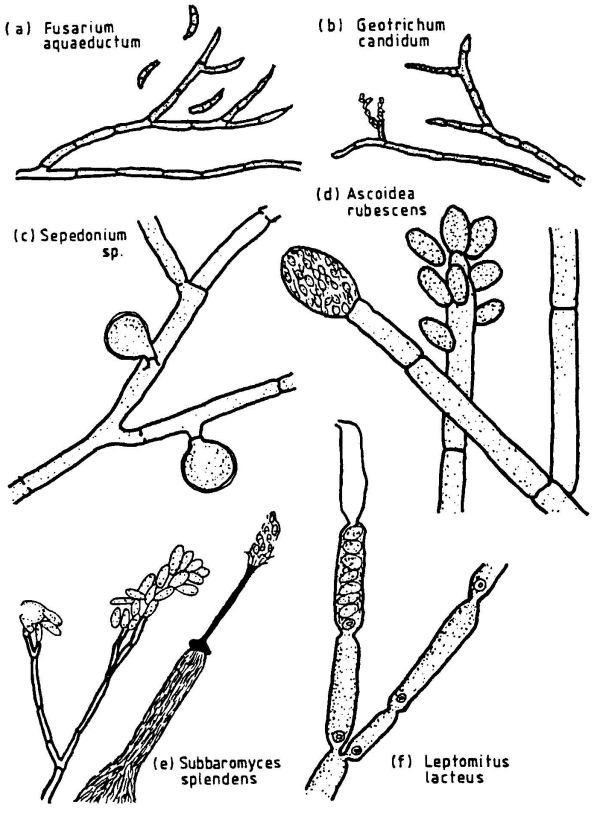


Fig. 4.15. The hyphae and spores of fungi commonly associated with percolating filters (Williams 1973).

and removed. Conversely, fungi dominate when the substrate concentration is high and usually at the surface of the filter bed before the strength of the influent has been reduced. Fungi have the ability to attach strongly to medium and are not easily dislodged by the wastewater. The mycelium physically entrains solids and fungal dominant films grow rapidly compared to bacterial films that rely on adsorption only. This results in very thick layers of fungal film developing, which, due to the reduced grazing activity during the winter, is why the interstices of the medium become clogged and ponding often results. The hyphae protrude into the wastewater passing over the surface of the film, and fungal-dominated films are less likely to be nutrient-limited as the nutrients can diffuse throughout the film, resulting in continuous growth. Fungal-dominated films are so thick that if the film was bacterial in nature, oxygen would become limiting in the deeper regions.

However, the nature of the hyphae allows oxygen transfer deep into the film layer by a process of protoplasmic streaming within the hyphae, preventing anaerobic conditions associated with thick bacterial-dominated films (Tomlinson and Snaddon 1966). Fungi are generally considered undesirable as dominant members of the film community, as they cause solids accumulation and eventual ponding (Hawkes 1963). Many authors have also associated heavy fungal films in filters with very large populations of fly larvae, which result in fly problems later on. However, the fungi have the same removal efficiencies as the bacteria, although the fungi produce a greater biomass per unit BOD removed resulting in faster film accumulation and, eventually, a greater sludge production (Water Pollution Research Laboratory 1955). The role of fungi in wastewater treatment and in percolating filters in particular is considered in detail in Sec. 3.3.1.

Fungi often dominate the film that develops on plastic media, and is able easily to colonise the smooth surface. One particular fungus, *Subbaromyces splendens*, grows particularly well on random plastic filter medium. The fungus is unusual because it has only ever been isolated from percolating filters, and its natural habitat is unknown (Hesseltine 1953), It is also unusual because it is associated with weak domestic wastewaters (Hawkes 1965; Hawkes and Shephard 1972; Wheatley 1976; Gray 1983b). Fungi are generally found in greatest abundance on the surface and in the top 150 mm of the filter bed (Hawkes 1963). They follow a seasonal pattern of abundance, which slowly increases in autumn and reaches a peak in winter or spring, becoming scarce by mid-summer. A comparative study by Gray (1983b) showed that *S. splendens* produced more extensive growth on random plastic medium than on conventional mineral medium. The fungus was not restricted to the upper 150 mm but produced extensive vegetative growth throughout the depth of the filter causing localised ponding both at the surface and inside the bed. An increase in the hydraulic loading normally leads to a reduction in the fungal growth at the surface of a filter, resulting in a more even distribution of film throughout the bed (Hawkes 1957). This was also the case in the comparative study, when the loading was increased from 1.68 $m^3m^{-3}d^{-1}$ (0.28 kg BOD $m^{-3}d^{-1}$) to 3.37 $m^3m^{-3}d^{-1}$ (0.63 kg BOD $m^{-3}d^{-1}$). However, at the higher loading the total biomass of the fungus in the filter containing the plastic medium was increased nine-fold, with ponding occurring in the lower half of the filter. Fungal films are structurally stronger than bacterial ones and less readily sloughed off (Tomlinson and Williams 1975). It is this aspect that may help fungi, and, in particular *S. splendens*, to dominate in filters containing the smoother plastic medium (Fig. 4.4). Other factors include: a higher medium surface area resulting in less competition for space with bacteria; the lower

diversity of niches within plastic media compared with mineral media for supporting grazing fauna; and the large voidage permitting a high ventilation rate resulting in the temperature of the bed being significantly cooler than a mineral filter bed and permitting a large diurnal fluctuation in temperature, which will be similar to the variation in the air temperature thus inhibiting bacterial growth. The ability of *S. splendens* to cause ponding at depth within the filter has a practical significance, apart from problems in operational control. It has generally been accepted that random plastic filter media can be housed in prefabricated or weaker structures than normally used for mineral media, due to the difference in bulk densities (Table 4.2). However, if ponding was to occur at depth, then the bulk density would increase proportionally to the extra volume of sewage retained. It may, therefore, be possible that under extreme conditions, ponding by the fungus might increase the bulk density of a filter, housing random plastic medium, in excess of the designed strength of the unit. This could result in the structure becoming damaged, weakened or even failing and collapsing. Three control options are suggested:

(i) *Recirculation or alternating double filtration*: Hawkes (1965) suggests that fungal growths in percolating filters can be controlled by these modifications. However, the study by Gray (1983b) did not show that the fungus was restricted at increased hydraulic loadings or that the growth of the fungus was related to the sewage strength at a particular depth.

(ii) *Periodic dosing*: Continuous dosing has been shown to favour the fungus *S. splendens*, and Hawkes and Shephard (1972) demonstrated that the fungus could be controlled by reducing the frequency of dosing. Although surface growths would be controlled, careful consideration would have to be given to the retention time as this may even out the flow at lower depths in the filter resulting in enhanced growth of the fungus deeper in the filter bed.

(iii) *Design*: The safest precaution, but the most expensive, is to ensure that the structure housing the medium is able to withstand maximum possible bulk density, i.e. total saturation with water (Table 4.2), making the filter completely safe. Such a design, however, would make random plastic filter media less attractive economically, because one of the major advantages of using such media is the saving of capital cost of engineering work (Gray 1983b).

The fungal ecology of percolating filters has been reviewed by Cooke (1954, 1963), Becker and Shaw (1955), and Tomlinson and Williams (1975).

The surface of the filter is the only part of the medium exposed to sunlight and photosynthetic algae and bacteria are restricted to the top 50 mm of the bed. Phytosynthetic species, and algae in particular, play a very minor role in the purification process, but can interfere with the efficient operation of the filter. Algae may be present as thin or dispersed incrustations of green unicellar algae (e.g. *Chlorella* spp.) and diatoms, or filamentous species can form thick luxuriant surface mats covering the surface of the medium (e.g. *Stigeoclonium* sp.). The felt-like algal mats impair distribution, decrease ventilation, and may even cause ponding. Cyanobacteria (blue-green algae) are classified as bacteria even though they are photosynthetic. In percolating filters they are present both as unicellular (e.g. *Chlorococcum* sp.) and filamentous forms (e.g. *Oscillatoria* sp., *Phormidium* sp.). The major species causing the dark-green, thick surface mats so often seen in percolating filters is the blue-green alga *Phormidium* sp., which forms a characteristic leathery mucilaginous sheet over the medium impeding both air and liquid movement through the bed (Benson–Evans and Williams 1975).

Mosses and liverworts are also occasionally found. Moss growth can become so extensive that the entire surface of the bed can become affected. The moss traps solids, forming a thick layer of debris in the interstices of the medium which interferes with percolation. The most frequently occurring species is *Leptodictyum* (*Amblystegium*) *riparium*, although Hussey (1982) recorded 12 species of moss in a survey of 64 filters. Factors that encourage moss growth include: a rough, pitted medium; wide spacing of the distribution nozzles providing inter-jet zones; a low organic loading; and the absence of strong industrial or inhibitory wastewaters. The ecology of the filter is altered, with significant changes in the grazing fauna occurring due to the presence of the moss (Table 4.13) (Gray 1984b). Raking may not effectively control the moss, and may even help to spread it over the rest of the surface of the bed. Herbicides are effective but can also destabilise the community structure of the filter bed by killing other species. Success has been obtained by excluding the light from the surface of the bed or, alternatively, by using a flame gun to burn off the moss. Increasing the organic strength of the influent by reducing the number of filters in operation eliminates moss growth over a period of six months, although there is also normally a deterioration of the final effluent quality. Control can be obtained by increasing the frequency of dosing, reducing the inter jet spacing on the distributors, or by using splash plates to eliminate the inter-jet zones on the surface of the medium. Replacing or covering the surface layer of medium with a smooth surfaced random plastic filter medium will also discourage

Table 4.13. Comparison of macro-invertebrate densities (no. l^{-1} medium) in filter medium with and without moss growth. The 95% confidence limits and the level of significance (P) of the Mann-Whitney test statistic U are also given.

Macro-invertebrate group	Medium		P
	Moss present $\bar{x}$ CL	Moss absent $\bar{x}$ CL	
Lumbricidae	50 ± 8	4 ± 6	< 0.001
Enchytraeidae	1040 ± 420	356 ± 205	< 0.05
Psychodidae (larvae)	1514 ± 370	809 ± 317	< 0.05
Tipulidae (larvae)	11 ± 3	0 ± 0	< 0.001

growth. Prevention is better than trying to eradicate extensive moss growth. Thus, when the surface of the filter is checked periodically for accumulated inorganic debris (plastic strips, etc.) any moss can be weeded out before it becomes established.

Nitrification

Nitrification is a two-stage process with ammonia oxidised to nitrite by bacteria of the genus *Nitrosomonas*, and nitrite to nitrate by *Nitrobacter* spp. (Sec. 3.5.2). In low-rate single pass filters containing 50 mm stone medium, virtually full nitrification can be obtained throughout the year with a specific ammonia removal rate of between 120–180 mg $m^{-2}d^{-1}$ when loaded at 0.1 kg BOD $m^{-3}d^{-1}$, resulting in a final effluent low in ammonia but rich in nitrate. In a percolating filter, nitrifying bacteria tend to become established later than heterotrophs, with *Nitrosomonas* becoming established before *Nitrobacter*, as ammonia is abundant. Therefore, the first sign of nitrification in a filter is the production of nitrite rather than nitrate. The number of nitrifying bacteria and the level of nitrifying activity increase with depth (Tomlinson 1942; Harkness 1966; Painter 1970). This results in the upper level of single pass filters being dominated by heterotrophs, and the lower section containing a proportionately higher number of nitrifying bacteria. The reason for this apparent stratification is due to a number of factors. The autotrophic bacteria responsible for nitrification are slow growing compared to heterotrophs and have an even more reduced growth rate in competitive situations. Therefore, in the upper layers of the bed where there is abundant organic matter, the heterotrophs will dominate. Nitrifiers are extremely sensitive to toxic compounds in the wastewater,

especially heavy metals, and so the presence of such compounds will limit the growth of the bacteria until the compounds have been removed from the wastewater by adsorption by the heterotrophic film as it passes through the filter bed. Research has shown that the process is also inhibited when the oxygen concentration in the influent wastewater is limited (Heukelekian 1947; Hawkes 1963; Tomlinson and Snaddon 1966). Nitrifying bacteria are strict aerobes and will be inhibited by reduced aerobic conditions caused by high heterotrophic activity, and as nitrification is a high oxygen consuming process, adequate supplies of air are required. Although Painter (1970) showed that organic matter did not *directly* inhibit nitrification, he indicated that the nitrifying bacteria needed to be attached to a stable surface, suggesting inhibition may be due to competition for space. When loadings are increased, extending the depth of heterotrophic activity, nitrifying bacteria are overgrown and killed by the more rapidly growing heterotrophic bacteria. Nitrification is virtually eliminated by hydraulic loadings of domestic wastewater in excess of 2.5 $m^3m^{-3}d^{-1}$. This enhances heterotrophic growth which can extend throughout the depth of the bed and exclude the nitrifying organisms (Bruce *et al.* 1970; Joslin *et al.* 1971; Bruce *et al.* 1975). Temperature also has a marked influence on nitrification (Painter 1970), and the large fluctuations in temperature seen in random plastic filters account for the low degree of nitrification that occurs with such media (Gray 1980). Although the threshold temperature for the process is 10°C, with domestic wastewater rarely falling to below 12°C (normal annual range 12–18°C), a few degrees reduction in the temperature below 10°C is likely to have a disproportionate reduction on nitrification (Bruce *et al.* 1975). This is clearly seen in comparative studies between mineral and plastic medium filters where the high voidage of the plastics medium allows a greater degree of ventilation and large diurnal changes in temperature similar to the air temperature.

Many workers have noticed discrepancies in the total nitrogen balance of their filters, with the decrease in ammonia concentration not corresponding to the increase in nitrate concentration (Bruce *et al.* 1975; Hemming and Wheatley 1979; Gray 1980). This is due to the ammonia being supplied not only in the applied wastewater, but also within the filter from the de-animation of organic nitrogen, endogenous respiration, and from cell lysis. Ammonia, on the other hand, is not only removed by nitrification, but also by volatilisation of free ammonia and by metabolism into new cellular material. Gray found that at times of low film accumulation, when the grazing fauna population was still large, the ammonia concentration in

the final effluent was high. He concluded that the observed increase in the ammonia concentration was due to the excretion products of the grazing fauna. The oxygen profile formed by diffusion through the film results in nitrate ions being lost by denitrification as gaseous nitrogen from the anoxic zone near the biomass-medium interface.

Meiofauna

Protozoa. Protozoa are a major group of unicellular micro-organisms that are well represented in all aerobic treatment systems. Many are mobile, utilising flagella, cilia, or pseudopodia for locomotion. In percolating filters, as in other systems, three classes of protozoa are common. Sarcomastigophorea are the smallest being < 10 μm in diameter. Known as flagellates, due to movement by one or two whip-like flagella, they can be broadly separated into two groups. The Phytomastigophorea contain chloroplasts and are able to photosynthesise, while the Zoomastigophorea are heterotrophs. Flagellates are unable to feed on particles but are osmotrophic, i.e. they absorb dissolved nutrients directly through the cell wall (Fig. 4.16). The Sarcodina (amoebae) are a variable group of protozoa with species varying in size from 10 to 250 μm. Amoebae are transparent cells that crawl or flow (or stream) extremely slowly over surfaces making them almost indistinguishable from the surrounding debris. Movement is achieved by extending pseudopodia into which the remainder of the cell flows. They feed on bacteria ingesting them inside contractile vacuoles. Amoebae are either naked or testate i.e. have a small shell that partially covers or houses the body (Fig. 4.16). The final group of protozoans, the Ciliatea (ciliates), are the best known due to their relatively large size (20–200 μm). They all possess cilia, which are small hairs usually dispersed over the entire surface of their body, although these hairs have become modified to form cirri (tufts) or even tentacles. They predominately feed on bacteria, although some are predatory on other protozoa, and are either free-swimming, attached to the film by a stalk, or crawl over the surface of the film (Sec. 5.5.3). The cilia are used both for locomotion and feeding (Fig. 4.17).

Protozoa are particularly abundant in percolating filters and over 218 species have been isolated. There are 35 species of Phytomastigophorea, 30 species of Zoomastigophorea, 31 species of Rhizopodea and 7 species of Actinopodea. However, the bulk of the species, some 116 of them, belong to the class Ciliatea. The abundance of ciliates, amoebae, and flagellated species in filters has resulted in most studies concentrating on these groups alone.

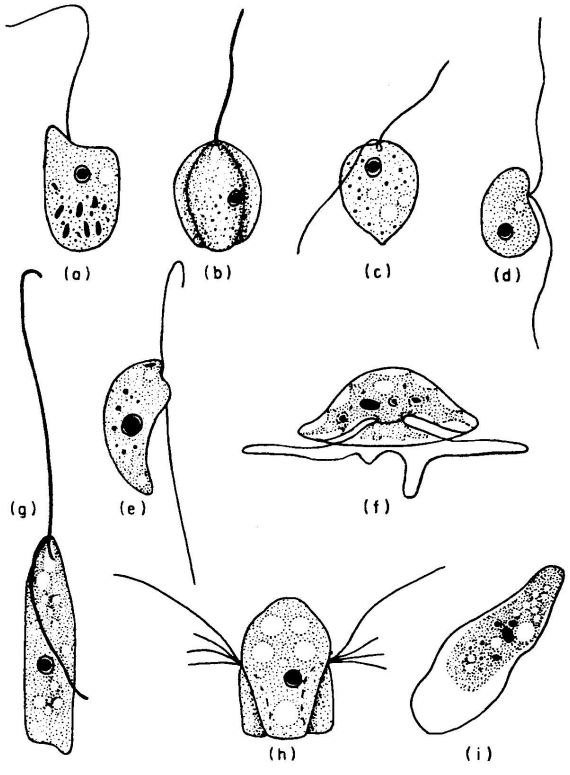


Fig. 4.16. Common flagellates and amoebae associated with percolating filters and other wastewater treatment processes: (a) *Oicomonas termo*; (b) *Notosolenus orbicularis*; (c) *Cercobodo crassicauda*; (d) *Pleuromonas jaculans*; (e) *Bodo caudatus*; (f) *Arcella vulgaris*; (g) *Peranema trichophorum*; (h) *Trepomonas agilis*; and (i) *Vahlkampfia limax* (Curds 1975).

The role of protozoans in percolating filters is similar to that of the activated sludge process, with species feeding mainly on free-living bacteria and clarifying the effluent as well as stimulating bacterial growth by reducing the population density (Sec. 5.5.3) Some protozoans are able to feed on non-living particulate, or soluble organic material, whereas a few species are predators of other protozoans. It is generally thought that the ciliates are numerically dominant over the flagellates (Frye and Becker 1929; Brink 1967), although Barker (1942, 1946) and Gray (1980) both found flagellates to be dominant in percolating filters. Gray found the ratio of Sarcomastigophora to Ciliophora to range from 3.7 to 4.3 in low- and high-rate mineral media filters and 2.0 to 4.4 in low- and high-rate random plastic media filters respectively. However, flagellates are significantly

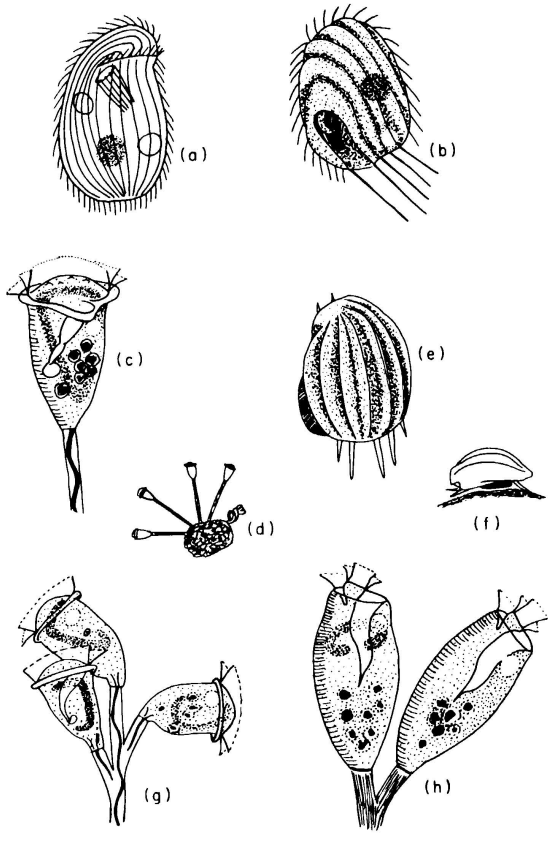


Fig. 4.17. Some common ciliates in percolating filters and activated sludge: (a) *Chilodonella unicata*; (b) *Cinetochilum margaritaceum*; (c) *Vorticella convallaria*; (d) *V. convallaria* growing on a sludge floc; (e) *Aspidisca costata*; (f) *A. costata* lateral view showing crawling habit; (g) *Carchesium polypinum*; (h) *Opercularia microdiscum* (Curds 1975).

smaller than ciliates both in overall dimensions and in volume, therefore, in terms of biomass, ciliates are always dominant. The population density of protozoans increase with organic loading, although the flagellated and free-swimming ciliate protozoans are more susceptible to being flushed out of the plastic medium filter at higher loading. It has been reported that the number of ciliate species in filters increases with depth (Baker 1949; Baines *et al.* 1953; Curds 1975; Wheatley 1976), and Hussey (1975) found that ciliate diversity was negatively associated with the film weight. Many of these workers suggested that ciliates are unable to compete effectively with the other organisms normally associated with the film in the low-dissolved

oxygen conditions prevalent at the filter surface. Therefore, the greatest diversity and largest abundance of ciliates occur in the lower depths of filters where there is an increasingly smaller concentration of organic matter in the partly treated sewage. Vertical distribution of various ciliate species within filter beds have been reported (Lackey 1924, 1925; Frye and Becker 1929; Cutler *et al.* 1932; Holtje 1943; Barker 1946; Ingram and Edwards 1960). Generally, they found that particular species tended to predominate at certain depths, stratification being dependent on anyone factor such as nutrition, or a mixture of environmental and biological interactions. Liebmann (1951) showed that whereas bacterial feeding species were present throughout the bed, being especially abundant in the upper areas, predatory species of protozoans were restricted to the lower half of the filter. Liebmann (1949) also put forward the saprobity theory, that as the wastewater passed through the filter it was gradually purified so that the saprobic nature of the liquid environment changed with depth. This was polysaprobic at the top, followed by α- and β-mesosaprobic zones as the depth increased. Thus, for example, *Vorticella microstoma*, *Glaucoma scintillans*, and *Colpidium colpoda* are restricted to the surface of the filter (polysaprobic), whereas *Paramecium caudatum*, *Chilodonella uncinata*, *Uronema nigricans*, *Opercularia coarctata*, and *Podophyra fixa* are all typical of the middle regions of the filter (α-mesosaprobic). *Aspidisca costata* is usually recorded in the lower regions of filters and is associated with the lowest levels of organic matter (β-mesosaprobic). Gray (1980) found that at the lower loading, ciliates were widely distributed throughout the depth of his pilot filters, and although certain species were limited to specific regions, that, generally, there was no increase in either diversity of species or abundance with depth. At the higher loading, maximum species diversity and the abundance of protozoans occurred in the lower half of the filters. The increased abundance of certain species in the lower depths of the filters at this loading, such as *Opercularia microdiscum*, was not due to the lower organic content of the influent sewage at that depth as suggested previously, because this species is tolerant of both organic load and film accumulation. It is more probable that the increased occurrence of protozoans at the lower depth was a consequence of the increased hydraulic loading. This is confirmed by a greater number of individuals being washed out with the final effluent at the higher loading. In conventional filters, there would be a greater tendency for the protozoan fauna to be forced deeper into the filters by using the normal instantaneous and heavy system of sewage application, compared to the finer distribution system used by Gray on his pilot filters (Gray and Learner 1983).

The class ciliatea is divided into four subclasses:

(i) *Holotrichia* are free-swimming protozoans, having a body covered in uniform ciliature. Typical Holotrichia include the genera *Chilodonella*, *Colpoda*, *Colpidium*, *Paramecium*, and *Uronema*.

(ii) *Spirotrichia*, in contrast, do not have cilia uniformly distributed over their bodies, but the cilia are bound together to form thick tufts known as cirri. Among the common genera are *Stentor*, *Aspidisca*, *Euplotes*, *Stylonychia*, and *Oxytrichia*.

(iii) *Peritrichia* are sesscile species with no cilia on the body but conspicuous oral ciliatures. The body is barrel or inverted bell-shaped and is borne on a stalk which may be contractile. In some species, the body can break free from the stalk to form a free-swimming unit (a telotroch). By closing the mouth the telotroch can use the ring of aboral cilia to propel the body through the water. Typical genera are *Vorticella*, *Carchesium*, *Opercularia*, and *Epistylis*.

(iv) *Suctoria* are predatory and do not possess cilia or locomotary organs, they catch other protozoans with tentacles. Two types of tentacles are seen, sharp tentacles that capture prey by piercing its body, or tentacles hold the prey by suction via an adapted terminal knob. Typical genera include *Tokophyra*, *Acineta*, *Podophyra*, and *Sphaerophrya*.

The taxonomy and identification of the protozoans is dealt with by Curds (1969). Examples of typical ciliates found in percolating filters are shown in Fig. 4.17.

In a survey of 52 percolating filters in the UK, Curds and Cockburn (1970a) found that all the filters including those treating industrial wastewater contained protozoans, with ciliates generally dominant. They found that the protozoan fauna of percolating filters closely resembled those of activated sludge but that certain species were found in either one or the other of the processes (Table 4.14). The ciliates identified in the survey consisted of 19 holotrichs, 20 peritrichs, 11 spirotrichs, and 3 suctorians. The most common ciliates found are listed in Table 4.14, with *Chilodonella uncinata* present in 90% of the samples, *Vorticella convallaria* in 83%, *Opercularia microdiscum* in 81%, and *Carchesium polypinum* in 62%. However, although species are widely distributed they are not always abundant or the dominant protozoan in a filter. Curds and Cockburn only recorded eight ciliates in large numbers, *Chilodonella uncinata* (4% of sites), *C. polypinum* (15%), *Vorticella alba* (2%), *Vorticella convallaria* (10%), *Vorticella striata* var. *octava* (2%), *Opercularia coarctata* (2%), *Opercularia microdiscum* (44%), and *Opercularia phryganeae* (4%). In his pilot-scale

Table 4.14. Characteristic ciliate protozoans identified in percolating filters and activated sludge plants (Curds and Cockburn 1970a).

Percolating filters	Activated sludge plants
Opercularia microdiscum	*Aspidisca costata*
Carchesium polypinum	*Vorticella convallaria*
Vorticella convallaria	*Vorticella microstoma*
Chilodonella uncinata	*Trachelophyllum pusillum*
Opercularia coarctata	*Opercularia coarctata*
Opercularia phryganeae	*Vorticella alba*
Vorticella striata var. *octava*	*Carchesium polypinum*
Aspidisca costata	*Euplotes moebiusi*
Cinetochilum margaritaceum	*Vorticella fromenteli*

Table 4.15. Most frequently observed species of protozoans recorded in percolating filters (Curds and Cockburn 1970a).

Class	Species
Phytomastigophorea	*Peranema trichophorum*
Zoomastigophorea	*Bodo caudatus*
	Trepomonas agilis
Rhizopodea	Small amoebae
	Arcella vulgaris
Ciliatea	*Aspidisca costata*
	Carchesium polypinum
	Chilodonella uncinata
	Cinetochilum margaritaceum
	Opercularia coarctata
	Opercularia microdiscum
	Trachelophyllum pusillum
	Vorticella convallaria
	Vorticella striata var. *octava*

study, Gray (1980) observed that species were restricted by a variety of factors including saprobity, flow rate, film accumulation, season, and medium type. He found that five species dominated his filters, four holotrichs, *Paramecium aurelia*, *Uronema nigricans*, *Glaucoma scintillans*, and *Colpidium colpoda*, and one peritrich *Opercularila microdiscum*, all of which feed on bacteria (Table 4.16). Under low-rate conditions, *Opercularia*

Table 4.16. Number of months when specific ciliate species were dominant in experimental filters loaded at 1.68 $m^3m^{-3}d^{-1}$ (low-loading) and 3.37 $m^3m^{-3}d^{-1}$ (high-loading) (Gray 1980).

Species	Low loading			High loading			Total		Total Dominance
	Filter			Filter					
	Slag	Mixed	Plastic	Slag	Mixed	Plastic	Low loading	High loading	
Uronema nigricans	2	5	5	1	1	2	12	4	16
Paramecium aurelia	5	1	4	1	0	1	10	2	12
Opercularia microdiscum	5	5	2	8	7	5	12	20	32
Colpidium colpoda	0	0	1	1	2	3	1	6	7
Glaucoma scintillans	0	1	0	0	1	0	1	1	2

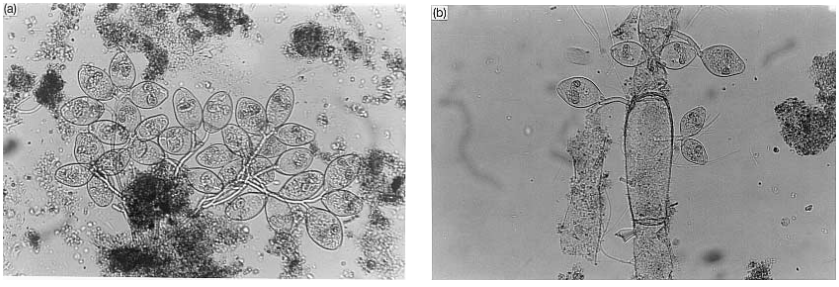


Fig. 4.18. (a) The ciliate protozoan *Opercularia microdiscum*; (b) Colonies of *O. microdiscum* attached to the hyphae of the fungus *Subbaromyces splendens*.

microdiscum, *Uronema nigricans*, and *Paramecium aurelia* were most frequently recorded, whereas under high-rate conditions, only *O. microdiscum* was regularly recorded as abundant (Fig. 4.18). *Paramecium aurelia* is inhibited by high hydraulic loadings, most likely due to its relatively large size (120–150 μm in length). It was present in large numbers, frequently comprising 70–80% of the total ciliate population. The abundance of this species is linked to the film accumulation, being more abundant in the winter months and scarce when the film was at its minimum thickness during May to August (Fig. 4.19). At the low-rate loading, *P. aurelia* was recorded throughout the bed, whereas at the higher rate it was restricted to the lower half of the filter until eventually washed out. *Uronema nigricans* is also unable to cope with high hydraulic loadings but is found in greatest abundance in the summer, with numbers declining as the film accumulates. The density of *Uronema* is negatively correlated with film accumulation, which is in contrast to *P. aurelia*. *Uronema's* preference for light film development results in *P. aurelia* and *U. nigricans* being rarely found together (Fig. 4.19). *Opercularia microdiscum* is a sessile organism attached to the medium or other suitable substrate by a non-contractile stalk and feeding passively on free swimming bacteria. It is unable to actively search for food and, unlike the holotrichs, it is unable to move away from any adverse environmental changes, predators or the activities of the macroinvertebrate grazing fauna, except in its telotroch phase. The population density of the opercularian remained relatively small under low-rate conditions, but when the loading was increased to high-rate, the mean population density increased five-fold, occasionally making up between 90–100% of the total ciliate population in all three pilot filters. However, at neither loading was *O. microdiscum* able to compete successfully with *U. nigricans* during periods of light film accumulation, but was found in greatest abundance

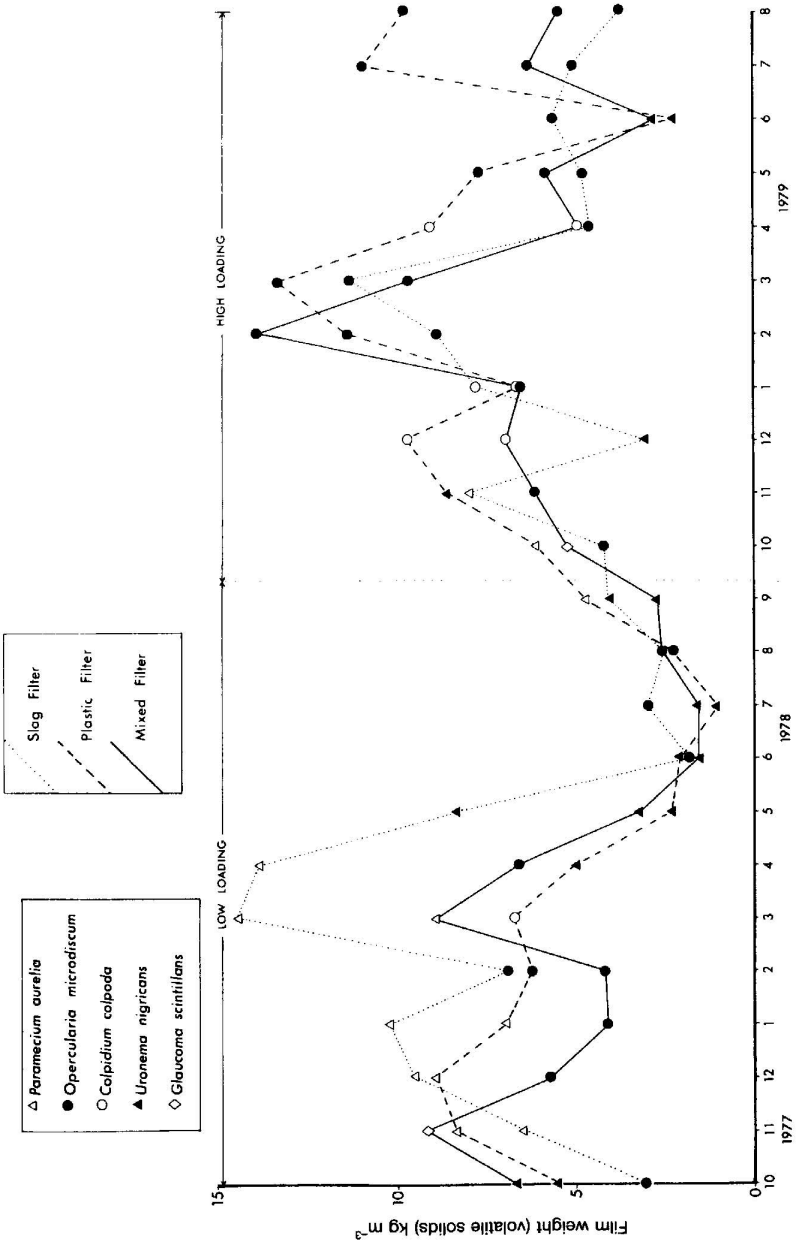


Fig. 4.19. Dominant ciliate protozoan species in pilot filters at various film accumulations (Gray 1980).

during January/February and July/August when film accumulation was heavy. The sedentary ciliates attach themselves to a variety of substrates including zoogloeal bacteria, fungal hyphae, insect debris, and the larger filamentous bacteria. *Opercularia microdiscum* was positively correlated with both zoogloeal bacteria and the fungus *Subbaromyces splendens*, which it used as a substrate for attachment. In the pilot filters, the peritrich was restricted by competition for nutrients and space under low-rate conditions, and limited only by the lack of suitable surfaces for attachment at the higher loading. In all the pilot filters, *O. microdiscum* was found throughout the depth of the beds during the low loading, except at depths with heavy film accumulation or high abundance of *P. aurelia*. With the increase in loading rate, however, the population increased mainly in the lower half of the beds, although still avoiding those areas of heaviest film accumulation. The clear association of the ciliate with *S. splendens*, the tough hyphae of which grow out into the liquid layer flowing over the film, provides an ideal niche for the sessile protozoan so that it is away from any danger of being overgrown by the rapidly developing film (Fig. 4.18). Where the film is bacterial in nature then no suitable substrates for attachment may exist when holotrichs dominate. Although *O. microdiscum* has been previously associated with low concentrations of organic matter (Barritt 1940; Barker 1946; Tomlinson and Snaddon 1966), more recent work has suggested that the species may have a more general distribution (Curds 1969; Curds and Cockburn 1970a; Hussey 1975). Learner (1975a) also noted the importance of this species in wastewater treatment and found it to be the dominant organism in the majority of filters examined. He recorded a positive correlation of the organic and hydraulic loading with *O. microdiscum*, noting that maximum abundance was recorded in filters receiving loads in excess of 0.25 kg BOD $m^{-3}d^{-1}$. Some years earlier, Bruce and Merkens (1970) had found large numbers of the species, but no other ciliate, in experimental filters receiving an organic loading of 2.0 kg BOD $m^{-3}d^{-1}$ at a hydraulic loading of 6 $m^3m^{-3}d^{-1}$. *Colpidium colpoda* occupies the same niche as *P. aurelia* and is present at the same times. The species is associated with the lower range of film accumulations at which *P. aurelia* dominates, and is found reaching maximum numbers at film weights of between 4 and 9 kg m^{-3}. At the higher loading, the density of *C. colpoda* increased due to the exclusion of *P. aurelia*, where it dominated at periods of moderate film accumulation. At the higher loading, the sequence of species from heavy to light accumulation can be seen quite clearly, from *O. microdiscum* to *C. colpoda* and then to *U. nigricans* at the lighter weights of film, with each peak in the population density clearly separated from the next. *Colpidum colpoda* and *O. microdiscum* are

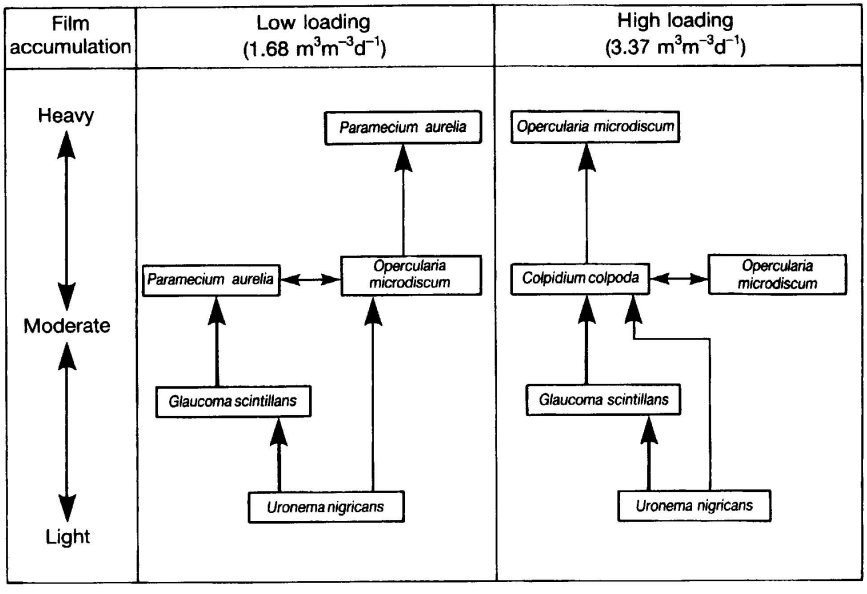


Fig. 4.20. Succession of dominant protozoan species with film accumulation (Fig. 4.19) at loadings of 1.68 and 3.37 $m^3m^{-3}d^{-1}$ (Gray 1980).

both high-rate species and compete directly; when one species is found in large numbers the other is present only in small numbers. It does seem, however, that *O. microdiscum* is more successful than *C. colpoda* when there are enough suitable surfaces for attachment. The succession of these species, which are either controlled by film accumulation or temperature, at low- and high-rate loadings, is summarised in Fig. 4.20. The final dominant species, *Glaucoma scintillans*, competes directly with *U. nigricans* and the former is more successful at moderate film weights (5–9 kg m^{-3}), whereas *U. nigricans* is dominant at low film weights (< 5 kg m^{-3}).

Curds (1973c, 1975) divided the wastewater protozoan fauna into three groups according to their habitat: those that swim freely in the liquid phase and are prone to being washed out; those that crawl over the surface of the film and are occasionally washed out; and those that are attached directly to the film, or some other material, and are only removed during sloughing. Obviously, habitat preference of those species found in percolating filters is important in survival terms and dictate which species are to be successful. Bungay and Bungay (1968) found that a peritrich, such as *Opercularlia microdiscum*, is always present even after sloughing in quite large numbers, and, therefore, potentially able to build up the population

rapidly. However, in any given situation, in the competition between species for food, the organism that is fastest both to grow and reproduce under the prevailing conditions will become dominant (Moser 1958).

Opercularia microdiscum and *P. aurelia* were found at all depths by Gray (1980) in his low-rate filters, whereas *C. colpoda* was found in the upper regions of the bed where the organic concentration of the wastewater was greatest. *Aspidisca costata* was limited to the lower half of the pilot filters and was continuously washed out of the filters in large numbers. The dominant species at the higher loading were restricted to particular depths in line with the saprobic index proposed by Liebmann (1949). *Colpidium colpoda* was found in the top and middle regions of the pilot filters, *O. microdiscum* in the middle and lower regions, whereas *A. costata* and *U. nigricans* were restricted to the lower portion of the filters. Suctoria, which are mainly predators on other protozoan species, were found in the middle and lower areas of the filters only where they would have maximum opportunity to come into contact with suitable prey. However, Gray concludes that no individual reason can account for the stratification of the various protozoan species, but that it is the result of a number of environmental (e.g. nature of wastewater, organic load, temperature, hydraulic flow, food availability, surface area) and biological factors (e.g. competition, predation, type of film), which change continuously and alters the distribution of the protozoans within the film. Mistri *et al.* (1994) have studied the effects of physico-chemical and plant operational variables on protozoan community structure.

Rotifera. The role of rotifers in wastewater treatment processes has been reviewed by Doohan (1975). Unfortunately, most of the research carried out on this group has been in connection with the activated sludge process (Curds and Vandyke 1966; McKinney 1957; Calaway 1968; Sydenham 1968, 1971), thus, relatively little is known concerning the rotifers found in the percolating filter environment (Donner 1966). In the completely mixed environment of the activated sludge process, rotifers have two distinct functions: they break up floc particles providing nuclei for new floc formation; and they clarify the effluent by removing non-flocculated bacteria that are in suspension. They are powerful feeders and the strong ciliary currents produced by rotifers allows them to feed effectively even when the concentration of bacteria on which they feed is very low. This is in contrast to protozoans such as Vorticellids, which have weaker ciliary action and therefore are unable to survive at such low bacterial concentrations. Thus, in the activated sludge process, Vorticellids are normally succeeded by rotifers,

and for the same reason rotifers are generally found in the lower sections of percolating filters. From field experiments Doohan (1975) supposed that it was food availability which exerted the greatest influence on the reproduction rate of the Rotifera, rather than other environmental factors, such as temperature. Therefore, their distribution within the filter environment would appear related to food availability, and ability to compete with other bacterial feeders. Rotifers have greater mobility compared to protozoans. For example, *Lecane* spp. have specialised feet that enable them to crawl over the film and medium. This specialisation allows rotifers to move within the filter bed and extends their distribution area when conditions permit. When protozoans are under stress because of food limitation, rotifers usually move into that area and compete for the available bacteria. Rotifers also help in the production of discrete faecal pellets that consist of undigested material bound together by mucus and which rapidly settle. A comprehensive list of species found in wastewater treatment processes is given by Doohan (1975) (Fig. 4.21).

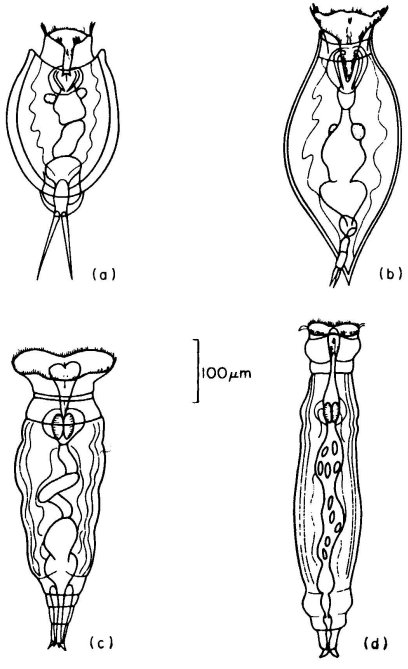


Fig. 4.21. Commonly reported rotifers in both percolating filters and activated sludge: (a) *Lecane* sp.; (b) *Notommata* sp.; (c) *Philodina* sp.; (d) *Habrotrocha* sp. (Doohan 1975).

Nematoda. Little is known regarding the role of Nematoda in wastewater treatment processes, but as they are present in such large numbers in percolating filters they must be important members of the filter community. Large populations of nematodes have been recorded in filters (Peters 1930; Lloyd 1945; Calaway 1968). The population of nematodes is dominated by bacterial feeders, although predator species feeding on other nematodes and rotifers are also present, but are far less abundant (Schiemer 1975). Weninger (1964) recorded maximum population densities of 180 individuals per millilitre, whereas Scherb (1968) found maximum densities of up to 280 ml^{-1} in a bench-scale-activated sludge plant. Schiemer (1975) suggested that experiments carried out by Pillai and Taylor (1968) showed that the maximum population in conventional low-rate filters could be in the order of 1000 ml^{-1}, which corresponds closely with the results obtained by Gray (1980), where a maximum population density of 940 ml^{-1} was recorded in his low-rate pilot filter containing slag medium (Fig. 4.14). The seasonal variation in abundance coincides with film accumulation, with maximum population densities of nematodes recorded during the early spring and with minimum population densities occurring immediately after sloughing. Weninger (1971), and Murad and Bazer (1970) observed that population density was inversely related to temperature, resulting in maximum populations between 7–10°C, although Chaudhuri *et al.* (1965) recorded maximum nematode densities between 17–18°C. Hawkes and Shephard (1972) regarded the Nematoda as being important grazers that were closely associated with film accumulation. Nematodes live in the film rather than on the surface or in the liquid phase and thus are directly related to film accumulation and film thickness. They were appreciably less abundant on smoother plastic media where film thickness is less, compared with rough mineral media. Another advantage of nematodes is their high reproductive potential (Schiemer 1975), which is similar to that of the astigmatid mites and the enchytraeid worms, all three groups being able to respond rapidly to increases in the available food. Schiemer considered the role of nematodes in the filter environment and estimated that the nematode population is responsible for 0.001% of the community's respiration. He identified that they had three functions: grazing of bacteria, which affects bacterial density and growth; decomposition of organic matter; and recycling of energy-rich substances inside the filter because of excretion products, faeces, and dead body tissues, although these may be lost to the system via the final effluent. Thus, although nematodes may have important effects on bacterial activity, they have probably only a minor role in the decomposition of organic matter in wastewater.

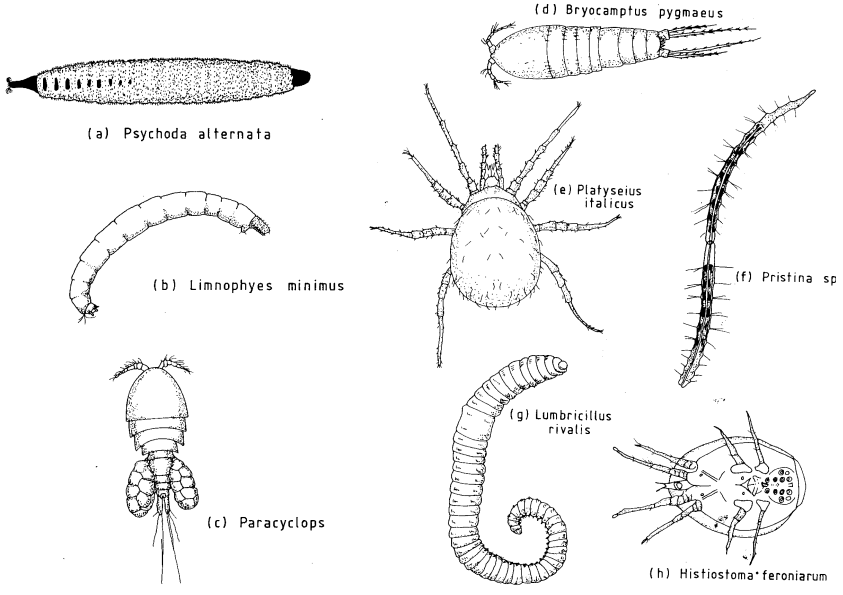


Fig. 4.22. Common invertebrates in percolating filters with approximate lengths in parenthesis: (a) *Psychoda alternata* larva, dorsal view (7 mm); (b) *Limnophyes minimus* larva, lateral (3.5 mm); (c) *Paracyclops* sp., dorsal view of female with egg sacs (0.8 mm); (d) *Bryocamptus pygmaeus* female, dorsal (0.5 mm); (e) *Platyseius italicus* female, dorsal (0.8 mm); (f) *Pristina* sp. showing asexual budding (5 mm); (g) *Lumbricillus rivalis*, lateral (20 mm); (h) *Histiostoma feroniarum* hypopus, ventral (0.2 mm).

Grazing fauna

Percolating filters are dominated by two phyla of grazers, the Annelida and Insecta. Other grazers from the Arachnida, Crustacea, and Mollusca are also present but are generally of less significance (Fig. 4.22). Learner (1975a) carried out a detailed survey of invertebrates at 67 separate filter beds at 48 sewage treatment plants throughout Britain. All were sampled in Spring while 59 were also sampled a second time during Autumn. A 2.5 litre sample of filter medium and film was removed from the top 300 mm of each filter bed and a 10 cm^3 solids sample was collected from the final effluent using a 400 μm mesh drift net. A total of 87 species were recorded of which 39 occurred in the surface samples. From the surface samples 24 species were particularly successful being both widespread and abundant (Table 4.17). Three species, the worm *Lumbricillus rivalis*, the moth fly *Psychoda alternata*, and the mite *Histiostoma feroniarum* were almost ubiquitous. All the 24 most common species grazed the film with one exception, the predatory mite *Platyseius italicus* (Learner and Chawner 1998).

Table 4.17. The principal macro-invertebrate species occurring the surface medium samples of 59 filter beds that were sampled in the spring and autumn by Learner (1975a). Average abundance of species (no. l^{-1} of medium) and percentage occurrence are given (Learner and Chawner 1998).

Species	Average abundance (nos l^{-1})	Occurrence (%)
Lumbricillus rivalis	6055	91
Psychoda alternata	3509	89
Histiostoma feroniarum	588	92
Pristina idrensis	314	48
Bryocamptus pygmaeus	146	61
Metriocnemus eurynotus	119	54
Enchytraeus coronatus	118	22
Platyseius italicus	113	85
Psychoda albipennis	109	72
Limnophyes minimus	98	61
Nais elinguis	88	52
Hypogastrura viatica	84	51
Histiogaster carpio	65	66
Enchytraeus buchholzi	47	57
Paracyclops fimbriatus	41	73
Chaetocladius perennis	31	24
Sylvicola fenestralis	25	49
Nais variabilis	21	54
Paracyclops chiltoni	8	18
Pristina aequiseta	8	42
Paracyclops poppei	5	12
Eiseniella tetraedra	3	52
Tomocerus minor	3	28
Dendrodrilus subrubicundus	3	43

Unusual invertebrates are also occasionally recorded. For example, seawater can enter damage sewers at coastal sites allowing estuarine and marine species to colonise filters. Jones and Wigham (1993) studied a population of the supralittoral amphipod *Gammarus duebeni* that had colonised and thrived in a percolating filter in Cornwall, England.

Annelida. Only two families of the phylum Annelida, the Enchytraeidae and the Lumbricidae, are common in wastewater treatment. They are almost exclusively found in percolating filters because, except for certain naids and lumbriculids, they are not active swimmers. The most frequently observed enchytraeid is *Lumbricillus rivalis* which was found in 91% of the filters surveyed by Learner (1975a). This species can make up 95–100% of the

total enchytraeid population in filters with maximum population densities reaching 10000 per litre of mineral medium (Gray 1980). *Enchytraeus buchholzi* is the next most frequently observed species, being recorded in low numbers in 57% of the filters examined by Learner. Gray observed that *L. rivalis* was distributed throughout the depth of his pilot filters at the low-rate loading, reaching maximum abundance in the central and lower areas of the filters, with the enchytraeid restricted to the lower half of the filters at the high-rate loading. Previous workers had found *L. rivalis* principally in the upper portions of the filters near the surface (Reynoldson 1947; Solbe *et al.* 1967; Williams *et al.* 1969). Maximum numbers of enchytraeids are found during February with minimum densities occurring in August, indicating the population density is directly linked to film accumulation. However, Enchytraeidae are restricted by the presence of Psychodid larvae. They reach maximum population densities the month preceding the occurrence of the maximum number of Psychodid larvae, but both reach maximum abundance in response to the heavy film accumulation. The *Psychoda* spp. and enchytraeids are in direct competition both for food and space in the filter, with the larvae being more successful because of their size and rapid growth rate. The increased competition from the larvae caused a dramatic decline in the numbers of enchytraeids present (Gray 1980). Enchytraeidae have also been found to dominate the grazing fauna under reduced competition from the fly larvae caused by higher hydraulic loadings or lower temperatures ($< 10°C$) (Tomlinson and Hall 1950; Hawkes 1955; Solbe *et al.* 1974). Hawkes (1955) demonstrated in his experiments on dosing frequencies that *L. rivalis* could withstand certain hydraulic flows because it possessed strong, curved setae and that as the cocoons were firmly attached to the medium they could withstand periods of even higher flow-rates. Enchytraeidae are restricted at higher flow rates (> 2 $m^3m^{-3}d^{-1}$) with large numbers lost in the effluent especially during sloughing (Reynoldson 1941, 1948), and are generally absent from high-rate filters using either plastic or mineral media at loading rates > 6 $m^3m^{-3}d^{-1}$ (Bruce and Merkens 1970).

Lumbricillus rivalis is capable of increasing its population density rapidly because of the large number of cocoons present in the filter at specific periods, and its high population growth rate (Learner 1972). This allows the Enchytraeidae to respond quickly to changes in the film accumulation and also to changes in community structure. The number of cocoons in the filter bed is controlled by the film accumulation. More are retained as the film increases, either by adhesion to the film or some other suitable substrate, and by being mechanically filtered out of the sewage by the film

and humus. At times of low film accumulation there is a corresponding low abundance of cocoons which are mainly adhering to the actual surface of the media. Reynoldson (1947) and Solbe *et al.* (1967) both found that the abundance of cocoons followed a seasonal pattern reaching maximum numbers in spring and autumn. In his pilot filters, Gray found that the seasonal abundance of cocoons followed a similar pattern to that of the adult enchytraeids, reaching maximum numbers during February to April and minimum numbers during the Summer months after sloughing. Both the adult enchytraeids and the cocoons are eaten by a number of predatory dipteran larvae *including Limnophyes minimus*, *Metriocnemus eurynotus*, and *Psychoda albipennis* (Lloyd 1945).

Only three lumbricids are frequently recorded in percolating filters, *Eisenia foetida* (31% of the filters surveyed by Learner), *Dendrobaena subrubicunda* (43%), and *Eiseniella tetraedra* (52%). Considerable information regarding these species in filters has been gathered (Tomlinson 1946a; Hawkes 1963; Solbe *et al.* 1967; Solbe 1971) and this has been reviewed in detail by Solbe (1975). Of the two commonest species, *E. tetraedra* is an amphibious species, whereas *D. subrubicunda* is terrestrial in nature being commonly found a compost heaps (Gerard 1964). Therefore, it is not surprising that *E. tetraedra* is more successful at higher hydraulic loading than the other species. Lumbricids tend to be more numerous in smaller media (Terry 1951), with worms rarely found near the surface in filters containing larger media or media with a high voidage, due to the flushing action of the applied wastewater. Solbe (1971) examining the depth distribution of lubricids reported that the population density of *D. subrubicunda* increased towards the base of the filters, whereas *E. tetraedra* is found in the middle regions of the bed. Therefore, the distribution of the lumbricids is apparently related to the hydraulic flow and the size of the interstices.

The presence of lumbricids is generally accepted as an indication that a filter has matured and that the community structure has become stable. However, during high rainfall large numbers of worms are flushed out of the soil on to paved areas and are washed away in the surface runoff. Thus, where combined sewerage systems are employed, these worms eventually arrive at the treatment plant and those not removed by primary sedimentation will become established in the filter, with large population densities becoming established quite quickly. The function of annelids in percolating filters is mainly one of film control. *Dendrobaena subrubicunda*, for example, can ingest film at a rate of 133 mg $g^{-1}d^{-1}$ at 15°C, with the entire lumbricid population of a mature filter capable of ingesting an amount of film equivalent to 55% of the daily input of carbonaceous material to the filter

(Solbe 1975). Annelids therefore prevent the accumulation of excess solids and may also be the prime cause of the sloughing of film in some filters during spring. Annelids are able to ingest a wide range of substances including those not readily degraded by the filter bacteria. Thus, Annelida are able to reduce the organic matter directly by absorption and indirectly by the formation of excreted aggregates (faeces) which readily settle in the humus tank. Although some heterotrophic bacteria flourish in the gut of certain annelids and are passed out with the aggregates, they are also responsible for the consumption of many pathogenic micro-organisms. The respiration of the phylum accounts for a small, but useful, proportion of the carbon dissipated by the filter, which in some filters may be as high as 8.5%. The group causes little nuisance in the filter, although the accumulation of large numbers on the surface can cause alarm to operators even though they are quite harmless. If large numbers are washed out into the humus tank they rapidly die and putrefy causing a reduction in the dissolved oxygen concentration that can cause problems. They do, however, compete directly with fly larvae and so reduce the potential nuisance caused when adult flies emerge from filters.

Insecta. The members of the various orders of Insecta are principally associated with percolating filters, rarely being found in other kinds, or at other stages, of wastewater treatment. Many species lists have been compiled (Lloyd 1945; Tomlinson 1946a; Terry 1951; Hawkes 1963; Solbe *et al.* 1967). However, the first comprehensive survey into the fauna of percolating filters was undertaken by Learner (1975a) and a comprehensive species list prepared, containing 186 species of insects belonging to 38 families (Learner 1975b).

Sixteen species of Collembola (springtails) have been recorded from percolating filters, the most important species being *Hypogastrura viatica* (*Achorutes subviaticus*), which was recorded in 57% of the filters surveyed by Learner (1975b), with *Tomocerus minor* (28%) and *Proisotoma minuta* (21%) also frequently observed. However, only *H. viatica* is found in large numbers, reaching maximum densities of 3800 individuals per litre of medium. They are active grazers found throughout the depth of the filter and feed on a wide variety of organic material including fungal hyphae and spores, bacteria, dead and decaying plant material, and algae (Learner 1975b). *Hypogastrura viatica* is extremely sensitive to increased rates of filtration (Hawkes and Jenkins 1955, 1958; Wheatley 1976). For example, Tomlinson and Hall (1950) recorded maximum abundance of the springtail at 1.5 $m^3m^{-3}d^{-1}$, with none found at loads > 3.0 $m^3m^{-3}d^{-1}$. *Isotoma olivaceaviolacea* is more abundant in random plastic than mineral media

(Gray 1980), because of its preference for drier areas, such as inter-jet zones in conventional filters (Hawkes 1959). Each module of random plastic media, such as Flocor RC or RS has a dry area suitable for such organisms as *Isotoma* sp.

Beetles (Coleoptera) are not common in percolating filters. Only members of the Hydrophilidae and Staphylinidae are frequently encountered, with the most widely occurring species being *Cercyon ustulatus* and *Platystethus arenarius*, which were observed by Learner during his survey. Coleoptera are rarely found as larvae in the filter. Only adults are found, and they presumably do not breed within the filter environment. The most important insects found in filters are the Diptera, with 28 species from 11 dipteran families regularly occurring (Learner's survey). The principal filter species belong to the genera *Psychodidae*, *Chironomidae*, and *Anisopodidae*, and can all breed successfully in the filter bed, with the larvae being abundant and feeding on the film. Many more species of Diptera are associated with filters and are captured in very large numbers on insect traps set on, or near to, filters. However, it is these species that are able to breed in the filter bed environment and which have an important role in the actual purification process.

The dipteran larvae and Collembola are very active grazers, preventing the filters from becoming blocked with excessive film accumulation (Williams and Taylor 1968). They are most active during the warmer months, although large populations of some species, such as *Sylvicola*, are still active even during winter. The function of the Insecta in the purification process is the same as that of the Annelida; they digest the film and absorb some of the organics, and form dense faecal pellets that rapidly settle. Williams and Taylor (1968) demonstrated that solids from filters containing *Psychoda* and/or *Lumbricillus* spp. settled far more rapidly, with 65–70% of the total settleable solids settling within one hour compared with 34% in the control filter without macro-invertebrates. The sludge from the filters containing macro-invertebrates contained a high density of animal fragments and faecal pellets.

There are three species of Psychodidae that occur in filters. *Psychoda alternata* is the most frequently observed species, occurring in 89% of the filters sampled by Learner and reaching maximum densities of 44,700 l^{-1} of medium (Fig. 4.22). Therefore, this species, together with *Lumbricillus rivalis*, must be considered the major grazer in percolating filters. *Psychoda albipennis* (formally *P. severini*) is also widely distributed (72%) but reaches lower densities (1240 l^{-1}) than *P. alternata*, whereas the third species, *Psychoda cinera*, is rare and is to be found in only 4% of filters and

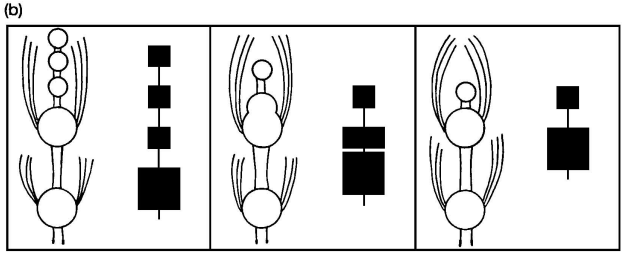


Fig. 4.23. The dipteran most associated with percolating filters are small moth-like flies less than 5 mm long of the genera *Psychoda*. (a) *Psychoda alternata* is extremely common and is known as the "sewage filter fly". It is readily distinguished from the other two species of *Psychoda* found in filters by dark tufts of hairs at the tips of some wing veins. *Psychoda cinerea* and *P. albipennis* have hair-less wings; (b) Flies can be positively identified by low-power microscopic examination of their antennae *Psychoda cinera* terminates in three small segments, *P. alternata* has two large segments broadly joined and a smaller terminal segment, while *P. albipennis* in a single small segment.

in low numbers (Fig. 4.23). *Psychoda albipennis* is parthenogenetic and, like *P. alternata*, is able to carry out its entire life-cycle without leaving the filter bed. It is therefore quite common to find adult flies deep within the medium. This is in contrast to many other dipterans where the adult flies need to leave the filter after pupation to swarm and mate before returning to lay eggs. These two species do not actively compete as *P. albipennis* is able to reproduce at temperatures below 10°C and is more abundant than *P. alternata* during the winter and spring, whereas the latter is dominant in summer. The abundance of *P. alternata* is positively correlated with temperature. Learner (1975a) clearly illustrated that the reproductive potential (life cycle) of *P. alternata* is controlled by temperature and that it had the most rapid development rate at temperatures in excess of 10°C

of any of the insects found in the percolating filter environment. Obviously, there is a lag phase between maximum food availability and the resultant increase in the number of grazers. In the case of *P. alternata*, this phase was a 1–2 months duration, depending on the temperature within the filter (Solbe and Tozer 1971). This explains why no direct correlation between film weight and density of Psychodid larvae was observed by Gray (1980). The restriction in the surface accumulation of *Psychoda* spp. is due partly to its sensitivity to the hydraulic flow (Tomlinson and Hall 1950; Hawkes 1955; Lumb and Eastwood 1958). The effect of various distribution systems was studied by Hawkes (1959), who found that splash plates produced an even distribution of sewage resulting in a large accumulation of film and a high density of *Psychoda* in the top 600 mm. By increasing the velocity of application, the *Psychoda* populations were reduced in the surface layers and were forced below 600 mm. This resulted in more film in the top layer. Tomlinson and Hall (1950) found that the abundance of the *Psychoda* larvae decreased if the hydraulic load exceeded 3.6 $m^3m^{-3}d^{-1}$, even though the film was thick. In their experimental filters, Bruce and Merkens (1970) found that large numbers of *Psychoda* larvae were present in filters loaded at 6.0 $m^3m^{-3}d^{-1}$. This was probably due to the higher voidage as it has been shown that smaller media restricted the natural life-cycle of the insects (Tomlinson and Stride 1945; Hawkes and Jenkins 1951, 1955). Using multivariate techniques Learner and Chawner (1998) were able to reanalyse the unique data set of invertebrates in British percolating filters collected by Learner (1975a). Using TWINSPAN multivariate analysis they found that *P. albipennis*, along with the copepod *Bryocamptus pygmaeus*, were indicators of low organic loading whereas abundant populations ($>$ 1000 larvae l^{-1} of medium) *of P. alternata* indicated high organic loadings.

Three chironomid species were frequently isolated by Learner (1975a), *Chaetocladius perennis* (formally *Hydrobaenus perennis*) (24%), *Metriocnemus eurynotus* (formally *Metriocnemus hygropectricus*) (54%), and *Limnophyes minimus* (formally *Hydrobaenus minimus*) (Pinder 1978) (61%), all being able to breed in filters with maximum larval densities reaching 1400, 1892, and 3460 l^{-1} medium respectively. The larvae of *Chaetocladius perennis* migrate in a general downward direction during their development. This results in the maximum abundance of larvae being found at the base of filters, high numbers of larvae being washed out in the final effluent and a scarcity of pupae in comparison with other species. The final larval instar finally burrows deep into the film to pupate (Lloyd *et al.* 1940). Like other chironomids, the species is common in all types of filter media although it is more readily flushed out of the smoother faced

media, and is abundant from February to August. Chironomid larvae are generally only found in large numbers in lightly loaded filters (Tomlinson and Stride 1945), when the film accumulation is thin (Terry 1956; Hawkes and Shepherd 1972). *Metriocnemus eurynotus* is found in greatest numbers during the autumn, and due to the upward migration of the species prior to pupation (Dyson and Lloyd 1936), the larvae are found in large quantities in the upper section of filters. *Limnophyes minimus* is restricted to low-rate filters. It is found from May to October throughout the filter, and is most successful when the film is thin. Chironomid larvae are only found in relatively small numbers in filters and have only a minor role in the purification process. However, the larvae are capable of successfully competing with the other macro-invertebrates of the filter in favourable conditions. Lloyd *et al.* (1940) reported that chironomid larvae were able to compete with psychodid larvae for the available food, reducing the population densities of *Psychoda* spp. at the surface of the filter and causing the extension of the species distribution deeper into the filter. In heavy film conditions, the *Psychoda* and *Sylvicola* larvae are more able to cope than the larvae of the chironomid species. They utilise their respiratory siphons when buried in the thick layer of film. Both *L. minimus* and *M. eurynotus,* like *P. albipennis*, have shorter life cycles than *P. alternata* at the lower range of temperatures recorded in filters. This may account for their relative success in the areas of the bed most affected by exposure to the air and which is frequently cold in comparison to the other areas of the filter. Examples of vertical and seasonal variation of some grazing organisms, including *Psychoda* spp. and *Chaetocladius* (*Hydrobaenus*) *perennis* larvae, are shown in Fig. 4.24.

Sylvicola (formally *Anisopus*) *fenestralis* is the only member of the Anisopodidae recovered from filters as it is widely distributed (49% of filters) and found in large numbers (1680 l^{-1} medium). The adult fly has extremely large wings in comparison with other filter flies and is a powerful insect with large larvae. Like *Psychoda*, it can complete its life cycle within the filter bed with maximum numbers occurring between April to June. The larvae are generally found in largest numbers in the top 600 mm of filters irrespective of the organic or hydraulic loading (Tomlinson and Hall 1950; Gray 1980) (Table 4.18), while the pupa reach maximum density between 300–900 mm. Hawkes (1963) found that the vertical distribution of the species could be altered by high instantaneous rates of domestic wastewater. *Sylvicola fenestralis* abundance has been shown to be closely and directly related to film distribution (Hawkes 1952a). However, recent studies suggest that the amount of film is not always an important factor

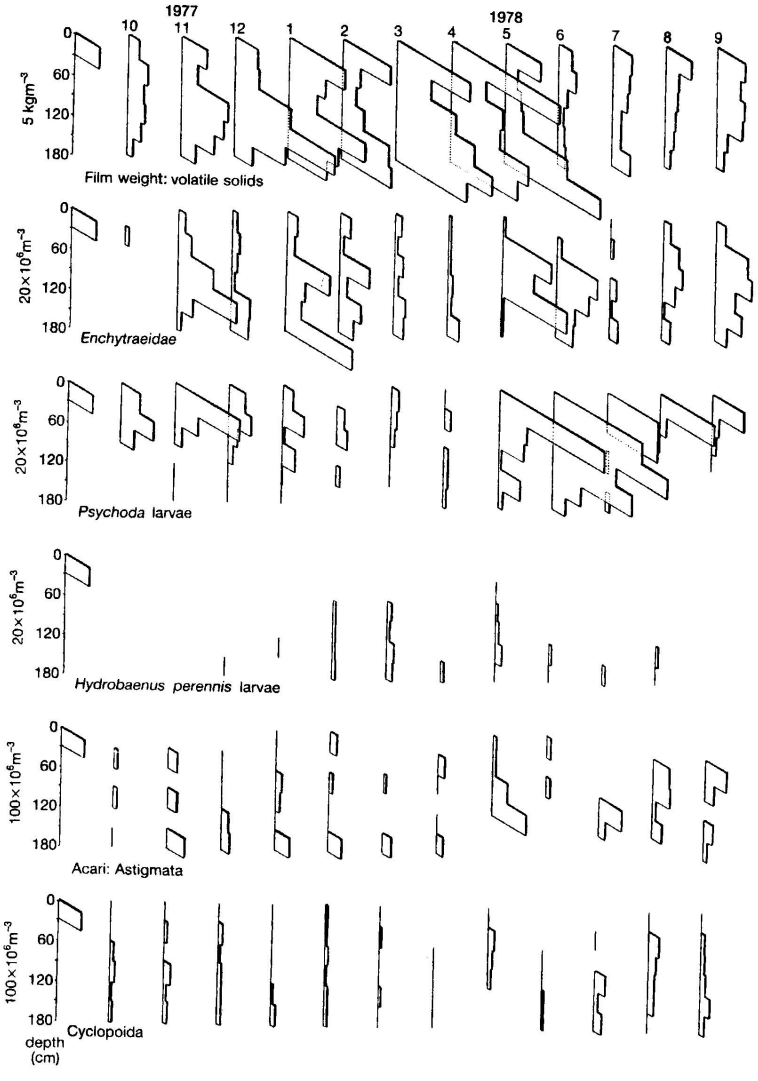


Fig. 4.24. Vertical distribution of film and macrofauna in a low-rate filter containing 50 mm blast-furnace slag medium.

determining the vertical distribution of this species. This species was reported as being more successful in random plastic medium rather than slag medium by Gray (1980), with the greatest number of larvae and pupae being recorded in the plastic filter. Each module of random plastic media has some of its surface area free from the film which is often quite dry,

Table 4.18. Vertical distribution of common insects at Minworth Wastewater Treatment Works (filter block B) in Birmingham during August 1968 (Learner 1975b). A dash is equivalen to zero.

	No. per liter of medium						
Depth (m)	*Anurida granaria*	*Hypogastrura viatica* and *H. purpurescens*	*Cercyon ustulatus*	*Sylvicola fenestralis*	*Psychoda alternata*	*P. cinerea*	*P. albipennis*
0.0–0.15	18	18	—	—	89	89	—
0.15–0.30	71	18	36	107	36	214	53
0.30–0.46	142	89	36	18	18	231	18
0.46–0.61	1175	53	53	71	—	303	18
0.61–0.76	552	125	18	—	36	71	—
0.76–0.91	445	71	—	—	—	89	—
0.91–1.07	677	142	18	—	—	36	—
1.07–1.22	944	71	—	—	18	36	18
1.22–1.37	427	53	—	—	—	53	18
1.37–1.52	374	—	—	—	—	18	—
1.52–1.68	214	71	—	—	—	—	—
1.68–1.83	641	89	—	—	—	—	—

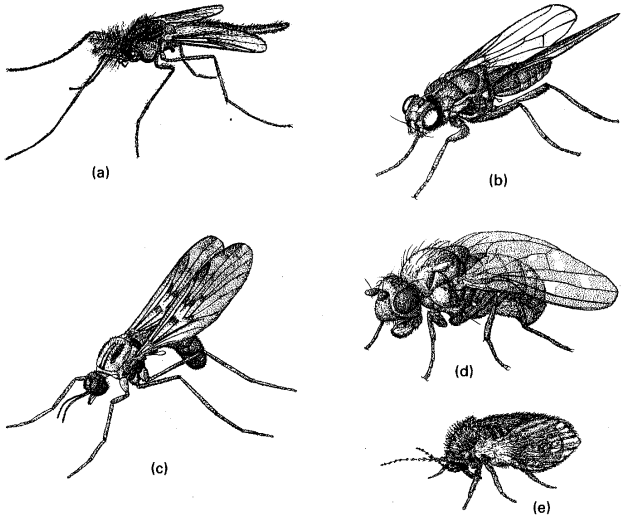


Fig. 4.25. The major species of flies associated with percolating filters (a) *Metriocnemus hygropectricus* ♂, (b) *Spathiophora hydromyzina* ♀, (c) *Sylvicola fenestralis* ♂, (d) *Scatella silacea* ♂, (e) *Psychoda alternata* ♂. (IWEM 1988).

and this may be the reason why the pupae in particular were found in comparatively large numbers in this filter. *Sylvicola fenestralis* requires a drier environment for successful pupation than that tolerated by the larvae (Hawkes 1952a), and this is thought to be the reason why the larvae are reported to migrate to drier areas in conventional plants (Learner 1975b). *Sylvicola fenestralis* appears unaffected by competition with either chironomid or psychodid larvae.

Many of the dipteran flies form dense swarms as they emerge from the filter bed, and for some chironomids, swarming is a prelude to mating (Fig. 4.25). The numbers of flies emerging can be so large that from a distance it has the appearance of smoke coming from the beds. Emergence is affected by temperature, light intensity, and wind velocity. Learner (2000) found that average air temperature was a major environmental factor determining the phenology of filter flies, allowing emergence patterns to be predicted. Insects will not fly unless the temperature is above a threshold value, for example 10°C for *P. alternata*, 7–8°C for *L. minimus*, and 4°C for both *Metriocnemus* spp. and *Sylvicola fenestralis* (Learner 1975b). Hawkes (1961b) found that for every 1.2°C rise in temperature above the threshold value up to 24°C the number of *Sylvicola fenestralis* flies in flight doubled. *Chaetocladius perennis* and *P. albipennis* are cold-adapted species that emerge principally in spring. *Psychoda alternata* and *P. cinera* are

warm-adapted species that emerge mainly in the summer, while *L. minimus* emerges principally in late summer to early autumn. *Metriocnemus eurynotus* is variable in its time of emergence with other environmental variables besides temperature being important (Learner 2000). Diel periodicity is observed for all the species with the peak of emergence for *P. alternata* and *P. albipennis* in the early afternoon, *S. fenestralis* at dusk, and a smaller peak at dawn. Wind velocity is an important factor with swarms quickly broken up and individuals unable to fly if the wind is too strong. In essence, the stronger the fly the greater the wind velocity it can withstand with *S. fenestralis* able to withstand velocities up to 6.7 m s^{-1} (Hawkes 1952b, 1961b). At the treatment plant, the density of flies can be problematic making working conditions unpleasant as flies are drawn into the mouth and nostrils, and are caught in the eyes. None of the commonly occurring flies, including *S. fenestralis*, bite. However, although harmless, the large size and intimidating appearance of *S. fenestralis* can alarm people, which results in frequent complaints from nearby residents. Psychodid flies are found up to 1.6 km away from the treatment plant, although this does depend on the direction of the prevailing wind. *Sylvicola fenestralis* are found in large numbers up to 1.2 km, although few reach farther than 2.4 km from the plant. However, flies are well known to cause both aesthetic and public health problems to those living or working close to treatment plants Woods *et al.* 1978; Painter 1980; Palfrey *et al.* 1992), with *P. alternata*, *P. albipennis*, *S. fenestralis*, *L. minimus*, and *M. eurynotus* being the main nuisance species. However, Learner (1975a) found that *P. alternata* comprised 80% or more of the total annual emergence of flies from 10 out of 17 filters where emergence traps were located. Details of the less common dipterans caught from fly traps (Fig. 4.26) placed on the surface of filter beds from ten wastewater treatment works around Britain by Learner (2000) are summarised in Table 4.19. June and July are the principal months of emergence.

Three control options are available for remedying fly nuisance. Physical methods have been least successful and are not recommended. In the USA filters were flooded to eliminate fly species. This approach damaged the ecology of the system and affected performance, providing at best just a temporary reduction in the number of flies emerging (Otter 1966). Covering the surface of the medium with a layer of finer media (13–19 mm) to a depth of 250 mm reduces the numbers of adult *Psychoda* and *Sylvicola* emerging, but results in severe ponding during the winter. Enclosing filters would appear effective but rather expensive. However, Painter (1980) suggests that this remedy may not always work and quotes an example when after covering a filter the extremes in temperature were reduced and led

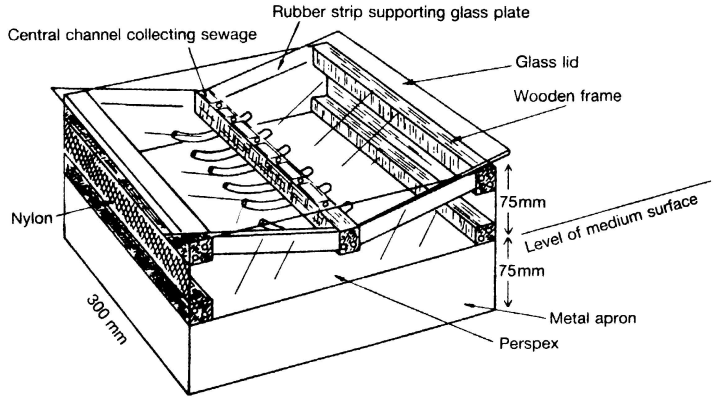


Fig. 4.26. Insect trap for determining the emergence of insects from the surface of percolating filters. The inclined glass or plastic sheets are covered with an adhesive and placed sticky side downwards so that the emerging insects become trapped (Solbe *et al.* 1967).

to an overall increase in fly production, with flies escaping via ventilation ports. The most widely adopted control method has been the use of chemical insecticides, especially DDT (dichlorodiphenyl trichloroethane) and HCH (1,2,3,4,5,6-hexachlorocyclohexane, also known as Gammexane). These insecticides were effective in killing the larger larvae without affecting the other grazers. However, such non-biodegradable pesticides are no longer permitted in Europe as they are non-specific and persist in the food chain. Also, some flies, such as *S. fenestralis*, become immune to HCH after prolonged use (Watson and Fishburn 1964). Subsequently, two insecticides were adopted for use in the UK for controlling filter flies, Actellic M20 (pirimiphos methyl) and Dimilin (diflubenzuran), which are *O*-2-diethylamine-6-methylpyrimidin-4-yl-*O*-*O*-dimethyl phosphorothioate and 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea respectively. However, neither insecticide appears particularly effective against *S. fenestralis*. These pesticides have since lost clearance for use on filters due to their persistence in the environment, as well as concerns about resistance developing within fly populations.

Current control methods use entomopathogenic bacteria (Houston *et al.* 1989a,b; Coombs *et al.* 1991) or insect growth hormones (Kamei *et al.* 1993, Coombs *et al.* 1996) against the larvae. The most widely adopted technique is the use the entomopathogenic bacterium *Bacillus thuringiensis* var. *israelensis*. The bacterium, which is available as a commercial preparation known as Teknor®, is effective against fly larvae only and does not affect

Table 4.19. Monthly occurrence of some less common insect species caught on fly traps by Learner (2000). Double crosses indicate periods when highest numbers were trapped.

Species	January	February	March	April	May	June	July	August	September	October	November	December
Trichocera maculipennis		+	++	+	+	+				+	+	
Sylvicola fenestralis				+	+	+	++	++	+	+	+	+
Metriocnemus hirticollis	+	+	+	+	+	+	+	+	+	++	+	
Coproica vagans					+	++	++	+	+			
Elachisoma aterrimum						++	+	+	+			
Halidayina spinipennis				+	+	++	++	+	+	+	+	
Ischiolepta pusilla				+		++	+	+	+	+		
Leptocera caenosa			+	+	+	++	++	+	+	+	+	
L. fontinalis			+	+		++						
L. limosa							+	++				
L. nigra				+		+	++	++	+	+	+	
Limosina silvatica										++	+	
Opacifrons coxata					+	+	++	+		+		
O. humida						++	+	+	+			

Table 4.19. (*Continued*)

Species	January	February	March	April	May	June	July	August	September	October	November	December
Opalimosina liliputana						++		+	+	+		
O. mirabilis						++	+	+	+	+		
Spelobia bifrons						++	+	+				
S. cambrica					+	++	+	+	++			
Sphaerocera curvipes				+		++	+					
Trachyopella atomus						++	+	+	+	+		
Scatella silacea				+	+	+	+	++	+	++	+	
S. stagnalis						+	++		+	+		
Culicoides vexans								++				
Themira putris					+	+	+	++				

other filter fauna or performance. The bacterium produces crystalline cell inclusions during sporulation that are highly poisonous when eaten by the dipteran larvae. Currently this is the only registered pesticide available for use on percolating filters. Insect growth hormones inhibit development of insect growth stages and have been successfully tried experimentally. Pyriproxifen has been used to control *Psychoda*. Normally the hormones are produced by the insects to regulate their development by inhibitory metamorphosis of the larval instars. A reduction of the hormone causes pupation and the absence of the hormone results in the emergence of the adult (imago). The addition of the hormone analogue permits the development of the larvae but prevents pupation or emergence, thus retaining maximum grazing activity but preventing fly emergence (Coombs *et al.* 1996). Due to the low persistence of these new methods, and their cost, they should only be used when a major egression of flies is expected which requires accurate prediction (Learner 2000).

Limited control can be obtained by changing the operational practice of the plant. For example, by limiting the amount of film accumulation, especially at the surface. This can be effected by reducing the f/m ratio or by using one of the modifications such as recirculation, double filtration, or ADF. Increasing the hydraulic loading may also make it more difficult for dipterans to complete their life-cycles in the filter. Continuous dosing using nozzles prevents emergence and severely reduces the available surface area for the flies to lay eggs. The problem of fly nuisance associated with percolating filters and the various possible control measures are fully reviewed by Painter (1980) and Learner (2000).

Astigmata mites (Acari) are extremely abundant in percolating filters and are associated with drier areas of the medium. They are found in the dry inter-jet zone of filters and are abundant in random plastic medium where each module supports a dry niche used by for such mites. Maximum abundance occurs in spring, with maximum densities in slag media reaching 3900 l^{-1}, although this exceeds 32,000 l^{-1} in random plastic media (Gray 1980). They are more abundant in low-rate systems but are found in reduced numbers in high-rate filters provided that a suitable niche is available. Four species are particularly common, *Histogaster carpio*, *Histiostoma feroniarum*, *Rhizoglyphus echinopus*, and the predatory mesostigmata *Platyseius italicus* (Baker 1961). The Astigmata are able to respond quickly to increases in film accumulation in comparison to the other macrograzers. The reproductive potential of the Astigmata and, in particular *H. feroniarum*, is far greater than that of the dipteran larvae (Learner 1975b) or the Enchytraeidae (Learner 1972). Hughes (1961) found that *H. feroniarum*

completed its life cycle in only 2 to 4 days at 20–25°C, whereas *R. echinopus* takes 9 to 13 days over a similar temperature range (Zachvatkin 1941). Initially, the mites take advantage of the large accumulation of film due to their fast reproductive rate, but the enormous numbers of Psychodid larvae that will develop subsequently force the mites into other areas of the filter bed or cause a reduction in the total population density. *Platyseius italicus* has been shown to feed on a wide variety of invertebrates, but mainly on *Lumbricillus rivalis*, although it does not eat the cocoons (Baker 1961). It is closely associated with the *Enchytraeidae* density, often being found together on the surface in large numbers (Tomlinson 1946a; Gray 1980).

Not all the macrofauna found in filters are grazing on the microbial film. The spiders and mesostigmatid mites are predators as are some of the chironomid larvae when the film becomes scarce. Duffey (1997) only recorded two spiders in large numbers in the filter beds at Minworth Wastewater Treatment Works in Birmingham. These were *Leptorhoptrum robustum* and *Erigone longipalpis* (*Linyphiidae*). Where humus (dead organic matter) accumulates within the filter saprophytic species, such as *Cercyon ustulatas* and the larvae of *Spaziphora hydromyzina*, are found. A number of the dipteran larvae are predated on or parasitised by other flies.

The ecology of percolating filters has been excellently reviewed by Hawkes (1983b). The physico-chemical nature of the filter bed environment is of prime importance in determining the community structure of each bed. Using TWINSPAN, a powerful multivariate programme for ecological spatial data, Learner and Chawner (1998) classified 59 filter beds into groups using species associations (Fig. 4.27). A second programme DECORANA ordination determined the abiotic variables likely to affect faunal composition (Fig. 4.28). From this study, the abiotic factors most likely to affect faunal composition were the organic loading, film accumulation, air temperature, size of the medium, and the age of the bed. All these variables, except air temperature, are under operator control giving the potential for the faunal community structure to be modified and even managed.

Biological analysis

Regular biological analysis of the film can often provide answers to operational problems as well as being an ideal environment for ecological research (Gray 1982b). Learner (1975a) used a single sample of medium collected from the surface of each filter plus a litre sample of the final effluent for biological analysis during his survey of percolating filters. However, only limited information can be obtained by taking surface samples. In practice,

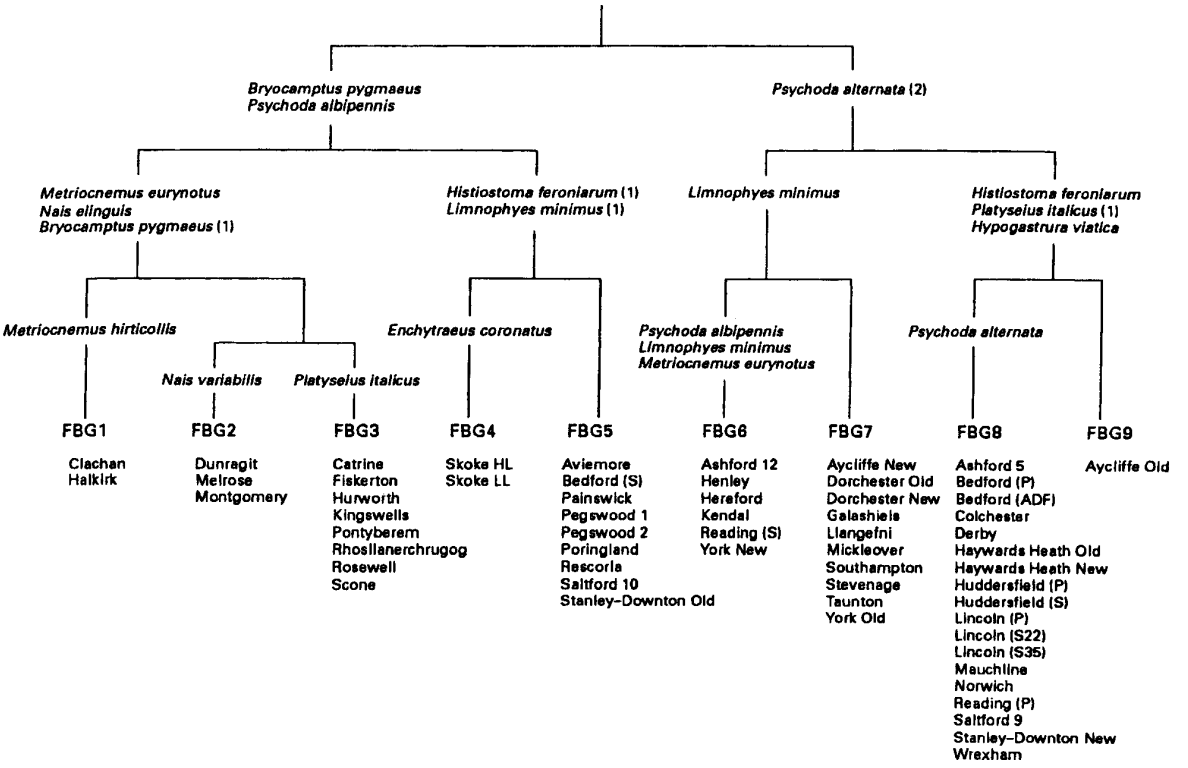


Fig. 4.27. TWINSPAN classification of the percolating filter beds surveyed by Learner (1975a). The indicator species for the various divisions are shown. The parentheses next to an indicator species denotes an abundance threshold. 1 => 100 l^{-1} medium; 2 => 1000 l^{-1} medium (Learner and Chawner 1998).

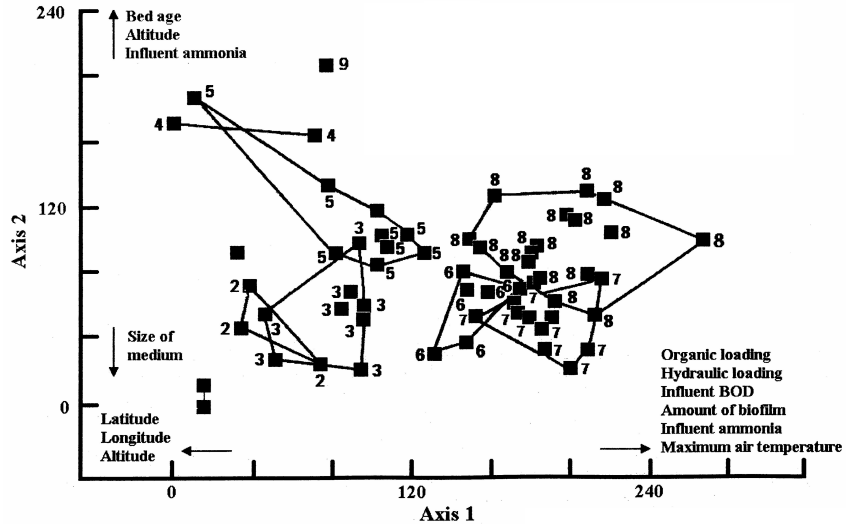


Fig. 4.28. DECORANA ordination of the filter-beds. The filter-bed group to which each bed belongs, as classified by TWINSPAN (Fig. 4.27), is indicated by the numerals. The environmental variables that correlated with the axes are also shown; the arrows indicate the direction of increasing magnitude.

it is not possible to collect medium from depths in excess of 500 mm by digging a hole in the surface of the bed. Thus, little information can be obtained regarding the vertical distribution of the film or of the organisms restricted to the lower half of the filter by this method. Also, where high hydraulic loadings are used or simple flow-on distributors, then little film and a restricted fauna will be present in the top layer of the medium anyway. It is important, therefore, to be able to sample the medium throughout the depth of the filter in such a way so as to cause as little disturbance and damage as possible. This is facilitated by the provision of sampling tubes which allows the vertical distribution of film and fauna to be measured both quantitatively and qualitatively.

Sampling tubes are perforated pipes into which closely fitting perforated baskets, containing medium, fit. The baskets can then be lifted out allowing the medium and associated film to be sampled at any depth. Gray and Learner (1983) used perforated thick-walled (25 mm) ABS plastic pipes with an internal diameter of 150 mm. The pipes were exactly the same depth as the bed with the top of the plastic pipe just below the surface of the medium. Each pipe was perforated with 38 mm diameter holes some 6 mm apart. Hawkes and Shephard (1972) used smaller holes, less closely spaced, in their sampling columns but examination of the voids within

full-scale percolating filters indicated that larger holes would simulate actual operating conditions more closely. The larger holes also allow greater redistribution of wastewater and movement of loose solids and filter fauna between the sampling column and the surrounding medium. However, the diameter of the perforations was not so large that the blast-furnace slag or random plastic media (50 mm nominal size) used by Gray and Learner would protrude through them sufficiently during settlement to interfere with the removal of the sampling baskets. Each sampling column contained six rigid plastic-coated wire-mesh baskets, each 300 mm long and 146 mm in diameter. Two plastic-coated wires were welded on to each sampling basket to aid their removal. The wires were just thin enough to pass between the baskets and the wall of the sampling column. Film accumulated on these wires and on pieces of medium that protruded slightly through the holes in the wall of the pipe, thus restricting the free vertical passage of sewage through the gap between the baskets and the pipe. Removable baskets have been used by many other workers. The baskets enable the medium to be sampled throughout the depth of a filter, although a wide variety of designs and materials have been used (Williams *et al.* 1969; Hawkes and Shephard 1972; Wheatley 1976; Rowlands 1979; HMSO 1988) (Fig. 4.29). Horizontal distribution of the biota can be investigated by using extra sampling baskets housed in shorter sections of perforated pipes positioned in the surface of each filter, thus providing details of distribution of film in the top 300 mm. This provides useful information on the development of film and of the possibility of ponding.

(a) (b)

Fig. 4.29. (a) Sampling tubes placed in a filter containing granite medium. (b) A sampling basket removed from a filter containing a random plastic filter medium.

Sampling procedure. Removing sampling baskets from their tube obviously causes some disturbance and so they should be left as long as possible (at least a month) between sampling. The sampling procedure should always be the same. The distribution system must be turned off prior to sampling, the baskets removed one at a time, and carefully labelled. The basket should be left for five minutes for excess liquid to drain before a subsample of the medium is taken. Gray and Learner (1983) carefully graded the medium within their sample baskets so that four pieces of medium were equivalent to 250 cm^3 of medium, and this was taken along with its attached film for analysis. The pieces were removed randomly from different points within the basket to give a representative sample and placed in a labelled plastic bag that was sealed to prevent evaporation and loss of material. The remaining medium was carefully replaced in the baskets in the same order in which they had originally been removed, with the medium from the previous sampling taking the place of the sampled pieces. The sampling procedure should not exceed 20 minutes for each filter in order to minimise the damage to the rest of the biota. It is important to carefully replace all the pieces of medium, matching up the disturbed surface film so that only a small quantity of film is dislodged and washed away when the distribution system is switched on again. The sample bag should be checked for leaks, double sealed, and then returned to the laboratory as quickly as possible where it can be weighed to give an estimate of the biomass of film on the medium (Sec. 4.1.4). The addition of preservatives such as formaldehyde or alcohol makes identification of the microfauna, especially the Protozoa, almost impossible, therefore it is advisable to store samples at 4°C until they can be processed further.

Sample processing. The film needs to be removed from the sampled medium within a few days of collection. The procedure used by Gray (1980) is summarised in Fig. 4.30. The sample bag is opened carefully and the pieces of medium removed individually, taking care not to allow any of the animals, including individual flies, to escape. The loose film and associated animals are removed from the medium by gently brushing the surface with a soft artist's paintbrush (red sable, bright) in a shallow dish of water. Any large animals present are removed at this stage, identified, and counted, in order to prevent them from being damaged during the more vigorous washing procedure later on. The piece of medium is then placed into a beaker and stirred vigorously using a magnetic stirrer, and the next piece of medium is removed from the bag and initially brushed clean. The first piece of medium is then removed from the beaker and put into a second dish of water and scrubbed with a short-haired paintbrush (hogs hair, flat), which

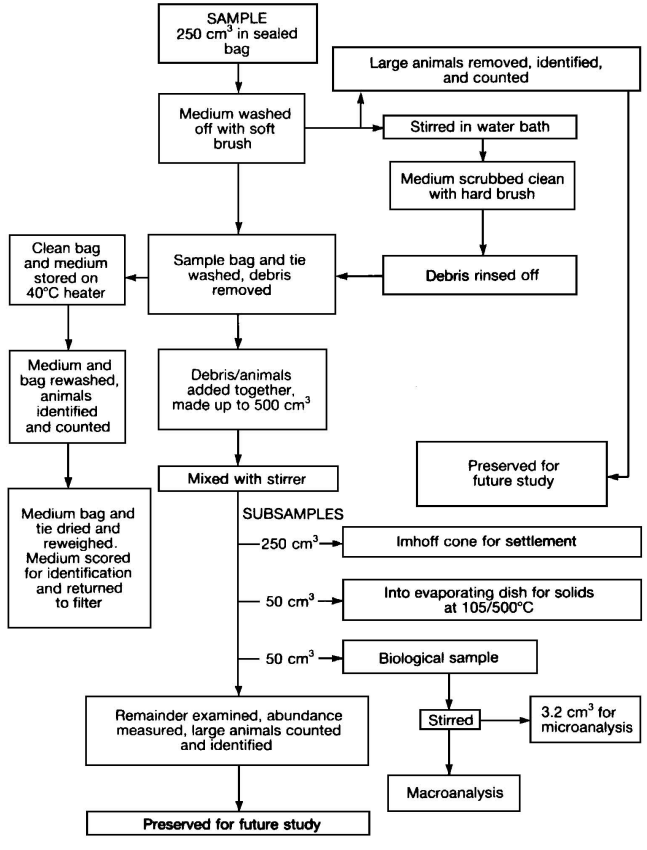


Fig. 4.30. Flow chart illustrating the processing of filter medium and associated film for chemical, physical, and biological analysis.

removes any tenacious film still adhering to the surface. The medium and the sample bag are finally rinsed with clean water to remove any remaining debris and the medium is then resealed in the bag. By standing the bags of freshly cleaned medium on a 40°C heater for about an hour, many animals, mainly enchytraeids and dipteran larvae, not removed from deep within the pores of pitted media such as slag or clinker, are driven to the surface by the heat. Both the bag and the medium are subsequently rewashed and the extra animals collected are identified and counted. The bags of clean medium are finally left to dry completely and then reweighed. The weight of the film is estimated by subtracting the dry weight of the bag and its contents from the wet weight which had been measured immediately after the samples had been returned to the laboratory.

All the debris, solids, and animals removed from the sample of medium collected from each basket are added together and the total volume made up to 500 cm^3 with distilled water. The liquid sample is then mixed thoroughly using a magnetic stirrer and subsamples taken for the various analyses summarised in Fig. 4.30. The subsamples taken for biological analysis are stored in glass bottles at 4°C. The remaining 150 cm^3 left after all the various subsamples are removed, is poured on to a white examination tray so that an assessment of the relative abundance of the various macro-invertebrates can be made. All the animals are identified and the larger ones are counted. Afterwards, as much of the sample as possible should be retained and preserved for future study and reference.

An assessment of the volume of solids present can be made using the Imhoff cone method (DoE 1972). The 250 cm^3 subsample (Fig. 4.30) is allowed to settle for 45 minutes in the Imhoff cone, which is then gently twisted to remove any debris adhering to the glass sides. The quantity of solids settled after one hour is recorded. Although most of the larger invertebrates should be removed prior to settlement, the samples contain large numbers of organisms and these should be included in this assessment of film accumulation.

Micro- and meiofauna analysis. The 50 cm^3 subsample taken for biological analysis (Fig. 4.30) is shaken to produce complete mixing within the container. Then, using a sterile pasteur pipette, a small volume is transferred to a haemacytometer type counting chamber of the Mod-Fuchs Rosenthal type. A total area of 36 mm^2 split up into 0.25 mm^2 squares is examined for each sample under a compound microscope. Three magnifications are generally required, ×100 for counting large micro-organisms such as *Paramecium caudatum*, nematodes and also large bacterial and fungal colonies, and ×200 for counting the other micro-organisms that are normally identified at ×400. By measuring the depth of the sample under the cover slip of the counting chamber using the microscope, the total volume of the sample examined per chamber can be calculated. Details of other counting chambers, staining methods, and separation methods for parasitic ova, cysts, and nematodes are given by Fox *et al.* (1981) and the American Public Health Association *et al.* (1985).

The microfauna can only be identified accurately when alive and this poses a practical problem regarding protozoans in particular, because of their greater mobility. This is partly overcome by the addition of 1% nickel sulphate to the sample, which has a narcotic effect on the protozoans. However, general use of this method should be avoided where possible as

the peritrichs and the suctorians are more easily identified when active. Alternatively, small amounts of 2% xylocaine can be used to slow down the movement of protozoans without significantly affecting their physical form, thus allowing identification. The xylocaine will eventually evaporate and therefore treated samples should be processed reasonably quickly (Norouzian *et al.* 1987).

Problems will arise in the identification of the fungi and filamentous bacteria, and in deciding how many cells or what length of filament constitutes the presence of a countable and reproducible unit. Gray (1983b) set minimum limits in his study. For a fungal hypha, this was 10 cells or 6 cells plus either a growing tip or conidium, and for bacteria only filaments in excess of 0.2 mm in length or complete zoogloeal colonies were counted. The main identification keys available for each major group are summarised in Table 4.20. Furthermore, Fox *et al.* (1981) have produced

Table 4.20. Identification keys to the micro- and macrofauna found in percolating filters and other wastewater treatment units.

Microfauna group	Key references
Bacteria	Farquhar and Boyle 1971a; Eikelboom 1975; Eikelboom and Van Buijsen 1981; Jenkins *et al.* 1984
Fungi	Cooke 1963; Tomlinson and Williams 1975
Algae	Belcher and Swale 1976; George 1976; Bellinger 1980; Sykes 1981
Protozoa	Kudo 1932; Martin 1968; Calaway and Lackey 1962; Bick 1972; Page 1976; Curds 1969, 1982; Curds *et al.* 1983; Foissner and Berger 1996
Nematoda	Tarjan *et al.* 1977
Rotifera	Donner 1966; Ruttner-Kolisko 1972; Pontin 1978
Annelida	Brinkhurst 1971; Nielson and Christensen 1959, 1961, 1963; Gerard 1964; Sperber 1950; Tynen 1966
Insecta	Lawrence 1970; Satchell 1947, 1949; Coe *et al.* 1950; Bryce 1960; Brindle 1962; Mason 1968; Bryce and Hobart 1972; Unwin 1981
Arachnida	Evans *et al.* 1961
Crustacea	Harding and Smith 1974
Mollusca	Janus 1965; Cameron *et al.* 1983
General reference work: Edmondson 1959; Armitage *et al.* 1979; Martin 1968; Macan 1959; Tomlinson 1946a; Armitage *et al.* 1979.	

a beautifully illustrated key, which covers many groups including parasitic helminths, parasitic, and free-living protozoa, algae, and many other meiofauna groups. However, this is not a key in the true sense but rather a collection of photomicrographs of micro-organisms isolated at the sewage treatment plants of the Metropolitan Sanitary District of Greater Chicago, USA. Photographs can be a useful aid to identification and prove invaluable for monitoring the changes in the surface film. By using colour transparencies, the photographs of the surface film growth in the filter can be enlarged so that the extent of film accumulation, dominant species, action of grazers, and effects of surface ponding can be carefully examined and recorded.

Macrofauna analysis. The remainder of the subsample used for the microfaunal analysis is shaken as before and poured into a large Hartley pattern Buchner funnel, and the sample container rinsed out. The sample is gently filtered at low pressure, so as not to damage the annelids, through Whatman 113 (150 mm diameter) filter paper. The filter paper is then cut into four equal sections and examined in a low form plastic dish under a stereo-microscope. The larger invertebrates, such as dipteran larvae and enchytraeid worms can be identified, counted and removed at ×5–10 magnification, whereas the other invertebrates have to be located before identification and counted by a systematic search using fine needles at ×15–20 magnification. Mites have to be identified and counted separately using a 1 cm^2 illuminated background plate that fits under the plastic dish containing the filter paper. This allows only a specific area to be illuminated which can then be carefully searched at ×40 magnification. A total area of 6 cm^2 is searched in this way, the 1 cm^2 areas being chosen at random on the four sections of the filter paper. Apart from the general key by Tomlinson (1946a), which covers only a few of the most common percolating filter grazing organisms and is now taxonomically very out of date, there is no general key available. Therefore, identification must be made using specialised keys (Table 4.20). The number of grazers is expressed as total number per litre or per cubic metre of medium.

Curds and Cockburn (1970a) found that a greater variety of protozoan species were to be found in the effluent from filters than in the film collected just from the surface of the filter. Similarly, Learner (1975a) recorded only 39 invertebrate grazing species in surface media samples (0–30 cm depth) and a further 48 in the final effluent. Therefore, in order to obtain a comprehensive list of species present in the filter and to discover which micro- and macro-organisms are being washed out of the filters, the effluent should be regularly examined. The same methods are used for the effluent as are used for the media. The effluent can be collected as spot 1 litre samples or

preferably by using a drift net with a 400 μm mesh with a collection bottle attached to the end.

In order to assess the rate at which flies emerge from a filter a special emergence trap is required (Solbe *et al.* 1967) (Fig. 4.26). Styles (1979) has developed a much simpler trap of smaller dimensions which is ideal for trapping Chironomidae. It is a rectangular perspex box 280 mm by 140 mm and 70 mm high, with a removable sliding lid that is covered with fly adhesive on the under surface and so acts as the trapping area. Each end of the box is made of nylon mesh to allow ventilation. It was designed specially to be placed in the inter-jet zone between the jets from the distributor and does not incorporate a system of redistributing the wastewater as seen in the larger traps. An accurate assessment of the aerial density of flies over or near the filter can be more easily obtained. A variety of traps have been designed and successfully used (Tomlinson and Stride 1945; Hawkes 1951; Taylor 1951, 1955; Hawkes 1983b). Although traps are by far the most accurate and efficient method of measuring aerial density they are expensive to construct. However, large sticky paper sheets stretched out between supports is simple and fairly accurate, although identification of the smaller species can be difficult because as they struggle, the flies become covered in the adhesive used on the paper. Fly abundance can also be assessed by using a large entomological aspirator to catch as many flies as possible over a unit period of time.

4.1.4. *Factors affecting performance*

A number of factors that affect the performance of percolating filters have already been discussed in some detail, especially the effect of hydraulic and organic loadings. It is important that the wastewater must be amenable to treatment by filtration and have a satisfactory C:N:P ratio for aerobic oxidation (Sec. 1.2). Other important factors include the media, temperature, retention time, depth, film, oxygen, and frequency of dosing, all of which are dealt with below.

Media

Performance is generally related to the specific surface area of the medium provided film accumulation does not result in blocking of the interstices. Therefore, smaller grades of media will produce a better quality final effluent than a larger grade of the same medium, when loaded at the same rate (Table 4.21) (Truesdale *et al.* 1962). The potential for film

Table 4.21. Mean results of analysis over a 12 month period, of influent settled sewage and settled effluents from filters containing different types and sizes of media. Mean rate of application of sewage 0.59 $m^3m^{-3}d^{-1}$. Figures in brackets are the specific surface area (m^2m^{-3}) of bulk medium as determined by the paint dipping test (Truesdale *et al.* 1962).

	Influent	Settled effluent from filter containing							
		25 mm nominal size				63 mm nominal size			
		Clinker (202)	Slag (196)	Rock (142)	Rounded (gravel) (146)	Clinker (123)	Slag (108)	Rock (91)	Rounded gravel (65)
BOD (mg l^{-1})	308	12	10	18	21	18	21	26	28
Ammonia (as N) (mg l^{-1})	61	4.0	1.8	13.1	13.5	13.3	18.6	32.5	40.6
Oxidized nitrogen (as N) (mg l^{-1})	—	52.7	50.7	43.3	44.0	40.1	34.2	24.7	18.3

accumulation is an important selection criterion for media, which is dependent on the size of the interstices, the specific surface area, surface texture of the medium, and the organic loading. Smoother media are slightly less effective than rough-surfaced media, although the latter is more susceptible to excessive accumulation of solids. Performance varies seasonally with the larger grades of media, producing a better effluent in winter, whereas the smaller grades perform best in summer (Hawkes and Jenkins 1955). Therefore, selection of media must be a compromise between having sufficient specific surface area and large enough interstices to prevent clogging in winter. The effects of media type on performance has already been considered in an earlier section (Sec. 4.1.1).

Temperature

Temperature is an important operational parameter in all biological wastewater treatment systems. In percolating filters, the metabolic rate of micro-organisms increases with temperature, with the rate of treatment doubling for every 10°C increase within the range 5–30°C. Also, the diffusivity of nutrients and oxygen increases with temperature and the solubility of oxygen decreases. As the BOD removal efficiency (E) falls with declining temperature (T) the relationship can be expressed as:

$$E_{\mathrm{T}} = E_{20} \cdot A_{\mathrm{T}}{}^{(\mathrm{T}-20)}$$

where E_{T} and E_{20} are the BOD removal efficiencies at T°C and 20°C respectively, and A_{T} is a temperature coefficient with a value between 1.035–1.047 (Roberts 1973; Shriver and Bowers 1975). Film accumulation is affected by temperature by reducing the rate of oxidation (Fig. 4.10) and the activity of grazers, which is severely restricted at temperatures below 10°C (Hawkes 1957; Bayley and Downing 1963; Solbe *et al.* 1974; Shephard and Hawkes 1976). Nitrification is also temperature-dependent with little activity below 10°C (Bruce *et al.* 1967) (Fig. 4.31).

The normal range of domestic wastewater is between 10–20°C, although Gray (1980) recorded a range of 6.5–18.0°C for settled influent wastewater loaded onto his pilot filters (Table 4.22). Thus the design loading for a percolating filter is restricted by the lowest winter temperature. The temperature of the influent wastewater and of the film varies seasonally (Fig. 4.32). Gray (1980) examined the effect of temperature on percolating filter performance in his pilot filters which were built above ground. The concrete walls, although thick enough to offer some insulation, allowed heat transfer between the filter medium and the air outside. Changes in the air

Table 4.22. Summary of the mean performance of three pilot filters, over a range of hydraulic loadings, containing 50 mm blast furnace slag (slag), 50 mm random plastic medium (Flocor RC®) (plastic), and 50 mm blast furnace slag with a surface layer of 50 mm random plastic medium on the surface (mixed).

Loading (duration)	1.68 $m^3m^{-3}d^{-1}$ (13 months)			3.37 $m^3m^{-3}d^{-1}$ (13 months)			5.72 $m^3m^{-3}d^{-1}$ (3 months*)		
Filter	Slag	Mixed	Plastic	Slag	Mixed	Plastic	Slag	Mixed	Plastic
Organic load (kg BOD $m^{-3}d^{-1}$)	0.280	0.280	0.280	0.628	0.628	0.628	0.854	0.854	0.854
Effluent BOD (mg l^{-1})	20.20	20.53	22.54	33.09	25.03	27.12	33.70	26.25	32.00
Percentage removal BOD	87.58	87.73	86.26	82.36	87.18	85.50	68.00	74.50	68.60
Suspended solids load (kg $m^{-3}d^{-1}$)	0.201	0.201	0.201	0.417	0.417	0.417	0.743	0.743	0.743
Effluent suspended solids (mg l^{-1})	24.73	24.72	27.53	31.08	26.32	28.79	71.00	39.50	27.00
Percentage removal suspended solids	77.82	77.67	75.66	72.20	77.00	78.30	41.60	60.10	66.40
Ammonia load (kg $m^{-3}d^{-1}$)	0.054	0.054	0.054	0.075	0.075	0.075	0.114	0.114	0.114
Effluent ammonia (mg l^{-1})	14.71	17.80	20.73	17.98	15.75	19.09	16.90	16.60	16.70
Percentage removal NH_3	52.81	44.97	34.86	21.08	32.42	17.75	7.75	8.45	8.2
Total oxidized nitrogen (mg l^{-1})	14.32	12.09	9.03	6.19	6.95	4.30	1.05	1.15	0.75
Effluent temperature (°C)	8.97	8.78	8.94	10.86	10.64	11.09	—	—	—

*These results were collected over a shorter period during maturation of the filters, and therefore are not directly comparable to the results collected during the other loadings.

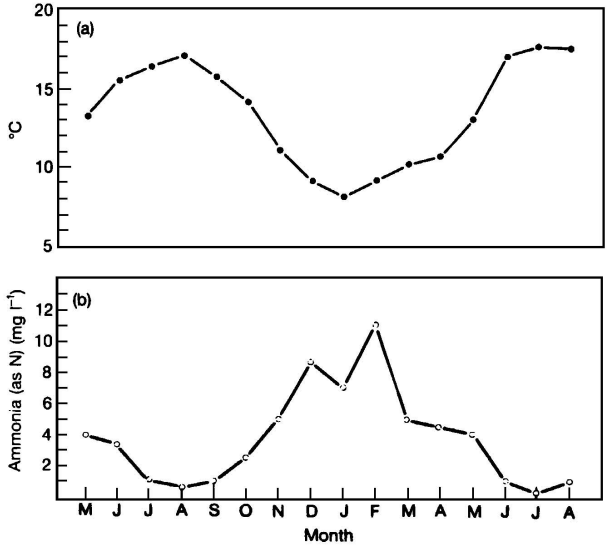


Fig. 4.31. Seasonal variation in the temperature of sewage applied to a percolating filter and the concentration of ammonia remaining in the effluent. Average concentration of ammonia over the whole period was 5.1 mg N l^{-1}. (a) Monthly average temperature of applied sewage; (b) Monthly average concentration of ammonia in filter effluent (Bruce *et al.* 1967).

temperature, wind velocity affecting heat loss from the walls, and the absorption of heat by solar radiation all caused temperature changes to occur in the medium immediately adjacent to the walls within the filters, which were closely recorded by the thermo-couples inside the pilot filters (Gray and Learner 1983). There was a clear diurnal variation in the temperature in the medium immediately adjacent to the wall, getting warmer during the day and cooler at night. The temperature of the central core of the filters remained far more constant. Gray compared 50 mm blast-furnace slag with a random plastic filter medium of the same nominal size (Flocor RC). He found that in fact the core temperature in the slag medium, as measured by the central thermo-couple, remained extremely constant, with small changes in temperature occurring over long periods, i.e. in excess of 6 h, whereas the temperature in the plastic medium changed more rapidly, often by 1°C within 30 minutes. During winter, the temperature gradually increased with depth due to heat produced from microbial activity, although the medium adjacent to the walls of the filters remained cooler. In the summer, the influent retained more of its original heat and the greater microbial activity meant that again the temperature of the influent sewage

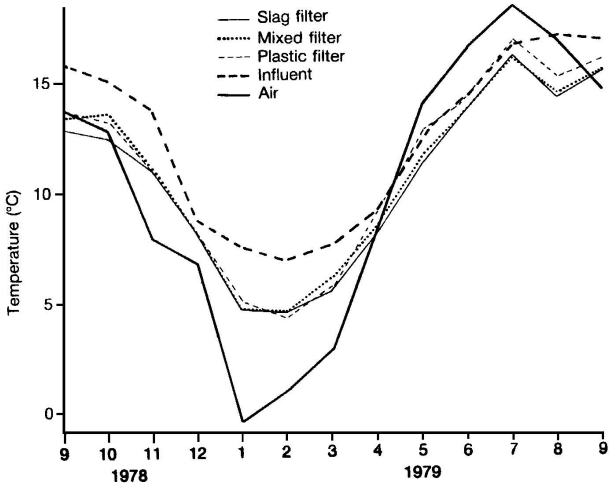


Fig. 4.32. Seasonal changes in influent, final effluents, and air temperatures during high-rate loading of pilot percolating filters 1.8 m deep and loaded at 3.37 $m^3m^{-3}d^{-1}$.

increased with depth but to a greater degree than before. The metabolic rate increases exponentially with temperature and, therefore, greater temperature productions are to be expected during the warmer months. The temperature of the slag filter was closely related at both low- and high-rate operation to the temperature of the influent. The temperature of the influent wastewater was affected by the air temperature (Fig. 4.32), and during extremely cold conditions the influent was also cooled considerably in the fall from the distributor to the surface of the medium.

Although the temperature of the surface layer of the medium was clearly related to the influent temperature, the core temperature of the filter remained constant due to heat production from biological oxidation. The slag filter comprised of 48.5% solid material that was able to retain the heat compared with 8.7% in the plastic filter. Therefore, with more heat-retaining material, less voidage and hence lower potential ventilation than the plastic filter, the slag filter was a far more effective buffer against changes in the air temperature; with the central core of the filter being maintained at a constant temperature by the heat produced by biological oxidation. The temperature of the plastic medium filter was influenced far more by the air temperature than that of the influent because of the greater voidage giving rise to excessive and therefore more heat exchange. The plastic filter was seen to respond quickly to changes in air temperature. This close association resulted in variations in the diurnal temperature of

the film that was not reflected in the seasonal data (Table 4.22). Bayley and Downing (1963) suggested that with high voidage synthetic media the air temperature and rate of flow of air influenced the temperature in the voids, but that the temperature of the influent was still the main factor in maintaining the temperature, and to a lesser extent, the rate of reaction within the microbial film. The temperature of the influent always prevented extremes of temperature within the plastic filter and this was seen clearly at the higher loading. In tall towers containing modular plastic media it is necessary to control the rate of ventilation through the filter, by adjusting the air vents at the base, in order to maintain the temperature, especially on cold windy days, to prevent excessive cooling.

Warm wastewaters are advantageous in reducing seasonal fluctuations in temperature, preventing low temperatures in the winter. In colder climates, filters are covered to conserve heat and to prevent freezing. Gebert and Wilderer (2000) compared two filters at the Ingolstat Wastewater Treatment Works in Germany to study the effects of heating the medium to increase the treatment capacity of filters during cold weather conditions. By embedding heat exchange pipes into the medium of one filter and comparing it to an identical filter operated under normal temperature conditions, they found that the performance of filters can not be constantly improved by heating the medium. They cite the decrease in oxygen solubility in the wastewater and mass transfer limitations caused by an increase in film thickness as the main reasons for no improvement in overall efficiency.

Retention time

The longer intimate contact is maintained between wastewater and the active film supported on the medium of percolating filters, then the better the final effluent quality. Clearly, the duration of liquid retention within the filter is potentially an extremely useful and important parameter (Eckenfelder 1961). However, prediction of the performance of filters using the retention time has proved to be largely unsuccessful. Although retention time is considered a major factor in filter efficiency (Tariq 1975), the exact meaning of such data has remained unclear, resulting in its infrequent use in the assessment of filter efficiency.

The importance of retention time, also referred to as residence or contact time, in the assessment of the efficiency of filters has been stressed from the earliest times (Royal Commission on Sewage Disposal 1908). The theory that increased retention allows more time for the wastewater to be in intimate contact with the film, therefore allowing greater adsorption of

particulate matter and maximum exchange of nutrients, has been widely studied (Eden *et al.* 1964; Craft *et al.* 1972; Craft and Ingols 1973; Cook and Katzberger 1977). Many of these workers found that the retention time was associated with, although not directly related to, performance. Eden *et al.* (1964), when examining the measurement and significance of retention time in filters, found that there was three controlling factors, the hydraulic flow, film accumulation, and the size and shape of the filter medium. The physical characteristics of the medium remain constant, and any variation in retention time of a particular filter will be due to daily changes in the hydraulic flow or seasonal changes in the film accumulation. Increases in hydraulic flows normally result in a decrease in retention time, except in certain random plastic filter medium where an increase in hydraulic loading results in better redistribution of the wastewater within the bed, thus maintaining the retention time (Bruce *et al.* 1975; Porter and Smith 1979). Escritt (1965) found a strong inverse relationship between the hydraulic loading and retention time in a conventional full-scale filter, 1.8 m deep. He was able to calculate the retention time (t_r) in minutes as:

$$t_r = 40 \ \mathrm{In}(9.4/L_v)$$

where L_v is the hydraulic retention time ($m^3m^{-3}d^{-1}$). Generally, the fluctuations in hydraulic flow to filters are small and will only have slight effects on the retention time. The effects of film accumulation on retention time are less clear, and as no direct relationship has been established it would appear that the film modifies the flow pattern of the wastewater at particular accumulations. This was demonstrated by Gray (1983c), who showed that film and humus had a pronounced effect on retention time, but that retention time was not directly related to removal efficiency. Large variations in the retention time had little effect on the organic removal efficiency of low-rate filters, whereas small variations in retention time had large effects on the removal efficiency of high-rate filters. The results of retention tests are commonly expressed as the time (in minutes) required for the recovery of 16% (t_{16}) and 50% (t_{50}) of the added tracer, taken from the plot of percentage tracer recovered against time on logarithmic paper on which a log normal distribution is a straight line. The 16 percentile and the 50 percentile (or median) values are chosen as $(t_{50}/t_{16}) = \sigma$, the standard deviation of the log normal distribution (Eden *et al.* 1964). Gray used t_{50}/t_{16} to predict the flow pattern of wastewater within the filter. He found that when channels are formed in filters because of excessive film accumulation, some of the tracer passed through the filter extremely quickly, causing the

rate of discharge to decrease after a short period producing a ratio (t_{50}/t_{16}) of > 3.125. Under this film condition, and during periods of normal film accumulation, the median retention time (MRT) increased as the film accumulation increased, at a ratio of 3.125 or less. It was during this period that the MRT was directly associated with the performance. When the MRT increased rapidly to a very high value and the ratio became more than 3.125, this indicated that the influent was being retained in reservoirs formed by excessive film conditions. Although in this instance the MRT is longer, it is not associated with increased performance. Because the influent is stored in reservoirs only a very small proportion of the liquid is in intimate contact with the film, thus nutrient transfer does not take place so efficiently. Also, wastewater stored in this way will be low in dissolved oxygen and effectively eliminates oxygen transfer into the film, severely reducing activity. An increase in film accumulation either leads to the production of channels or if the excess film is not sloughed or removed by the grazing fauna, the filter becomes completely blocked. These processes are summarised in Fig. 4.33.

The size, shape, and surface area of the media are also important, with the MRT increasing with the specific surface area of the media (Fig. 4.34). However, as the medium within a filter remains constant, it can be ignored in terms of operation. Work on the periodicity of dosing has shown that retention time can be increased by large intermittent doses of influent (Shephard 1967) due to storage of liquid in horizontal chambers within the filters. These reservoirs are found in filters at times of high accumulations of film where the tracer enters quickly but is slowly discharged as

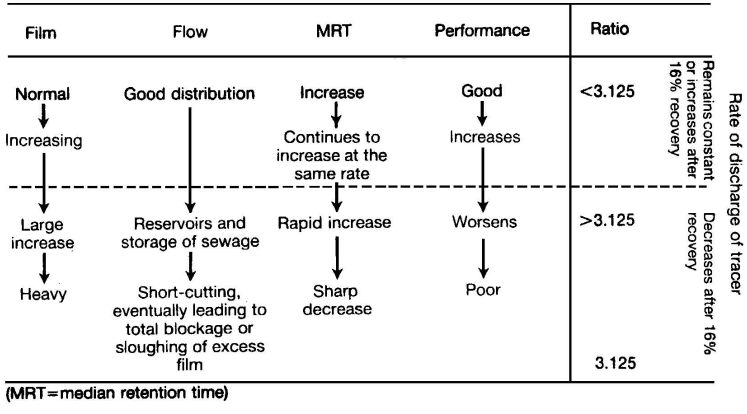


Fig. 4.33. Summary of observed flow characteristics in relation to the ratio of the 16 percentile to the 50 percentile (t_{50}/t_{16}) of the retention time in pilot percolating filters.

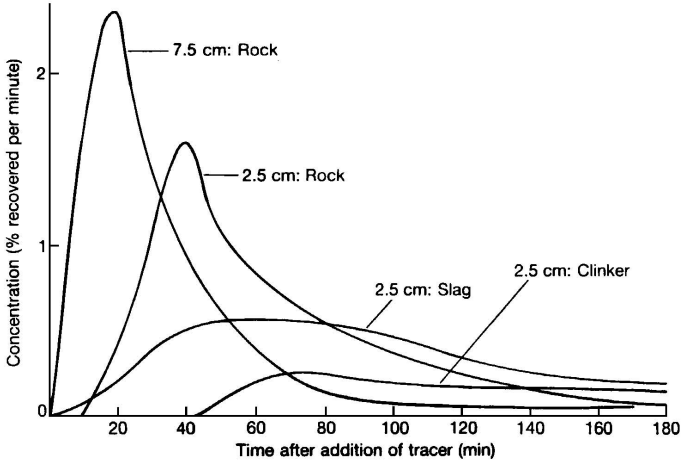


Fig. 4.34. Comparison of the retention characteristics of various clean media (i.e. without any film development) in pilot filters 1.8 m deep and loaded at 0.6 $m^3m^{-3}d^{-1}$ (Eden *et al.* 1964).

more influent wastewater replaces and dilutes it. This explains why the large increase in MRT recorded before the rapid decline in MRT is not directly associated with performance. In contrast, a number of workers consider that retention is probably irrelevant to percolating filter performance (Atkinson *et al.* 1963; Mehta *et al.* 1972).

Tracers can only measure their own retention and not necessarily the retention characteristics of waste liquids (Eden *et al.* 1964). Originally, dyes, salt solutions or ammonium salt were used (Tomlinson and Hall 1950), but these often suffered from prolonged retention due to adsorption on to the biological film. In the late 1950s, however, experiments with radioactive tracers showed them to be ideal on retention analysis. This was because: (a) only small quantities of such tracers are required; (b) of the ease and sensitivity of detection; and (c) of the negligible tendency of some of the radioactive substances to be adsorbed on to the film (Eden and Melbourne 1960). Most of the research at that time on retention analysis has involved the use of radioactive tracers, although Tariq (1975) published a method to calculate the mean time of retention by measuring the influent and drainage rates. A major disadvantage of Tariq's method is that the filter has to be shut down in order to measure the drainage characteristics, thus taking it out of operation for a number of hours, which in a smaller works would prove very difficult. Due to the technical and financial problems of using radioactive isotopes, and also the possible environmental and health aspects

of using them as tracers for the measurement of retention time, Gray (1981) used a simple technique using the traditional tracer sodium chloride and continuously monitored the conductivity of the final effluents to determine its recovery. He found the method to incur negligible cost, extremely quick, and produced reproducible results, making it possible to include the regular measurement of retention time in the routine assessment of filter efficiency.

Depth

Most filters are built between 1.5–2.5 m in depth, with the majority approximately 1.8 m deep. It is traditional in some countries, such as Germany, to build them slightly deeper (3–5 m) but there seems little justification for constructing conventional filters deeper than 2.5 m. Depth does not appear to be a major factor in determining performance, although total available surface area of the media is critical. Therefore, a shallow filter will give the same performance as a deeper filter of the same volume. Bruce and Merkens (1970) found no significant difference in performance between two filters of different depths containing plastic media, one 7.1 m deep the other 2.1 m, treating the same high volumetric loading.

Shallow filters (< 1 m) do have significant disadvantages. The risk of ponding and short circuiting is far more serious in shallow filters. Without the extra depth to compensate, as the depth increases it becomes easier to obtain uniform distribution within the medium. Although shallow filters are structurally cheaper to construct they require more land area and the distribution cost will be increase. In contrast, tall filters take up less area and there is a significant increase in retention time with the greater depth. However, increased pumping costs and increased structural costs make depth selection critical and design engineers should avoid adding an extra 0.5 m or so for good luck.

Gray (1980) studied the performance of low- and high-rate filters at various depths. In single-pass low-rate filters three-quarters of all the available BOD is removed in the top 900 mm of the bed, with between 45–50% removal occurring in the top 300 mm. The removal rate is affected by the film accumulation, with large removals of BOD associated with relatively thin active films. This association continues until the film becomes so thick that it begins to restrict the flow of wastewater through the filter, resulting in a short retention time due to channelling and a subsequent decrease in removal efficiency. At depths > 900 mm, little BOD removal occurs (Table 4.23). More depth is utilised at high-rate loadings, illustrating the need for a greater surface area to oxidise the increased organic load. This is

Table 4.23. The percentage removal of the total BOD, in relation to depth, in three experimental filters containing a variety of different media (Table 4.22) operated under low-rate conditions (1.68 $m^3m^{-3}d^{-1}$) (Gray 1980).

	Slag filter				Mixed filter				Plastic filter			
Depth (mm)	0–300	300–900	900–1500	1500–1800	0–300	300–900	900–1500	1500–1800	0–300	300–900	900–1500	1500–1800
Nov. 77	69.6	16.4	5.7	5.9	58.9	32.5	2.8	0.0	63.1	26.0	0.0	2.8
Dec. 77	30.0	17.1	27.5	4.3	26.9	15.4	29.7	0.0	30.0	27.1	16.3	0.0
Jan. 78	20.6	61.6	12.2	0.0	40.0	46.1	0.0	3.4	47.5	45.4	3.7	1.5
Feb. 78	32.7	21.6	45.6	0.0	43.7	46.1	0.0	4.5	66.9	31.9	0.0	0.8
Mar. 78	40.8	33.8	13.3	6.5	53.2	19.4	22.5	0.3	58.7	22.5	3.4	6.6
Apr. 78	—	—	—	—	—	—	—	—	—	—	—	—
May. 78	59.0	27.9	0.0	2.7	62.0	19.3	14.4	0.0	56.9	24.6	6.9	2.8
Jun. 78	38.8	37.3	0.0	12.4	34.4	50.3	0.6	4.5	68.3	0.4	10.1	3.9
Jul. 78	58.6	25.7	3.7	7.5	57.2	26.8	3.0	6.8	45.5	27.1	19.0	0.0
Aug. 78	73.1	15.9	0.0	1.9	48.1	39.5	3.6	1.9	45.7	37.9	9.9	0.0
Sep. 78	44.2	20.3	17.9	7.9	68.4	6.8	12.3	0.0	42.2	27.3	10.2	8.1
Mean	46.74	27.76	12.59	4.91	49.28	30.22	8.89	2.14	52.48	27.02	7.95	2.65
S.d.	17.56	13.96	14.69	3.91	13.08	14.96	10.47	2.50	12.29	11.62	6.43	2.84

why most high-rate filters using plastic media are > 5 m in height although they rarely exceed 7 m. Higher loading rates will increase the recirculation within filters using random plastic filter media, due to the high specific area of the medium, which reduces the possible increase in depth required. Suspended solids removal occurs mainly in the top 900 mm at both low- and high-rate loadings, and is clearly linked with the adsorption capacity of the film. In the same filters, nitrification was restricted to depths > 900 mm which increased to > 1500 mm, or even excluded altogether from the filter in the high-rate filters. Therefore, a minimum depth of 1 m is required to ensure adequate BOD and suspended solids removal in low-rate filters, although if nitrification is also required a minimum depth of 1.5 m is required. At higher loadings a greater depth is required if enough surface area is to be made available for maximum BOD removal. If random plastic medium is used, sufficient depth can also result in nitrification. Examples of preferred depths of percolating filters in various countries are given by Pike (1978).

Film

Removal of organic matter occurs in four stages: (i) adsorption to the surface of the film; (ii) extracellular breakdown of adsorbed material; (iii) absorption by heterotrophic micro-organisms; and (iv) mineralisation. As the first step in purification is adsorption, any limitation on the availability of adsorption sites will reduce purification. Removal efficiency of both BOD and suspended solids in wastewater is linked to thin actively growing film. The grazing fauna are very important in helping to maintain an actively growing film, thus ensuring maximum rates of adsorption. The rate of adsorption has already been shown to be independent of temperature, whereas the rate of oxidation is temperature-dependent (Fig. 4.10).

The factor that most influences retention time, assuming the hydraulic flow remains constant, is the film accumulation. Eden *et al.* (1964) demonstrated that retention time increased directly with film accumulation up to an optimum weight, after which retention characteristics changed due to excessive film. Heavy weights of film alter the flow pattern resulting in the storage of effluent as localised reservoirs within filters, channelling of the wastewater, and eventual ponding, which results in a loss of performance (Gray 1983c). The film accumulation also follows a seasonal pattern, becoming thicker during winter months due to reduced bioxidation and reduced grazing at the lower temperatures (Gray 1983b) (Fig. 4.9). Therefore, the most important influence on filter efficiency is the volume and growth rate of film, which not only controls the retention time, but also the

rate of adsorption of suspended and dissolved nutrients, the rate of bioxidation, and the sludge production. In operational terms, a knowledge of the film accumulation within a filter is required if maximum performance efficiency is to be maintained (Honda and Matsumoto 1983). Ideally, the quantity and quality of active biomass within the filter should be controlled as in the activated sludge process, which can only be achieved in percolating filters by having a greater fundamental understanding of the kinetics of film growth and accurate methods of determining film accumulation. A number of modifications of the percolating filter process have been developed and some examples are random and modular plastics media, recirculation, alternating double filtration (ADF), control of the frequency of dosing, and modifications to the distribution system. One major advantage of these modifications is they attempt to achieve optimal film growth. However, even with such modifications, it is desirable to have some indication of the film accumulation within the filter, especially as depth studies have shown that the surface film accumulation rarely resembles the film accumulated within the filter (Gray 1983b). With modifications such as ADF and recirculation, the stage of film development is an important factor in deciding whether the filter series should be changed or the ratio of returned effluent modified.

Although a number of possible methods of estimating the degree of film accumulation are available, for example, the adsorption of dyes (Smith and Coackley 1983), or comparative retention time analysis, the relationship between these parameters and film weight is far from clear. The information obtained in this way is also rather imprecise, with one value obtained for the condition of the entire filter but no estimation of film accumulation at specific depths or areas of the filter being possible. Two approaches to film measurement are generally used at present. Direct measurement using sampling tubes containing baskets of medium which can be removed and film estimated by a number of gravimetric methods, or an indirect assessment method where the film is measured within the filter without disturbing the medium using a neutron modulation technique.

The film is comprised mainly of water between 92–97% in plastic media filters and 95–98% in mineral media filters (Gray 1980). This allows film accumulation to be estimated in filters by measuring the abundance of hydrogen atoms present in the water molecules using neutron modulation. Fast neutrons emitted from a radioactive isotope (beryllium-americium) form a cloud of slow neutrons on collision with hydrogen atoms. By using a slow neutron detector, the hydrogen atoms in any water present in the filter can be measured (Bell 1973) (Fig. 4.35). There are several commercial

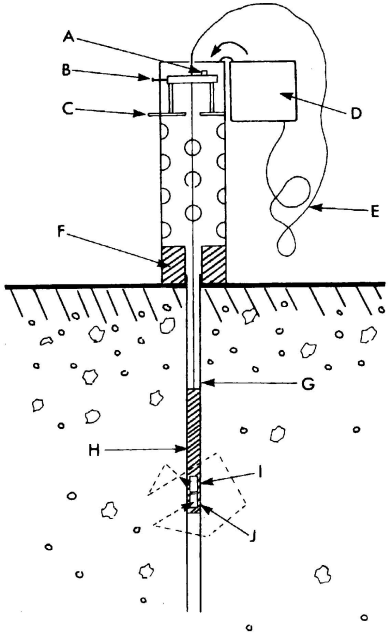


Fig. 4.35. Diagram showing the neutron probe in use. A, depth indicator; B, cable clamp; C, lock; D, rate meter; E, cable; F, plastic shield containing isotope; G, filter access tube; H, probe; I, slow neutron detector; J, neutron source.

neutron probes available, although the most widely used probe in wastewater studies is the Wallingford Soil moisture probe (manufactured by D. A. Pitman Ltd, Weybridge, Surrey). The probe is lowered into the filter via a 50 mm diameter aluminium access tube, which is sealed when not in use to keep it perfectly dry. The probe has to be calibrated for each medium tested using a 210 litre drum containing the medium over a range of moisture levels. Specific and significantly different calibration curves are obtained for each medium used, with the difference between the slope of the calibration curves for different media because of absorption of thermal neutrons by the media (Harvey *et al.* 1963). Therefore, the appropriate calibration curve must be used when converting the probe readings into percentage saturation of the voids. The use of neutron modulation in the estimation of film accumulation has been fully described by Harvey *et al.* (1963) and Gray (1984c).

Gray and Learner (1984b) compared direct gravimetric measurements of film weight with the neutron scattering technique. Four gravimetric methods of film accumulation were used, total film weight, total dry solids,

volatile solids, and percentage settlement of solids, which incorporated both weight and volume measurements. Total film was the weight increase of the sampling basket and the medium due to accumulated film. The wet weight of the clean medium in each sampling basket was measured at the start of the investigation. Once a month, each basket was removed from the sampling tube, excess liquid drained off for 5 minutes and then weighed, the increase in weight being due to film growth. Total dry solids were measured by evaporating a 50 cm^3 subsample of film, removed from a 1 litre sample of filter medium, in a weighed evaporating dish, to dryness at 105°C for 24 hours (DoE 1972). The dish was reweighed after cooling to room temperature. The quantity of volatile solids was determined by removing the organic fraction of the dried sample by burning in a muffle furnace at 500°C for 1 hour and then reweighing the dish after cooling to room temperature (Allen 1974). An assessment of the volume of solids present was made using the Imhoff cone method (DoE 1972). A 250 cm^3 subsample of the removed film was allowed to settle for 45 minutes in the Imhoff cone, which was then gently twisted to remove any debris adhering to the glass sides. The quantity of solids which settled after 1 hour was recorded as the percentage settlement. Although any large lumbricid worms were removed prior to settlement, all the samples contained large numbers of invertebrates and these were included in this assessment of film accumulation. All the gravimetric estimations were made at monthly intervals at six depths within each filter corresponding with the depths at which the film was estimated by the neutron scattering technique. Before neutron scattering can be used, all the excess liquid has to be drained out of the filters once the distribution system has been shut off. Readings were taken at 150 mm intervals throughout the depth of the filters, and each value was a mean of five readings, of which each was a mean count per second integrated over a 16 second sample period. Replicate access tubes were used to allow the film accumulation to be studied from either side of each filter and the mean of the two average readings at each depth was taken to represent the mean percentage saturation of the voids. Neutron probe readings were always taken within 24 hours of the gravimetric sampling. The results of the comparative study by Gray and Learner (Table 4.24) showed good correlations ($P < 0.001$) between all the methods tested in low-rate conditions (1.68 $m^3m^{-3}d^{-1}$ and 0.28 kg BOD $m^{-3}d^{-1}$), although the neutron scattering results were not significantly correlated ($P > 0.05$) with any of the gravimetric methods at the higher loading (3.37 $m^3m^{-3}d^{-1}$ and 0.63 kg BOD $m^{-3}d^{-1}$). They concluded that although neutron scattering provided a rapid and sensitive measure of hydrogen atoms in the filter, the results expressed as percentage

Table 4.24. Summary of the range of film accumulations measured by the neutron scattering technique (NP), and also as total film (TF), volatile solids (VS), total solids (TS), and percentage settlement (PS) in mineral and plastics media at two different loading rates (Gray and Learner 1984b).

	Mineral medium					Plastics medium				
	NP (% sat)	TF	VS	TS	PS (%)	NP (% sat)	TF	VS	TS	PS (%)
		(kg m^{-3})					(kg m^{-3})			
					Low loading rate (1.68 $m^3m^{-3}d^{-1}$)					
Mean	23.0	257	7.25	10.08	16.0	3.5	104	5.01	6.60	11.0
Minimum	11.3	96	1.04	1.48	2.4	1.1	28	0.36	0.44	0.6
Maximum	39.0	616	23.08	31.44	60.0	15.6	315	16.44	21.28	37.2
Range	27.7	520	22.04	29.96	57.6	14.5	287	16.08	20.84	36.6
Skewness	0.09	0.75	0.99	0.96	1.37	2.22	0.72	0.69	0.60	0.69
n	66	54	66	66	66	66	54	66	66	66
					High loading rate (3.37 $m^3m^{-3}d^{-1}$)					
Mean	26.5	216	5.91	7.68	11.1	4.5	173	8.34	10.91	18.8
Minimum	16.9	83	1.32	1.88	2.2	1.3	34	1.20	1.36	1.4
Maximum	40.2	494	23.68	32.56	48.0	12.2	474	25.08	33.92	78.4
Range	23.3	411	22.36	30.68	45.8	10.9	440	23.88	32.56	77.0
Skewness	0.25	1.31	2.22	2.37	1.85	1.06	0.91	1.20	1.28	1.62
n	12	72	72	72	72	12	72	72	72	72

saturation of the voids are not directly transferable to film weights and should be treated separately, not as a true measure of film accumulation. Neither could the technique provide qualitative information about the film that was obtained by visually inspecting the exposed cores of film covered medium. It does, however, clearly indicate to the operator areas of high or low water saturation. It therefore appears to be ideal for use in the routine operational control of filter modifications, such as ADF and recirculation, and also in predicting the onset of ponding, especially below the surface of the medium. However, accurate measurement of film weight and a detailed analysis of the condition and composition of the film is only possible by using gravimetric analyses.

Film accumulation data is therefore useful in indicating where biomass is building up within the filter and can inform the operator if potential ponding problems exist, if the filter is being over or underloaded, or if film development is inhibited. There are a number of ways of visually interpreting such data. Traditionally, kite diagrams have been used to illustrate film accumulation at various depths, with a separate kite plot for each month or sampling period (Fig. 4.36). A development of this is the horizontal histogram, which, when turned on its side, gives a three-dimensional view of the changes over the sampling period of vertical film accumulation within the filter bed (Fig. 4.23). More recently, contour mapping of film data allows

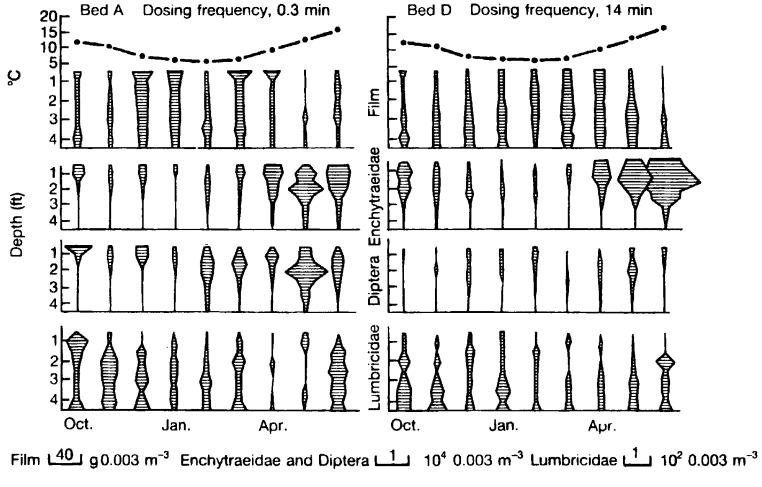


Fig. 4.36. Kite diagram showing seasonal changes in the abundance and vertical distribution of film and grazing fauna in high-frequency dosed filter A and low-frequency dosed filter D (Hawkes and Shephard 1972).

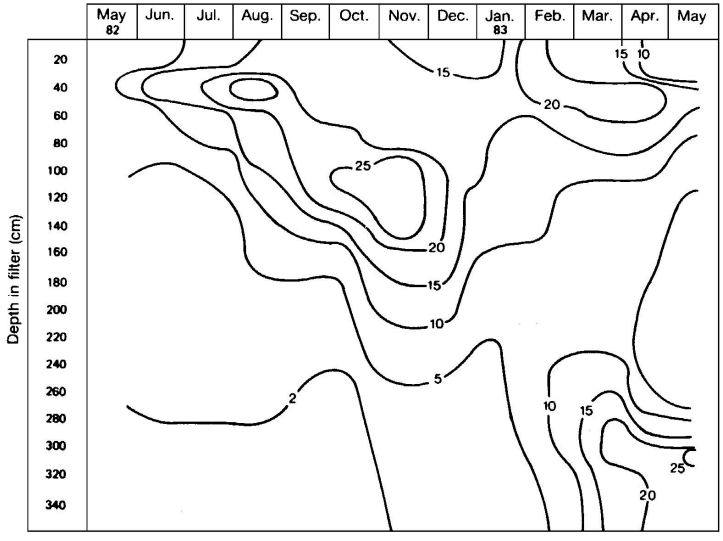


Fig. 4.37. Contour mapping of the film accumulation (as percentage saturation of the voidage) in a random plastic filter medium (Flocor RC®) receiving finely screened sewage (Hoyland and Roland 1984).

film concentration contours to be plotted with depth and time being the only axes. This approach allows interpolation between the available data points, normally by simple linear regression. By selecting the number of concentration contour levels to be plotted, either simple or very detailed views of seasonal film accumulation within the filter can be obtained (Fig. 4.37).

Ventilation

Oxygen is supplied to the aerobic micro-organisms comprising the film almost exclusively by absorption from air as it passes through the filter by natural ventilation. When ventilation ports are blocked or partially restricted, the performance of the filter is seriously affected; therefore good ventilation is vital for the successful operation of percolating filters. Ventilation currents are caused by the temperature difference between the air outside and the liquid and film inside the filter, and the resultant flow of air may be in either direction. In the winter, the air entering the filter will be warmed by the wastewater passing through the bed causing a net upward flow while in the summer the opposite may occur with the air possibly being cooled in the filter resulting in a downward flow of air. The rate of flow of air through the filter is directly proportional to the difference in the

temperature between the outside air and the wastewater, with a difference of 10°C inducing a rate of flow of air of about 20 $m^3m^{-2}d^{-1}$. Other factors such as wind currents (Mitchell and Eden 1963) and humidity, also affect ventilation. The humidity of the air inside the filter will increase as it passes through, and as an increase in humidity reduces the density of air, this encourages an upward flow. While the oxygen concentration of air in the interstices within the filter remains very close to that of normal air (20.9% v/v), reduction of the oxygen concentration is possible during periods of high carbonaceous oxidation. It is unlikely that normal heterotrophic activity will be affected by low oxygen concentrations, with no reduction in BOD removal at oxygen concentrations of 2% v/v, although nitrification is far more sensitive to oxygen limitation (Department of Scientific and Industrial Research 1956).

The rate of oxygen uptake in percolating filters varies from 0.273 kg $m^{-3}d^{-1}$ in low-rate filters, where 0.187 kg m^3d^{-1} is required for heterotrophic oxidation and 0.086 kg $m^{-3}d^{-1}$ for nitrification (Montgomery and Borne 1966), to 1.490 kg $m^{-3}d^{-1}$ for high-rate filters (Bruce and Merkens 1970). The rate of oxygen utilisation depends on the specific surface area of the media on which the film develops, thus, for a medium with a specific surface area of 200 m^2m^{-3} the potential uptake rate of oxygen could be as high as 1.900 kg $m^{-3}d^{-1}$. The rate of oxygen utilisation is fairly uniform throughout low-rate filters with demand from heterotrophs in the upper layer declining with depth, and the demand from nitrifiers increasing with depth. The rates at which oxygen and organic nutrients diffuse into the film depends on their relative concentrations in the liquid phase covering the film. For example, when the nutrient concentration is high then the concentration gradient is large, causing more rapid diffusion to a much greater depth. In fact, the depth of penetration into the film is approximately proportional to the substrate concentration (Atkinson and Fowler 1974). The depth of penetration of organic nutrients into the film doubled from 0.06 to 0.15 mm when the substrate concentration was increased from 10 to 500 g COD m^{-3}. In practice, the maximum substrate concentration is found in the upper 900 mm of filters, where the maximum film accumulation is also recorded. The rate of diffusion in this area of the filter may exceed the rate at which oxygen can diffuse into the film for aerobic metabolism, resulting in incomplete oxidation. Therefore, in the top section of the filter, the oxidation rate will be oxygen-limited, whereas with depth, the rate of oxidation will decrease due to a reduction in the substrate concentration. At lower substrate concentrations, the organic nutrients can only penetrate a short distance into the film before being consumed so here the rate of

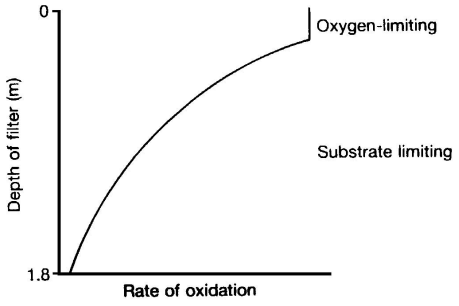


Fig. 4.38. Rate of oxidation in relation to the depth of a percolating filter indicating where oxygen and substrate concentration are major limiting factors.

oxidation is substrate limited (Fig. 4.38). The remaining thickness of film is important as it acts as a buffer against environmental and operational changes. For example, if the temperature fell, the nutrients would penetrate further into the film before being consumed thereby using more of the microbial population. This has been modelled by Harris and Hansford (1976) who suggested that oxygen limitation occurs when the substrate concentration exceeded 400 g COD m^{-3}. Williamson and McCarty (1976) have also modelled this phenomenon and suggested that oxygen becomes the limiting factor in film when:

$$CK_c < SK_s$$

where C and S are the concentrations of oxygen and BOD in the film respectively, and K_c and K_s their respective saturation coefficients.

Numerous methods have been employed to improve ventilation including leaving gaps between stonework, using special air bricks, providing ventilation tubes through the medium, and even using fans and pumps to force air through the bed. However, it appears that such systems are unnecessary as normal ventilation systems using air vents attached to the under drains will provide adequate amounts of oxygen for biological activity unless the voids are excessively clogged with excessive film accumulation.

Normally odour is not a problem associated with low-rate filtration so long as the plant is in good working order. Occasionally, when the air and influent temperature are the same, especially when there is little air movement, ventilation may be suppressed for short periods resulting in stale air being produced when air movement recommences. Likewise, when the filter is shut down for any length of time during warm weather, odours will result due to loss of aerobic conditions within the bed. Odours can

be produced when: (i) the filter treats septic wastewater, (ii) the filter treats industrial wastewater containing high concentrations of sulphate, and (iii) the wastewater contains a significant portion of seawater. In all three cases hydrogen sulphides and other associated malodours may be produced. If the filter ponds or becomes choked with film so that insufficient ventilation results in anaerobic areas occurring, malodours will result.

In contrast, high-rate filters can cause odour problems as they act as very efficient vapour strippers, especially of ammonia. This is a particular problem with industrial installations, such as pharmaceutical factories, where solvents are widely employed in the manufacturing process. To control this, industrial high-rate filters are normally sealed, requiring a forced ventilation system to be used. This has the advantage that the temperature within the filter and the oxygen availability can be controlled to ensure maximum oxidation rates. Slow air movement through deep filters operating at high loading rates may also lead to odours and loss of performance, requiring the use of forced ventilation systems (Suschka 1987). Some odour production is inevitable from high-rate filters due to the nature of the plastic medium. The film rapidly develops and regularly sloughs away from the surface of the smooth medium, usually when the inner layer of the film becomes anaerobic. This results in the release of malodours everytime sloughing occurs.

Frequency of dosing

Influent wastewater is distributed on to the surface of percolating filters via sprayers or nozzles mounted on either a moving distributor or a system of static distributor pipes. Although the same loading rate is applied to both types of filters, the former distributor arrangement doses each specific area intermittently whereas the latter doses the entire surface of the filter continuously. It is general practice to use moving distributors on all filters, except very small installations where a fixed system of distributor pipes is used and intermittent dosing is achieved by using balancing tanks that are emptied at the required interval, or tipping-trough systems. Filters containing plastic media that have a high voidage are generally loaded continuously from a fixed distribution system as this gives better distribution of wastewater within the filter. Under low-rate conditions the best performance is obtained using large doses at infrequent intervals, i.e. low speeds of rotation. In this way, each segment of the filter bed is subjected to a surge of liquid followed by a long period without any flow before the next dose. The slower the speed of rotation, the greater the surge of wastewater and

the longer the intervening period between doses. In theory, the long period between dosing ensures that the micro-organisms comprising the film are in a nutrient-limited condition so that they are in the endogenous respiration growth phase, i.e. a low f/m condition, which limits film accumulation. This has been found to be especially effective in preventing fungal growths in the winter. The surge of wastewater ensures a more uniform distribution of organic nutrients within the bed, extending the depth of heterotrophic activity and encouraging a more even distribution of film. The mechanical scouring effect also limits film development, but this is of minor significance compared to the limitation of nutrients. By lowering the frequency of dosing, the retention time is also reduced. Therefore, dosing frequency must be a compromise between maximising the retention time so that a sufficient wastewater-film contact time is maintained in order that maximum adsorption of organic nutrients can occur, and excessive film accumulation controlled. High frequency dosing approaches the conditions associated with continuous dosing resulting in a large accumulation of microbial film at the surface. High frequency dosing is ideal for random plastic medium or media with a high voidage that is able to support a high film accumulation in the top 900 mm. It is also suitable for mineral media filters that have been uprated by replacing the surface layer of medium with a random plastic medium (Gray and Learner 1984b).

The optimum frequency of dosing for low-rate filters is between 15–30 minute intervals (Tomlinson and Hall 1950). This prevents ponding in the winter, reduces the population of the fly *Sylvicola fenestralis* emerging during the warmer months, and encourages the enchytraeid worms to become the dominant grazer replacing the dipteran larvae (Hawkes 1955). However, the optimum frequency of dosing for a specific filter varies with the operating conditions, the type of medium, and the nature of the wastewater. The optimum frequency of dosing will also change seasonally, being much lower in the winter in order to control excessive film accumulation, compared to the summer when other factors control the film (Bruce and Hawkes 1983).

To control the frequency of dosing, the rotating arms need to be propelled using a motor driven rotor or a peripheral wheel driven by the wastewater flow using a water-wheel arrangement. Although the speed of rotary arms driven by the reaction of the spray jets alone can be controlled to a certain extent by altering the angle of the jets, reaction driven rotation requires a minimum head of 0.5–1.0 m and is severely affected by changes in wind direction. Some form of mechanical drive is required therefore, for accurate periodic dosing of filters.

4.1.5. *Nitrifying filters*

Because of design or loading, many biological treatment processes are able to complete the carbonaceous oxidation of wastewater but are unable to successfully oxidise the ammonia-nitrogen (NH_3-N) present. In the activated sludge process, failure to provide full nitrification of wastewater may be due to inadequate retention time or aeration capacity. In percolating filtration, modifications such as ADF, insufficient surface area, or high organic loadings may result in incomplete oxidation of ammonia to nitrate. Ammonia-nitrogen is very toxic and many discharge licences will include an upper limit for ammonia being discharged to fresh waters. Therefore, a separate purification stage just to remove ammonia is often required to meet such standards.

The sole purpose of nitrifying filters is to remove ammonia from effluents that have undergone complete carbonaceous oxidation. It is based on the stratification of microbial species in conventional low-rate filters, where nitrifying bacteria are located towards the base of the bed. In this way, separate filters were developed for carbonaceous oxidation and nitrification, although nitrifying filters can be used to nitrify the effluent from other types of treatment units. Nitrifying filters are very similar to percolating filters in design except smaller grades of media are used ($<$ 25 mm) in order to maximise the specific surface area (Lekang and Kleppe 2000). The use of smaller grades of media is made possible as the wastewater contains very little organic matter and only minimal development of heterotrophic film can occur. Therefore, excessive film accumulation that could cause clogging does not occur. The nitrifying bacteria themselves are very slow-growing and produce only a small volume of biomass in comparison with heterotrophs. For example the cell yield of *Nitrosomonas* spp. is 0.053 g per g NH_3–N oxidised. The film accumulation is also restricted by high hydraulic loadings of between 2–6 $m^3m^{-3}d^{-1}$. Wastewater is distributed on to the filter via a fixed distribution system using fine spray nozzles to ensure maximum utilisation of the media and adequate oxygen. Using spray nozzles also allows maximum loss of ammonia by volatisation. Removal rates of ammonia-nitrogen can rise to 120 g $m^{-3}d^{-1}$, although this depends on the specific surface area of the media, so removal rates of ammonia-nitrogen per unit surface area are in the order of 500–1000 mg $m^{-2}d^{-1}$ (Barnes and Bliss 1983). Wik (2000) has studied strategies to improve the efficiency of nitrifying filters. The nitrification process has been fully examined in Sec. 3.5.2.

4.2. Rotating Biological Contactors

Rotating biological contactors (RBC) became commercially available in 1965, although the system was first developed in the late 1920s (Doman 1929). They are now widely used throughout the world and although particularly well suited for treating wastewater from small communities, they are now also being used to treat large domestic and industrial loads. RBCs can be operated under aerobic, anoxic, or anaerobic conditions and can be used for a wide variety of applications. However, in practice, they are almost exclusively used for aerobic secondary treatment.

RBCs are widely available as packaged plants under a variety of commercial names such as Biosurf, Biodisc, Biodrum, and Biospiral. The basic design consists of a series of flat or corrugated discs 2–3 m in diameter mounted on a horizontal shaft and driven mechanically so that the discs rotate at right angles to the flow of settled wastewater (Fig. 4.39). The discs are usually plastic (MDPE), corrugated polythene, PVC, GRP, or expanded polystyrene, although other materials such as asbestos cement and expanded metal are also used. The surface area of the medium is normally between 150–200 m^2m^{-3} with the higher surface area medium used at the outlet end for nitrification. Each disc is 10–20 mm thick and spaced about 20 mm apart (Fig. 4.40). The mounted discs are placed in a contoured tank that fits fairly closely to the rotating medium so that

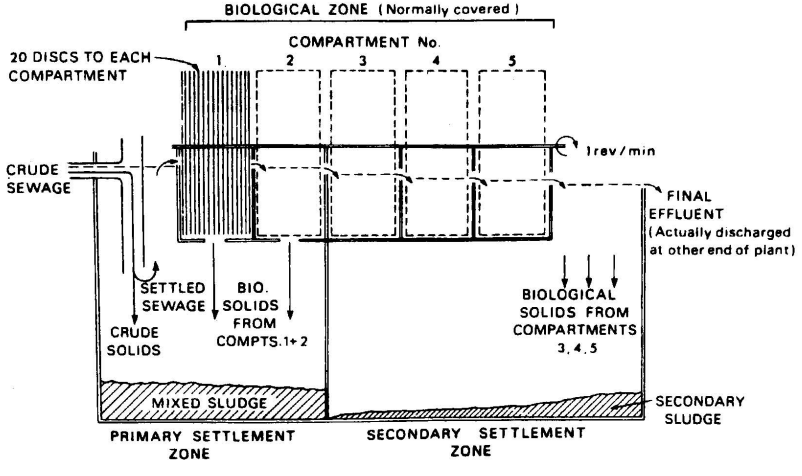


Fig. 4.39. A rotating biological contactor (Mann 1979).

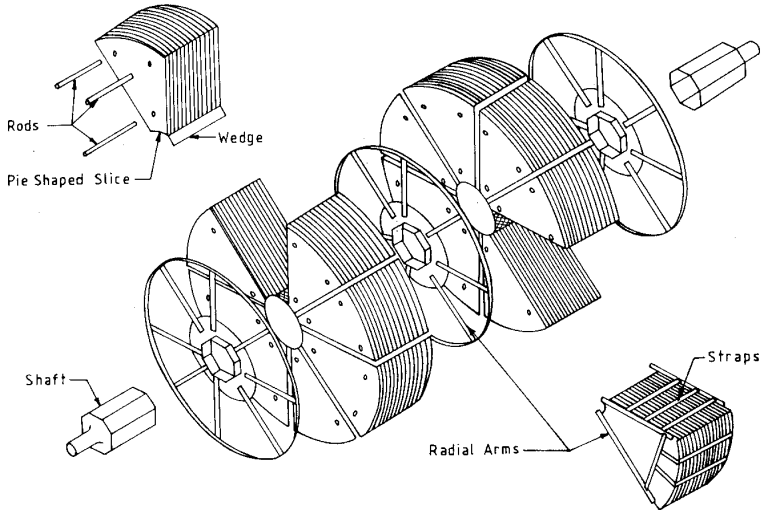


Fig. 4.40. Schematic view of disc assembly in a rotating biological contactor showing support rods that ensure exact spacing between discs (IWEM 1988).

35–40% of their area is immersed, and are slowly but continuously rotated. At immersion depths > 40%, supplementary air may be required to maintain aerobic conditions. Discs are usually but not exclusively used as support media. Other configurations, such as lattice constructions or wire mesh containers filled with random plastic media have also been used successfully. The flow of wastewater through the tank and the action of the rotating medium produces a high hydraulic shear on the film that ensures efficient mass transfer from the liquid into the film and, at the same time, controls excessive film accumulation. Discs are arranged in groups separated by baffles to minimise short circuiting and the effect of surges of flow. Normally, a minimum of four compartments, separated by baffles, are used to simulate plug flow with a small head loss of between 10–20 mm between each compartment. With large installations, plug-flow conditions are achieved by having several complete RBC units in series (Steels 1974). The system consumes very little energy as the lightweight plastic media is evenly balanced throughout the drive shaft and a small motor is used to drive the rotor, which results in a low power consumption for the amount of BOD removed. For example, in a 300 PE unit, the power consumption of the motor is only 0.3 kW. RBC units are invariably covered, with small units covered with a moulded plastic or GRP cover and larger units housed in buildings. Covering the media is important in order to protect the exposed

film on the discs from the weather, especially frost and wind which can damage the film. Wind can also increase the load on the motor driving the rotor. Covering insulates the system and reduces heat loss and increases the rate of oxidation. By insulating the cover such units can operate successfully even in arctic conditions. The cover also eliminates fly nuisance and even controls odours to some extent. Using green covers makes such plants inconspicuous, especially as they have the same head loss as a septic tank (< 100 mm), and therefore are low-form structures. They are ideal for use in open areas where landscape quality is important, for example, golf clubs, hotels, and general amenity areas.

As the discs rotate, wastewater enters the spaces between the discs when immersed and is then replaced with air when out of the liquid. In this way, a biological film builds up on the discs in the same way as it develops on the medium in a percolating filter. Therefore, purification occurs with the film alternately adsorbing organic nutrients from the wastewater and then obtaining oxygen from the atmosphere for oxidation.

Separation of the discs varies between 15–30 mm which is a compromise between reducing the spacing to maximize surface area per metre length of rotor, while preventing film from bridging the gap between discs so reducing the effective surface area and leading to low dissolved oxygen within the film. This is especially a problem when shearing velocities are too low to dislodge excess film. A survey conducted on 114 RBCs by Severn-Trent Water showed film thickness to vary between 0.5–4.5 mm with significantly thicker films developing on inlet discs compared to those at the outlet (Fig. 4.41) (Griffin and Findlay 2000). Allowing a minimum spacing of 15 mm for

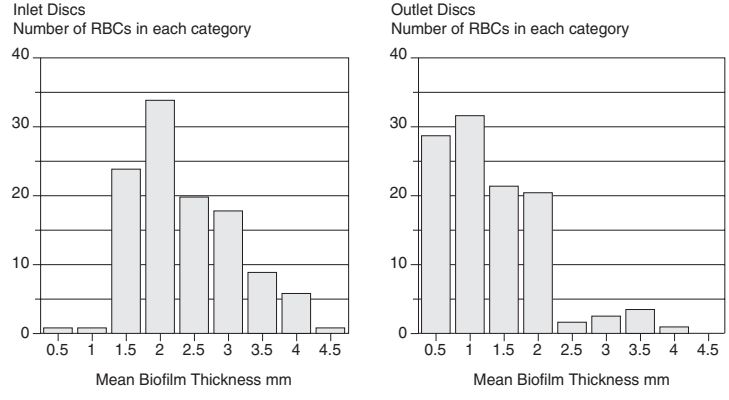


Fig. 4.41. Mean film thickness on the inlet and outlet discs at 114 rotating biological contactor sites (Griffin and Finday 2000).

water to pass between discs and 5 mm or 3 mm for maximum permissible film development on the inlet and outlet discs respectively, they used 25 and 21 mm for inlet and outlet disc separation distances respectively. The thickness of the film is managed by preventing organic overloading. Using these spacings the approximate surface area for a 1.0 m diameter disc unit is 50–80 m^2m^{-1} rotor length rising to 700 m^2m^{-1} for a 3.0 m diameter disc unit The average depth of disc immersion was 40%.

After the biological phase, the wastewater passes through a settlement tank where any solids are removed before discharge.

The film that develops on the discs is a complex and diverse community of bacteria, filamentous bacteria, protozoa, and metazoa. There is a gradation of microbial species along the reactor path with the first discs developing a heavy growth of heterotrophic bacteria and fungi. Filamentous bacteria, especially *Sphaerotilus natans*, *Nocardia*, and *Beggiatoa* are commonly recorded (Hildlebaugh and Miller 1981; Kinner *et al.* 1983). While both are indicative of heavy organic loading, *Beggiatoa* oxidises reduced sulphur compounds. So while its presence at the inlet is normal, if it extends through several stages or becomes excessively luxuriant, then this indicates overloading. The bacterium forms a smooth but dense white layer over the existing biological film and can become quite thick. It contrasts dramatically to the normal thin chocolate-brown film that is normally found on the majority of discs and so the extensive white growth often causes concern to operators when discs are periodically inspected. *Beggiatoa* out competes the normal heterotrophic non-filamentous bacteria for both space and oxygen resulting in very heavy film growths, which adds to the mechanical stress on the RBC shaft and bearings, as well as significantly reducing the BOD removal efficiency. Below the *Beggiatoa* film is a black layer, the discolouration being caused by ferrous sulphide precipitation, that is rich in *Desulfovibrio* spp. which are sulphate-reducing bacteria. There is an interesting relationship between *Beggiatoa* and *Desulfovibrio* (Fig. 4.42). In the inner anaerobic layer, fermentative bacteria provide the end products used by sulphate reducing bacteria (e.g. organic acids, alcohols). *Beggiatoa* in the outer aerobic zone uses the hydrogen sulphide, produced by the sulphate reducing bacteria in the lower anaerobic zone, as an electron donor oxidising it to elemental sulphur (Alleman *et al.* 1982).

A succession of protozoan species are seen on successive discs following the sapropic rating of species (Liebmann 1951). These can be used as indicators of the state of organic loading and RBC effluent quality (Kinner *et al.* 1988). This succession is similar to that seen in the activated sludge process (Kinner and Curds 1989):

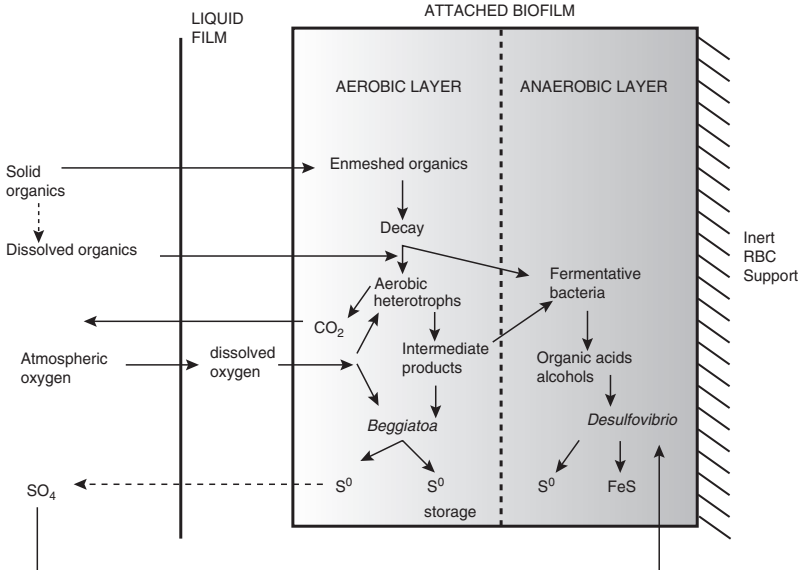


Fig. 4.42. Relationship between the bacteria *Beggiatoa* and *Desulfovibrio* in the film of rotating biological contactors (Bitton 1999).

Bacteria colonisation → Flagellates → Amoebae → Free swimming bacteriovorous ciliates (e.g. *Colpidium* spp.) → Nematodes → Stalked ciliates (e.g. *Vorticella* spp.) → Rotifers.

Depending on the organic loading, nitrification can occur on the first group of discs, although nitrifying activity is usually limited. Like percolating filters, nitrifying bacteria in RBCs are found towards the end of the system on the discs in the third and fourth compartments of a four-compartment unit. Due to the plug flow nature of the system there is a tendency for nitrite to accumulate in the compartment where nitrification first occurs. There is a net loss of nitrogen through the system which approaches 20% because of denitrification at the media-film interface (Ellis and Banaga 1976). Some designs incorporate recirculation of final effluent back to the first stage. The idea behind this is to supply combined oxygen in the heavily loaded first stage to prevent septicity and to allow denitrification. The nitrogen oxidation rate is similar to that found in filters at approximately 1 g NH_3-N $m^{-2}d^{-1}$ at 20°C (Antonie 1976). Major variables affecting nitrification include total disc area, wastewater temperature, influent nitrogen, and BOD concentrations and flow rate (Barnes and Bliss 1983). For example, based on the Van't Hoff-Arrhenius relationship,

nitrification at 5°C may require 2.5 times more medium surface area than at 13°C. The speed of rotation is limited by the shearing of the film at the periphery of the discs, but the normal range is between 0.5–10 rpm, typically 0.5–1.5 rpm depending on the diameter of the disc. Film is sloughed off continuously and will remain in the liquid phase adding to the overall treatment process. Eventually, sloughed film is carried away to a separate sedimentation tank although in smaller units the secondary settlement tank is built into the chamber. Most units also have a digestion chamber below the discs to reduce the load to the secondary settlement tank, thereby reducing the quantity and sludge produced and the frequency of desludging. The solids settle and collect in the digestion chamber where they undergo anaerobic digestion (Fig. 4.43). Periods between desludging are normally between 1–3 months and failure to maintain a regular desludging programme results in odour production.

An electric motor is not always necessary to drive the rotor and it is possible to use air-induced drive systems. For example, air is sprayed into the rotating medium from below and to one side of the horizonal shaft. The liquid movement and asymmetric buoyancy from air collecting in one side of the medium induces it to rotate (Winkler 1981). This method reduces the risk of anaerobiosis in the first compartment or in the first RBC unit of a multiple unit system used for large populations. It is also used to uprate the activated sludge process. The disc system can be placed in an existing activated sludge aeration tank to increase significantly the biomass concentration within the tank without the need to increase the aeration capacity in order to satisfy the oxygen demand of an increased biomass (MLSS) concentration (Guarino *et al.* 1980).

Small plants are designed on the basis of BOD loading per unit area of discs using both sides of each disc in the calculation. Bruce and Merkens (1975) found that a Royal Commission effluent (20:30) could be produced at organic loading rates of < 6 g BOD $m^{-2}d^{-1}$. A conventional low-rate percolating filter containing 50 mm mineral medium and loaded at 0.10–0.12 kg BOD $m^{-3}d^{-1}$ can be loaded at a surface loading of 1.0–1.2 g BOD $m^{-2}d^{-1}$, which is five or six times less than an RBC, to produce a 20:30 effluent (Pike 1978). Higher loading figures have been given for 80–90% BOD reductions, ranging from 6–20 g BOD $m^{-2}d^{-1}$, although the organic loading can be increased by a factor of 10 for efficient partial treatment (Hao and Hendricks 1975a, b; Forster 1971). Loadings to larger units are based on hydraulic loading per unit area of disc. The calculation of loadings is complex and explained in detail by Steels (1974) and Wilson (1981). For obvious reasons, manufacturers feel that loading to RBCs are too conservative,

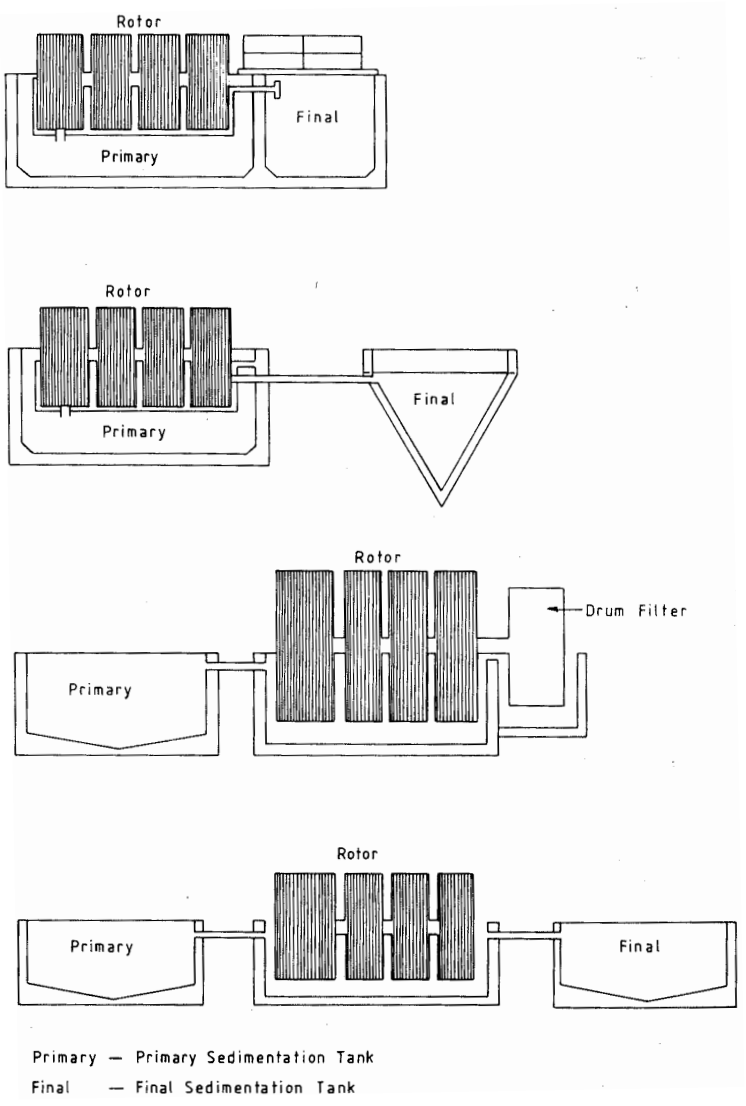


Fig. 4.43. Some rotating biological contactor configurations (IWEM 1988).

making them less attractive when compared to the activated sludge process. Loadings and their calculation has been reviewed by Lumbers (1983). The recommended loading for RBCs to give a 20:30 effluent should not exceed 5 g BOD $m^{-2}d^{-1}$ (BSI 1983). In practice, design loading are significantly lower than this at 4 g BOD $m^{-2}d^{-1}$ in order to achieve a 20:30 effluent.

Experience has shown that if full nitrification is required, then loading should not exceed 2.5 g BOD $m^{-2}d^{-1}$. These values refer to the total medium surface area of the RBC unit; however, if the organic loading to the first stage exceeds 31 g BOD $m^{-2}d^{-1}$ then problems with excessive *Beggiatoa* development will follow (Brenner *et al.* 1984). A minimum HRT of 0.6–1.5 h is required for carbonaceous oxidation and 1.5–3.8 h for nitrification (Wang *et al.* 1984). Although Cheung and Mack (1982) showed that a HRT in excess of 3 h did not improve carbonaceous removal. Maximum flows to RBC units are restricted to 6 DWF by the provision of a storm sewage overflow, often linked to a dedicated storm water reed bed. When pumped from a holding tank, wastewater should be applied to the RBC unit little and often. This is done by using a simple timer control that starts the pump. For example, every hour for only 1 to 2 minutes, although this must be overridden by level controls. A minimum dissolved oxygen concentration of 1.5–2.0 mg l^{-1} is required to ensure maximum BOD removal and to minimise nuisance. The dissolved oxygen concentration in the wastewater is a function of the oxygen utilisation by the film and the oxygen transfer between the film and the liquid. Where the organic loading is too high, oxygen uptake rate of the film exceeds the oxygen transfer rate, resulting in low dissolved oxygen concentrations, loss of performance, and development of *Beggiatoa*.

The RBC is the intensification of the percolating filtration process with the density of biomass on the discs reaching 200 g (DW) m^2, which is equivalent to a sludge loading of 40–60 kg MLVSS m^{-3} in the activated process. RBCs have many advantages over the percolating filter process, for example: complete wetting of the media; no clogging of the media due to excessive film accumulation reducing the retention time (in practice the retention time of an RBC is the same as a percolating filter); and regular exposure to the air ensures unlimited oxygen and the area of land used is only 10% of that required by the equivalent treatment capacity supplied by low-rate filtration. Rotating biological contactors are available as package units, that can be installed very quickly, with integral primary and secondary settlement compartments. When designed correctly, inspection periods can be extended to several weeks making them ideal for remote locations that may become cut off for short periods in the winter. Other advantages include low sludge production, excellent process control, ease of operation, high degree of BOD removal including substantial nitrification (Barnes and Bliss 1983), good settleability of solids, low power consumption, no distribution problems, no fly problems, and no recirculation is required to ensure wetting of the film as is often necessary with small percolating filters.

There are some potential disadvantages, which include frequent motor drive and bearing maintenance. Also, the process is affected by surges of flow and is sensitive to overloading. Power failure causes total loss of efficiency and where the discs are left standing in the wastewater for any length of time, uneven film distribution results with thicker film developing on the submerged surfaces and the film on the exposed surfaces drying out. This causes imbalance of the discs and severe motor wear, which results in eventual failure. While the active depth of film on the discs is < 600 μm, and 200 μm for nitrifying film, thick growths give rise to anaerobic conditions at the film-medium boundary. Such heavy film development results in odour when the film sloughs and also mechanical problems due to the excessive weight such as collapsed medium, failed bearings and drive mechanisms, and also broken shafts. Finally, RBCs are very expensive compared to other biological units, especially for small treatment plants. But their inconspicuousness, lack of odour, low fly nuisance, and quietness make them ideal where treatment must be done close to houses.

Rotating biological contactors have been used for secondary treatment for populations between 50–2,000 PE by Severn Trent Water since 1989. Since then, over 335 units have been constructed on 223 sites. Griffin and Findlay (2000) have outlined their design and operation. The RBCs use high density polyethylene (HDPE) discs submerged 40% of their diameter and rotated between 0.5–2.0 rpm, although 1 rpm is standard. The RBC design is particularly robust with the disc unit consisting of two mild steel end frames connected by through rods that support the discs. A minimum of three separate compartments are required in the biozone to promote plug flow characteristics. To avoid excessive film thickness and nuisance organisms such as *Beggiatoa* in the first compartment, a maximum loading of 20 g BOD $m^{-2}d^{-1}$ is specified. Maximum specific surface area of the medium is 150 m^2m^{-3} in the first compartment and 220 m^2m^{-3} in the remaining compartments. Performance is linked directly to disc loading (Fig. 4.44) with 4 g BOD $m^{-2}d^{-1}$ used when a consent of > 15 mg l^{-1} ammoniacal nitrogen is set, reducing to 2.5 BOD $m^{-2}d^{-1}$ when it is < 10 mg l^{-1}. This lower loading has been found to meet the 95 percentile ammonical N standard of 3 mg l^{-1} currently set for sensitive receiving waters. Long term monitoring has shown that the RBCs used by Severn Trent Water do not reliably achieve a 95 percentile compliance with BOD standards < 25 mg l^{-1}. Therefore they have adopted a strategy of using tertiary treatment reed beds that can produce final effluents with a BOD < 3 mg l^{-1}, suspended solis < 4 mg l^{-1}, and fully nitrified (Upton *et al.* 1995).

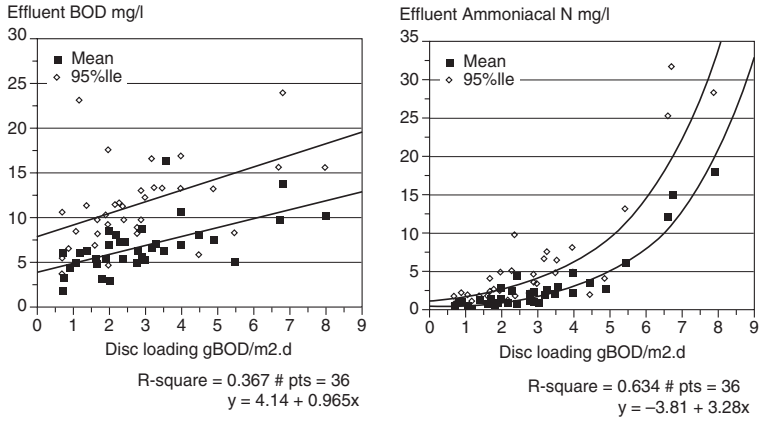


Fig. 4.44. Annual mean and 95 percentile concentrations for BOD_5 and ammoniacal nitrogen at different disc loadings (g $BOD_5 m^{-2} d^{-1}$) for 26 different rotating biological contactor sites (Griffin and Finday 2000).

4.3. Submerged Fixed Film Systems

4.3.1. *Introduction*

One of the limiting factors to increasing the efficiency of percolating filtration is the limited surface area available for film development without reducing the size of the interstices so that they become blocked with film growth and so restrict flow and aeration. While the introduction of plastic media improved the situation, the total area of fixed active biomass still remains much lower in perolating filters than in the activated sludge process per unit volume of reactor. In the 1980s the use of very fine media, such as expanded clay nodules or even sand, and passing the wastewater upwards through the media at a sufficient velocity to fluidise the media was proposed (Sec. 4.3.2). In this way, the interstices could not become blocked no matter how much biomass developed. The small media offered specific surface areas of up to 3,500 $m^2 m^{-3}$ allowing biomass concentrations equivalent to an MLSS concentration of 30–40,000 mg l^{-1}. The use of such fine media providing very high specific surface areas allowed more compact and versatile systems to be designed. From this original concept two further submerged systems emerged. Biological aerated flooded filters (BAFF) (Sec. 4.3.3) and submerged aerated filters (SAF) (Sec. 4.3.4) both use submerged solid medium with oxygen provided by diffusers or other mechanical devices. There is considerable confusion between the two systems and the terms are often used interchangeably. This is because there

is such a wide variation between individual systems that the term BAFF or SAF is often not sufficient on its own. However, most authors define BAFF technology as those filters that achieve solids separation by backwashing, while SAF technology are filters that require a settlement tank for solids separation. This definition is also used here.

4.3.2. *Fluidised bed reactors*

In order to provide sufficient surface area in a percolating filter to act as support for microbial film development, a medium is used. However, when 50 mm blast-furnace slag is used, 48.7% of the volume of the filter is taken up with the medium itself. This falls to 8.7% for the random plastic medium Flocor RC. The problem of providing sufficient fixed biomass for complete treatment is overcome by using sand as the medium and passing the wastewater through the medium in an upward direction at sufficient velocity to fluidise the sand. The sand provides a large increase in specific surface area compared to other media (3300 m^2m^{-3}), which allows considerable biomass to develop, equivalent to a MLSS concentration of up to 40,000 mg l^{-1}. In addition, fluidisation of the sand particles prevents clogging or excessive film accumulation as the interstices are not stable. This mode of operation allows the density of the biomass to be maintained at high concentrations without having to recycle solids as in the activated sludge process. Also, the quantity of biomass is controllable so there are no wide seasonal fluctuations in biomass concentration as seen in other fixed-film reactors.

The basic layout of a fluidised bed system is shown in Fig. 4.45. The high biomass concentration results in a very high oxygen demand that can only

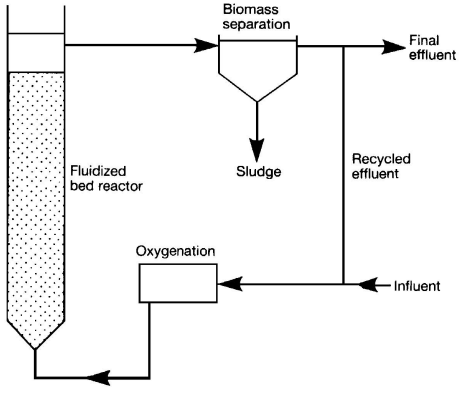


Fig. 4.45. Schematic diagram of a fluidised bed reactor system.

be satisfied by injecting pure oxygen into the influent wastewater stream as it enters the base of the reactor, giving an oxygen concentration approaching 100 mg l^{-1}. Even distribution of the influent wastewater into the medium is achieved by injecting the wastewater downwards into a conical base (Cooper and Wheeldon 1982). The expansion of the bed within the reactor is controlled by the rate of wastewater input, so that the effluent can be removed from the reactor below the level of the expanded layer without loss of media. A secondary sedimentation tank can be supplied but is not necessary as the biomass can be retained within this upflow system by careful operation. The sand-covered biomass is regularly removed and the biomass separated from individual sand particles by passing it through a hydroclone where a high shear strips away any attached microbial growth. The clean sand is returned to the reactor and the sludge can be further treated before disposal.

The above systems are ideal for treating high strength wastewater and are particularly useful for treating industrial wastewaters where the loading is variable (Cooper and Wheeldon 1980, 1982). They operate at short retention times with 95% removal of BOD achieved within 16 minutes. When operated as a nitrifying process, a 20 mg l^{-1} concentration of ammonia-nitrogen can be reduced by 99% in 11 minutes at 24°C, which represents an ammonia-nitrogen loading of 900 mg $m^{-2}d^{-1}$ at a hydraulic loading of 140 $m^3m^{-3}d^{-1}$. This is similar to that of a standard nitrifying filter (Sec. 4.1.5) (Jeris *et al.* 1977).

Fluidised beds are compact systems compared to activated sludge, therefore land requirement and capital cost is lower. However, operating costs are significantly greater because high purity oxygen and pumping are used. The sludge is more concentrated at 10% solids compared with 2% in gravity-thickened activated sludge. There are no odour or fly nuisances either. The fluidised bed can be operated aerobically, anoxically, or anaerobically. Anaerobic beds tend to expand rather than fluidise and are used for the conversion of carbonaceous wastes into gaseous end-products, therefore these reactors require a gas/liquid separation stage. Operation is in the mesophilic temperature range and heating is required (Moteleb *et al.* 2001) (Sec. 7.3.4). Anoxic beds have been developed for denitrification (Gauntlett and Craft 1979).

Although sand (0.2–2.0 mm diameter) is the most common support media in aerobic systems, anthracite, carbon, and glass are also used. Small particles have a higher specific surface area but a lower settling velocity than large particles. Therefore, larger fabricated media which are porous have been developed, allowing the biomass to grow within the

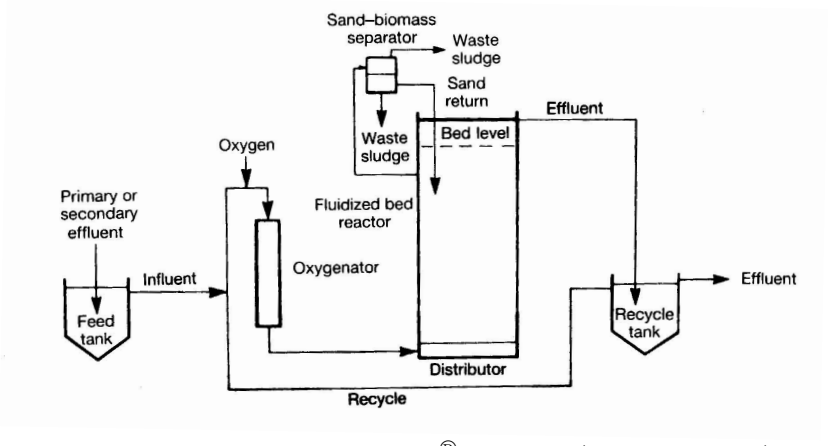


Fig. 4.46. The Dorr-Oliver Oxitron® pilot plant (Sutton *et al.* 1981).

media as well as on the outer surface. One example is reticulated polyester foam pads ($25 \times 25 \times 10$ mm). There are two full-scale aerobic fluidised beds on the market. Oxitron® is manufactured by Dorr-Oliver Inc and uses sand particles 250–550 μm in diameter with a specific surface area > 3000 m^{2}m^{-3}. It is able to support a large biomass of between 10–17 kg MLVSS m^{-3}, with the excess biomass removed by shear. Oxygen is supplied by dissolving high purity oxygen into the influent wastewater stream (Fig. 4.46) (Sutton *et al.* 1981; Hoyland and Robinson 1983). Capitor® is the only commercially available bed using plastic foam pads. Manufactured by Simon-Hartley Ltd, the excess biomass is removed by passing the pads through a compression roller, which releases a concentrated sludge with the pads returned to the reactor with a reduced microbial population. Oxygen is supplied by using a proprietary oxygenator (Fig. 4.47) (Walker and Austin 1981).

On-site bioreactors, such as fluidised beds, submerged aerated filters, or biological aerated flooded filters, are widely used for the treatment of industrial and agricultural contaminants in groundwater such as petroleum hydrocarbons, monoaromatic hydrocarbons, chlorinated alipatics, and aromatics (Langwaldt and Puhakka 2000). An unusual application of a fluidised bed to remove algae from Lake Kasumigaura in Japan, a eutrophic lake, is described by Tanaka *et al.* (2001). The system is floated on the lake and is powered by solar panels. Three reactors are connected sequentially through the head tanks (Fig. 4.48). Using 0.5 mm diameter granular media, a 50:50 mixture of cristobalite and activated carbon particles, the volume of the media expands about 30% with a HRT for the entire system of 1 h. No

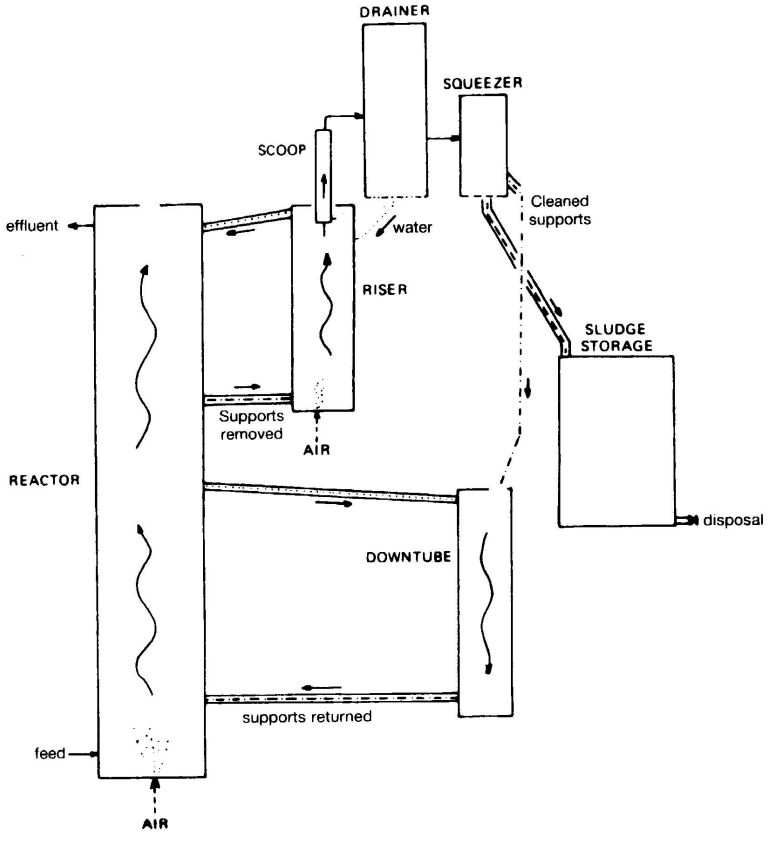


Fig. 4.47. Biomass recovery scheme from the Simon-Hartley Ltd Captor® process (Walker and Austin 1981).

process air is injected. Instead, the dissolved oxygen in the influent water and that supplied by the cascades in the head tanks mounted on the inlets of the second and third reactors. The system was operated at a flow rate of 1500 m^3d^{-1} and during the summer (August to September) when algal blooms occurred the average efficiency of chlorophyll *a* removal was 64% at an average influent chlorophyll *a* concentration of 137.8 $\mu g\ l^{-1}$. Almost all of the algae removed by the reactor was biologically degraded with 3.47 mg of oxygen required for the aerobic degradation of 1 mg C of algae. Aerobic degradation of algae can be expressed as:

$$C_{106}H_{263}O_{110}N_{16}P + 138O_2 \rightarrow 106CO_2 + 16N_3^- + HPO_4^{2-} + 122H_2O + 18H^+$$

(Kobayahi *et al.* 1990).

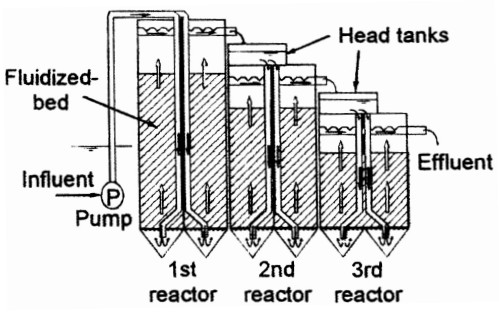


Fig. 4.48. Schematic diagram of the experimental fluidised bed biofilm reactor of Tanake *et al.* (2000) used to treat eutrophic lakes.

4.3.3. *Biological aerated flooded filters*

Introduction

Although the correct name for the process is biological aerated flooded filters (BAFF), this name has been universally shortened to biological aerated filter (BAF). Experiments in aerating flooded fixed film reactors have been going on since 1913, although the process as we recognise today was first introduced in the late 1980s (Hodkinson *et al.* 1999). With the introduction of the Urban Wastewater Treatment Directive, there is pressure to uprate existing sewage treatment plants, especially at coastal sites, where land is at a premium, in order to meet new standards. The potential of BAFF technology was immediately recognised because of its compactness, the quietness of operation, and the low odour potential of the process. There are many proprietary BAFF systems available, all of which are slightly different in design. However, the principle of the process is common to all (Lilly *et al.* 1992).

Process design

BAFF technology can be used in the same way as the activated sludge process for carbonaceous removal, nitrification, denitrification, and biological phosphorus removal (Yoon and Suzuki 1991; Smith and Hardy 1992). In fact, whenever activated sludge is appropriate then BAFF should also be considered.

Primary sedimentation or fine screening (1–3 mm) is required prior to secondary treatment by BAFFs. They are constructed above ground in either concrete or steel and must be strong enough to support the media when expanded during backwashing. The system is made up of a number of individual cells or compartments. Mineral or artificial granular medium,

generally in the range of 1–5 mm in diameter, is used. Granular media with a density less than water is termed floating (e.g. polyurethane, polystyrene) while media with a density greater than water is termed sunken (e.g. sand, pozzolanic stone). The selection of the medium is a compromise between maximum surface area and retention efficiency of the medium during backwashing. Like fluidised beds, the granular mineral media in BAFFs are subject to abrasion so that fine particles formed are lost during backwashing. This leads to media losses of 2–5% per annum. Some proprietary systems employ fixed lattice type medium, more commonly associated with submerged aerated filters (SAF). The advantage of this modular is that there is lower head loss and no loss of media. However, modular media has significantly lower surface areas, poorer solids removal, and is unable to produce high quality (< 10 mg l^{-1} BOD) final effluents. Multi-layer granular media beds, similar to rapid sand filters are employed where primary sedimentation is omitted (Fig. 4.49). Screened and degritted raw wastewater enters the bottom of the filter and rises through graded layers of media. Solid removal is achieved by gravity flushing, upflow air and water scouring, and flushing.

The depth of the medium varies from 2–4 m for sunken granular media used in downflow mode (now largely replaced by upflow systems), 3–5 m for upflow granular media systems, and 4–6 m for fixed laminar media, making all BAFFs tall units that are highly visible. A separate backwash water holding tank is required to store the final effluent used for backwashing, and another tank for holding the dirty backwash water which is fed back to the inlet is also required. Air blowers are used for both aeration and air

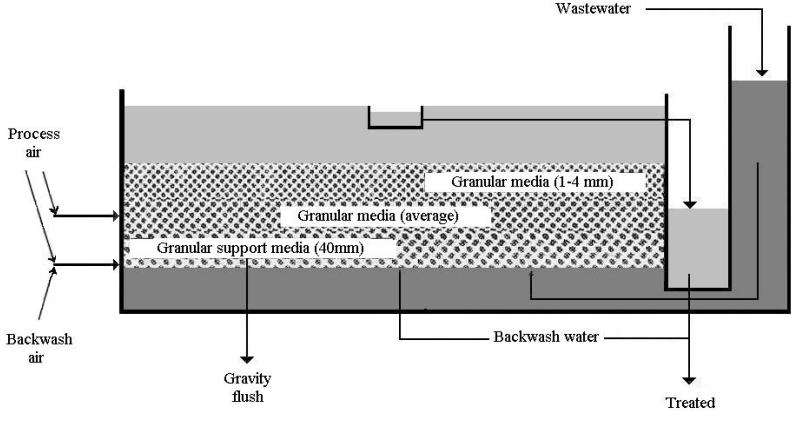


Fig. 4.49. A typical multi-layer biological aerated flooded filter (BAFF) (IWEM 2000).

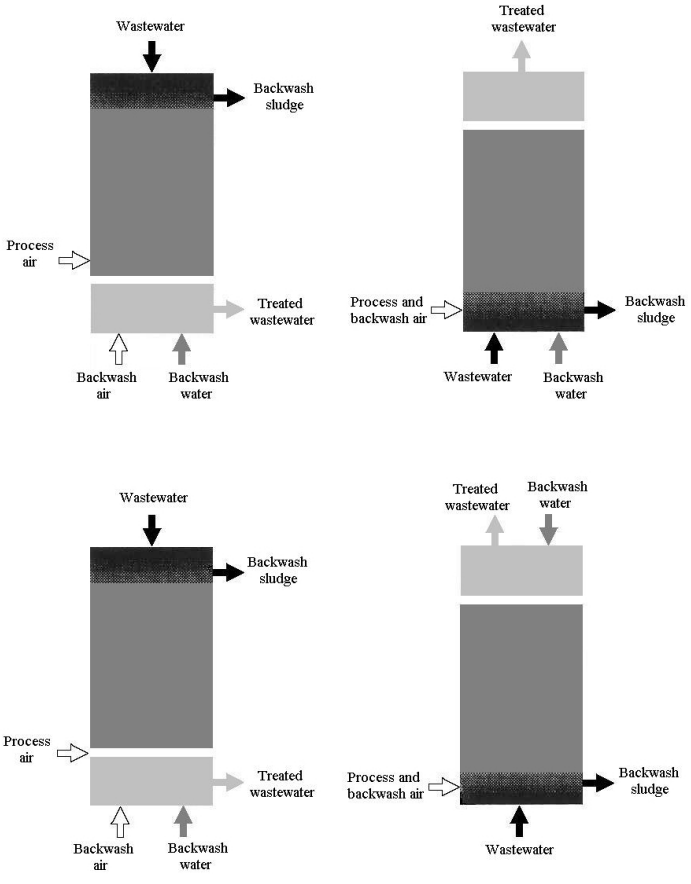


Fig. 4.50. Directions of flow and backwash in BAFFs (IWEM 2000).

scouring during the backwash cycle. The direction of wastewater flow and backwashing can be in either direction and varies widely (Fig. 4.50).

Two sets of air blowers are required: fine bubble diffusers supply processed air at a constant rate to satisfy the oxygen demand of the biomass, while coarse bubble diffusers supply higher rates of air for short periods during backwashing. This often requires two sets of pipework and diffusers, and even two sets of blowers. In smaller plants, both fine and coarse bubble aeration can be supplied using perforated latex rubber sheets covered by perforated metal diffuser plates. The size of the bubble and the rate air is supplied is controlled by varying the pressure within the pipework which causes the pores within the latex to expand or contract, altering the size of the bubble created.

Backwashing is very important in BAFF systems as it reduces excess biomass from the filter, prevents head loss due to solids retention, and collects the solid for treatment. The frequency of backwashing is controlled by the accumulation rate of the film which results in head loss through the cell. Typical backwash cycles vary from 12–24 h for carbonaceous BAFFs to 4–5 d for nitrification only systems. Backwashing takes between 30–60 minutes and has four phases. (i) Influent feed to the cell is shut off. (ii) Water and air is passed upward through the bed of granular medium expanding it 1.3–1.5 times its operational depth. (iii) The medium is agitated by air scouring to detach excess biomass. (iv) Backwash water is passed upward through the medium to remove the detached biomass. Phases (ii) and (iii) are repeated several times to ensure adequate solids removal. The velocity of backwash water is 5–30 $m^3m^{-2}h^{-1}$ for short periods of 5–20 minutes, the larger the media the higher the rate. The backwash air flow varies from process to process, but a typical range is 20–60 m^3 air m^2 of bed area per backwash. The volume of backwash water used ranges from 5–15% of the average flow, although 10% is typical. It is similar in nature to surplus activated sludge being a mixture of biomass and trapped solids, but with a much lower dry solids content (0.2–0.5%). The backwash water is pumped to the primary sedimentation tank for solids separation, or where there is no primary sedimentation then the backwash water is thickened and the sludge treated as normal. Where medium losses are high, the backwash water should be returned up stream of the grit separation stage of the plant.

As BAFFs are truly aquatic ecosystems, the biology is similar to that of activated sludge (Sec. 5.5). Although the concept of sludge age is inappropriate, the nature of the system does not favour the development of higher trophic levels such as invertebrate grazers which are common in percolating filters. The backwashing process also physically restricts the size and type of biota found.

Loading and performance

Typical organic loadings are between 2–3 kg BOD m^3d^{-1}, although this can be increased to 5 kg BOD m^3d^{-1} for high rate treatment producing lower effluent quality (70% BOD). High hydraulic loadings are beneficial because of the improved physical kinetics within the cell, although if the bed becomes fluidised, there will be a significant loss in effluent quality. Assuming one cell out of operation for backwashing, then hydraulic loadings of 4–12 $m^3m^{-2}h^{-1}$ are normal. Sludge production is between 0.6–1.0 kg suspended solids per kg BOD removed, while the oxygen requirement of

BAFFs is similar to that of activated sludge at 1.0–1.5 kg O_2 per kg BOD removed. Design criteria for nitrification are 1.0 kg amm.N $m^{-3}d^{-1}$ with an additional oxygen requirement of 4.3 kg O_2 kg amm.N removed. The current practice is to nitrify in a two-stage BAFF as conditions are more appropriate for nitrifying bacteria in the second stage. An example of a ten cell steel BAFF unit producing a fully nitrified effluent with an average BOD of 2.7 mg l^{-1} and a suspended solids concentration of 3.7 mg l^{-1} is described by Robinson *et al.* (1994).

Performance falls immediately following backwashing due to a reduction in the physical filtration performance of the medium due to a reduction of the solids that reduce the size of the interstices of the medium. Thus for up to an hour after backwashing, both suspended solids and BOD will be higher than normal. Nitrification is unaffected by backwashing. When 95 percentile effluent quality better than 10 mg l^{-1} BOD and 15 mg l^{-1} suspended solids is required, tertiary treatment is recommended.

Although primarily used for standard secondary treatment, nitrification-denitrification is possible with BAFF employing similar design criteria as used for nitrifying filters (Meaney and Strickland 1994). An anoxic zone is required for denitrification with carbon supplied either by recirculation of nitrified effluent or the addition of a separate carbon source (Ryhiner *et al.* 1994; Chen *et al.* 2000; Ouyang *et al.* 2000). Volumetric nitrification rates in BAFFs are up to 3.5 and 8 times higher than RBCs and percolating filters using plastic media respectively (Boller *et al.* 1997). Tertiary nitrification using BAFFs has also been successfully combined with chemical phosphorus removal. Pujol *et al.* (1995) give an example of a sewage treatment works treating 1,450,000 PE at Köln in Germany. Here BAFFs were used to uprate the existing activated sludge plant for simultaneous nitrification and tertiary phosphorus removal. Removal rates of 70% P and $> 95\%$ nitrification were achieved with final effluent concentrations < 0.2 mg N-NH_4 l^{-1} and 0.5 mg TP l^{-1}.

Operational control

It takes between 3–8 days for a BAFF system to become operational in terms of BOD removal, although it may take up to 20 days for excellent quality effluents to be achieved. This rapid start up is particularly useful in areas subject to seasonal variation in population. The high concentration of biomass (15,000–25,000 mg l^{-1}) enables high volumetric loading rates compared to the activated sludge process, thereby permitting much smaller tanks to be used.

The operation of BAFF systems is more complex than either activated sludge or percolating filtration due to the frequent backwashing that is required. Therefore BAFF plants are generally highly automated and operated by computer. Backwashing is operated on a timed basis with an automated head loss override to prevent the cells from overflowing. The quantity of backwash water is significant and so should be returned to the inlet when the hydraulic loading to the treatment plant is low. In small plants this is done during the night, while at larger plants, cells are backwashed one at a time on a continuous basis. Whenever possible, backwash water should be returned upstream of grit separation to ensure that any displaced medium can not damage mechanical equipment.

In terms of noise, BAFF plants are similar to a fine bubble diffused air activated sludge plant. Noise can be reduced even further by using acoustic covers and buildings. Odour potential is low in upward flow BAFF systems but can be significant in downward flow systems where the settled sewage remains on the surface of each cell. The use of diffused air also increases odour potential, but the odour produced from the BAFF unit will be small compared to the odour production of the plant as a whole.

Foaming is a common problem in BAFFs, with the foam similar in nature to that found at activated sludge plants. Foaming is normally caused by low loading, so control is achieved by increasing the organic loading by taking some of the cells out of operation. Other control options include recirculation of flow, reduction in the frequency of backwashing, or the use of anti-foaming agents.

The compactness of the BAFF process has led to its widespread use at coastal locations. However, a common problem at such sites is that where the sewerage network is in poor conditions, seawater enters the sewer and becomes mixed with the wastewater. Varying concentrations of chloride, or very high concentrations of chloride, cause the bacteria in the BAFF unit to form extra-cellular excretions of polysaccharides which rapidly block the interstices of granular media. Head loss quickly follows, triggering backwashing prematurely of the timer resulting in short run times and the production of large quantities of backwash water. Operational problems are discussed by Wheale and Cooper-Smith (1995).

4.3.4. *Submerged aerated filters*

Submerged aerated filter (SAF) and biological aerated flooded filter (BAFF) technologies are similar, i.e. the media is submerged and air is supplied via blowers to maintain the biomass on the medium surface, except

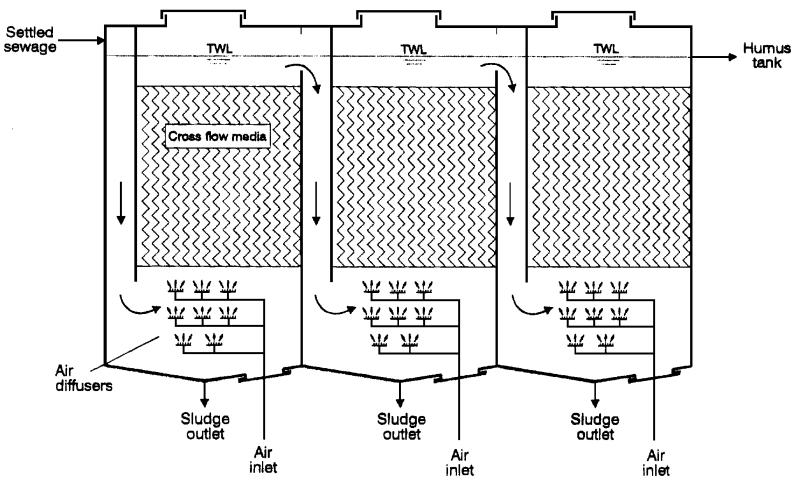


Fig. 4.51. Schematic diagram of submerged aerated filters in series (IWEM 2000).

that SAF systems do not employ backwashing but require a sedimentation tank for solids separation and removal. The medium used is normally modular plastic, 3–6 m in depth, which reduces head loss and prevents blockages (Fig. 4.51). The surface area of the modular medium is significantly lower than the granular media used in BAFFs, resulting in lower solids retention which eliminates backwashing. Typical specific surface areas of SAF modular media varies from 250–350 m^2m^{-3}, with the higher surface areas are being used for nitrification. Air is supplied by diffusers and although only process air is required some designs incorporate the provision of air scouring at similar pressures as used during backwashing of BAFFs. This provides greater control of sludge removal as otherwise the biomass sloughs off in large clumps causing problems during final settlement.

Submerged aerated filters are packaged units designed for typical organic loadings of 3 kg BOD $m^{-3}d^{-1}$ and hydraulic loadings of 2–8 $m^3m^{-2}h$. Oxygen requirements and sludge production rates are similar to those for BAFF. If a 95 percentile effluent quality < 25 mg l^{-1} BOD and 35 mg l^{-1} suspended solids is required, then tertiary treatment must be used.

Simple to operate and robust, SAFs are ideal packaged units for small treatment plants ($< 5,000$ PE). They require little maintenance making them suitable for unmanned and isolated works. SAF units are used to uprate existing works, for example by reducing the loads to existing percolating filters in order to promote nitrification. The period for set up is much shorter than for activated sludge, being only 3–8 d for BOD removal,

making them very flexible in terms of expanding treatment capability. Schlegel and Teichgrber (2000) describe the successful application of SAFs for the pre-treatment of industrial wastewaters including wastewaters from food processing, carpet dyeing, pharmaceutical and tar processing factories. An upflow SAF system was used by Timur (2001) for denitrification with maximum denitrification rates achieved at COD:NO_3-N ratios of 5–6, independent of hydraulic loading rate or influent NO_3-N concentrations. Denitrification followed half-order kinetics, with removal efficiencies of 71–99% reported.

4.3.5. *Moving bed biofilm reactor*

The principle of the moving bed biofilm reactor (MBBR) is small plastic media, normally polyethylene, 10–20 mm in diameter of a density close to that of water (1 g cm^{-3}) on which biofilm develops. The density of the medium is critical as it allows it to move freely within the reactor even at filling fractions (i.e. the volume occupied by the medium in an empty reactor) of 70%. In contrast to other fixed-film systems, the reactor is completely mixed, so that the whole reactor volume is biologically active resulting in higher biomass activity (Ødegaard *et al.* 1994). It has all the advantages of a submerged filter with no clogging problems and a lower head loss. Compared to the activated sludge process, MBBRs can operate with more reactors in series, at higher f/m ratios, and with more selected biomass for each step (Pastorelli *et al.* 1997; Rusten *et al.* 1997). Over 100 plants are now using the system throughout the world treating both municipal and industrial wastewaters for BOD/COD removal, nitrification and denitrification (Ødegaard *et al.* 2000).

Andrettola *et al.* (2000) compared an MBBR system containing Flocor-RMP® plastic media with a specific surace area of 160 m^2m^{-3} (Table 4.25)

Table 4.25. Characteristics of a typical plastic medium (Flocor RMP®) used in moving bed biofilm reactors.

Material	Polypropylene (density = 0.94 g cm^3)
Shape	Corrugated cylinder
Dimensions	Length: 20–30 mm
	Diameter: 15–20 mm
Specific surface	160 m^2/m^3
Filling rate	70%

with activated sludge. As only 70% of the tank was occupied by the medium, the actual active medium surface area, termed the specific MBBR surface, was 112 m^2m^{-3}. The performance of the two processes was almost identical and they concluded that media with higher surface areas were needed to achieve better performance by increasing the biomass. However, MBBRs require no sludge recycling, do not require close control as with activated sludge, nor has the associated bulking problems. Specialised biomass for carbon and nitrogen removal can be selected by using multi-reactor configurations.

Ødegaard *et al.* (2000) have analysed the influence of media size and shape on performance. They found that MBBRs should be designed based on surface area loading rates (g COD m^2d^{-1}), while the shape and size of the media is not a significant factor as long as an effective surface area is provided. Design values as high as 30 mg soluble COD m^2d^{-1} can be used. Problems were reported by the authors of the settleability of the biomass decreasing with increasing organic loading.

Further reading

Percolating filters: Bruce and Hawkes 1983; Pike 1978; Fitch *et al.* 1999; Parker 1999.

Design and operation: Pike 1978; Allen and Kingsbury 1973; IWEM 2000.

Process modifications: Pike 1978; Bruce and Hawkes 1983; Hawkes 1983b; IWEM 2000.

Organisms and ecology: Curds and Hawkes 1975; Hawkes 1983b.

Factors affecting performance: Bruce and Hawkes 1983; Hawkes 1983b; Adin *et al.* 1985.

Rotating biological contactors: Antonie 1976; Steels 1974; Lumbers 1983; Gould 1994; Griffin and Findlay 2000.

Fluidised beds: Cooper and Atkinson 1981.

Nitrifying filters: Barnes and Bliss 1983; Schlegel 1988; Wik 2000.

Biological aerated flooded filters: Pujol *et al.* 1992; Lazarova *et al.* 2000; Regall and van Loosdrecht 2000.

Submerged aerated filters: Regall and van Loosdrecht 2000.

Moving bed biofilm reactors: Ødegaard *et al.* 1994; Pastorelli *et al.* 1997.

5

Activated Sludge

The activated sludge process is the most widely used biological wastewater treatment process today, treating both domestic and industrial wastewaters. Since its conception in the late nineteenth century and subsequent development into a full-scale unit process in 1913 by Ardern and Lockett at the Davyhulme Treatment Works in Manchester, the basic process has been widely adopted and further developed, giving it a unique flexibility of operation (Alleman and Prakasam 1983). The process is more than simply a refinement of percolating filtration (Chapter 4), it is conceptually a very different treatment system. Whereas fixed-film reactors can be compared with the periphyton growing over the surface of stones and submerged plants in rivers, the activated sludge process utilises the dispersed bacterial flocs and free-living micro-organisms that are suspended within the body of the water.

The activated sludge process relies on a dense microbial population being mixed with the wastewater under aerobic conditions. With unlimited food and oxygen, extremely high rates of microbial growth and respiration can be achieved, resulting in the utilisation of the organic matter present either as oxidised end-products (CO_2, NO_3, SO_4, PO_4) or the biosynthesis of new micro-organisms. Purification occurs as a number of successive steps, but as the microbial biomass is mixed with the wastewater within a single reactor (the aeration tank), the individual steps are not discernible but occur simultaneously. In settled sewage, the organic material is present partly as soluble material (< 1 mμm) and partly as finely suspended (1–100 μm) and colloidal (1 mμm–1 μm) particulate matter, with the organic material predominantly associated with the particulate fraction (Heukelekian

and Balmat 1959). In sewage, 64% of the total solids are classed as soluble, whereas 34% are particulate, with 80% of the particulate fraction organic, compared with only 20% in the soluble fraction. Therefore, the majority of the organic loading to the activated sludge unit will be in the form of colloidal or larger sized particulate solids with little present as simple low molecular weight nutrients. In the first stage of purification, the particulate and colloidal material is rapidly adsorbed or agglomerated onto the microbial floc, so that initially treatment is primarily physical in nature. Although the soluble material present can be utilised immediately, the colloidal or suspended matter has to be broken down extracellularly before becoming available for microbial oxidation. Rickert and Hunter (1967, 1971, 1972) have demonstrated that particulate solids are removed more efficiently than the soluble fraction by biological treatment, which supports the importance of the physico-chemical processes in wastewater treatment. The varied nature of the organic matter present ensures that it is oxidised at different rates. For example, high respiration rates are measured at the inlet of the tank reaching up to 500 mg O_2 g^{-1} h^{-1}, with a gradual decline in respiration rate with retention time falling to below 50 mg O_2 g^{-1} h^{-1} at the outlet. Part of the organic matter is oxidised to simple end-products (mineralisation), while the remainder is converted into new cellular material (assimilation).

The activated sludge process consists of two phases, aeration and sludge settlement (Fig. 5.1). The main components comprising the process are summarised in Table 5.1. In the first phase, wastewater is added to the aeration tank containing the mixed microbial population and air is added either by surface agitation or via diffusers using compressed air.

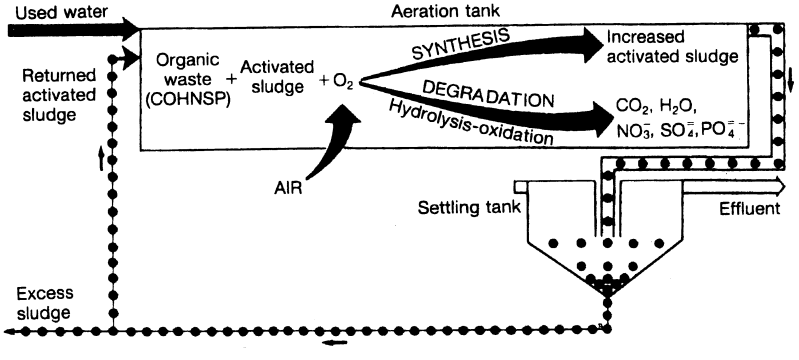


Fig. 5.1. Development and control of biomass in the activated sludge process (Hawkes 1983a).

Table 5.1. Main components of all activated sludge systems.

1. *The reactor* This can be a tank, lagoon, or ditch. The main criteria of a reactor is that the contents can be adequately mixed and aerated. The reactor is also known as the aeration tank or basin.
2. *Activated sludge* This is the microbial biomass within the reactor which is comprised mainly of bacteria and other microfauna and flora. The sludge is a flocculant suspension of these organisms and is often referred to as the mixed liquor. The normal concentration of mixed liquor expressed as suspended solids (MLSS) is between 2000–5000 mg^{-1}.
3. *Aeration/mixing system* Aeration and mixing of the activated sludge and incoming wastewater are essential. While these tasks can be done independently, they are normally done using a single system, either surface aeration or diffused air is used.
4. *Sedimentation tank* Final settlement (or clarification) of the activated sludge displaced from the aeration tank by the incoming wastewater is required. This separates the microbial biomass from the treated effluent.
5. *Returned sludge* The settled activated sludge in the sedimentation tank is recycled back to the reactor to maintain the microbial population at a required concentration in order to ensure continuation of treatment.

The aeration has a dual function, to supply oxygen to the aerobic micro-organisms in the reactor for respiration and to maintain the microbial flocs in a continuous state of agitated suspension, which ensures maximum contact between the surface of the floc and the wastewater. This continuous mixing action is important, not only to ensure adequate food, but also a maximum oxygen concentration gradient to enhance mass transfer and to help disperse metabolic end-products from within the floc. Ardern and Lockett (1923) originally developed a batch process known as the fill and draw method, with aeration and settlement taking place within the same tank. Subsequently, a continuous system was developed with no settlement allowed within the aeration tank. As the settled wastewater enters the aeration tank, it displaces the mixed liquor (the mixture of wastewater and microbial biomass) into a sedimentation tank. Here, the flocculated biomass settles rapidly out of suspension to form a layer of sludge with the clarified effluent, which is virtually free from solids, discharged as the final effluent. In the conventional activated sludge process (Sec. 5.3.1) between 0.5 and 0.8 kg dry weight of sludge is produced for every kg BOD_5 removed. The sludge is rather like a weak slurry containing between 0.5 and 2.0% dry solids, and therefore is pumped easily. As the solids content increases the viscosity rapidly becomes greater, although, as discussed in Chapter 2, activated sludge is difficult to consolidate to above 4% dry solids by gravity alone. However, most of the activated sludge is returned to the aeration tank to act as an inoculum of micro-organisms, ensuring that there is an

adequate microbial population to fully oxidise the wastewater during the period of retention within the aeration tank. The excess sludge requires further treatment prior to disposal.

The most important function in the activated sludge process is the flocculent nature of the microbial biomass. Not only do the flocs have to be efficient in the adsorption and subsequent absorption of the organic fraction of the wastewater, but they also have to rapidly and effectively separate from the treated effluent within the settlement chamber. Any change in the operation of the reactor will lead to changes in the nature of the flocs, which can adversely affect the overall process in a number of ways, most notably in poor settlement resulting in turbid effluents and a loss of microbial biomass (Sec. 5.4).

Although some variations of the activated sludge process are used to treat raw sewage (Sec. 5.3), most activated sludge processes use settled sewage. The sludge produced from the process should not be confused with primary sludge as it is entirely microbial biomass and adsorbed particulate matter, and does not contain coarse organic or inorganic solids. For this reason, the activated sludge process is similar to normal fermentation processes except, of course, it is not aseptic. Compared to industrial fermenters, activated sludge is different in:

(i) that there is no product being synthesised while material is being broken down;
(ii) only a small proportion of the activated sludge is actually viable and capable of reproduction; and
(iii) the nutrient concentration in the aeration tank is much lower than the nutrient medium used in a conventional fermenter.

Ideally, the activated sludge process should be operated as close to a food-limited condition as possible in order to encourage endogenous respiration when the micro-organisms utilise their own cellular contents, thus reducing the quantity of biomass produced. During the endogenous respiration phase, the respiratory rate will fall to a minimum that is sufficient for cell maintenance only. However, under normal operating conditions the growth of the microbial population and accumulation of non-biodegradable solids results in an increase in the amount of activated sludge produced.

The removal mechanism, assimilation or mineralisation, can be selected by using specific operating conditions with certain advantages and disadvantages. For example, the most rapid removal of nutrients is achieved by removing organic matter by assimilation only, where it is precipitated in the form of biomass. Such processes produce considerable surplus sludge, which

requires a higher proportion of the operating costs to be spent on sludge separation and disposal. Complete oxidation (mineralisation) of wastewater is much slower and requires long aeration periods. Thus, although much less sludge is produced, the saving on sludge handling and aeration costs will be much higher (Winkler 1981). The selection of a particular design of activated sludge plant depends largely on the size of the population served. For example, packaged plants are used for populations of 100–4,000; extended aeration systems such as oxidation ditches 100–20,000 or Carrousel plants 10,000–100,000; while conventional activated sludge plants are generally used for populations of 10,000–2,000,000.

The process is more intensive than in fixed-film reactors, treating up to 10 times more wastewater per unit reactor volume. Although this makes the activated sludge process cheaper in terms of capital cost, they are considerably more difficult to operate than percolating filters, have high operating costs, and produce comparatively large quantities of surplus biomass or sludge. The two process are compared in Table 4.1. The design of the activated sludge processes has been extensively studied and has also been extensively modelled (Niku *et al.* 1979; Ouano and Mariano 1979; Vavilin 1982; Henze *et al.* 1999; Olson and Newell 1999; Dochain and Vanrolleghem 2001; Henze 2002).

5.1. Flocculation

The basic operational unit of activated sludge is the floc. Under the microscope, activated sludge comprises discrete clumps of micro-organisms known as flocs, which vary both in shape and size. Good flocculant growth is important for the successful operation of the process, so that suspended, colloidal, and ionic matter in the wastewater can be removed by adsorption and agglomeration, and subsequently for the rapid and efficient separation of sludge from the treated effluent. There is a rapid agglomeration of suspended and colloidal matter onto the flocs as soon as the sludge and wastewater are mixed (enmeshment), which results in a sharp fall in the residual BOD of the wastewater (Guellil *et al.* 2001). The volatile matter content of flocs is generally high, between 60 and 90%, although this depends on the nature of the waste and the amount of fine suspended and colloidal inert matter present. The percentage of organic material is also dependent on sludge loading rate, the higher the sludge loading rate the greater the percentage of organic matter comprised in the floc, and also whether primary sedimentation is incorporated into the plant design. Where

primary sedimentation is not employed, the flocs will contain a higher proportion of inorganic material with the organic fraction being as low as 40%, compared to 70–75% for plants incorporating primary sedimentation. The adsorption capacity of the floc depends on the availability of suitable cell surfaces. Once all the adsorption sites are occupied, the floc has a very reduced capacity for adsorbing further material until it has metabolised that already adsorbed. Breakdown and assimilation of the agglomerated material proceeds more slowly (stabilisation). Therefore, if the hydraulic retention time of the wastewater (HRT) in the aeration tank is too short, there will be a progressive reduction in BOD removal as there will have been insufficient time for adsorbed material to be stabilised. Although all flocs have a specific gravity greater than 1.0, in the sedimentation tank, only well-formed flocs settle out of suspension with smaller flocs, dispersed micro-organisms and solids, carried out of the tank with the final effluent. Removal efficiencies in sedimentation tanks are significantly lower for smaller particles, especially those less than 5 μm in diameter (Parker 1983) (Fig. 5.2). The process depends on continuous reinoculation with recycled settled sludge, so the system will only select floc-forming organisms that rapidly settle in the sedimentation tank. Thus, the process is self-regulating with the best flocs recirculated.

Using a multi-exposure photographic method, Li and Ganczarczyk (1987) were able to determine the settling velocity and size of activated sludge flocs. They reported that the settling velocity of a floc is a linear

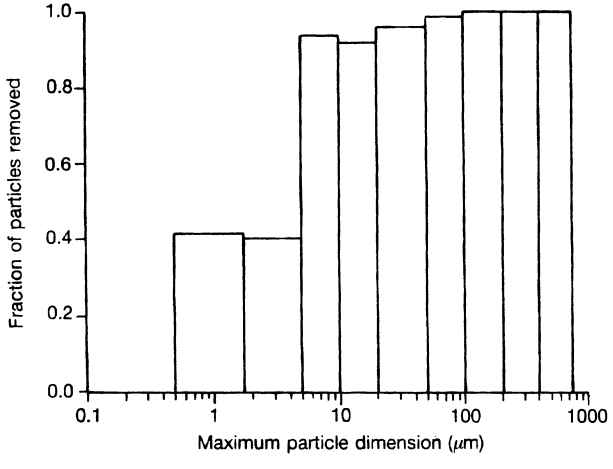
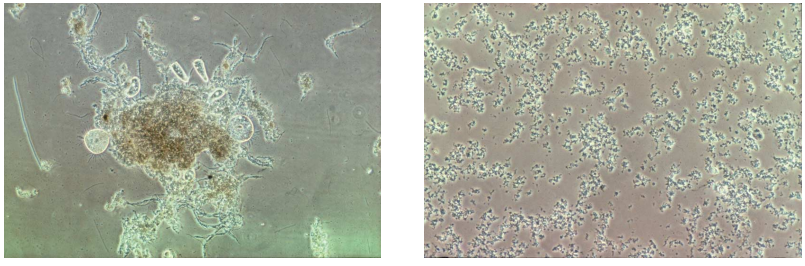


Fig. 5.2. The settling efficiency of particles of different sizes comprising the mixed liquor under quiescent conditions (Parker 1983).



(a) (b)

Fig. 5.3. Example of typical flocs: (a) A mature floc with secondary colonisation typical of activated sludge treating domestic wastewater with good settlement characteristics; (b) Small dispersed flocs typical of activated sludge treating pharmaceutical wastewater with poor settlement characteristics.

function of the cross-sectional diameter of the largest dimension of the floc. Also, that the porosity of the flocs increases as a function of the largest dimension, the rate of increase falling dramatically after the floc exceeds 200 μm.

Individual flocs are complex biochemical units. Each floc is a cluster of several million heterotrophic bacteria bound together with some inert organic and inorganic material (Figs. 5.3 and 5.4). There is a wide range of particle sizes in the activated sludge process ranging from individual bacteria of between 0.5 and 5.0 μm, up to large flocs which may be greater than 1 mm (1000 μm) in diameter. Parker *et al.* (1971) found that there was a bimodal particle size distribution of flocs in activated sludge (Fig. 4.32), and suggested that the smaller particles were either individual micro-organisms or small aggregates that had not flocculated or had been sheared off larger flocs. The maximum size of flocs is dependent on their physical strength and the degree of shear exerted by the turbulence caused by the aeration system in the aeration tank. The process of floc formation is far from being understood. Originally, it was thought due to the slime-forming bacterium *Zoogloea ramigera*, however, many other bacteria and protozoa are now known to be associated with floc formation (McKinney and Weichlein 1953; Kato *et al.* 1971). Bacteria, protozoa, and detritus are either attached to the surface of the floc or embedded in some form of material forming a matrix. The exact nature of this flocculating material is still not known although it appears largely bacterial in origin. The material can be readily extracted from activated sludge (Brown and Lester 1979) and constitutes a significant portion of the dry weight of the sludge, up to 10%. All the studies have shown that the material is a polymer, which can be composed

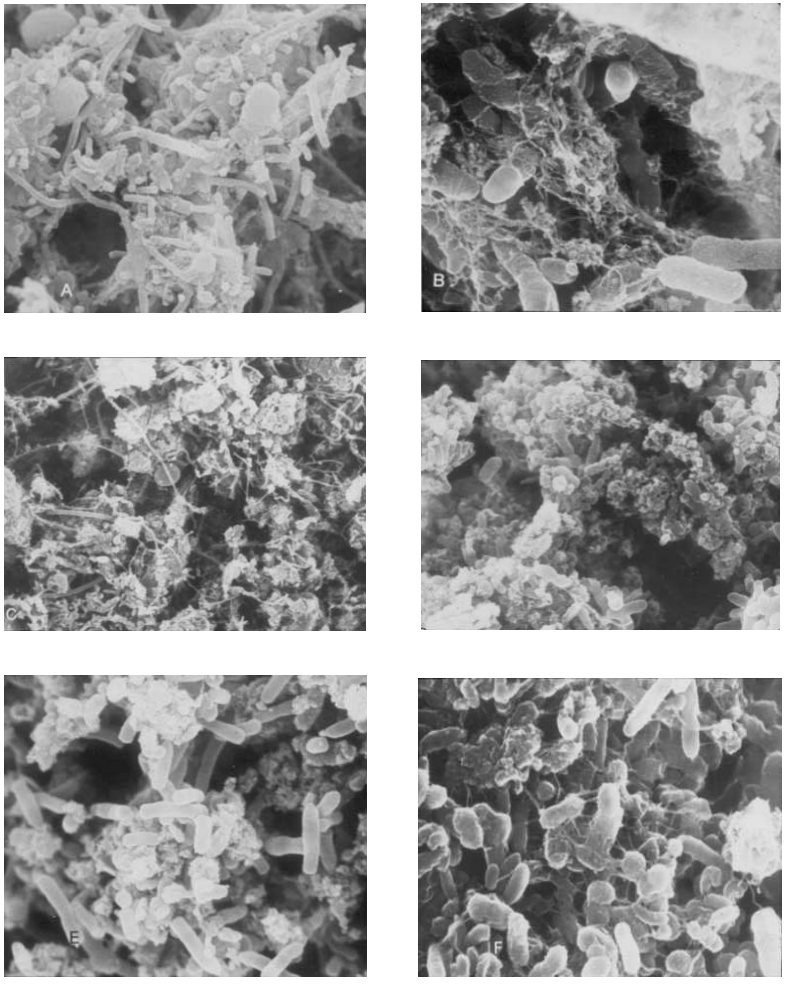


Fig. 5.4. Scanning electron micrographs of sludge flocs from five different plants treating petrochemical wastewaters. Where A is from Plant I (×5,000), B is also from Plant I (×20,000), and C is from Plant II (×1,000). Plants I and II have filamentous flocs. D is from Plant III (×5,000), E is from Plant IV (×5,000), and F is from Plant V (×20,000). The mixed liquor from Plants III, IV, and V are non-filamentous in nature (Salanitro and Rader 1982).

of a number of organic compounds, such as polysaccharides, amino polysaccharides, and protein (Sato and Ose 1980). Lipids may also be present with minor amounts of nucleic acids and other biopolymers, but the exact nature of these flocculating polymers will depend on the species of bacteria or protozoa producing it (Dignac *et al.* 1998; Flemming and Wingender 2001a).

Each polymer will have varying surface properties and charges that will influence not only the settling characteristics but also the water binding properties of the floc. The polymer does not only give the floc components cohesion, it also allows suspended particles in the waste to bind to the floc by adsorption. Cations and anions, including phosphorus and a range of pollutants and toxic compounds, are known to be adsorbed by such polymers (Beech and Cheung 1995; Loaëc *et al.* 1997; Cloete and Oosthuizen 2001). Therefore, the polymer has a critical role in the operation of the activated sludge process (Harris and Mitchell 1973; Forster 1976; Unz and Farrah 1976; Farrah and Unz 1976; Tago and Aiba 1977; Flemming and Wingender 2001a,b). These extracellular polymers (ECPs), also referred to as extracellular polymeric substances (EPSs), are not food reserves, like poly-β-hydroxybutrate, and are not easily decomposed. The EPS matrix is a dynamic system that enables cells in flocs to function in a manner similar to multi-cellular organisms. They have unique sorption properties, especially for metals (Liu *et al.* 2001). Decho (2000) has suggested that sorption of heavy metals is a strategy to protect the bacteria against toxic effects. The surface charges on the microbial cells and bridge formation by polyvalent cations also contribute to flocculation (Deinema and Zevenhuizen 1971; Steiner *et al.* 1976).

Cations, such as calcium and magnesium, have a significant effect on the bulk properties of activated sludge improving both settling and dewatering properties (Higgins and Novak 1997a,b). Ion exchange occurs between the flocs and cations in the wastewater. Divalent ions in the mixed liquor are replaced with monovalent ions leading to weaker polymer bonds and poor sludge characteristics (Novak *et al.* 1998). This is shown where the addition of sodium causes significant deterioration of sludge characteristics (Higgins and Novak 1997b). The exact mechanism is still uncertain with a number of different theories postulated. Zita and Hermansson (1994) suggest that the presence of cations reduces the separation distance between negatively charged bacteria promoting flocculation. However, it appears more likely that cations are involved with flocculation through ionic bridging (Kakii *et al.* 1985; Eriksson and Alm 1991). Biggs *et al.* (2001) used a unique experimental technique to allow on-line analysis of the size of flocs during flocculation. They found that at calcium concentrations below 8 meq l^{-1}, no significant increase in floc size was observed while at concentrations above 8 meq l^{-1}, a dramatic increase in floc size was recorded. Floc density also increased resulting in enhanced settlement rates. However, Cousin and Ganczarczyk (1999) have reported that as floc density increases with the addition of polyvalent cations, there is a decrease in floc porosity.

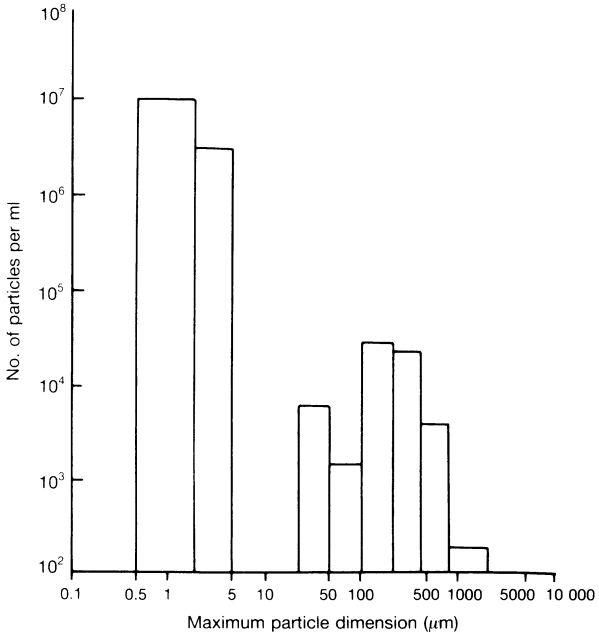


Fig. 5.5. Example of a typical particle size distribution in activated sludge (Parker *et al.* 1971).

Examination of flocs by electron microscopy has shown that granular and amorphous materials are present, and that fine cellulose fibrils are formed. These cellulose fibrils can only be seen by taking ultra-thin sections of sludge flocs and examining them using transmission electron microscopy at magnifications in excess of × 20,000. Young flocs contain actively growing and dividing heterotrophic bacteria with a high rate of metabolism. Older flocs, in contrast, have a lower proportion of viable cells, being composed mainly of dead cells surrounded by a viable bacterial layer. Although the majority of these cells are no longer viable, they retain active enzyme systems. Older flocs have a reduced rate of metabolism but as they are physically larger, they settle far more readily than younger flocs, which are often associated with poor settleability. Weddle and Jenkins (1971) have suggested that the viability of micro-organisms making up flocs is quite low, estimating between 5 and 20% viability. As the floc ages, the slower growing autotrophs become established, especially the nitrifying bacteria, therefore, the concept of sludge age is important in terms of overall efficiency (Sec. 5.2.1). Flocs undergo a secondary colonisation by other micro-organisms such as protozoans, nematodes, and rotifers (Fig. 5.6). The

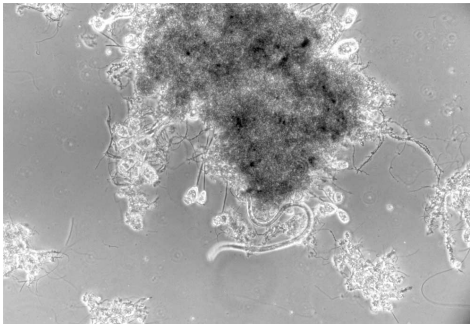


Fig. 5.6. Typical mature floc with secondary colonisation. This particular floc, which has filamentous bacteria growing out into the liquid has been colonised by stalked protozoa (*Vorticella* spp.) and nematodes ($\times$ 200).

ciliate protozoans are considered particularly important as they feed on dispersed bacteria, thus reducing the turbidity of the final effluent, whereas the higher trophic levels present graze on the floc itself, which reduces the overall biomass (Sec. 5.5). A well-flocculated sludge is in a state of dynamic equilibrium between the flocs aggregating into larger flocs and being broken up into smaller flocs by the shear stress imposed by the aeration system (Parker *et al.* 1972; Tench 1979). As flocs have strong binding forces, based on calcium, magnesium, or other multivalent cations, they can tolerate quite high shear stresses. The surface area of flocs has been calculated by dye adsorption and found to range between 43 and 155 m^2 g of floc. The radius of flocs can then be calculated from the specific surface area, assuming a floc to be a homogenous mass of micro-organisms. Therefore, the lowest surface area of 43 m^2 g^{-1} will have a floc radius of 0.58 μm. However, in practice, flocs are considerably larger than this, indicating that the high specific surface area is due to flocs being porous. This is confirmed by electron microscopy that shows flocs to have a spongy appearance (Fig. 5.4). The porous nature explains why flocs are so good at adsorbing particulate matter, and also why the diffusion rate of nutrients and oxygen into the centre of the floc is greater than if flocs were homogenous masses of bacteria (Coackley 1985). There are, however, areas of the floc that are either food or oxygen limited. For example, Matson and Characklis (1976) were able to measure an oxygen concentration profile towards the centre of flocs, with the availability of oxygen limited by the rate of diffusion in larger flocs. The oxygen uptake rate of mixed liquor is significantly increased by breaking up the flocs by homogenisation, thus effectively removing any diffusion limitation (Baillod and Boyle 1970). The role of diffusion in activated sludge

flocs and the effect on plant performance is reviewed by Neethling (1988). The structure of flocs is reviewed by Li and Ganczarczyk (1990) while the dynamic equilibrium between floc growth and floc breakup is examined by Chaignon *et al.* (2002).

The floc morphology between lightly and heavily loaded activated sludge plants varies in a characteristic way. For example, lightly loaded plants have compact flocs with a darker central core or inclusions which are made up primarily of inorganic material such as iron hydroxide, calcium phosphate, and aluminium hydroxide, along with non-biodegradable organic material. The lighter, less dense, outer regions of such flocs are composed of active micro-organisms (Fig. 5.7). This is due to these flocs being older and the floc having undergone repeated periods of active growth and subsequent starvation resulting in a compact floc with the older non-degraded material in the centre. In contrast, heavily loaded plants often form finger-like growths (zoogloeal) in which individual bacteria are embedded in a transparent matrix (Fig. 5.8). It is assumed that growth is rapid with the age

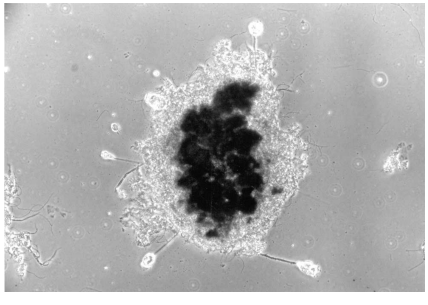


Fig. 5.7. Compact floc with dark central inclusions from an oxidation ditch ($\times 200$).

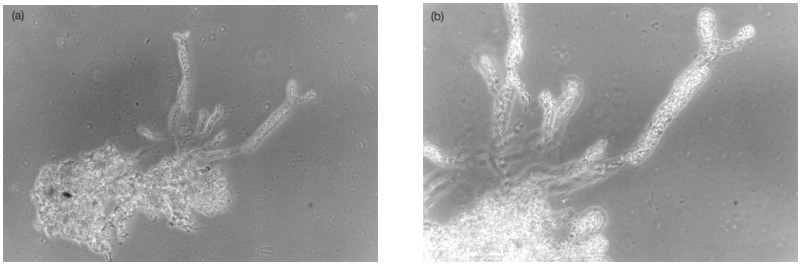


Fig. 5.8. Loosely formed flocs with zoogloeal bacteria forming characteristic finger-like growths from an overloaded conventional activated sludge plant at (a) $\times 200$ and (b) $\times 400$.

of flocs relatively short and so comprised almost entirely of active bacteria. In practice, variability is so great that both inert inclusions and finger-like bacterial growths radiating from the flocs are often seen together. While the diagnostic value of such features remains uncertain, they can, with experience, be an extremely useful diagnostic tool.

In general, good flocculation is associated with low-rate, and poor flocculation with high-rate processes. However, considerable variation is seen between flocs from different sludges and it is this variation that causes operational difficulties such as bulking, deflocculation, pinpoint floc, foaming, and rising sludge (Sec. 5.4). For example, toxic discharges, nutritional imbalance or changes in the microbial ecology of the process can all alter the surface chemistry of the flocs, which in turn will influence the settlement characteristics of the activated sludge.

5.2. Operating Factors

5.2.1. *Process control*

5.2.1.1. *Mixed liquor suspended solids*

The concentration of suspended solids in the aeration tank, commonly referred to as the mixed liquor suspended solids concentration (MLSS), is a crude measure of the biomass within the aeration tank. It is measured in the same way as suspended solids in wastewater: by filtering a known volume of the mixed liquor sample through Whatman GF/C filter paper and weighing it after drying in an oven at 105°C (Department of the Environment 1983a). The MLSS is a basic parameter used in the calculation of a number of other operating parameters and is expressed in mg l^{-1} or gm^{-3}. Some of the MLSS may be inorganic, and under certain circumstances, this may represent a significant proportion of the solids present. To remedy this, many operators estimate the organic fraction of the sludge by measuring the combustible matter present in the MLSS by burning the dried sludge in a muffle furnace at 500°C. This is also expressed in mg l^{-1} and is termed the mixed liquor volatile suspended solids (MLVSS). However, this does not distinguish between the biochemically active and the inert material and, therefore, a more complex technique must be employed to measure the biochemical sludge activity. The proportion of active micro-organisms in the MLVSS will vary depending on operating conditions of the activated sludge unit as well as the amount of volatile solids in the wastewater. Thus, it must be used with caution and not as an accurate estimation of microbial activity.

For day-to-day operational control, the MLSS is quite adequate with the MLVSS and the other measures of sludge activity used mainly in research and development work. Normal MLSS concentrations range from 1,500 to 3,500 mg l^{-1} for conventional activated sludge units rising to 8,000 mg l^{-1} for high-rate systems. The MLSS concentration is controlled by altering the sludge wastage rate. In theory, the higher the MLSS concentration in the aeration tank the greater the efficiency of the process, as there is a greater biomass to utilise the available food. However, high values of MLSS are limited by the availability of oxygen in the aeration tank and by the capacity of the sedimentation unit to separate and recycle activated sludge (Hawkes 1983a).

5.2.1.2. *Sludge residence time or sludge age*

The sludge residence time (SRT) affects the character and condition of the activated sludge flocs within the aeration basin. It is calculated as the total amount of sludge solids in the system divided by the rate of loss of sludge from the system. In practical terms, it is impossible to take into account all the sludge in the various stages of the activated sludge process, including the pipework and sedimentation tank as well as the aeration basin, so a simplified equation is used:

$$t_s = VX/[(Q_w X_w) + (Q_e X_e)]\,,$$

where V is the volume of liquid in the aeration tank (m^3); X the MLSS (mg l^{-1}); Q_w the sludge wastage rate (m^3 d^{-1}); X_w the MLSS (mg l^{-1}) in the waste sludge stream; Q_e the effluent discharge rate (m^3 d^{-1}); X_e the effluent suspended solids concentration (mg l^{-1}), and t_s the SRT in days (Fig. 5.9).

If the proportion of microbial cells in the MLSS is assumed constant, the SRT can be referred to as either the mean cell residence time (MCRT) or the sludge age. If the system is balanced with the amount of sludge in the aeration tank constant VX, then the sludge wastage $Q_w X_w$ will represent the net sludge production of the system (kg dry solids d^{-1}). SRT is an operational factor giving control over sludge activity because SRT is the reciprocal of the net specific growth rate of the sludge and thus can be considered as a measure of sludge activity. A low SRT (< 0.5 d) indicates a sludge with a high growth rate as used in high-rate units for pretreatment or partial treatment; a high SRT (> 5 d) indicates a low growth rate sludge, such as extended aeration systems (Table 5.2). Conventional activated sludge has a SRT of between 3 and 4 d, and has good settling properties.

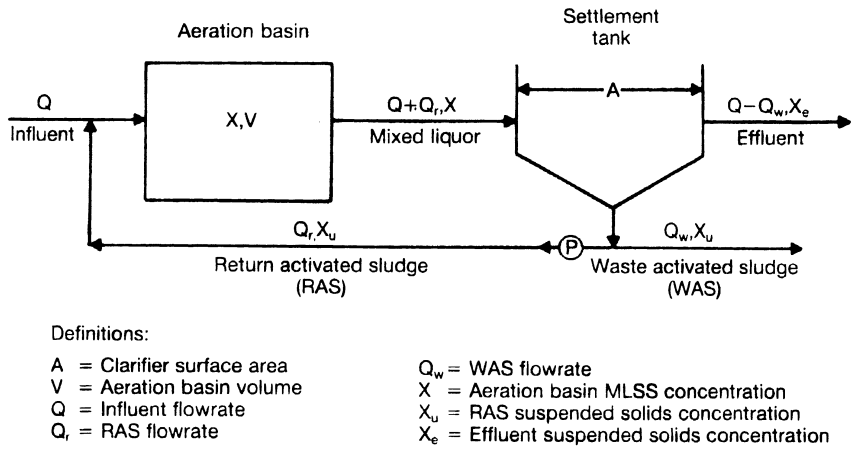


Fig. 5.9. Schematic diagram of the contiuous flow activated sludge process.

However, at SRT above 6 d or between 0.5 and 3 d, there is a reduction in settleability. SRT is controlled by altering the sludge wastage rate.

5.2.1.3. *Plant loading*

Three loading factors are used in the design and operation of activated sludge. The volumetric loading, which is the flow of wastewater in relation to the aeration tank capacity; organic loading, which is the BOD load in relation to the aeration tank capacity; and sludge loading, which is the BOD load in relation to the total biomass of activated sludge.

Volumetric loading

The retention time of the wastewater in the aeration tank, the hydraulic retention time (HRT), is expressed as:

$$\mathrm{HRT} = (V \times 24)/Q \ \mathrm{h}\,,$$

where V is the liquid capacity of the aeration tank (m^3) and Q the rate of flow of influent wastewater to the tank ($m^3\ d^{-1}$), so that the hydraulic retention time is expressed in hours.

The HRT as measured here does not take into account the flow of recycled activated sludge returned to the aeration tank which may represent between 25 and 50% of the overall flow, making the actual retention time much less than calculated. For this reason, the HRT is often referred to as the "nominal retention time". The HRT must be sufficiently long to

Table 5.2. Comparison of loading and operation parameters for different activated sludge treatment rates (Hawkes 1983a).

Treatment rate	Retention period (h)	BOD loading per capacity kg BOD $m^{-3}d^{-1}$	Sludge loading (f/m) kg BOD $kg^{-1}d^{-1}$	Sludge age (d)	Sludge production kg dry sludge per kg BOD removed	Application
Median	5–14	0.4–1.2	0.2–0.5	3–4	0.5–0.8	Conventional treatment for medium and large works to produce 20:30 effluent with or without nitrification depending on loading within range
High	1–2	> 2.5	> 1.0	0.2–0.5	0.8–1.0	For pretreatment or partial treatment
Low	24–72	< 0.3	0.1	> 5–6	0.4	Extended aeration for full treatment on small works. Effluent highly stabilised but may contain fine solids

allow the required degree of adsorption, flocculation, and mineralisation. In conventional activated sludge systems, the HRT will be at least 5 hours at DWF. However, because of fluctuations in flow and the recycling of sludge, the actual retention time is much less and during a storm, when maximum volumetric loadings are being received (3 DWF), the sludge recycle is as much as 1.5 DWF, and the actual retention time may be as short as 1 h (Hawkes 1983a).

Organic loading

Wastewaters have different organic contents and it is useful to express loadings in terms of kg BOD per tank capacity per day:

$$\text{Organic loading} = (Q \times \text{BOD})/(V \times 1000) \text{ kg BOD}_5 \text{ m}^{-3} \text{ d}^{-1},$$

where BOD_5 is the biochemical oxygen demand of the influent wastewater (mg l^{-1}). The organic loading is expressed as kg BOD_5 m^{-3} d^{-1}, therefore, an activated sludge unit with a retention time of 4.5 h and a settled sewage BOD_5 of 240 mg l^{-1} will have an organic loading of

$$(240 \times 24)/(4.5 \times 1000) = 1.28 \text{ kg BOD}_5 \text{ m}^{-3} \text{ d}^{-1}.$$

In essence, the higher the organic loading the greater the BOD_5 of the final effluent (Table 5.2).

The rate of BOD_5 removal (g $BOD_5 d^{-1}$) in the aeration tank can be calculated as

$$(\text{Influent BOD}_5 - \text{Effluent BOD})/\text{HRT}.$$

Although this works for completely mixed reactors that are continuously loaded, in plug-flow systems the influent wastewater is diluted by the recycled activated sludge. Thus, the BOD_5 concentration at the inlet must be calculated separately as

$$[(Q \times \text{Influent BOD}_5) + (Q_\text{r} \times \text{Effluent BOD})]/(Q + Q_\text{r}),$$

where Q_r is the sludge recycle rate (m^3 d^{-1}). The BOD_5 removal rate can then be calculated in the normal way.

Sludge loading

With the biomass actively removing the organic fraction of the wastewater, it follows that the BOD_5 loading should be related to the amount of

activated sludge in the aeration tank. The sludge loading is referred to as the food (f) to micro-organism (m) ratio and is calculated as

$$\begin{aligned} f/m &= \text{Organic loading/Volume of sludge} \\ &= (Q \times \text{BOD})/(V \times X) \text{ g g}^{-1} \text{ d}^{-1}, \end{aligned}$$

where X is the MLSS in the aeration tank. The f/m ratio is expressed as grams BOD_5 per day per gram MLSS (g g^{-1} d^{-1}) or alternatively kg kg^{-1} d^{-1}. It can also be calculated using the expression

$$(\text{BOD}/X)(24/\text{HRT}).$$

Thus, for a conventional activated sludge with a HRT of 4.5 h, influent BOD_5 of 240 mg l^{-1} and a MLSS concentration of 2500 mg l^{-1}, the sludge loading is

$$(240/2500)(24/4.5) = 0.51 \text{ g g}^{-1} \text{ d}^{-1}.$$

When the f/m ratio is high, the micro-organisms are in the exponential growth phase. With excess food, the rate of metabolism is at a maximum with large BOD_5 removals achieved. However, under these conditions the micro-organisms do not form flocs but are generally dispersed making, it difficult to settle and form a recyclable sludge. Because food is in excess, not all the organic material will be utilised and the remainder will pass out in the final effluent, giving a high BOD_5. In contrast, low f/m ratios put the micro-organisms into a food-limited environment even though the rate of metabolism may be high when the recycled micro-organisms are first mixed with the incoming wastewater. Once food becomes limiting, the rate of metabolism will rapidly decline until the micro-organisms are in the endogenous respiration phase with cell lysis and resynthesis taking place. Under low sludge loadings there is almost complete oxidation of organics resulting in a high quality effluent with the micro-organisms flocculating and settling rapidly (Fig. 5.10).

In theory, the sludge loading is related to SRT because sludge activity increases if the organic loading is increased, resulting in an increased rate of sludge growth. Therefore, in order to maintain a constant sludge concentration (MLSS), the sludge wastage rate must be increased, thus reducing the SRT. Therefore, it appears that the f/m ratio is approximately inversely proportional to SRT, although the work of Ekama and Marais (1979) does not support this.

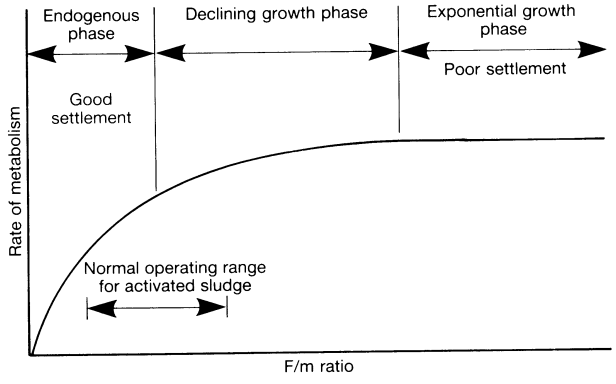


Fig. 5.10. The rate of metabolism of the micro-orgamism in the activated sludge aeration tank is controlled by the f/m ratio (adapted from Viesmann and Hammer 1985).

5.2.1.4. *Sludge settleability*

Most problems associated with the activated sludge process involve poor settleability. Therefore, a rapid method of assessing settleability is vital if good separation of the sludge in the secondary tank is to be maintained, ensuring adequate sludge recycle and a final effluent with a low suspended solids concentration. Four indices can be used to assess the settling qualities of activated sludge, the sludge density index (SDI) and the sludge volume index (SVI), the latter being the most widely used (Keeper 1963; Dick and Vesilind 1969), the diluted sludge volume index (DSVI), and the specific sludge volume index (SSVI) (Jenkins *et al.* 1993). All these methods have been described in detail in Sec. 2.2.3.

The SDI and SVI are both calculated by settling the mixed liquor in a cylinder for 30 minutes and then measuring the volume of settled sludge. However, it has been demonstrated that within 30 minutes of quiescent settlement, the process of sedimentation will have proceeded past the hindered settlement stage in which all the sludge flocs are evenly distributed (Type III settlement), and that transitional or even compression settlement phases may have started (Type IV) (Sec. 2.2.1). To minimise the effects of consolidation under compression, shorter periods of settlement for the test have been proposed (Finch and Ives 1950), or taking a series of readings over a slightly longer period and plotting the settlement curve. However, the problem has been adequately overcome by a modification of the SVI where settlement is measured while the mixed liquor is gently stirred at a constant temperature. This more accurate assessment of sludge settleability, known

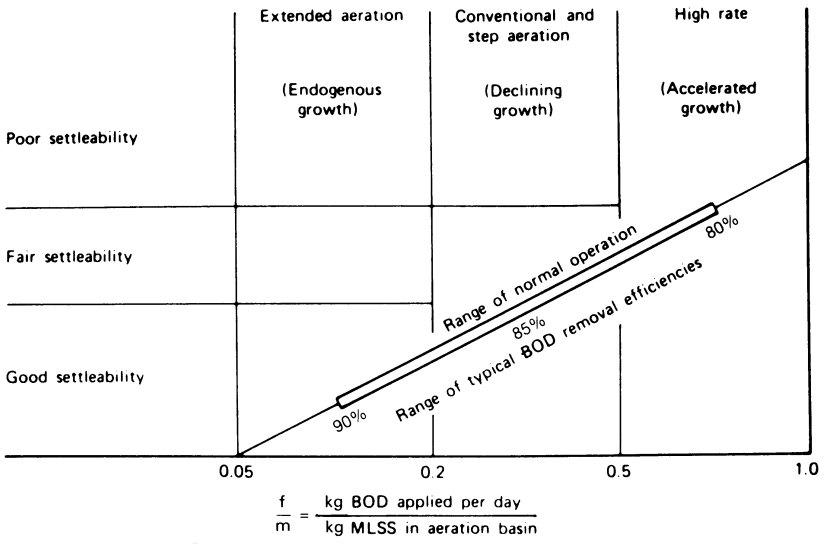


Fig. 5.11. Settleability of activated sludge related to the f/m ratio (Hammer 1977).

as the stirred specific volume index (SSVI), is described and compared with the SVI test in Sec. 2.2.3 (White 1976; Rachwal *et al.* 1982).

Sludge settleability is dependent on the microbiological, biochemical, and physico-chemical properties of the sludge. For example, a direct relationship between the bound water content of flocs and the SVI has been reported by Heukelekian and Weisberg (1956), whereas Forster (1968, 1971) has shown that the surface charge on flocs is directly proportional to the SVI. Under normal operating conditions, activated sludge settleability depends on the sludge loading, although good settling sludges are obtained from both conventional and high-rate loaded units. In the USA a functional relationship between sludge loading and SVI has been developed (Fig. 5.11), although the ranges and values quoted are very approximate.

Activated sludge will settle well if the SVI < 100 (SDI > 1), whereas a SVI > 150 (SDI < 0.66) indicates settling problems and possibly bulking (Fig. 5.12). In terms of SSVI, a good sludge has a value below 120 ml g^{-1}, whereas a bulking sludge or one with poor settleability has a value above 200 ml g^{-1}.

5.2.1.5. *Sludge activity*

Although theories and working models of activated sludge behaviour and performance have been developed using measures of microbial biomass

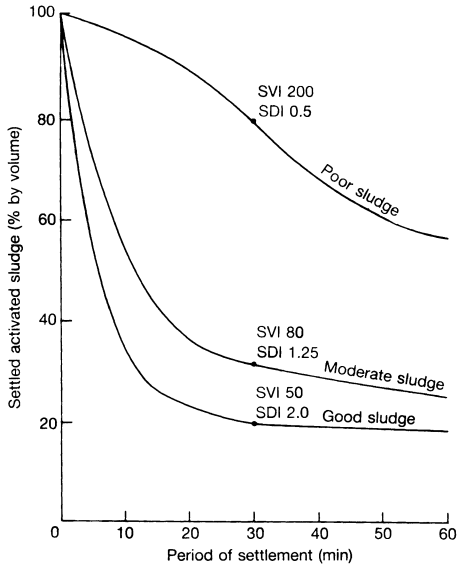


Fig. 5.12. Comparison of settling characteristics of different activated sludges using the sludge volume index and sludge density index (Hawkes 1983a).

such as MLSS or MLVSS, it has not been possible to relate bacterial numbers directly to performance. The problems of estimating bacterial numbers, of differentiating viable and non-viable cells, and of estimating levels of activity due to age of the cell, have all proved extremely difficult with the mixed populations of bacteria associated with activated sludge. Therefore, other methods of assessing biological activity have been developed using chemical analyses rather than direct counting techniques. The most widely used biochemical measure of biological activity is adenosine triphosphate (ATP), which is used to measure the number of viable cells present in activated sludge (Roe and Bhagat 1982). Deoxyribonucleic acid (DNA), although less sensitive than ATP, appears to be a function of the type and the number of organisms present in activated sludge (Gentelli 1967; Bardtke and Thomanetz 1976; Raebel and Schlierf 1980). Enzymatic activity, especially protease activity, has also been used (Thiel and Hattingh 1966; Sridhar and Pillai 1973; Vankova *et al.* 1980). The electron transfer process associated with oxidation offers a range of co-enzymes that could be used as potential indicators of sludge activity. The rate of electron transfer is reflected by the activity of nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), both of which can be used as a measure of metabolic activity in activated sludge (Jones and Prasad 1969; Dickson

1983). Dehydrogenase activity in activated sludge can be measured using the dye triphenyl tetrazolium chloride (TTC), which is reduced to a red dye triphenyl formazan (TF) in the presence of dehydrogenase enzymes. It is readily extracted from activated sludge and its concentration determined using a spectrophotometer. Unlike the other techniques for measuring sludge activity, which are complicated and based in the analytical laboratory so that they cannot be used for routine operational control, TF analysis is more straightforward and can be used for routine activated sludge operation (Coackley and O'Neill 1975). Also, the dehydrogenase response to changes is very rapid and Coackley and O'Neill (1975) observed that the increase in dehydrogenase activity, in response to the addition of organic matter to activated sludge, occurs much faster than can be accounted for simply by an increase in bacterial numbers. Another method of determining sludge activity is based on the assimilation of glucose. A standard glucose solution is added to the sample of activated sludge, which is continuously aerated in a small reactor. A glucose selective electrode is used to measure its assimilation by the activated sludge and sludge activity is then calculated on the basis of the reaction time required for 50% assimilation of glucose. There is a problem with interference with the dissolved oxygen concentration, therefore the dissolved oxygen concentration also has to be measured so that the electrical response of the glucose electrode can be corrected with that of the oxygen electrode (Olah and Princz 1986). Respirometry, defined as the measurement and interpretation of the oxygen uptake rate, is one of the most popular methods for the characterisation of wastewater composition and activated sludge biokinetics (Spanjers *et al.* 1998; Vanrolleghem *et al.* 1999; Gernaey *et al.* 2001; Peterson *et al.* 2002). Oxygen uptake rate (OUR) is a widely used measure of biomass activity and has been related to estimations of viable biomass (Jörgensen *et al.* 1991). Ziglio *et al.* (2002) have proposed the use of fluorescent dyes and multi-parameter flow cytometry for rapid and direct viability/activity assessments of activated sludge samples.

By being able to estimate the biological capacity of the activated sludge, system loadings including toxic substances, can be based on the functional relationship between the concentration of the waste and sludge activity, thus optimising performance and reducing the possibility of overloading or inhibition. Direct toxicity assessment (DTA) is now widely used to protect biological treatment processes, such as activated sludge, from toxic compounds in wastewater influents (Sec. 3.6). For example, Dalzell and Christofi (2002) describe a simple and cheap ATP luminescence method that can be used as a toxic bioassay to protect activated sludge systems. Practical applications of toxicity control are discussed by Ko *et al.* (2002).

5.2.1.6. *Recirculation of sludge*

Sludge is returned to the aeration tank in order to maintain sufficient microbial biomass for oxidation of the wastewater. Assuming no sludge wastage, this relationship is expressed as

$$Q_{\mathrm{r}}/(Q + Q_{\mathrm{r}}) = V/1000\,,$$

where Q is the mean flow of influent wastewater to the aeration tank ($\mathrm{m}^3\ \mathrm{d}^{-1}$), Q_{r} the sludge recycle rate ($\mathrm{m}^3\ \mathrm{d}^{-1}$), and V the volume of settled solids after 30 minutes in a 1000 ml graduated cylinder. Using this relationship, the volume of recirculated activated sludge Q_{r} can be calculated by

$$Q_{\mathrm{r}} = (V \times Q)/(1000 - V)\,,$$

as can the solids concentration in the recirculated sludge (X_{r}) and in the wasted sludge (X_{w}),

$$X_{\mathrm{r}} = 1.0 \times 10^6/\mathrm{SVI}\,.$$

The ratio of sludge returned to the aeration tank Q_{r} is normally expressed as a percentage of the influent wastewater Q,

$$Q_{\mathrm{r}}/Q = [V/(1000 - V)]\,100\,.$$

Thus, if the return activated sludge rate is 20% and the influent wastewater into the aeration tank is 1 $\mathrm{m}^3\ \mathrm{s}^{-1}$, then the recycled sludge is equivalent to 0.2 $\mathrm{m}^3\mathrm{s}^{-1}$.

For example, for a conventional plant with a retention time of 4.5 h and an influent wastewater flow rate of 1 $\mathrm{m}^3\ \mathrm{s}^{-1}$, an influent BOD of 240 mg l^{-1}, a MLSS of 2500 mg l^{-1}, a sludge loading of 0.519 $\mathrm{g}^{-1}\ \mathrm{d}^{-1}$, and a volume V of settled solids in a 1000 ml cylinder after 30 minutes of 240 ml, the SVI, the volume of recirculated activated sludge Q_{r}, the solids concentration of the sludge X_{r} and the ratio of returned sludge to influent wastewater Q_{r}/Q are

Sludge volume index

$$\mathrm{SVI} = (240 \times 1000)/2500 = 96\ \mathrm{ml}\ \mathrm{g}^{-1}\,;$$

Volume of recirculated sludge

$$Q_{\mathrm{r}} = (240 \times 86,400)/(1000 - 210) = 27284\ \mathrm{m}^3\ \mathrm{d}^{-1}\,;$$

Solids concentration in the recirculated sludge

$$X_{\mathrm{r}} = (1,000,000/96) = 10417\ \mathrm{mg}\ \mathrm{l}^{-1}\,;$$

Ratio of returned sludge

$$(Q_{\mathrm{r}}/Q) = 240/(1000 - 240) \times 100 = 32\%\,.$$

5.2.2. *Factors affecting the process*

The activated sludge process is affected by the nature of the wastewater being treated as well as environmental, climatic, and hydrological factors.

Biological activity of sludge flocs and their settling characteristics are affected by wastewater composition. In conventional activated sludge, a BOD:N:P ratio of 100:6:1 is required to maintain the optimal nutrient balance for heterotrophic activity which is equivalent to an operational range of 0.03–0.06 kg nitrogen and 0.007–0.01 kg potassium per kg BOD. The presence of toxic or inhibitory substances affects the metabolic activity of aerobic heterotrophs, although activated sludge does become acclimatised to low concentrations with time, and can be used to treat potentially toxic wastes such as phenolic wastewaters. Unlike fixed-film reactors, activated sludge systems are sensitive to fluctuations in the organic strength of the wastewater, which seriously affects sludge characteristics and the SVI, with carbohydrates in particular encouraging filamentous and zoogloeal growths in the sludge, causing a rapid increase in SVI. Thus, an important aspect of operational management of activated sludge systems is to ensure that strong organic loads due to the circulation of stormwater or the return of supernatant liquor from sludge consolidation or digestion are prevented. When hard detergents were being used widely, deep layers of foam would accumulate on the surface and around the aeration basin, especially those using diffusers. Foaming due to detergents made operation difficult and dangerous, with a loss of MLSS from the aeration tank as the flocs became entrained in the foam. Since the introduction of soft detergents, detergent foaming has been markedly reduced except where hard detergents still need to be used for specialised industrial purposes. Other chemicals, such as polyglycols and alkyl phenoxy compounds, can also cause foaming and where it occurs, foam suppression is required, by spraying with recycled effluent or the application of oil-based anti-foam compounds (Sec. 5.4).

Increase in the temperature of the mixed liquor during the summer results in an increase in metabolic activity. In the activated sludge system, all the biochemical reaction rates, such as organic substrate stabilisation, production of cellular material, maintenance energy requirements, oxygen utilisation, auto-oxidation of cellular mass, and nitrification, follow the Arrhenius relationship over the 5–20°C range. However, except for the substrate utilisation rate, they do not conform to this relationship above 25°C (Randall *et al.* 1982). Whereas increases in the temperature of the mixed liquor enhance the BOD removal of activated sludge systems, in practice it leads to problems of increased oxygen demand, which can lead to the system becoming oxygen-limited. Also, in warmer weather there is a

significant increase in the incidence of bulking (Sec. 5.4). Lin and Heinke (1977a,b) considered the temperature to be of major importance in explaining the variation in performance of activated sludge systems and found that aeration tank values should be larger for lower temperature operation. They suggested that a much higher performance could be achieved if hot compressed air was used for aeration, although whether this would be economically feasible at normal treatment plants is uncertain.

Oxygen is only slightly soluble in water, having a saturation concentration of 9.07 mg l^{-1} at 20°C (Table 5.3). It is this low saturation concentration that limits the rate at which oxygen can dissolve from the atmosphere into water. The solubility of oxygen will also decrease when either the water temperature or salinity are increased, or will also decrease as the pressure decreases. The transfer of oxygen is represented by the two-film theory. When air and water are in contact, either at the surface of the liquid or as a bubble submerged within the liquid, five discrete regions can be identified (Fig. 5.13). The bulk liquid; a thin static liquid film; the bulk gas (possibly the atmosphere); a thin static gas film; and the interface between the two films. The transfer of oxygen through the gas and water films is by molecular diffusion, a mass transfer phenomenon. The transfer of oxygen can be assumed to be in three phases: the diffusion of oxygen from the bulk gas through the gas film to the interface; the passage across the interface; and finally diffusion through the liquid film into the bulk of the liquid.

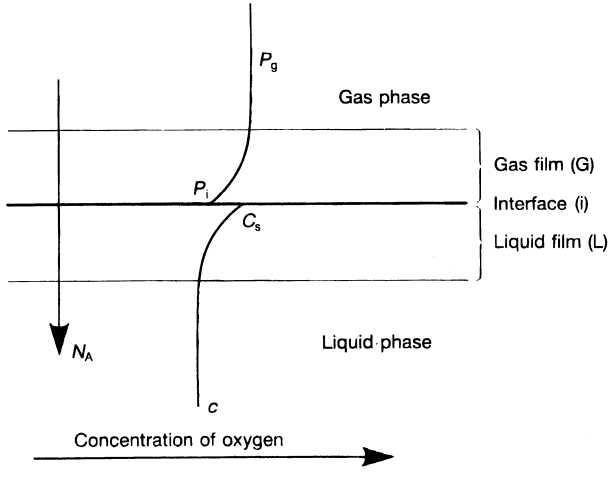


Fig. 5.13. The rate of oxygen transfer (N_A) between the gas bubble and the water via the air/water interface is illustrated, showing the variation in both the oxygen pressure and concentration.

Table 5.3. Variation in the dissolved oxygen saturation concentration with temperature.

Temperature (°C)	Dissolved oxygen (mg l^{-1})
0	14.6
1	14.2
2	13.8
3	13.4
4	13.1
5	12.8
6	12.4
7	12.1
8	11.8
9	11.6
10	11.3
11	11.1
12	10.8
13	10.5
14	10.3
15	10.1
16	9.9
17	9.7
18	9.5
19	9.3
20	9.1
21	8.9
22	8.7
23	8.6
24	8.4
25	8.2
26	8.1
27	8.0
28	7.8
29	7.7
30	7.5

The resistance to the transfer of oxygen is therefore the summation of the gas film resistance, interfacial resistance and the liquid film resistance. The resistance to diffusion through the gas film is negligible when compared to the resistance through the water film. Due to the high diffusion resistance through the liquid film, the partial pressure gradient, which is the driving force of diffusion on the atmospheric side of the interface $P_g - P_i$,

approaches zero. So the main driving force responsible for oxygen transfer is the concentration gradient between the liquid film and the bulk liquid $C_s - C$ (i.e. oxygen transfer is liquid film controlled).

Based on this theory the rate of oxygen transfer dm/dt can be expressed as

$$dm/dt = (D_L/Y_L)A(C_s - C) = K_L A(C_s - C)\,,$$

where dm/dt is the rate of mass transfer of oxygen, D_L the molecular diffusivity of oxygen through the liquid film, Y_L the film thickness, A the interfacial area through which diffusion is occurring, K_L the mass transfer coefficient (i.e. the oxygen diffusivity divided by the film thickness), C_s the saturation concentration of dissolved oxygen (mg l^{-1}), and C the concentration of dissolved oxygen in the bulk liquid (mg l^{-1}).

The rate of mass transfer is directly proportional to the interfacial area and the oxygen deficit $C_s - C$, while inversely proportional to the thickness of the water film. Thus, maximum rates of transfer will occur when $C = 0$. Turbulence past the water film will reduce the film thickness $[Y_L]$, which increases the rate of transfer. The degree of turbulence is controlled by the size and the geometric configuration of the aeration tank, the aeration device, and the rate of aeration in terms of submergence (mechanical aerators) or air pumpage (air diffusers). An increase in the interfacial area (i.e. the ratio of air water contact) will also increase the oxygen transfer efficiency (Table 5.4).

The rate of mass transfer can be expressed in terms of concentration by dividing the previous equation by the volume of liquid in the aeration tank,

$$(1/V)(dm/dt) = dc/dt = (K_L A/dt)(C_s - C)\,,$$

where dc/dt is the rate of change in the oxygen concentration and V the volume of liquid aerated.

The problem in solving either equation is the difficulty of measuring the total interfacial area $[A]$. This is overcome by substituting an overall value

Table 5.4. The volume of air required and the oxygen transfer efficiency both vary for different bubble sizes in diffused air systems.

Bubble size (mm)	Air volume required (m^3) per kg BOD removed	Oxygen transfer efficiency (%)
Fine (1.5)	36–72	11
Medium (1.5–3)	60–120	6.5
Coarse (3)	70–140	5.5

$[a]$ for A/V so that the oxygen transfer rate is $[K_{\mathrm{L}}a]$. Thus

$$\mathrm{d}c/\mathrm{d}t = K_{\mathrm{L}}a(C_{\mathrm{s}} - C)\,.$$

Integrating this equation gives

$$^{C}\int_{c_0} \frac{\mathrm{d}c}{C_{\mathrm{s}} - C} = K_{\mathrm{L}}a\ ^{t}\int_{t_0} \mathrm{d}t\,.$$

So

$$K_{\mathrm{L}}a = \frac{1}{t - t_0}\, l_{\mathrm{n}}\, \frac{C_{\mathrm{s}} - C_0}{C_{\mathrm{s}} - C}\,.$$

Here C_0 is the dissolved oxygen concentration at time t_0 and C the dissolved oxygen concentration at time t. Using this equation, the rate of oxygen, transfer can be calculated.

The oxygen transfer rate $[K_{\mathrm{L}}a]$ is dependent upon the temperature, increasing as the temperature increases. It is convention, however, to quote the $[K_{\mathrm{L}}a]$ value at a standard temperature of 20°C. It is possible to calculate the oxygen transfer rate $[(K_{\mathrm{L}}a)_2]$ for any temperature T_2 if the corresponding quantity $[(K_{\mathrm{L}}a)_1]$ is known for some other temperature T_1 by

$$\theta^{(T_2 - T_1)} = (K_{\mathrm{L}}a)_2/(K_{\mathrm{L}}a)_1\,,$$

where θ is the temperature coefficient which approximates to 1.024 over the range 10–30°C.

As $[K_{\mathrm{L}}a]$ is normally expressed at 20°C, then

$$(K_{\mathrm{L}}a)_T = (K_{\mathrm{L}}a)_{20}(1.024)^{T-20}\,,$$

where T is the temperature (°C) at which $[K_{\mathrm{L}}a]$ has been measured, so that

$$(K_{\mathrm{L}}a)_{20} = (K_{\mathrm{L}}a)_T/(1.024)^{T-20}\,.$$

Values of 1.024^{T-20} for different temperatures are given in Table 5.5. However, in calculating $[K_{\mathrm{L}}a]$ the effect of the temperature on the oxygen saturation concentration $[C_{\mathrm{s}}]$ is also important (Table 5.3). The effect of the temperature on $[C_{\mathrm{s}}]$ is about equal and opposite to that on $[K_{\mathrm{L}}a]$, so that for practical purposes, the effect of the temperature on $\mathrm{d}c/\mathrm{d}t$ can be neglected. This is clearly shown in Table 5.6.

The oxygen requirement in conventional activated sludge depends on the SRT and the operating temperature. Benjes (1980) gives a typical value of 1.1 kg of oxygen per kg BOD at a SRT of 7 d at 10–20°C. If the SRT is long enough for nitrification to occur, then the oxygen requirement will be greater due to nitrogenous demand. Therefore, at a SRT of 5 d, 1 kg BOD

Table 5.5. Temperature correction factors θ.

Temperature (°C)	$(1.024)^{T-20}$	Temperature (°C)	$(1.024)^{T-20}$
0	0.622	16	0.909
1	0.637	17	0.931
2	0.653	18	0.953
3	0.668	19	0.977
4	0.684	20	1.000
5	0.701	21	1.024
6	0.717	22	1.048
7	0.735	23	1.074
8	0.752	24	1.100
9	0.770	25	1.126
10	0.789	26	1.153
11	0.808	27	1.181
12	0.827	28	1.209
13	0.847	29	1.238
14	0.867	30	1.267
15	0.888		

Table 5.6. The effect of temperature on oxygen transfer rate $\mathrm{d}c/\mathrm{d}t$ over a temperature range typically found in activated sludge aeration tanks. Where C and $[(K_\mathrm{L}a)_{20}]$ are assumed to be 2 mg l^{-1} and 5 h^{-1}, respectively.

Temperature (°C) (T)	C_s(mg l^{-1})	$(K_\mathrm{L}a)_\mathrm{T}$	$\frac{\mathrm{d}c}{\mathrm{d}t}$
14	10.29	4.34	35.95
12	10.77	4.14	36.27
10	11.28	3.94	36.60
8	11.84	3.76	37.01
6	12.45	3.59	37.49

Mean value for $\mathrm{d}c/\mathrm{d}t = 36.66 \pm 1.65\%$

requires 1 kg O_2 for carbonaceous oxidation, and a further 4.3 kg of oxygen will be required to oxidise 1 kg ammonical nitrogen (Lister and Boon 1973). In the aeration tank, aerobic activity is independent of dissolved oxygen concentration above a minimum "critical" concentration, below 1.0 mg l^{-1} for carbonaceous oxidation, below which metabolism is limited by a reduced oxygen supply. Minimum critical concentrations range from 0.2 to 2.0 mg O_2 l^{-1}, depending on the MLSS and other operating factors. As biological activity is just as great at low as at high dissolved oxygen concentrations

and as the oxygen transfer rate is proportional to the oxygen deficit in the mixed liquor, it is logical to operate the aeration tank as close to the critical minimum dissolved oxygen concentration as possible (Fig. 3.22). There is a reduction in concentration gradient as oxygen diffuses into sludge flocs. However, in practice, the dissolved oxygen concentration should not be allowed to rise above 2.0 mg O_2 l^{-1} but should never fall below 0.5 mg O_2 l^{-1} for carbonaceous oxidation, whereas for nitrification the mixed liquor should be maintained as near to the 2.0 mg O_2 l^{-1} concentration as possible. To maintain the critical oxygen concentration in aeration tanks, the dissolved oxygen concentration is automatically maintained by oxygen electrodes which match the rate of aeration to the rate of oxygen utilisation by the mixed liquor. While the most accurate control of the aeration rate, using a dissolved oxygen electrode, is achieved by variable or multi-speed drives to the aerators, or by adjustable outlet weirs to alter the immersion depth; the cheapest and most widely used method is the intermittent switching on or off of selected aerators when the dissolved oxygen concentration falls below or exceeds the critical oxygen limits set. The latter system normally incorporates a timer override so that the aerators will automatically be switched on again, regardless of the dissolved oxygen concentration, after a specified period, usually 30 minutes. Such systems normally pay for themselves by savings in energy consumption within 12 months (Ainsworth and Gill 1987). The oxygen electrodes are normally positioned in the corner of the tank where lowest oxygen concentrations can be expected, or near the inlet where the oxygen demand is greatest (Lwein and Henley 1972). The dissolved oxygen concentration tends to increase towards saturation concentration as the tank outlet is approached. Therefore it is vital, if energy is to be saved, to match oxygen supply to actual demand by treating aeration tanks as a series of independently controllable zones, each with an associated dissolved oxygen probe with appropriate settings (Barnard and Meiring 1988). The positioning of oxygen electrodes in plug-flow systems such as oxidation ditches is also dependent on other factors. Such a control system is vital for optimum and economic operation, as shown in Fig. 5.14. Supplying extra oxygen for nitrification is very expensive and can be overcome by the use of an anoxic zone. Here, the recycled nitrified effluent is denitrified while in admixture with the settled sewage in a separate anoxic area before entering the main aeration tank. The low oxygen conditions allow facultative anaerobes to use nitrates as a source of oxygen. The reduction of 1 mg l^{-1} of nitrate to nitrogen gas provides 2.85 mg l^{-1} of oxygen, which is used to reduce BOD loading to the main aeration tank.

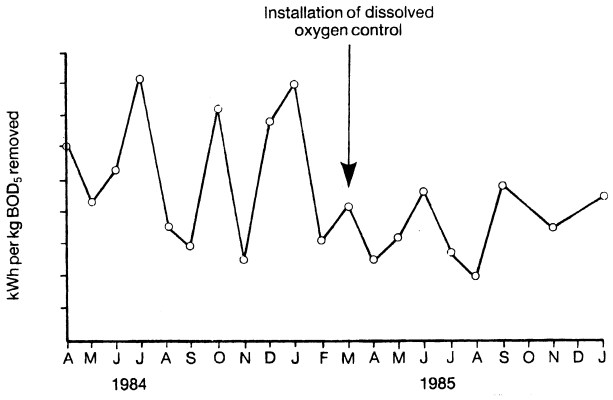


Fig. 5.14. Energy utilisation at Rochdale Sewage Treatment Works before and after installation of oxygen control (Ainsworth and Gill 1987).

A common problem in activated sludge design is that the aerators are not capable of supplying sufficient oxygen for the level of treatment required. In practice this leads to aerators being left on for twenty-four hours a day at maximum immersion. Even then, oxygen stress or even anaerobic conditions may occur during high organic loading period each day. Boon (1983) has estimated R, the oxygen required for carbonaceous oxidation in a conventionally loaded plant as

$$R = 0.75\,B + 0.048\;MV(\text{kg O}_2\ \text{d}^{-1})\,,$$

where B is the BOD removal rate (kg d^{-1}), M the MLSS (kg m^{-3}), and V the aeration tank capacity (m^3). A similar method of estimating the oxygen required in an aeration ditch, which incorporates nitrification and denitrification, has been developed by Johnstone and Carmichael (1982).

A sudden increase in the hydraulic loading to the aeration tank, due to storms or recirculation of wastewater within the plant, will increase the discharge of mixed liquor to the sedimentation tank. Where there is a constant rate of return of sludge, this will result in a reduction of the MLSS in the aeration tank, with more sludge stored within the sedimentation tank. This will result in the sludge being stored for longer periods within the sedimentation tank before being recycled, which may adversely affect the viability of the micro-organisms comprising the sludge.

Increased flows also reduce the effectiveness of the sedimentation tanks by increasing the upward flow rate, which will extend the sludge blanket towards the surface with the possibility that some of the sludge will be discharged with the final effluent. This will be particularly significant if the

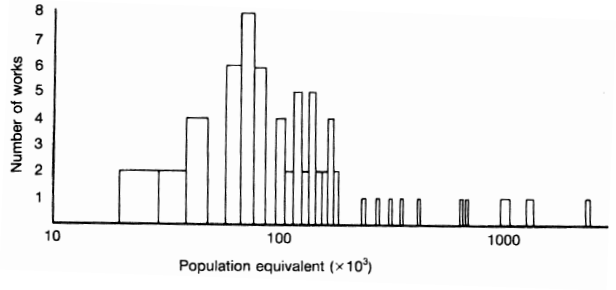
SVI is high. Sudden storms will scour out the deposited stale accumulations in the sewerage system and carry it to the treatment plant. This will result in a shock load of organically rich wastewater to the aeration tank, which will adversely alter the characteristics of the sludge. In practice, the flow to an activated sludge aeration basin is normally greater than the design loading (i.e. 3 DWF for large and 6 DWF for small treatment plants), thus any increase in the hydraulic or organic loading will have a serious effect on operation.

5.2.3. *Aeration methods*

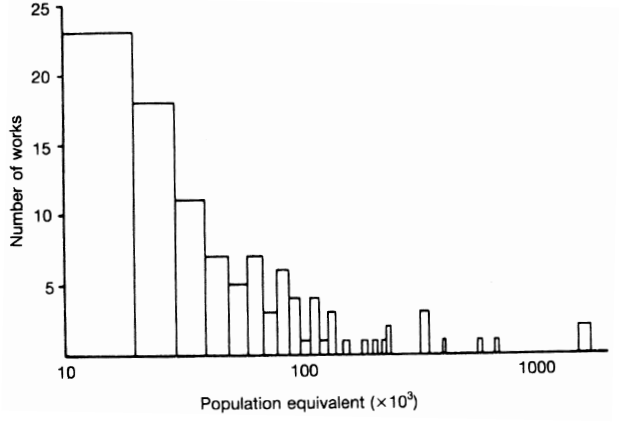
The main functions of the aeration mechanism are summarised in Table 5.7. In essence, it is to provide sufficient oxygen in order to satisfy the respiratory demand of the microbial biomass and to maintain this biomass in suspension. Aeration and mixing in the aeration tank is normally achieved by using either mechanical aeration, that is using surface aerators with either a vertical or horizontal shaft, or by air diffusion. Surface aerators using vertical shafts or air diffusion are widely used for conventional and high-rate systems, while surface aerators using horizontal shafts are used for extended aeration systems employing oxidation ditches. Of the 250 activated sludge plants serving populations in excess of 10,000 in the UK, approximately 40 use fine bubble diffused air aeration while 100 use vertical shaft systems. The remainder use a variety of other systems, although only a few employ coarse bubble diffused air. The size range of plants employing these two types of aeration are shown in Fig. 5.15, where it is can be seen that surface aerators are preferred for smaller plants. For example, according to Chamber and Jones (1988), while there is only one diffused air plant serving a population of below 30,000, over 50% of the surface aeration plants serve populations of below 40,000. The total population served by each system is very similar at 11.2 and 11.5 million persons respectively. In recent years there is an increasing trend to use diffused aeration in all new activated sludge plants regardless of size.

Table 5.7. Main functions of aeration units in the activated sludge process.

1. To ensure an adequate and continuous supply of dissolved oxygen for bacteria.
2. To keep the mixed liquor in suspension.
3. To mix the incoming wastewater and the mixed liquor.
4. To remove from solution excess CO_2 resulting from the oxidation of the organic matter.



(a)



(b)

Fig. 5.15. The distribution of UK activated sludge plants in 1988 employing (a) diffused air and (b) surface aeration (Chambers and Jones 1988).

5.2.3.1. *Surface aeration*

Surface aeration achieves aeration and mixing by the use of blades or vanes that are rotated at speed. The aerator, which rotates about a vertical or horizontal shaft, is positioned at or near the surface of the liquid in the aeration tank. The action of the aerator produces considerable turbulence and spray, resulting in enhanced movement and oxygen transfer. Aeration with vertical shafts, i.e. the blades rotate in a horizontal plane, sets up a circulatory movement within the entire reactor; while those with horizontal shafts, i.e. the blades rotate in a vertical plane, create laminar flow in a particular direction. Oxygenation is achieved by the mechanical action of the aerator which entrains bubbles of air in the liquid. The air is drawn into the mixed liquor in the region of high turbulence, which is immediately

behind the blade as it rotates. The droplets of liquid which comprise the spray have a high surface area enabling a rapid uptake of oxygen as it passes through the air and eventually falls back into the liquid. The shearing action of the aerator is also important in continuously creating new gas/liquid interfaces which increase the oxygen transfer rate. The rate of aeration is governed by either varying the aerator speed; there are normally just two fixed speeds, or more commonly by varying the depth of immersion. The depth of immersion, however, has a critical range within which optimum performance is achievable.

Vertical shaft aerators

The most widely used aeration method is the vertical shaft aerator. These are used for the treatment of both domestic and industrial wastewaters at plants serving populations of any size. They can be incorporated into any operational mode of the activated sludge process.

Essentially these are inverted metal or plastic cones with blades welded or cast onto the surface and turned individually by a large electric motor using a suitable reducing gear. These motors vary in size from 1 to 120 kW depending on the size of the aerator. The motors must be weather proof and are generally fan-cooled. The whole apparatus is mounted on a permanent support structure over the aeration tank with the cone immersed in the mixed liquor. The cone is operated on a vertical shaft at between 35 and 60 rpm and is normally positioned at the centre of the tank to ensure maximum efficiency of action. A strong upward flow of mixed liquor from the centre of the tank is induced that is thrown over the surface of the tank as a dense spray, entrapping air and causing considerable turbulence which maintains the floc in suspension. The oxygen transfer rate is approximately proportional to the power absorbed at the aerator shaft, which in turn is dictated by the size, speed, and immersion depth of the aerators. The rate of aeration is normally increased to compensate for an increase in organic loading by varying the level of the mixed liquor in the aeration tank by raising the outlet weir or sluice gate of the tanks, thus increasing the depth of immersion of the cone. Where the motor and cone are attached to a floating platform or floats, which is widely used in lagoons and for the re-oxygenation of rivers and lakes, the rate of aeration is controlled by increasing the rate of rotation of the cone.

There are now an enormous range of cone aerators on the market. However, two types are generally employed. A simple cone for medium depth tanks or for those using floating platform or floats, such as the Simcar®

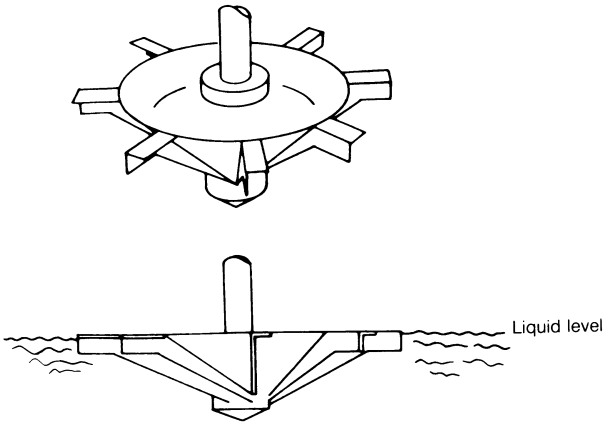
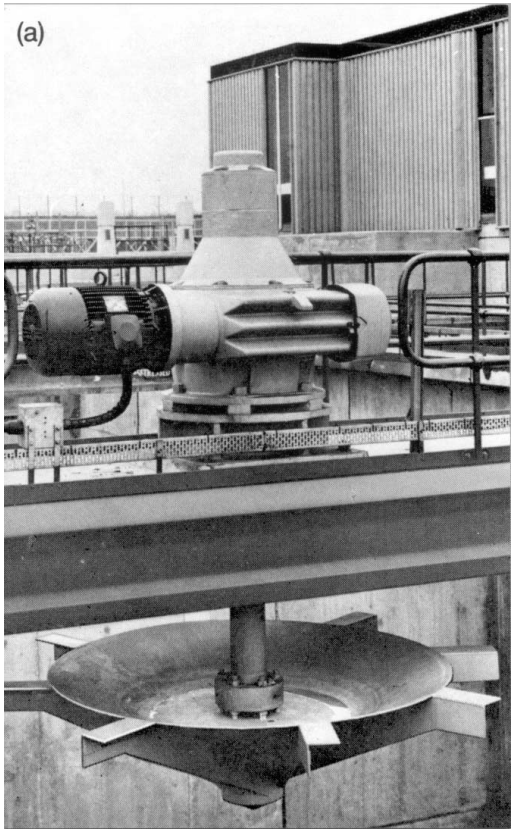


Fig. 5.16. One of the most frequently used aeration cones the Simcar® aeration cone manufactured by Simon-Hartley Ltd (Winkler 1981).

aeration cone manufactured by Simon–Hartley Ltd. (Figs. 5.16 and 5.17). This cone does not require a fixed draught tube and therefore can be used for a variety of functions. The diameter of the aerators can vary from 0.4 to 3.6 m and can transfer between 1 and 18 kg O_2 h^{-1}. For deeper tanks of 10 m or more the Simplex® cone developed by Ames, Crosta and Babcock, now part of the Biwater Group, is mounted above a vertical draught tube which extends to just above the floor of the aeration tank. The updraught of mixed liquor caused by the cone ensures aeration and complete mixing (Fig. 5.18). These aerators are very robust, manufactured in 0.5–4.0 cm mild steel plate and able to supply between 1.5 and 2.3 kg O_2 kW h^{-1}, though the siting of the cone is critical to ensure maximum aeration and mixing. Vertical shaft aerators can be installed centrally in single square aeration tanks or in rows in rectangular tanks and compartments in large tanks. However, where there are no dividing walls within an aeration tank employing more than one aerator, then short-circuiting can be prevented by using contra-rotating aerators. The design of the blades on cones is ingenious, and each manufacturer incorporates specific design features. For example, as the Simcar aerator revolves, the liquid is drawn up along the blades and then flung outwards at a low trajectory with only a small energy loss. Air is drawn in behind the blades and mixed with the liquid in the region of maximum turbulence, whilst the front of the blades disperse the aerated liquid over a large surface area thus entraining more air (Fig. 5.19). This action, coupled with the displacement of mixed liquor directly below the aerator, creates a powerful vortex which pulls more of the mixed liquor from



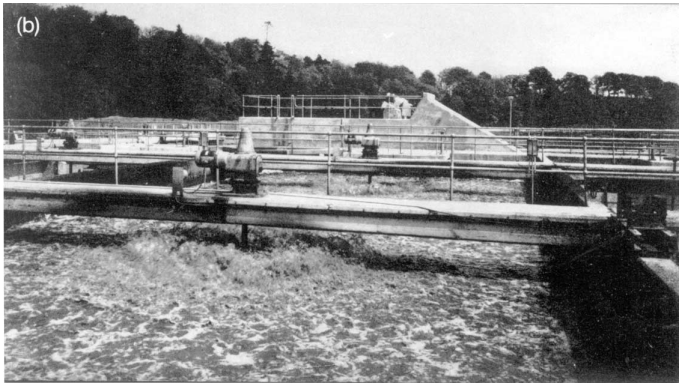


Fig. 5.17. A Simcar® aerator (a) *in situ* showing a direct-mounted drive arrangement and (b) in action (with permission of Simon-Hartley Ltd.).

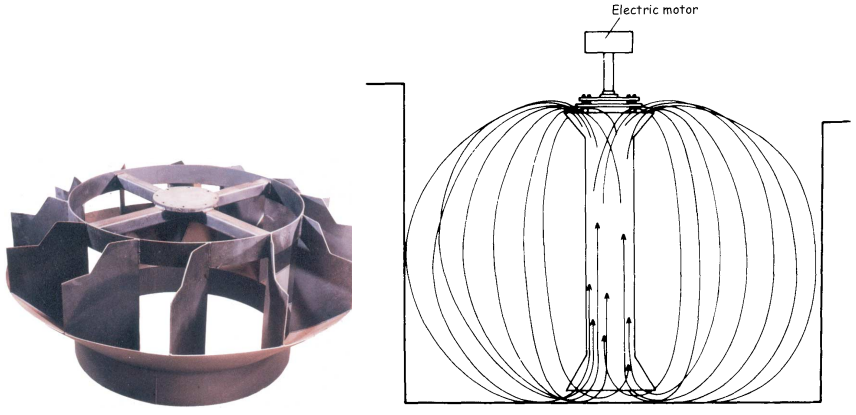


Fig. 5.18. The Simplex® cone aeration system employing an updraught tube to stabilise the flow pattern within the aeration tank (with permission of Biwater Treatment Ltd; Hawkes 1983a).

Fig. 5.19. Platform mounted Simcar® aerator in action.

the base of the tank creating a continuous aeration cycle (Fig. 5.20). All aerators come in a range of sizes to provide specific oxygenation capacities (Table 5.8).

Horizontal shaft aerators

These aerators are generally used at small to medium sized plants operated as extended aeration systems. Horizontal shaft aerators are used in elongated tanks, usually oval in plan with a central baffle or island to separate

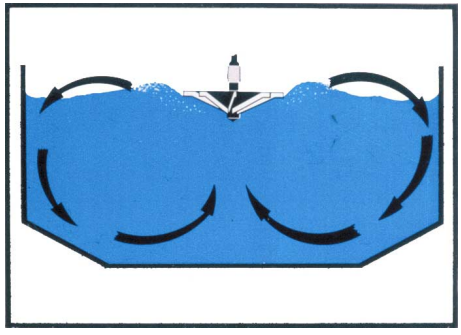


Fig. 5.20. The action of the Simcar® aerator ensuring continuous mixing and aeration of the entire contents of the tank (with permission of Simon-Hartley Ltd.).

the two main channels. The action of the aerator is essentially the same as vertical shaft aerators, except that the horizontal motion of the mixed liquor within the tank is induced by the aerator ensuring mixing and preventing settlement. There are three well known designs of horizontal shaft aerators: the Kessner® brush, the TNO cage rotor, and the Mammoth® rotor.

Kessner® brush aerators consist of a horizontal shaft into which rows of steel combs have been attached. Overall brush diameters range between 400 and 800 mm, and are designed to operate at shallow immersions of less than 10 mm at rotational speeds of 50–70 rpm. From this basic design the TNO cage, often referred to simply as the cage aerator, and the Mammoth® rotors were subsequently developed. The TNO cage has T-shaped bars arranged around a central horizontal shaft. Onto each of these, a series of rectangular metal plates or blades are attached. This operates at a greater depth of immersion than the brush aerator at approximately 150 mm with a rotation speed of 70 rpm. The Mammoth® rotor was developed to increase the oxygenation capacity per unit length of rotor. In this design, individual blades are attached directly to the horizontal shaft. The blades are mounted in different planes so that they enter the water in sequence as the shaft rotates. These rotors are mounted on a hollow central shaft and are 1 m in overall diameter (Fig. 5.21). They operate at 70–80 rpm at a typical immersion depth of 300 mm. In comparison with other rotors, they are extremely powerful and are used for both conventional and extended aeration systems, where rotors of up to 9 m in length are employed (Fig. 5.22).

The rotor is supported at each end by cylindrical bearings, although long rotors may require intermediate support also using suitable bearings. Brushes and rotors are extremely well balanced, with the central shaft often

Table 5.8. Operational details of the Simcar® range of aerators. Where * is at zero dissolved oxygen conditions, 760 mm Hg, pure water at 10°C; + indicates that dimensions are nominal (Permission of Simon-Hartley Ltd.).

'Simcar' Model No.	Aerator diam. mm	Oxygen transfer * kg d^{-1}	Preferred liquid depth + mm	Motor hp	Motor kW	Unit weight (nominal) kg	Clearance from bridge mm	Clearance to platform support leg mm	Minimum blade immersion + mm	Immersion range mm
40	1016	26–40	1400–2000	1	0.75	260	340	510	30	90
45	1143	52–80	1600–2300	2	1.50	300	380	580	35	100
50	1270	104–160	1750–2600		3	400	430	640	35	110
56	1422	145–240	2000-2850	5.5	4	490	480	720	40	115
64	1626	265–400	2300–3250	10	7.5	730	550	820	45	135
72	1829	400–600	2600–3700	15	11	1110	610	920	50	160
80	2032	530–800	2850–4100	20	15	1290	680	1020	55	170
90	2286	800–1230	3200–4600	30	22	1950	770	1150	60	190
110	2540	1000–1600	3600–5100	40	30	2340	850	1280	70	210
112	2845	1300–2000	4000–5500	50	37	3730	950	1430	80	240
128	3251	2000–3000	4500–5800	75	55	5500	1100	1630	90	270
144	3658	2600–4000	5100–6000	100	75	6470	1250	1830	100	300

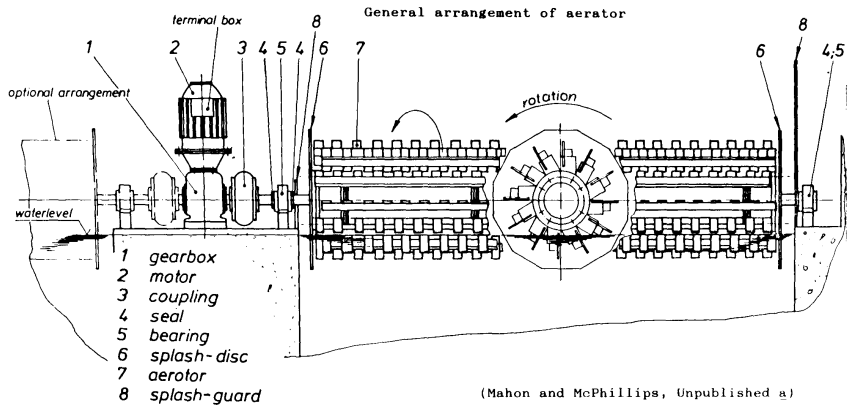


Fig. 5.21. General arrangements of a Mammoth® rotor (O'Leary 1988).

hollow to minimise weight. Therefore relatively small motors can be used to drive them. Fan-cooled electric motors, which are totally enclosed, operate via reduction gear boxes. The degree of oxygenation can be controlled by either using a variable speed motor, normally two speed, or by adjusting the depth of immersion by altering the level of the mixed liquor in the aeration tank.

Kessner® brushes can provide up to 2 kg O_2 kW h^{-1}, while cage rotors which have a more intense action can operate at higher efficiencies of up to 2.5 kg O_2 kW h^{-1}. Oxygenation capacity is often expressed as kg O_2 delivered per unit time per length of rotor (kg O_2 h^{-1} m^{-1}). So the TNO cage rotor can provide up to 2.5 kg O_2 h^{-1} m^{-1} compared with a maximum of 8.0 kg O_2 h^{-1} m^{-1} for the Mammoth® rotor.

5.2.3.2. *Air diffusion*

Oxygen can be supplied directly to the mixed liquor by pumping air, under pressure, into the aeration tank and releasing it as a stream of bubbles. Circulation and mixing is achieved by currents set up by the rising bubbles, while oxygen transfer takes place between the air bubble and the surrounding liquid. The release of bubbles under pressure results in turbulence at the surface of the tank, which also encourages enhanced oxygen transfer. Air diffusion is categorised as either fine or coarse bubble diffusion systems, which depends on the size of the bubbles formed. In general terms fine bubbles are less than 1.5 mm in diameter, coarse bubbles are greater than 3.0 mm, while a medium range of between 1.5 and 3.0 mm is also recognised. The finer the bubbles the larger the air-liquid interfacial area

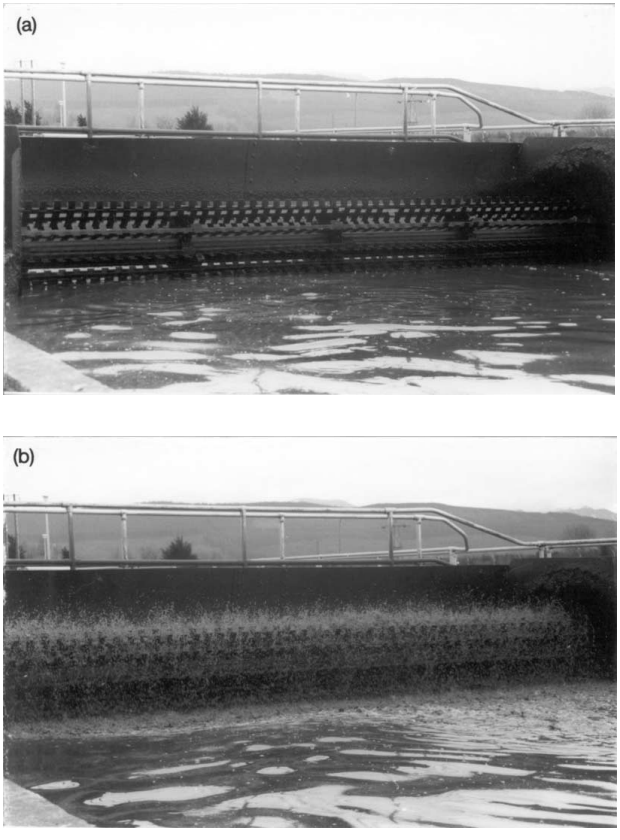


Fig. 5.22. Mammoth® rotor (a) switched off and (b) in action (O'Leary 1988).

per unit volume of air supplied, resulting in a more efficient transfer rate of oxygen into the mixed liquor from the bubbles. Therefore, the larger the bubble size the greater the volume of air required per kg BOD removed within the aeration tank (Table 5.4).

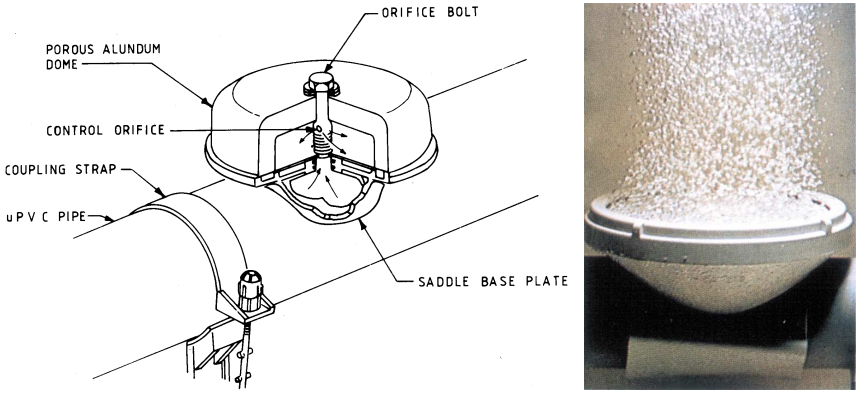
Air is supplied from blowers, although the smaller the pores in the aeration dome or exit holes in the pipework from which the air is released, then the greater the pressure required. For fine bubble systems each aeration dome can release anything between 0.25 and 0.75 litres of air per second, so that large volumes of air are required. Obviously compressors are required for continuous air supply, so standby-units and generators are required. These are usually based in permanent buildings which are soundproofed to reduce noise. The air must be filtered to provide dust free air to the system using either viscous impingement, dry barrier, or electrostatic filters. The

air supplied to the aeration domes should contain less than 3.5 mg of dust per 1000 m^3. In coarse bubble systems, where large exit holes are used, the need for air filtration and a powerful compressor is considerably reduced.

Fine bubble diffusion

This method of aeration is widely used for all modes of activated sludge operation treating both industrial and domestic wastewaters. They are used for small to very large treatment plants. For example, fine bubble diffusion is used at Thames Water's Beckton works in London that treats a population equivalent in excess of 4 million. Such systems are the most efficient aeration technique employing air, reaching oxygenation efficiencies of 2.0 to 2.5 kg O_2 kW h^{-1}. Air is pumped into the aeration basin via a network of pipes anchored to the bottom of the tank and released into the mixed liquor via a series of diffusers. These are fitted onto the air supply pipework by a single bolt, with the dome sat on a closely fitting saddle. The dome itself can be made out of a variety of porous materials. For example, the Alundum® diffuser has an average pore size of 0.15 mm and is made out of a fixed crystalline aluminium silicon ceramic (Fig. 5.23). Porous polyethylene is also widely used.

The small pore size inevitably results in blockages both from the inside, due to dust in the air supply, or sloughed material from the pipework or air ducts. This has been reduced by filtering air supplies and replacing steel and iron air pipes with uPVC, although the plastic pipework must be



(a) (b)

Fig. 5.23. Dome diffuser (a) schematic diagram (Institute of Water pollution Control 1987) and (b) a dome in action (with permission of Carborundum Abrasives GB Ltd.).

protected above water from ultraviolet light from the sun. The outer surface of diffuser domes eventually becomes blocked due to deposition of solids, especially if the air is switched off for maintenance or due to failure of the power supply, development of chemical scale and bacterial slimes. Blockages are clearly visible from the surface as a reduction in turbulence, and cleaning requires the aeration tank to be drained and the domes individually cleaned. Depending on the material, they may be cleaned by sand blasting, acid washing, soaking in various solvents, or using an ultrasonic bath. The main cause of blockages is interruption in the power supply. The air, which is under pressure, is warm and once the air blower stops then the warm air in the distribution system will rapidly cool, creating a small vacuum which will cause deposited material or slime growth to be pulled into the pores. This is overcome by ensuring that a standby generator is available which automatically cuts in whenever the main power supply fails, thus preventing any interruption in air supply. Alternatively a system to ensure positive pressure within the pipework at all times has to be employed.

Most fine bubble diffused air systems are placed in flat bottomed rectangular aeration tanks. The depth is variable, between 2 and 5 m depending on the contact time required between the air bubbles and the mixed liquor. A high density of diffuser domes are required to ensure sufficient aeration, with each line of diffusers resulting in a circulatory mixing pattern (Fig. 5.24). An attempt to increase the contact time between the air bubbles and the mixed liquor is the spiral flow design. The spiral flow aeration tank consists of long channels 2 m $\times$ 2 m with the diffusers placed on one side of the channel floor only. The rising bubbles cause a circular motion in the mixed

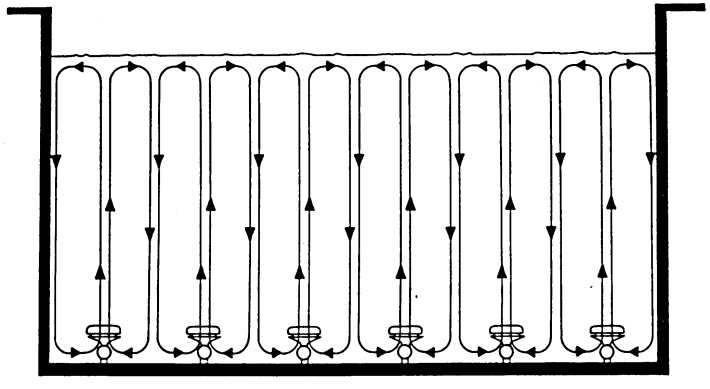
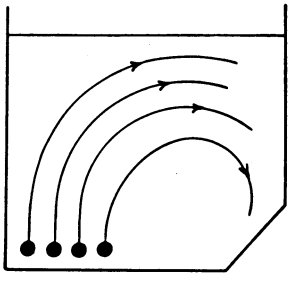


Fig. 5.24. Mixing cycle set up by diffusers within the aeration tank (Institute of Water Pollution Control 1987).



Spiral flow

Note: Each line of diffusers shown thus: •

Fig. 5.25. The spiral flow arrangement of diffusers (Institute of Water Pollution Control 1987).

Fig. 5.26. Disc aerator manufactured by Rosewater Engineering Ltd. (with permission).

liquor which, as the mixed liquor flows along the channel, develops into a spiral or helical flow pattern producing more efficient oxygen transfer rates than conventional designs (Fig. 5.25). Due to the necessity of having to clean the domes periodically, several aeration tanks are required so that one tank can be taken out of service for maintenance.

Apart from domes, there are a number of other diffuser designs available. Rosewater Engineering Limited , for example, produce a range of non-dome diffusers that reduce the risk of fouling as well as prevent the backflow of liquid into the air supply pipework during shutdown. The diffuser is a flexible porous membrane mounted either on a rigid disc (Fig. 5.26) or a tube manufactured either in PVC or stainless steel. Each disc or tube is mounted on a framework of air supply pipes (Fig. 5.27). The design is such that two one-way valves are incorporated into each aerator. When the pressure in the air supply pipeline is lost, the membrane perforations

(a) (b)

Fig. 5.27. (a) Fine bubble tube aerators mounted on a framework of air supply pipes. (b) Aeration in operation (with permission of Rosewater Engineering Ltd.).

close and the non-porous membrane area sits tightly over the incoming air supply orifices. In practice this means that the air supply can be shut down completely without any risk of fouling. Air-borne dirt is expelled by regularly increasing the air pressure slightly to open up the orifices in the membrane. Tube aerators vary in length between 500 and 2000 mm, while the disc is 520 mm in diameter, which is equivalent to a 1.5 m tube aerator. Very fine bubbles can be produced by this aeration system which is widely used in the UK. Each disc or tube is mounted on a ring main which is fed by a central set of blowers. Such a system is described by Wallis (1989) at the Hawick Sewage Treatment Works, where aeration tubes have been used to replace TNO rotors in longitudinal plug-flow aeration tanks. Each aeration tank consists of two lanes. In the first lane 41 aeration tubes have been used, with 29 in the second, providing a degree of matching aeration to oxygen demand (Fig. 5.28).

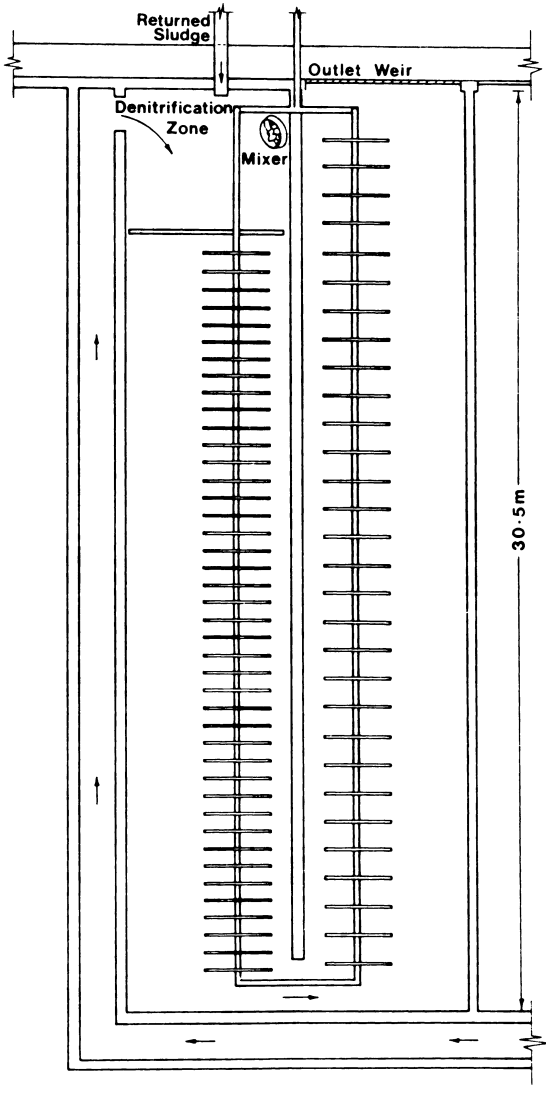


Fig. 5.28. Layout of fine bubble tube aerators at Hawick Sewage Treatment Works (Wallis 1989).

Coarse bubble diffusers

Large bubbles up to 12 mm in diameter are produced using perforated plastic or stainless steel pipework with exit holes of up to 6 mm in diameter. The large diameter holes ensure that there are no problems with blockages,

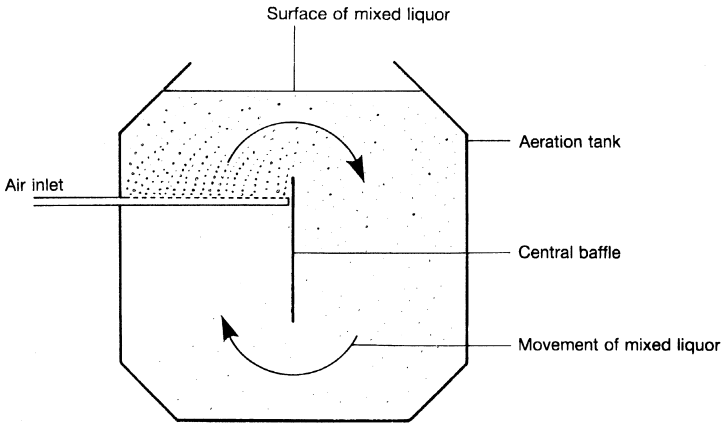


Fig. 5.29. The INKA process.

so there is no need to filter the air before use. The air in the pipework is maintained at a much lower pressure than fine bubble diffusion systems so that high efficiency fans can be used instead of compressors to supply the large volumes of air required. They have a low oxygenation efficiency (0.8–1.2 kg O_2 kW h^{-1}) (Table 5.4) and are used primarily for small packaged extended aeration plants treating populations up to 3,000. The oxygen transfer efficiency is relatively low due to the small specific air/water interfacial area which is due to the low surface area to volume ratio of the bubbles. For this reason coarse bubble systems are used in deeper tanks, up to 4 m in depth, to maximise the contact time between the bubbles and the mixed liquor.

The Swedish Inka process employs coarse bubble diffusion. The aeration tank is separated into two equal sections by a vertical baffle. A grid of stainless steel pipes perforated with 2.5 mm holes on their underside is positioned about 0.8 m below the surface of the liquid in the tank. The bubbles act as an air lift pump causing a circulating mixing of the mixed liquor around the central baffle (Fig. 5.29).

5.2.3.3. *Testing aerators*

Rachwal and Waller (1982) compared vertical cone and horizontal aerators, used in oxidation ditches from various plants operated by Thames Water, by plotting aeration efficiency against immersion depth (Fig. 5.30). Both types of aerator showed that there was an optimum immersion depth for maximum aeration efficiency. Also the cone aerators had a greater peak

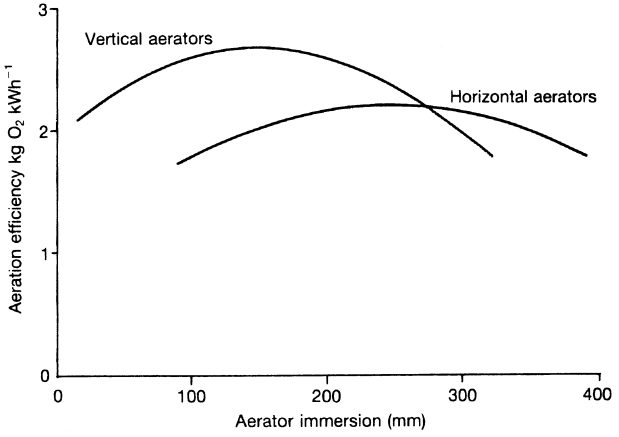


Fig. 5.30. Comparison of the aeration efficiency compared to depth of immersion of vertical and horizontal aerators (Rachwal and Waller 1982).

efficiency compared to the horizontal aerators at 2.5 and 2.2 kg O_2 kW h^{-1} respectively. While power consumption increases linearly with immersion depth, oxygenation capacity does not. This was demonstrated at the Carrousel plant at Cirencester by Rachwal and Waller (1982), who found that after two thirds immersion, no applicable increase in oxygenation capacity occurred while power consumption could be increased by a further 30% (Fig. 5.31). In order to prevent wasted power consumption, aeration efficiency should be calibrated against immersion depth and the "stop limit" on the aerator immersion system adjusted to coincide with the apex of the curve. So during commissioning or optimisation of aeration equipment, it is important to check that it complies with the specification.

Two methods are employed to test the oxygen transfer rate and overall efficiency of aeration systems. Steady state techniques measure the amount of oxygen required to achieve a mass balance between supply and utilisation by the mixed liquor during normal operation, although constant operating and loading conditions are required. Steady state methods are only used for completely mixed systems. Unsteady-state techniques are more commonly used and measure the rate of change of dissolved oxygen concentration during the reaeration of deoxygenated clean water. The water used for unsteady-state assessments of aeration efficiency must be clean, and the aeration equipment itself free from all traces of oil, grease, and sludge. Synthetic detergents also effect the transfer of oxygen and so the aeration equipment must be cleaned with high pressure hoses using sodium bicarbonate to scour surfaces where necessary. The water is deoxygenated by adding

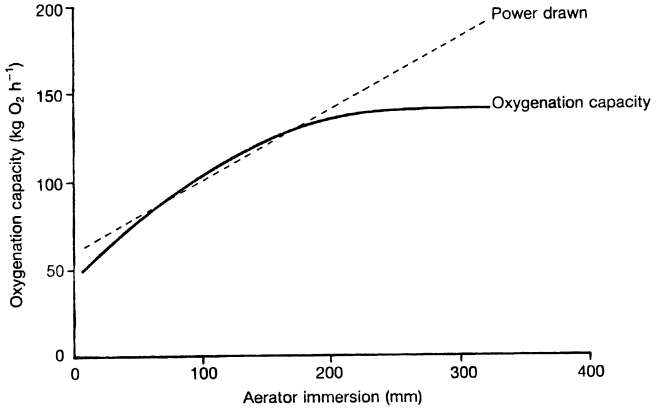


Fig. 5.31. The oxygenation capacity and power used by a cone aerator at Cirencester Sewage Treatment Plant at various depths of immersion (Rachwal and Waller 1982).

sodium sulphite in solution with cobalt chloride (or nitrate) to catalyse the deoxgenation reaction. Once all the unreacted sulphite has been utilised the test can be carried out. Using a number of dissolved oxygen (DO) probes positioned throughout the tank, the aerator is switched on and the dissolved oxygen concentration monitored over time until the water is saturated with oxygen or steady-state dissolved oxygen conditions are achieved. (Fig. 5.32).

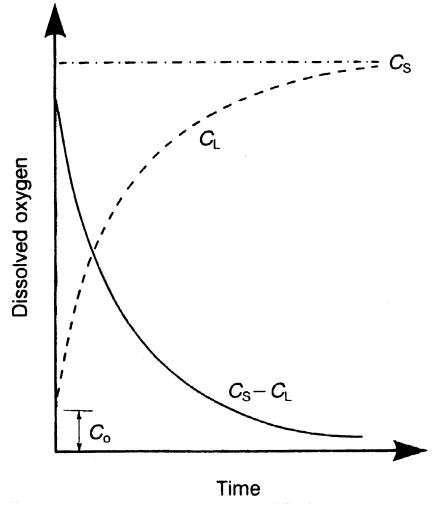


Fig. 5.32. The relationship between the saturated dissolved oxygen concentration C_s, the dissolved oxygen concentration at time zero (C_0), the concentration at time t, C_L, and the dissolved oxygen deficit $C_s - C_L$.

Using the dissolved oxygen readings, a graph of $\log_e$ of the dissolved oxygen deficit $C_s - C$ is plotted against time for each probe, only using dissolved oxygen values between 20 and 80% saturation. The slopes of these graphs are then used to calculate separate values for the oxygen transfer rate ($K_L a$) at temperature T

$$K_L a(T) = \frac{60}{t_2 - t_1} \log_e \frac{(C_s - C_1)}{(C_s - C_2)} \quad (h^{-1}),$$

where C_s is the saturated DO concentration (mg l^{-1}), C_1 and C_2 the DO concentrations (mg l^{-1}) at times t_1 and t_2 (min) respectively. The mean $K_L a$ value is taken as the $K_L a$ quantity for the whole system. The oxygenation capacity (OC) of the aeration system is calculated as

$$\mathrm{OC} = K_L a(T) V C_s(T) \times 10^{-3} \quad (\mathrm{kg\ h^{-1}}),$$

where V is the volume of water used in the test, T is the temperature of the water, and $C_s(T)$ the oxygen saturation concentration of clean water at test temperature T.

The transfer rate coefficient ($K_L a$) is dependent on temperature and is expressed at a standard temperature of 20°C, i.e. $(K_L a)_{20}$. Therefore it must be converted using

$$(K_L a)_T = (K_L a)_{20} (1.024)^{T-20},$$

where T is the temperature at which $K_L a$ is measured.

The values for $K_L a$ obtained using clean water by this method will be higher than those obtained using activated sludge. The impurities in waste water have significant effects on $K_L a$. For example, both fatty and surface active materials such as detergents reduce the rate of oxygen transfer. However, detergents and fatty acids, when present as soaps (pH > 6), are able to increase $K_L a$ by preventing bubble coalescence, thus maintaining the mean bubble size at a lower level than if such chemicals were absent. This increases the total interfacial area, thus increasing the overall mass transfer of oxygen. These two opposing effects do not necessarily cancel each other out and so must be considered in the calculation of $K_L a$. Other impurities in water can also alter $K_L a$, and not always in a predictable way. Therefore the effects of all impurities must be calculated together by measuring the α factor (IWPC, 1987):

$$\alpha \text{ factor} = \frac{(K_L a) \text{ waste water}}{(K_L a) \text{ cleanwater}}.$$

The value of $K_L a$ varies for each type of waste water and also the duration of aeration. For example α varies from 0.3 at the beginning of the aeration period for domestic sewage to 0.8 after 4 hours aeration. Typical α values for mixed liquor vary from 0.46 to 0.62. The impurities in waste waters will also affect the oxygen saturation concentration compared with clean water at the same temperature. This is adjusted by the β factor where:

$$\beta \text{ factor} = \frac{C_s \text{ in waste water}}{C_s \text{ in clean water}} .$$

The β value normally approximates to 0.9. Once the α and the β factors are known for a particular waste water, then the clean water test results can be used to predict the expected field results. Details of testing aerators, including a worked example, are given in IWPC (1987). However, testing aerators is extremely expensive and full scale trials present considerable logistic problems. For example, Rachwal and Waller (1982) state that up to six separate tests may be required for a typical oxidation ditch aeration tank, which if it has a typical volume of 7,000 m^3, may require 10 tonnes of concentrated sulphite to deoxygenate it! An interesting example is given by Reinius and Hultgren (1988) who describe in detail how the fine bubble aeration system at the Henriksdal Sewage Treatment Works in Stockholm, which has a design flow of 370,000 $m^3\ d^{-1}$, was evaluated. A similar example is given by Gillot and Héduit (2000).

Aeration efficiency in terms of mass of oxygen transferred to the mixed liquor per unit of energy expended is expressed as kg O_2 kW h^{-1}. An estimate of aeration efficiency can be made by measuring the oxygen demand (OD) exerted by carbonaceous oxidation and nitrification, taking into account the flow rate, influent and effluent BOD concentrations using the equation below:

$$\begin{aligned} \text{OD} = {} & 0.0864 q_s [0.75(\text{BOD}_i - \text{BOD}_e)] \\ & + [(5.25 \times 10^{-4} \times C_{\text{MLSS}} \times V)/q_s] + 4.3(N_i - N_e) \quad (\text{kg d}^{-1}) , \end{aligned}$$

where q_s is the mean flow rate of settled influent (L_s^{-1}), BOD the mean biochemical oxygen demand of the influent (i) and effluent (e) (mg l^{-1}), C_{MLSS} the mean MLSS concentration (mg l^{-1}), V the aeration tank volume (m^3), and N the mean ammonia concentration in the influent (i) and effluent (e) (mg l^{-1}). The calculated oxygen demand can be converted to aeration efficiency by dividing by the daily power consumption (kW h d^{-1}). While this assessment is not as accurate as standard performance tests, it

does allow an excellent comparison between plants without the prohibitive expense of *in-situ* tests (Houck and Boon 1980; Chambers and Jones, 1988).

5.3. Modes of Operation

By using different combinations of the main operating parameters, various different rates and degrees of treatment are possible. This flexibility in design, allowing operation over a wide range of loadings to suit specific treatment objectives, is the major advantage of the activated sludge process over other treatment processes (Table 5.9). Although the activated sludge system is primarily designed to remove carbonaceous BOD, with sufficient

Table 5.9. Major uses of the more widely used modifications of the activated sludge process.

Mode of operation	Major function
Conventional completely mixed systems	BOD removal
Conventional plug flow	BOD removal, nitrification
Contact stabilisation	BOD removal
Extended aeration	BOD removal, nitrification
Oxidation ditch	BOD removal, nitrification, denitrification
Anoxic zone system	BOD removal, nitrification, denitrification

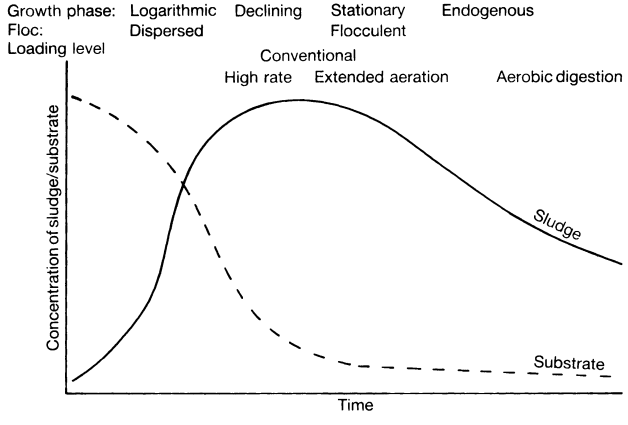


Fig. 5.33. The microbial growth curve showing the operational growth phase used in different modes of activated sludge (Winkler 1981).

operational control using suitable modifications it can also achieve nitrification, denitrification, and phosphorus control. The relationship between the substrate and sludge concentration, the f/m ratio, is a fundamental one in the operation of activated sludge and is summarised in Fig. 5.33. The different regions of this growth curve are analogous to activated sludge systems operating at different loading levels with corresponding degrees of substrate removal. In general, high-rate processes have a high sludge loading, a short SRT, a high sludge activity and a short retention time. Although large weights of BOD_5 can be removed per unit volume of reactor, there is a relatively high concentration of organic matter remaining in the final effluent. In contrast, low-rate processes have a low sludge loading, a long SRT, a low sludge activity and a long retention time. The sludge is in the endogenous respiration phase so food is limited, resulting in a low residual concentration of organic matter in the final effluent, and as the rate of microbial decay is high compared with the rate of microbial growth, there is little excess sludge produced. Conventional operation falls between these two extremes (Winkler 1981).

Activated sludge operation can be categorised as either conventional, high-rate or extended aeration, although the delineation between these categories is by no means precise and the terms are used only in their broadest sense (Table 5.2).

5.3.1. *Conventional activated sludge processes*

Conventional activated sludge is the most widely used operational mode as it gives full treatment to wastewaters, particularly domestic sewage, producing a 20:30 effluent with or without nitrification, suitable for discharge to inland waters. The term conventional comes from the median-rate of treatment with a BOD loading range between 0.5 and 1.5 kg BOD m^{-3} d^{-1}, a sludge age of between 3 and 4 d, a HRT of 5–14 h, a sludge loading rate (f/m) of 0.2–0.6 kg BOD kg^{-1} d^{-1}, and a sludge concentration in the aeration tank (MLSS) of between 2 and 3 kg m^{-3} (2000–3000 mg l^{-1}). These figures are for domestic wastewater; the loadings for dairy or maltings wastes will be at the lower end of the range and those containing highly biodegradable substrates, such as simple alcohols, carbohydrates, and organic acids will be in the upper loading range (Calley *et al.* 1977). The sludge production is about 0.5 kg per kg BOD removed and a dry solids content below 1%. This represents over 50 kg wet sludge produced per kg BOD removed, indicating the problem of handling waste sludge in the activated sludge process. The HRT is critical for nitrification with

only about 6 h required for adequate carbonaceous oxidation and up to a further 4 h required for nitrification. However, the more recalcitrant the waste the longer the HRT required. For example, Cox (1977) found that phenolic wastewater required an HRT of 75 h to obtain a 96% reduction in the organic matter. In general, conventional activated sludge is expected to produce BOD reductions of between 90 and 95%.

Within this category is a wide variety of systems both in structure and operation. The most important difference is the mixing regime used (Bode and Seyfried 1984), with conventional activated sludge being nominally plug-flow or nominally completely mixed. Plug-flow conditions are difficult to maintain as the mixed liquor has to be aerated, which results in a significant degree of mixing; while the problem of completely mixing liquid, sludge flocs, and gas bubbles makes a truly completely mixed system difficult to achieve. Therefore, activated sludge systems have a tendency towards a particular mixing regime rather than exactly fulfilling the criteria for a particular mixing regime. The difference between plug-flow and completely

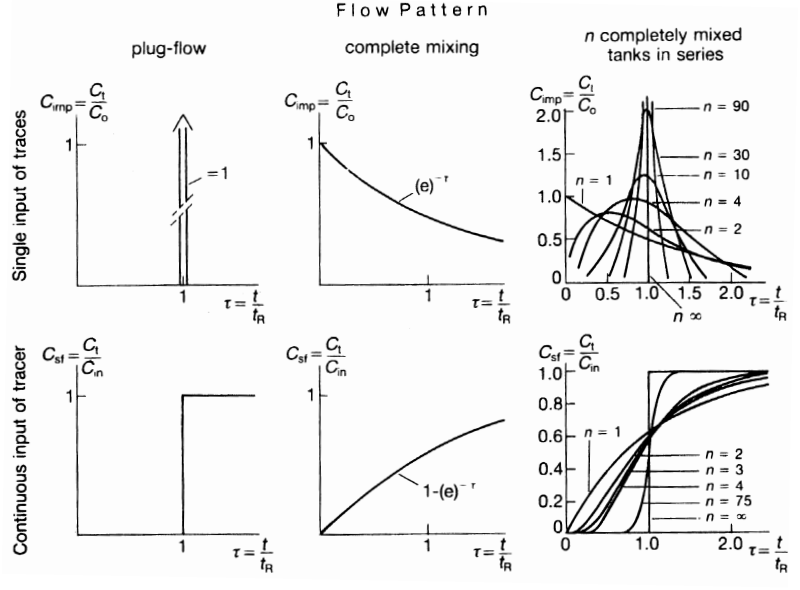


Fig. 5.34. Comparison of the output tracer response curves from plug-flow, completely mixed and n completely mixed tanks in series, receiving a single or a continuous input of tracer. Where the effluent concentration C_t measured at time t is set in proportion either to the calculated initial concentration C_0 for a single input or to the inflow concentration C_{in} for continuous input of tracer. The relative effluent concentrations calculated as C_{imp} and C_{sf} are plotted against the relative time $\tau = t/t_{\mathrm{R}}$, where t is the time (h) and t_{R} the theoretical mean retention time (h) which is the volume of the tank (m^3) divided by the wastewater flow ($\mathrm{m}^3\ \mathrm{h}^{-1}$) (Bode and Seyfried 1984).

mixed tanks can be clearly demonstrated by tracer techniques using radioactive isotopes such as bromine-82, sodium-24 or gold-198, which can all be detected at concentrations at least two orders of magnitude below that acceptable in drinking water (Nixon and Belcher 1985). Figure 5.34 shows that the tracer added to a plug flow aeration tank does not mix with the tank contents while, in contrast, the tracer is immediately and uniformly distributed in a completely mixed tank. The mixing characteristics of tanks in series lie between these two theoretical extremes and approach those of plug-flow as the number of tanks in series increases (Fig. 5.34). The return sludge cycle has a strong influence on the tracer response curves, and so special procedures must be followed to evaluate the mixing regime in the field (Bode and Seyfried 1984). Although different modes of operation of conventional activated sludge are preferred by certain workers, comparative studies show that there is very little difference in performance of such systems when operated at particular sludge loadings (Knop 1966). Plug-flow systems do however lead to an improvement in sludge settleability when compared to completely mixed systems (Poole 1987). Examples of various mixing regimes are shown in Fig. 5.35.

5.3.1.1. *Plug-flow systems*

Theoretically, the term plug-flow describes a situation where the order in which the substrate leaves the reactor is the same as it is added. It can be demonstrated by having a series of completely mixed reactors in series, with the plug-flow nature of the system increasing as the number of reactors increases (Erickson and Fan 1968; Scuras *et al.* 2001). In practice oxidation ditches incorporating a central baffle, thus providing a single extended longitudinal tank with a single cage rotor, are the type of activated sludge configuration most closely approaching the ideal plug-flow design (Fig. 5.36). Although plant configuration is important, both aeration and sludge recycle will affect the degree of mixing within any aeration tank.

In the conventional plug-flow system, both the influent wastewater and the returned sludge are added at the end of an elongated rectangular aeration tank, typically 6–10 m wide, 30–100 m long, and 4–5 m deep (Fig. 5.37). The tank is equipped either with diffusers or, less commonly, surface mechanical aerators to provide oxygen to the mixed liquor along the length of the tank. As the mixed liquor proceeds along the tank the organic matter is utilised, with the desired level of removal being controlled by the time it takes to reach the outlet at the far end. In theory, the sludge growth curve should be discernible as the mixed liquor moves along the tank, as in a batch system, with an initially rapid rate of removal becoming

progressively slower as it makes its way along the tank. However, because of longitudinal mixing, the plug-flow effect is hidden so that the removal rate remains fairly uniform over the first third of the tank at least. There is also a discernible BOD concentration gradient along the tank (Fig. 5.38). The rate of oxygen utilisation also changes along the length of the aeration tank and the oxygen supply may be deficient at the inlet where demand is greatest, and be in excess (> 5 mg O_2 l^{-1}) at the outlet, where the demand is lowest. This is overcome by a modification known as tapered aeration. The advantage of plug-flow systems is the low risk of short-circuiting of the tank. The risk can be reduced even further by incorporating dividing walls or baffles into the design of the tank; although this may increase localised mixing within the divided chambers. Toxic and shock organic loads are not diluted or buffered as in completely mixed systems, but pass through the

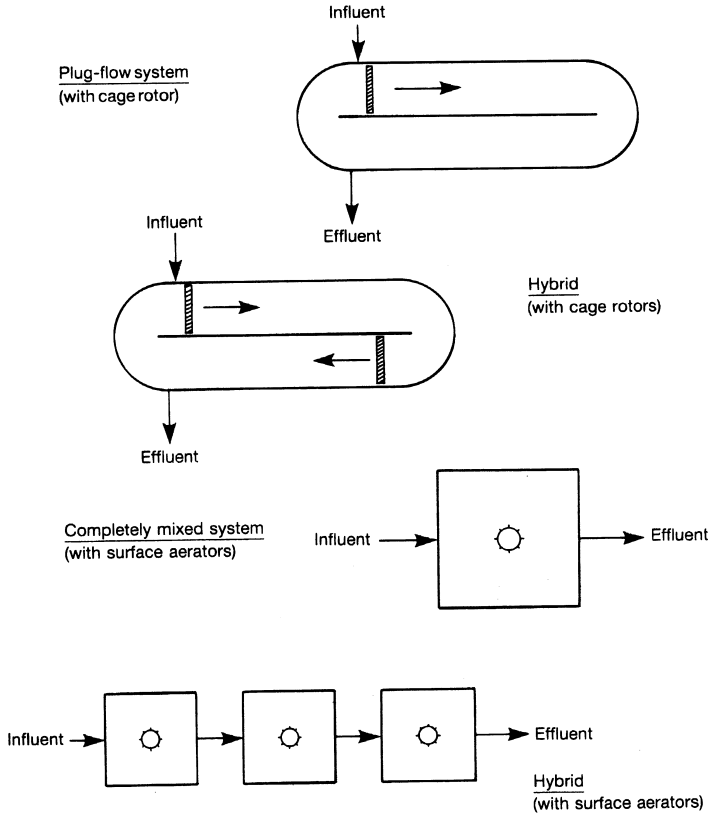


Fig. 5.35. Comparison between plug-flow and completely mixed systems, and hybrids of each.

tank as a discrete plug, resulting in serious deterioration of effluent quality. Plug-flow systems produce sludges with good settling properties, although as the degree of mixing increases, sludge settleability deteriorates.

Accumulation–regeneration theory

The reason why plug-flow systems offer better sludge settleability is due to a lower incidence of filamentous growth in the mixed liquor. Chudoba *et al.* (1973a) have suggested that this is due to differences in the metabolism of

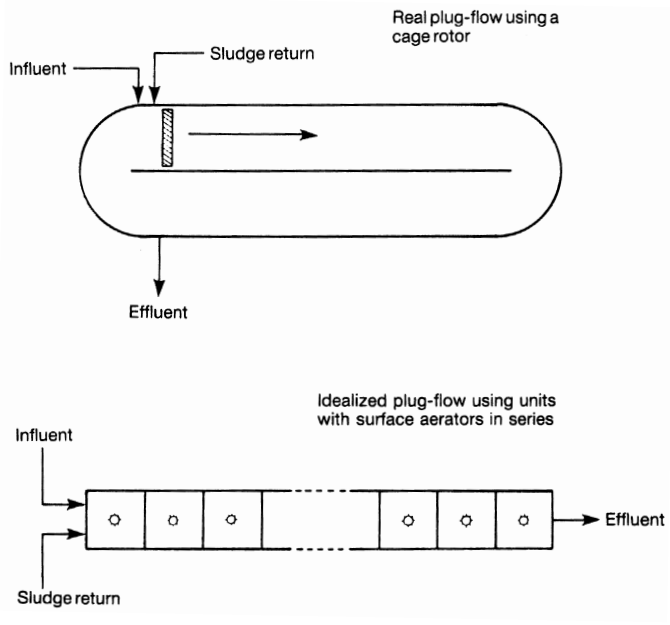


Fig. 5.36. The oxidation ditch with a single rotor is as close to the idealised design of a plug-flow reactor generally used in the treatment of wastewater.

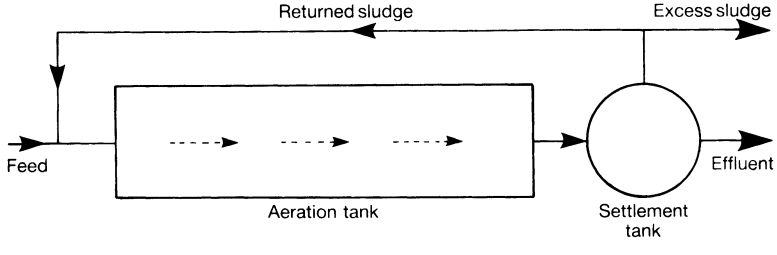


Fig. 5.37. Schematic flow diagram of a plug-flow activated sludge system.

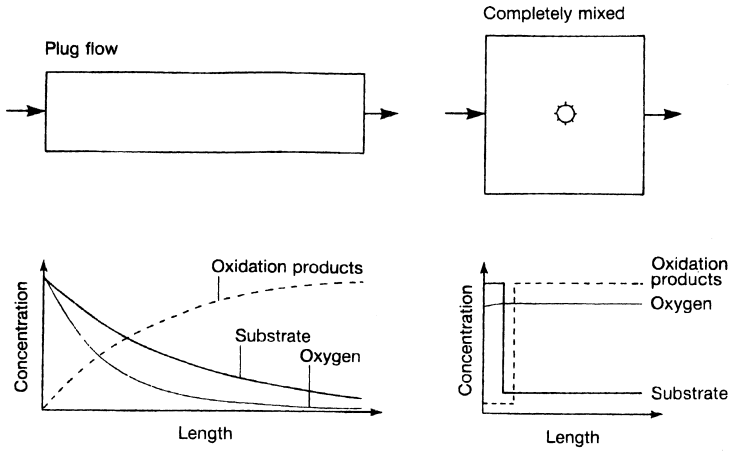


Fig. 5.38. Comparison of the utilisation of substrate and oxygen, and the production of oxidation products, through a plug-flow and completely mixed reactor.

filamentous and floc-forming bacteria, i.e. the accumulation-regeneration theory (Grau *et al.* 1982). Initially soluble substrate is rapidly absorbed where it is stored within the cell (accumulation). After the cell can no longer absorb substrate, it then proceeds to digest the material and produce new cells (regeneration). Chudoba *et al.* (1973a) proposed that within an aeration tank, conditions should exist to enable all the micro-organisms to go through both of these phases in their feeding cycle. In a plug-flow reactor a substrate concentration gradient is formed (Fig. 5.38), so that immediately below the aerator there is excess substrate and oxygen available. Maximum biological activity occurs here and it is in this zone that maximum accumulation occurs. Under these conditions both the floc-forming and filamentous bacteria are able to complete the accumulation phase. So in a plug-flow situation there is no competition between the two groups of bacteria. In the remainder of the tank, the accumulated substrate is subsequently oxidised and new cells are produced (regeneration). This whole cycle is repeated numerous times each day. In comparison, the substrate concentration in a completely mixed reactor is uniformly low (Fig. 5.38), so that the filamentous and floc-forming bacteria are actively competing for the available substrate. The filamentous bacteria are more efficient and should dominate, as at low substrate concentrations the specific growth rate (μ) of filamentous bacteria is higher then for floc-formers; but in practice the floc-formers predominate as they are selectively returned by the sedimentation phase of the process. However, if conditions in the

aeration tank become slightly stressed, such as reduced oxygen, increased substrate loading, or low nutrient concentration, then the filamentous micro-organisms will dominate and sludge settleability will deteriorate.

The accumulation–regeneration theory is based on the substrate being soluble and so available for immediate absorption by bacteria. In practice, hydrolytic enzymes released by both viable and non-viable bacteria result in more substrate being released into solution over time. Thus, in a plug-flow reactor, there should in reality be a continuous supply of oxidizable substrate at a low concentration which should favour the development of filaments. Yet plug-flow systems do not appear to favour filamentous development. It has been shown that certain reactor configurations such as plug-flow, fill and draw (batch systems), and contact (selector) tanks stimulate the specific growth rate of floc-forming bacteria, so that they have higher specific growth rates than filaments even at low substrate concentrations. So using such modifications ensures that the floc-formers will always have a competite advantage over filamentous bacteria, regardless of the substrate concentration. Hence for floc-formers the maximum specific growth rate (μ_{m}) in the Monod expression,

$$\mu = \mu_{\mathrm{m}}[S/(K_{\mathrm{s}} + S)]$$

is dependent on plant operation, so that where a contact zone is present (i.e. high f/m ratio) μ_{m} will be high whereas when the f/m ratio is low, as in a completely mixed reactor, then μ_{m} will be low. As the saturation constant (K_{s}) does not vary, then for a given substrate concentration S, the specific growth rate μ will be directly proportional to μ_{m}. Typical values of μ_{m} and K_{s} (1 d^{-1} and 1 mg l^{-1} COD respectively) are lower for filamentous than for floc-forming bacteria (2–5 d^{-1} and 4 mg l^{-1} COD respectively). So while the μ_{m} values show that floc-formers grow faster than filaments, the K_{s} values demonstrate that filaments can perform more efficiently at lower substrate concentrations. This is the basis of the mass transfer proposal theory where filaments have a higher surface area to volume ratio and are therefore more effective than floc-formers in utilising low concentrations of substrate, oxygen and nutrients. This is also the basis of the accumulation–regeneration theory of Chudoba *et al.* (1973a,b). However, the disadvantage that floc-formers have at low S values, due to a high K_{s} value, is overcome if μ_{m} is increased, and it has been shown that certain plant modifications can in fact do this. This is graphically illustrated in Fig. 5.39 where μ is plotted against S. Three lines have been plotted; the two lines for floc-formers have typically higher μ_{m} and K_{s} values than filaments. However, if lines

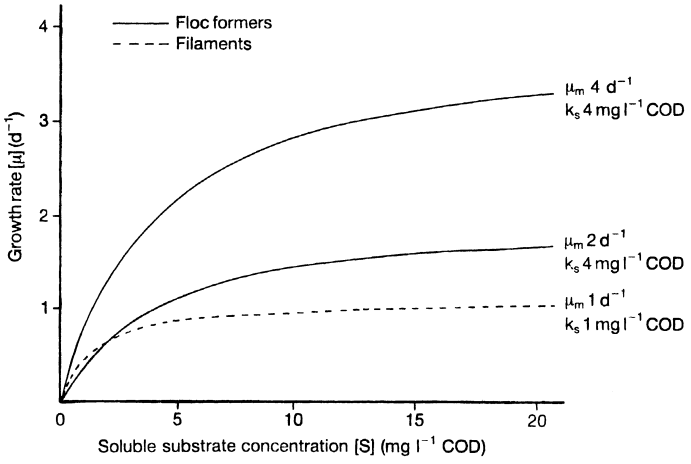


Fig. 5.39. Comparison of the growth rate of floc formers and filamentous microorganisms at various substrate concentrations.

are plotted for floc-formers with the same K_s value (4 mg l^{-1} COD) but different μ_m values (2 and 4 d^{-1}), then very different lines are observed. At the lower μ_m (2 d^{-1}) the plot for floc-formers crosses that for filamentous growth at a substrate concentration of 2 mg l^{-1}. This demonstrates that at low substrate concentrations, filaments have higher specific growth rates. This is the accumulation–regeneration theory and can be considered as a special case in the modified theory (Ekama and Marais 1986). However, at higher μ_m values, the specific growth rate of floc-formers always exceeds that of the filaments regardless of the substrate concentration (Fig. 5.39). This has been elegantly modelled by Cenens *et al.* (2000a,b).

Tapered aeration

Where constant aeration is provided along the length of a plug-flow system, problems will arise due to the decreasing oxygen demand gradient that occurs along the length of the tank. The result is under-aeration at the inlet and over-aeration at the outlet. This can be overcome by tapering the aeration according to the respiratory requirement of the mixed liquor. Although the term tapered aeration suggests a gradual reduction in the aeration along the tank (Fig. 5.40), in practice, it occurs as a series of steps or movements. Lister and Boon (1973) estimate that the oxygen requirement in a plug flow tank is twice as high in the first half, thus, where three steps are used, about 45% of the aeration is supplied to the first third of

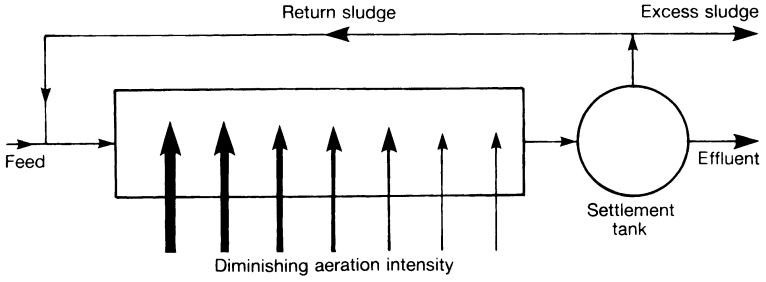


Fig. 5.40. Schematic flow diagram of tapered aeration in a plug-flow activated sludge system.

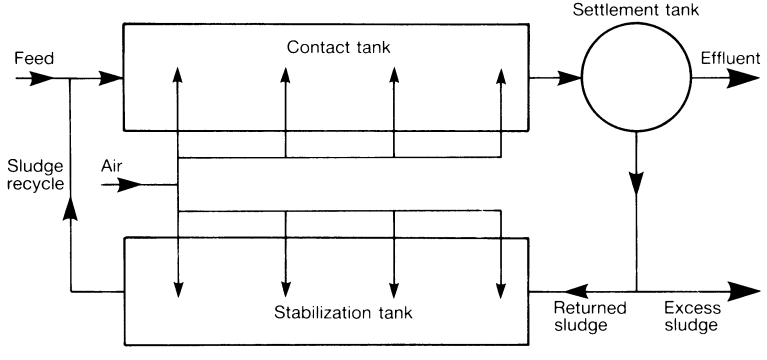


Fig. 5.41. Schematic flow diagram of contact stabilisation.

the tank, 30% in the next third, and 25% in the final third. It is vital to ensure that the aeration at the outlet end of the tank is also sufficient to maintain the sludge in suspension as well as supply the required oxygen. It is easiest to introduce tapered aeration where surface aerators are used, where the different aeration rates are achieved by altering their depth of immersion or by their speed of rotation.

Contact stabilisation

In the contact stabilisation, or biosorption process, adsorption and oxidation are carried out in separate tanks. The influent wastewater enters the contact tank where it is mixed with mixed liquor to give a MLSS concentration of between 2,000 and 3,000 mg l^{-1} for between 0.5 and 1 h (Fig. 5.41). During this short contact period, the organic material present is adsorbed on to the activated sludge flocs. The mixed liquor is then settled in the sedimentation tank and the separated sludge pumped into the aeration

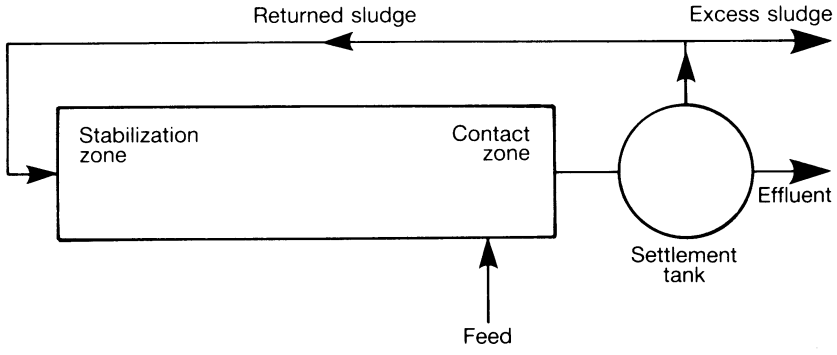


Fig. 5.42. Schematic flow diagram of a single basin contact stabilisation activated sludge system.

tank where it is aerated at a high MLSS concentration of between 4,000–10,000 mg l^{-1} for 5–6 h so that the adsorbed material can be fully oxidised. By having such a short HRT in the contact tank, it is not necessary to provide aeration tank capacity for the entire wastewater flow, as is the case with other conventional activated sludge processes. In fact, approximately 50% less capacity is required and considerable savings in both capital and aeration costs are made. In some designs, the aeration tank is of the same capacity as a conventional plant, which allows a long SRT which gives a greater degree of oxidation. With the sludge in the endogenous respiration phase, less sludge is produced that is of better quality, although the larger aeration capacity will result in higher aeration costs. Moore and Todd (1968) obtained very low sludge production figures of 0.39 kg DS per kg BOD applied, which is significantly less than the 0.5–0.8 kg kg^{-1} BOD expected from conventional plants. Once oxidation is completed in the aeration tank; which is often referred to as reconditioning or reactivation, the mixed liquor is returned to the contact tank to be mixed with the incoming wastewater. The aeration and contact tanks can be incorporated into a single unit, with the influent wastewater entering the tanks towards the outlet zone but the recycled sludge entering at the far end (Fig. 5.42). In this way, two distinct zones are created within the aeration tank. The sludge loading rate (f/m) for contact stabilisation is at the lower end of the range for conventional processes, between 0.1 and 0.2 kg BOD kg^{-1} d^{-1}. The process is particularly suited to strong industrial effluentshaving a large proportion of the biodegradable BOD present as colloidal

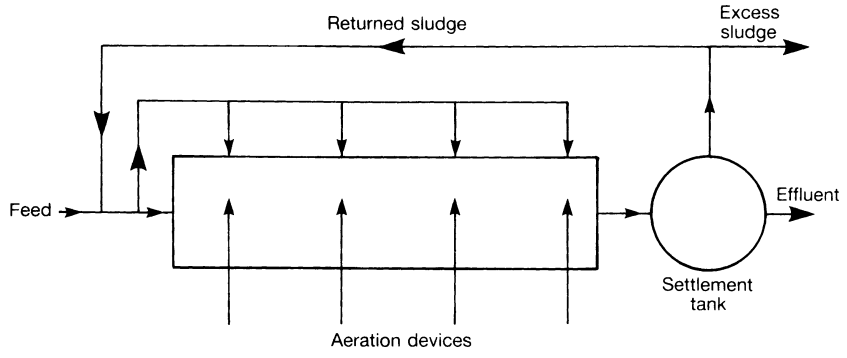


Fig. 5.43. Schematic flow diagram of step aeration in a conventional plug-flow activated sludge system.

or suspended matter. This process can also be used to uprate or expand existing activated sludge systems.

Incremental feeding

The same "levelling out" of oxygen demand along the length of the aeration tank achieved by tapered aeration can also be done by introducing the influent wastewater incrementally at several points along the length of the tank (Fig. 5.43), with all the recycled sludge still introduced into the aeration tank at the inlet end. This results in a more even distribution of the BOD load and therefore a more even oxygen demand along the tank. Thus with a uniform aeration system the BOD:dissolved oxygen ratio should remain more constant, ensuring efficient use of the air supply (Hawkes 1983a). A useful advantage of this system is that the proportion of influent wastewater entering the tank at each stage can be varied according to changes in the organic or hydraulic loadings. This gives the process a considerable degree of operational flexibility. Even though the oxygen demand still rises at the inlet and falls at the outlet end of the tank, the conditions in the aeration tank are approaching those of a completely mixed system, and is generally considered as intermediate between a plug-flow and completely mixed process. Although the effects of shock loads are less than in strictly plug-flow reactors, the chance of short-circuiting is increased and the sludge settleability is slightly reduced. The process is known as incremental or stepped feeding, but is also widely known by the confusing term "step aeration", which would appear to be a more appropriate description of tapered aeration.

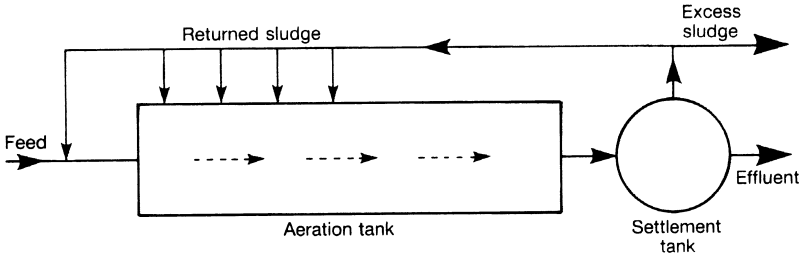


Fig. 5.44. Schematic flow diagram of an incremental sludge feed aeration activated sludge system.

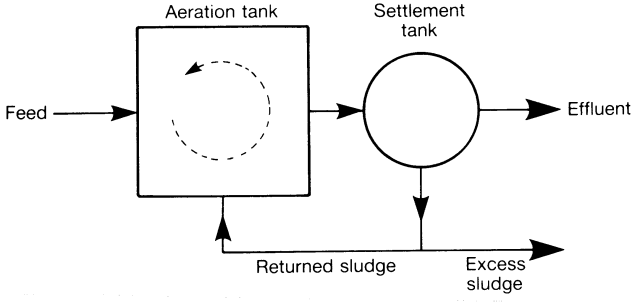


Fig. 5.45. Schematic flow diagram of a completely mixed activated sludge system.

Incremental sludge feeding

A similar effect to tapered aeration or incremental feeding can be achieved by feeding the returned sludge incrementally at several points along the length of the tank, while the influent wastewater enters at a single point at the inlet and aeration is uniform along the length of the tank (Fig. 5.44). The theory is that the sludge activity is balanced with the dissolved oxygen concentration resulting in maximum oxygen utilisation (Balmer *et al.* 1967). When compared with the other incremental modifications, it appears to be only advantageous when the system is oxygen-limited at the inlet stage.

5.3.1.2. *Completely mixed systems*

The theoretical basis of completely mixed systems is that the influent wastewater and the returned sludge are immediately amalgamated with the mixed liquor as they are introduced into the aeration tank (Fig. 5.45) and are therefore diluted with purified effluent, which ensures uniform loading conditions throughout the tank (Fig. 5.38). The immediate dilution effect

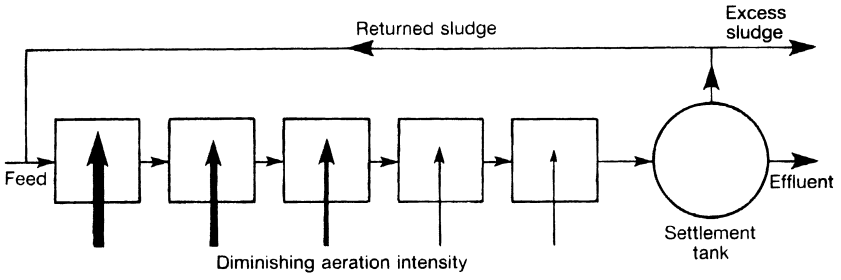


Fig. 5.46. Schematic flow diagram of step aeration in a number of tanks in series.

buffers the system to shock loadings of strong organic or toxic wastes. However, although short-circuiting is possible, completely mixed systems are considered to produce low-density sludge which is difficult to settle compared with plug flow systems. In terms of operation, the most important factor in a completely mixed reactor is efficient mixing and aeration of the mixed liquor within the aeration tank. The MLSS of completely mixed reactors are generally higher (3,000–6,000 mg l^{-1}) than conventional plug flow reactors (2,000–3,000 mg l^{-1}), allowing for considerably higher BOD loadings. Critically low dissolved oxygen concentrations can result in some parts of the tank because of under-aeration or overloading, which prevents nitrification and also subsequently causes anoxic conditions in the settling tanks, resulting in problems of rising sludge due to denitrification. Some of these disadvantages are overcome by attempting to utilise such reactors in a plug-flow manner by using a number of completely mixed tanks in series providing a "stepped" plug-flow system. This is really a series of independent treatment stages that can be individually controlled in terms of loading and aeration. The most widely used adoption of such systems is as a modification to tapered aeration (Fig. 5.46) and step aeration (Fig. 5.47) systems, and also in nutrient removal systems employing anaerobic, anoxic and aerobic tanks or zones (Sec. 5.6).

Although other designs are used, completely mixed reactors are usually single tanks, square in cross-section, with a flat or slightly centrally inclined base. The tank normally allows for a liquor depth of between 3.0 and 6.0 m and a freeboard of 1.0–1.5 m to contain spray. There are many different types of mechanical surface aerators, but those used for completely mixed conventional activated sludge systems are of the vertical shaft type, i.e. rotating in a horizontal plane (Sec. 5.2.3).

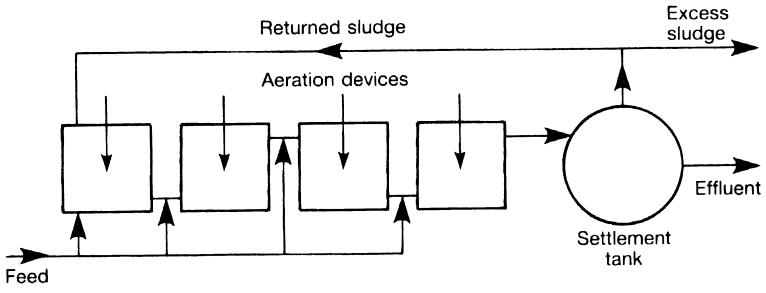


Fig. 5.47. Schematic flow diagram of step aeration in a number of tanks in series.

5.3.1.3. *Sequencing batch reactor technology*

The original design of activated sludge was a single tank batch reactor where aeration and settlement took place in the same tank (Arden and Lockett 1914; Alleman and Prakasam 1983). This design was developed into the conventional systems used today, where aeration and settlement occur in separate tanks. However, renewed interest in batch operation has led to the introduction of sequencing batch reactor (SBR) systems (Irvine and Ketchum 1983). Using a single tank the sequence of operational steps, as outlined in Fig. 5.48, is carried out repeatedly, with each complete cycle taking between 4 to 48 hours, and with the SRT varying from 15 to 70 days. The f/m ratio will vary according to the length of the cycle but typically ranges from 0.03 to 0.18 kg BOD kg^{-1} d^{-1}. A single tank permits better operation management of the mixed liquor with excellent control over oxygen and redox conditions. Sequencing batch reactors are also used for nitrification, denitrification, and biological nutrient removal (Sec. 5.6). Sequencing batch reactor technology is used throughout the world for scientific studies, bench-scale testing, and full-scale applications for small to medium sized populations. Full-scale treatment for large populations is not feasible using standard SBR units. Instead a modification of the process is used, cyclic activated sludge technology (CAST), which is a continuously-fed batch process. They are often referred to under a trade name such as CASS® (Goronszy 1991). In contrast to SBRs where there are strict time cycles for separate aerobic, anoxic, and anaerobic sequencing, requiring influent equalisation and mechanical mixing, CAST has very different process features. For example:

(i) short cycle times of typically below 4 h;
(ii) biological selector zones using transverse partial baffle walls;
(iii) full sequence regulation for bulking control and concurrent nitrification/denitrification;

(iv) no requirement for mixing sequences or equivalent;
(v) process control using in-basin respiration rates, so that process control is demand, rather than time, oriented; and
(vi) high-rate decanting of final effluent from reactor basins.

There are many examples of this type of technology. For example, Potsdam, Germany (PE 90,000) (Demoulin *et al.* 2001) and Neubrandenburg, Germany (PE 140,000) (Demoulin and Goronszy 1999). The application, design, operation and modeling of SBR and CAST processes are reviewed by Delgenès *et al.* (2001) and Wilderer *et al.* (2001). A comparison between batch and conventional activated processes has been made by Hopkins *et al.* (2001).

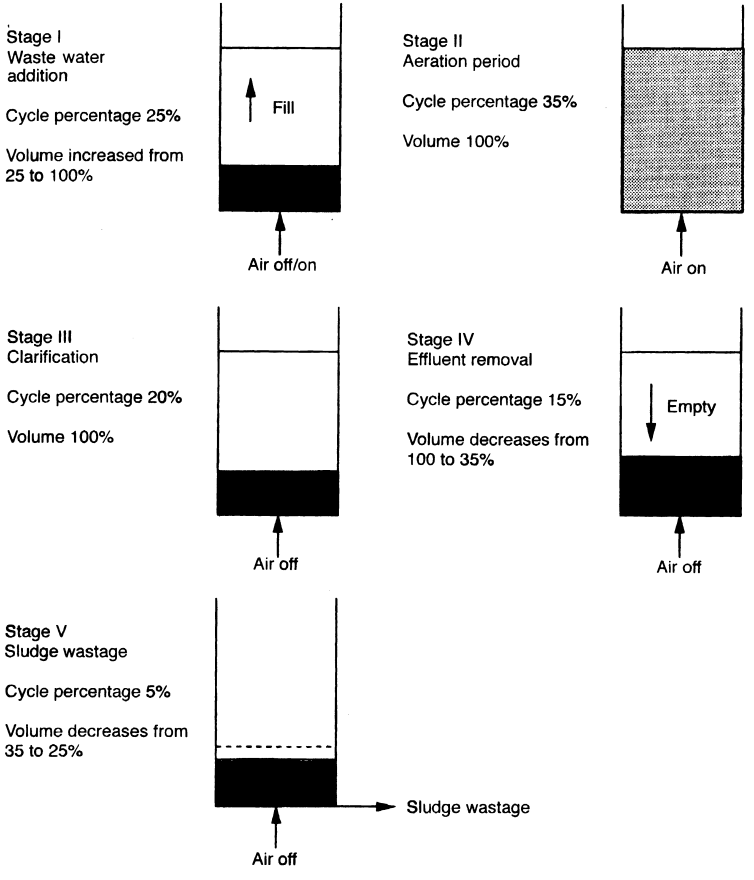


Fig. 5.48. Typical cycle and configuration of a sequencing batch reactor (SBR).

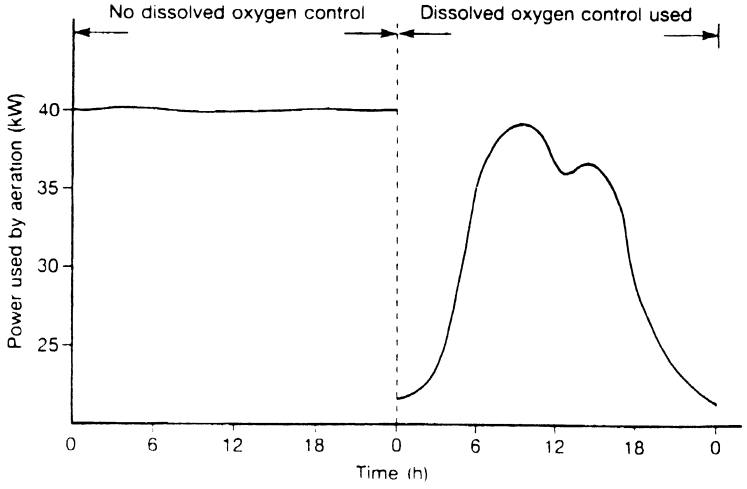


Fig. 5.49. The effect on power consumption by aerators in an oxidation ditch when dissolved oxygen control is used (Cox *et al.* 1982).

5.3.2. *Extended aeration*

In extended aeration, the sludge loading level is very low, between 0.03 and 0.15 kg BOD kg^{-1} d^{-1}, which results in the process being food-limited, forcing the micro-organisms, which comprise the activated sludge, into the endogenous respiration phase of activity (Fig. 5.33). Therefore, the waste is fully oxidised (95% BOD reduction) and less sludge is produced (0.2–0.4 kg DS kg^{-1} BOD removed), which is also more stabilised and more easily dewatered than conventional activated sludge. Organic loadings range from 0.24 to 0.32 kg BOD m^{-3} d^{-1}. This type of low-rate activated sludge process has a long HRT of between 1 and 3 d and as the sludge activity is very low, it requires less intensive aeration than conventional or high-rate processes. Oxygen control of aerators is still required to ensure optimum energy usage (Fig. 5.49). The sludge age of extended aeration processes as expected is quite long, above 5–6 d (Table 5.2).

5.3.2.1. *Oxidation ditches*

The first extended aeration system was developed in 1953 by Pasveer. Known as the Pasveer or oxidation ditch, it is a simple, single-stage oxidation process with no primary sedimentation (Pasveer 1959). In its basic design, it is an oval ditch, usually with a central island, with the sewage agitated by horizontal paddles or cages that aerate the wastewater as well as

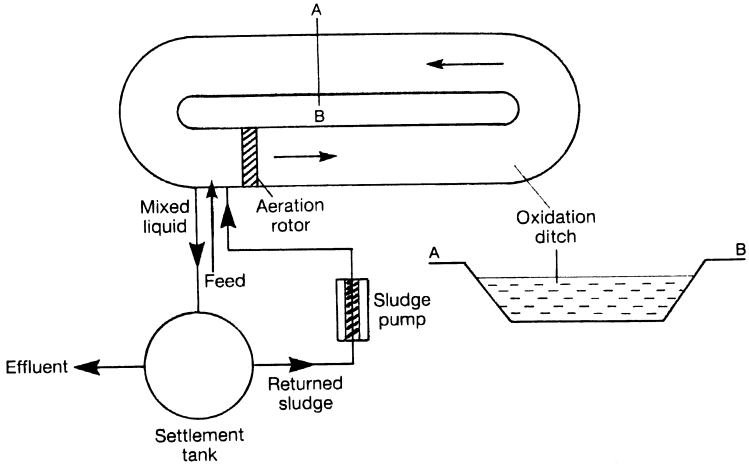


Fig. 5.50. Typical layout of a Pasveer oxidation ditch, with a cross-sectional profile across the main channel between points A and B.

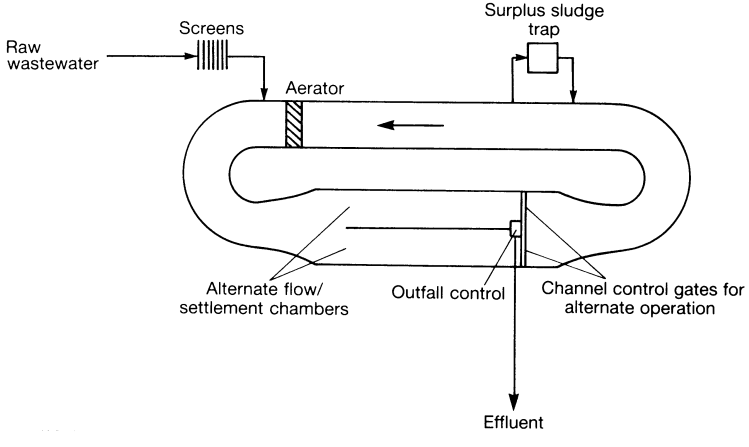


Fig. 5.51. Layout of a continuous split channel Pasveer oxidation ditch.

giving it directional flow (Fig. 5.50). The ditch is normally between 1.0 and 3.5 m in depth and is either rectangular or trapizoidal in cross-section. This basic system represents 95% of the oxidation ditches operating in the UK. The influent wastewater enters the ditch upstream of the aerator ensuring maximum dilution; therefore, oxidation ditches are completely mixed systems, as well as supplying the oxygen where oxygen demand will be greatest. The mixed liquor is drawn off by displacement into the sedimentation tank

and, after separation, most of the sludge is returned. The aeration rate is controlled by the depth the horizontal paddles or cage are immersed, and is altered by controlling the depth of the mixed liquor in the ditch by using an adjustable weir. Two developments of this system are also used.

The continuous split channel system has the channel on one side of the ditch widened and split into two so that, by using control gates, the divided channels can be used alternatively as a settlement tank and flow channel (Fig. 5.51). By shutting the gate, the channel acts as a horizontal sedimentation tank with the final effluent discharged over the end weir, and the settled sludge is resuspended once the control gate is re-opened. Channels are operated alternatively every 4 hours. Excess sludge is removed by means of a trap, or a small sedimentation tank, through which a small portion of the flow continuously passes.

In intermittent flow systems the ditch acts as the aeration and sedimentation tank. It is used for small populations of less than 500 or from strong wastewaters with an intermittent pattern of flow. The operation of the ditch is quite straight forward and is fully automated. The influent enters a balancing tank where it is stored, when full, the ditch aerator switches off and the sludge is allowed to settle for 30 minutes. Influent wastewater is then pumped into the ditch causing the level to rise and a siphon to operate and draw off the same volume of settled final effluent at the same rate. Once the balancing tank is emptied the pump switches off, the siphon breaks, and the aerator restarts so that the whole cycle recommences. These systems are designed to operate on a 4–6 hour cycle.

The lower loading level gives a far greater reserve of dissolved oxygen to cope with surges in organic load, needs little maintenance compared to conventional or high-rate activated sludge and suffers less from sludge bulking or odour problems. Although the settleability of activated sludge tends to improve with increased SRT, after a critical period so much of the microbial mass has been broken down during endogenous respiration that the floc particles become too small to settle efficiently, thus causing problems. Basic design is based on simple formulations, for example, in the calculation of ditch capacity 4.73 m^3 of ditch is required per kg BOD load (PE = 0.055 kg BOD). The HRT (h) is calculated as:

$$\frac{\text{Ditch capacity } (m^3) \times 24}{\text{DWF } (m^3\ d^{-1})}\ \text{h}$$

which is normally in excess of 24 h. In terms of aeration, about 2 kg of oxygen is required to remove 1 kg BOD d^{-1}. Using a horizontal cage aerator at 70 rpm immersed to a depth of 127 mm, a 1 m length of cage will remove

Table 5.10. Basic design criteria for oxidation ditches.

Determinand	UK	USA
Volumetric load		
g m^{-3} d^{-1}	210	161–332
Sludge load		
(kg kg^{-1} d^{-1})	0.05–0.15	0.03–0.10
MLSS (mg l^{-1})	2000–6000	3000–5000
Sludge residence time (d)	—	20–30
Recycle ratio	—	0.25–0.75
kg O_2 per kg BOD $removed^{-1}$	—	1.5–1.8

29.4 kg BOD d^{-1}. Thus, the length of aerator cage (m) required is

$$\frac{\text{Daily BOD load (kg)}}{29.4} \text{ m.}$$

The power requirement (kW) is very low, and at maximum immersion it can be approximated as

$$\text{Length of cage aerator (m)} \times 1.6 \text{ kW.}$$

The rate of flow in the channel is important and should be maintained at between 0.3 and 0.5 ms^{-1} in order to prevent settlement of solids. Flows in excess of 0.6 ms^{-1} are undesirable because excess turbulence affects the stability of the flocs. The sludge loading should be below 0.15 kg BOD kg^{-1} d^{-1}, and if too high, organic matter in the form of sludge will be removed from the channel before being completely oxidised, resulting in a surplus sludge production. However, if sludge loading is kept low then most of the organic matter is oxidised, thus reducing the sludge volume. The normal range for MLSS in extended aeration systems is between 2,000 and 6,000 mg l^{-1} (Table 5.10). Although the area of land required for oxidation ditches is large, this is offset by their cheap construction cost, especially as there is no primary sedimentation. Also, operating costs are low with regard to reduced sludge handling and power requirement (Cooper and Evance 1981). This makes them ideal for small communities and for third world installations. Oxidation ditches are also widely used for treating dairy and other difficult organic wastewaters where a long SRT is desirable. The design and operation of oxidation ditches have been reviewed by Barnes *et al.* (1983), Heide (1982) and Forster *et al.* (1984).

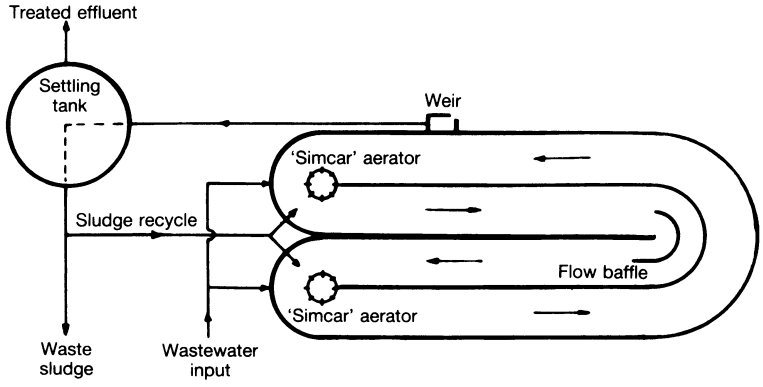


Fig. 5.52. Schematic diagram showing plan for the carrousel type oxidation channel developed by Simon-Hartley Ltd. (Winkler 1981).

The problem of using extended aeration to treat domestic wastewaters from large populations of more than 10,000, when loaded at below 0.3 kg BOD m^{-3} d^{-1}, is the cost of maintaining the sludge in suspension. This has been overcome by using two rotor aerators positioned equidistant from each other. A development of this system is the carrousel-type of extended aeration process which comprises of two rectangular tanks with an aerator at each end (Fig. 5.52) (Zeper and De Man 1970). The tanks are up to 4 m deep and 8 m wide with aeration and turbulence supplied by "Simcar"-type vertical cone aerators. By siting one at each bend, the rotary motion is diverted to provide linear motion. There are a number of carrousel-type plants in operation throughout Europe, with the performance reported as being excellent. For example, the Ash Vale plant of Thames Water treats a PE of 25,000. At a sludge loading of between 0.05 and 0.15 kg kg^{-1} d^{-1}, a 10:10 final effluent was produced (Pay and Gibson 1979). A carrousel-type system in the Cotswold division of Thames Water has been studied over a number of years and is fully examined by Rachwal *et al.* (1983) (Fig. 5.53; Table 5.11).

Three features of oxidation ditches in general, and of carrousel-type plants in particular are: (1) ease of operation; (2) low production of a highly stabilised sludge; and (3) the ability of such systems to nitrify and denitrify within a single tank. Denitrification occurs by allowing the part of the ditch, most remote from the aerators, to become anoxic (Johnstone and Carmichael 1982; Matsui and Kimata 1986) (Fig. 5.54).

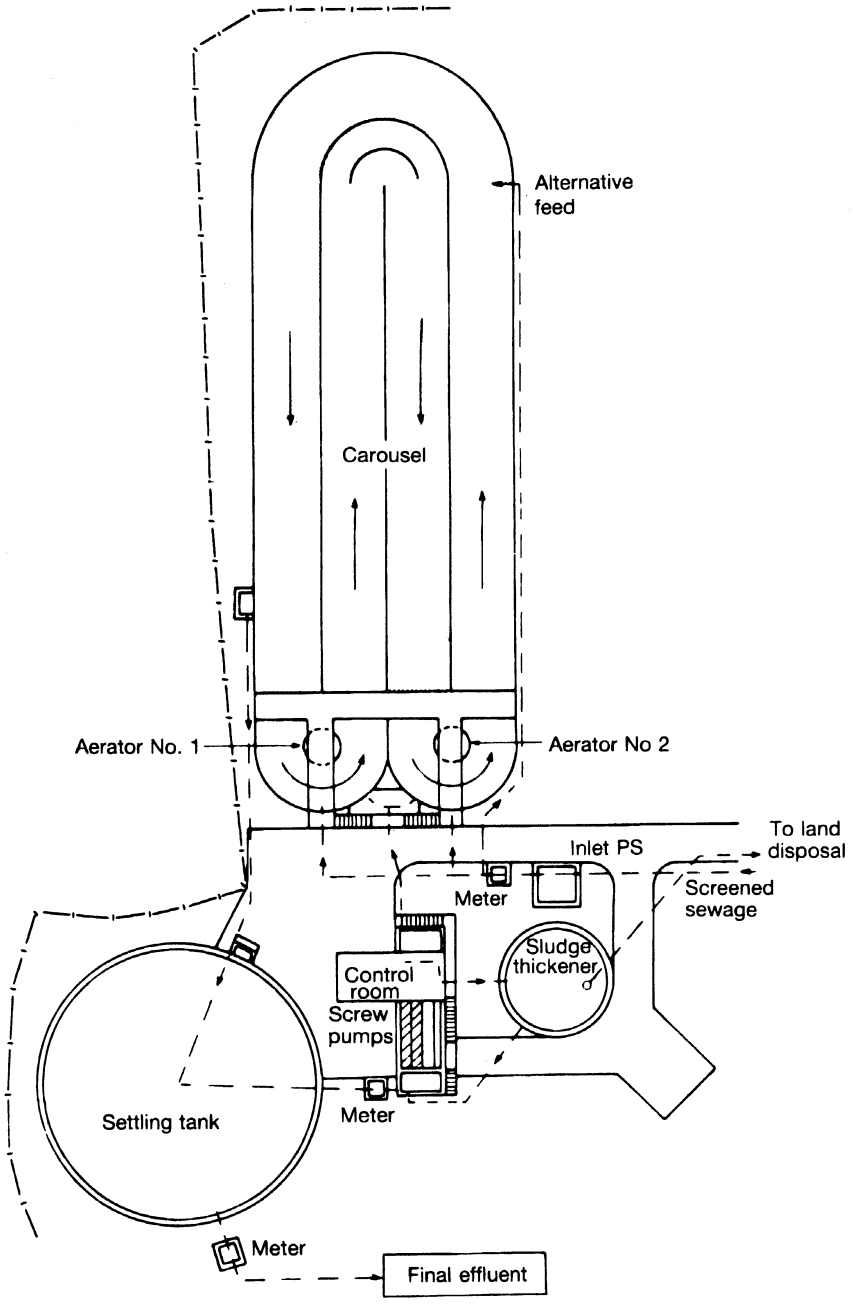


Fig. 5.53. Carrousel activated sludge plant at Cirencester (Rachwal *et al.* 1982).

Table 5.11. Performance summary of the Cirencester carrousel activated sludge plant operated by Thames water, with a population equivalent of 25,000 (Johnstone and Carmichael 1982).

	Carrousel effluent (mg l^{-1})			
	BOD (atu)	SS	NH_3-N	NO_3-N
1978–79				
95 percentile	7.4	17.5	5.3	23.3*
No. results	314	332	332	327
Average percentage removal	98.4	97.1	94.5	—
1979–80				
95 percentile	9.2	12.5	10.9	24.4*
No. results	279	286	289	283
Average percentage removal	96.9	97.5	82.5	—
1980–81				
95 Percentile	10.9	12.6	6.9	31.9*
No. results	341	343	342	315
Average percentage removal	95.7	96.7	86.1	—

* *Maximum values*

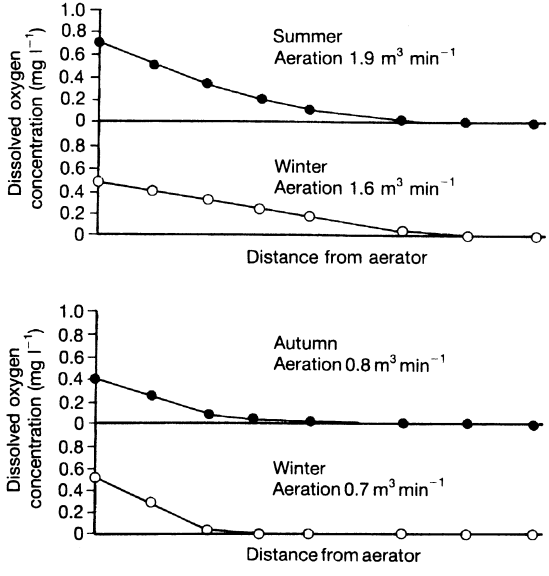


Fig. 5.54. Variation of dissolved oxygen concentration downstream of the aerator in an oxidation ditch at different aeration levels and different seasons (Matsui and Kimata 1986).

5.3.2.2. *Packaged plants*

In Ireland, as in other parts of Europe, small sewage treatment plants serving isolated communities, which have traditionally used a septic tank and percolating filter, are now installing package activated sludge units as an alternative. These self-contained treatment units, which are prefabricated either in concrete or steel, are designed to treat screened sewage that is directly fed into the aeration tank, therefore a primary sedimentation unit is not required. Sludge separation and recirculation facilities are often built into the unit, and aeration is usually supplied by coarse bubble diffusers that also ensure adequate mixing (Fig. 5.55) although small horizontal

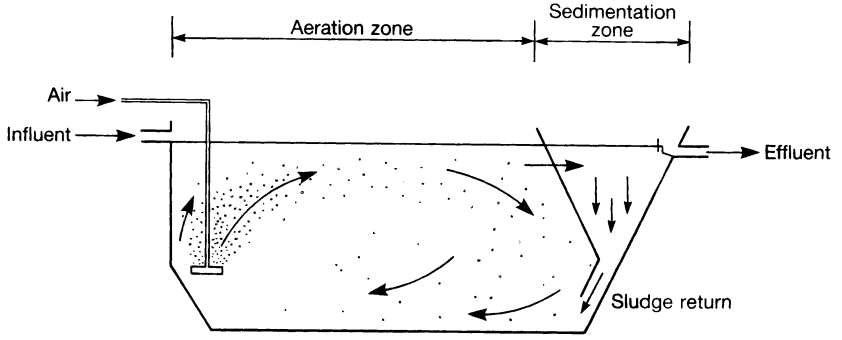


Fig. 5.55. Schematic diagram of extended aeration activated sludge package plant with a clarifier incorporated into the same tank.

Fig. 5.56. Packaged activated sludge plant for a population of 500 with a small horizontal rotor aerator ready to be taken to site for installation.

aerators can also be used (Fig. 5.56). With the HRT above 24 h, the activated sludge is in the endogenous respiration phase so that the minimum amount of surplus activated sludge is produced, thus, a sludge loading of between 0.05 and 0.15 kg kg^{-1} d^{-1} is required. Approximately 0.23 m^3 of aeration tank volume is allowed per person, which is equivalent to a volumetric loading of about 0.24 kg BOD m^{-3} d^{-1}. Although each manufacturer has slightly different design features, sludge separation normally takes place in a small chamber within the aeration basin, with the settled sludge returned to the main tank through a slot at the base (Fig. 5.55), or by using an airlift pump, although this makes the overall operation and management more difficult. Units used for villages, schools or hospitals have a very exaggerated diurnal variation in flow with often no flow at all between 01.00 and 06.00 h. Although flow balancing may be desirable, the long HRT of such units ensures that they can accept intermittent loads without upsetting the overall operation. These units are relatively easy and quick to install, and require only periodic desludging to remove surplus activated sludge. This is done by shutting off the aeration, allowing the solids to settle, and then simply pumping the surplus sludge out of the tank using a gully sucker and tanker. The MLSS concentration ranges from 1,000 mg l^{-1}, after desludging, to a maximum of 10,000 mg l^{-1}, when desludging is required. It is estimated that the MLSS will increase in concentration by about 50 mg l^{-1} per day, so that desludging is only required approximately every six months, if loaded correctly. As the MLSS builds up, there may be periodic losses of sludge in the effluent and many manufacturers recommend installing tertiary treatment to intercept any suspended solids before discharge to the receiving water. Like all packaged plants installation is extremely simple, requiring a concrete platform either above, or in this case below ground (Fig. 5.57).

The nature of these units makes them ideal in many ways for isolated communities. However, experience in Ireland has shown that it is these very communities that often become cut off in winter because of snow and have frequent and often prolonged cuts in electricity. Although short periods without power to operate the aerators will not adversely affect the operation of package activated sludge units, long periods of more than 24 hours will begin to kill off the floc micro-organisms. Where this is a problem, the mechanically operated septic tank and percolating filter system is probably the best treatment option. Other activated sludge processes, such as contact stabilisation, are also available as package plants. In the UK the installation of packaged activated sludge plants reached a peak during the 1960's. However, due to their high operating and maintenance

Fig. 5.57. Newly sited aeration tank for 1000 people, with a Euromatic aerator. Sludge separation is by an upflow clarifier with a wedgewire filtration system. Sludge is returned from the centre of the clarifier to the aeration tank by an airlift pump.

costs, coupled with frequently inconsistent effluent quality many are being phased out (Ainsworth and Gill 1987).

5.3.3. *High-rate activated sludge processes*

High-rate treatment is used, as is high-rate filtration, as a partial or roughing treatment for medium to strong wastes prior to further biological treatment or discharge to a sewer (Boon and Burgess 1972). They are widely used in the food processing and dairy industries (Bruce and Boon 1971), although they are also used for partial treatment of domestic wastewaters.

The loading levels are several times greater than those in conventional activated sludge processes (Table 5.2), which give a rapid but only partial removal of BOD. The BOD loading is 1.5–3.5 kg BOD m^{-3} d^{-1}, with a sludge loading of 1.0–2.5 kg BOD kg^{-1} d^{-1} (Emde 1963). To maintain such a high sludge loading rate, the MLSS concentration must be maintained at 5,000–10,000 mg l^{-1}. This is a major design factor requiring a generous separation provision allowing for returned sludge facility of up to 100% of the influent wastewater. With a short SRT of below 0.5 d, the micro-organisms are in an environment where both food and oxygen are non-limiting, resulting in a rapid growth rate and a high sludge production

rate of 0.8–1.2 kg DS per kg BOD removed. These conditions tend to favour the dispersed bacteria that are able to grow faster than the floc-forming species and results in a less flocculent activated sludge, which may cause problems in the separation stage. The dewatering characteristics of high-rate activated sludge are considered to be inferior to conventional activated sludge (Gale and Baskerville 1970), although, in practice, there is very little difference between the two. Where problems do exist, Bruce and Boon (1971) have suggested that coagulants will help to dewater the sludge. In the aeration tank, the MLSS concentration is higher than in conventional plants, and with the increase in sludge activity there is a greatly enhanced BOD removal rate requiring a higher critical concentration of dissolved oxygen (above 2 mg O_2 l^{-1}). The high oxygen demand of the mixed liquor combined with the higher MLSS concentration requires a highly effective aeration and mixing system. Although vertical cones can be used, fine or coarse bubble diffusers are favoured. With a HRT of only 1–2 h, BOD reduction is limited to 60–70% in high rate systems, although this represents a very high BOD removal per unit volume of aeration tank per unit time compared with other processes. The process is similar to contact stabilisation, with an initial rapid adsorption of organic waste over the first 10–15 min of aeration, followed by a second period of adsorption after about 1 h, giving a considerable degree of BOD removal almost entirely by adsorption on to the flocs (Kehr and Emde 1960). Because of this, the HRT is largely unimportant so long as it is above 1 h (Hawkes 1983a). However, problems with process instability and a risk of odour production have led to the adoption of a longer HRT, normally 8 h based on DWF.

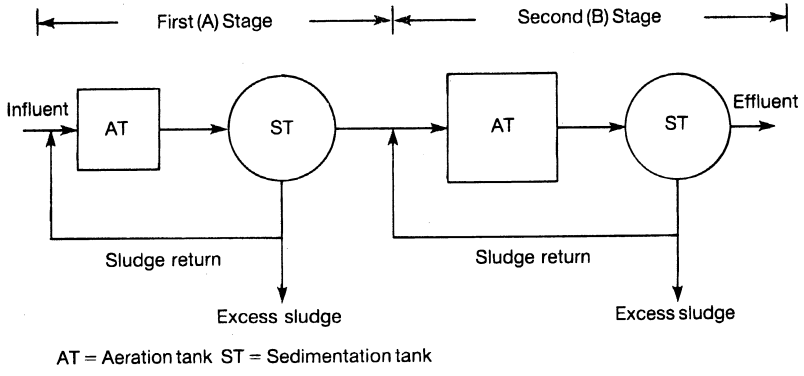


Fig. 5.58. The A–B process.

5.3.3.1. *A–B process*

This is a two-stage activated sludge system developed by Bohnke (1983) of the Technical University of Aachen. The process comprises two separate plants in series, a highly loaded first (A) stage followed by a low loaded second (B) stage (Fig. 5.58). There is no primary sedimentation and the influent enters the first aeration tank after receiving preliminary treatment only. Sludge loading rates in the A-stage are up to 3–6 kg kg^{-1} d^{-1}, with up to 70% of the influent BOD removed at this stage. The B-stage is operated at a normal sludge loading of between 0.15–0.30 kg kg^{-1} d^{-1}. The A-stage effectively buffers the B-stage from variations in either flow or influent quality, especially toxic, pH, or organic shock loadings, thus ensuring a high degree of process stability in the second stage. The wastewater entering the B-stage is extremely constant in terms of quality making this second stage very stable. The lower sludge production in the second stage permits longer sludge ages, resulting in greater BOD removal and nitrification. The average sludge age in the second stage is 20–25 d, which is about twice the sludge age of a conventional single stage plant operated at the same sludge loading. The A–B process does not require primary sedimentation and only requires about 60% of the aeration tank capacity of a conventional plant. This can result in a reduction in capital (constructional) costs of up to 30%, while the lower aeration costs can be in the order of up to 20% less than a single stage activated sludge plant.

Existing activated sludge plants can be uprated by incorporating an A-stage, converting the plant to an A–B process. For example, the treatment plant at Haan–Gruiten was uprated by converting the existing aerated grit removal system into a combined A-stage and grit chamber, and utilising the existing primary sedimentation tank for intermediate settlement. All that was required was a new sludge return pumping station for the A-stage (Eitner 1983). Comparative plant performance has been discussed by Versprille *et al.* (1984). They compare five A–B plants in Germany (Table 5.12) in which the mean BOD removal at the A-stage ranged from 45 to 62%, while overall BOD removal was 97.2 to 99.4%.

While A–B plants have been widely built in Germany, one of the most interesting examples was commissioned in August 1987 at Dokhaven in Rotterdam. This plant, which serves a population of 470,000, was built on the site of a disused harbour. Because the area was primarily residential, it was decided to construct the plant entirely below ground. From the surface, all that is visible is the main central buildings which are set in a large expanse of parkland. As with most high-rate systems, the A-stage at the

Table 5.12. Operating data for five full-scale A-B plants in West Germany (Versprille *et al.* 1984).

	Krefeld	Pulheim	Rhein-hausen	Bad Gruiten	Bad Honnef
Capacity (PE)					
Design	800,000	80,000	170,000	10,000	35,000
Actual	505,000	26,700	84,000	4,500	50,000
Influent (mg BOD l^{-1})	480	412	214	288	620
Effluent (mg BOD l^{-1})	5–7	6	6	5	4
Effluent (mg COD l^{-1})	30–60	42	52	50	30
Reduction rate A-stage (%)	55	59–62	44	43	55–60
Sludge load B-stage $\left(\frac{\text{kg BOD}}{\text{kg MLSS} \cdot \text{day}}\right)$	0.13	0.05	0.18	0.15	0.13
SVI A-stage (ml g^{-1})	37	40–58	60	50	40–60
SVI B-stage (ml g^{-1})	130	50–70	93	65	70–100

Dokhaven plant uses air diffusers and is loaded at 10 kg BOD m^{-2} d^{-1}. This gives a sludge loading of only 2.5 kg kg^{-1} d^{-1}, which is low compared to other plants where a mean sludge loading of 5.0 kg kg^{-1} d^{-1} is normal. The B-stage employs surface aerators. Due to the closeness to residential housing the air vented from the plant, some 80,000 m^3 h^{-1}, has to be purified before discharge via a 50 m high chimney at the nearby sludge handling plant. The air is purified by a three-stage wet chemical scrubber system that effectively removes odour and any air-borne pathogens (Anon 1987a; Meijer 1988).

Enhanced BOD and solids settling have been achieved by introducing plastic media into aeration tanks thus combining suspended and fixed growth processes. Such hybrid systems have been reviewed by Tyagi and Vembu (1990). Gebara (1998) reported considerable improvements in performance by fitting plastic nets vertically inside the aeration tank.

5.3.4. *Advanced activated sludge systems*

The main objective of advanced activated sludge systems has been to increase the amount of dissolved oxygen available for biological activity. This has been achieved by increasing the rate of oxygen transfer from the gas phase to the liquid phase by increasing the partial pressure of oxygen

in the gas phase and so increasing the saturation concentration of dissolved oxygen. Two methods have received considerable attention: the deep shaft process in which the total pressure of the system is increased; and the use of pure oxygen instead of air to increase the proportion of oxygen in the gas phase. These advanced processes, such as the ICI Deep Shaft® process, the Wimpey Unox®, BOC Vitox®, and the Megox® processes, have all intensified the activated sludge process making it even more compact both in terms of design and operation.

5.3.4.1. *ICI Deep Shaft® process*

The deep shaft system, developed by ICI as a spin-off from their single cell protein production, is perhaps one of the most interesting advanced treatment systems in operation today. By using a deep shaft or well (30–220 m), the hydrostatic pressure in the base of the unit reaches pressures of 5–10 atmospheres, resulting in high oxygen transfer efficiencies without a high energy consumption. The increase in pressure results in an increase in oxygen solubility by as much as a factor of 10. Although there is a high degree of mixing in the process there is little dispersion or back mixing, making the process essentially plug-flow in nature (Dunlop 1978).

The shaft is between 0.8–6 m in diameter and is divided vertically to produce a central downflow pipe and an outer upflow section that are connected at the base (Fig. 5.59); although alternative arrangements have been used, such as a more simple U-tube configuration (Fig. 5.60) (Cox *et al.* 1980). Raw sewage is screened and degritted, and before it enters the shaft it is mixed with returned activated sludge (Fig. 5.60) (1). Once in the top chamber above the actual shaft it is mixed with the mixed liquor already in the unit (2), before it is drawn down the central shaft. Compressed air is added at about a third to a half of the depth of the shaft, and as the downflow liquor velocity exceeds the bubble rise rate, the air is carried down the shaft and gradually dissolves as the pressure increases (3). The high pressure near the base of the shaft ensures that almost all the air present is in solution, providing an oxygen-rich environment for the activated sludge micro-organisms (4). At the bottom of the shaft, mixing is induced by the configuration of the base but a minimum current velocity of 1.5 m s^{-1} must be maintained to prevent settlement (5). The MLSS concentration can be as high as 5,000 mg l^{-1} so oxidation is rapid (6). The turbulence due to the high liquid circulation rate results in a high liquid Reynolds Number (above 10^5) and an increase in the rate of mass transfer. The contact time between the air bubbles and the liquid is also very much higher being up to 5 minutes compared to 15 seconds in a conventional

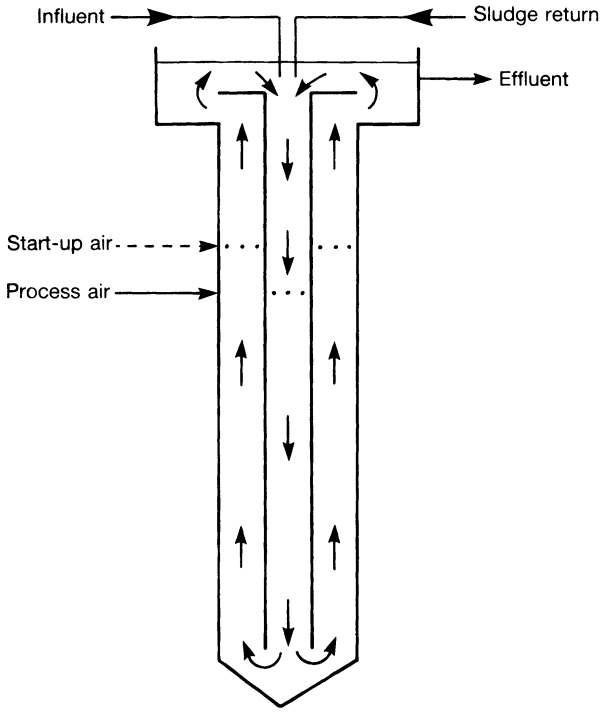


Fig. 5.59. Schematic diagram of the Deep Shaft® process developed by ICI plc using a central tube configuration.

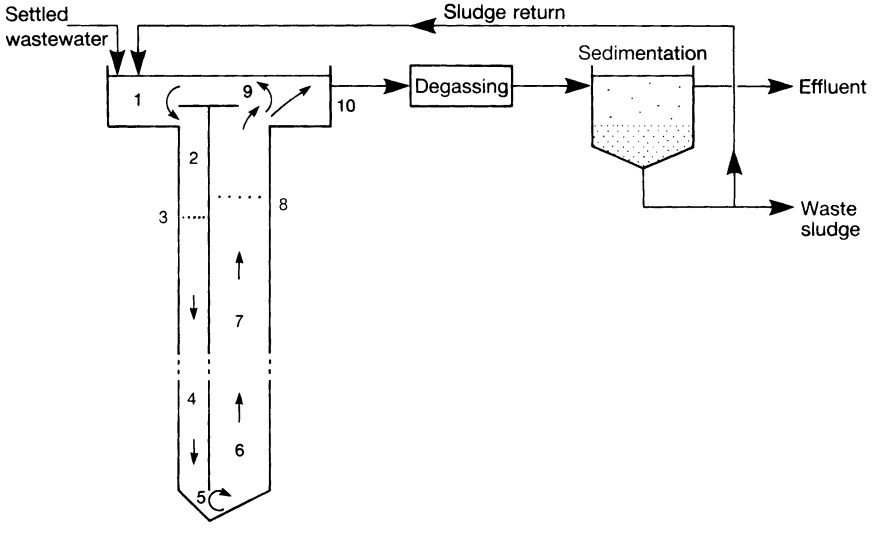


Fig. 5.60. Schematic diagram of the Deep Shaft® process employing a U-shaped reaction shaft.

diffused air system (Hemming *et al.* 1977). The pressure decreases as the mixed liquor moves up the upflow section releasing the air, which is mainly carbon dioxide, nitrogen, and some residual oxygen, from solution forming bubbles. As the bubbles near the surface they increase in size thus increasing their upward velocity. The presence of the bubbles lowers the specific gravity in the upflow section, forcing the mixed liquor to rise, which gives the process its overall circulation (7). The upflow air injection is used to start up the system and to control the flow rate; however, once circulation is established the release of air from solution as the pressure changes in the upflow section is enough to keep the circulation in operation (8). Once at the surface, the air escapes to the atmosphere (9), but due to the amount of air in solution it may be necessary to use vacuum degassing to ensure that the air in the mixed liquor does not interfere with the separation of sludge from the purified effluent in the sedimentation tank (10).

Pilot-scale trials have shown that the deep shaft process is able to achieve a good BOD removal rate (90%), but little nitrification. The normal organic loadings range between 3.7–6.6 kg BOD m^{-3} d^{-1}, with a sludge loading rate (f/m) of 0.8–0.9 kg kg^{-1} d^{-1}, a sludge age of 4–5 d, an HRT of 1.17–1.75 h, which is even shorter than in high-rate processes, and a MLSS of between 2,000–6,000 mg l^{-1} for the treatment of mixed industrial and domestic wastewater. The sludge has good settling characteristics, with SSVI values between 30 and 100 l^{-1} g^{-1}, which is readily dewatered. In terms of sludge activity and microbial ecology, sludges from deep shaft units are very similar to those from conventional plants, with sludge flocs about 0.1 mm in diameter (Dunlop 1978; Hemming 1979). The process is seeded by using activated sludge from a conventional plant and whereas the microbial population takes 2–3 weeks to stabilise, a good BOD removal can be expected within 1–2 days (Hemming 1979). However, the effluent does contain high concentrations of suspended solids, therefore overall the sludge production is lower than conventional activated sludge plants at 0.50–0.85 kg kg^{-1} BOD removal (Bolton and Ousby 1977; Hemming *et al.* 1977; Cox *et al.* 1980). Clearly, the deep shaft process provides an excellent first stage for a two-stage process with the second stage providing nitrification. Cox *et al.* (1980) used a percolating filter as a second stage in their pilot trials which achieved a 95% reduction in ammonia when loaded at 1.7 m^3 m^{-3} d^{-1}. Full-scale plants have been used to partially treat strong wastes. Collins and Elder (1980, 1982) describe a plant treating a strong domestic wastewater, with a mean BOD of 1,060 mg l^{-1}, for discharge directly into the Thames Estuary. This plant was of the concentric tube design, 130 m deep and 1.86 m in diameter. Operated with a HRT of 1.5 h

and at an MLSS concentration of 5,000 mg l^{-1}, the average BOD reduction was above 85%, with good sludge settleability (SSVI 40–70 ml g^{-1}). By filtering the final effluent to remove the suspended solids, the BOD reduction was increased to 95.5%.

In terms of loading, the deep shaft system falls between conventional and high-rate processes. It has a small space requirement, taking up 50% less land area than conventional activated sludge processes because of not having primary sedimentation and the aeration tank volume being largely underground (Irwin *et al.* 1989). The power requirement is low, requiring approximately 0.85 kW h kg^{-1} BOD removed, which includes both aeration and recirculation of sludge. But in order to be competitive with other activated sludge processes, the deep shaft process has to overcome a number of fundamental problems. For example, constructing deep shaft systems is difficult, and the stability of the soil and the bedrock is extremely important. Two options are available, to build a deep narrow shaft or a shallow wide shaft. The former gives higher hydrostatic pressures and increased oxygen transfer, but increased excavation and constructional costs, whereas the latter option provides a lower hydraulic resistance, which requires a lower specific power requirement than the narrower shafts and is also easier and cheaper to build. Other problems include foam production (Wheatland and Boon 1979), degassing, presence of fine organic solids in the final effluent that significantly increases the BOD, the possibility of a reversal of flow within the shaft, and the accumulation of dense solids in the bottom of the shaft due to inadequate screening or grit removal. The removal of the microbubbles from the activated sludge after it has left the shaft can be difficult. Vacuum degassing, flotation and air stripping are the most commonly used methods. However, it is becoming more common to design plants where smaller shafts are used to remove only a proportion of the organic load. Subsequent aeration in plug-flow reactors not only strip out the microbubbles but oxidise the remaining organic matter. Normal sedimentation tanks are used with the separated sludge returned directly to the shaft and not the secondary aeration tanks.

5.3.4.2. *Pure oxygen systems*

Atmospheric air contains only 21% oxygen and using pure oxygen instead of air will increase the saturation concentration of oxygen by a factor of five, thereby significantly increasing the oxygen transfer rate. In theory, this should increase treatment capacity of the aeration stage, or by switching from atmospheric air to pure oxygen at times of high organic loading it can

be used to temporarily increase the capacity of the aeration tank at peak loadings.

The theory is quite straightforward. The rate oxygen is absorbed by water from air is controlled by the rate of diffusion across the air-water interface. The rate of oxygen transfer can be expressed as

$$\mathrm{d}c/\mathrm{d}t = [K_\mathrm{L} A(C_\mathrm{s} - C)]/V\,,$$

where K_L is the liquid film mass transfer coefficient; A the interfacial area; V the volume of mixed liquor; C_s the equilibrium saturation concentration of dissolved oxygen; and C the actual concentration of dissolved oxygen in the mixed liquor (mg O_2 l^{-1}). When the mixed liquor is aerated, the total interfacial area A cannot be realistically measured, therefore the overall oxygen transfer coefficient ($K_\mathrm{L}a$) is used,

$$K_\mathrm{L}a = K_\mathrm{L}(A/V)\,,$$

thus, a represents the interfacial area per unit volume of mixed liquor. Thus,

$$\mathrm{d}c/\mathrm{d}t = K_\mathrm{L}a(C_\mathrm{s} - C)\,.$$

The oxygen transfer coefficient ($K_\mathrm{L}a$) in mixed liquor varies with the type of wastewater being treated, with detergents and the method of aeration adopted of particular importance. The effect on the oxygen transfer coefficient is complex. For example, in diffused air systems detergents tend to reduce $K_\mathrm{L}a$ as the interfacial area per unit volume (a) is increased by affecting the surface tension and bubble size, whereas the concentration of surface active material at the interface decreases the mobility of the surface film thus reducing K_L (Lister and Boon 1973; Boon 1976). With high-shear surface aerators the presence of detergents has the opposite effect on $K_\mathrm{L}a$ by increasing a to a greater degree, then K_L is reduced (Winkler 1981).

Therefore, in air systems, the maximum rate of oxygen transfer is achieved by maximising $K_\mathrm{L}a$ by optimal use of the aeration system and by maintaining a maximum oxygen deficit $C_\mathrm{s} - C$ by operating the aeration tank at the critical dissolved oxygen concentration ($C < 2.0$ mg O_2 l^{-1}). Higher oxygen concentrations can only be obtained by increasing the rate of aeration, thereby increasing the $K_\mathrm{L}a$ rate, although this will be expensive in terms of energy and in loss of settleability due to shear damage to the floc structure as turbulence is increased. The advantage of pure oxygen systems is that the oxygen deficit $C_\mathrm{s} - C$ is so great that high operating oxygen concentrations can be maintained (6–10 mg O_2 l^{-1}) without increasing $K_\mathrm{L}a$. In fact, the $K_\mathrm{L}a$ value is decreased in practice in order to achieve the same

mass transfer, which results in a lower intensity of agitation (Lewandowski 1974). Oxygen transfer kinetics, and improved methods of controlling the dissolved oxygen concentration in pure oxygen activated sludge systems, are discussed by Cliff and Barnett (1988), and also by Clifft and Garrett (1988).

As lower $K_L a$ values result in less disruption of the floc due to shear forces, which will improve sludge settleability, ideal designs have aimed to reduce the level of agitation in the aeration tank to the minimum so that the sludge is just maintained in suspension. The main advantages are: flexibility in operation to cope with surge flows; reduction in energy usage to drive aerators; higher organic loading per unit volume of reactor able to be treated; and better performance. Enhanced performance including nitrification, is due to the higher oxygen concentration, which ensures maximum penetration of oxygen into the floc as well as a smaller proportion of the activated sludge deprived of oxygen. Pure oxygen systems have been adopted because of three important advantages over air systems: (i) improved rates of BOD removal; (ii) reduced sludge yields; and (iii) improved settling characteristics of the sludge (Sidwick and Lewandowski 1975). However, comparative studies between pure oxygen and air systems have not always supported all these claims (Huang and Mandt 1978; Suominen 1980). Therefore, pilot-scale trials are necessary before the adoption of such an expensive system. The cost of producing oxygen is the limiting factor for this advanced process and, in recent years, new methods of producing oxygen have been continuously developed so that the cost of oxygen has steadily fallen (McWhirter 1978a,b).

There are two main types of pure oxygen activated sludge systems, those that operate in closed oxygen-rich atmospheres that generally employ surface mechanical aerators, and open systems that employ fine bubble diffusers.

Closed systems

By enclosing the aeration tank, a pure oxygen atmosphere can be maintained above the mixed liquor. There is, of course, an increased risk from having a pure oxygen atmosphere as many materials that may occur in wastewaters are highly combustible under these conditions, even though the gas phase is saturated with water, which reduces the hazard (Baker and Carlson 1978). Also, the materials used for the construction of such systems must be safe for use in a high oxygen environment. Safety equipment to monitor hydrocarbon accumulation in the space above the mixed liquor, and

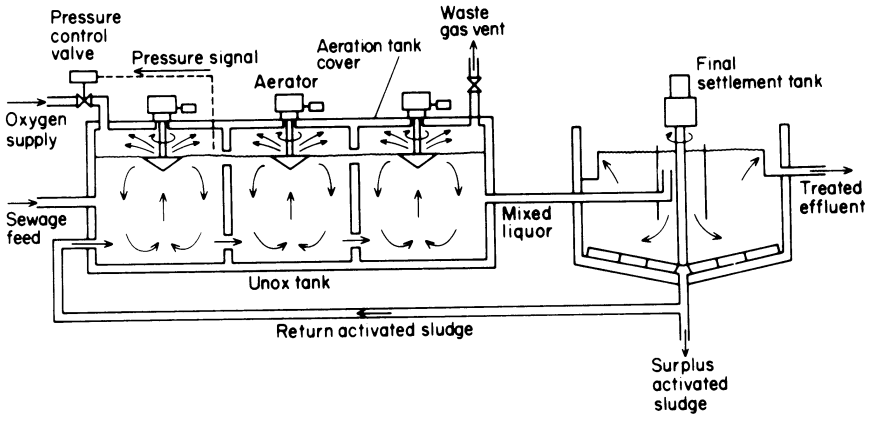


Fig. 5.61. Schematic diagram of the Unox® activated sludge system developed by Wimpey-Unox Ltd. which uses pure oxygen (Hawkes 1983a).

for rapidly venting the oxygen and replacing it with air, is standard. These systems operate at low pressures and at normal ambient temperatures. Like all enclosed treatment systems, odours are greatly reduced and problems associated with spray are contained.

Although marketed in Britain and Ireland by Wimpey Unox®, the Unox Process was originally developed by the Union Carbide Corporation in the USA. Most plants are in the USA and Japan and treat a variety of domestic, industrial, and chemical wastewater (McWhirter 1978c). The enclosed aeration tank is split up into a number of compartments by baffles, each served by a mechanical surface aerator (Fig. 5.61). The number of compartments depends on the level of treatment required and the nature of the wastewater, but the optimum number for domestic and municipal wastewaters appears to be between four and six. However, the greater the number of compartments the greater the oxygen utilisation (Mc Whirter 1978b). Using submerged inlets, the settled wastewater and returned sludge are fed into the final compartment, and the oxygen is pumped into the space above the mixed liquor. The oxygen and mixed liquor flow from one compartment to another so that the aeration tank becomes a series of completely mixed units. The oxygen concentration in the gas phase falls in each compartment as it is utilised by the micro-organisms, with 80–90% of the oxygen supplied utilised overall, which reduces the volume of gas to about 20% of the original volume. In the last compartment, the gas only contains 40–50% oxygen as it has been diluted by carbon dioxide, and is finally vented to the atmosphere. As

the nutrient concentration in the mixed liquor also falls from stage to stage, the design ensures that oxygen transfer is greatest where the oxygen demand from the mixed liquor is greatest. When treating typical municipal wastewater, the MLSS is maintained at 5,000–6,000 mg l^{-1}, which is about 50% higher than conventional activated sludge processes using air. The working concentration of dissolved oxygen in the mixed liquor is 4–8 mg l^{-1} compared to only 1–2 mg l^{-1} in air-operated plants. The high oxygen concentration limits the sludge loading even at high BOD loadings, which are 3–4 times higher than air systems at 2.5–4.0 kg BOD m^{-3} d^{-1}. The sludge loading rate (f/m) can be double the conventional loading rate (Table 5.2) at 0.4–1 kg kg^{-1} d^{-1}. Pure air systems have up to 40% shorter retention times and generally produce less sludge per unit mass of BOD removed with better settling properties. A most interesting Unox pilot plant study has been undertaken by the Scottish Development Department (1977) using a mixed domestic and industrial effluent.

Other closed systems include the Oases® system developed by Air Products and Chemicals Inc (USA), and the Forced Free-Fall® oxygenation system by Airco Inc (USA) (Wyatt *et al.* 1975; McWhirter 1978a).

Open tank systems

The use of pure oxygen in open systems requires a highly effective mechanism to ensure maximum dissolution of oxygen so that wastage is minimised. The Vitox® aeration system, developed by the British Oxygen Company, was originally devised for re-aerating lakes and rivers that had become oxygen depleted. The potential of the system to uprate overloaded activated sludge plants was quickly realised and many new specifically designed systems are now available. The main advantage of these systems is that they can be used with existing aeration tanks with no need to construct additional tanks or even modifying existing ones. The use of pure oxygen can be used to replace the existing air system of aeration, to supplement the existing aeration to provide extra oxygen at peak flows, or when the system is overloaded because of seasonal increases in loading. Other advantages of open systems include: no hazard because of an enclosed oxygen-rich atmosphere; no monitoring of potentially combustible compounds; and better access to the tank for maintenance.

The British Oxygen Company has developed two high-efficiency oxygenation devices known as Vitox 1® and Vitox 2®. The Vitox 1® system involves increasing the pressure of the settled sewage flow to 2–3 bars and then pumping it through a vent, when it is then injected with oxygen. The

oxygen is entrained in the flow as fine bubbles that are then broken up into micro-bubbles as the sewage is discharged into the mixed liquor within the aeration tank at a high velocity through an expansion nozzle (Fig. 5.62a). Mixed liquor can be used instead of the incoming settled sewage to carry the oxygen into the aeration tank (Fig. 5.62b). Mixed liquor is withdrawn directly from the aeration tank, injected with oxygen and returned to the

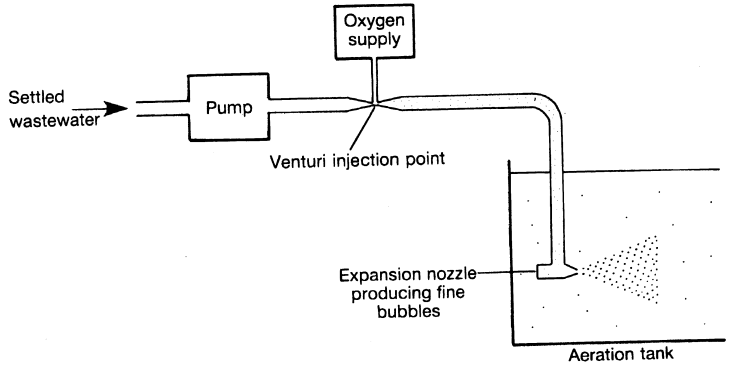


Fig. 5.62a. The Vitox 1® high pressure side stream oxygenation system developed by BOC Ltd.

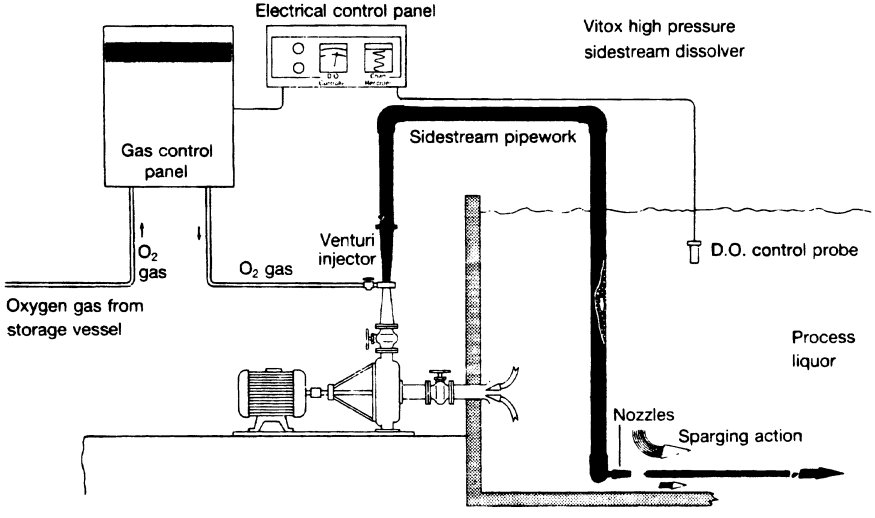


Fig. 5.62b. Alternative layout for Vitox 1® system, with the mixed liquor recirculated through the wall of the aeration tank and then injected with oxygen via the venturi injector before return to the tank via the expansion nozzle.

aeration tank via an expansion nozzle. The high velocity of the discharge causes considerable turbulence ensuring that the oxygen is rapidly mixed with the contents of the aeration tank (Boon 1978). Units are essentially made to measure and are able to inject up to 7 tonnes O_2 d^{-1}. Multiple units can be used to provide more oxygen if required. The system has been adopted at a number of holiday resorts to cope with seasonal increases in the organic loading (Rees 1978). Under these circumstances, the cost of installing a Vitox 1® system will be only a few percent of the cost of extending a conventional activated sludge plant.

A modification of the Vitox 1® system is the use of a downflow bubble contactor, a process known as Vitox 2®. The oxygen is pumped into the main wastewater stream, which then flows downwards through the contactor at a low velocity. The reduced velocity ensures that only very small bubbles with an extremely low upward flow velocity are carried out of the contactor and injected into the aeration tank. The larger bubbles remain in the contactor until their size is reduced sufficiently by oxygen dissolution to be carried out of the wastewater stream (Fig. 5.63).

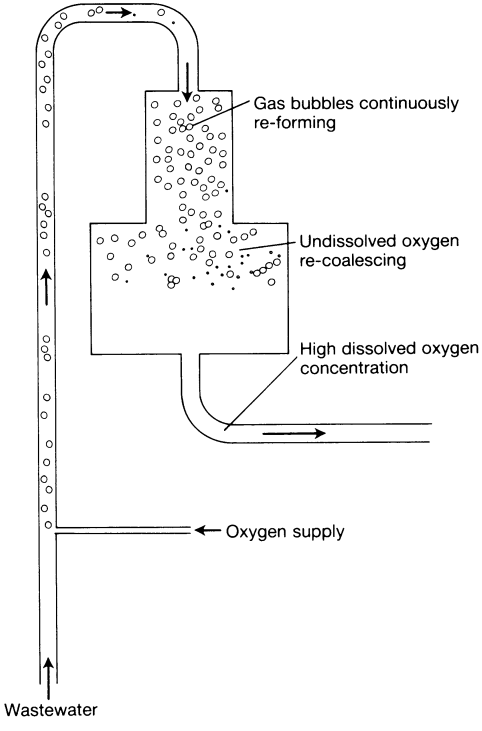


Fig. 5.63. The Vitox 2® bell diffuser system developed by BOC Ltd. (Winkler 1981).

The Vitox 2® oxygenation system is used in the Megox® treatment process, which has been particularly designed to treat difficult and nutrient-rich wastewaters, especially those from the food-processing industry. The Megox® process incorporates both the biological and sludge separation phases into a single tank. The influent wastewater is mixed with the recycled activated sludge and injected with oxygen via the downflow bubble contactor. This oxygen rich mixed liquor is discharged into the central well of the tank, and as the oxygen concentration is already very high only a minimum amount of mixing is required to maintain the microbial flocs in suspension, which is provided by a rotating sludge consolidator with a very slow rotation speed. There is an outer concentric zone separated from the central reaction zone by a deep baffle, where the sludge is discharged at the

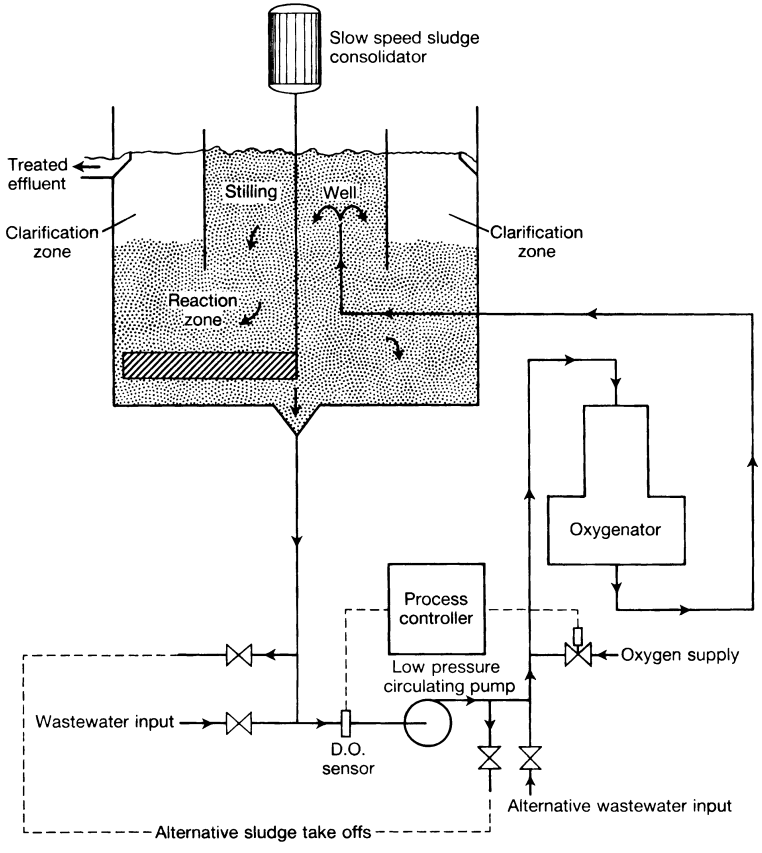


Fig. 5.64. The Megox® process developed by BOC Ltd. (Winkler 1981).

base of the tank and recirculated (Fig. 5.64). The amount of oxygen injected into the influent stream is strictly controlled using a dissolved oxygen sensor linked to the injection system. This allows the process to cope with shock loads as well as conserving oxygen usage.

5.4. Sludge Problems

The most common operational difficulties associated with activated sludge are those concerned with the separation of sludge from the clarified wastewater in the sedimentation tank. The ability of activated sludge to separate is normally measured by an index of settleability, such as the sludge volume index (SVI), the sludge density index (SDI) or the stirred specific volume index (SSVI) (Sec. 2.2.3). Problems in sludge settlement can be caused by bulking, deflocculation, pin-point flocs, foaming or denitrification. These terms describe the effects, although their definitions are rather imprecise and there is some overlap.

With the exception of denitrification, all settleability problems can be traced back to the structure of the activated sludge floc. There is a wide range of particle sizes in the activated sludge mixed liquor ranging from individual bacteria of between 0.5 and 5.0 μm up to large flocs that may be greater than 1 mm (1,000 μm) in diameter. Parker *et al.* (1971) found that there was biomodal particle size distribution of flocs in activated sludges (Fig. 5.5) and suggested that the smaller particles represented individual micro-organisms or small aggregates that have not flocculated or have been sheared off larger flocs. The maximum size of a floc is dependent on their cohesive strength and the degree of shear exerted by the turbulence within the aeration tank. Floc structure has been subdivided into two distinct categories, micro- and macrostructure (Sezgin *et al.* 1978) (Fig. 5.65). Microstructure is where the flocs are small (below 75 μm in diameter), spherical, compact, and relatively weak. They are composed of floc-forming bacteria and formed by aggregation and bioflocculation where individual micro-organisms adhere to one another to form large aggregates. The structure of such flocs is termed weak because in the turbulent conditions of the aeration tank, they can easily be sheared into smaller particles. Although such flocs rapidly settle, the smaller aggregates that have been sheared from the larger flocs, which take longer to settle, may well be carried out of the sedimentation tank in the final effluent increasing the BOD and giving the clarified effluent a high turbidity. When filamentous micro-organisms are present, the flocs take on a macrostructure, with the micro-organisms

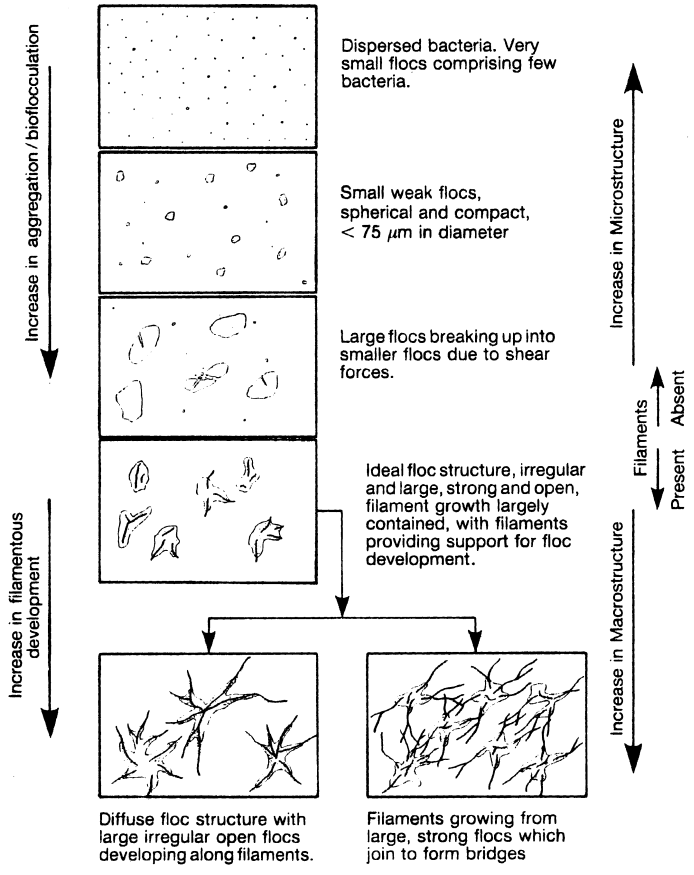


Fig. 5.65. Diagrammatic representation of floc structure in terms of micro- and macrostructure.

aggregating around the filaments making larger flocs of irregular shape and able to withstand high shear forces within the aeration tank because of this extra support. This distinctive micro- and macrostructure of activated sludge flocs was demonstrated experimentally by Lau *et al.* (1984a) who grew a floc-forming bacterium *Citromonas* sp. in dual culture with the filamentous bacterium *Sphaerotilus natans*. When grown on their own, each formed compact spherical flocs with typical micro-structural properties, whereas when grown together in roughly equal proportions irregularly shaped flocs were formed with *S. natans* acting as the support structure on which the other bacterium aggregated (Fig. 5.66). A useful checklist of sludge settleability problems has been produced by the Water Research

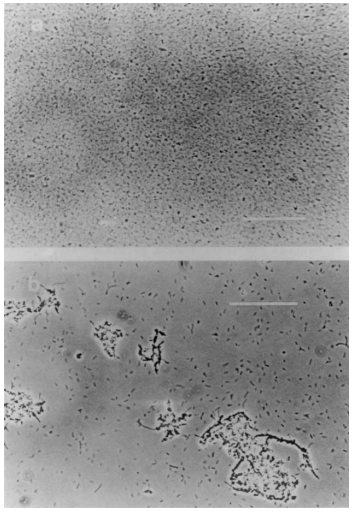


Fig. 5.66. The effect of filamentous organisms on floc structure: (a) Aggregates observed for a pure culture of an activated sludge floc-former, and (b) aggregates observed for a dual culture of a single floc-former and a single filamentous organisms. Photographs taken at ×100 using phase contrast, with the bar representing 100 μm (Lau *et al.* 1984a).

Centre (Chambers and Tomlinson 1981), which gives a step-by-step guide to identifying and solving sludge problems. The main causes and effects of the main sludge problems are summarised in Table 5.13.

5.4.1. *Deflocculation*

Deflocculation or dispersed bacteria is a phenomenon caused by the microstructure of flocs failing either by the flocs becoming less stable and breaking up, as would be the case in severe turbulence caused by over-aerating (Parker *et al.* 1972; Parker 1983; Tuntoolavest *et al.* 1983), or the bacteria failing to aggregate into flocs. The micro-organisms are present as individual cells or as very small clumps which remain in suspension in the liquid phase in the sedimentation tank and are continually washed out with the final effluent. Although some of the sludge continues to settle, the final effluent becomes progressively turbid as the remaining flocs break up. It is mainly a problem of activated sludge with a microstructure character, but it is also associated with bulking. If deflocculation is not controlled, it will become gradually more difficult to maintain the MLSS concentration in the aeration tank, as the microbial biomass is washed out, with the sludge loading (f/m) gradually increasing as a consequence. Deflocculation is thought

Table 5.13. Causes and effects of activated sludge separation problems (Jenkins *et al.* 1984).

Name of problem	Cause of problem	Effect of problem
Dispersed growth	Microorganisms do not form flocs but are dispersed, forming only small clumps or single cells.	Turbid effluent. No zone settling of sludge.
Slime (jelly) Viscous bulking; (also referred to as Non-filamentous bulking).	Microorganisms are present in large amounts of exocellular slime. In severe cases the slime imparts a jelly-like consistency to the activated sludge	Reduced settling and compaction rates. Virtually no solids separation in severe cases resulting in overflow of sludge blanket from secondary clarifier. In less severe cases a viscous foam is often present
Pin floc or pinpoint floc	Small, compact, weak roughly spherical flocs are formed, the larger of which settle rapidly. Smaller aggregates settle slowly.	Low sludge volume index (SVI) and a cloudy, turbid effluent.
Bulking	Filamentous organisms extend from flocs into the bulk solution and interfere with compaction and settling of activated sludge.	High SVI — very clear supernatant. Low RAS and WAS solids concentration. In severe cases overflow of sludge blanket occurs. Solids handling processes become hydraulically overloaded.
Blanket rising	Denitrification in secondary clarifier releases poorly soluble N_2 gas which attaches to activated sludge flocs and floats them to the secondary clarifier surface.	A scum of activated sludge forms on surface of secondary clarifier.
Foaming/scum formation	Caused by (i) non-degradeable surfactants and (ii) by the presence of *Nocardia* sp. and other foam associated species.	Foams float large amounts of activated sludge solids to surface of treatment units. *Nocardia* and *Microthrix* foams are persistent and difficult to break mechanically. Foams accumulate and can putrify. Solids can overflow into secondary effluent or even overflow out of the tank free-board onto walkways.

to be due to low dissolved oxygen concentrations, low pH, or shock loads, although Pipes (1979) suggests that sludge loadings above 0.4 kg kg^{-1} d^{-1} will tend to lead to deflocculation. It would appear that if the sludge is microstructural, then a high sludge loading will eventually cause deflocculation. Certain toxic wastewaters can also cause flocs to disintegrate into small aggregates resulting in very turbid effluents and a total breakdown of operations as the MLSS is rapidly reduced. High population densities of free-swimming protozoans, such as *Colpoda* spp. and *Paramecium* spp., are also known to cause the final effluent to appear turbid, although sludge settleability is unaffected.

5.4.2. *Pin-point floc*

Activated sludge flocs without any filamentous micro-organisms present have a pure microstructure. Because of the lack of macrostructure the flocs are small, compact, and spherical, with a weak structure so that they are readily broken up into smaller flocs within the aeration tank. This total dependence on microstructure and the cohesive forces of individual bacteria to hold the agglutinated mass of cells together, means that the floc becomes progressively unstable as it grows. Thus, there is a high proportion of flocs with a small particle size that take much longer to settle than the larger flocs and can be carried out of the sedimentation tank in the final effluent. These small flocs, known as pin-point or pin floc, do not cause high turbidity in the effluent, as is the case with deflocculation, because the particles are much larger and visible to the naked eye as discrete flocs in the final effluent. The SVI usually remains low with much of the activated sludge continuing to settle, although the continued loss of microbial biomass due to pin-point floc may eventually pose a problem of maintaining sufficient MLSS in the aeration tank. Pin-point floc is mainly associated with a long sludge age above 5–6 d and with low organic loadings below 0.2 kg m^{-3} d^{-1} (Pipes 1979) and is therefore a problem associated with extended aeration systems (Forster 1977). It has been suggested that as pin-point floc is associated with long sludge ages and low organic loadings that the small flocs are most likely to be the non-biodegradable fraction of the aerobically digested mixed liquor. This is supported by reports that the presence of such flocs in the final effluent did not significantly increase the BOD. However, this does not appear the case with all continuously loaded activated sludge plants.

Activated sludge plants treating industrial wastewaters, especially from the chemical and pharmaceutical sectors, produce small weak flocs without any filaments present, leading to typical pin floc problems. This is due

to the action of toxic compounds in the liquid phase preventing filament development outside flocs. Jobbágy *et al.* (2000) found that simple changes in bioreactor configuration and operation significant improvements could be made to floc structure reducing the loss of fine floc particles in the final effluent.

5.4.3. *Foaming*

Since the ban on the use of non-degradable (hard) detergents, white frothy foam, forming deep banks often covering the aeration tanks, is now rarely encountered at activated sludge plants. However, the intensity of the aeration system produces small quantities of light, transient foam that quickly disperses. A foam is defined as a dispersion of gas (air) bubbles in a liquid (water) or solid. In water the air is the dispersed phase and the water the continuous phase. Eventually the water layer separating the bubbles thins until the bubbles burst releasing the air. Surface active

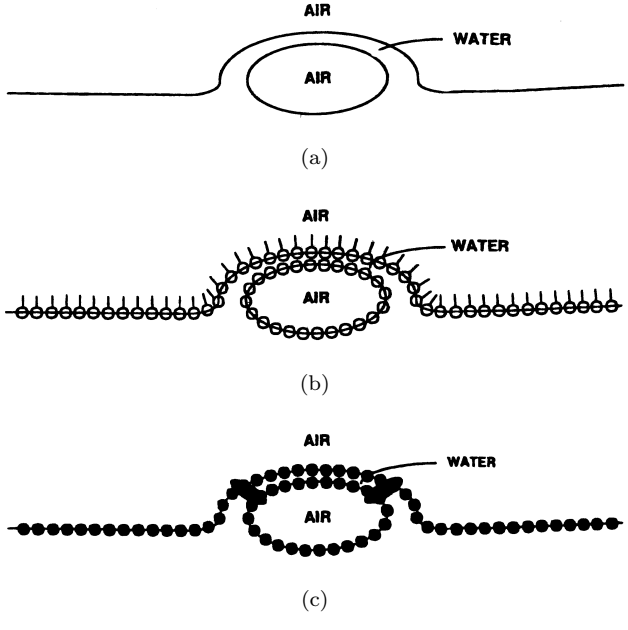


Fig. 5.67. Various types of air-water foams. (a) No surface active materials present; (b) Surface active materials present that accumulate at the air-water interface and stabilise it. (c) Hydrophobic solids present that accumulate at the interface and stabilise it, with larger solid particles forming bridges across the air bubble preventing drainage of water from it (Jenkins *et al.* 1984).

agents (i.e. surfactants) stabilise foams by their molecules being attracted to the air-water interface making the water layer stronger so that it has to become much thinner than normal before rupturing. Therefore any water that contains surfactants produces more stable and longer lasting foams than water without surfactants present. Hydrophobic particles also form foams. These particles are poorly wetted by water producing complex foams made up of dispersed phases of the hydrophobic particles and air within the continuous water phase. The particles congregate at the air-water interface, making the foam even stronger. Jenkins *et al.* (1994) describe how if the hydrophobic particles are large enough, they can bridge the water film between air bubbles creating what is in essence a dam that prevents the water draining away, thus preventing the film from thinning and rupturing (Fig. 5.67). A dense foam, very similar to chocolate mousse both in texture and colour, occasionally occurs on aeration tanks and it is this phenomenon which is referred to as activated sludge foaming (Al-Diwamy and Cross 1978; Pipes 1978b; DhaliwaI1979). This type of foaming is caused by the excessive growth of certain hydrophobic filamentous micro-organisms usually of the genus *Nocardia*. The presence of long-chain mycolic acid on

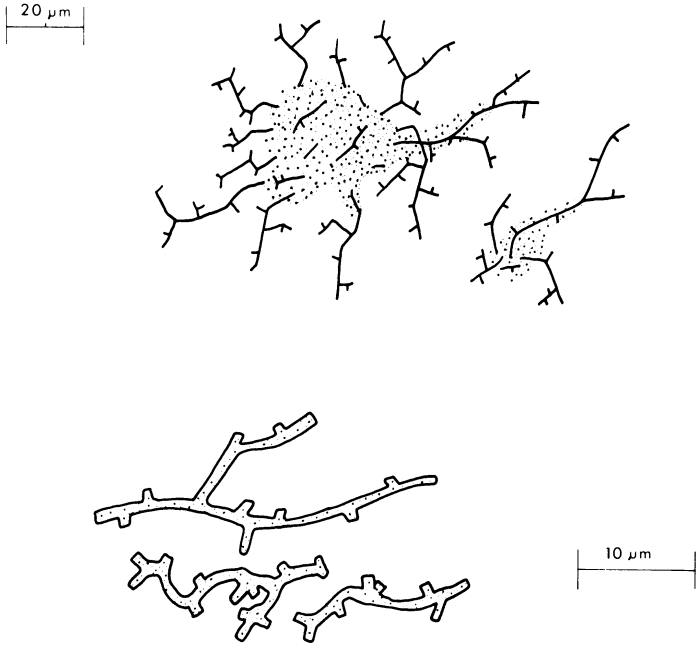


Fig. 5.68. The filamentous bacterium *Nocardia* sp.

the surface of their cell walls makes *Nocardia* spp. hydrophobic (Minnikin 1982; Chun *et al.* 1996; Stratton *et al.* 1998). Their presence make flocs hydrophobic so that they attach to the bubbles in the aeration tank such that they are buoyed to the surface where a dense stable foam or a thick scum is formed. The foam can be carried over to the sedimentation tank and be discharged with the final effluent. Although the population density of this filamentous micro-organism is also very high in the mixed liquor, it does not necessarily affect the settling qualities of the sludge which generally remain good. This is because *Nocardia* is highly branched (Fig. 5.68), giving the flocs a strong macrostructure that produces large firm flocs which readily settle. Unlike other filamentous micro-organisms, the hyphae are largely retained within the floc and do not extend from its surface, which aids settlement. The depth of foam can be considerable, covering walkways, mechanical equipment, overflowing the outlet weirs, and generally making maintenance difficult and dangerous. Foaming in closed aeration tanks reduces the available head-space and has even reduced the available hydraulic head for gravity flow through the tank. The MLSS becomes trapped in the foam giving it a dark brown colour, and between 30 and 50% of the total activated sludge can be entrained in the foam making it operationally difficult to maintain adequate MLSS in the mixed liquor in order to continue treatment. The foam contains considerable air, as well as *Nocardia* and activated sludge flocs, and has a bulk density of about 0.7 g ml^{-1} (Lechevalier and Lechevalier 1975). As expected, the density of *Nocardia* is enriched in the foam with up to 10^{12} microcolonies ml^{-1} compared to only 10^6 microcolonies ml^{-1} in the mixed liquor (Wheeler and Rule 1980). This has been confirmed by Pitt and Jenkins (1990). Using laboratory scale plants, Vega-Rodriquez (1983) observed that up to 90% of *Nocardia* filaments, on a filament length basis, are potentially transferred from the mixed liquor and into the foam. De los Reyes and Raskin (2002) have identified two foaming thresholds based on filament length of Nocardioforms. The formation threshold (2×10^8 μm m^{-1}) and a stability threshold (1×10^9 μm m^{-1}). These have been measured by a simple foaming potential test. Two tablets of Alka-Seltzer® (two tablets containing 650 mg sodium acetylsalicylate, 3,832 mg heat treated sodium bicarbonate, and 2,000 mg citric acid; Bayer Corp, Elkhart, IN) are added to 250 ml of well mixed samples in 500 ml graduated cylinders. The foaming potential is the maximum volume of foam produced, while foam stability is determined by measuring the foam half life (i.e. the time it takes for half of the maximum volume of foam generated to dissipate (Oerther *et al.* 2001). Other problems include odour production in the summer, and if the wasted sludge

is digested, the *Nocardia* can subsequently cause foaming in the anaerobic digester (Jenkins *et al.* 1984).

Although the exact incidence of *Nocardia* foaming is not known, the actinomycete is one of the most commonly observed filamentous micro-organisms in activated sludge in the USA, South Africa, and Europe (Eikelboom 1977; Gray 1982b; Richard *et al.* 1981; Wagner 1982; Stronl and Jenkins 1984; Blackbeard and Ekamma 1984; Ekamma *et al.* 1985; Byron 1987) (Table 5.14). The actinomycetes of the genus *Nocardia* occurring in activated sludge are very diverse, with *N. amarae* the most commonly occurring species in the USA (Lechevalier and Lechevalier 1974), and like the fungus *Subbaromyces splendens* in percolating filters, it has not been isolated from any other environment except activated sludge. Other species isolated from activated sludge include *N. rhodochrus*, which is common in West Germany and Switzerland (Lemmer and Kroppenstedt 1984), *N. asteroides*, *N. caviae*, and strains of *Mycobacterium*, *Rhodococcus*, *Tsukamurella*, and *Skermania* (de los Reyes *et al.* 1998; Goodfellow

Table 5.14. Ranking of dominant filamentous organisms recorded in major activated sludge surveys.

Filamentous micro-organisms	South Africa	USA	Europe	Germany	Ireland
Type 0092	1	11	4	—	12
M. parvicella	2	7	1	2	3
Type 1851	3	9	12	—	6
Type 0675	4	6	—	—	4
Type 0914	5	—	—	—	—
Type 0041	6	5	6	3	1
Nocardia spp.	6	1	14	5	13
Type 0803	7	7	9	10	10
Type 1701	8	2	5	8	8
N. limicola	8	8	11	7	5
Type 021N	—	3	2	1	2
H. hydrossis	9	9	3	6	9
S. natans	—	6	7	4	11
Thiothrix spp.	9	4	17	—	—
Type 0581	9	12	8	—	—
Type 0961	—	10	10	9	—
Beggiatoa spp.	—	12	—	—	14
Number of plants	56	190	200	315	36
Number of samples	56	300	1100	3500	36

et al. 1998). Soddell *et al.* (1998) has carried out a taxonomic survey of foam-forming actinomycetes in activated sludge using 16S rDNA sequencing. They show that *Nocardia* are only a small portion of the mycolic-acid producing actinomycetes found in activated sludge, and recommend the term Nocardioform foam should replace *Nocardia* foam to highlight the role of other foam forming organisms. *Microthrix parvicella*, which is now thought to be an actinomycete (Slijkhuis and Deinma 1982; Blackall *et al.* 1994; Kocianova *et al.* 1994; Tandoi *et al.* 1998), is also commonly associated with foaming (Jenkins *et al.* 1993; Mamais *et al.* 1998). Blackbeard *et al.* (1988) surveyed 33 activated sludge plants, which incorporated nutrient removal, in South Africa. Of these, 18 had foam development on the surface of the aeration tank indicating a potential foaming problem. The most frequently recorded dominant filamentous micro-organims were type 0092 (78% of the plants surveyed), *M. parvicella* (50%), and type 0041 (33%), with types 0675, 0914, and *Nocardia*, all occurring in 22% of

Table 5.15. Comparison of the frequency of occurrence and dominance of filamentous micro-organisms in foam from nutrient and non-nutrient removal activated sludge plants in South Africa (Blackbeard *et al.* 1988).

Filamentous organisms	Nutrient removal plants		∗All activated sludge plants	
	†Dominance	‡Occurrence	†Dominance	‡Occurence
Type 0092	78 (1)	89	46 (1)	73
M. parvicella	50 (2)	67	46 (2)	59
Type 0041	33 (3)	61	14 (4)	73
Type 0675	22 (4)	72	8 (6)	51
Type 0914	22 (5)	67	11 (5)	46
Nocardia	22 (6)	44	30 (3)	41
Type 1851	17	22	5	43
H. hydrossis	5	17	3	14
Type 0803	5	11	11	27
Type 1863	5	11	0	5
Type 1701	5	11	0	22
N. limicola	0	5	0	1
Type 1702	0	5	0	5
Type 021N	0	5	0	22
Number of plants	18		37	

∗ Data from Blackbeard *et al.* (1986)
† Percentage of plants in which particular filamentous organisms were dominant
‡ Percentage of plants in which particular filamentous organisms were present
() Denotes rank in descending order.

the plants examined. Evaluation of the filamentous micro-organisms in the foam was compared to an earlier survey of activated sludge plants not removing nutrients, also in South Africa (Blackbeard *et al.* 1986) (Table 5.15). By comparing the frequency of occurrence in the foam to the mixed liquor, it could be seen that *M. parvicella* and *Nocardia* were the only species that were selectively accumulated in the foam rather than being incidentally entrapped in the foam from the mixed liquor (Table 5.16). Of the 167 activated sludge plants surveyed in Italy by Madoni *et al.* (2000), 84 suffered from foaming, 81 from bulking, while 55 suffered from both. *Microthrix parvicella* was the most common filamentous micro-organisms associated with both foaming and bulking, being dominant in 75% of the plants with foaming compared to 16% for Nocardioforms (GALOs) (Table 5.17). Mamais *et al.* (1998) have found that low temperatures and substrates in the form of long chain fatty acids favour the growth of *M. parvicella* in nutrient removal

Table 5.16. Comparison of the frequency of dominance of filamentous micro-organisms in foam and mixed liquor samples from nutrient and non-nutrient removal activated sludge plants (Blackbeard *et al.* 1988).

Filamentous organism	Frequency of dominance in			
	Nutrient removal plants in		∗All activated sludge plants in	
	Foam	Mixed liquor	Foam	Mixed liquor
Type 0092	78	82	46	34
M. parvicella	50	33	46	20
Type 0041	33	39	14	14
Type 0675	22	45	8	16
Type 0194	22	33	11	24
Nocardia	22	15	30	14
Type 1851	17	21	5	17
H. hydrossis	5	12	3	1
Type 0803	5	17	11	9
Type 1863	5	6	0	0
Type 1701	5	—	0	—
N. limicola	0	6	0	2
Type 1702	0	0	0	3
Type 021N	0	—	0	—
Number of plants	18	33	37	96

∗ Data from Blackbeard *et al.* (1986)

Table 5.17. Dominant filamentous micro-organisms in bulking and foaming activated sludge from different countries (Madoni *et al.* 2001).

Country	Ranking		
	1	2	3
Bulking			
Czech Republic	*M. parvicella*	Type 0092	*N. limicola*
Denmark	*M. parvicella*	Type 0041	*N. limicola*
Germany	Type 0092	*M. parvicella*	Type 0041
South Africa	Type 0092	Type 0675	Type 0041
Switzerland	*S. natans*	Type 021N	Type 0961
The Netherlands	*M. parvicella*	Type 0041	Type 021N
USA	GALOs	Type 1701	Type 021N
Italy	*M. parvicella*	Type 0041	Type 021N
Foaming			
Australia	*M. parvicella*	GALOs	Type 0092
Czech Republic	*M. parvicella*	GALOs	*N. limicola*
France	*M. parvicella*	Type 0675	GALOs
South Africa	Type 0092	*M. parvicella*	GALOs
The Netherlands	*M. parvicella*	GALOs	*N. limicola*
UK	*M. parvicella*	*N. limicola*	GALOs
Italy	*M. parvicella*	GALOs	Type 0675

systems. They recommended the best control strategy for suppressing the micro-organism is the adoption of continuous plug flow reactors. This is due to (i) utilisation of the higher sorption capacity of floc formers to remove greater amounts of the long chain fatty acids present, and (ii) avoidance of the dispersion of the soluble products of colloidal material hydrolysis. Mori *et al.* (1988) identified *Rhodococcus* spp. as producing an extremely stable foam in an activated sludge aeration tank. It was shown experimentally that this actinomycete had a greater hydrophobic strength than *N. amarea*.

It should be remembered that many of these species are pathogenic and so there may be a risk of infection to humans through aerosol formation (Sec. 9.4.3). Causes of *Nocardia* growth are not known, with contradictory evidence relating to such factors as temperature ($> 18°C$), high loading levels, and long sludge ages ($>$ 9d) (Pipes 1978*a*, *b*). The actual formation of foam depends on the mixed liquor containing adequate suspended solids (MLSS $> 5{,}000$ mg l^{-1}) (Wheeler and Rule 1980), with the amount of foam produced directly proportional to the amount of *Nocardia* present on a total

filament length basis (Vega-Rodriguez 1983). Increases in temperature or in the air flow will both increase the height of the foam layer.

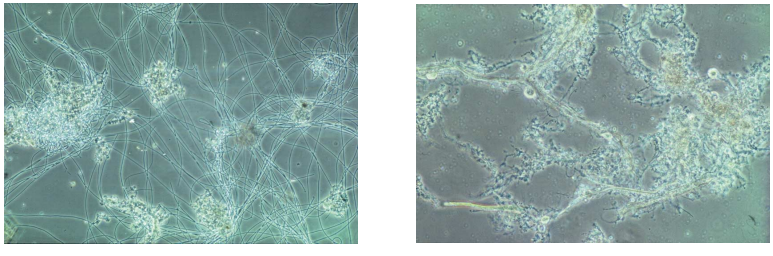
The most widely used method to control *Nocardia* growth in activated sludge is to reduce the sludge age. By increasing the sludge wastage rate it is possible to wash *Nocardia* out of the system, although the effective sludge age is a function of temperature, with the higher the temperature the lower the sludge age required. If nitrification is carried out in the aeration tank then this approach must be ruled out as the minimum sludge age for the retention of the nitrifying bacteria is much greater than that required to wash out *Nocardia*. Wilson *et al.* (1982) have successfully achieved *Nocardia* wash-out at a sludge age of 1 d. Anaerobic digester supernatant is toxic to *Nocardia* (Lechevalier and Lechevalier 1975; Lemmer and Kroppenstedt 1984) and some success has been achieved in controlling it by adding the supernatant to full-scale plants (Lechevalier *et al.* 1977), although this is in contrast to the findings of Wheeler and Rule (1980). Antifoaming devices employing water sprays that burst the air bubbles are ineffective against these stable foams and merely dilute it. However, by diluting it and reducing the thickness of the foam in the sedimentation tank, it may be possible for the scum trap to cope with the foam and effectively contain and possibly even remove it. Another problem is that the activated sludge sedimentation tanks have small scum traps drained by small diameter pipes that do not allow the foam to freely drain away. It would be advantageous if such tanks could be fitted with full-width scum traps with larger diameter pipework. Some control has been obtained by removing the foam from the sedimentation tank via the scum traps and ensuring it is not fed into the system. Among other control options are the use of anti-forming chemicals (Stratton *et al.* 1998), surface chlorination (Albertson 1991), and flotation to selectively remove the foam (Pretorius 1987). Control strategies employed in the Czech Republic are discussed by Wanner *et al.* (2000). The control options for *Nocardia* growth and foaming have been reviewed by numerous authors including Wheeler and Rule (1980), Pitt and Jenkins (1990), Jenkins *et al.* (1993), Madoni and Davoli (1993) and Mamais *et al.* (1998).

The genus *Nocardia* has recently been reviewed and renamed *Gordona*. The assessment of these filaments have been based on Gram staining and filament counting. However, the filaments lose their Gram staining characteristics when subjected to competitive stress in selectors or anaerobic digesters (Cha *et al.* 1992; Hernandez *et al.* 1994). Therefore molecular techniques are now widely employed e.g. immuno-fluorescent probes (Hernandez *et al.* 1994), oligonucleotide hybridisation probes targeting the small-subunit of rRNA of the mycolata (de los Reyes *et al.* 1997), and fluorescent *in situ* hybridisation (de los Reyes *et al.* 1998).

5.4.4. *Filamentous bulking*

Bulking is a phenomenon where filamentous organisms extend from the flocs into the bulk solution, interfering with settlement and subsequent compaction of the activated sludge with a SVI > 150 ml g^{-1} (Pipes 1967; Anon 1979*b*). Although bulking sludges settle more slowly than normal sludges, they are normally quite efficient in purifying the wastewater and so produce good effluents. Even when bulking is quite severe, a very clear supernatant is obtained as the larger number of extended filaments filter out the small particles that cause turbidity. Whereas poor settleability extends the sludge blanket so that larger flocs are carried out from the sedimentation tank with an increase in both suspended solids and BOD of the final effluent, the major problem associated with bulking is poor sludge compaction. This results in much thinner sludges being returned to the aeration tank with a low MLSS that leads to difficulty of maintaining the desired operational MLSS in the aeration tank with a subsequent fall in effluent quality. Attempts to control the height of the sludge blanket within the sedimentation tank by wasting more sludge than normal results in the MLSS concentration in the aeration tank rapidly declining.

In structural terms, bulking is due to flocs having a strong macrostructure, so much so that filamentous organisms are present in large numbers. In the ideal floc, where the SVI is between 80 and 120 ml g^{-1} and the final effluent is largely free from suspended solids and turbidity, the filamentous and floc-forming organisms are balanced. The filamentous organisms are retained largely within the floc giving it strength and a definite structure. Although a few filaments may protrude from the floc they are sufficiently scarce and of a sufficiently reduced length not to interfere with settlement. In contrast, the flocs comprising a bulking sludge have large numbers of filaments protruding from the floc, with two types of bulking flocs discernible.



(a) (b)

Fig. 5.69. Bulking in activated sludge is caused by either (a) bridging or (b) diffuse growth.

Fairly compact flocs with long filaments growing out of the floc and linking individual flocs together (bridging) forming a meshwork of filaments and flocs; or alternatively, flocs with a more open (diffuse) structure, which is formed by bacteria agglomerating along the length of the filament forming rather thin, spindly flocs of a large size (Fig. 5.69). The type of floc formed, the type of compaction and settling interference caused, depends on the type of filamentous organisms present. Bulking is predominantly caused by bacterial species, with bridging caused by type 021N, *Sphaerotilus natans*, type 0961, type 0803, *Thiothrix* sp., type 0041 and *Haliscomenobacter hydrosis*. Open floc structure is associated with type 1701, type 0041, type 0675, *Nostocoida limicola*, and *Microthrix parvicella* (Anon 1979*b*).

About 25 different filamentous bacteria are known to be able to cause activated sludge bulking. A number of fungi and algae are also known to cause bulking, although fungi and algae are not normally found as dominant organisms in activated sludge (Farquhar and Boyle 1971*b*). The fungi *Geotrichium candidum* (Hawkes 1963) and *Zoophagus insidians* (Cooke and Ludzack 1958) have been identified (Tomlinson and Williams 1975), and the blue-green alga *Schizothrix calciola* was observed in an activated sludge unit treating a wastewater rich in acetate (Sykes *et al.* 1979). The same bacterial species has been observed worldwide, causing bulking, with approximately 10 bacterial species accounting for at least 90% of all bulking incidents. The frequency of occurrence and the frequency of dominance of filamentous micro-organisms in bulking sludge has been measured in the USA (Richard *et al.* 1981; Strom and Jenkins 1984), in South Africa (Blackbeard and Ekama 1984; Blackbeard *et al.* 1985; Ekama *et al.* 1985), and in Europe

Table 5.18. The ten most frequently occurring filamentous micro-organisms recorded in activated sludge plants in the USA, the Netherlands, Germany, South Africa, and Ireland based on the data in Table 5.14.

Rank	USA	Netherlands	Germany	South Africa	Ireland
1	*Norcadia*	*M. parvicella*	021N	0092	0041
2	1701	021N	*M. parvicella*	0041	021N
3	021N	*H. hydrossis*	0041	0675	*M. parvicella*
4	0041	0092	*S. natans*	*Nocardia*	0675
5	*Thiothrix*	1701	*Nocardia*	*M. parvicella*	*N. limicola*
6	*S. natans*	0041	*H. hydrossis*	1851	1851
7	*M. parvicella*	*S. natans*	*N. limicola*	0914	Fungi
8	0092	0581	1701	0803	1701
9	*H. hydrossis*	0803	0961	*N. limicola*	*H. hydrossis*
10	0675	0961	0803	021N	0803

(Eikelboom 1977; Wagner 1982; Byron 1987). The most frequently encountered species observed in different countries are summarised in Table 5.14. Using the data on dominant filamentous micro-organisms in bulking sludge from 525 samples taken from 270 treatment plants in the USA (Richard *et al.* 1984; Strom and Jenkins 1984), 1,100 samples taken from 200 plants in The Netherlands (Eikelboom 1977), 3,500 samples from 315 treatment plants in West Germany (Wagner 1982), 36 plants in Ireland (Byron 1987), and 60 samples from 50 plants in South Africa (Blackbeard and Ekama 1984), the top 10 species can be compared (Table 5.18). Sixteen species, excluding fungi, were recorded in all, with only three species, types 021N, 0041, and *M. parvicella* occurring in the top 10 species from all five countries, and a further three species were recorded in the top 10 from four countries examined. Using a mean ranking procedure where only the top three values are used, the most frequently occurring species can be identified. In order of occurrence, the top four species are: type 0641, *Nocardia*, 021N, and *M. parvicella*. More recent studies identify the same organisms as major causes of bulking (Eikelboom *et al.* 1998; Wanner *et al.* 1998; Madoni *et al.* 2000).

According to Jenkins *et al.* (1984), the following filamentous micro-organisms are usually observed in domestic wastewater treatment plants at conventional organic loading rates: *S. natans*, *Thiothrix* I and II, *Beggiatoa* spp., *Nocardia* spp., *N. limicola* II, *H. hydrossis*, types 1701, 021N and 1863. Plants treating industrial wastewaters or domestic plants operated a low organic loading included: types 0041, 0675, 021N, 0914, 1851, 0803, 0092, 0961, 0581, *Thiothrix* I and II, *Beggiatoa* spp., *M. parvicella*, *Nocardia* spp., *H. hydrossis*, *N. limicola* I, II and III. Those species infrequently observed include fungi, *Cyanophyceae*, types 1702, 1852, 0211, 0411, *Bacillus* spp., and *Flexibacter* spp. Detailed descriptions of all these filamentous micro-organisms are given by Eikelboom (1975) and Eikelboom and van Buijsen (1981). There are a number of keys to these micro-organisms including Farquhar and Boyle (1971*a*), Eikelboom (1975), Eikelboom and van Buijsen (1981) and Jenkins *et al.* (1984, 1992). The latter two keys are beautifully illustrated. Each identification key is based on a number of morphological features that can be identified using simple staining techniques. For example, the key of Eikelboom and van Buijsen relies on just three staining techniques, the Gram, Neisser, and sulphur storage test (Fig. 5.70). Jenkins *et al.* (1984) have extended this key making use of three more simple stains (India ink reverse, polyhydroxbutyrate (βH), and crystal violet sheath stains) to aid identification (Fig. 5.71, Table 5.19).

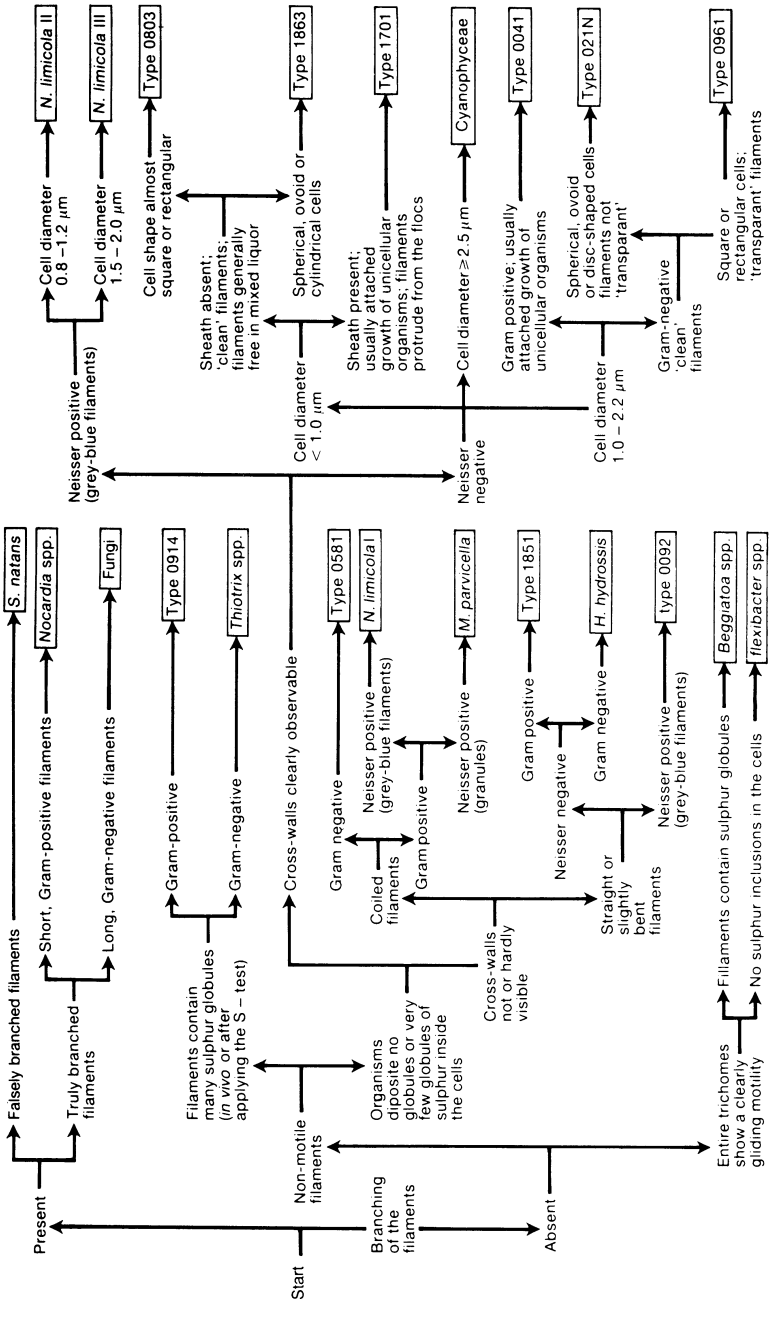


Fig. 5.70. Identification key for filamentous micro-organisms causing bulking and foaming based on three biochemical tests (Eikelboom and van Buijsen 1981).

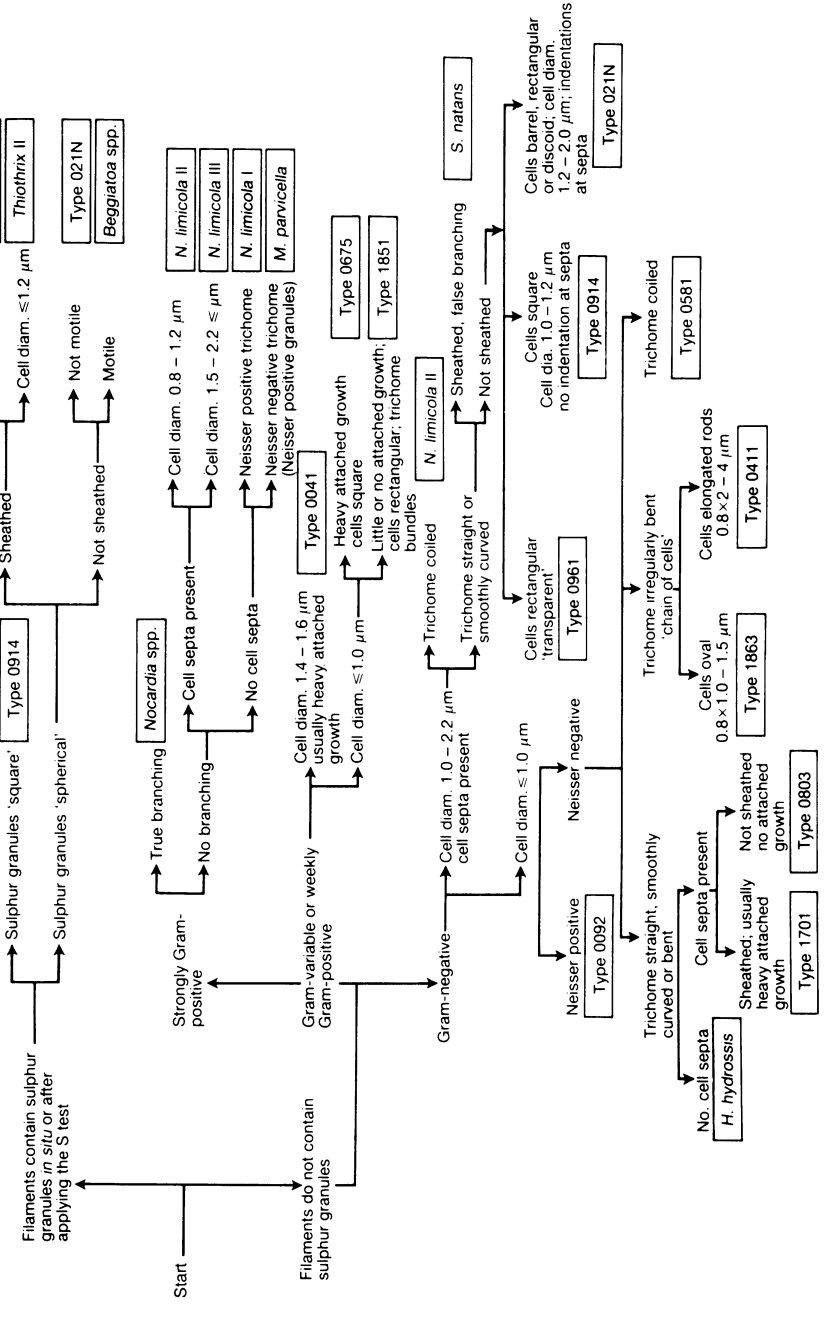


Fig. 5.71. Alternative identification key for filamentous micro-organisms causing bulking and foaming based on six biochemical tests (Jenkins *et al.* 1984).

Table 5.19. Morphological and staining characteristics of filamentous organisms associated with activated sludge bulking (Jenkins *et al.* 1984).

Bright field observation				Phase contrast observation 1000×												
	Gram stain	Neisser stain		Sulphur granules		Other cell inclusions	Trichome diameter μm	Trichome lenght μm	Trichome shape	Trichome location	Cell septa clearly observed	Indentations at cell septa	Sheath	Attached growth	Cell shape and size μm	
Filament type		trichome	granules	*In situ*	S test											Notes
S. natans	–	–	–	–	–	βH	1.0–1.4	> 500	St	E	+	+	+	–	Round-ended rods 1.4 × 2.0	False branching
Type 1701	–	–	–	–	–	βH	0.6–0.8	20–80	St,B	I,E	+	+	+	++	Round-ended rods 0.8 × 1.2	Cell septa hard to discern
Type 0041	+,V	–	–,+	–	–	–	1.4–1.6	100–500	St	I,E	+	–	+	++,–	Squares 1.4 × 1.5–2.0	Neisser positive reaction occurs
Type 0675	+,V	–	–,+	–	–	–	0.8–1.0	50–150	St	I	+	–	+	++,–	Squares 1.0 × 1.0	Neisser positive reaction occurs
Type 021N	–	–	–,+	–,+	+	βH	1.0–2.0	50–> 500	St,Sc	E	+	+	–		Barrels, rectangles, discoid 1–2 × 1.5–2.0	Rosettes, gonidia
Thiothrix I	–,+	–	–,+	+,–	+	βH	1.4–2.5	100–> 500	St,Sc	E	+	–	+	–	Rectangles 2.0 × 3–5	Rosettes, gonidia
Thiothrix II	–	–	–,+	+,–	+	βH	0.8–1.4	50–200	St,Sc	E	+	–	+	–	Rectangles 1.0 × 1.55	Rosettes, gonidia
Type 0914	–,+	–	–,+	–,+	–	βH	1.0	50–200	St	E,F	+	–	–	–	Squares 1.0 × 1.0	Sulphur granules square
Beggiatoa spp.	–,+	–	–,+	+,–	+	βH	1.2–3.0	100–> 500	St	F	–,+	–	–	–	Rectangles 2.0 × 6.0	Motile: flexing and gliding
Type 1851	+ weak	–	–	–	–	–	0.8	100–300	St,B	E	+,–	–	+	–,+	Rectangles 0.8 × 1.5	Trichome bundles

Table 5.19. (*Continued*)

Bright field observation			Phase contrast observation 1000×													
	Gram stain	Neisser stain		Sulphur granules		Other cell inclusions	Trichome diameter μm	Trichome lenght μm	Trichome shape	Trichome location	Cell septa clearly observed	Indentations at cell septa	Sheath	Attached growth	Cell shape and size μm	
Filament type		trichome	granules	*In situ*	S test											Notes
Type 0803	–	–	–	–	–	–	0.8	50–150	St	E,F	+	–	–	–	Rectangles 0.8 × 1.5	
Type 0092	–	+	–	–	–	+	0.8–1.0	20–60	St,B	I	+,–	–	–	–	Rectangles 0.8 × 1.5	
Type 0961	–	–	–	–	–	–	0.8–1.2	40–80	St	E	+	–	–	–	Rectangles 1.0 × 2.0	‘Transparent’
M. parvicella	+	–	+	–	–	βH	0.8	100–400	C	I	–	–	–	–	–	Large ‘patches’
Nocardia spp.	+	–	+	–	–	βH	1.0	10–20	I	I	+,–	–	–	–	Variable 1.0 × 1–2	True branching
N. limicola I	+	+	–	–	–	–	0.8	100	C	I,E	–	–	–	–	–	
N. limicola II	–,+	+,–	–	–	–	βH	1.2–1.4	100–200	C	I,E	+	+	–	–	Discs, ovals 1.2 × 1.0	Incidental branching Gram and Neisser variable
N. limicola III	+	+	–	–	–	βH	2.0	200–300	C	I,E	+	+	–	–	Discs, ovals 2.0 × 1.5	
H. hydrossis	–	–	–	–	–	–	0.5	20–100	St,B	E,F	–	–	+	–,+	–	‘Rigidly straight’
Type 0581	–	–	–	–	–	–	0.5–0.8	100–200	C	I	–	–	–	–	–	
Type 1863	–	–	–,+	–	–	–	0.8	20–50	B,I	E,F	+	+	–	–	Oval rods 0.8 × 1–1.5	‘Chain of cells’
Type 0411	–	–	–	–	–	–	0.8	50–150	B,I	E	+	+	–	–	Elongated rods 0.8 × 2–4	‘Chain of cells’

Key: + = positive; – = negative; V = variable; Single symbol invariant; +, – or –, +, variable, the first being most observed. Trichome shape: St = straight; B = bent; SC = smoothly curved; C = coiled; I = irregularly-shaped; βH = poly-β-hydroxybutyrate. Trichome location: E = extends from floc surface; I = found mostly within the floc; F = free in liquid between the flocs.

Strom and Jenkins (1984) compared three methods of identifying filamentous micro-organisms in bulking sludge. They found that biochemical identification using *Bergey's Manual* was least successful, as many of the species found in activated sludge were not included in the text. Also the method was extremely time consuming to use requiring filaments to be isolated and cultured. The key by Farquhar and Boyle (1971a) was developed from *Bergey's Manual* so that although based on morphology and simple staining techniques, many of the species included in this key are not found in activated sludge and that several important species are absent. This leads to misidentification, although, it was found by Strom and Jenkins to be a considerable improvement over biochemical testing. While the latter two identification schemes identify filaments to species, the key developed by Eikelboom (1975) only goes to type, not genus or species. This level of identification is adequate for routine work, especially when looking for indicators to the problem of bulking, but is inadequate for research work on the species themselves. This is because different species may have more than one growth form and so be considered different types, and also some types may contain two or more distinct species. Strom and Jenkins found this key to be more accurate and rapid. So the keys of Eikelboom (1975) and Jenkins *et al.* (1984a) provide the operator with a rapid identification system with a detailed breakdown of species within thirty minutes to an hour. The method is not subject to errors in calculating or sampling as is chemical and physical analyses, assuming that identification is correct, and integrates all the factors affecting the treatment system over a period of time. Significant progress has been made using 16S rRNA based techniques, in particular whole cell hydridisation or fluorescence *in situ* hybridisation (FISH) (Amman *et al.* 1995), polymerase chain reaction (PCR), and denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.* 1993). These techniques exploit differences between the ribosomal gene sequence of different micro-organisms. In whole cell hybridisation, individual bacterial cells can be identified and even quantified using fluorescent probes targeted at specific signature sequences in ribosomal RNA (Ballinger *et al.* 1998; Curtis and Craine 1998; van der Waarde *et al.* 1998; Juretschko *et al.* 1998). The application of these techniques to optimise activated sludge populations is discussed by Yuan and Blackall (2002). The detection and cultivation of filamentous bacteria from activated sludge is reviewed by Kämpfer (1997).

Blackbeard *et al.* (1988) surveyed 33 activated sludge plants incorporating nutrient removal in South Africa. Of these, 27 had bulking problems. The most frequently occurring filamentous micro-organisms were types 0092, 0675, 0041, *M. parvicella*, and type 0914 (Table 5.20). Wanner and

Table 5.20. Frequency of dominance and frequency of occurrence of filamentous micro-organisms in mixed liquor samples from nutrient removal plants (left) and all activated sludge plants (right) in South Africa (Blackbeard *et al.* 1988).

Filamentous organism type	South Africa			
	Nutrient removal plants		†All activated sludge plants	
	†Dominance	§Occurrence	‡Dominance	§Occurrence
Type 0092	82 (1)	94 (1)	34 (1)	78 (2)
Type 0675	45 (2)	73 (4)	16 (5)	68 (3)
Type 0041	39 (3)	85 (2)	14 (6)	86 (1)
M. parvicella	33 (4)	76 (3)	20 (3)	48 (5)
Type 0914	33 (5)	70 (5)	24 (2)	41 (6)
Type 1851	21 (6)	58 (6)	17 (4)	51 (4)
Type 0803	17	27	9	29
Nocardia	15	24	14	33
H. hydrossis	12	21	1	32
N. limicola	6	21	2	21
Type 1863	6	9	0	1
Thiothrix	3	6	1	6
Type 0961	0	3	2	8
Type 1702	0	3	3	4
Number of samples	33		96	
Number of plants	33		96	

∗ For a comparison with the frequencies of dominance of filamentous organisms in USA, Holland, Ireland and West German Plants, see Table 5.18.
† Data from Blackbeard *et al.* 1986 for bulking and non-bulking sludges.
‡ Percentage of plants in which particular filamentous organisms were dominant.
§ Percentage of plants in which particular filamentous organisms were present.
() Denotes rank in descending order.

Grau (1988) found that the presence of anaerobic or anoxic zones in such nutrient removal systems suppressed filamentous growth, with the development of filamentous micro-organisms largely restricted to the aerobic aeration tank only.

There is a direct relationship between the density of filamentous organisms in the mixed liquor and the settling properties of activated sludge (Finstein and Heukelekian 1967: Pipes 1979). This can provide a more sensitive measure of the onset of bulking. A useful assessment of filament density is the method developed by Sezgin *et al.* (1978) for estimating total extended filament length. Good correlations between total extended

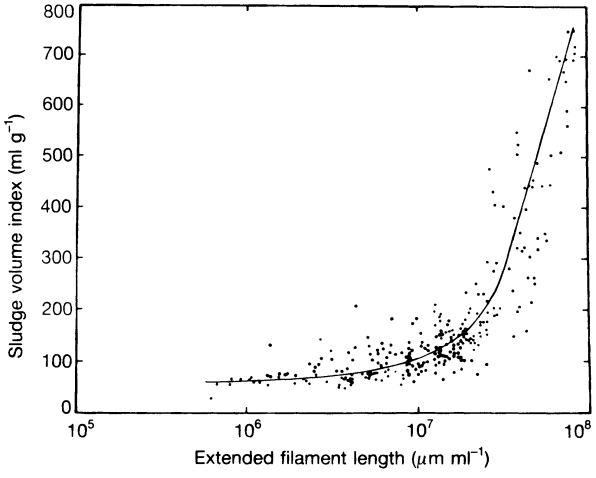


Fig. 5.72. Effect of total extended filament length on SVI (Palm *et al.* 1980).

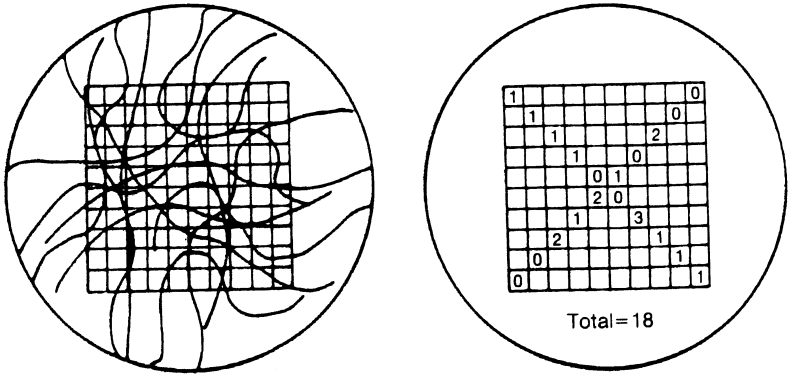


Fig. 5.73. Assessment of filament length using a Lund cell developed by Walker (1982). The diagram shows the microscopic field of view with (a) the eyepiece graticule superimposed on filamentous bacteria, and (b) the counts ascribed to this field by the diagonal counting technique.

filament length (TEFL) and settleability indices have been obtained (Sezgin *et al.* 1978; Palm *et al.* 1980; Lee *et al.* 1982; Baker and Veenstra 1986), with SVI increasing rapidly above 100 ml g^{-1} when the TEFL value was above 10^7 μm ml^{-1}, resulting in filament to filament or floc to filament aggregates being formed that have loose structures of low densities, which do not settle

rapidly (Fig. 5.72). This method is, however, fairly time-consuming compared to more recent assessments of filament length (Walker 1982; Green 1982). For example, in the method developed by Walker (1982), a sample of mixed liquor is placed in a Lund Cell and using a Whipple eyepiece graticle, which superimposes a grid of 100 square on the image, the number of filaments is counted that cross each of the 10 squares of each diagonal. Only filaments that are in the square for at least two-thirds of a single square width are counted (Fig. 5.73). This is repeated five times giving a minimum count of 100 squares. The filament length is expressed in cm per fig of MLSS,

$$\text{Filament length} = \frac{N \times F \times 1000}{\text{MLSS}} \text{ cm mg}^{-1},$$

where N is the total count of filaments per 100 squares and F is the width of one square (cm)/area of 100 squares (cm^2) $\times$ cell depth (cm). F will, of course, remain constant for any specific microscope/cell combination at fixed magnification.

A more simplified filament counting technique uses a 40 μl subsample of mixed liquor. This is placed on a glass slide and covered with a 22×22 mm cover slip. The number of separate fields required to examine the entire area under the cover slip once, at $\times$100 magnification, is calculated. This is a 144 for the microscope in my own laboratory. Using an eyepiece with a single hair-line fitted across the centre, the number of times that any filamentous micro-organisms intersect the hair line is counted. This is repeated as many times as practicable, in my case I have selected 20 times, working over the entire area of the cover slip selecting sites randomly. A total filament count per μl is calculated as

$$\text{Total filament count} = (C/40)(144/20) \ \mu\text{m } \mu\text{l}^{-1},$$

where C is the sum of all the intersections of the hairline from all the fields of view examined. Obviously different size cover slips and subsamples of mixed liquor can be used as long as the calculation is altered accordingly.

However, for rapid routine assessment, it is possible to use a subjective scoring index using an abundance scale (e.g. absent, rare, common, frequent or abundant), where the abundance of filaments can be compared to a set of drawings or photographs at $\times$100 magnification (Farquhar and Boyle 1971b; Rensink 1974; Forster and Dallas-Newton 1980; Eikelboom and van Buijzen 1981; Eikelboom 1982) (Fig. 5.74). This scoring system can also be correlated with a more precise assessment such as total extended filament length. There is much interest in the development of automated systems for

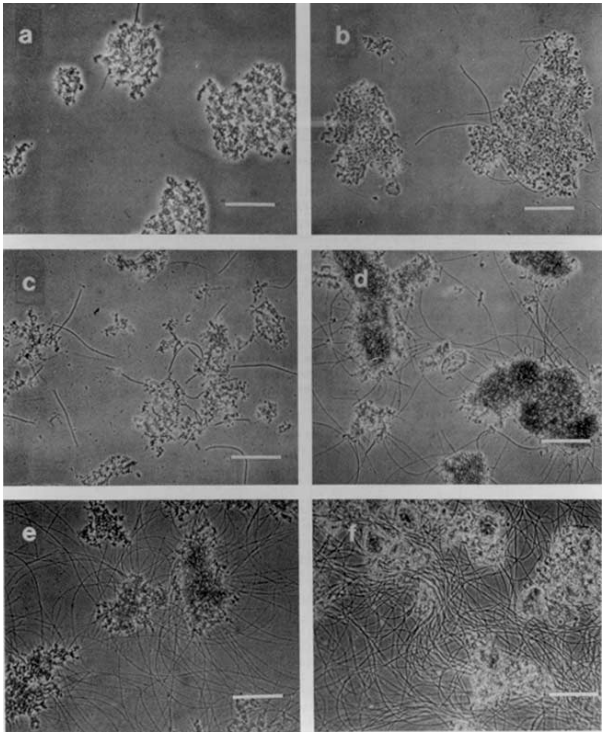


Fig. 5.74. Filament abundance categories using subjective scoring system: (a) few; (b) some; (c) common; (d) very common; (e) abundant; (f) excessive. (The photographs were taken by Jenkins *et al.* (1984) using phase contrast at $\times 100$ magnification. The bar indicates 100 μm.

the quantitative analysis of floc size and filament development. Grijspeerdt and Verstraete (1997) and Motta *et al.* (2001) have used image analysis to assess the shape of flocs, while Cenens *et al.* (2002) have devised new image analysis techniques to determine the ratio of flocs to filaments.

A number of theories explaining the predominance of filaments in the aeration tank have been proposed. These can be loosely categorised as either being related to process operation or wastewater characteristics. The major causes of bulking are thought to include low dissolved oxygen concentration due to insufficient aeration capacity in relation to sludge loading, low sludge loading rates in completely mixed systems, septic wastes, nutrient deficiency, and a low pH (< 6.5). Filaments tend to dominate whenever there is a shortage of substrate, dissolved oxygen, or essential nutrients, especially nitrogen and phosphorus. It has been suggested that predominance

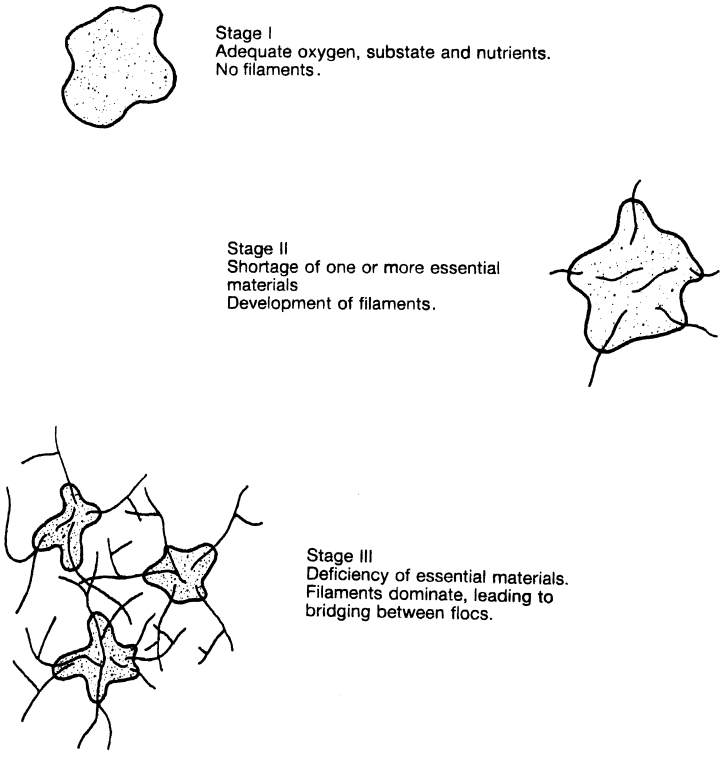


Fig. 5.75. A diagrammatic representation of the three stages in the development of excessive macrostructure and subsequent bulking.

of filaments under these conditions is due to their high surface area to volume ratio, which maximises filamentous development at the expense of floc-forming micro-organisms. Sezgin, Jenkins and Parker (1978) describe this phenomenon as a three-stage process (Fig. 5.75).

(i) Adequate concentrations of substrate, oxygen, or nutrients in solution results in a sludge dominated by flocs with no filamentous growth present. Settleability of the sludge will be good.

(ii) A shortage of one or more of these key materials in solution will reduce the rate of diffusion of these materials into individual sludge flocs. There will be a concentration gradient of these materials in the floc with the lowest concentrations in the centre, and it is here that filamentous growth will predominate. The filaments will develop from the centre and radiate out of the floc. The competitive advantage of the filaments over the floc-forming micro-organisms will increase rapidly

Table 5.21. The use of dominant filament types as indicator of conditions causing activated sludge bulking (Strom and Jenkins 1984).

Suggested causative conditions	Indicative filament types
Low dissolved oxygen	Type 1701, *S. natans*, *H. hydrossis*
Low f/m	*M. parvicella*, *H. hydrossis*, *Nocardia* sp. types 021N, 0041, 0675, 0092, 0581, 0961, 0803
Septic wastewater/sulphide	*Thiothrix* sp., *Beggiatoa*, type 021N
Nutrient deficiency	*Thiothrix* sp. *S. natans*, type 021N, and possibly *H. hydrossis*, and types 0041 and 0675
Low pH	Fungi

as their strands of cells, which have a large surface area to volume ratio, enter the liquid phase. There will a reduction of settleability at this stage.

(iii) Filamentous growth will continue to increase until bridging occurs which reduces the settleability of the sludge even further.

The major causes of bulking are thought to include low dissolved oxygen concentration due to insufficient aeration capacity in relation to sludge loading, low sludge loading rate in completely mixed systems, septic wastes, nutrient deficiency, and a low pH (< 6.5). By examining large numbers of bulking sludges and relating the dominant bulking organism to various operational parameters, including wastewater characteristics, correlations are gradually becoming evident, and as the database grows, more associations will emerge. Strom and Jenkins (1984) have summarised some of the major associations in Table 5.21, and by examining the filamentous organisms present in a bulking sludge, some indications of the cause of bulking can be obtained. Some of these associations are extremely well correlated. For example, fungi indicates that the wastewater contains a strong acid discharge which has reduced the pH of the aeration basin. Type 1701 and *S. natans* both indicate the dissolved oxygen concentration is too low due to a high sludge loading, with type 1701 indicative of even more severe dissolved oxygen limitations than *S. natans*. Although the oxygen concentration may still appear within the critical limits, the presence of these filamentous organisms indicates that the sludge loading is too high for the existing oxygen conditions (Hao *et al.* 1983; Lau *et al.* 1984a,b). Other associations involving *M. parvicella* (Slijkhuis and Oeinema 1982; Slijkhuis 1983) and type 021N (Richard *et al.* 1984) have also been observed. For example, *M. parvicella* thrives in periods or regions of low dissolved oxygen

concentration, but not anoxic or anaerobic conditions, under long sludge age conditions where there is a low f/m ratio (Gabb *et al.* 1987; Slijkhis 1983).

The most frequently encountered problem in activated sludge operations is the separation of the mixed liquor in the final settlement tank, when the mixed liquor itself is carried out of the tank with the final effluent. This has two general effects:

(i) It reduces the quality of the final effluent in terms of BOD and suspended solids, although if it was not for the presence of these solids, the effluent would be of excellent quality. Filaments that cause bulking, for example, are extremely efficient in removing BOD, however, their inability to rapidly separate from the treated effluent in the final sedimentation tank leads to their being discharged with the final effluent.
(ii) This loss of mixed liquor results in a very thin sludge with a low MLSS concentration. Therefore, problems occur in recycling enough sludge back to the aeration tank in order to maintain the MLSS at a satisfactory concentration resulting in a gradual reduction in treatment efficiency as the f/m ratio increases.

Not all problems are associated with inadequate separation. For example, operation problems such as increased BOD or suspended solids in the final effluent, low MLSS concentrations in aeration tanks, or reduced oxygen utilisation rate by the mixed liquor, can all be caused by factors other than those associated with filamentous micro-organisms.

5.4.5. *Identifying problems*

It is desirable not only to identify the extent of an existing problem but also to detect the onset of a problem so that remedial action can be taken quickly to prevent a serious operational failure. It is also important to distinguish between true bulking and other solid-liquid separation problems. The regular use of various chemical, physical, and microscopic analyses are required to indicate to the operator that settling efficiency is impaired. Regular chemical analysis will indicate when there is a reduction in treatment efficiency, however, loss in treatment efficiency is generally the final symptom of the problem. Earlier signs of impending failure can be obtained by the presence of filaments in the sludge, a high sludge settleability index (e.g. SVI), or an increased turbidity of the final effluent.

Presence of filaments

Using a microscope at ×100 or ×200 magnification, a rapid assessment of filament development in the sludge can be made. Such an assessment takes only a few minutes (Fig. 5.74). More accurate assessments of filamentous development can be made by the measurement of total filament length. Either of these methods will give an early prediction of sludge bulking before the SVI has significantly increased.

Sludge settleability

If a steady increase in the measured SVI (or SSVI) occurs exceeding 150 ml g^{-1} (200 ml g^{-1}) then bulking is imminent.

Cone test

During the measurement of SVI (SSVI) the supernatant should be examined. The sludge should form a discrete blanket with a clear supernatant. If there is a large volume of sludge (> 650 ml) in the one litre cylinder or cone with a clear supernatant then bulking is occurring. However, if the supernatant is turbid, then another sludge settlement problem is the cause (Table 5.22).

Once a problem has been identified, then remedial measures can be attempted. The most widely occurring sludge settlement problem is that of bulking and it has been suggested that over 50% of the treatment plants in the UK, for example, regularly suffer bulking problems (Tomlinson and Chambers 1984).

Table 5.22. Distinguishing sludge problems from other solid–liquid separation problems.

	Bulking	Pin floc	Deflocculation	Denitrification (rising sludge)
Filaments present	Yes	No	No	No
High SVI	Yes	Possibly	Possibly	Possibly
Supernatant clear	Yes	No	No	Possibly
Rising sludge that covers surface	No	No	No	Yes
Turbid effluent due to small particles	No	Yes	Yes	No

More specific aids to diagnosis may help to identify the causative factors more rapidly. For example, protozoa can help to rapidly identify such operational conditions as organic overloading, underaeration, or insufficient solids retention time (Sec. 5.5). A general guide is given in Table 5.23. Identification of filaments that are responsible for bulking has also proved useful, as many specific operational factors are associated with characteristic filament types (Table 5.21); although several species are associated with more than one possible cause of bulking. For example, the presence of *M. parvicella* is indicative of low dissolved oxygen concentrations occurring where there are long sludge ages and a low f/m ratio. So where this micro-organism occurs in a reactor using intermittent aeration, such as an extended aeration plant, it can be effectively eliminated by switching to continuous aeration for a period of time to maintain the dissolved oxygen concentration at 2–3 mg l^{-1} (Gabb *et al.* 1987). In Ireland, *M. parvicella* has only been recorded from activated sludge plants using surface aerators and not from those using diffused air systems. Blackbeard *et al.* (1988) suggest that such a difference is due to a more uniform dissolved oxygen distribution and better mixing than is achieved by using mechanical aerators.

Table 5.23. Biological summary of mixed liquor.

Heavily loaded (temporary overloaded/starting)	
Flagellates dominate	
Amoebae (naked)	
Normally loaded	
Irregular flocs	
Ciliates present in large numbers	
Typical species	*Vorticella convallaria*
	Opercularia coarctata
	Aspidisca costata
	Euplotes affinis
Flagellates, Amoebae	present in small numbers
Lightly loaded	
Small compact flocs	
Ciliates present in small numbers	
Typical species	*Vorticella communis*
	Epistylis rotans
	Stentor roeseli
Amoebae (testate)	
Rotifers and nematodes present	
Suctorians abundant	

Regular microscopic examination of the mixed liquor will provide a useful data base from which the presence of particular filament types will be clearly associated with particular environmental or operational factors. Such associations are more easily discernible in individual plants and so more readily applied to operational management. Early detection of filament development by regular use of a subjective scoring index where the abundance of filaments are compared with a set of drawings or photographs at $\times 100$ magnification, followed by identification, will give the operator early warning that bulking is developing and enough time to adjust the relevant operational factor before the SVI is affected. There may be sufficient time to try several possible corrective measures before the full severity of bulking occurs.

Even if the filamentous organism responsible is known, there is no specific control method for bulking sludges. Indeed, many of the control methods suggested are contradictory and others are quite bizarre (Chambers and Tomlinson 1982a). Therefore, operators must try a series of corrective options, starting by correcting the possible cause as indicated by the filamentous organisms present, until the problem disappears (Waller and Hurley 1982). In practice rapid solutions are required which consists of adding a substance directly to the sludge to improve settleability (Jenkins *et al.* 1993; Wanner 1994). Three main groups of chemicals are used each with a different mode of action: Biocides (mainly chlorine based), ballasting agents (mainly talc based), and coagulating or flocculating agents (mainly synthetic polymers). Among the large number of possible control options that have been suggested the more commonly employed include:

(1) *Controlling the sludge loading ratio*: Normal plant sludge loading is between 0.2 and 0.45 with bulking problems occurring if the sludge loading falls outside this range, although this has been shown to depend on the mixing regime with the critical sludge loading in plug flow systems almost twice the value than for completely mixed reactors (Tomlinson and Chambers 1979). In order to avoid bulking, the sludge loading should be maintained within the operational range of 0.2–0.45 kg kg^{-1} d^{-1}. The sludge loading can be altered by changing the influent flow rate, the BOD strength, the aeration tank volume, or the MLSS concentration. In practice, the MLSS concentration is the only operational variable that can easily be altered, so by reducing the MLSS concentration in the aeration tank by wasting more sludge from the system the sludge loading can be increased. Conversely, the sludge loading is decreased by reducing the wastage rate, thereby increasing the aeration tank MLSS concentration. It is advisable

not to lower the MLSS below 2,000 mg l^{-1}, while the upper limit is dependent on aeration capacity and the ability of the secondary settlement tank to handle solids.

(2) *Nutrients*: A BOD:N:P ratio of 100:5:1 is required to prevent bulking (Pipes 1979). Filaments are able to store essential nutrients when the BOD:N or BOD:P ratios are high, making them available when the nutrient concentration falls. This gives filamentous organisms a strong competitive advantage over floc-forming bacteria. Nutrient deficiency is detected by analysing the final effluent. If no excess N and P is present, then addition is required. Agricultural fertiliser is normally used as a ready source of these nutrients, or urea as a source of nitrogen only, being added directly to the aeration tank. Many operators prefer to dissolve the fertiliser first to prevent deposition within the tank. If bulking is a result of a high concentration of low molecular weight carbohydrate in the wastewater then some form of roughing treatment (e.g. biotower) or trade effluent control will be required.

(3) *Oxygen concentration*: To prevent filamentous growth a minimum dissolved oxygen concentration of 2 mg O_2 l^{-1} must be maintained (Pipes 1979). Although from the work of Palm *et al.* (1980) it is clear that the minimum dissolved oxygen concentration required to prevent bulking is a function of the sludge loading, increasing as the sludge loading increases (Fig. 5.76).

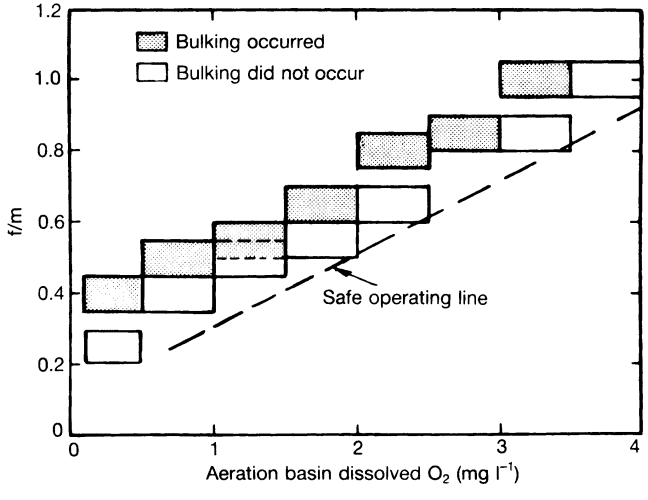


Fig. 5.76. The minimum dissolved oxygen concentration in the aeration tank required to prevent bulking as a function of sludge loading (Palm *et al.* 1980).

(4) *Introduction of anoxic zone*: Although anoxic zones are principally used for denitrification, they have been found to improve sludge settleability (Tomlinson and Chambers 1979; Price 1982). The reason for this is unclear but may be due in part to a reduction in the overall oxygen demand within the aeration tank, enhanced oxygen availability due to the release of oxygen bound in oxidised nitrogen, and finally due to a reduction in the degree of longitudinal mixing in the system. The use of anoxic zones are primarily restricted to nitrifying plants, although their effectiveness in improving settleability can only be determined by pilot scale studies. The function and operation of anoxic zones are fully discussed in relation to nutrient removal (Sec. 5.6).

(5) *Mixing pattern*: Changing the mixing pattern in completely mixed aeration tanks to a more plug flow type, i.e. reducing the degree of dispersion, produces sludges with better settling characteristics (Chudoba *et al.* 1973; Tomlinson and Chambers 1979; Chambers 1982; Humohries 1982). This also reduces the competitive advantage that filaments have over floc-forming bacteria due to their higher surface area to volume ratio which makes them more efficient in obtaining nutrients in conditions of low nutrient and low dissolved oxygen concentration.

It is very difficult to determine how closely a particular aeration tank approaches the idealised plug-flow system, i.e. an aeration tank with a length to width ratio greater than 20:1, or ten or more completely mixed reactors in series. The degree of longitudinal mixing is used to estimate mixing pattern, using a tracer technique (Thomlinson and Chambers 1979b), with a plug-flow system having a low degree of longitudinal mixing. The degree of longitudinal mixing is expressed in terms of dispersion number (DN), where DN = 0 for a plug-flow system and DN = 1 for a completely mixed system. It is clear from comparing DN with settleability that the lower DN the better the sludge settling characteristics of the sludge (Chudoba *et al.* 1973a; Tomlinson and Chambers 1979c) (Fig. 5.77).

(6) *Selector*: This process modification is based on the modified accumulation: regeneration theory. Filamentous growth can be controlled by ensuring that the most efficient micro-organisms adsorb the bulk of the substrate under high substrate concentrations, that favours the floc-forming bacteria, in a special zone before entering an area of low substrate concentration where the adsorbed material is subsequently metabolised. Hence the most favourable microbial population is selected. A contact tank or selector is a small chamber preceding the aeration tank or in an oxidation ditch it can be an actual zone, normally immediately after the cage rotor (Fig. 5.78).

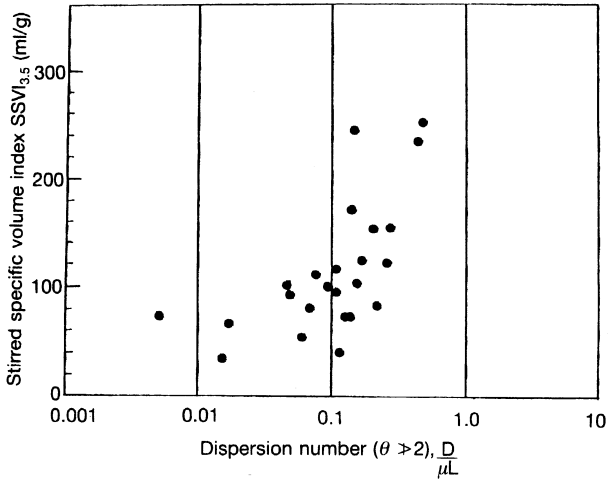


Fig. 5.77. Relation between settleability and modified dispersion number (Tomlinson 1982).

Here the substrate and the returned sludge are introduced under conditions of high aeration to ensure continuous aerobic conditions. The main aeration tank will have a much lower substrate concentration thereby ensuring there is always a concentration gradient (Chudoba *et al.* 1973b). The size of the contact tank has been estimated as between 1/74 to 1/15 the size of the aeration tank, although precise design criteria have yet to be developed (Lee *et al.* 1982; Ekama and Marais 1989). Wakefield and Slim (1988) used a selector to successfully restore a badly bulking sludge at a municipal treatment plant, while Ruider *et al.* (1988) successfully used a selector at a plant treating sugar beet wastewater.

(7) *Biocides*: The theory behind the use of biocides to control bulking is a simple one. The filaments associated with bulking sludge flocs grow out from the mass of micro-organisms forming the floc into the surrounding liquid. Therefore filaments are more exposed than the floc-forming organisms to any biocide added to the mixed liquor. The objective is to selectively kill the filaments while having minimum toxic effect on the floc-forming micro-organisms. Therefore, the dosing rate is critical with overdosing leading to a significant reduction in BOD removal efficiency and loss of nitrification (Hwang and Tanaka 1998).

Two biocides are in general use. Chlorine and chlorine-based compounds such as sodium hypochlorite are very toxic to micro-organisms at low concentrations, while hydrogen peroxide and ozone are also very reactive but

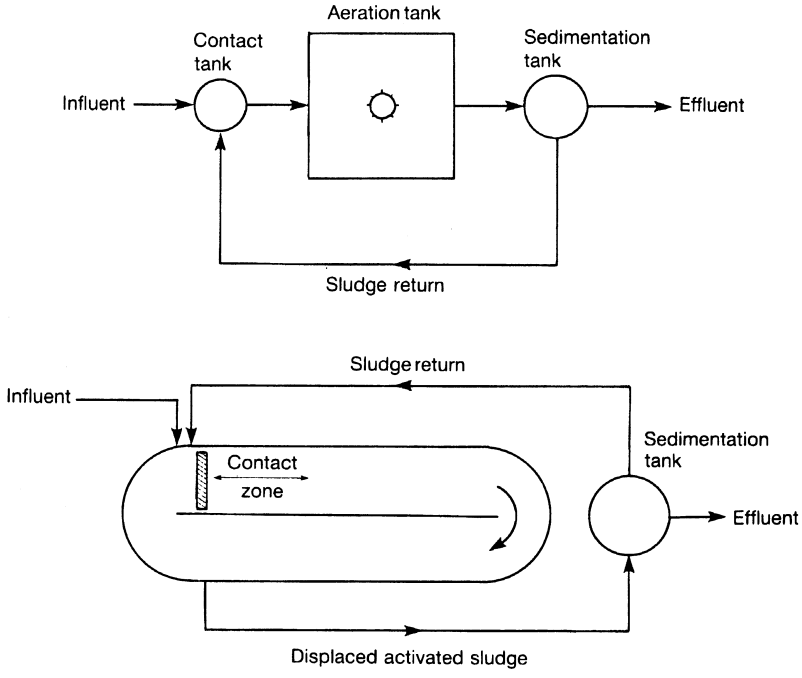


Fig. 5.78. The incorporation of a contact tank in a completely mixed system or a contact zone in a plug-flow system.

also add oxygen to the mixed liquor as they decompose. It is best to start dosing at a low concentration and gradually increase the concentration until the required improvement in sludge settleability is achieved. Signs of overdosing are inhibition of nitrification, death of the protozoa, and the production of a turbid effluent due to cell lysis and disintegration. Reported dosing rates range from 0.1–2.5 g Cl_2 per kg of returned sludge, while incoming wastewater have also been successfully dosed at 10–20 mg Cl_2 per litre of wastewater (Chambers and Tomlinson 1981; Jenkins *et al.* 1982). Much work has been done on this form of control using chlorine (Tapelshay 1945; Smith and Purdy 1936; Pipes 1974; Jenkins *et al.* 1982; Jenkins *et al.* 1984a; Wakefield and Slim 1988). Hydrogen peroxide is less frequently used and the calculation of the effective dosage rate more complex. Keller and Cole (1973) reported that 0.1 kg H_2O_2 per kg MLSS caused a 50% reduction in SVI within 7 days, while 0.4 kg H_2O_2 kg^{-1} MLSS had the same effect in about 24 h.

While many case studies have been reported using hydrogen peroxide (Cole *et al.* 1973; Keller and Cole 1973), chlorine appears to be more widely

used. Ozone is more powerful than either chlorine or hydrogen peroxide in its reactivity. Unlike chlorine, it does not interfere with nitrification or biological phosphorus removal, and does not add chlorinated hydrocarbons, residual chlorine or chloramines to the final effluent. Leeuwen and Pretorius (1988) have successfully used ozone to control bulking at a continuous dosage rate of 4 g per kg sludge in the aerobic zone of a three stage activated sludge process of the Bardenpho type (Sec. 5.6). Such control measures, however, can only be temporary while some other plant modification is carried out to permanently solve the problem.

(8) *Chemical coagulants*: Inorganic coagulants, such as lime or ferric chloride, or polyelectrolytes are used to improve flocculation and increase floc strength and settleability (Carter and McKinney 1973; Renskink *et al.* 1979; Thomanetz and Bardtke 1977). Metal salts have a metabolic effect on the micro-organisms in the sludge at low concentrations, so at least one sludge age must elapse before a significant improvement in settling ability is observed. Coagulation is also important at higher dosage rates when metal hydroxide flocs are formed. A secondary effect of using metal salts is that phosphorus removal also occurs. Also there is a significant increase in sludge production and possible sludge dewatering problems. Ferric chloride or ferric sulphate can be added directly to the aeration tank, as can hydrated aluminium sulphate (alum) (Carter and McKinney 1973). Pilot trials are required to determine exact dosage rates, which alter with effluent strength and plant loading, although as a rule of thumb guide 10 mg l^{-1} as Fe is a normal dose. Alum is added at a lower concentration, but as with all chemicals it is best to start at a low dosage rate and gradually increase it. Polyelectrolytes are flocculating agents with a rapid response. These should be dosed into the overflow from the aeration tank as it passes to the settlement tank. A mixing chamber will increase efficiency, with normal rates of dosing 1–2 mg l^{-1}. As with metal salts, dosing should commence at a low concentration and if no improvement in sludge settleability is observed, then the dosage should be increased. If no improvement is seen then a different chemical should be considered. There is such a large range of synthetic polyelectrolytes available that it is worthwhile to carry out laboratory scale trials using the Jar Test to find the best polyelectrolyte and the most effective dosage rate. Most suppliers are happy to carry out such tests. While the addition of flocculating or coagulating agents result in larger and firmer flocs that yield immediate improvement in sedimentation, the immediate positive effect on the sludge is of relatively short duration, requiring repeated additions to maintain any improvement (Vanderhasselt and Vertraete 1999).

(9) *Ballasting agents*: As the term suggests, ballasting agents work by weighting the sludge and reinforcing floc structure (Clauss *et al.* 1999). Like flocculating agents, their addition results in an immediate improvement in settleability, has no effect on filament growth, does not result in long term improvement but requires repeated application of agent. Rates of addition of balancing agents, such as talc (alumino–silicate), are up to 70–100% of the MLSS which results in a large increase in waste sludge in terms of dry solids (Eikelboom and Grovenstein 1998). Seka *et al.* (2002) found that using a combination of biocide (cetyltrimethyl anmmonium bromide), ballasting agent, and a coagulant to control bulking sludges gave immediate improvement in settleability as well some degree of filament destruction, thereby providing long term improvement.

Full details of control methods are given by Chambers and Tomlinson (1982a) and Jenkins *et al.* (1983, 1984, 1993).

5.4.6. *Non-filamentous bulking*

Bulking occasionally occurs without filaments being present. This is associated with deflocculation where the dispersed bacteria then produce a zoogloeal or viscous bulking, it is due to a failure of the microstructure of the floc with excess extra-cellular polymer (ECP) being produced, resulting in the mixed liquor having a slimey or even jelly-like consistency. It can be clearly seen by reverse staining using India ink. When normal flocs are stained in this way, the ink penetrates deep into the flocs, whereas the extracellular material prevents the penetration of the ink so the flocs remain unstained.

5.4.7. *Denitrification*

In the sedimentation tank, the dissolved oxygen is rapidly utilised by the sludge flocs as it separates from the clarified effluent, so the oxygen conditions within the tank are usually anoxic. This can become a problem when the sludge residence time in the sedimentation tank is long and the effluent has been fully nitrified within the aeration tank, so that nitrates and nitrites can be reduced to nitrogen gas or nitrous oxide by denitrifying bacteria (Sec. 3.4.5). The gas released becomes entrained in the flocs making them buoyant so that they rise to the surface and are carried out of the tank with the final effluent. Known also as rising sludge, the rate of gas production can be extremely high in warmer weather causing significant turbulence within the tank inhibiting normal settlement. Apart from sludge

being clearly visible on the surface of the sedimentation tank forming a thin scum, gas bubbles can also be seen rising to the surface. This problem is not associated with sludge structure but rather with operational practice. However, rising sludge will be more serious if flocs have a macrostructure because their irregular shape entrains gas bubbles more readily than the smaller, spherical flocs, and as flocs with macrostructure are considerably larger more sludge can be lost from the system.

Rising sludge can be overcome by ensuring that the settled sludge is not retained too long in the sedimentation tank before being recirculated or wasted. The problem can be the result of poor sedimentation tank design. Flat-bottomed tanks with central sludge take-off are particularly susceptible to denitrification problems as the sludge at the periphery of these tanks may remain there for long periods. If nitrification is not required, the easiest remedy is to prevent nitrification occurring in the aeration tank so that there is no oxidised nitrogen available in the secondary sedimentation tank. This can be done either by decreasing the aeration intensity or increasing the sludge loading to the aeration tank. Where nitrification is required, the oxidised nitrogen can be removed from the effluent by denitrification using an anoxic zone prior to aeration. Denitrification has also been controlled by increasing the sludge recycle rate from the sedimentation tank or ensuring the mixed liquor is well aerated before it enters the sedimentation tank (Chambers and Tomlinson 1981). If the sludge is retained for even longer periods in the sedimentation tank the liquid may become completely deoxygenated resulting in the sludge becoming anaerobic and decomposing, producing hydrogen sulphide gas and an unpleasant putrescent sludge.

5.5. Ecology

Like all biological treatment processes, the activated sludge system relies on a mixed culture of bacteria to carry out the basic oxidation of the organic material present, with higher grazing micro-organisms also present, thus forming a complete ecosystem with various trophic levels. The activated sludge aeration tank is a truly aquatic environment, although the high BOD and high level of bacterial activity make it unlike any natural aquatic habitat. The constant aeration and recirculation of sludge makes it inhospitable for most aquatic species, especially those larger than the smaller mesofauna, such as rotifers and nematodes or those with long life-cycles. The main biological groups present are bacteria, fungi, protozoans, rotifers,

and nematodes, with flocs a heterogeneous mixture of them all, together with organic and inorganic material. Other groups such as *Cyclops*, *Aelosoma*, and even larvae of some dipterans are occasionally observed, although they are not major components of the community structure. Algae are also present in the mixed liquor but rarely become established. In contrast to percolating filters (Sec. 4.1.3), where the active biomass is attached to a static support medium and the wastewater flows over the surface, activated sludge is a dispersed growth system with the microbial biomass present as discrete flocs that are suspended in the wastewater and mixed together by the aeration system. Due to the physical difference between a static and dispersed growth system, fewer trophic levels are present in the activated sludge process (Fig. 5.79). The larger macro-invertebrate grazers associated with percolating filters are largely absent as they cannot be supported within the floc. Thus, apart from certain protozoans and nematodes, most of the larger grazers are found swimming in the liquid phase between the flocs. A simplified food web for conventional activated sludge is given in Fig. 5.80.

As the floc ages, it becomes colonised by bacteria-feeding organisms, such as ciliate protozoans, nematodes, and rotifers. Also, the proportion of the floc comprised of dead cells or accumulated inert solids increases, with the living cells clustered in the outer surface of the spongy structure of the floc. Material continues to be adsorbed on to the floc and although complete oxidation is only possible by the living cells, the dead cells remain capable of enzyme secretion. As the floc ages, the rate of oxidation gradually declines. A reduction in substrate removal with increasing sludge age has been reported by many workers (Keefer and Meisel 1953; Wuhrmann 1956). However, the slower growing nitrifying bacteria can only become established in the floc if long sludge ages are used (Sec. 5.6). Anaerobic bacteria are largely absent from activated sludge and those present have been introduced from the incoming wastewater or anaerobic activity either in the primary or secondary sedimentation tanks. As the floc increases in size, the rate of diffusion of nutrients and dissolved oxygen into and the movement of metabolic waste products out of the floc becomes more difficult. Concentration gradients of both nutrients and oxygen occur throughout the floc, with the centre of larger flocs becoming anoxic or even anaerobic. In theory, it is possible that the activated sludge ecosystem can be completely balanced so that nearly all the available organic matter is utilised by the primary heterotrophic activity, which in turn is used by the grazers so that there is no excess microbial biomass. However, in practice there is a significant increase

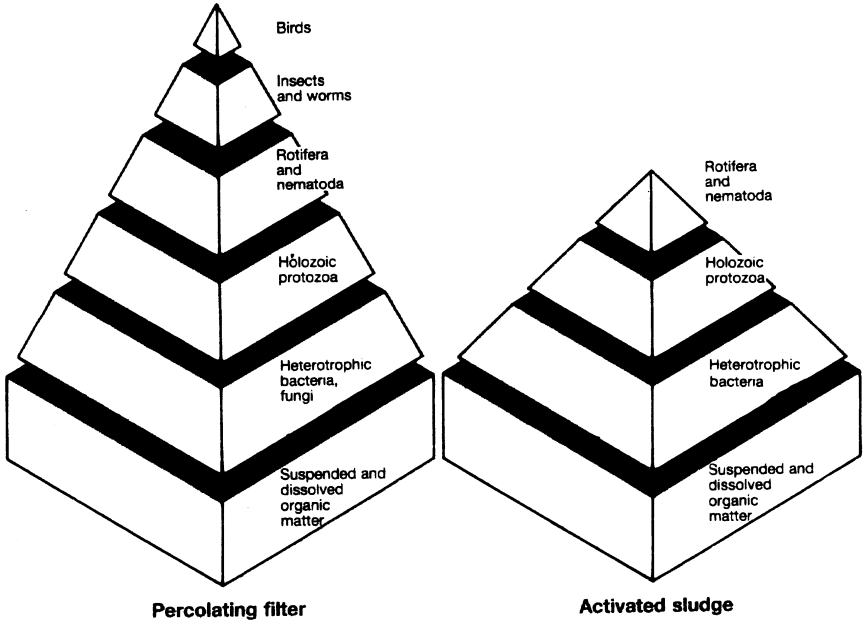


Fig. 5.79. Comparison of the food pyramids for percolating filter and activated sludge treatment systems.

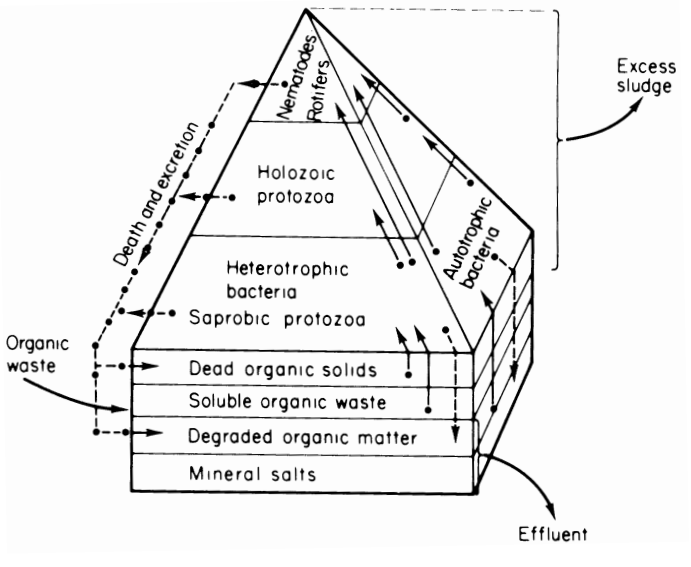


Fig. 5.80. Food pyramid representing feeding relationships in the activated sludge process: ——→, synthesis; – – – →, degradation (Hawkes 1983a).

in the microbial biomass, composed mainly of heterotrophic bacteria, which is surplus to that required to maintain the microbial population density in the aeration tank via the returned sludge. Therefore, the excess microbial biomass must be disposed of separately as unwanted sludge (Fig. 5.1). Under steady-state conditions, the growth rate of the micro-organisms will be equivalent to the specific sludge wastage rate (Curds 1971b).

Activated sludge is a complex ecological system made up of species forming several trophic levels, which compete for food resources, with predator-prey and parasite-host relationships clearly discernible. The microbiology of activated sludge processes has been extensively studied and excellently reviewed (Pike and Curds 1971; Curds and Hawkes 1975; Hughes and Stafford 1976; Pipes 1966, 1978a). Ecological considerations have also been dealt with by Hawkes (1963; 1983b) and Pipes (1966).

5.5.1. *Bacteria*

Bacteria in the activated sludge process are present as individual free-swimming cells dispersed in the liquid phase, floc-forming forms, and as dispersed non-floc-forming bacteria associated with the floc. As already discussed in Sec. 5.1, heterotrophic bacteria form the basis of flocs, which form the basic ecological unit of the activated sludge process, with individual cells agglutinating together or on to filamentous bacteria to form them. The biological condition of the flocs determines the rate of substrate removal, and their physical structure will determine the efficiency in being separated from the clarified effluent in the sedimentation tank. The principal species present will be those most able to reproduce in the activated sludge environment. In terms of bacteria, the species making up the mixed liquor are significantly different to those present in the incoming wastewater (Dias and Bhat 1964). The process itself selects the most efficient flocculating bacteria by retaining those flocs that rapidly separate from the clarified liquor and settle in the sedimentation tank, which are then returned to the aeration tank to maintain the correct MLSS concentration. Those species associated with flocs or with other settleable material in the sedimentation tank will also be returned to the aeration tank and thrive. The rate at which bacteria multiply is also critical and, in order to survive, their reproduction rate must exceed the rate at which they are removed from the system by the wastage of excess sludge. The type of bacteria present also depends largely on the wastewater being treated, environmental factors in the aeration tank such as pH, temperature, dissolved oxygen, nutrient concentration, degree of turbulence, and the operating factors, in

Table 5.24. Genera of aerobic heterotrophic bacteria and yeasts from activated sludge excluding zoogloeal strains (Benedict and Carlson 1971).

Genera	Laboratory activated sludge	Renton Metro sludge	Genera	Laboratory activated sludge	Renton Metro sludge
Acinetobacter	9[a]	4[a]	*Debaromyces*	0	4
Alcaligenes	4	2	*Flavobacterium*	7	1
Bacillus	0	5	*Hyphomicrobium*	0	2
Brevibacterium	17	7	*Microbacterium*	2	0
Caulobacter	2	0	*Pseudomonas*	8	16
Comomonas	7	5	*Sphaerotilus*	0	3[b]
Cytophaga	1	8	Unidentified	2	12[c]

[a] Number of isolates. [b] Only three colonies picked. [c] Did not grow in Difco nutrient broth or could not be keyed to genus.

particular, sludge loading and sludge age. For example, high sludge wastage rates or dilution rates in the aeration tank will encourage a high growth rate. This will select faster-growing bacteria, suppress the higher trophic levels, and result in incomplete nutrient removal. Therefore, in high-rate activated sludge processes, protozoans and rotifers are generally absent as will be the slower growing bacteria, such as the nitrifying species *Nitrosomonas* spp. and *Nitrobacter* spp. (Sec. 5.6). Dispersed, free-swimming bacteria are also present in large numbers but are constantly being removed from the system by either being discharged with the final effluent or by mesofaunal grazing mainly by protozoans. Earlier studies had assumed that floc formation was due to a particular bacterium *Zoogloea ramigera* (Pike 1975); however, bacteria from a large number of genera are able to form flocs, especially at the low nutrient concentrations associated with wastewater treatment (McKinney and Weichlein 1953; Kato *et al.* 1971). The majority of the bacteria isolated from the process are Gram-negative species belonging to the genera *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Bacillus*, *Alcaligenes*, and *Micrococcus* (Fig. 5.81). Many other genera are also found, but less frequently (Pike 1975). There is also a wide variety of filamentous bacteria isolated from activated sludge, which are associated with bulking problems (Sec. 5.4). Benedict and Carlson (1971) compared the aerobic heterotrophic bacteria from a laboratory and full-scale domestic plant. They recorded species from 11 different genera (Table 5.24) including a yeast *Debaromyces* sp., which occurred in significant numbers in the full-scale plant.

Bacterial growth kinetics have been used to model the activated sludge process and as bacteria are the most important group in terms of nutrient

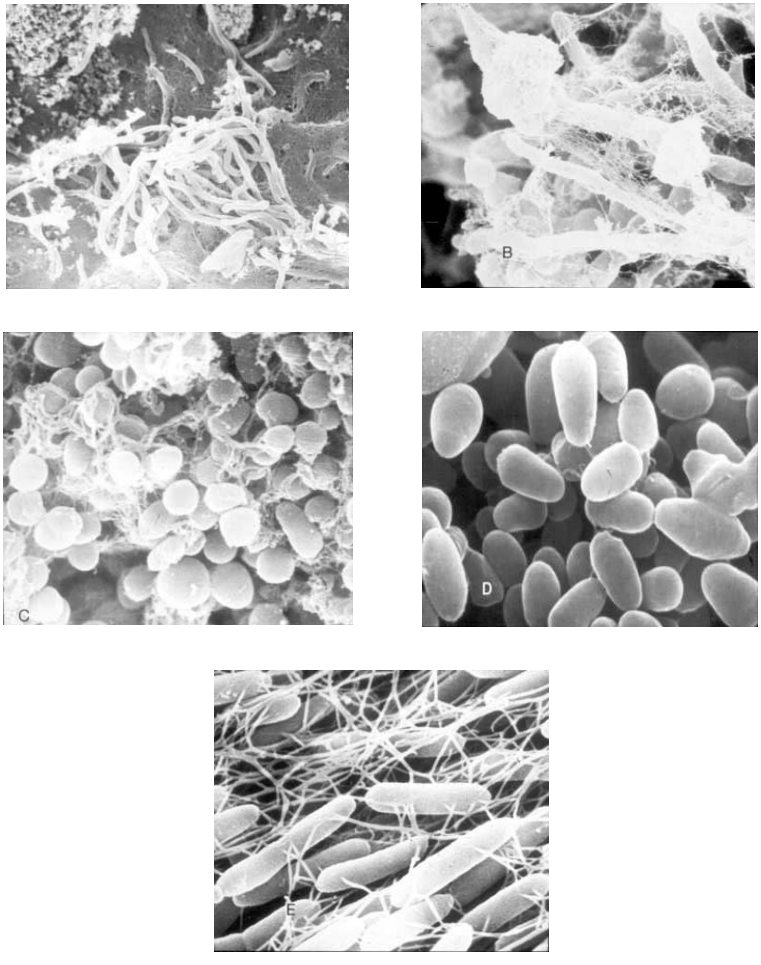


Fig. 5.81. Scanning electron micrographs of predominant bacterial strains isolated from sludges. All isolates were cultured on tryptone-glucose-meat and yeast extract-vitamins agar (TGEVA) or sludge extract media. A. *Nocardia* (× 20,000); B. *Sphaerotilus* (× 5,000); C. *Pseudomonas* (20000); D. *Arthrobacter* (× 20,000); E. *Zoogloea* (× 20,000) (Salanitro and Rader 1982).

removal, some idea of bacterial density is often required. Three approaches have been used: direct counting, plate counts, and biochemical assessment of activity. These are reviewed in Sec. 3.3.1.

The number of viable bacteria estimated to be in mixed liquor from activated sludge units varies between 0.02×10^{10} to 59×10^{10} cells g^{-1}, although a working figure of $1–5 \times 10^{10}$ cells g^{-1} is normally taken (Banks

et al. 1976; Takii 1977). The number of bacteria varies according to operational factors such as sludge age, environmental variables, and wastewater characteristics. Numbers of bacteria at different stages during the treatment process are summarised in Table 5.25. The number of viable bacteria in mixed liquor constitutes only 1–2% of the total biomass.

5.5.2. *Fungi*

Fungi are rarely present as a dominant organism in activated sludge, although fungal hyphae is often seen associated with flocs. When the pH of the mixed liquor is lowered to below 6.0, then bacteria are inhibited and fungi will begin to dominate. Therefore, in general terms, the presence of fungi as a dominant organism indicates acidic industrial effluents. Studies on the occurrence of fungi in activated sludge plants have found them to be rare with *Geotrichium candidum* and *Trichosporon* sp. being the most abundant (Table 3.8, Fig. 4.15) (Cooke and Pipe 1968; Cooke and Pipes 1970) (Sec. 3.3.1).

A predacious fungus, which captures and consumes rotifers, *Zoophagous insidians* was identified by Cooke and Ludzack (1958) in a laboratory aeration tank, and by Pipes (1965) in a pilot-scale plant. These fungi can capture a range of mesofauna including protozoans and nematodes. Gray (1985b) found both endoparasitic and predatory nematophagous fungi from a number of activated sludge plants in Ireland, where they had a significant role both in floc formation and in the regulation of the nematode population density (Fig. 5.82).

5.5.3. *Protozoa*

Protozoa are common components in activated sludge with population densities reaching up to 50,000 ml^{-1}, which can represent as much as 5–12% of the dry weight of the mixed liquor (Hopwood and Downing 1965; Pike and Curds 1971). This is similar to the total mass of bacteria, both viable and non-active, in the mixed liquor (HMSO 1971) and protozoans can represent a major proportion of the total biomass. Curds (1975) lists 228 species of Protozoa from activated sludge plants with 70% of them from the class Ciliatea. Flagellates, both Phyto- and Zoomastigophorea, are far more common in percolating filters, being found in small numbers in activated sludge units and being associated with overloaded plants (Fig. 4.16). Although species diversity is dominated by ciliate Protozoa (Fig. 4.17), testate, and naked forms of *Amoeba* sp. can occasionally be

Table 5.25. Mean numbers of total and viable bacteria recorded at various stages of domestic wastewater treatment (Pike and Carrington 1972).

Source (and number) of samples[b]	Bacterial counts[a]				Viability (%)	Total suspended solids (mg l^{-1})
	In samples (No. ml^{-1})		In suspended solids (No. g^{-1})			
	Total	Viable	Total	Viable		
Settled sewage (46)	5.6×10^8	6.3×10^6	3.0×10^{12}	3.4×10^{10}	1.1	190
Activated sludge mixed liquor, conventional rate (18)	5.9×10^9	4.9×10^7	1.3×10^{12}	1.1×10^{10}	0.83	4,600
Activated sludge mixed liquor, high-rate (24)	1.4×10^{10}	2.4×10^8	3.0×10^{12}	5.0×10^{10}	1.7	4,800
Filter slimes (18)	6.2×10^{10}	1.5×10^9	1.3×10^{12}	3.2×10^{10}	2.5	54,000
Secondary effluent (16)	5.4×10^7	1.1×10^6	1.9×10^{12}	4.1×10^{10}	2.1	28
Effluents, high rate activated sludge plants (24)	4.8×10^7	1.4×10^6	3.3×10^{12}	1.0×10^{11}	3.0	14
Tertiary effluents (11)	2.9×10^7	6.6×10^4	3.0×10^{12}	6.8×10^9	0.23	9.7

[a] Total counts obtained with Helber counting chamber, viable counts by plate dilution frequency method on CGY agar, incubation for 6 days at 22°C; viability expressed as percentage ratio of viable count to total count.
[b] Samples from sewage works and laboratory pilot plants; high-rate plants are pilot scale, working at loadings of 0.46–2.5 kg BOD removed/kg MLSS day, secondary effluents from nine filters and seven activated sludge plants, tertiary effluents from 10 lagoons, one grass plot.

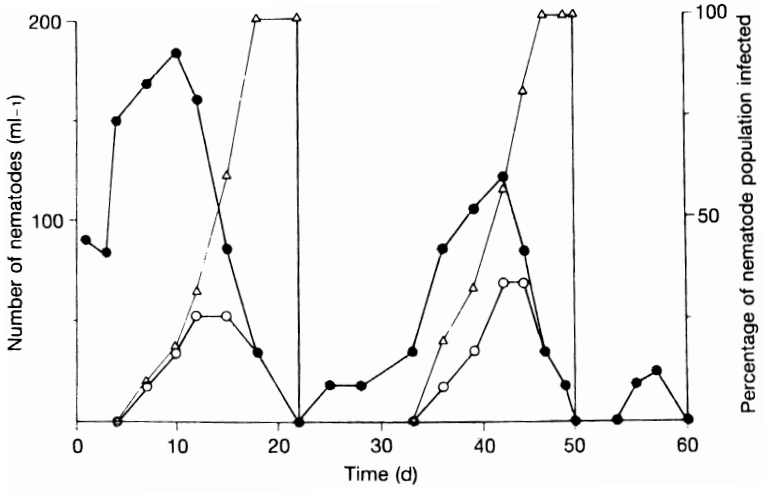


Fig. 5.82. The numbers of nematodes infected by the endoparasite *Catenaria anguillulae* (○) and the percentage of nematode population infected (%) compared with the total number of nematodes (●) in the mixed liquor from Naas Sewage Treatment Works during the 60-day experimental period.

dominant numerically (Schofield 1971; Sydenham 1971). In general terms, naked amoebae are associated with heavily loaded plants and testate forms with lightly loaded plants.

In a survey of 56 activated sludge plants in the UK, Curds and Cockburn (1970a) found the commonest species were *Vorticella microstoma* (75% of plants), *Aspidisca costata* (69%), *Trachelophyllum pusillum* (64%), *Vorticella convallaria* (58%), *Opercularia coarctata* (54%), and *Vorticella alba* (38%). The most frequently observed Protozoa recorded by two other workers (Brown 1965; Schofield 1971) are compared to the results of Curds and Cockburn (1970a) in Table 5.26. The protozoan fauna of activated sludge differs from that of percolating filters because of the significant difference between the two environments. For example, Peritrichida, mainly vorticellids, are far more common in activated sludge, with a greater diversity of suctorians also recorded (Fig. 5.83). The reason why opercularians are less common in activated sludge is that these stalked protozoans, unlike the vorticellids, do not have a contractile stalk and can be damaged when flocs come into close contact in the aeration tank. However, the static film environment in percolating filters is ideal for them. Identification of ciliates to species level requires some degree of taxonomic expertise, however, the major subclasses, the Suctoria, Peritrichia, Holotrichia, and Spriotrichia,

Table 5.26. The most frequently observed protozoa in surveys of activated sludge plants (adapted from Curds 1975).

Class	Brown (1965)	Curds and Cockburn (1970*a*)	Schofield (1971)
Phytomastigophorea			
		Peranema trichophorum	
Zoomastigophorea			
Rhizopodea	*Euglypha alveolata*	Small amoebae	*Amoeba* sp.
		Arcella vulgaris	*Cochliopodium* sp.
		Euglypha sp.	
Ciliatea	*Aspidisca costata*	*Aspidisca costata*	*Aspidisca costata*
	Aspidisca lynceus	*Carchesium polypinum*	*Chilodonella uncinata*
	Aspidisca robusta	*Euplotes moebiusi*	*Drepanomonas* sp.
	Chilodonella cucullulus	*Opercularia coarctata*	*Epistylis* sp.
	Epistylis plicatilis	*Trachelophyllum pusillum*	*Hemiophrys* sp.
	Euplotes aediculatus	*Vorticella alba*	*Vorticella convallaria*
	Litonotus fasciola	*Vorticella convallaria*	*Vorticella microstoma*
	Vorticella campanula	*Vorticella fromenteli*	
	Vorticella convallaria	*Vorticella microstoma*	
	Vorticella microstoma		
	Vorticella nebulifera var. *similis*		
	Vorticella striata var. *octava*		

and many of their component genera, are easily discernible (Table 5.27). There are a number of excellent keys to the group, the most useful being Curds (1969). Other more specialist keys include Bick (1972), Curds (1982,1983), Kudo (1966) and Foissner and Berger (1996).

Ciliate Protozoa in activated sludge can be categorised according to their habits. Three categories are discernible in the mixed liquor: sessile species, which are attached to the individual flocs (*Vorticella* spp., *Opercularia* spp., *Epistylis* spp.); crawling species, which move over the surface of the floc (*Aspidisca* spp., *Euplotes* spp); and free-swimming species, which live in the liquid phase of the mixed liquor and are not

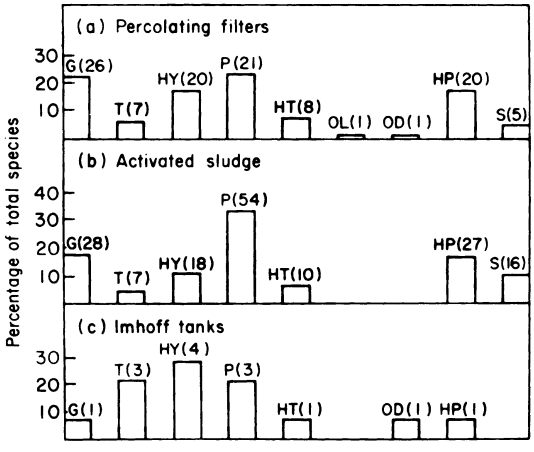
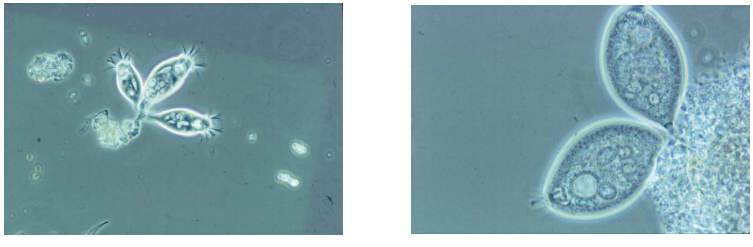


Fig. 5.83. Percentage distribution of species between the orders of the class Ciliatea found in used water treatment processes. G, Gymnostomatida; T, Trichostomatida; HY, Hymenostomatida; P, Peritrichida; HT, Heterotrichida; OL, Oligotrichida; OD, Odonstostomatida; HP, Hypotrichida; S, Suctorida. Numbers in parenthesis indicate actual numbers of species represented in each order (Curds 1975).



(a) (b)

Fig. 5.84. *Opercularia* sp. (a) × 400; (b) × 1000.

Table 5.27. Characteristics of the main subclasses of the Class *Ciliatea*.

Subclass	Diagnostic features	Habit	*Common spp.*
Suctoria	No cilia present, hollow tentacles formed	Generally attached to flocs	*Tokophyra mollis*, *Podophyra maupasi*, *Acineta foetidia*
Peritrichia	No cilia on the body, but around oral aperture only. Body barrel, bell or vase shaped supported on a stalk	Attached to flocs	*Vorticella microstoma*, *Carchesium polypinium*, *Epistylis rotans*
Spirotrichia	Cilia bound together to form thick pointed spikes	Free swimming or crawling over floc surface	*Aspidisca costata*, *Euplotes patella*, *Oxytricha fallax*
Holotrichia	Cilia uniformly spread over body	Free swimming	*Paramecum caudatum*, *Colpidium colpoda*, *Trachelophyllum pusillum*

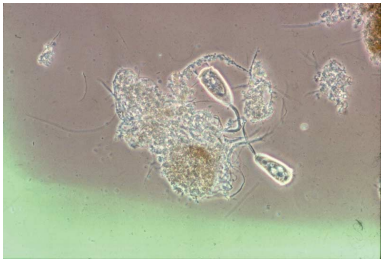


Fig. 5.85. *Vorticella* sp. (× 400).

directly associated with floc (*Paramecium* spp., *Colpidium* spp., *Litonotus* spp. (Figs. 5.84–5.87). Whereas the Phytomastiophora are primary producers, the Zoomastigophora compete with the bacteria at the basic trophic level (Fig. 5.80). The holozoic forms of Protozoa, present mainly as ciliates, form the next trophic level. All three groups of ciliates feed predominantly on bacteria, although there are several attached (Suctoria) and free-living species (e.g. *Trachelophyllulm pusillum*), which are predatory on protozoans. Although bacteria are primarily responsible for the removal of organic matter, protozoans also play an important role in the purification process as bacterial feeders. This has two major effects. First, it

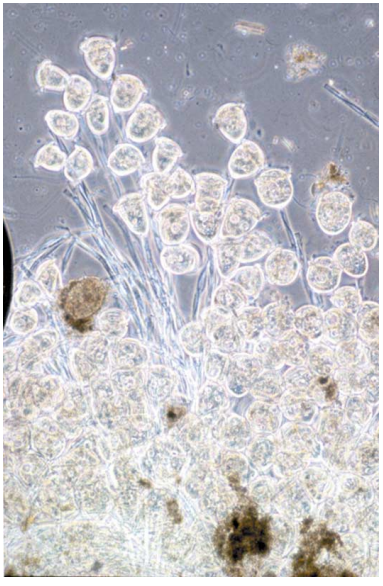


Fig. 5.86. *Carchesium polypinum* (× 400).

regulates bacterial density, preventing bacteria from reaching self-limiting numbers, both dispersed and floc-forming bacteria, causing enhanced bacterial activity in non-food-limited plants as well as reducing the overall bacterial biomass. Secondly, protozoans clarify the effluent by removing suspended material. When the nutrients are limiting in activated sludge, protozoal grazing will reduce the bacterial population, resulting in an increase in substrate concentration, which will cause either an enhanced nutrient uptake rate by the remaining bacteria or an increase in the substrate concentration of the effluent (Curds 1971b). Stalked protozoans attached to the flocs, and the free-swimming protozoans both feed on dispersed bacteria and organic particulate matter that is in suspension (Curds and Vandyke 1966). This was elegantly demonstrated by Curds *et al.* (1968), who compared the turbidity and chemical quality of the final effluent from six bench-scale activated sludge plants. When operated without Protozoa, the effluent was turbid with a high BOD and suspended solids concentration. However, when a mixed population of Protozoa was added to three of the plants, leaving the other three as protozoan-free control plants, a significant reduction in the turbidity, BOD, and suspended solids concentration was recorded (Fig. 5.88). Protozoa also feed on pathogenic bacteria, which was demonstrated by Curds and Fey (1969) using *Escherichia* coli.

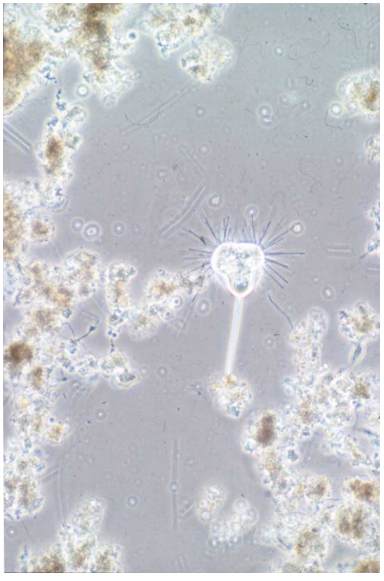


Fig. 5.87. The suctorian *Tokohyra mollis* (× 400).

They obtained a 95% removal of *E. coli* when Protozoa were present, compared to just 50% removal when absent, removal being due to flocculation and adsorption on to flocs (Fig. 5.89). Under certain food-limiting conditions, the dispersed bacteria may well be in competition with the floc-forming bacteria for the soluble substrate present. By removing the dispersed bacteria, the protozoans will reduce the competition for the remaining substrate, allowing the floc-forming bacteria to produce larger flocs, which will improve settleability of the mixed liquor as well as reducing suspended material in the effluent, thereby enhancing overall quality (Downing and Wheatland, 1962; Curds *et al.* 1968).

Although no spatial variation in protozoan species is discernible in the completely mixed environment of the activated sludge aeration tank where the sludge is continuously recycled, which is in contrast to percolating filters, temporal succession of species has been well documented. The succession follows a similar pattern in all activated sludge plants as the mixed liquor matures with flagellates dominating initially followed by free-swimming ciliates, crawling ciliates, and attached ciliates (Agersborg and Hatfield 1929; Horosawa 1950; McKinney and Gram 1956; Curds 1966) (Fig. 5.90). Both the crawling and attached ciliates are closely associated with the flocs, and once established their population densities will be

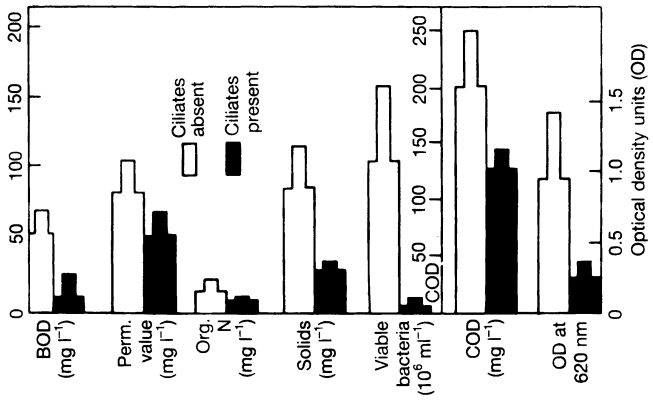


Fig. 5.88. Effluent qualities from experimental activated sludge units operating in the absence and presence of ciliated protozoa. The 'shoulders' on blocks indicate ranges of means of final effluent quality (Curds *et al.* 1968).

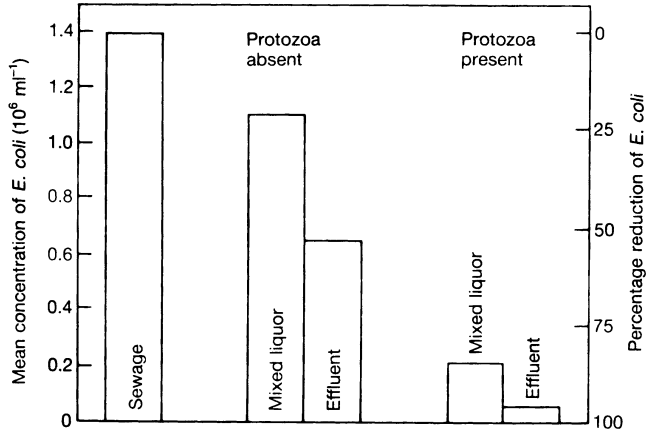


Fig. 5.89. Removal of *Escherichia coli* in experimental activated sludge plants operating in the absence and presence of ciliated protozoa (Curds and Fey 1969).

maintained by being recirculated from the sedimentation tank with the settled sludge. The wash-out rate for the flagellates and the free-swimming ciliates will be much greater than the other types of protozoans. This association has been shown by a computer model by Curds (1971b). Once the mixed liquor has matured, the peritrichs and crawling species appear to be inversely related (Brown 1965; Curds 1966). As the mixed liquor matures and the protozoan population develops, the effluent quality gradually

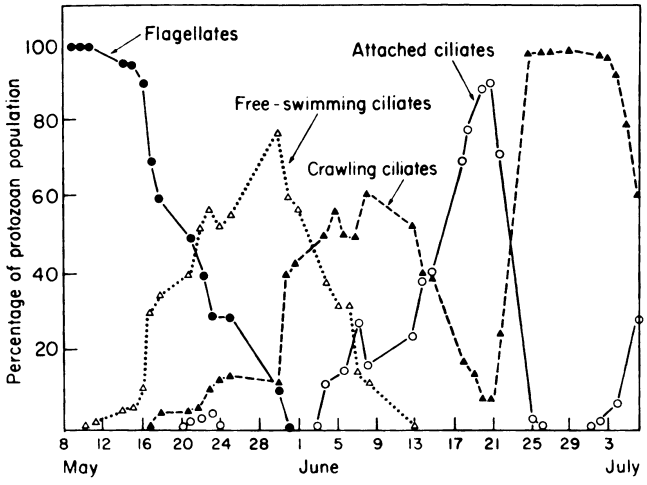


Fig. 5.90. Succession of protozoa in the MLSS of an activated sludge plant (Curds 1966).

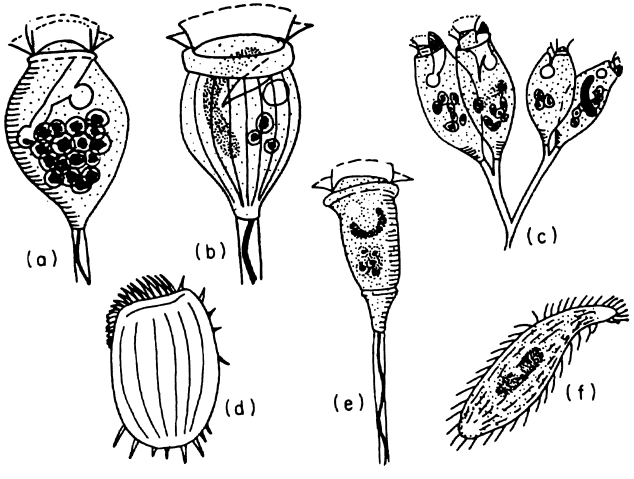


Fig. 5.91. Frequently encountered ciliates in the activated sludge process. (a) *Vorticella microstoma*, (b) *V. alba*, (c) *Opercularia coarctata*, (d) *Euplotes moebiusi*, (e) *V. fromenteli*, and (f) *Trachelophyllum pusillum* (Curds 1975).

improves. This is due to the transition from a dispersed bacterial population to a flocculated bacterial population in the aeration tank (Fig. 5.91). A new mixed liquor or a plant that is in poor condition producing an inferior quality effluent contains mainly flagellates and rhizopods, with few

ciliates present. In contrast, satisfactory mixed liquor producing a reasonable quality effluent contains mainly ciliates with some flagellates and amoebae. The ciliates are dominated by species, such as *Chilodonella* spp., *Colpoda* spp., *Colpidium* spp., *Aspidisca* spp., and certain *Carchesium* spp. and *Vorticella* spp. Activated sludge plants producing nitrified effluents of excellent quality contain very few flagellates or amoebae and are dominated by ciliates, such as *Carchesium* spp., *Vorticella* spp., *Aspidisca* spp., *Loxophyllum* spp., and *Chaenea* spp. (Curds 1975). This association of certain species with the condition of the sludge and effluent quality has led to the use of protozoans to indicate the effluent quality of activated sludge plants (Table 5.23). By utilising the large data base collected in their survey of UK plants, Curds and Cockburn (1970b) have been able to correlate species diversity to effluent quality. Using four effluent quality categories based on BOD, very high (0–10 mg l^{-1}), high quality (11–20 mg l^{-1}), inferior (21–30 mg l^{-1}) and low quality (> 30 mg l^{-1}), the frequency of occurrence of each species in the activated sludge plants sampled in the survey was recorded. The frequency of occurrence recorded in each of the four categories were then totalled and a value out of a total of 10 was awarded to each category depending on the proportion of the total occurring in each category (Table 5.28). This gave a weighting to the effluent quality categories that were most associated with a particular species. This was done for all the 288 species identified by Curds and Cockburn (1970a), a full list of which has been published by them (Curds and Cockburn 1970b) (Table 5.29). Effluent quality of activated sludge plants is then calculated by producing a comprehensive species list and adding up the various

Table 5.28. Percentage frequency occurrence of some common Protozoa in activated sludge plants producing final effluents in four BOD ranges (Curds and Cockburn 1970b).

BOD range (mg l^{-1})	Frequency of occurrence (%) and points awarded (in brackets)			
	0–10	11–20	21–30	> 30
Vorticella convallaria	63 (3)	73 (4)	37 (2)	22 (1)
Vorticella fromenteli	38 (5)	33 (4)	12 (1)	0 (0)
Carchesium polypinum	19 (3)	47 (5)	12 (2)	0 (0)
Aspidisca costata	75 (3)	80 (3)	50 (2)	56 (2)
Euplotes patella	38 (4)	25 (3)	24 (3)	0 (0)
Flagellated protozoa	0 (0)	0 (0)	37 (4)	45 (6)

Table 5.29. Association ratings for ciliate protozoa found in activated sludge. These ratings are used to predict the quality of effluent from activated sludge plants and are based on data collected from 80 activated sludge plants in the UK (Curds and Cockburn 1970b).

Species	Association rating for effluent BOD range			
	0–10	11–20	21–30	> 30
Subclass 1. Holotrichia				
*Coleps hirtus**	10	0	0	0
Trachelophyllum pusillum	3	3	3	1
*Amphileptus claparedei**	10	0	0	0
*Litonotus anguilla**	10	0	0	0
*Litonotus carinatus**	10	0	0	0
Litonotus fasciola	0	10	0	0
Hemiophrys fusidens	3	4	3	0
Hemiophrys pleurosigma	10	0	0	0
Spathidium spathula	5	5	0	0
Trochilia minuta	0	10	0	0
Chilodonella cucullulus	4	4	1	1
Chilodonella uncinata	3	6	1	0
Drepanomonas revoluta	1	4	5	0
Uronema nigricans	2	4	4	0
Tetrahymena pyriformia	1	3	3	3
*Sathrophilus oviformis**	10	0	0	0
Glaucoma scintillans	2	2	3	3
Colpidium campylum	2	2	2	4
Colpidium colpoda	0	0	4	6
Paramecium aurelia	10	0	0	0
Paramecium caudatum	2	5	3	0
Paramecium trichium	4	3	2	1
Cinetochilum margaritaceum	7	3	0	0
Subclass 2. Peritrichia				
Vorticella aequilata	2	2	3	3
Vorticella alba	3	3	3	1
Vorticella campanula	8	2	0	0
*Vorticella communis**	10	0	0	0
Vorticella convallaria	3	4	2	1
Vorticella elongata	10	0	0	0
Vorticella fromenteli	5	4	1	0
Vorticella hamata	7	2	1	0
Vorticella microstoma	2	4	2	2
Vorticella nebulifera var. *similis*	5	5	0	0
Vorticella striata var. *octava*	3	3	2	2
Zoothamnium mucedo	10	0	0	0
Zoothamnium pygmauem	10	0	0	0
Carchesium polypinum	3	5	2	0
Epistylis plicatilis	0	4	4	2

Table 5.29. (*Continued*)

Species	Association rating for effluent BOD range			
	0–10	11–20	21–30	> 30
Epistylis rotans	10	0	0	0
Opercularia coarctata	2	2	4	2
Opercularia phryganeae	0	0	0	10
*Telotrochidium henneguyi**	0	0	0	10
Subclass 3. Spirotrichia				
Stentor roeseli	10	0	0	0
*Spirostomum teres**	0	10	0	0
Aspidisca costata	3	3	2	2
Aspidisca lynceus	5	5	0	0
Aspidisca turrita	10	0	0	0
Opisthotricha similis	5	5	0	0
Tachysoma pellionella	0	10	0	0
*Hypotrichidium conicum**	0	0	10	0
Histriculus similis	10	0	0	0
Euplotes affinis	6	4	0	0
Euplotes carinatus	2	4	4	0
Euplotes eurystomus	2	4	4	0
Euplotes moebiusi	3	3	3	1
Euplotes patella	4	3	3	0
Subclass 4. Suctoria				
*Acineta cuspidata**	10	0	0	0
Acineta grandis	10	0	0	0
Acineta foetida	0	0	10	0
Podophrya fixa	0	2	7	1
Podophrya maupasi	0	10	0	0
Podophrya carchesii	2	2	3	3
Tokophrya mollis	0	5	5	0
Tokophrya quadripartita	4	3	3	0
Discophrya elongata	0	10	0	0
Sphaerophrya magna	0	2	7	1
Flagellated protozoa	0	0	4	6
Ciliated protozoa absent	0	0	0	10

* Based on few observations; ratings to be regarded with caution.

weightings for each species in each of the four effluent quality categories. The category with the highest total indicates the predicted quality (Table 5.30). Curds and Cockburn (1970b) tested this method on their original data and on a further 34 plants not included in the original survey and found the predicted effluent quality was correct in 85% and 83% of the cases

Table 5.30. Association ratings used to predict the final effluent quality from an activated sludge plant (Curds and Cockburn 1970b). In this example, the highest points are awarded to BOD range 0–10 mg l^{-1}, which corresponds to the measured BOD of the final effluent which was 8 mg l^{-1}.

Protozoa in sludge	Effluent BOD ranges			
	0–10	11–20	21–30	> 30
Trachelophyllum pusillum	3	3	3	1
Hemiophrys fusidens	3	4	3	0
Chilodonella cucullulus	4	4	1	1
Paramecium trichium	4	3	2	1
Vorticella communis	10	0	0	0
Vorticella convallaria	3	4	2	1
Vorticella fromenteli	5	4	1	0
Vorticella microstoma	2	4	2	2
Opercularia coarctata	2	2	3	2
Carchesium polypinum	3	5	2	0
Zoothamnium mucedo	10	0	0	0
Aspidisca costata	3	3	2	2
Euplotes moebiusi	3	3	3	1
Euplotes affinis	6	4	0	0
Euplotes patella	4	3	3	0
Total points	65	46	28	11

respectively. A lower rate of success has been achieved by the author, who found that the prediction success rate was improved by weighting the scores of individual species obtained from the list compiled by Curds and Cockburn according to their relative abundance in the mixed liquor. In this way, in a very good quality mixed liquor, the presence of a poor quality indicator species in low numbers is outweighed by an abundant good quality indicator. However, the method of evaluated plant performance using the mixed liquor fauna proposed by Curds and Cockburn is limited by two factors. The technique relies on identification of ciliate species, which requires some degree of specialist taxonomic expertise, also, the results will only suggest the BOD of the effluent. To some extent this has been overcome by Poole (1984), who compared nitrifying and non-nitrifying activated sludge systems, using a greater range of ciliate taxa including some non-ciliate protozoan and some metazoan groups. From this, he was able to identify a number of reliable indicators of performance such as the holotrich *Prorodon* spp., testate

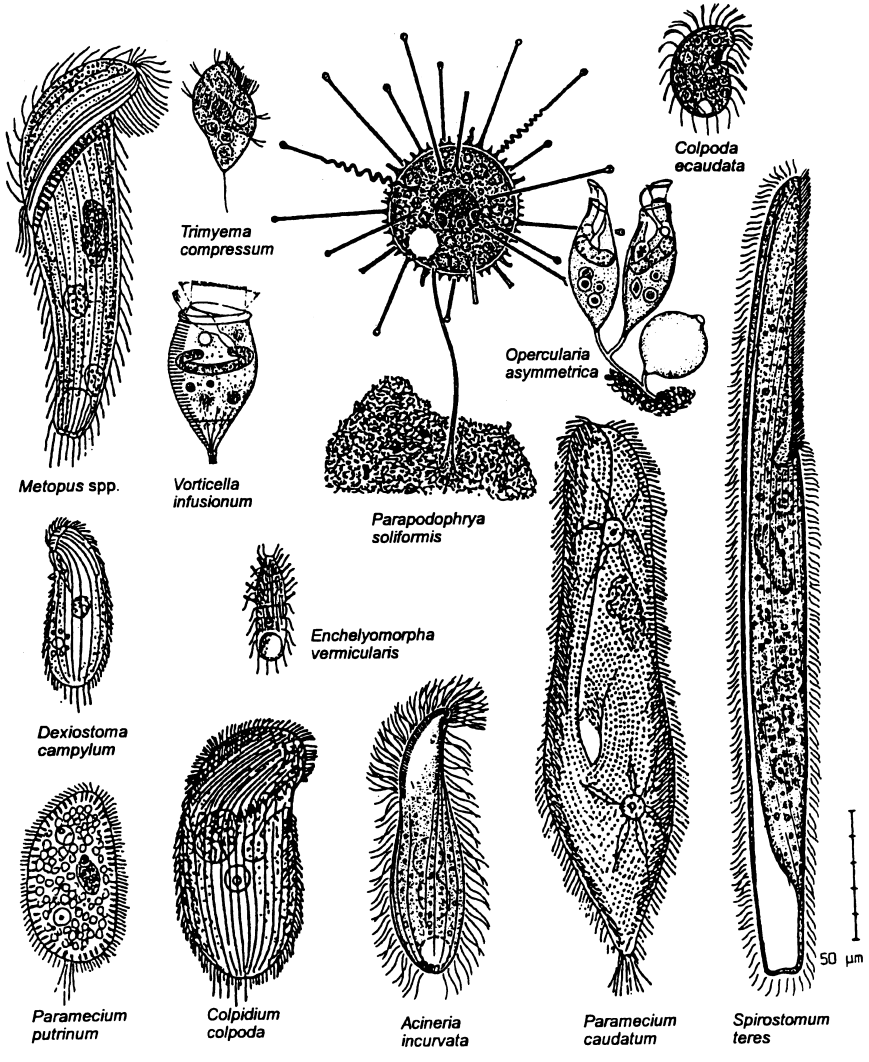


Fig. 5.92. Typical ciliate species of alpha-mesosaprobic and polysaprobic conditions indicative of overloaded (e.g. *Colpidium*, *Dexiostoma*, *Paramecium*), anaerobic (e.g. *Metopus*, *Trimyema*) or oxygen deficient (e.g. *Vorticella infusionum*, Dexiostoma) activated sludge. Scale bar division 10 μm (Foissner and Berger 1996).

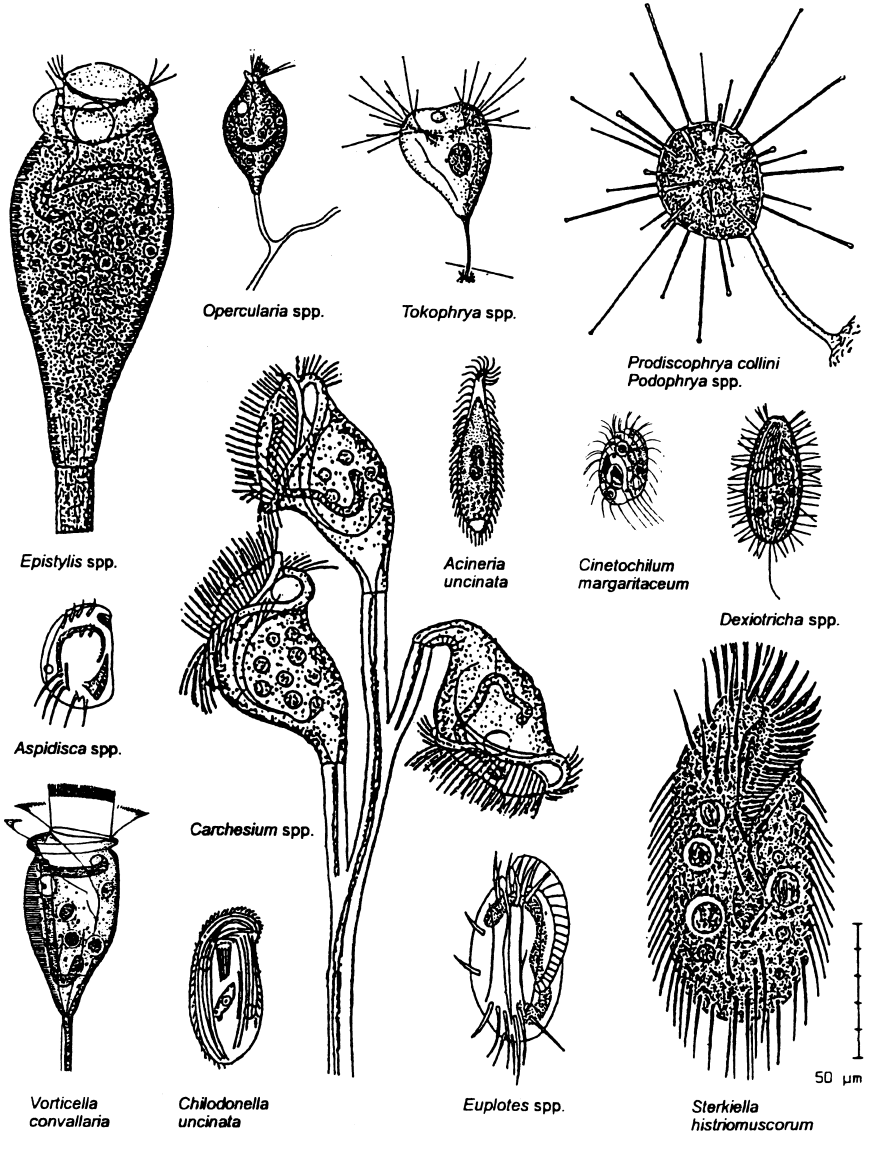


Fig. 5.93. Typical ciliate species of beta-mesosaprobic and alpha-mesosaprobic conditions indicative of normal and activated sludge. Scale bar division 10 μm (Foissner and Begger 1996).

amoebae, and the *Monogononta* rotifers all of which are associated with low final effluent $BOD_{(ATU)}$ and ammonia-nitrogen concentrations with a high MLSS concentration. Conversely, the peritrichs, *Vorticella microstoma*, *Opercularia coarctata*, and *Opercularia microdiscum* are associated with high final effluent $BOD_{(ATU)}$ and ammonia-nitrogen concentrations and a low MLSS concentration. Many other workers have used protozoa to indicate changes in the performance of activated sludge plants (Esteban *et al.* 1990; Al-Shahwani and Horan 1991) or loading (Salvado and Gracia 1993), including the development of various indices (Maldoni 1994). Foissner and Berger (1996) have produced two slides showing comparing the species associated with overloaded or oxygen deficient activated sludge plants (Fig. 5.92) and healthy plants (Fig. 5.93).

The bacterial-protozoal relationship has been modelled by several workers (Curds 1971b; Curds 1973a,b; Canali *et al.* 1973), but although these models are of considerable interest ecologically, they do not appear to have been utilised in plant design or operation. This indicates that perhaps designers still regard the activated sludge process as an essentially bacterial process and model it in those terms, rather than as a more dynamic ecosystem functioning on several different trophic levels. In terms of modelling, it is certainly easier to consider it as a single trophic level system.

5.5.4. *Other groups*

The largest grazers in the activated sludge process are the nematodes and rotifers. Little is known of their role in the activated sludge process and generally they represent only a minor part of the microbial biomass. Nematodes are not abundant in mixed liquor as there is no suitable niche for them in the aeration tank (Fig. 5.94). Also, the population doubling time is so long

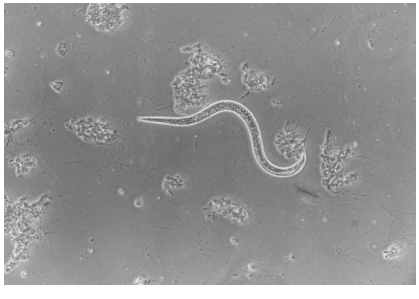


Fig. 5.94. Nematode among activated sludge flocs (×200).

that the normal HRT and sludge ages of conventional plants do not allow nematodes to develop within the process, making them of little importance (Chaudhuri *et al.* 1965; Schiemer 1975). This is supported by the study by Woombs and Laybourn-Parry (1987), who studied the seasonal variation in both species composition and species diversity. They found that nematode

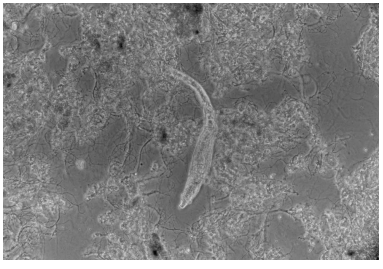


Fig. 5.95. Rotifers are common in extended aeration plants (×200).

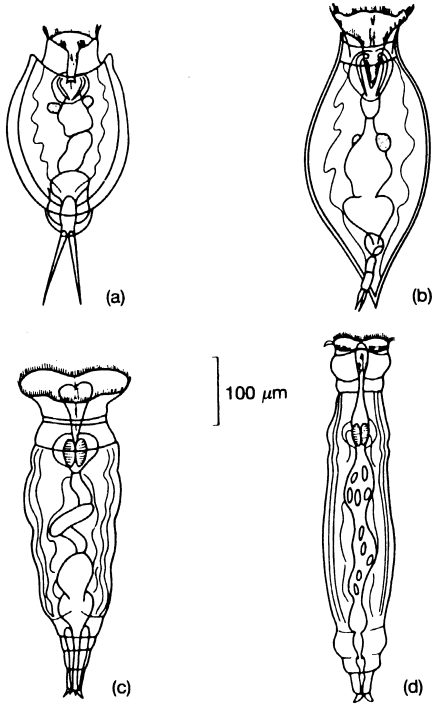


Fig. 5.96. Some frequently observed rotifers in activated sludge: (a) *Lecane* sp., (b) *Notommata* sp., (c) *Philodina* sp. and (d) *Habrotrocha* sp. (Doohan 1975).

density and species composition were both independent of all environmental, operational, and performance variables, except BOD; the density of nematodes increasing with an increase in the influent BOD concentration. They estimated that the total daily energy contribution was so low that their role in bacterial grazing and decomposition in activated sludge was negligible.

Rotifers are far more common in activated sludge than nematodes (Fig. 5.95), with a wide diversity of species recorded (Godeanu 1966) (Fig. 5.96). They appear to have a number of important roles. For example, they break up flocs into smaller particles, which encourages new floc formation as well as contributing to floc formation directly by the production of faecal pellets consisting of undigested material held together by mucus, which form the basis of new flocs. Also, they clarify the effluent by removing dispersed bacteria which are in suspension. Details of morphology and taxonomy are given in the key by Pontin (1978). They appear more efficient than protozoans in filtering suspended material, not only because of their size, but also because of their strong ciliary currents (Calaway 1968). Due to the longer development time, rotifers, unlike protozoans, are generally more abundant in extended aeration plants or other processes with long sludge ages (Doohan 1975), and in conventional plants their presence

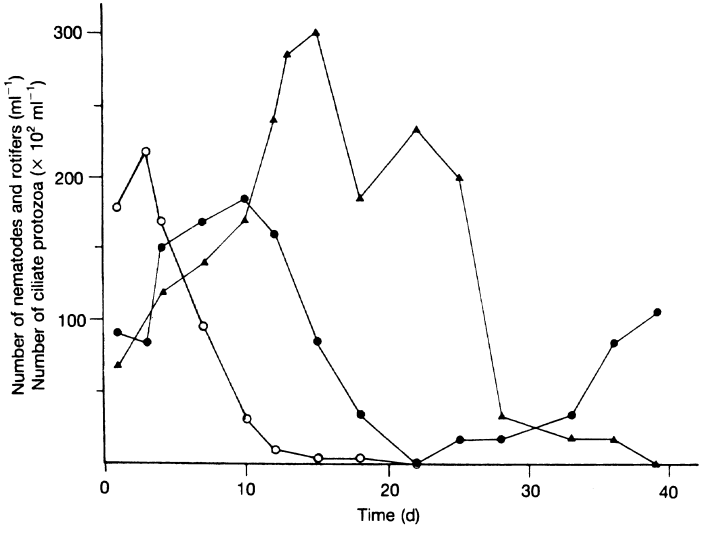


Fig. 5.97. Changes in the population densities of the ciliate protozoa (○), nematodes (●), and rotifers (ρ) in the mixed liquor from Naas Sewage Treatment Works during the fierst 40 days of the experimental period.

indicate stable operating conditions (Fig. 5.91). In batch reactors clear successions of micro- and macrofaunal groups are seen (Fig. 5.97).

Other mesofauna are rarely recorded, although Copepods and the annelid *Aelosoma* spp. are both seen occasionally. Dipteran larvae are very occasionally recorded, but they develop most probably in the slime covering the walls of the aeration tank and are found in the mixed liquor when they become dislodged from their more stable environment.

5.6. Nutrient Removal

The bacterial process of nitrification and its kinetics is dealt with in detail in Sec. 3.5.2. Nitrifying bacteria have much lower specific growth rates than heterotrophic bacteria, therefore nitrification in the activated sludge process is dependent on the sludge age of the microbial biomass (Marais 1973; Jones and Sabra 1980). Like all species of micro-organisms, the nitrifying bacteria will only be able to grow and reproduce within the aeration tank if their specific growth rate in activated sludge is greater than the specific sludge wastage rate. Using *Nitrosomonas*, as it is the rate-limiting species in the two-stage nitrification process (Sec. 3.5.2), the reaction can be summarised under steady-state conditions as

$$\frac{C_{\mathrm{N}} - C_{\mathrm{N0}}}{C_{\mathrm{N}}} > \frac{\Delta S}{S}\,,$$

where C_{N} is concentration of *Nitrosomonas* in the effluent mixed liquor, C_{N0} the concentration of *Nitrosomonas* in the inlet mixed liquor, ΔS the increase in MLSS, and S the MLSS at the inlet, so that $\Delta S/S$ is the specific sludge wastage rate. In operational terms, nitrification can only be expected below a critical sludge loading (f/m) rate of 0.15 kg kg^{-1} d^{-1} or a sludge age of above 4 d. This may pose a problem for the operator because in order to provide sufficient time for nitrification to occur, the sludge age may no longer be optimal for settlement. Settlement may also be seriously affected if denitrification occurs in the sedimentation tank causing the sludge to rise. In this case, denitrification may be necessary by the provision of an anoxic unit before the aeration tank (Sec. 3.4.5). Downing *et al.* (1964) found that as the MLSS concentration increased there was a significant decrease in the period of aeration required to achieve nitrification (Fig. 5.98), so the greater the MLSS the better the degree of nitrification. The rate of ammonia oxidation in activated sludge is approximately 0.5–3.0 mg g^{-1} h^{-1} compared to 250 mg g^{-1} h^{-1} in pure culture studies (Painter 1977). Therefore, nitrifying

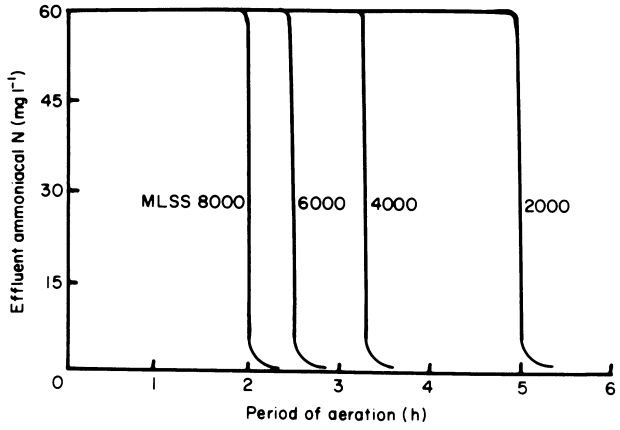


Fig. 5.98. Predicted relations between nitrification and aeration period at different MLSS concentrations (Downing *et al.* 1964).

bacteria comprise only a very small proportion of the microbial biomass; no more than a few per cent. Environmental factors are also important in nitrification once the bacteria have become established (Sec. 3.5.2).

Painter (1978) suggests that the critical dissolved oxygen concentration should not be less than 2.0 mg O_2 l^{-1} in the aeration tank in order to ensure that the oxygen concentration for nitrifiers within the sludge floc does not fall below the inhibitory 0.3 mg O_2 l^{-1} (Wild *et al.* 1971). The extra aeration costs to maintain this high critical oxygen concentration as well as satisfying the high oxygen demand of nitrification can be considerable. Linked to the larger tank capacity that is required due to the longer aeration period compared to that needed for carbonaceous oxidation only, the overall cost of nitrification is very high. The rate of growth of the nitrifying bacteria increases considerably with temperature over the range 8–30°C, with *Nitrosomonas* having a 9.5% increase in growth rate per °C rise. This can be important in terms of the aeration period, as the minimum period of aeration for nitrification can be reduced by 50% if the temperature is increased by 7°C over the range 7–25°C (DoE 1963a). The size of the aeration tank is dependent on achieving the required rate of nitrification. However, with this dependence on temperature, the aeration tank capacity may need to be doubled during the winter, assuming a 10°C difference in sewage temperature, to achieve the same degree of nitrification. In practice, this is avoided by increasing the MLSS in the tank during the winter months in order to increase the sludge age (Table 5.31). It has been demonstrated that at high temperatures (> 20°C), nitrification is inevitable, even with

Table 5.31. Minimum periods (h) of aeration of sewage (BOD 250 mg l^{-1}) to produce a fully nitrified effluent at different temperatures and MLSS (Institute of Water Pollution Control 1987).

MLSS (mg l^{-1})	7°C	12°C	17°C
2,000	11–23	8–16	5.5–11
4,000	6–13	4.5–9	3.6–5
6,000	4–9	3–6.5	2–4.5

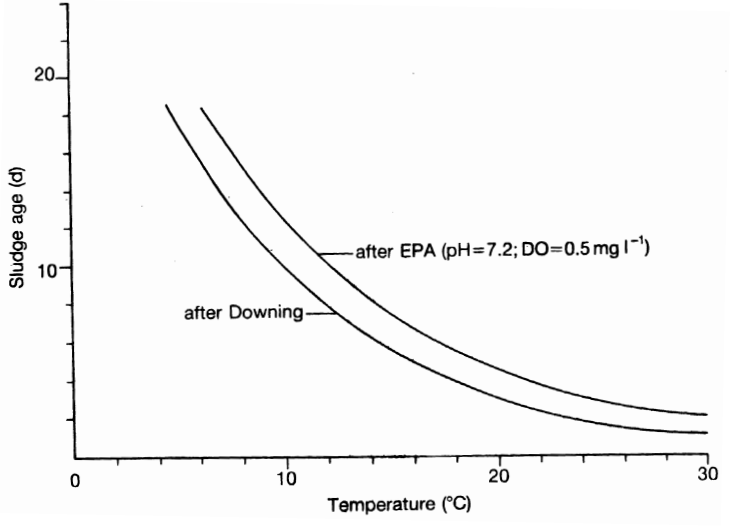


Fig. 5.99. The required sludge age for nitrification at various temperatures.

short sludge ages (Fig. 5.99). The minimum sludge age required to achieve complete nitrification can be calculated by

$$(\mathrm{SA})_{\mathrm{T}} = 3.05 \times (1.127)^{20-T}$$

where SA is the minimum sludge age at temperature T. The effect of other environmental variables, including pH (Wild *et al.* 1971; Haug and McCarty 1972) and inhibitory substances (Downing *et al.* 1964), has already been discussed in Sec. 3.5.2.

In general, therefore, nitrification favours those biological processes that are low-rate and have long MCRTs, which is associated mainly with attached growth systems, such as percolating filters and fluidised

beds. Although extended aeration systems such as the Pasveer ditch and carrousel-type systems have long MCRTs and so achieve a high degree of nitrification (Stensel *et al.* 1978; Pay and Gibson 1979), conventional and high-rate activated sludge processes usually do not nitrify their effluents. In order to produce a fully nitrified effluent, a completely mixed conventional system requires a sludge loading of less than 0.25 kg kg^{-1} d^{-1} with a sludge age above 7 d at 18°C. In open pure oxygen systems, good nitrification is possible, whereas in closed systems, nitrification is suppressed because of the accumulation of carbon dioxide in the gas phase above the mixed liquor, which reduces the liquid pH to 6, inhibiting the nitrifying bacteria. In terms of cost effectiveness, nitrification can be carried out separately by a nitrifying filter after the activated sludge stage. This allows a smaller aeration tank to be used, reduces the aeration costs, reduces the sludge age, and also prevents denitrification occurring in the sedimentation tank ensuring good sludge separation. The use of a two-stage activated sludge process using two separate aeration tanks, one for carbonaceous removal and the second for nitrification has also been examined (Sutton *et al.* 1977; Young *et al.* 1979), although the system is less reliable and robust than nitrifying filters. Due to the long sludge age required for nitrification, large reactors occupying large areas of land are required. This problem has been overcome at the Dokhaven Watewater Treatment Plant in Rotterdam which was originally designed as an A–B activated sludge process (Sec. 5.3.1). At this plant, sludge digester effluent rich in ammonia (1.0–1.5 g NH_4–N l^{-1}) contributes 15% of the total plant loading but only 1% of the hydraulic load (Dongen *et al.* 2001). This ammonia rich wastewater is treated using the combination of a partial nitrification process (SHARON®) and an anoxic ammonium oxidation (Anammox®) process. The ammonia is partially oxidised to nitrite, then the nitrite is denitrified with ammonium as electron donor (van Loosdrecht and Jetten 1998). Oxygen requirement for nitrogen removal is reduced by 60%, no COD is required, sludge production is reduced as are the net CO_2 emissions. Nitrification in the activated sludge process has been reviewed in considerable depth by Barnes and Bliss (1983).

The SHARON® process

This process is generally used to produce an ammonium-nitrite feed effluent for the Anammox® process (Sec. 5.6.1), where only 50% of the influent ammonium needs to be converted to nitrite:

$$NH_4^+ + HCO_3^- + 0.75\ O_2 \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O$$

The process is used to treat ammonia rich effluents from anaerobic sludge digestion. The stoichiometry implies that no extra addition of a base is required since sludge liquor from anaerobic digestion generally contains sufficient alkalinity in the form of bicarbonate to compensate for acid production where only 50% of the ammonia is oxidised. The process operates without any biomass retention, so the SRT equals the HRT. Effluent concentration is only dependent on the growth rate (1/SRT) of the bacteria involved, and is independent of the influent concentration (Hellinga *et al.* 1998). Fast-growing ammonium oxidisers are selected by the process when operated at temperatures $> 25°C$. As these micro-organisms have a low affinity for ammonia (affinity constant 20–40 mg NH_4–N l^{-1}), final effluents will always have a relatively high ammonia concentration (50–100 mg N l^{-1}). Thus the SHARON® process is only applicable to wastewaters with high ammonia concentrations ($>$ 500 mg N l^{-1}) and where final effluent quality is non-critical. The micro-organisms involved are diverse with *Nitrosomonas* spp. identified using a 16S rRNA targeted fluorescent oligonucleotide probe. The commonest species is very similar to *Nitrosomonas eutrophia*, which is known to be a fast growing nitrifier able to grow at high ammonia and nitrite concentrations (Logemann *et al.* 1998). When treating sludge digester effluents, the SHARON® process is operated at 30–40°C without any biomass retention. The dilution rate is set to ensure that the ammonium oxidisers grow fast enough to stay within the reactor, while the slower growing nitrite oxidisers are washed out (Mulder *et al.* 2001). At Dokhaven Wastewater Treatment Plant in Rotterdam, the SHARON® process is used to treat sludge digester liquor. Loaded at 1.2 kg N m^3 d^{-1} without pH control, ammonium is oxidised by 53% to nitrite with the ammonium oxidising bacteria tolerating high concentrations of nitrite ($>$ 0.5 g NO_2–N l^{-1} at pH 7). The ratio of ammonium to nitrite can be altered by changing the reactor pH over the range of 6.5 and 7.5 (van Dongen *et al.* 2001). Typical design data for full-scale reactors are given in Table 5.32.

5.6.1. *Denitrification*

Nitrate can be converted via nitrite to gaseous nitrogen under low dissolved oxygen conditions by the process known as denitrification. The process occurs in any nitrified effluent when deprived of oxygen. So unless a specific denitrifying reactor is constructed, denitrification is only likely to occur in the sludge layer of the sedimentation tank after an aerobic biological reactor where nitrification is occurring. The process can only proceed under

Table 5.32. Dimensions of a full scale Sharon®-Anammox® process for three different cases (van Dongen *et al.* 2001).

Reactor	**Parameter**	**Unit**	**Case 1**	**Case 2**	**Case 3**
General	N-Load	kg N/day	1,200	1,200	1,200
	NH_4-N Concentration	kg N/m^3	500	1,200	2,000
	Influent flow	m^3/day	2,400	1,000	600
Sharon-reactor	Volume	m^3	3,120	1,300	780
	Oxygen Demand	kg O_2/day	2,181	2,181	2,181
	Air Demand	Nm^3/day	56,000	56,000	56,000
Moving bed	Volume	m^3	450	450	450
Anammox-reactor	HRT	Hour	4.5	11	18
Granular Sludge	Volume	m^3	75	75	75
Anammox-reactor	HRT	Hour	0.75	1.8	3

anoxic conditions, (i.e. when the dissolved oxygen conditions are very low but not necessarily zero), and when a suitable carbon source is available. Denitrification can occur at dissolved oxygen concentrations up to, but not exceeding, 2 per cent saturation (Sec. 3.4.5). The denitrifying bacteria remove significant amounts of BOD during the reduction of nitrate. While some effluents will contain enough residual organic carbon to allow denitrification to proceed, untreated settled sewage can be used as a supplement. The preferred source of organic carbon is methanol, which is also used for potable supplies, as the input of organic carbon into the reactor can be accurately controlled.

The amount of methanol, or organic carbon equivalent, can be estimated from

$$C_{\mathrm{m}} = 2.47N_0 + 1.52N_{\mathrm{i}} + 0.87D_0\,,$$

where C_{m} is the concentration of methanol required (mg l^{-1}), N_0 the initial nitrate concentration (mg N l^{-1}) and N_{i} the initial nitrite concentration (mg N l^{-1}), while D_0 is the initial dissolved oxygen concentration (mg l^{-1}) as the reaction will take place under anoxic as well as anaerobic conditions.

The biomass produced can be estimated as

$$C_{\mathrm{b}} = 0.53N_0 + 0.32N_{\mathrm{i}} + 0.19D_0\,,$$

where C_{b} is the biomass production (mg l^{-1}).

So when N_{i} and D_0 are zero, the methanol:nitrate ratio is 2.47 and the biomass production is $0.53N_0$. In these calculations only the amount of

nitrogen present is used, so for a sample containing 15 mg l^{-1} of nitrate ions at 0 mg l^{-1} nitrite ions and dissolved oxygen, then the amount of methanol required will be $(2.47 \times 15 \times 14/62) = 8.4$ mg l^{-1} with $(0.53 \times 15 \times 14/62) = 1.8$ mg l^{-1} of biomass produced. If however the sample contained 15 mg l^{-1} of nitrate-nitrogen, then 37 mg l^{-1} of methanol would be required producing 8 mg l^{-1} of biomass. It is important to provide the correct dose of methanol to the reactor as if it is under estimated then denitrification will be incomplete, and if over estimated methanol will remain in the final effluent and cause further pollution.

The overall effect of the denitrification reaction is to raise the pH by the formation of hydroxide ions. This replaces about 50% of the alkalinity consumed by the oxidation of ammonia during nitrification. For each mg of ammonia oxidised to nitrate, 7 mg of alkalinity are utilised, and 3 mg are produced during denitrification. The denitrification reaction is sensitive to pH with an optimum range between pH 6.5 and 7.5, but falling to 70% efficiency at pH 6 or 8. Temperature is also important with the reaction occurring between 0–50°C, but with optimum reaction rate between 35–50°C. Denitrification and nitrification process kinetics are explained in detail in IAWQ (1995).

The denitrifying filter

The major denitrification process is the denitrifying filter which is a submerged filter containing an inert medium on which a film of denitrifying bacteria develop. This contact system provides the necessary MCRT for the development of these slow-growing bacteria and depending on the voidage of the medium, the SRT can be very short, often less than 1 h. The most frequent operational problem is excessive film accumulation or entrapped gas bubbles which alter the internal flow pattern restricting the flow causing a significant head loss or reducing the HRT by channelling the effluent through the filter. Nitrate removal efficiency increases with the accumulation of biological solids, however removal efficiency sharply falls off as the flow is restricted and the pressure builds up until finally the liquid forces it way through the bed scouring much of the film away. Therefore it is better to opt for a larger medium (15–25 mm diameter) with larger voids and a slightly reduced surface area so that film accumulation is less of a problem. In this way the filter will operate more consistently. Better film control has been achieved using fluidised bed systems, although this has been designed primarily for potable water treatment (Croll *et al.* 1985).

The Anammox® process

The Anammox® process occurs under anoxic conditions when nitrite is converted to nitrogen gas by autotrophic bacteria using ammonium as electron donor

$$NH_4^+ + NO_2 \rightarrow N_2 + 2H_2O\,.$$

As this is an autotrophic reaction no external carbon source (e.g. methanol) is required (Jetten *et al.* 1998), with activity inhibited at oxygen concentrations above 0.5% air saturation (Strous *et al.* 1997). The process in normally used in conjunction with the SHARON® process which produces an ammonium–nitrite feed effluent ideal for the Anammox® process. The growth rate (doubling time 11 d) and growth yield (0.11 g VSS g^{-1} NH_4–N) for these micro-organisms are very low, resulting in a low sludge production. Therefore efficient biomass retention is required to maintain suitable Anammox biomass concentrations within reactors, thus favouring SBR systems. Anammox reactors are very compact due to a high maximum specific nitrogen consumption rate (0.82 g N g^{-1} VSS d^{-1}), a high affinity for ammonium and nitrite, and the granular growth allowing efficient biomass retention. Anaerobic/anoxic ammonium oxidation may be widespread in nature, and the phenomenon has been widely reported in conditions of very high nitrogen loads and a limited air supply (Strous *et al.* 1999b) The exact identities of the micro-organisms responsible for the Anammox® process are still being investigated. Strous *et al.* (1999a) found 70% of their Anammox micro-organisms to be of one morphological type. It had four properties in common to the order Planctomycetales, i.e. cell division by budding, internal cell compartmentalisation, the presence of crateriform structures in the cell wall, and the presence of unusual lipids in the membranes. Based on 16S rRNA analysis the organisms responsible for the Anammox® process have been provisionally named *Brocadia anammoxidans*. The Anammox® process has been used successfully at the Dokhaven Wastewater Treatment Plant in Rotterdam, where it is operated as a granular sludge SBR process. Ammonium conversion rates to nitrogen gas exceed 80% at loading rates of 1.2 kg N m^{-3} d^{-1} (van Dongen *et al.* 2001). Typical design data for three different loading rates is given in Table 5.32.

Activated sludge systems

In activated sludge systems denitrification occurs in anoxic zones or reactors with a simultaneous loss in BOD. For each 1 g of nitrate N utilised 2.9 g of

BOD is also removed (Muyima *et al.* 1997). In plug flow systems both BOD oxidation and nitrification occur in the same aerobic zone. A high degree of nitrification is achieved with only approximately 10% of the original influent BOD remaining. This will limit the degree of denitrification in the subsequent anoxic zone due to a lack of available organic carbon to just 20% of the available nitrate. In completely mixed systems this is overcome by constructing a separate anoxic zone in front of the aerobic zone (Figs. 5.100 and 5.101) with the degree of denitrification dependent on the quantity of effluent and sludge returned to the anoxic zone. Denitrification efficiency (De) can be estimated by

$$\mathrm{De} = \frac{Q_\mathrm{r} + Q_\mathrm{re}}{Q + Q_\mathrm{r} + Q_\mathrm{re}},$$

where Q is the volume of influent waste water ($\mathrm{m^3\ h^{-1}}$), Q_r the return sludge rate ($\mathrm{m^3\ h^{-1}}$) and Q_re the recirculated effluent rate ($\mathrm{m^3\ h^{-1}}$). Large quantities of treated effluent must be recirculated in order to achieve high denitrification efficiencies (Table 5.32). This is very expensive due to extra reactor volume and pumping costs. However, in oxidation ditches, simultaneous nitrification and denitrification occurs to a high degree achieved by natural internal recirculation (Fig. 5.102). The internal recirculation factor (R) can be calculated using the equation

$$R = \frac{Q_\mathrm{r} + (V/t_\mathrm{r})}{Q},$$

where V is the volume of the oxidation ditch ($\mathrm{m^3}$) and t_r the recirculation

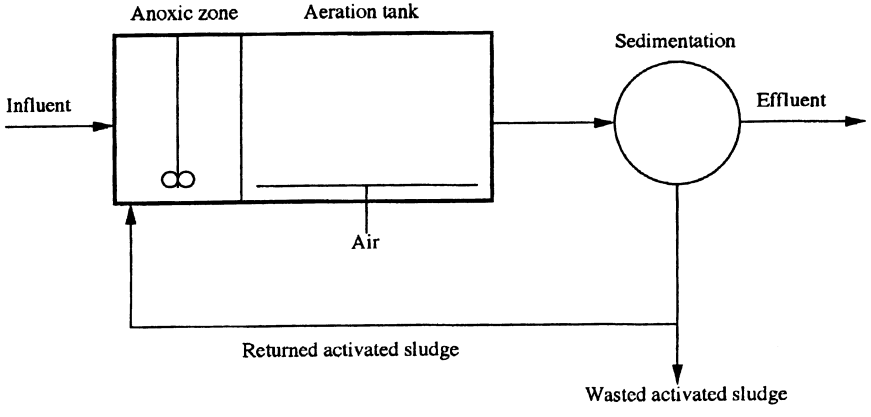


Fig. 5.100. Schematic layout of a conventional pre-nitrification system.

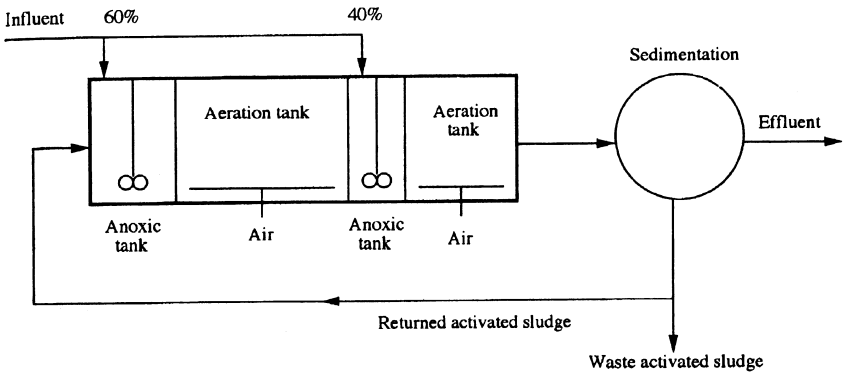


Fig. 5.101. Schematic layout of a two-stage nitrification-denitrification systems.

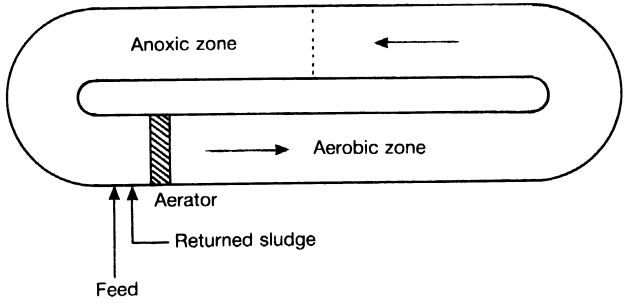


Fig. 5.102. Aerobic and anoxic zone for dual nitrification-denitrification within single oxidation ditches.

time (h). In most oxidation ditches R is larger than 20 which, according to Table 5.33, is equivalent to above 95% denitrification. A detailed case study is presented by Cooper *et al.* (1977a,b) while the design of anoxic zones is reviewed by Barnard (1973).

The benefits of denitrifcation are primarily that:

(i) it reduces the concentration of oxidised nitrogen in the water, not only as nitrate but also nitrite which may be present in high concentrations in certain industrial wastewaters if *Nitrobacter* is inhibited;
(ii) it utilises some of the residual organic matter during nitrification; and
(iii) it releases oxygen into solution which can be used by heterotrophs thereby offsetting the extra aeration cost for nitrification.

Secondary advantages of using an anoxic zone at the inlet of a nitrifying activated sludge plant include:

Table 5.33. Typical relationship between denitrification and the rate of recirculation.

Recirculation factor (R)	Denitrification (%)
1	50
2	67
3	75
5	83
10	91
20	95
30	97

(iv) reduces in the problem of denitrification in secondary sedimentation tanks thereby preventing high suspended solids concentrations in the final effluent;
(v) improves the settleability of the mixed liquor;
(vi) reduces the incidence of bulking; and
(vii) it increases the pH ensuring that nitrification proceeds without pH adjustment.

5.6.2. *Phosphorus removal*

Phosphorus is present in wastewater as orthophosphate (PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$ and H_3PO_4), polyphosphates and organic phosphate. The average phosphorus concentration in sewage is between 5–20 mg P l^{-1} as total phosphorus of which 1–5 mg P l^{-1} is organic, the rest inorganic. Normal secondary treatment can only remove 1–2 mg P l^{-1} and so there is a large excess of phosphorus that is discharged in the final effluent that gives rise to eutrophication in surface waters (Sec. 1.2.2). New legislation requires effluents discharged into sensitive waters to incorporate phosphate removal to bring final effluent phosphorus concentrations to below 2 mg P l^{-1} (Sec. 1.1.2). Traditionally phosphorus is removed by the addition of coagulants to wastewater, although recent advances in the understanding of how phosphorus is taken up by micro-organisms has led to the development of biological removal processes.

Chemical precipitation is used to remove the inorganic forms of phosphate by the addition of a coagulant (lime, aluminium salts or iron salts).

The selection of a coagulant depends on a number of factors:

(i) influent P concentration;

(ii) influent suspended solids concentration and alkalinity;
(iii) cost of the chemical used at treatment plant;
(iv) availability and reliability of chemical supply;
(v) sludge handling facilities at plant or cost of new system;
(vi) disposal method of sludge and cost. For example if sludge is disposed to agricultural land then iron or aluminium sludges are less favoured than those treated with lime;
(vii) compatibility with other unit processes used at the plant; and
(viii) the potential environmental impact of using coagulants.

Lime addition removes calcium ions as well as phosphorus from waste water, along with any suspended solids. The lime $Ca(HO)_2$ reacts first with the natural alkalinity in the waste water to produce calcium carbonate which is primarily responsible for enhancing suspended solids removal.

$$Ca(HCO_3)_2 + Ca(OH)_2 \longrightarrow 2CaCO_3 + 2H_2O$$

After the alkalinity is removed calcium ions combine with the orthophosphate present under alkaline conditions (pH 10.5) to form insoluble and gelatinous calcium hydroxyapatite ($Ca_5(OH)(PO_4)_3$),

$$5Ca^{2+} + 4OH^- + 3HPO_4^{2-} \longrightarrow Ca_5(OH)(PO_4)_3 + 3H_2O\,.$$

The lime dose required can be approximated at 1.5 times the alkalinity as $CaCO_3$. Neutralisation may be required to reduce the pH before the aeration tank to protect the micro-organisms. This is done by injecting carbon dioxide to produce calcium carbonate although the carbon dioxide produced by the micro-organisms themselves is often sufficient to maintain a neutral pH in the aeration tank. A disadvantage is that large quantities of lime sludge are produced possibly doubling the normal volume of sludge requiring disposal.

Alum or hydrated aluminium sulphate ($Al_2(SO_4)_3 \cdot 14.3H_2O$) is widely used precipitating phosphates as aluminium phosphate ($AlPO_4$).

$$Al_2(SO_4)_3 \cdot 14.3H_2O + 2PO_4^{3-} \longrightarrow 2AlPO_4 + 3SO_4^{2-} + 14.3\ H_2O$$

This reaction causes a reduction in the pH and the release of sulphate ions into the wastewater. Although the natural alkalinity accounts for some of the coagulant, the dosage rate of alum is a function of the degree of phosphorus removal required. The efficiency of coagulation falls as the concentration of phosphorus decreases. So for phosphorus reductions of 75, 85 and 95% the aluminium to phosphorus ratio will have to be increased to approximately 13:1, 16:1 and 22:1 respectively. In practice a 80–90%

removal rate is achieved at coagulant dosage rates of between 50–200 mg l^{-1} (Fig. 5.103). Sodium aluminate ($NaAlO_2$) can be used instead of alum but it causes an increase in the pH and the release of sodium ions. Aluminium coagulants can adversely affect the microbial population in activated sludge, especially protozoa and rotifers, at dosage rates above 150 mg l^{-1}. However, in practice such high dosage rates have little effect on either BOD or suspended solids removal as the clarification function of the protozoa and rotifers is largely compensated by the enhanced removal of suspended solids by chemical precipitation.

Ferric chloride $FeCl_3$ or sulphate ($Fe_2(SO_4)_3$) and ferrous sulphate $FeSO_4{\cdot}7H_2O$, also known as copperas, are all widely used for phosphorus removal, although the actual reactions are not fully understood. For example ferric ions combine to form ferric phosphate. Ferric chloride reacts slowly with the natural alkalinity and so a coagulant aid such as lime is normally added to raise the pH in order to increase the hydroxyl ion concentration as well as enhance coagulation overall. The ferric ion reacts with both the natural alkalinity and the lime to precipitate out as ferric hydroxide. The overall reaction is

$$FeCl_3 + PO_4^{3-} \longrightarrow FePO_4 + 3Cl^- .$$

The main design modifications to the activated sludge process that permit the addition of coagulants for the removal of phosphorus are shown in

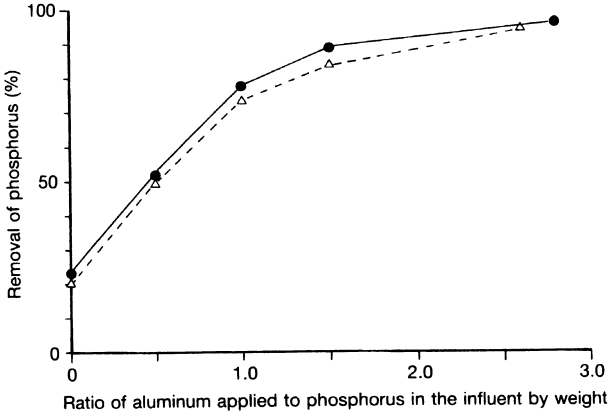


Fig. 5.103. Removal efficiency using different loadings of alum in a bench scale activated sludge plant. The substrates used were • settled municipal wastewater and △ synthetic wastewater comprising glucose and glumatic acid, both with 10 mg l^{-1} of phosphorus added (Anderson and Hammer 1973).

Fig. 5.104. In terms of efficiency, post coagulation is by far the most effective phosphorus removal system. The coagulant is added after biological treatment, so that nearly all the phosphorus present has been hydrolysed to orthophosphate and so is potentially removable, but before final sedimentation which removes the chemical precipitate. A portion of the chemical floc is returned to the aeration tank along with the returned sludge. This results in the mixed liquor having a greater inorganic content (i.e. lower MLVSS), so the operational MLSS must be increased to maintain adequate BOD removal. The problem of returning chemical sludge back to the aeration tank can be overcome by post precipitation. The coagulant is added after final sedimentation but it requires an extra mixing tank and sedimentation tank, making it essentially an advanced treatment process. Post precipitation will also remove fine suspended solids thereby increasing clarity of the effluent and reducing the final BOD.

Conventional activated sludge mixed liquor contains 1.0–2.5% of phosphorus as dry weight. This can be increased to 5% or more in biological phosphorus removal processes due to enhanced biological (Luxury) uptake by bacteria (Liss and Langen 1962). Luxury uptake of phosphorus is achieved by subjecting the mixed liquor to a cycle of anaerobic and aerobic conditions (Carberyy and Tenny 1973). In an anaerobic environment, the bacteria of the mixed liquor are conditioned or stressed so that once back in an aerobic environment with the stress removed, the bacteria will rapidly take up phosphorus as long as an adequate supply of BOD is available. In the anaerobic environment volatile fatty acids (VFAs), which are products of fermentation, are rapidly absorbed by the mixed liquor, accumulating as storage products within bacterial cells while at the same time phosphorus is released from the cell into solution. Once in an aerobic environment, the bacteria oxidise the absorbed VFAs, causing a simultaneous uptake of phosphorus from solution (Jones *et al.* 1987). The phosphorus is stored within the cell as polyphosphate granules. Phosphorus enters bacterial cells either by diffusion or biochemically via an active transport mechanism such as ATP. The latter is more important in activated sludge as the concentration of phosphorus is normally higher in the cell than in the waste water. Commonly anaerobic zones do not generate sufficient volatile fatty acids, so in line fermentors may be employed. The genus *Acinetobacter* is especially successful at absorbing phosphorus, being able to accumulate polyphosphate up to 25% of their cell mass (Buchan 1983; Lotter 1985; Muyima *et al.* 1997). However, the use of fluorescent *in situ* hybridisation (FISH) probing using rRNA directed oligonucleotide has shown that other bacteria, especially the beta proteobacteria and high mol% G+C Gram

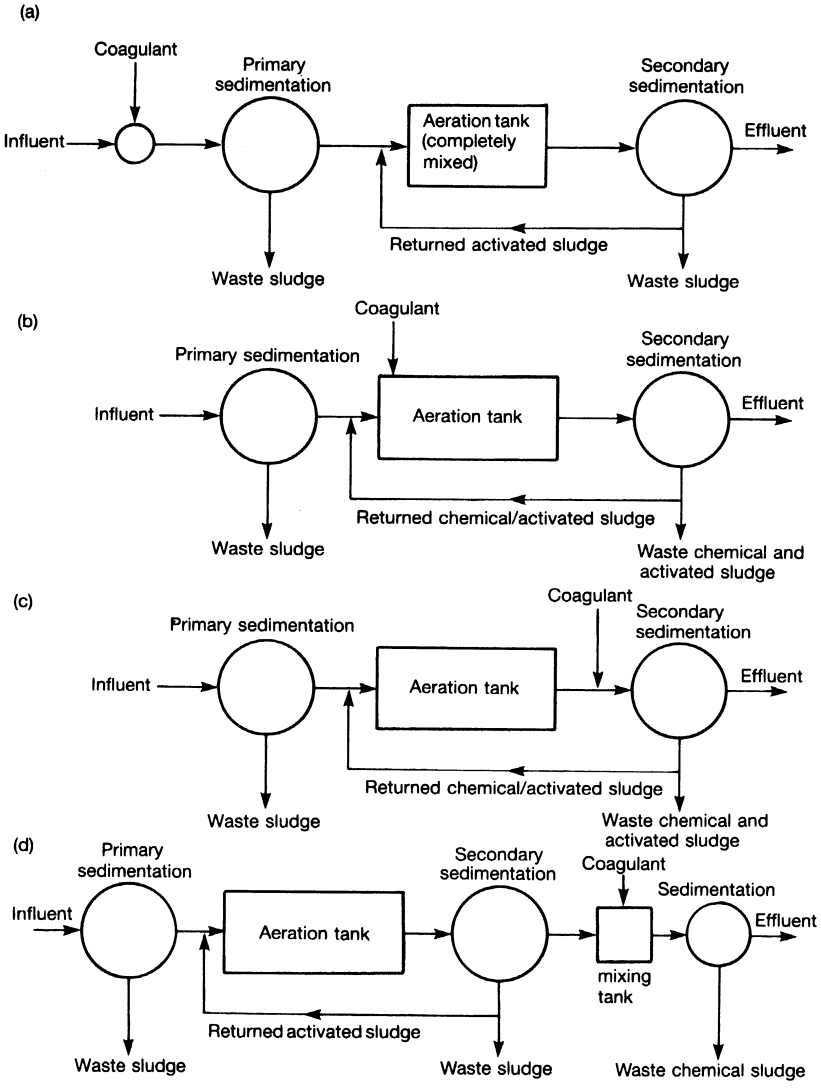


Fig. 5.104. Phosphorus removal using coagulants. (a) primary coagulation, (b) simultaneous coagulation, (c) post coagulation, and (d) post precipitation.

positive bacteria are abundant in enhanced biological phosphorus removal (EBPR) sludges (Bond *et al.* 1999). It is now thought that these other micro-organisms play a more important role in phosphorus uptake than *Acinetobacter*. The accumulated phosphorus is removed from the system with the waste activated sludge stream. To be effective the final effluent

suspended solids concentration must be kept as low as possible as suspended solids have a high phosphorus content. Also if the excess sludge is not kept aerobic before wastage is complete then phosphorus will be released back into the process water.

The main biological phosphorus removal system is the A–O process which stands for anaerobic-oxic (Fig. 5.105). In the anaerobic tank the bacteria are conditioned and any phosphorus present becomes soluble. This is then absorbed by the bacterial biomass in the aeration tank. Biological phosphate removal is largely independent of temperature over the range of 5–20°C. McGrath *et al.* (2001) have identified acid stimulated luxury uptake in activated sludge, with uptake optimised between pH 5.0 to 6.5. Phosphorus uptake increased between 50 and 143% at pH 5.5 compared to 7.5. The system depends on a BOD:P ratio > 20 in order to achieve final effluent concentrations of less than 1 mg P L^{-1}, and if the ratio falls below this critical limit then chemical coagulation will be required to remove the residual phosphorus from the final effluent. The anaerobic reactor should have an HRT of 1–2 h, although this can be reduced if the influent waste water is either septic or has a high BOD:P ratio. Whereas if the BOD is not readily degradable then an extended retention time will be required in the anaerobic tank. Nitrate inhibits the fermentation processes producing VFAs in the anaerobic zone and must be either removed by denitrification before any recycling takes place or nitrification should not be allowed to occur. The aeration tank is of standard design, although if a long sludge age is needed to permit nitrification a separate denitrification zone will be required.

The Phostrip® process combines biological and chemical removal. Phosphorus is accumulated biologically by luxury uptake then released back into solution in the anaerobic (stripper) tank (Fig. 5.106). The concentrated

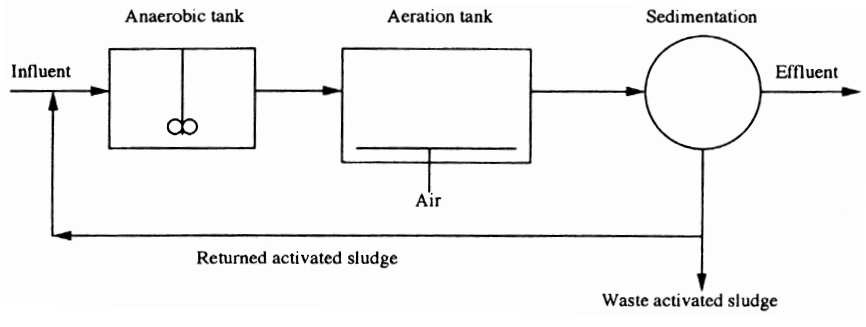


Fig. 5.105. The two-stage (A–O) biological phosphorus removal process.

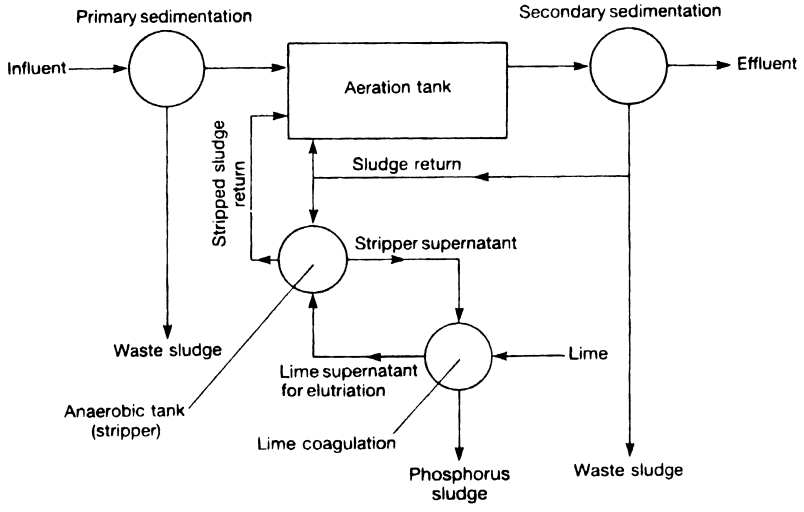


Fig. 5.106. The phostrip process.

solution of phosphorus produced is treated by lime coagulation. The anaerobic tank with an HRT of 10 h also conditions the mixed liquor so that it is able to take up the phosphorus in the aeration tank as well as carry out carbonaceous oxidation. The process is continuous with only 10–15% of the total wastewater flow passing through the anaerobic tank, so that only a small proportion of the flow is actually treated with the coagulant, resulting in a considerable saving. The higher phosphorus concentration of the liquid from the stripper makes chemical precipitation much more cost-effective. There is no nitrogen removal in this process. However if nitrification does occur in the aeration tank due to temperature or sludge age then a separate anoxic zone is required for denitrification prior to the anaerobic tank.

Biological nutrient removal systems

Biological nutrient removal (BNR) is widely used in Australia, South Africa and the US, and in response to the stricter discharge limits for nutrients in the EC Urban Waste Water Treatment Directive such systems are becoming increasingly common in Europe. There are many different designs of BNR systems, and some of the commonest are described below. Recent developments and problems in BNR technology are reviewed by Ekama and Wentzel (1999).

The Bardenpho® process incorporates nitrification, denitrification, phosphorus removal and carbonaceous oxidation by providing a series of

aerobic, anoxic and anaerobic zones (Fig. 5.107). Influent wastewater enters an anaerobic zone where the mixed liquor is conditioned for phosphorus uptake. It then flows in to an anoxic zone where denitrification occurs and then enters the aeration tank where carbonaceous oxidation, nitrification and luxury uptake of phosphorus occurs simultaneously. Mixed liquor is constantly recycled back to the previous anoxic zone resulting in up to 80% denitrification before leaving the aeration tank to the final anoxic zone to

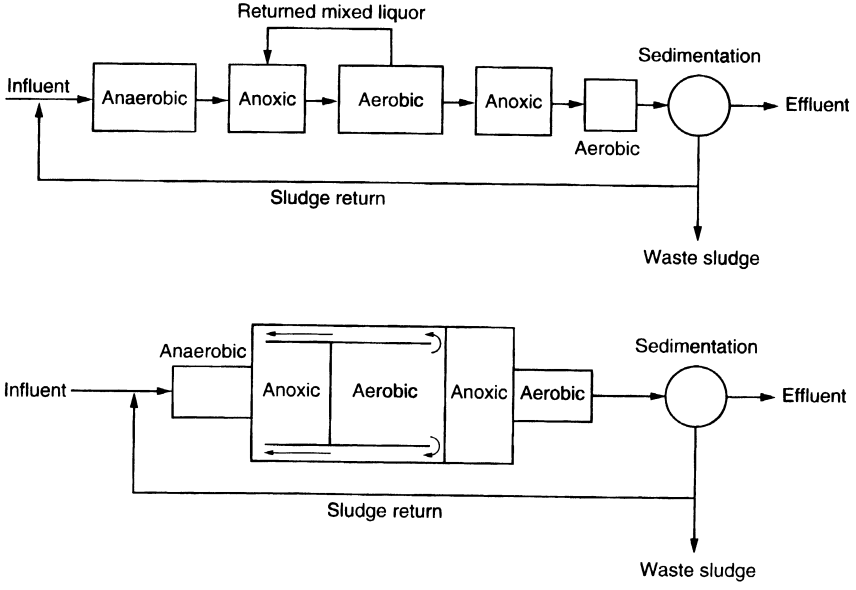


Fig. 5.107. Typical schematic designs of the Bardenpho process.

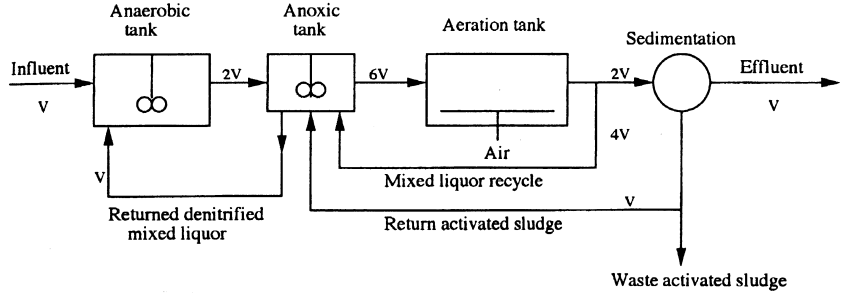


Fig. 5.108. Schematic layout of the University of Cape Town-Virginia Initiative (UCT-VIP) process.

complete denitrification. The final small aerobic zone is to prevent anaerobic conditions developing in the final sedimentation tank and to prevent loss of absorbed phosphorus from the mixed liquor. With a typical SRT of 18 d, an HRT of 22 h, and an anaerobic zone HRT of 1.5 h, the phosphorus concentration in the effluent is below 1 mg l^{-1}. The sludge can accumulate between 6–8% phosphorus dry weight, although the sludge must be rapidly dewatered before it becomes anaerobic and phosphorus is resolubilised. There are a large number of different BNR systems based on the Bardenpho concept (Figs. 5.108–5.109) mostly of the AAO design, which is an acronym for anaerobic, anoxic and oxic (Fig. 5.110).

Sequencing batch reactors have been described in Sec. 5.3.4. When used for nutrient removal all the processes (aerobic, anaerobic and anoxic) occur within a single reactor. The process cycle outlined in Fig. 5.111 is changed to: I. Waste water addition, II. Anaerobic mixing, III. Aeration, IV. Anoxic mixing, V. Clarification, VI. Effluent removal, and VII. Sludge wastage (Fig. 5.112). The batch nature of the process provides an almost ideal plug

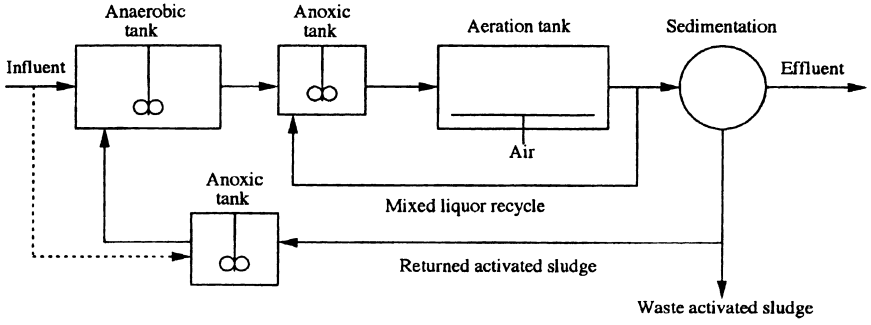


Fig. 5.109. Schematic layout of the AAO Johnannersburg (JHB) process.

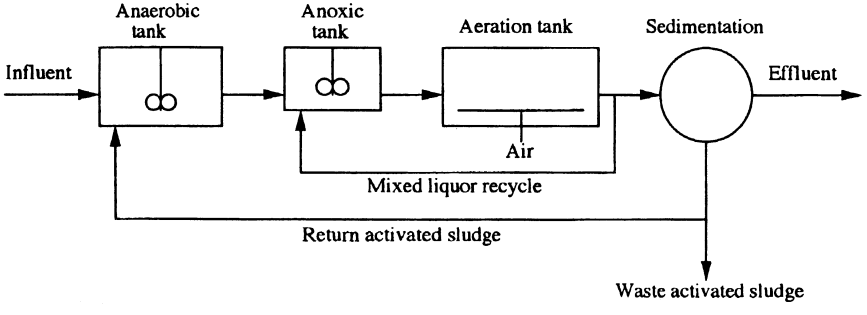


Fig. 5.110. Schematic layout of the AAO activated sludge process.

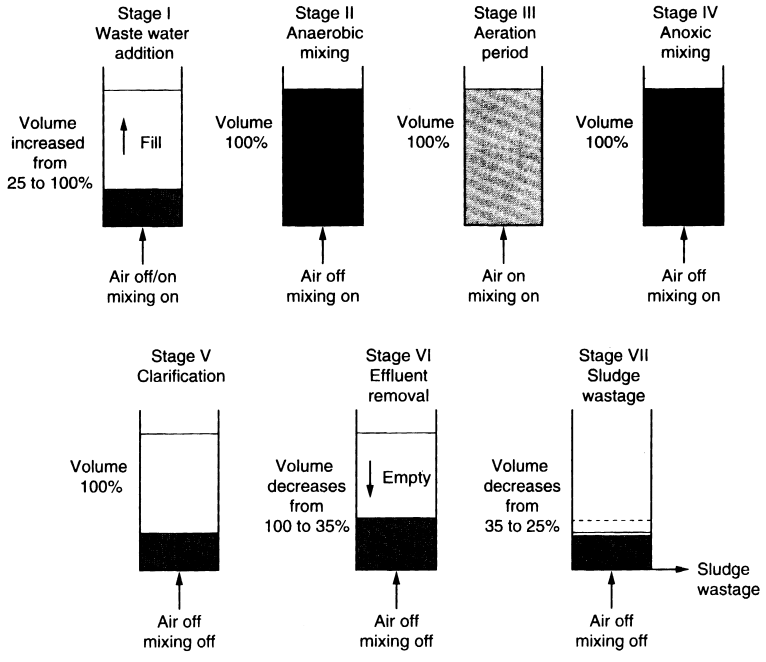


Fig. 5.111. Sequencing batch reactor system for nutrient removal.

flow configuration that prevents the development of filamentous bacteria, but such systems are not good at dealing with storm water flows. This design is particularly effective and is becoming increasingly popular. For example the new plant serving North Brisbane in Queensland, Australia treats a population equivalent of 40,000 and is achieving a final effluent of 10 mg l^{-1} BOD, 10 mg l^{-1} suspended solids, 5 mg l^{-1} total nitrogen and 1 mg l^{-1} total phosphorus (Hayword, 1998). Tasli *et al.* (2001) have shown that it is possible to retrofit existing SBR systems for nutrient removal. They demonstrated that SBRs used for small tourist areas posses the ability to provide above 60% N and 90% P removal by appropriate adjustment of the cyclic operation. Nocardioforms do not compete well with other micro-organisms such as *Acinetobacter* under anaerobic/aerobic cyclic conditions in SBRs. Therefore with careful operation foaming problems can be eliminated (Kim and Pagilla 2000).

Models

Activated sludge reactors are complex physical-chemical-biological systems with numerous internal interactions between process variables and dynamic

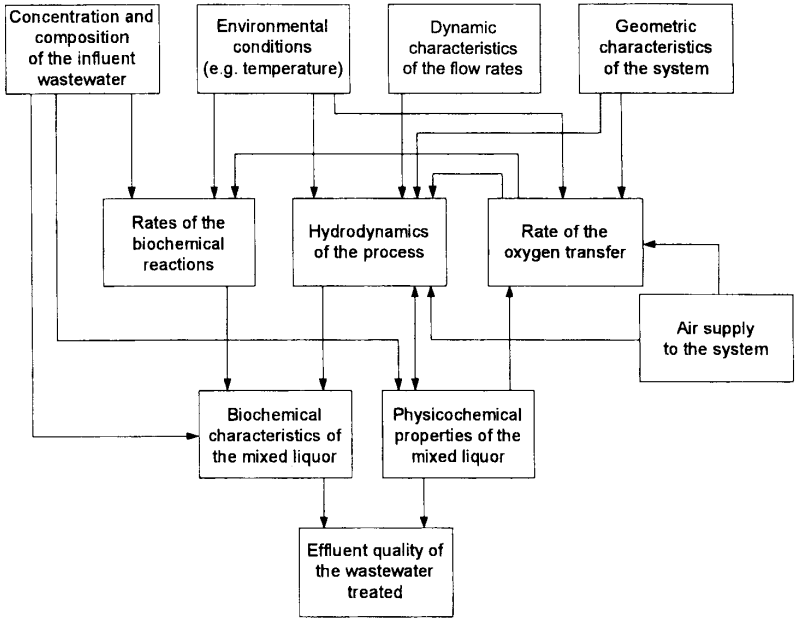


Fig. 5.112. Internal interactions in the activated sludge process (Makina and Wells 2000).

changes in influent wastewater characteristics (i.e. flow rate, composition, and concentration) (Makinia and Wells 2000) (Fig. 5.113). Over the years the complexity of the process has increased to include carbon oxidation, nitrification, denitrification, and phosphorus removal. There have been many attempts to model the activated sludge process. Such models have proved extremely useful as they not only require the modeller to ask critical questions about the system, but they also allow general design guidelines to be developed and refined (Water Research Commission 1984). In 1986 the International Association of Water Pollution Research and Control (now the International Water Association) produced the first in a series of activated sludge models (Henze *et al.* 1987). Activated sludge model No. 1 (ASM1) was to become a major landmark in wastewater modelling, producing a user friendly, logical model. This has been followed by revised models ASM2 (Henze *et al.* 1995), ASM2D (Henze *et al.* 1999), and now the latest version ASM3 (Gujer *et al.* 1999). The kinetics and stoichiometry used to describe the processes have been chosen as simply as possible. For example, Monod kinetics were used for those components that can influence reaction rates. However, with increasing computing power more realistic descriptions of

processes have been included in the later models (Gujer *et al.* 1999). The application of these models have been examined by Henze *et al.* (2002) and Dudley *et al.* (2002).

This is a complex and rapidly changing area with refinements and new developments being published monthly (Henze 2002).

Further reading

General: Hawkes 1983a; Bode and Seyfried 1984; Cech *et al.* 1985; Henze *et al.* 1987; Chambers and Jones 1988; Muyima *et al.* 1997.

Extended aeration: Barnes *et al.* 1983; Matsui and Kimata 1986.

Advanced systems: Hemming *et al.* 1977; McWhirter 1978a; Cox *et al.* 1980; Hayward 1998.

Sludge problems: Pipes 1979; Eikelboom and van Buijsen 1981; Chambers and Tomlinson 1982b; Jenkins *et al.* 1993; Wanner 1994.

Ecology: Curds and Hawkes 1975; Hawkes 1983a,b; Richard 1989.

Bacteria: Pike 1975. Fungi: Tomlinson and Williams 1975.

Protozoa: Curds 1975; Madoni and Ghetti 1981.

Nitrification: Poduska and Andrews 1975; Barnes and Bliss 1983.

Nutrient removal: Yeoman *et al.* 1988; Mino 1998; Keller 1999; Strickland 1999.

6

Natural Treatment Systems

'Natural treatment systems' include the application of wastewater on to land; the controlled mass culture of higher plants and animals; the use of natural and artificial wetlands; and anaerobic, high-rate and facultative stabilisation ponds (Reed *et al.* 1988). Natural treatment systems differ from conventional wastewater treatment systems in terms of sustainability. They use natural renewable energy, rely on atmospheric diffusion and/or photosynthesis as the major source of oxygen, and are constructed using the minimum of man-made materials. They provide silent, normally odour free, robust treatment processes. They do, however, require much larger areas of land than conventional systems (Table 6.1). Such natural systems play an important role in the treatment of wastewaters, especially in warmer countries. For example, fish ponds have been used for many centuries throughout Asia, with the organic wastewater promoting algal growth which has resulted in increased yields of fish, feeding either directly on the algal biomass (phytoplankton) or on intermediate grazers (zooplankton). In recent years, the search for low-cost treatment systems yielding effluents low in nitrogen and phosphorus has renewed interest, especially in the southern USA, in the use of plants for treatment. Apart from adequately treating the wastewater, the use of plants results in the production of excess biomass, which can be used for a variety of purposes such as energy production, animal feed, and even protein production; thus offsetting the cost of treatment (Chap. 10). The use of wetlands, bogs (peatland) and other types of land for treatment is also currently being revived.

Table 6.1. Comparison of natural treatment systems with a conventional wastewater treatment system (e.g. activated sludge).

	Natural treatment system	Conventional treatment system
Energy	Renewable, natural (solar, kinetic wind energy)	Non-renewable, fossil fuel derived electricity
Action of treatment	Micro-organisms, plants, soil	Micro-organisms
Prime construction materials	Soil bunds, liner possibly required	Concrete, steel, plastic
Aeration	Atmospheric diffusion and/or photosynthesis	Power intense mechanical aeration
Layout	Dispersed	Compact
Chemical usage	None/low	Moderate/high
Land area required	High	Low
Noise	None/very low	High
Odour	Low	Moderate/high
Aerosol formation	None	High
Costs: Capital	Moderate	High
Operational	Low	High
Controllability	Low/moderate	High
Sludge production	Low, on-site disposal	High, off-site disposal
Sustainability	High	Low
Environmental impact	Low	Moderate
Typical use	Rural, small populations	Urban, large populations

6.1. Land Treatment

Land treatment of wastewater dates back to the sixteenth century and became widely used in Europe during the nineteenth century. At the time of the British Rivers Pollution Prevention Act of 1876, new methods of sewage treatment were being investigated. At this time, land treatment remained the only alternative method of disposal of sewage apart from directly discharging it to a watercourse. However, land treatment itself had been improved. Instead of simply flooding land, the soil was ploughed deeply and the sewage allowed to flow into furrows. The waste matter decayed and made the soil very fertile, allowing vegetables of excellent quality to be grown on top of the furrows, hence the term 'sewage farms'. Sewage farming became a lucrative business, with the rights to a town's sewage often costing farmers considerable amounts of money. However, because of the amount of land required, the occurrence of unpleasant smells, and the problem of flies in warmer months, sewage farms were superseded by other biological treatment methods. Various improvements in land treatment were introduced in an attempt to reduce the area of land required. The most successful was the 'Latham soil-filter system', which comprised three layers of soil separated by layers of coke breeze overlying land drains. This method reduced the area of land required by up to a factor of 10 compared with traditional sewage farm methods. However, even with these new developments the loading rate to land was restricted to a maximum of 0.05 $m^3m^{-2}d^{-1}$, which is roughly equivalent to 1000 persons per acre. In 1900, the City of Birmingham (UK) was using 2800 acres of land to treat its sewage, an area that stretched for 2.5 miles along the banks of the River Tame. The area available for treatment had to be increased at a rate of 1.5 acres per week to keep pace with the growing population (Bruce and Hawkes 1983). However, sewage farms were slow to disappear and several major European cities still relied partly on such systems up to the 1990s. The large area of flooded land also proved an excellent feeding ground for many birds, especially waders, and studies have suggested that the demise of the sewage farm may be closely linked to the reduction in the population of certain birds (Fuller and Glue 1978, 1980, 1981). Although now not widely practised on a large scale in Europe for domestic wastewaters, spray irrigation of animal slurry and some food processing wastewaters remain common. Spray irrigation of dairy wastewaters is widely practised in New Zealand, whereas in Germany, at the Braunschweig Land Treatment Plant, the treated effluent from a population equivalent of 270 000 is reused on 300 ha of farmland by sprinkler irrigation (Boll and Kayser 1986). Land

treatment using on-site subsurface infiltration or percolation areas is still widely used in conjunction with septic tank systems (Sec. 7.2.1). In areas of the world where water is in short supply, raw and treated wastewater is used for crop irrigation (Israel) or watering public parks and green areas (southern USA) (Sec. 10.2). Agricultural land is a major recipient of sewage sludge in Europe and this is fully examined in Sec. 8.2.

There are four distinct types of land application of wastewater: on-site subsurface infiltration, slow-rate land application, rapid infiltration and overland flow; although there is some overlap between these categories (Environmental Protection Agency 1981). The important factors in the design of land treatment systems are land availability, permeability and depth of soil, type of bedrock and nature of ground water, climate including precipitation, evapotranspiration and temperature, plant cover, topography, wastewater type and required final effluent quality (Johnson 1973). All four types of system are widely used in the USA, with irrigation systems most widely used for secondary treatment and rapid infiltration used primarily for ground water recharge. In Europe, land treatment is less widely used and is generally restricted for secondary or tertiary treatment of wastewater from small communities.

6.1.1. *Purification process*

Wastewater is purified as it percolates through the soil by a range of physical, chemical and biological processes. Suspended material, including micro-organisms, are physically filtered out of solution as the water percolates through the upper soil layer. Organic material trapped in the soil is rapidly utilised by the high density of heterotrophs present. For example, a single plate count using just one gram of soil can yield 10^7 bacteria, 10^6 actinomycetes and 10^5 fungi, which in turn is supporting a large and diverse micro- and macro-fauna (Miller 1974). The humus, silt, and clay particles which comprise the soil provide a very large surface area for ion-exchange with mineral ions, especially cations, becoming strongly bonded on to soil particles. In practice, this results in a high removal efficiency of metals, phosphorus and ammonia from the water. Any nitrogen present is normally fully utilised by either plant uptake and subsequent harvest, the nitrification–denitrification process or volitisation. Discharge of excess sodium (Na^+) ions to land disperses the soil particles and inhibits plant growth (Ellis 1974) and as it accumulates, percolation is severely impaired and the tilth of the soil is reduced. In contrast, the divalent cations calcium (Ca^{2+}) and magnesium (Mg^+) will rectify the effects of excess sodium. The

ratio between sodium and both calcium and magnesium is known as the sodium absorption ratio (SAR) which is used to characterise the suitability of a soil for plant growth:

$$\mathrm{SAR} = \mathrm{Na}^{+}/\sqrt{(\mathrm{Ca}^{+} + \mathrm{Mg}^{+}/2)} \tag{6.1}$$

where all the cation concentrations are given as millequivalents l^{-1} (i.e. concentration in mg $\mathrm{l}^{-1} \times 0.044$, 0.050, and 0.082 for Na, Ca, and Mg respectively).

Irrigation of soil with wastewater leads to complex chemical changes, such as precipitation and oxidation-reduction. Immobilization of cations is enhanced at a soil water pH > 7 but acidic conditions lead to some mineral solubilisation and leaching. In conjunction with conductivity, which is used as a measure of total dissolved solids and hence the salinity hazard to crops, the SAR, which is a measure of the sodium or alkali hazard to crops, can be used to assess the suitability of wastewaters and effluents for irrigation (Sec. 10.2.2) (Fig. 6.1). Overloading land treatment sites can result in the

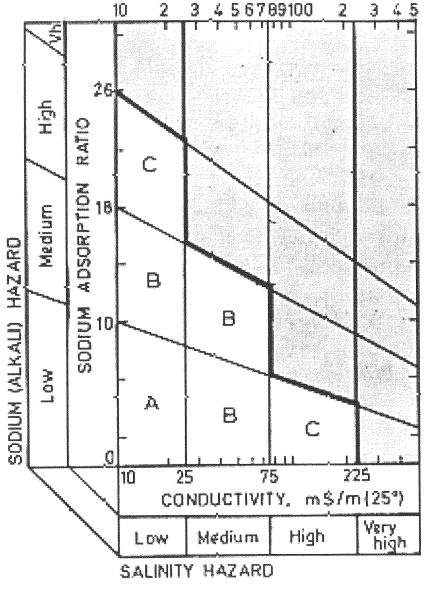


Fig. 6.1. The suitability of wastewaters and effluents for irrigation can be classified using the sodium adsorption ratio (Eq. (6.1)) and the conductivity (millisiemens per metre at 25°C). Waters in areas A and B are suitable for almost all irrigation purposes; waters in area C should be avoided if possible and only used in conjunction with expert advice; and waters in shaded areas are not suitable for irrigation under any circumstances (USDA 1954).

land becoming clogged with organic matter. This is unsightly, produces strong and unpleasant odours and often an associated fly nuisance, but as the soil is anaerobic, the pH gradually falls until minerals are released and leaching occurs. Bankside infiltration is practised in some European countries as a method of tertiary treatment for secondary treated effluents that are discharged to surface waters subsequently used for water treatment abstraction.

6.1.2. *On-site subsurface infiltration*

On-site subsurface infiltration systems are known more widely as leach fields or percolation areas. These are employed for individual homes or small communities to allow the settled waste water from either a septic tank or Imhoff tank to seep through the soil and into the water table. This is achieved by a system of narrow trenches (0.6–1.5 m deep) containing perforated or open jointed distribution pipes which are laid on a porous medium, 20–30 mm diameter gravel or clean crushed stone, to allow the water to freely drain away (Fig. 6.2). The role of the medium is to maintain the structure of the trenches, to provide partial treatment of the effluent by acting as a substrate for the growth of heterotrophs, to ensure even distribution of the effluent to the soil interface and to provide for temporary storage of

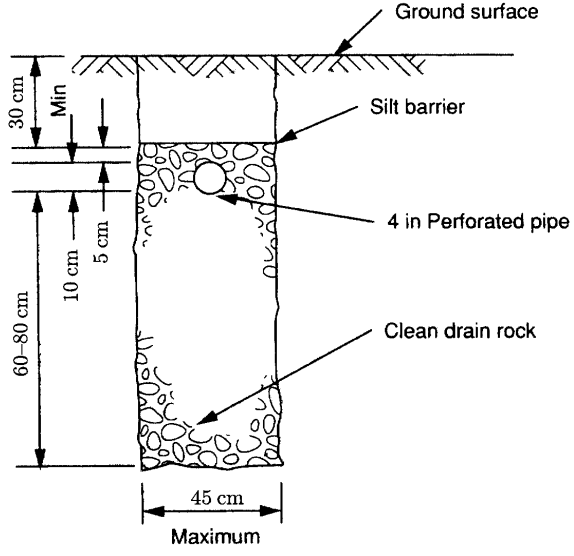


Fig. 6.2. Cross section of a percolation area disposal trench (Metcalf and Eddy 1991).

effluent during peak flows from the tank. Problems associated with the use of gravel or crushed stone media (18–64 mm diameter) have been reported by May (1996). These include physical damage to the infiltrative surface, compaction, negative effects of entrained fines, poor storage volume, native soil intrusion into the trench, and the promotion of excessive biomat development within the interstices of the medium reducing leaching rates. According to May, this leads to a significantly reduced long term acceptance (infiltration) rate (LTAR) of natural soil. However, the use of such media is universal in subsurface infiltration systems. The effluent is normally fed by gravity to subsurface infiltration areas serving individual homes, the flow being intermittent. Larger systems must be dosed periodically using either a siphon or pump (Laak 1986).

When newly constructed, a layer of organic debris and micro-organisms forms over the base of the trenches at the interface with the soil. This is the biomat which is the primary biological treatment mechanism (Kreissel 1982). The layer that develops is similar in nature to the film in low rate percolating filters, although the grazers comprise less insect species (Sec. 4.1.3). Fine solids are physically strained out of the effluent, while the micro-organisms metabolise the organic fraction. Over time, the biomat slowly covers the entire base of the trench system, increasing in thickness and forcing the effluent to infiltrate into the soil through the side walls. An equilibrium develops between biomat accumulation and biomat reduction through microbial metabolism and grazing by soil invertebrates. Therefore, except where the soil has a very high or low permeability (i.e. coarse gravel or clay respectively), it is the hydraulic characteristics of the biomat that control the LTAR of the system and not the permeability of the soil. If infiltration areas are rested and allowed to dry out, the biomat will be largely removed by the action of the grazers. The remaining biomat will eventually dry and crack, exposing the soil below and restoring its original permeability. For continuously loaded systems, resting is especially important if acceptable infiltration rates are to be maintained.

Treatment occurs as the effluent passes over the porous medium in the trench, as it flows through the biomat into the soil, and as it percolates through the unsaturated soil layer. Metabolism will be aerobic when the medium is loaded intermittently, but anaerobic if it is continuously submerged. Overall biological activity within the infiltration system depends on a number of factors: quality and quantity of wastewater applied, the hydraulic gradient, whether the system is adequately rested, oxygen availability within the trench and at the soil interface, and most importantly the temperature. To date, it has not been possible to predict the degree of

treatment provided by these systems. In colder climates, biological activity may be so limited that infiltration areas become primarily a system for hydraulic dispersal of effluents.

Although subsurface infiltration systems provide some degree of treatment of septic tank effluents, this is extremely variable, making ground water contamination common. Elevated concentrations of metals, nutrients and pathogens have all been reported (DeBorde *et al.* 1998; Robertson *et al.* 1998; Schwartz *et al.* 1998; Khwaja *et al.* 1999). Subsurface infiltration systems located in coarse sand soils are particularly at risk (Scandura and Sobsey 1997). A study by Harman *et al.* (1996) on a 44-year-old septic tank system serving a school in Ontario, Canada, showed a residence time of 7–14 d for the septic tank effluent as it passed through the 1.6 m deep unsaturated zone below the infiltration trench. This allowed enough time for complete oxidation of all the nitrogen present to nitrate. Within the ground water, a 15 m wide plume emanated from the site for over 110 m. The plume had detectable dissolved oxygen present, high nitrate (20–120 mg l^{-1} as N), chloride (42–209 mg l^{-1}), sodium (34–101 mg l^{-1}), calcium (120–249 mg l^{-1}) and above background concentrations of sulphate and potassium. The phosphate concentrations present in plumes are controlled by mineral precipitation reactions that occur in the unsaturated zone, resulting in elevated concentrations in the soil. Once in the ground water, phosphate plume migration is controlled by sorption processes (Robertson *et al.* 1998).

The area required for infiltration is based on initial soil permeability and ground water conditions. In the USA, it is calculated as:

$$A = 1.5Q/k \tag{6.2}$$

where A is the infiltration area (m^2), Q the average wastewater flow (m^3d^{-1}) and k the permeability of the soil below the infiltration trench ($m^3m^{-2}d^{-1}$) (WPCF 1990). The US Environmental Protection Agency (1980) give guidelines for hydraulic loading rates based on approximate percolation rates (Table 6.2). As soil infiltration rates can fall quite dramatically over time, there is some concern about the long term validity of using percolation tests to determine hydraulic loading rates. However, in practice, percolation tests are useful in identifying unsuitable and problem soils, as well as giving useful guidance about the relative infiltration area required for particular soil conditions.

The British Code of Practice (BS6297:1983) uses a percolation test to determine the value V_p which is the average time (in seconds) required for the water level to drop 1 mm. A test hole is dug to 250 mm below the

Table 6.2. US Environmental Protection Agency recommended hydraulic loading rates for on-site subsurface infiltration systems (US Environmental Protection Agency 1980).

Range of soil types	Percolation rate (min per 100 mm)	Hydraulic loading based on base area of trench ($l\ m^{-2}d^{-1}$)
Gravel, coarse sand	< 4	Not recommended
Coarse, medium sand	4–20	48
Fine sand, loam sand	21–60	32
Sandy loam, loam	61–120	24
Loam, porous silt loam	121–240	18
Silty loam, clay loam	241–480	8
Clays, colloidal clays	> 480	Not recommended

proposed invert level of the land drain, filled with water and the time then measured in seconds for the water to seep away completely. This is used to calculate the floor area of the subsurface drainage trench A_t in m^2:

$$A_t = P \times V_p \times 0.25 \tag{6.3}$$

where P is the population equivalent. For effluents that have received secondary treatment followed by settlement, this area is reduced by 20%:

$$A_t = P \times V_p \times 0.20 \tag{6.4}$$

In the UK, subsurface infiltration areas are permitted only where the geology and hydrogeology are suitable. Drainage trenches should have a uniform gradient no greater than 1:200, and be from 300 to 900 mm wide with a minimum of 2 m between parallel trenches. Perforated or porous pipes (75–110 mm diameter) are laid on a 150–250 mm layer of clean gravel or crushed stone (20–50 mm grade) and then covered by 50–150 mm of the same material. The trench is sealed by a strip of air permeable sheeting to prevent the entry of silt and filled with soil. Pipes should be a minimum of 500 mm below the surface. Where high percolation values are obtained, the soil is not suitable for the construction of an infiltration area. In this case, a complete treatment system must be employed or an infiltration area constructed above ground level. Where V_p is between 140 to 100 s (i.e. between 10 hours to 7 hours to fall 250 mm), underdrains are required to prevent the infiltration system from flooding. In this system, a second system of drainage pipes are laid on the bottom of the trench to convey the surplus

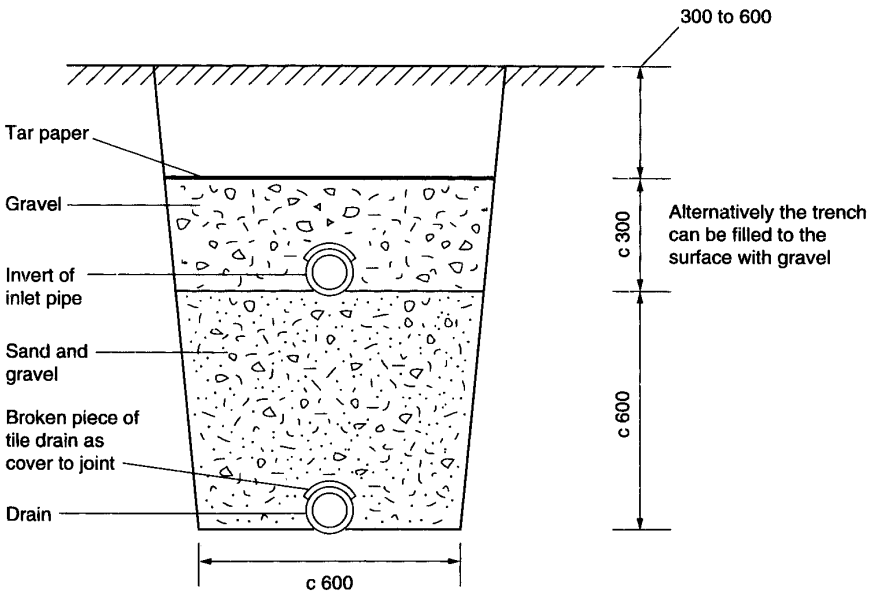


Fig. 6.3. Cross section of typical underdrain used in percolation areas where there is insufficient percolation (British Standards Institution 1983).

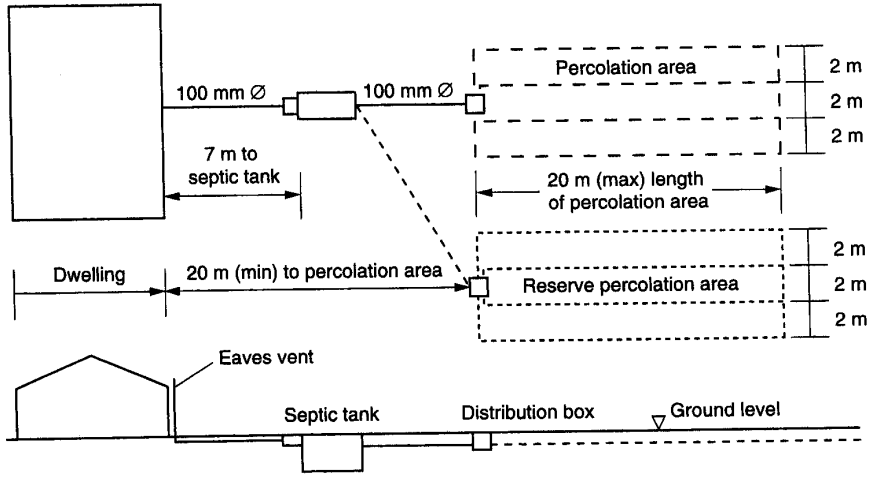


Fig. 6.4. Typical plan and section of septic tank and percolation area (National Standards Authority of Ireland 1991).

water via outfall to a ditch or watercourse (Fig. 6.3). A typical layout of an infiltration area is shown in Fig. 6.4. Full details of site suitability assessment and the construction of infiltration areas are given in Environmental Protection Agency (2000) and BSI (1983).

In most cases, a reserve infiltration area is also required which is used in rotation with the main area and can be used in the event of a failure of the primary infiltration area. Surface runoff from paved areas or roofs is piped to a separate infiltration area or, where the water is clean and not subject to contamination, to a soakaway (soak or disposal pit). Soakaways must not be used for the disposal of septic tank effluents as this invariably leads to severe ground water pollution (Gondwe *et al.* 1997). When the percolation rates are either too slow or rapid, or the water table too close to the base of the infiltration trench (i.e. normally < 1.5 m), or the site too steep, new infiltration areas can be constructed by raising the level of the existing land using a suitable soil. Alternatively, intermittent sand filters can be employed. These are either buried or open shallow beds of coarse sand 0.6–0.8 m in depth. The wastewater is applied periodically using a simple distribution system with the treated effluent collected by an underdrain system at the base of the filter (Metcalf and Eddy 1991). Sand filters have been shown to be more efficient in removing pathogens and nutrients than other media, thereby offering a greater degree of protection to ground waters (Harrison *et al.* 2000). Peat is increasingly being used as an alternative filter medium with a number of commercial units available. It has a high adsorption capacity and water holding characteristics that make it an ideal treatment medium for a wide range of wastewaters. The design of peat filters are similar to intermittent sand filters (Kennedy and Geel 2000).

6.1.3. *Slow rate land application*

Irrigation is the most widely used land treatment system and involves the disposal of screened and primary settled wastewater to agricultural or recreational land. Slow-rate irrigation is used to supplement rainfall and for maximising crop production by utilising the weak nutrient content of the wastewater as a growth promoter. It is also used for tertiary treatment of secondary treated effluents, although the degree of pretreatment required is dependent on the treatment objectives and design of the irrigation system. Slow-rate irrigation is unsuitable for shallow soil or soil primarily comprised of clay. The ideal soil is either loam, or silt, has good permeability and a minimum depth of 1.75 m to the ground water (Fig. 6.5). The rate of

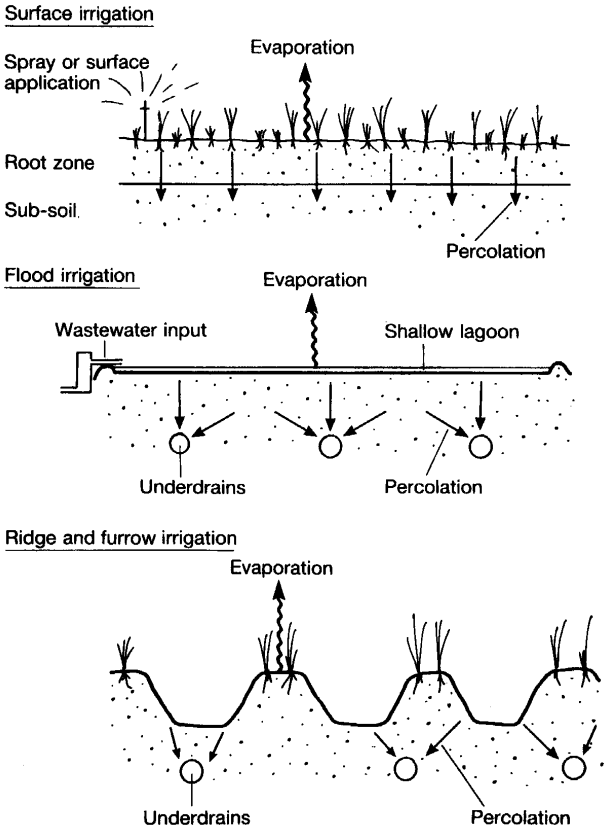


Fig. 6.5. Land treatment by surface irrigation.

application of wastewater depends on soil permeability, evapotranspiration rates, and precipitation. Particle-size distribution of soil is important in the removal efficiency of wastewater constituents; smaller particles are most effective as they reduce the percolation rate (Tare and Bokil 1982). Soil profile and crop cover have been identified as major factors in nutrient removal. Although pasture grass species are normally used, trees, legumes, and corn are also utilised as vegetative cover for such systems. Complete treatment can be obtained with all the BOD and suspended solids removed. The slow irrigation rate is important to ensure most of the phosphorus is immobilised and for the bulk of the nitrogen to be mineralised. The wastewater, after treatment, eventually enters the ground water in the aquifer below, percolates a side wall to enter surface water, or is intercepted by underground drainage pipes. Irrigation is not continuous, therefore, storage is

required, unless sufficient area is available to allow the land to be rested between application periods. This is to ensure that aerobic soil conditions are re-established which are essential for growth of non-wetland vegetation, maintenance of a permeable soil structure and optimum nutrient removal. Application cycles are normally every 4 to 10 days with hydraulic loading rates varying between 0.15–1.6 cm d^{-1} which is equivalent to an area of 6–67 ha per 1000 m^3d^{-1} (Pound *et al.* 1977; Metcalf and Eddy 1991; WEF 1992). Also, irrigation may not be possible during periods of high rainfall or when the land is frozen. Wastewater is distributed on to the land either by surface flooding or by spraying. Surface flooding is only possible where the land is level and may involve flooding specific areas for several days and then resting it for a couple of weeks, or using conventional furrows as were used on the original sewage farms (Fig. 6.5). Where deep furrows are preferred, each furrow is fed by a siphon connected to an open supply channel or simply filled with wastewater several times a day. In all surface flooding operations, it is necessary to contain the wastewater on the site by surrounding it by a dyke to prevent runoff. Spraying is done from either a fixed set of pipes or rotary sprayers, with different areas of the system rested intermittently, or by using a mobile rig which is moved manually from area to area. Spraying allows unlevel ground to be used, although there is a health risk from aerosols (Bausum *et al.* 1982), even though in the USA chlorination has been successfully used to reduce downwind levels of microbial and coliphage aerosols. Although soil damage is unlikely, unless severely overloaded organically, ground waters are at risk. Studies have shown that the total dissolved solids concentration and the nitrate concentration immediately below the site will be enhanced, although viruses and bacteria will be filtered out by the soil. However, prolonged application of wastewater to land may result in the translocation of some viruses through the soil (Shaub *et al.* 1982) (Sec. 9.4). Metals will be immobilised on to the soil particles, and the major metals, cadmium, copper, nickel, lead and zinc in secondary effluents have been rarely shown to migrate deeper than 1.5 m (Brown *et al.* 1983). However, some persistent pesticides have been detected (Koerner *et al.* 1978; Weaver *et al.* 1978). The largest slow-rate irrigation system in operation is Werribee Farm in Melbourne (Australia) which treats the wastewater from 800,000 people (546,000 m^3d^{-1}). This is sprayed on to pasture which is used for fattening livestock. Since it was opened in 1897, no adverse effects have been recorded to the soil, vegetation or the livestock (Dinges 1982). The vegetation, coupled with the active soil layer, makes slow-rate irrigation systems the most effective biological treatment process available, as long as wastewater hydraulic loading rates

are kept low. This makes them particularly suitable for tertiary treatment of wastewaters.

6.1.4. *Rapid infiltration land treatment systems*

High-rate irrigation and infiltration–percolation systems are very similar, except that the former is used on natural sandy soils which are irrigated at very high rates using sprayers, whereas the latter uses specially constructed basins in suitable soils so that the wastewater can soak away into the very permeable soil to the ground water. High-rate irrigation uses hydraulic loading rates of between 1.6–25 cm d^{-1} which is equivalent to an area of 0.4–6 ha per 1000 $m^3 d^{-1}$ (Metcalf and Eddy 1991; WEF 1992). Operation is continuous and due to the small area of land used, factors such as evapotranspiration and precipitation are unimportant. Irrigation sites can have vegetation, but as they are regularly ploughed to prevent accumulation of organic matter on the surface which could restrict percolation, they are normally bare. The small amount of clay, silt or humic material severely limits the capacity of the soil to remove metals, phosphorus and other wastewater constituents. Thus, the ground water is much more vulnerable and receives a significant volume of mineral contamination. At these high loading rates, there is only partial removal occurring with pathogens, especially viruses, reaching the ground water although both primary and secondary wastewaters have been successfully treated (Carlson *et al.* 1982). Although widely used in the USA as a secondary treatment process, high-rate irrigation is generally used for aquifer recharge using secondary treated effluents.

Irrigation–percolation is also restricted to sandy and sandy-loam soils, which have a high permeability allowing the applied wastewater to pass quickly through the soil to the ground water (Mottier *et al.* 2000). Lagoons or basins are specially constructed to enhance rapid percolation, and beneath them ground water mounding occurs (Fig. 6.6). This makes a stable infiltration rate difficult to maintain as the rate of infiltration becomes a function of the ratio between the bottom surface area of the basin and the length of its edges. Thus, high length to width ratios or smaller basin areas increase infiltration rates. In practice, multiple basins are used and are rotated ensuring sufficient time for the basin to dry and the recharge mound to fall. While the basin is rested, the permeability of the soil can be improved by harrowing or rotavating. Where a layer of sand has been used to line the basin, the top surface layer will need to be renewed periodically, depending on the organic loading, and replaced with clean sand. Where partially treated wastewater is used, basins are flooded only for a couple

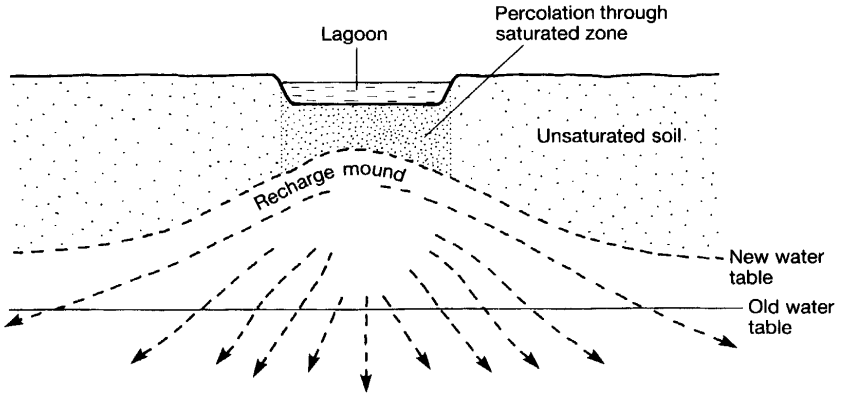


Fig. 6.6. Land treatment by infiltration–percolation.

of days before being rested for one or two weeks As with high-rate irrigation, the limiting factor for infiltration processes is the degree of ground water contamination. For example, an infiltration system has been used in Milton, Wisconsin (USA) for the past 30 years. The wastewater receives primary treatment before being discharged into a set of four basins which are continuously operated in series. The soil is glacial sands and gravels with inclusions of silt and clay. Test boreholes have shown that the quality of the ground water below the site has become contaminated, with a BOD of 4.2 mg l^{-1}, ammonia-N at 13.7 mg l^{-1}, nitrate at 1.5 mg l^{-1}, total phosphorus of 4.0 mg l^{-1} and faecal coliforms reaching 258 per 100 ml (Anon 1979b). Ground water contamination by trace organics have also been associated with rapid infiltration systems (Tomson *et al.* 1981). However, the most suitable natural sites are found in the flood valleys of rivers ensuring little environmental damage is done. Where there is a risk of contaminating water supplies, this treatment process should be avoided.

Due to over pumping, aquifers have become depleted in many countries, which has resulted in attempts to recharge aquifers from other, usually less pure, water sources such as rivers. There has been growing interest in using primary and secondary wastewater to replenish ground water, relying on the soil and the aquifer itself to provide a high degree of purification (Idelovitch 1978; Idelovitch and Michail 1980; Roberts and McCarthy 1978). However, it has proved very difficult to predict or model the movement of the recharge effluent within the aquifer. Thus, in practice, the water quality has often become impaired. There may be a lag period of several years between commencement of recharge and contamination of observation

boreholes, with a much longer period required for recovery of water quality once recharge has ceased. As recharge commences, a mixture of recharge effluent and natural ground water will eventually be pumped from the supply boreholes, therefore regular monitoring of water quality is essential to ensure potable standards are maintained. Because of the potential long term risks to important ground water resources where infiltration basins are used for ground water recharge, it is advisable only to use wastewater treated to as near drinking water quality as possible, and to regard the basin system solely as a means of recharging the aquifer much in the same way that injection wells are used. Therefore, any benefits from the potential treatment capacity of the soil as the water percolates to the aquifer should be regarded as incidental (Idelovitch and Michail 1984).

6.1.5. *Overland flow*

Overland flow, which is also widely known as grass filtration, is a development of the irrigation system, except that the underlying soil has a low permeability, being comprised of clay or clay loam soils. When wastewater is applied, the bulk of the water flows over the surface rather than percolates in to the soil, resulting in the effluent having to be collected for reuse or discharge to a watercourse. Flat land is preferred in order that it can be carefully regraded to give a slope of between 2–6%, although naturally sloping land can also be used. A low slope may result in ponding causing depletion of soil oxygen and death of the vegetation. Excessive slopes may lead to erosion and a high mineral suspended solids concentration in the final effluent. Each sloped section is between 30–60 m in length, the width is governed by the total area of land required. The wastewater is screened and the settleable solids removed by primary settlement. It is then applied to the top third of the gently sloping land either by overflow pipes or by spraying and allowing it to slowly flow by gravity down the slope (Fig. 6.7). It is important to ensure that an even sheet flow of wastewater is maintained over the vegetation to obtain maximum treatment. The ground is planted with a suitable species of grass so that purification occurs by filtration and bacterial decomposition as the wastewater moves through the vegetation. The grass is regularly cut to remove nutrients taken up by the plants, from the system which could be released if the vegetation is allowed to mature and die back. The addition of lime has been found to enhance the removal of phosphorus from overland flow systems (Khalid *et al.* 1982). These systems are very dependent on the weather and the hydraulic loading is normally kept to between 1–10 cm d^{-1}, which is equivalent to an area of 1–10 ha

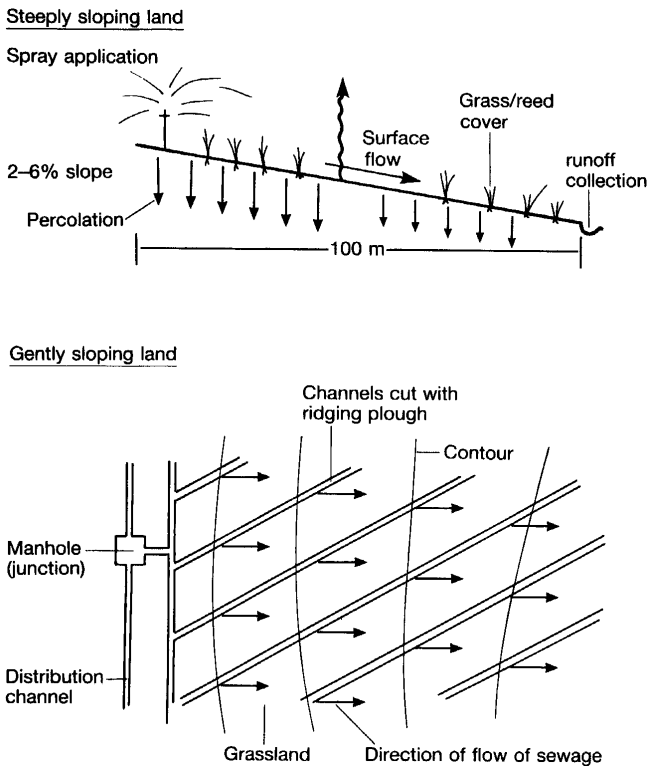


Fig. 6.7. Land treatment by overland flow.

per 1000 m^3d^{-1}, (Pound *et al.* 1977; Metcalf and Eddy 1991; WEF 1992). Application of wastewater is not continuous but for 8–12 hours each day. This allows the retained organic matter to be microbially oxidised and the nutrients to be adsorbed onto the soil particles, utilised by plant growth, or to be nitrified. Performance of these systems is good for primary and secondary wastewaters, but unlike BOD or suspended solids, the removal of ammonia decreases as the wastewater application rate increases (Wightman *et al.* 1983). Problems have arisen when overland flow systems have been used as a secondary treatment phase for lagoon treated wastewaters, as the algae in the applied wastewater is not removed by the land system resulting in a high suspended solids concentrations in the final effluent (Abernathy 1983; Witherow and Bledsoe 1983). The grass plots are normally 100 m in length and often several separate plots can be operated on a single slope. The use of grass plots in series has been beneficial in terms of enhanced effluent quality but less efficient in overall removal per unit area of land.

The design of overland flow systems has been reviewed by Hegg and Turner (1983) for use with animal wastes and by Smith and Schroeder (1983) for domestic wastewaters. The most up to date design details have been published by the Water Pollution Control Federation (WPCF 1990) and the Water Environment Federation (WEF 1992).

6.2. Macrophyte-Based Systems

The controlled culture of aquatic plants (macrophytes) is now widely used in wastewater treatment both as secondary and tertiary treatment systems. Not only do macrophytes accumulate nutrients and other soluble compounds from the water, but they also act as a food source for a variety of other organisms such as zooplankton, small crustaceans such as the brine shrimp (*Artemia salina*), and a wide variety of fish. The excess plant biomass itself can be harvested and used for various purposes.

The plants used in wastewater treatment systems are all natural wetland species and can be loosely classified as submerged algae and macrophytes, floating macrophytes, and emergent vegetation. The advantage of using plants is that the capital investment in the treatment works is low, and as little equipment, energy and chemicals are required, the operational costs are also very low. The operational cost of harvesting either the plant or the secondary animal biomass is reduced by its value as a source of energy or protein (Dinges 1982). Much work has been done in evaluating plants for wastewater treatment in terms of treatment efficiency, biomass production and value of the resultant biomass. The main criteria for such plants are: (1) ease of harvesting; (2) low water content; (3) high protein content; (4) low fibre and lignin content; (5) high mineral absorption capability; (6) extended growing and harvesting periods; (7) non-toxic either to humans or livestock; (8) capable of being processed into a useful by-product; and (9) have few natural pests. Selection of plants for wastewater treatment should, if possible, satisfy all of these criteria (Culley and Epps 1973).

Constructed wetlands provide the optimum treatment conditions found in natural wetland but can be built at almost any location. They can be used for secondary and tertiary treatment of sewage, for treating storm water and other wastewaters including acid mine drainage, landfill leachate, agricultural and industrial wastewaters (Hammer 1989; Mulamoottil *et al.* 1998; Kadlec *et al.* 2000). They can also be successfully employed for dewatering and stabilising sewage sludge (Sec. 8.1.1). In the USA, constructed wetland systems are classified into two basic designs: free water surface

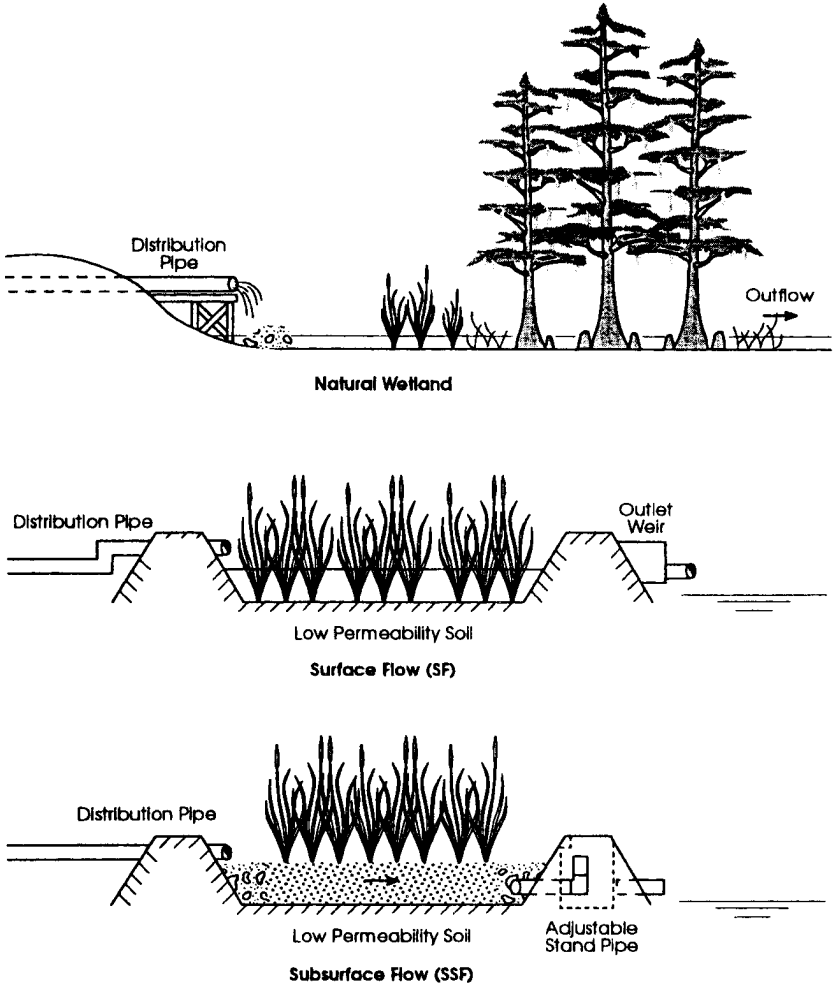


Fig. 6.8. Three basic wetland systems are employed in wastewater treatment. Natural wetlands, and constructed wetlands employing surface or subsurface flow (Kadlec and Knight 1996).

(FWS) or surface flow (SF) systems and subsurface flow (SSF) systems (Fig. 6.8). Free water surface treatment wetlands are densely vegetated and characterised by a significant depth of permanent water above the media surface. They can be further categorised by the type of vegetation used: (i) submerged macrophytes (Sec. 6.2.1), (ii) free floating macrophytes (Sec. 6.2.2), (iii) floating leaves but bottom rooted macrophytes such as water lilies (*Nymphaea* spp.), lotus (*Nelumbo* spp.), and cowlily (*Nuphar*

spp.), (iv) macrophytes forming mats, and (v) emergent macrophytes. Category (iii) systems are still experimental, whereas (iv) and (v) have currently only limited application (Sec. 6.2.3). Free water systems may not offer many advantages over waste stabilisation ponds (Sec. 6.3), and as mosquito breeding, especially *Mansonia* spp., can be a serious problem in FWS systems then waste stabilisation ponds should always be considered as an alternative (Ringuelet 1983).

The climatic conditions in Europe limit the type of constructed wetlands that can be employed successfully throughout the year. For that reason, European constructed wetlands are almost exclusively SSF systems using emergent plant species commonly referred to as reed beds (Sec. 6.2.3). A bed of soil or gravel 0.6–1.0 m depth is used as a substrate for the growth of rooted emergent species. Settled wastewater flows through the substrate by gravity, either horizontally or vertically, where it comes into contact with a mixture of facultative micro-organisms living in association with the substrate and plant roots.

6.2.1. *Algae and submerged macrophytes*

The role of algae in wastewater treatment is fully discussed in Sec. 6.3.2. In facultative ponds, the algae use the inorganic compounds, released by aerobic and facultative bacteria, for growth using sunlight for energy. They release oxygen into the solution, which in turn is utilised by the bacteria completing the symbiotic cycle (Fig. 6.9). The algae in facultative ponds are restricted to the euphotic zone, which is often only a few centimetres deep, depending on the organic loading and whether it is day or night. High-rate aerobic stabilisation ponds are not designed for optimum purification of wastewater but for algal production. Research is aimed at producing sufficient biomass from such ponds to make them a useful and economic source of single cell protein (Sec. 10.3.2). Mono-species cultures of green algae, such as *Chlorella* or *Scenedesmus*, have a protein content of 50% (dry weight (DW)) compared to 60–70% for the blue-green alga *Spirulina*. Light availability is the most critical factor controlling algal growth and as it is uneconomical to use artificial light, ponds must be located in areas where there is maximum sunshine. Shallow lagoons are normally used for algal production because as the density of cells increases, so the light penetration is reduced. Also, the cells nearest the surface can become damaged by constant exposure to sunlight. Ideally, deeper lagoons are used, which are gently mixed to maintain the algae in suspension, ensuring intermittent transport of the cells into the euphotic zone. Large-scale algal ponds are

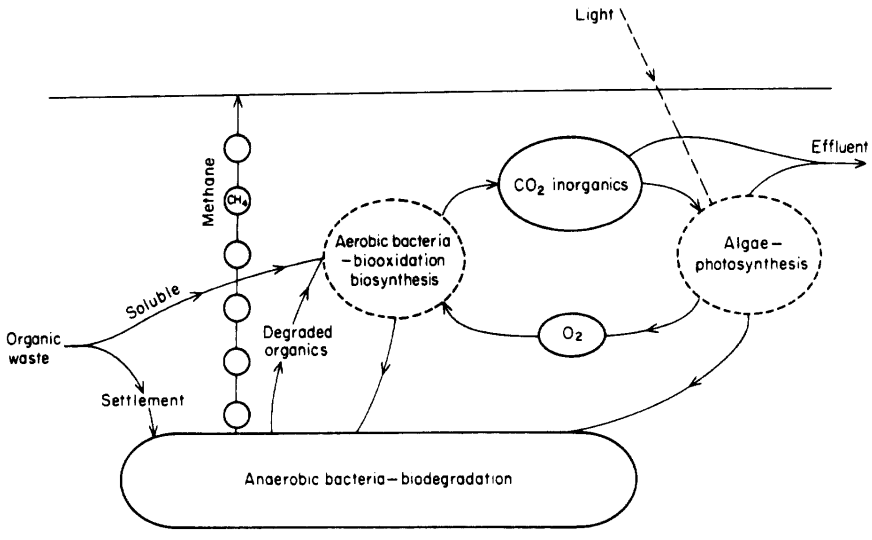


Fig. 6.9. Basic biological interactions in a facultative pond (Hawkes 1983a).

Table 6.3. Comparison of digestibility and biogas production of various plant material (Braun 1982).

Material	Hydraulic residence time (d)	Volumetric loading (kg TS $m^{-3}d^{-1}$)	Gas yield ($m^3kg^{-1}TS$)
Sea grass	20	2	0.1
Seaweed	20	2	0.17
Water hyacinth	10	4	0.1
Algae[a]	10	4	0.25
Algae[b]	3–30	1.4–11.2	0.26–0.5
Silage	15	6.7	0.45

[a]Freshwater algae.

[b]Sewage lagoon algae.

being used in several countries as an effective means of sewage treatment, as well as producing valuable algal biomass which can be used for many different purposes such as biogas production (Table 6.3), fodder and fertilizer (Oswald 1972; Shelef *et al.* 1978; Taiganides *et al.* 1979). The most recent use, and potentially the most valuable, is the removal and recovery of heavy metals from effluents. The ability of certain algae to absorb and

accumulate metals suggests the possibility of removing the low concentrations of heavy metals found in normal domestic wastewaters (Filip *et al.* 1979; Harding and Whitton 1981; Nakajima *et al.* 1981). However, Becker (1983) indicates that removal of metals by algae may be possible where long retention periods are possible, for example, for the pre-treatment of certain metal wastes. In ordinary algal ponds with relatively short retention times, metal removal will only be marginal. Thus, complete removal and recovery does not appear feasible at present (Sec. 10.2.3). The use of algae in stabilisation ponds has been fully discussed in Sec. 6.3.2.

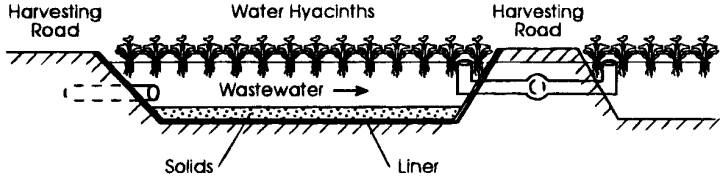
Submerged plants or macrophytes are used for tertiary treatment only as they require high clarity to ensure adequate light penetration and an aerobic environment for plant respiration. Only a few of the many submerged aquatic plants found in natural waters can tolerate enriched waters, for example *Potamogeton berchtoldii*, *P. filiformis*, *P. foliosus*, *P. pectinatus*, *P. zosterformis*, *Ceratophyllum demersum*, *Elodea canadensis*, *Ludwigia repens* and *Najas marina*. The macro-algae *Hydrodictyon* and the ubiquitous *Cladophora* are also associated with wastewaters. They remove soluble nutrients and some other compounds from the water but also provide a solid substrate for the development of an active periphyton slime, including heterotrophic bacteria. Submerged plants grow well in the summer but die back in the winter. For example, in Michigan (USA) the plant biomass degenerated by 98–99% in the winter (McNabb 1976). Plants will compete with each other in ponds, so where mixed cultures are used, *E. canadensis*, *C. demersum* or *P. foliosus* will dominate the others present.

In order that the euphotic zone extends to the bottom of the pond, the depth should not exceed 2 m. Four experimental ponds 1.8 m deep and covering a total area of 16 ha are being used to treat 1900 m^3d^{-1} of final effluent from an activated sludge plant serving Michigan State University (Bahr *et al.* 1977). The ponds contain *E. canadensis*, *C. demersum*, *P. foliosus* and the epiphytic algae *Cladophora*. Harvesting of the filamentous alga *Cladophora* and the macrophytes is done four times a year during the growing season, resulting in a biomass production of 400 g (DW) m^{-2}. After two years of operation, the nitrate concentration reduced from 15 to 0.01 mg l^{-1} and the concentration of phosphorus from 4 to 0.03 mg l^{-1} by using all four ponds in series. The macrophytes can be used for fish food, feeding livestock or for composting. *Elodea canadensis* contains 16.3–16.9% ash, 2.89–3.86% N and 0.092–1.180% P on a dry weight basis (Jewell 1971) (Table 10.13). Macrophytes can be harvested using a commercial weed harvester, although herbivorous fish can be used to crop the excess vegetation (Sutton 1974). Over-stocking with fish will lead to over-grazing,

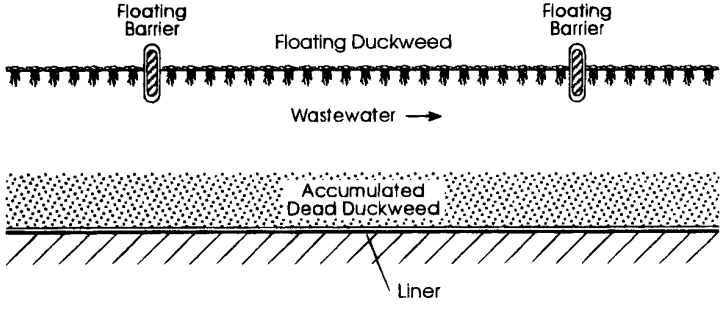
therefore care must be taken with stocking levels. The fish can then be harvested annually and used for a much wider range of uses than the plant material (Dinges 1982).

6.2.2. *Floating macrophytes*

This group of aquatic plants use atmospheric oxygen and carbon dioxide, but obtain the remaining nutrients they require from water. Three groups of floating macrophytes are used in wastewater treatment, the water hyacinth (*Eichhornia crassipes*), duckweeds (*Spirodela* spp., *Lemna* spp., *Wolffia* spp. and *Wolffiella* spp.) and water ferns (*Salvernia* spp. and *Azolla* spp.) (Jackson and Gould 1981; Mitchell 1978, 1979; Finlayson 1981). Treatment systems using floating plants are often confused with facultative ponds (Sec. 6.3.2). However, they are functionally very different as the floating plants release oxygen above the water surface, unlike the submerged planktonic algae in facultative ponds (Fig. 6.10). The dense layer of floating leaves reduces atmospheric oxygen diffusion making such systems normally oxygen deficient, with aerobic processes largely restricted to the plant root zone. The degree of oxygen depletion within the water phase of a pond



(a)



(b)

Fig. 6.10. Basic design of wastewater treatment systems employing floating macrophytes: (a) water hyacinth and (b) duckweed (Kadlec and Knight 1996).

containing floating plants is dependent on the organic loading rate, but is normally anaerobic. Three primary treatment processes can be identified: (1) Aerobic, anoxic and anaerobic metabolism by micro-organisms on the plant roots and in the settled debris on the bottom of the pond; (2) sedimentation of wastewater solids and autochtonous material (i.e. dead plant and animal debris) on the bottom; and (3) uptake of nutrients by the plants and their removal by harvesting. The application of floating plants for treatment has been limited because they rely on just a one or two species to colonise the pond. This makes the system vulnerable if the plant is either inhibited or eliminated. For example, water hyacinths are subject to attack by a number of pests and are susceptible to cold weather. Although duckweeds are generally more tolerant to low temperatures, they can still be eliminated by extreme cold events. Once floating plants are lost, it may take months for plants to become re-established.

Water hyacinth

Although originally discovered in the Amazon River basin, Brazil in 1824, the water hyacinth is now found growing wild throughout the warmer parts of the world including North America, Africa, Portugal, South and South East Africa, Australia and New Zealand (Holm *et al.* 1968; Pieterse 1978). The water hyacinth is the largest of the floating plants and the most studied macrophyte used in wastewater treatment. It is a rhizomatous plant with large glossy green leaves and a feathery unbranched root system. The pale lavender flowers with their beautiful splashes of yellow are very short-lived. The plant is able to reproduce asexually by the formation of stolons, a type of vegetative propagation, as well as sexually. New plants grow from the ends of each extended stolon which can grow up to 30 cm in length depending on the available space. The plants grow rapidly in both vertical and horizontal directions, although initially the hyacinths grow horizontally until the entire water surface is covered when they begin to grow vertically, increasing the overall plant height. When hyacinths are grown in ponds containing wastewater, they rapidly multiply and cover the available water surface. This crowding causes vertical growth with the plants growing in excess of 1 m in height. It is interesting that the more nutrient rich the water, the smaller the root system that will develop, and in wastewater lagoons the root system may be restricted to only 10 cm in length. The biology of the plant is reviewed by Pieterse (1978).

Hyacinths are very much a tropical plant requiring a minimum light intensity of 1400 lm m^{-2} and preferring full sunlight for maximum growth,

when their photosynthetic conversion efficiency approaches 4% (Penfound and Earle 1958; Wolverston and McDonald 1979). They have a growth range of between 10–35°C with optimum growth occurring between 25.0–27.5°C, but they cannot tolerate cold temperatures and the stem and leaves killed by frost. The central structure of the plant, from which the stem, roots and stolons arise, is the rhizome which is similar in shape to a carrot and grows up to 20 cm in length. The rhizome is critical to the plant and if just 4 cm of the rhizome tip is removed, then the plant dies. The tip floats just a few centimetres below the water surface, therefore it is very vulnerable to the cold, the tip is killed and the plant subsequently decays if the water temperature approaches freezing. If the stem and leaves are killed by the frost, the plant rhizome will normally remain viable. However, as the dead vegetation dries, the bulk weight of the plant is reduced so that the rhizome is displaced less and rises closer to the water surface making it more susceptible to possible damage from low temperatures. The northern limit of the plant in shallow waters has been shown to be where the mean air temperature for the coldest month never falls below 1°C. Therefore, in practice all the water hyacinth systems currently treating wastewater are located in tropical or warm climates (Middlebrooks *et al.* 1982). When hyacinth systems have been attempted in cooler climates, they have had to be housed in heated greenhouses with the temperature maintained within the optimum range. Although it has been suggested that methane produced from digesting the waste biomass in anaerobic digesters could be used to heat the greenhouses, it is doubtful if such a system could be cost-effective. Also, where the system only operates for part of the year, there is the added expense of operating a culture unit to maintain sufficient hyacinths to introduce into the system each spring. Hyacinths are freshwater plants and are inhibited by salinities of $> 1.6\%$ (Haller *et al.* 1974), but they can tolerate a wide pH range of 4–10 (Haller and Sutton 1973). They are very disease resistant, with only three exotic insects being able to exert any significant effect on growth: the hyacinth weevils (*Neochetina eichhorniae*, *N. bruchi*) and the leaf mining mite (*Orthogalumna terebrantis*). These species have been introduced in some areas of the world to control the plant, but other indigenous pests have had little effect on plant growth. However, insect-feeding on hyacinths is generally thought to be beneficial as it stimulates the formation of new growth.

The hyacinth is one of the most productive macrophytes in the world, with its biomass able to double every 7 days, which is equivalent to a daily gain in biomass of 108 kg (DW) $ha^{-1}d^{-1}$. Standing crops of hyacinth can reach 2500 g (DW) m^{-2}, although the exact biomass will depend on nutrient

availability and the degree of crowding. When grown in domestic wastewater, the standing crop reaches over 3000 g (DW) m^{-2}, which is equivalent to 30 tonnes (DW) $ha^{-1}d^{-1}$ (Wooten and Dodd 1976; Dinges 1982). With a moisture content of 95%, this represents a vast quantity of biomass to be handled and eventually processed. The dense growth of hyacinths in a wastewater lagoon provides a unique and very stable environment. The extensive root system prevents horizontal movement, and particulate solids rapidly settle. Oxygen is released into the water via the roots, resulting in an active heterotrophic and autotrophic (nitrification) community, and the lowest depths of the lagoon will be anoxic allowing denitrification. There is a complex micro- and macro-fauna associated with the root area of the lagoons but this has not been extensively studied (Dinges 1982).

Hyacinth lagoons are primarily used for tertiary treatment and nutrient removal, and because they require warm temperatures they would seem particularly suitable for use as a tertiary treatment phase after facultative stabilisation ponds which are widely used in warmer climates. They are also used for secondary treatment for small communities. In terms of treatment, most improvement in BOD and suspended solids is due to settlement, whereas nitrogen and phosphorus removal is by the plants, with the former unaffected by the season but the latter severely reduced in the winter. The plants exhibit luxury uptake of nutrients, far in excess of normal requirements, but also accumulate other components of wastewater, such as heavy metals and synthetic organic compounds. The uptake and accumulation of such compounds can be disadvantageous where the concentrations in the plant tissue prohibit the subsequent use of biomass for conversion into energy by digestion or use as a feed supplement.

The design of hyacinth lagoons is reviewed by the US Environmental Protection Agency (1988). Treatment objectives are important to ensure sufficient retention time, but the limiting factors in design appear to be the periodic removal of settled solids and the harvesting of hyacinths. Lagoons are normally large, varying in size from 0.4 to 4 ha. Long narrow channels with plug flow appear most efficient as it prevents short-circuiting and allows easy harvesting of the macrophytes, although settlement is inefficient. Various modifications to this basic design have been made (Dinges 1978; 1982), for example, a zig-zag channel configuration has been used to increase settlement as well as ensuring adequate access for harvesting equipment (Fig. 6.11) (Wolverton *et al.* 1976).

Adequate root depth must be provided for the hyacinths to enable the roots to come into contact with the majority of the liquid passing through the lagoon. For example, a depth of 0.4 m ensures complete wastewater

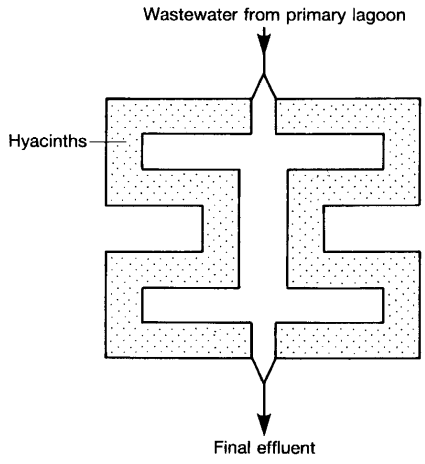


Fig. 6.11. NASA culture basin design for water hyacinth (Wolverton *et al.* 1976).

contact with the root system ensuring maximum nutrient removal. However, the depth can vary from 0.4–1.2 m with the deeper lagoons allowing space for settlement and anoxic activity. Although deeper ponds reduce the total land area required, if they are completely covered with plants, they tend to become completely anaerobic at night. Hydraulic loading is more critical than organic loading for wastewaters that have already received some treatment. In practice, loading varies from 240–3570 $m^3ha^{-1}d^{-1}$ for domestic effluents. However, to produce a final effluent with a final BOD < 10 mg l^{-1}, suspended solids < 10 mg l^{-1}, total kejldhal nitrogen < 5 mg l^{-1}, and total phosphorus < 5 mg l^{-1} from a secondary effluent, then the loading rate should not exceed 1870 $m^3ha^{-1}d^{-1}$. Stronger effluents require lower loading rates. The retention time should not be less than 5 days. For secondary treatment, organic loading should not exceed 100 kg BOD $ha^{-1}d^{-1}$ or 35 kg BOD $ha^{-1}d^{-1}$ for tertiary treatment. To achieve maximum nutrient removal, the hyacinth biomass necessary to absorb the nutrients is maintained by harvesting at a rate which balances net plant productivity. Frequent harvesting is necessary to ensure that the hyacinth population is kept in an active growing phase. Initial uptake of nutrients is rapid, with the amount absorbed related to the concentration of nutrients in the water. The removal rate of nutrients can be directly related to lagoon area. For example, a 2.1 ha hyacinth lagoon in Florida receiving 3800 m^3 of treated domestic wastewater each day removed 80% of the nitrogen and 44% of the available phosphorus (Cornwell *et al.* 1977). The productivity of the plant fluctuates seasonally according to temperature and other climatic

factors such as light intensity and day length, therefore removal efficiency will fluctuate and harvesting has to be adjusted accordingly.

Numerous pilot studies on hyacinth lagoons have been conducted and there is a considerable amount of performance data available. The comparative pilot study by Hauser (1984) at Roseville, California is typical. Three separate ponds were operated using secondary effluent from the Roseville Sewage Treatment Works. Pond 1 was unmanaged, with the hyacinths allowed to control their own density on the pond, whereas Pond 2 was aerated using a submerged perforated tubing connected to a compressor, to ensure an aerobic aquatic environment, but the hyacinths were not harvested. Only the first two-thirds of the pond were aerated with the pipes spread three feet apart, whereas the remaining third was left to allow settlement and filtration. In Pond 3, the plants were harvested but not aerated. Harvesting in Pond 3 was carried out every 2 weeks during the growing season with 20% of the surface area cleared each time. By alternating the harvesting location and not redistributing the plants after harvesting, the period between disturbance of the hyacinths was maximised. Initially, all three ponds were operated in an unmanaged mode, thus any difference in performance during maturation was due solely to unequal plant cover. All three ponds produced good final effluents with significant reductions in BOD and suspended solids; between 30–50% reduction in the winter and 70% for the warmer growing season (May–October). The final effluent concentration was independent of the influent concentration, suggesting that in these ponds, the potential BOD reduction was only partially utilised. Efficiency in suspended solids removal varied between the ponds more than BOD removal. The unmanaged pond (Pond 1) produced a consistently good effluent with low suspended solids concentration throughout the year, whereas in the other two ponds, high suspended solids concentrations were occasionally recorded. As the BOD was not high on these occasions, it suggested that the high solids in Pond 2 were inert particles disturbed by the aeration system as they accumulated, and in Pond 3, as the high suspended solids concentration was associated with harvesting, they were probably fragments of water hyacinths (Table 6.4). To control this 'harvest-induced' rise in suspended solids, Hauser (1984) suggests that a portion of the pond near the effluent end should be left unharvested or, alternatively, a separate non-harvested polishing pond could be used. Dinges and Doersam (1987) have described a full-scale hyacinth treatment plant in Austin, Texas. Analândia Water Treatment Plant in Brazil uses hyacinths to remove turbidity from impounded river water, and improve overall quality, before supply. Hyacinths are grown in a canal with a total area of 1590 m^2, depth

Table 6.4. Performance summary of three experimental hyacinth ponds. Pond 1 was unmanaged, Pond 2 was aerated, and Pond 3 was harvested (Hauser 1984).

	Influent concentration, mg l^{-1} (except pH)		Mean pond loading (all ponds)	Effluent concentration, mg l^{-1} (except pH)					
				Pond 1 (unmanaged)		Pond 2 (aerated)		Pond 3 (harvested)	
Parameter	Mean	S.d.	kg ha^{-1})	Mean	S.d.	Mean	S.d.	Mean	S.d.
Nov 1981–*Apr* 1982									
BOD_5	11.6	6.5	18	4.9	3.6	3.6	2.5	3.5	1.9
SS	10.7	6.5	16	2.8	2.4	4.6	6.9	3.0	2.1
pH	7.8	0.2	—	6.9	0.3	6.9	0.3	7.1	0.3
NH_4 as N	14.1	2.7	21	6.1	4.6	1.8	2.4	9.5	3.3
Organic N	2.3	1.0	3.5	1.4	0.7	0.9	0.5	1.3	0.6
NO_3 + NO_2 as N	0.3	0.3	0.5	3.6	3.5	7.1	2.9	2.7	2.2
Total N	16.7	2.7	25	11.1	2.9	9.9	4.1	13.5	3.0
Alkalinity	132	14	200	97	35	64	17	109	22
May 1982–*Oct* 1982									
BOD_5	8.6	4.2	13	2.3	1.3	1.9	0.3	2.5	0.9
SS	7.1	4.2	11	2.0	2.0	2.6	1.0	5.4	7.0
pH	7.8	0.2	—	6.5	0.2	6.6	0.2	6.6	0.2
NH_4 as N	15.8	3.1	24	4.6	2.4	0.2	0.1	3.7	1.2
Organic N	2.3	1.9	3.5	1.1	0.5	1.2	1.3	1.2	0.6
NO_3 + NO_2 as N	0.2	0.1	0.3	2.7	2.7	3.8	2.4	3.2	2.4
Total N	18.2	2.5	28	8.4	3.0	5.2	3.0	8.0	3.1
Alkalinity	117	15	178	78	17	51	11	67	9

of 0.7 m, and an HRT of 21 hours. The water subsequently flows through soil filters planted with rice (*Oriza sativa*) (Salati *et al.* 1999).

The two major mechanisms for ammonia reduction in hyacinth systems are bacterial nitrification and plant uptake. The primary function of water hyacinths in the bacterial nitrification process is to provide sufficient support for the development of the nitrifying bacteria. The nitrogen is converted from ammonia to nitrate with the possibility of denitrification in the lower anoxic layers of the ponds. However, nitrogen is removed by plant uptake and is incorporated into the plant biomass. Harvesting is thought to encourage plant uptake by eliminating the inhibitory effects of crowding on plant growth, although it may limit nitrification by reducing the availability of support structures as well as removing a portion of the nitrifying bacteria with the harvested plants (Hauser 1984).

Water hyacinths are easily harvested as they float on the surface of the water. The leaves of the plant act as sails and where there is open water, the wind blows the plants to the leeward side of the lagoon. Hyacinths are generally collected and chopped manually on experimental lagoons, although for larger installations there are a variety of mechanical harvesters commercially available. These normally operate from special platforms and combine a feed or collection system such as a rotary head, a chopper, and a conveyer to transport the material to trucks.

There are numerous potential uses for harvested hyacinths, although these depend to some extent on the composition of the wastewater used to culture the plants (Table 6.5). Hyacinths grown on a domestic wastewater can be used for livestock feed, a protein supplement, compost or soil amendment, paper manufacture, alcohol production, livestock bedding, pyrolytic conversion to oil, and incineration to produce steam for energy or for the generation of methane (Table 6.5). Chopped, pelleted or ensiled hyacinths can be fed to cattle and sheep. Ensiling requires the addition of about 4% citrus pulp or corn to act as a readily available carbohydrate source and can be fed directly to dairy cattle. The main value of hyacinth for livestock is as a source of roughage, minerals and energy (Bagnall *et al.* 1974; Zerinque *et al.* 1979). A valuable compost can be produced from whole or chopped hyacinths by storing wet plants aerobically for 1–6 months followed by drying and grinding. Growth is poor in pure compost, but when mixed with sand at a ratio of sand to compost of 3:1, excellent growth is obtained with the composted hyacinths able to retain water unusually well.[a] Hyacinths

[a]Water hyacinths have also been successfully vermicomposted (Balasubramanian and Bai 1995; Gajalakshmi *et al.* 2001) (Sec. 10.3.3).

Table 6.5. Conversion of vascular aquatic plants to useful products (Tourbier *et al.* 1976).

Pollution removal applications	Harvested plants: processing alternatives		Products
Removal of heavy metals from chemical and industrial wastewaters	Anaerobic fermentation		Methane gas
	Residual sludge	Metal extraction processes	Silver, gold, Cadmium, mercury, lead, etc., base metals
Removal of nitrates and phosphates from domestic sewage	Anaerobic fermentation		Methane gas
	Residual sludge	Dried: utilizing Methane gas or solar energy as source of thermal energy	Agricultural fertilizer (bagged or bulk)
	and/or		
	Chopped and dried plant material	Animal-feed processing	Additive for cattle, swine and poultry feeds
		Potable food-processing	Protein supplement flour or meal cereal ingredient
	and/or		
	Composted		Yard and garden mulch (bagged or bulk)

grown on industrial wastewater accumulate high concentrations of heavy metals, making them unsuitable for consumption or disposal to land as a compost or amendment. As long as the heavy metal concentration does not inhibit digestion, methane can be produced with the residual sludge used for metal recovery of particularly valuable or toxic metals such as silver, gold, cadmium, mercury, lead and all the base metals. Hyacinths can be readily digested in conventional mesophilic stirred digesters at low loadings and at high retention times (Klass and Ghosh 1980; Ghosh *et al.* 1980; Chin and Goh 1978) (Table 6.3). Better yields have been obtained using hyacinths mixed with primary sewage sludge and by using digesters in conjunction with upflow anaerobic filters (Chynoweth *et al.* 1981, 1982) (Table 6.5).

Other systems

Two other groups of floating plants are also used for wastewater treatment, duckweed and the water fern. Duckweeds (*Lemnaceae*) are widely distributed and found in all parts of the world except deserts and the polar regions. There are over 40 species in four genera: *Spirodela*, *Lemna*, *Wolffia*, and *Wolffiella*. Duckweeds are comprised of a flattened leaf-like frond without a stem, many species even lacking roots. Reproduction is primarily by vegetative multiplication, with biomass doubling times of between 1.0–5.3 days under optimum conditions. They are resistant to diseases or pests and will grow in slightly saline waters. They float on the surface of the water forming dense mats, but unlike hyacinths they do not provide much support for underwater micro-fauna. The fronds form a single layer on the water surface until crowding occurs, then the thickness of the surface layer may increase to form an extensive mat on the water surface several centimetres thick. The water beneath a dense mat will have a lowered dissolved oxygen concentration and if the organic loading is high, anaerobosis may occur. Duckweed will grow well even on raw wastewater, improving the water quality by direct plant uptake of organic and mineral material, suppression of algal growth and by creating a suitable habitat for the growth of zooplankton, especially *Daphnia* (Dinges 1982). Harvey and Fox (1973) grew *Lemna minor* in laboratory-scale units to evaluate nutrient removal efficiency by using 80 aquaria filled with secondary wastewater to a depth of 450 mm providing a surface area of 500 cm^2. The temperature was maintained at 24°C with the light intensity held at 11,022 lm m^{-2} using 12 hour photoperiods. Over a 10 day test period, frond doubling occurred every 4 days, with the harvested plants containing 4.6% nitrogen and 0.8% phosphorus (DW). There is growing interest in FWS systems using *Lemna minor*

in combination with SSF constructed wetlands for nutrient removal when discharges are to nutrient sensitive receiving waters (Masi *et al.* 1999).

A high nutrient content is essential in order to maintain optimal uptake by the duckweed as well as maintaining biomass production. Removal rates for phosphorus remain fairly constant at between 90–95%. Experimental studies have indicated that duckweed may be more effective as a preliminary treatment phase, with hyacinths scavenging for nutrients in treated wastewaters. Little is known of the design for duckweed ponds, but as they readily grow on all types of natural water bodies, the design may not be critical as long as the water surface is sheltered and preferably still (Al-Nozaily *et al.* 2000a,b). Thus, the actual shape and depth of ponds will be determined primarily by the harvesting method employed. The plant is easily harvested using a skimming device similar to those used for removing oil from the surface of water. Microstrainers can also be used. Much interest has been shown in the use of plants for poultry and livestock feeds as well as for the removal of nutrients from secondary treated wastewaters (Culley and Epps 1973; Harvey and Fox 1973; Hillman and Culley 1978). However, a high water content (the average water content for *Lemna* and *Spirodela* varies between 92–97%) and problems in removing the water have restricted their full development as an agricultural crop.[b]

The water fern (*Salveniaceae*) consists of some 16 species in two genera, *Salvenia* and *Azolla*. Little work has been done on this group, although several workers have indicated that they are less suitable than other macrophytes as they produce large amounts of detritus within the pond. Also, while they are not readily digested, they compost well (Harvey and Fox 1973). This group of plants has been reviewed by Dinges (1982).

6.2.3. *Emergent macrophytes*

Although there are several hundred potential emergent species, only a few have been selected as being potentially suitable for wastewater treatment, such as the bulrushes *Scripus lacustris*, *S. acutus* and *S. validus*; the reeds *Phragmites communis* and *P. australis*; and the yellow flag *Iris pseudocorus*. Reeds have underground rhizomes and jointed stems which root at the nodes, and both reeds and rushes multiply vegetatively from adventitious buds located on the rhizomes. Rushes grow between 2–3 m in height and are restricted to shallow water < 0.5 m deep, whereas reeds are able to grow at depths of up to 2.5 m, with some species growing to heights of 5 m

[b]A full-scale system at Mirzapur, Bangladesh, is described by Alaerts *et al.* (1996).

(Dinges 1982). Emergent species are generally very hardy and resistant to both pests and diseases. They are able to withstand cold temperatures and even freezing, making them ideal for more temperate climates. These plants are perennials, and with proper management stands of rush or reeds can be maintained indefinitely.

Natural wetlands

The position of natural wetlands has resulted in many of these habitats receiving wastewater for centuries. Since the early 1970s, however, considerable interest has been shown in the use of wetlands for complete, secondary or tertiary treatment of wastewaters. Natural wetlands include freshwater and saltwater marshes, swamps, fens, peat bogs and cypress domes, all of which have been used for wastewater treatment (Tilton *et al.* 1976; Tourbier and Pierson 1976; Felter *et al.* 1978; Good *et al.* 1978; Dolon *et al.* 1981; Dierberg and Brezonik 1983). In recent years, natural wetlands have come under considerable pressure from man for a variety of purposes, especially agriculture, when they have been drained or filled. With the increasing appreciation of fragile nature and the intrinsic value of wetlands, many wetland habitats are now considered just too vulnerable to be modified by the addition of nutrients in the form of wastewater. Therefore, constructed wetlands have been developed for treatment purposes (Seidel 1976; Small 1976; Sloey *et al.* 1978; Gersberg *et al.* 1983, 1984, 1986). Where natural wetlands have been adequately managed, the damage has been minimal and in some particular wetland sites, the addition of extra nutrients has increased the productivity of the habitat, resulting in an improved wildlife habitat and enhancing its overall aesthetic value (Dinges 1982). It has been argued that the use of wetlands for this purpose may be a significant factor in the survival of these increasingly rare habitats which are under pressure from other sectors.

Wetlands are habitats where the water table lies above or close to the surface of the root substrate for a significant part of the year (Etherington 1983). Depending on their location, they can be flooded with either fresh or saline waters, the former normally but not exclusively associated with, high rainfall regions, whereas the latter are situated on the coast or along the fringes of inland salty lakes. Freshwater wetlands can be subdivided into those where the decomposition rate is low resulting in a continuous accumulation of organic matter (peat) to form bogs and fens, or those where negligible accumulation of organic matter occurs and where the rooting medium is mainly mineral such as marshes and swamps (Jones 1986). Fens,

marshes and swamps are characterised by the presence of emergent aquatic species, and although the wetland communities of the tropics are floristically different from those of the temperate zones, the actual diversity of emergent species is not great. The primary productivity of wetlands, excluding bogs, is among the highest for any natural community (Westlake 1963; Lieth 1975; Keddy 2000).

Wetlands are normally dominated by a single species which restricts the establishment and growth of other plants. Jones (1986) cites several examples, including the papyrus swamps of East and Central Africa that are almost monospecific communities of *Cyperus papyrus*, and the extensive stands of *Phragmites australis* or *Typha latifolia* that dominate many wetland areas in temperate regions, although these species are extending into much warmer climates. Although some wetlands can have a high species diversity, species rich communities are normally less productive than wetlands with few species (Grime 1979; Wheeler and Giller 1982). Three genera of emergent macrophytes dominate wetlands, *Typha*, *Phragmites* and *Cyperus*, although other species such as *Misacanthidium*, *Cladium* and *Echmochloa* can be important locally. Examples of dominant emergent species in tropical, subtropical and temperate zones are given in Table 6.6.

Marine wetlands include mangrove swamps which are restricted between the latitudes 30°N and 30°S, whereas elsewhere salt-tolerant grasses (*Spartina* spp.) are the primary vegetative cover (Valiela and Vince 1978; Whigham and Simpson 1976). Cypress domes are a common palustrine wetland throughout the pine-palmetto woodlands of the Southern Atlantic and Gulf coastal plain. They are topographic depressions that vary in size from 1–10 ha which are dominated by two species of tree, the tall pond cypress (*Taxodium distictium* var. *nutans*) and the smaller black gum (*Nyssa sylvatica* var. *biflora*) (Monk and Brown 1965; Coultas and Calhoun 1975; Dierberg and Brezonik 1983; Ewel 1983; Keddy 2000).

The purification process in wetlands is complex and the most obvious biological component of the habitat, the emergent macrophytes, play an important, albeit minor role in the treatment of wastewater (Stephenson *et al.* 1980; Nichols 1983). As discussed in Sec. 6.2, the major removal mechanisms are bacterial and fungal transformations and physico-chemical processes such as adsorption, precipitation and sedimentation (Chan *et al.* 1982). The emergent plants have a number of important functions. The plant rhizomes provide a stable surface for heterotrophic growth as well as enhancing sedimentation of solids by ensuring flocculation and maintaining near quiescent conditions. Emergent macrophytes dominate wetlands due to the hostile root environment, which has a restricted oxygen supply allowing

Table 6.6. Emergent species of some wetland areas (Jones 1986).

Location	Dominants
Tropical, subtropical	
Nyumba ya Mungu reservoir, Tanzania	*Typha domingensis, Cyperus alopecuroides*
Lake Naivasha, Kenya	*Cyperus papyrus,* Cyperus immensus, Cyperus digitatus* spp. *auricomus, Cyperus alopecuroides*
Kashambya swamp, Kigezi, Uganda	*Cyperus papyrus*, *Cladium mariscus* (pH > 6.3), *Mischanthidium violaceum* (pH4.5), *Cyperus latifolius*, *Typha sp.*
S.E. Lango, lowland West Nile, Uganda. On alkaline clay-like soils in valleys draining into Lake Kyoga (seasonal swamps)	*Echinochloa pyramidalis,* Oryza barthii* (sometimes codominant with *E. pyramidalis*), *Leersia hexandra*
River Amazon, Manaus, Brazil (seasonal swamp)	*Echinochloa polystachya,* Oryza perennis, Paspalum repens, Hymenachne amplexicaulis*
Namiro swamp, northen end Lake Victoria, Uganda, landward zone (disturbed?)	*Miscanthidium violaceum,* Loudetia phragmatoides*; (towards water's edge), *Cyperus papyrus,* Miscanthidium violaceum*
Northern Sudd, Sudan	*Cyperus papyrus, Phragmites karka, Vossia cuspidata, Typha domingensis*
Temperate	
Huntingdon Marsh, Lake St. Francis, P.Q., Canada	*Carex aquatilis,* Carex Lanuginosa,* Calamagrostis canadensis, Typha angustifolia*
Prairie glacial marsh, Eagle Lake, IA, USA	*Typha glauca, Scirpus fluviatilis, Scirpus validus, Sparganium eurycarpum, Sagitaria latifolia*
Freshwater tidal marsh, Augustine Creek, GA, USA	*Zizaniopsis miliacea,* Zizania aquatica, Peltandra virginica, Pontederia cordata*
Norfolk Broadland, UK	*Cladium mariscus,* Phragmites australis,* Calamagrostis canescens, Carex acutifomis, Carex lasiocarpa, Carex paniculata, Glyceria maxima, Juncus subnodulosus, Phalaris arundinacea, Typha angustifolia*

*Most common species.

facultative and anaerobic bacteria to flourish (Armstrong 1982). The plants are able to translocate oxygen from their shoots to the roots making the rhizosphere (the root zone) an area where aerobic micro-organisms can survive. Heterotrophic and nitrifying bacteria flourish with nitrates diffusing to the oxygen limited zones (anoxic) of the wetland where they are removed from the system by denitrification (Sherr and Payne 1978; Iizumi *et al.* 1980; Gersberg *et al.* 1986). Nutrients are trapped in wetlands, which are often referred to as nutrient sinks, not only in the sediment but in the actual plant biomass by nutrient uptake. The phosphorus in the wastewater does

not move far in the soil as it is retained near the surface. The phosphate ions are chemically adsorbed onto the surface of hydrous oxides of iron and aluminium, and onto silicate clay minerals, where the phosphate binds to the aluminium atoms exposed at the edges of the clay particles. The chemical and physical adsorption of phosphate on to the surface of soil minerals is a rapid process, which in the laboratory occurs mostly within the first few minutes. In addition to this initial fast reaction, slower reactions continue to remove phosphate from solution for periods of several days to several months. Adsorption–precipitation by soils is not necessarily a permanent sink for the phosphorus in wastewater and is partially reversible by a reduction in the phosphate concentration in the water in contact with the soil, by plant uptake or by flushing or dilution with low phosphate concentration water which will release some phosphorus back into solution. The soil therefore regulates the concentration of phosphate in solution. It is interesting that soils which adsorb phosphorus least readily are those very same soils that typically release phosphorus most readily. Peaty material, made up of plant remains, can adsorb minerals by ion exchange and retain them indefinitely. Organic soils are typically low in phosphorus and consequently have a high C:P ratio. The phosphorus content of peat is commonly less than 0.05–0.10%. Microbial immobilisation has been suggested as a mechanism for retention of wastewater phosphorus where the C:P ratio is high. Initial immobilisation of phosphorus will occur in response to wastewater application but as the C:P ratio of wastewater is low compared to peat, continued application would soon satisfy microbial requirement for phosphorus. Therefore, immobilisation by micro-organisms is not likely to play a significant role in the long-term fixation of phosphorus by peat but will be important initially.

The nitrogen cycle in wetlands is extremely complex. Nitrogen exists in a multitude of organic forms and as ammonia, nitrite and nitrate as well as gaseous forms of ammonia, nitrogen and nitrogen oxides (Boyt *et al.* 1976). It is converted from one form to another in wetlands (Fig. 6.12). Denitrification occurs in the anoxic sediments although it occurs much more slowly under acid compared with either neutral or alkaline conditions. Below pH 5, chemical rather than biochemical reactions convert nitrogen to gaseous forms. Algae and bacteria associated with wetlands can fix atmospheric nitrogen into available forms which can reduce the overall removal efficiency of wastewater nitrogen of wetlands. The application to a wetland of secondary wastewater effluent, which typically has a N:P ratio of less than 10 may stimulate nitrogen fixation, although this is dependent on the amount of wastewater applied and other nutrient sources.

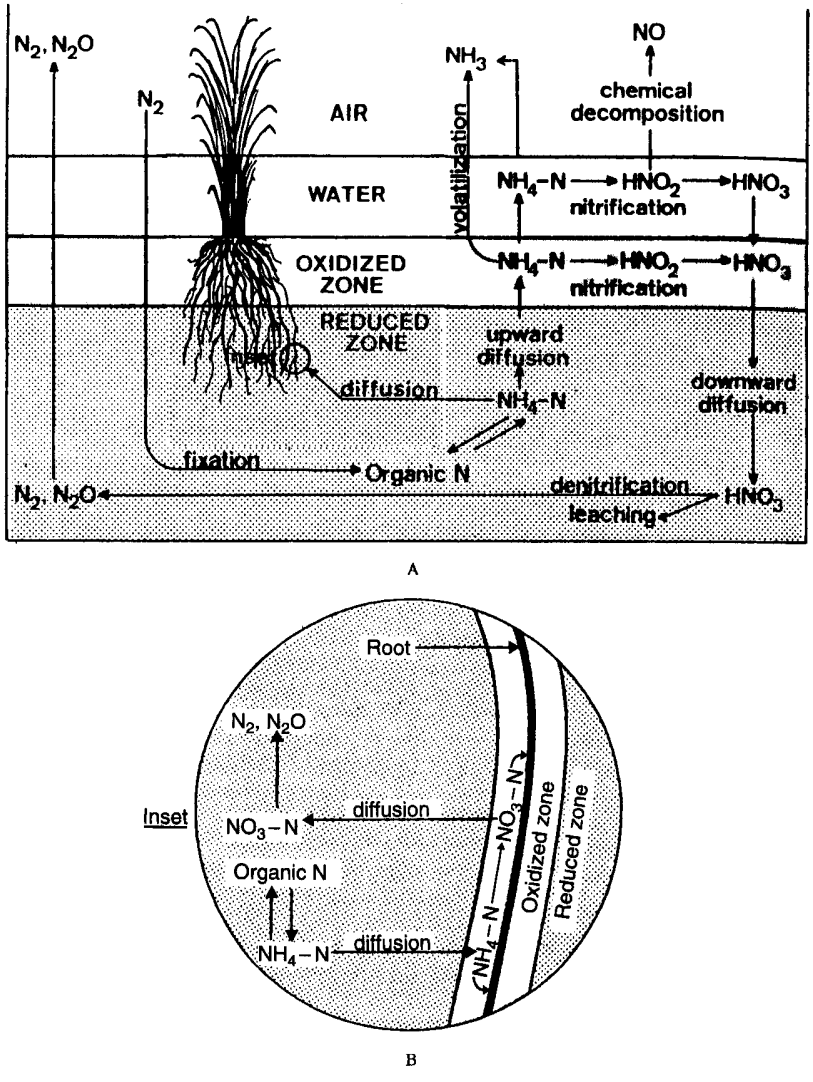


Fig. 6.12. The key steps of the nitrogen cycle in flooded soils and sediments. The major stock of sediment nitrogen is the organic N component, and the rate of release of this nitrogen is largely dependent upon oxygen availability (Reddy and Patrick 1984).

The soil rather than the water is the major source of nutrients for emergent vegetation, non-rooted plants such as algae and duckweeds which may also be present which obtain their nutrients directly from the water, and the subsequent incorporation of their detritus into the soil as a net transfer

of nutrients from the water to the soil. Nutrient retention is greatest during periods of active vegetative growth and is low during the non-growing season. Unless the vegetation can be harvested, much of the stored nutrients will be released during the seasonal cycle of die-back and decomposition. Subsequently, there is often a release of nutrients from the decaying biomass in the winter followed by the rapid release into the water of 35–75% of the phosphorus from the plant tissue, although less of the nitrogen is released. A long retention time is an important factor in many of the physico-chemical processes and also ensures maximum removal of pathogens. Metals present in the wastewater become immobilised in the anaerobic mud, forming metallic sulphides. Release of carbon dioxide and methane from the anaerobic mud will also occur.

Nearly all the water, both precipitation and surface water, entering the wetland leaves it by surface discharge with little entering the ground water strata. The quantity of water leaving the wetland will vary seasonally, with little or no discharge in the summer, but high in the winter and spring. Most natural wetlands contain open channels to enhance the movement of water, and much of the water never comes into contact with either the soil or vegetation, whereas salt marshes may be regularly inundated with high tides. In a natural system, there will be periodic flushes of accumulated solids and nutrients from the area. It is clear, therefore, that a natural wetland must be managed to ensure that the wastewater comes into maximum contact with the soil and the vegetation. During winter, the loading must be reduced as the emergent vegetation dies back and the rate of biological activity of the associated micro-organisms in the rhizosphere is greatly reduced. Loading capacity varies, with a natural wetland able to treat the wastewater from about 100 people ha^{-1}, although the same area can remove the nitrogen produced by 125 people, but the phosphorus from only 25 people (Dinges 1982).

To ensure maximum removal, it is important that the wastewater comes into maximum contact with the soil and vegetation, which can be optimised by ensuring a shallow depth < 30 cm. In essence, this is the basic design objective of constructed wetlands which use long shallow channels or several ponds in series. Nichols (1983) has listed several natural wetlands which receive applications of secondary wastewater (Table 6.7). At low loading rates, wetlands have the capacity to remove much of the phosphorus applied and continue to do so unless the loading rate is increased, when removal efficiency declines rapidly. It is not known how long a wetland can continue to remove phosphorus from wastewater but if sufficient nutrient is added, the adsorption capacity of the soil can become saturated. In addition, a

Table 6.7. Removal of total nitrogen and total phosphorus from wastewater applied to natural wetlands (Nicols 1983).

Types of wetland	Location	Size (ha)	Years wastewater applied	Hydraulic loading (cm y^{-1})		Nutrient loading (g $m^{-2} \cdot y^{-1}$)		Nutrient removal	
				Wastewater	Other	Total P	Total N	Total P	Total N
(1) Shrub sedge fen	Michigan	1[b]	1[c]	70[c]	—	1.7[c]	1.9[c]	95[c]	96[cd]
(2) Forest shrub fen	Michigan	18.2	1[e]	36.8[e]	—	0.9[e]	1.5[ed]	91[e]	75[ed]
			2[f]	74.1[f]	205[f]	2.6[f]	6.5[fd]	88[f]	80[fd]
			3[g]	65.2[g]	183[g]	1.7[g]	9.3[gd]	72[g]	80[gd]
			4[h]	55.7[h]	116[h]	1.8[h]	6.2[hd]	64[h]	77[hd]
			5[h]	57.3[h]	97[h]	1.7[h]	9.3[hd]	65[h]	75[hd]
(3) Blanket bog	Ireland	—	1	i	—	5.0	7.4[d]	96	82[d]
			2	i	—	13.1	15.4[d]	72	87[d]
			3	i	—	8.1	10.3[d]	43	68[d]
(4) Hardwood swamp	Florida	204	20	10.2	83	0.9	—	87	—
(5) Cattail marsh	Wisconsin	156	55	23.4	558	15.2	—	32	—
(6) Cattail marsh	Massachusetts	19.4	69	684	159	7.1	53.6	47	31
(7) Cattail	Massachusetts	2.4	69	5526	—	63.6	428	20	1
(8) Deepwater marsh	Ontario	162	55	231	5569	11.6	78.6	58[j]	41[j]
(9) Glycena marsh	Ontario	20	55	1870	—	77	404	24[j]	38[j]

[a]Secondary effluent. [b]Area affected by study, entire wetland is 710 ha
[c]May–September [d]Inorganic N only, organic N not measured
[e]August–October [f]March–November [g]April–November [h]June–November
[i]Chemical fertilizers, not wastewater applied
[j]Wastewater applied on a year-round basis but percent removal measured during the growing season only. Percent removed would likely have been more calculated on a year-round basis.

wetland soil can release a proportion of the phosphorus previously adsorbed if the concentration of phosphorus in the water is reduced. The nitrogen pattern of wetlands is similar to that for phosphorus, with high removal efficiency, $> 70\%$, at low loading rates (< 10 g N $m^{-2}y^{-1}$), rapidly declining as the loading rate increases. The hydraulic conditions in a wetland can also affect the removal of wastewater nutrients. Higher nitrogen and phosphorus loading rates are obtained by higher hydraulic loadings (Table 6.7), thus retention times in the wetland are reduced and less time for nutrient removal reactions is allowed. The morphology of wetlands is also important, for example, as the depth increases the chance for reactions to occur between wastewater nutrients and the underlying soil decreases. Deeper waters will, however, provide longer retention times than shallower wetlands at the same hydraulic loading.

Spangler *et al.* (1976) evaluated a natural wetland in Wisconsin. Using a 156 ha portion of a marsh with a total surface area of some 18 km^2, the marsh was loaded with a secondary effluent. The BOD was reduced from 26.9 to 5.3 mg l^{-1} (80%), with a 86.2% reduction in total coliforms and a 51.3% and 13.4% removal of nitrate and total phosphorus respectively. They observed that young shoots of emergent vegetation had the highest concentration of phosphorus which decreased as the tissue aged. They concluded that the quality of phosphorus removal could be greatly increased by frequent harvesting as the tissue was not permitted to mature completely or subsequently die and decompose, thereby re-releasing the accumulated nutrients.

A natural littoral reed belt on the southern edge of Lake Borrevannet in south east Norway is being used to prevent nutrients and suspended solids in surface runoff from agricultural land entering the eutrophic lake. Over the monitoring period (1994–1996) the total phosphorus concentration fell by 65%, orthophosphate by 85%, total nitrogen by 44% and suspended solids by 94%. Retention of suspended solids and phosphorus by the reed belt was unaffected by season; whereas total nitrogen retention was higher in the summer (50–85%) than winter (30%) (Bratli *et al.* 1999).

Unlike constructed wetlands, it is not possible, or desirable in terms of conservation, to harvest natural wetlands. Gopal (1999), while recognising the need to restore lost and degraded wetlands, particularly river floodplains, lake littoral zones, and coastal wetlands that reduce pollution from non-point sources, warns of their over-exploitation. He stresses the problems of eutrophication and pollution of wetlands, and sees it as ironical that natural wetlands should have ever been considered as alternatives for wastewater treatment.

Constructed wetlands

As discussed at the beginning of Sec. 6.2, constructed wetlands are categorised as either free water surface (FWS) or subsurface flow (SSF) systems. The former most resemble natural wetlands such as swamps and marshes, and develop a complex and diverse ecology. In contrast, SSF systems are normally monocultures of reeds that do not have permanent areas of open water, thus resembling a fen where the water movement is normally through the soil and not over the surface (Keddy 2000). In contrast to natural wetlands, constructed systems are subject to close control and designed to maximise treatment efficiency and biomass production, with the shape of constructed wetlands made to facilitate harvesting. They can be used in all climates and provide a valuable habitat for wildlife. In temperate climates, they function best in the warmer months. Therefore, there is a need to reduce organic loading during the winter and possibly even store effluent until the following spring. However, research in moderately cold climates, such as Ontario (Canada), has shown they can be operated successfully all the year round and produce good quality final effluents even when covered with ice (Wile *et al.* 1982; Reed *et al.* 1984). In terms of cost, they require: a large area of land; a high degree of labour to construct the bed and plant the vegetation; and periodic harvesting. However, work in the USA has shown that they are much more cost effective on a long-term basis than natural wetlands (Kadlec and Knight 1996).

Free water surface treatment wetlands

Free water surface wetlands utilise emergent macrophytes in two distinct ways. Most commonly, they are planted in shallow basins of soil or other medium with the water level controlled to ensure that the sediment, leaf litter and soil are always submerged and only the living and dead stems of the plants are above the water level. This is similar to a natural marsh with an oxygen gradient formed from the air–water interface with most of the medium anaerobic. Bulrushes (*Scripus* spp.), spike rush (*Eleocharis* spp.), sedges (*Cyperus* spp. and *Carex* spp.) and rushes (*Juncus* spp.) are most frequently used along with cattails (*Typha* spp.) and the common reed (*Phragmites australis*). A shallow depth of the medium, penetrated by roots and rhizomes, and the liquid layer above the medium surface are the major pathways for the wastewater. Young shoots protrude up from the medium into the water. Therefore, a dense matrix of roots develops above and below the water medium interface where a range of biochemical transformations occur (Khatiwada and Polprasert 1999). The main treatment

process is settlement. The influent wastewater spreads slowly through the shallow water, the suspended solids removed by settlement or entrapment by the vegetation and litter. Attached and suspended micro-organisms remove soluble BOD. Nitrification and denitrification occurs in the aerobic and anoxic areas respectively, whereas phosphorus removal is at a low but sustained rate by various sorption, complexation and precipitation reactions (Morshiri 1993; Kadlec and Knight 1996). These systems are particularly attractive to wildlife.

Constructed systems can be lined to prevent exfiltration from the wetland, with the vegetation planted in a shallow layer of gravel. Where the substrate is suitable, emergent vegetation can be planted directly into the soil. Where wetlands are also constructed for wildlife use, then it is important to include a diversity of habitats in the overall design such as areas of deep, as well as shallow water, islands and adequate areas of open water for wildfowl. Such a system has been constructed at Martinez in California, where the wetland was created primarily for wildlife. The system is 6.1 ha in area and has a retention time of 10 days. Some 60% of the wetland is open water with stands of *Scripus* and *Typha*. It has been necessary to use a further 2.1 ha around the edge of the existing wetland for growing plants, which the wildlife using the wetland can use as a food source.

Constructed wetlands function in the same way as natural ones except more nitrogen and phosphorus is lost from the system by harvesting. Such systems have been studied using plastic lined beds 18.5 m $\times$ 3.5 m and 0.76 m deep, with emergent macrophytes grown in gravel (Gersberg *et al.* 1986). Four beds were used to treat primary municipal wastewater from Santee, California. This is a particularly ideal climate for wetlands as the mean air temperature rarely falls below 12°C, and the plants continuously grow throughout the year. Three plants were compared for their ability to treat wastewater, *Scripus validus* (bulrush), *Phragmites communis* (common reed), and *Typha latifolia* (cattail). During the experimental period, the mean ammonia concentration was reduced from 24.7 mg l^{-1} in the influent to 1.4 mg l^{-1} in the bulrush bed, 5.3 mg l^{-1} in the reed bed, 17.7 mg l^{-1} in the cattail bed and only 22.1 mg l^{-1} in the control bed which did not contain any emergent plants. The ability of the bulrush and reed to remove ammonia from water is attributed to the ability of these plants in being able to translocate oxygen from the shoots to the rhizosphere, which stimulated nitrification–denitrification (Armstrong 1964). Similarly, BOD removal was significantly higher in the bulrush and reed beds at 5.3 and 22.2 mg l^{-1} respectively, compared to the cattail bed at 30.4 mg l^{-1}, or the control bed

at 36.4 mg l^{-1}, where the mean BOD of the influent was 118.3 mg l^{-1}. This showed that these two species were able to treat wastewater to an equal or even better quality than conventional secondary treatment and that the plants have a significant function in the removal of nitrogen separate to assimilation into plant tissue. Thus, the mean percentage removal of BOD was 96, 81, 74 and 69% for the bulrushes, reeds, cattails and control bed respectively. The poor performance of cattails can be attributed to the fact that it is less able to transport oxygen to the root zone, although this ability is seen in many similar plants such as *Spartina* and *Zostera*, and has a shallow root zone with most of the root mass confined to the top 30 cm of the substrate compared to > 60 cm for bulrushes and > 75 cm for reeds. This provides less root surface area for the development of heterotrophic as well as nitrifying bacteria. In this study, a hydraulic loading rate of 4.7 cm d^{-1} produced a 10:10 (BOD:SS) final effluent. At this loading rate, 5 ha of wetlands would be able to treat 2300 m^3 of primary wastewater each day.

In Flevoland (the Netherlands), an experimental rush pond of 1 ha in area was constructed to cope with the seasonal influx of tourists at an isolated camping site (de Jong 1976). The experimental pond had a star-shape layout to ensure maximum utilisation of the available area (Fig. 6.13) and was 0.4 m deep. To minimise maintenance, this design was later modified to long narrow channels 375 m in length which could be maintained mechanically. Rushes were rooted in the mud of the unlined basin and the results showed that the BOD of the raw wastewater was reduced by 95.7%,

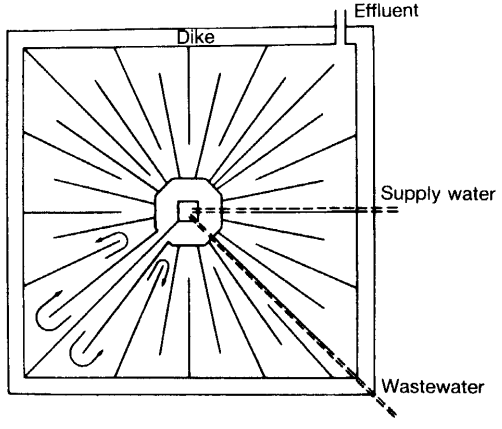


Fig. 6.13. Experimental rush pond covering an area of 1 ha and 0.4 m deep. The star shape was chosen to ensure maximum utilisation of the available area although it made mechanical harvesting and maintenance difficult (Tourbier *et al.* 1976).

Table 6.8. Performance of an experimental rush pond (de Jong 1976).

		Week number					Mean week
		26	28	30	32	34	26–34
BOD	Influent	285	331	347	276	127	257
(mg l^{-1})	Effluent	12	8	18	17	7	11
COD	Influent	661	734	900	590	285	530
(mg l^{-1})	Effluent	48	54	94	83	66	70
Coliforms	Influent	43×10^4	40×10^4	38×10^4	52×10^4	41×10^4	36×10^4
(MPN m l^{-1})	Effluent	1	64	2670	8	14	313

COD by 80.7% and faecal coliforms (MPN) by 99.9% (Table 6.8). Although the removal of nitrogen and phosphorus was rapid initially, the removal efficiency rapidly declined as the supply of nutrients exceeded the uptake rate, with mature rush stands removing between 300–500 kg N $ha^{-1}y^{-1}$ and 50–75 kg P $ha^{-1}y^{-1}$. De Jong (1976) observed that purification was achieved not only by the bacteria associated with plant roots but also by infiltration of the wastewater into the soil. In the channels, the performance of reeds and rushes was compared, and while there was little difference in their ability to purify wastewater, the reeds required less maintenance. However, reeds are harvested annually during the summer, whereas rushes can be gathered only every second or third year. Stem densities are about the same for both groups at 120–150 m^2 but reeds have no commercial value, unlike rushes, which are highly sought after as wicker for basket and furniture manufacture and can also be used for feeding livestock (Pomoell 1976). The total biomass of rushes produced in a treatment pond can reach between 5–10 tonnes $ha^{-1}y^{-1}$.

Spangler *et al.* (1976) compared the efficiency of *Iris versicolor* (the blue flag), *Scripus validus* (soft-stem bulrush) and *S. acutus* (hard-stem bulrush) in bench scale ponds over a winter period. They were planted in pea-sized gravel 70 mm deep in plastic lined basins (800 × 900 mm). Primary effluent was fed into the basins each day during Monday to Friday and retention times of 3, 5 and 15 days were studied. They found that there was no difference between the various ponds, including the control pond without the plants, in reducing BOD or COD. However, the presence of bulrushes made a significant difference in the removal of both total phosphorus and orthophosphate, being more effective than the control or *Iris* ponds. Removal efficiency increased with the longer retention times, with total phosphorus removal reaching 98% on occasion (average > 80%).

The efficiency of four emergent species *Typha domingensis*, *T. orientalis*, *Phragmites australis* and *Scripus validus* to treat the effluent from a poultry abattoir were compared by Finlayson and Chick (1983). The plants were grown in a gravel substrate in plastic lined trenches. Using a retention time of between 2.7–3.6 d^{-1}, the effluent was allowed to percolate through the trenches. All three genera were able to significantly reduce the suspended solids (83–89%) and turbidity (58–67%) of the effluent, whereas *Phragmites* and *Scripus* were both able to oxygenate the anaerobic inflow. *Scripus* was more effective than the other genera in reducing the concentrations of total nitrogen and phosphorus, and also reduced the concentrations of sodium, potassium and chloride. The genus *Typha* comprises of nearly 20 species and three species in particular have been used for treatment studies *Typha latifolia*, *T. domingensis* and *T. orientalis* (Chick *et al.* 1983).

Free water surface ponds are widely used in combined systems with other constructed wetlands, normally SSF beds planted with emergent macrophytes. Wastewater from a restaurant and swimming pool resort in Nairobi (Kenya) is treated by a SSF horizontal flow reed bed planted with *Typha* followed by three FWS ponds planted with indigenous reeds (*Cyperus alternifolius*, *Cyperus latifolius*), ornamental plants (*Arundo donax varigata*, *Pontederia* spp., *Sagittaria* spp.) and flowering ornamentals (*Ajuga remota*, *Aspilia mossambicensis*). Treating a population equivalent of 1200, the system achieves high removal rates of BOD 98%, suspended solids 85%, COD 96%, faecal coliforms 99%, ammonia 92% and orthophosphate 88%. The constructed wetland, as well as being aesthetically pleasing, has attracted over 120 bird species and amphibians (Nyakang'o and van Bruggen 1999).

Although emergent plants are an inexpensive treatment system for many types of wastewaters, some stronger effluents appear to be phytotoxic (Mitchell 1978). Piggery effluents in particular severely affect the vigour of emergent plants, especially *Typha domingensis* (Finlayson and Mitchell 1982; Bowmer 1985).

The second category of FWS treatment wetland employs a range of macrophytes that form floating mats. Cattails, giant sweet manner grass (*Glyceria maxima*), pennywort (*Hydrocotyle umbellata*) and the common reed can all colonise mats of dead plant debris that can become quite thick ($>$ 200 mm) and float on the surface of the water. The mat or raft is stabilised by the rhizomes or roots that intertwine and grow into the water phase (van Oostrom 1995).

Sub-surface flow treatment wetlands

It is in the construction of SSF treatment wetlands that emergent macrophytes are most widely used. The original concept was developed by Seidal and his colleagues at the Max Planck Institute in Germany between 1960–1980 (Seidal 1976). Originally known as the root zone method, the system is now universally known as the reed bed treatment system (RBTS). Reed beds are water tight basins designed to generate either a horizontal (HF) or vertical flow (VF) of wastewater through the rooting medium (Brix 1994b; Reed *et al.* 1995; Vymazal *et al.* 1998; Kadlec *et al.* 2000). The main application of RBTS is the secondary treatment of domestic wastewater from small communities (PE $<$ 500) or individual houses (PE $<$ 10). Very few European systems treat loads $>$ 1000 PE (Vymazal *et al.* 1998). In 1998, there were over 5,000 systems in operation in Europe with 3,500 in Germany alone (Börner *et al.* 1998). Design and treatment guidelines have been published by Copper *et al.* (1996) and also Gschlößl and Stuible (2000).

In horizontal flow, RBTS emergent macrophytes are grown in beds of soil or gravel retained by an impermeable subsurface barrier (clay or synthetic

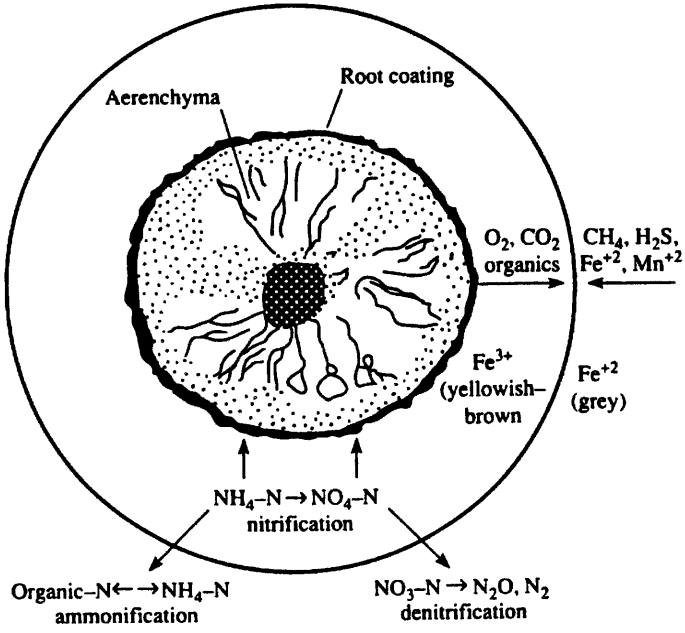


Fig. 6.14. Cross section of an oxidising root growing in a reduced sediment. The oxidised rhizosphere is depicted, along with some processes that occur as a result of this aerobic-anaerobic interface (Mendelssohn and Postek 1982).

liner). The base of the bed has a slope of 2–8% to encourage settled sewage to pass horizontally through the soil (Fig. 6.15). The outlet control pipework is designed to allow the water level in the bed to be adjusted to permit near saturation of the substrate during the establishment of the reeds, but lowering the level as the reeds mature. The depth of the bed depends on the root penetration of the plants used, e.g. cattails 300 mm, reeds 600 mm, bulrushes 750 mm. The most widely employed emergent aquatic macrophytes are *Phragmites communis*, *P. australis* and *Typha latifola*. *Glyceria maxima* and reed canary grass (*Phalaris arundinacea*) are also used in Europe normally in combination with *Phragmites* spp., whereas in the USA, *Scripus* spp. are popular. The substrate and the roots provide a large potential surface area for the growth of heterotrophic micro-organisms (Table 6.9).

The long roots of the vegetation penetrate deep into the substrate.

Table 6.9. Predominant removal mechanisms in reed bed treatment systems.

Wasterwater constituent	Removal mechanism
Suspended solids	Sedimentation
	Filtration
Soluble organics	Aerobic microbial degradation
	Anaerobic microbial degradation
Nitrogen	Ammonification/Nitrification
	Denitrification
	Plant uptake
	Matrix adsorption
	Ammonial volatilisation
Phosphorus	Matrix sorption
	Plant uptake
Metals	Adsorption and cation exchange
	Complexation
	Precipitation
	Plant uptake
	Microbial oxidation/reduction
Pathogens	Sedimentation
	Filtration
	Natural die-off
	Predation
	UV irradiation
	Root excretion of antibiotics

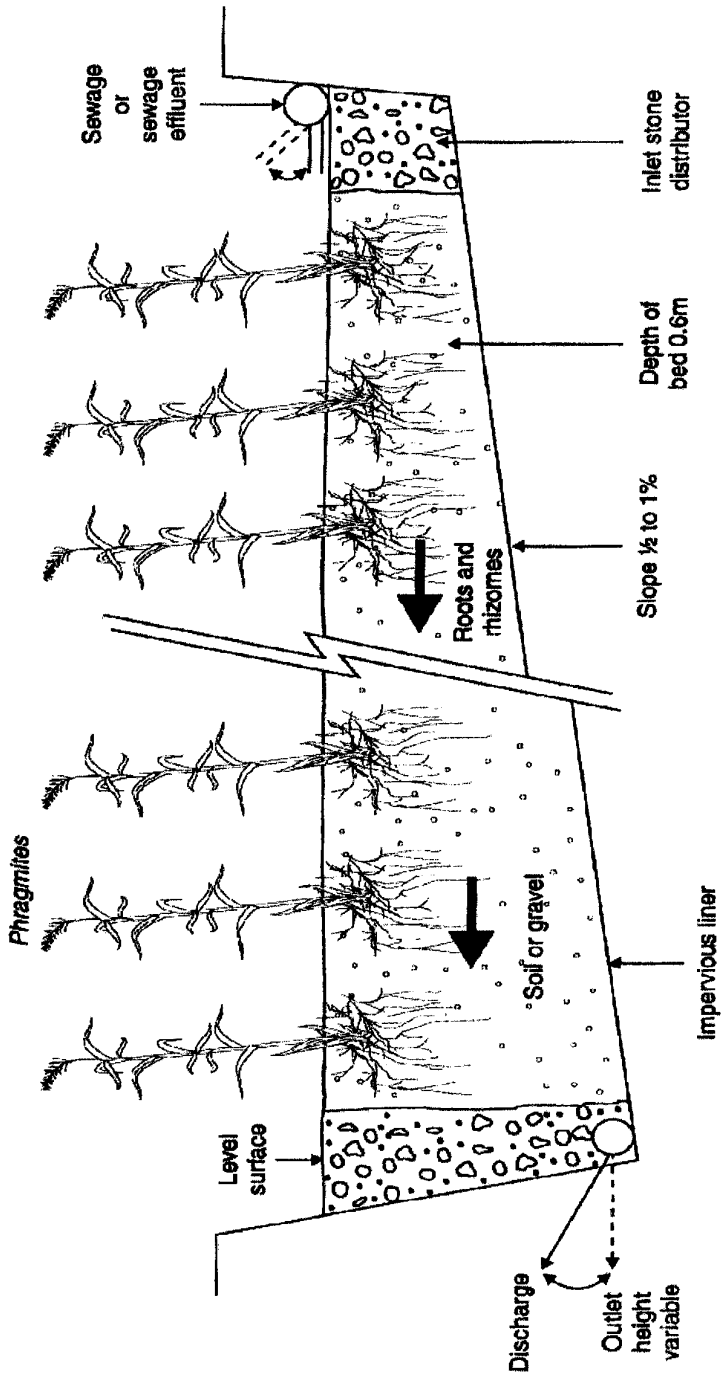


Fig. 6.15. Typical design of a horizontal flow reed bed treatment system (Cooper 1990).

The internal gas spaces of the plant, an adaptation to its partially submerged existence, transport oxygen to the saturated rhizomes and roots (i.e. aerenchyme tissue). Thus an aerobic zone is created within an otherwise water-logged sediment. Within the rhizosphere, there are also anoxic and reduced areas (Fig. 6.14). This combination allows both aerobic and anaerobic bacteria to survive, allowing carbonaceous oxidation, nitrification, denitrification and anaerobic degradation to occur. Ciliate protozoa also play an important role in SSF RBTS. In a study of 4 reed bed systems, Decamp *et al.* (1999) found 22 different ciliate species with the abundance being greatest in the first third and decreasing towards the outlet. The main function of ciliates is to graze the bacteria present. They estimated that grazing rates in gravel media was in the order of 49 bacteria per ciliate per hour. Assuming a mean abundance of 600 ciliates per ml of interstitial liquid, they estimated ciliates by their predatory activities were capable of removing up to 2.35×10^5 *E. coli* per ml of liquid per day in the first third of the bed. Other bacteriavores including rotifers, nematodes and amoebae are also known to remove *E. coli* (Decamp and Warren 1998).

Performance data of European HF systems is given by Coombes (1990) Schierup *et al.* (1990) and Brix (1994b), and North American systems by Kadlec and Knight (1996) (Table 6.10). The plants take up nutrients and store them in their tissues which are removed from the system when harvested. Finally, the substrate has a sorption potential to remove metals from solution. Removal potential exceeds 90% for most metals and these become

Table 6.10. Mean influent and effluent concentrations, and mass loading rates, in gravel-based subsurface horizontal flow constructed reed beds in the Czech Republic (Vymazal 1998b).

Parameter	n	Influent		Effluent	
		Mean	SD	Mean	SD
Concentrations (mg l^{-1})					
SS	37	71.9	47.2	10.8	7.1
BOD_5	39	87.4	65.7	11.9	11.4
TN	26	46.1	18.5	27.6	9.7
TP	27	6.4	3.8	3.1	2.1
Mass loading rates (g $m^{-2}d^{-1}$)					
SS	31	3.34	3.11	0.44	0.42
BOD_5	35	3.36	2.86	0.53	0.67
TN	26	1.39	0.91	0.80	0.16
TP	24	0.30	0.18	0.18	0.16

Table 6.11. Metal removal mechanisms in constructed reed beds.

VEGETATION:	Ion uptake/translocation
	Adsorption
	Organic decomposition
WATER:	Evaporation
	Dilution
	Complex formation
	Decomposition
	Microbial oxidation/reduction
	Precipitation
SUBSTRATE:	Microbial oxidation/reduction
	Ion exchange:
	Precipitation
	Adsorption
	Chelation
	Chemical (inorganic) decomposition
INORGANIC SUBSTRATE AND SEDIMENT:	Microbial oxidation/reduction
	Precipitation
	Adsorption

immobilised in the anaerobic mud layer in the base of the filter as metal sulphides (Table 6.11). Excessive accumulation will eventually inhibit the process. During winter, the emergent part of the vegetation dies back and treatment is restricted to the root zone only, but in practice performance is not severely affected. They do not require daily attention, although during the first year of operation, beds do require weeding, either by hand or by flooding, before the reeds are fully established.

A minimum hydraulic conductance of 10^{-3} to 10^{-4} m s^{-1} is required to prevent surface flow and channelling occurring, therefore the selection of the correct substrate or medium is vital. Once the reeds are established and the rhizomes have penetrated the entire root zone, the hydraulic conductance is secured by the network of micro-channels left after dead rhizomes have decayed (5–15 mm in diameter). Therefore, porosity increases with the age of the bed. The best results are achieved using gravel (5–32 mm diameter) rather than soils, although this medium has a low potential for the removal of heavy metals and phosphorus. Different substrate compositions have different qualities. For example, substrates with a low nutrient content encourage direct uptake of nutrients from waste water by the

plants rather than from the substrate itself. A high Al or Fe content provides effective phosphorus adsorption (Davies and Cottingham 1993). This is achieved by ligand exchange reactions in which phosphate displaces water or hydroxyl ions from the surface of Fe and Al hydrous oxides. Other specific phosphorus-sorbing media are available (Mann and Bavor 1993; Drizo *et al.* 1997; Zhu *et al.* 1997). As normal gravel media does not contain Fe, Al or Ca in any great quantities, removal of phosphorus is generally low (Cooke 1992; Kadlec *et al.* 2000). A high organic or clay content ensures a good removal of heavy metals. Crushed limestone is used in the treatment of acidic waste waters and also gives enhanced phosphorus removal.

Due to their ability to withstand a wide range of operating conditions and their high potential for wildlife conservation, reed beds have become popular with the industrial sector as part of an overall environmental management strategy. However, their main application is in the treatment of domestic sewage from isolated developments such as schools, housing estates, etc. Due to the relatively low loading rates that can be applied, constructed wetlands require large areas of land compared to conventional processes, and thus are normally restricted to treating domestic waste water from small communities. Waste waters require primary settlement before being applied to a reed bed. Average design loading for domestic waste waters is 5 m^2 per capita. The precise area of a HF reed bed required is calculated using Eq. (6.5):

$$A = \frac{Q(\ln C_o - \ln C_1)}{k_1} \tag{6.5}$$

where A is the area of the bed (m^2), Q the influent flow rate ($m^3 d^{-1}$), C_o the influent BOD concentration (mg l^{-1}), C_1 the required effluent BOD concentration (mg l^{-1}) and k_1 the BOD reaction rate constant (Sec. 1.4.2.1). Cooper (1990) has recommended that a k_1 value of 0.1 can be used in most European (temperate) situations. The equation tends to produce an area of 5 m^2 PE^{-1} for secondary treatment HF beds loaded with settled sewage. This will produce a 20:20 effluent but little ammonia reduction. This equation can also be used for HF beds used for tertiary treatment. The area can be reduced to 0.5 to 0.7 m^2 PE^{-1} and will often achieve full nitrification (Cooper 1999). Severn and Trent Water have installed tertiary treatment SSF wetlands at many of their small treatment plants (Green and Upton 1995). Using a design figure of 1.0 m^2 PE^{-1}, a final effluent of < 5 mg BOD l^{-1} and 10 mg TSS l^{-1} has been obtained. This figure has now been revised downward to 0.7 m^2 PE^{-1}. Performance data for a tertiary treatment HF RBTS at Leek Wooton in England is given in Table 6.12.

Table 6.12. Mean annual performance data for Leek Wooton, UK, tertiary treatment horizontal flow reed bed treatment system (Cooper *et al.* 1996).

	BOD_5 (mg l^{-1})		COD (mg l^{-1})		TSS (mg l^{-1})		NH_4-N (mg l^{-1})		TON (mg l^{-1})	
Year	In	Out	In	Out	In	Out	In	Out	In	Out
1990/1991	11.6	4.8	75.7	32.1	27.6	6.1	7.6	5.8	32.8	23.4
1991/1992	11.9	2.0	76.7	34.0	19.1	3.7	5.4	1.9	29.7	20.8
1992/1993	15.4	2.7	109.0	55.5	24.2	5.3	7.0	2.8	20.4	8.7
1993/1994	9.1	1.5	93.8	48.3	16.3	4.4	7.2	3.0	25.6	16.8
1994/1995	9.1	1.0	82.1	46.6	18.4	4.5	6.6	1.9	25.7	18.4

Abbreviation: TON, total oxidised nitrogen (NO_2-N + NO_3-N)

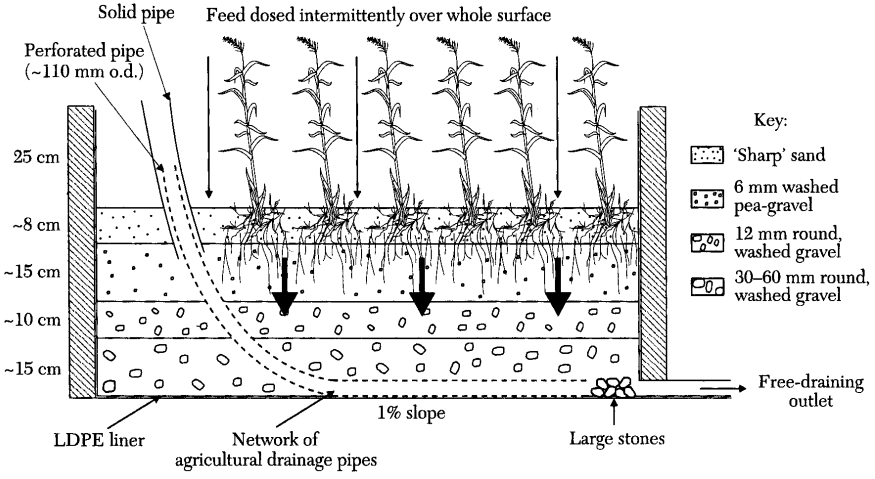


Fig. 6.16. Typical design of a vertical flow reed bed treatment system (Cooper 1996).

During the growing season, between 1.5–1.8 m^3 of water is lost by evapotranspiration per m^2 of fully developed *Phragmites* significantly reducing the effluent volume. Reed beds can be designed to operate with the waste water flowing in either horizontal or vertical direction, with the latter achieving better nitrogen removal overall.

The vertical flow RBTS was also developed by Seidal and the process takes its original name from the institute where the research was done, the Max Planck Institute Process (MPIP). Vertical flow beds are made up of a number of graded layers of gravel media (Fig. 6.16), through which the

wastewater percolates. The surface of the bed must be level and is normally topped with sharp sand. The bed is loaded intermittently with wastewater that floods the surface and slowly drains vertically through the media to a drainage system at the base. The bed is allowed to drain completely to allow the interstices to fill with air. The next dose of wastewater traps this air, ensuring good oxygen transfer into the microbial film that develops on the roots and media. Although the reeds do transfer some oxygen into the root zone via the rhizomes, this is not as important as in HF beds, the majority of oxygen transfer in VF beds provided by the dosing system. Tertiary treatment HF systems produce well nitrified effluents (Green 1997), whereas secondary treatment HF systems do not, due to their limited oxygen transfer capability. In contrast, VF systems provide sufficient aerobic conditions to ensure good BOD oxidation and nitrification. They are considerably more compact (1–2 m^2 PE^{-1}) than HF systems (5–10 m^2 PE^{-1}) for secondary treatment. Vertical flow beds are normally planted with the common reed, although cattails and bulrushes are occasionally used. The precise area of VF reed bed required for small systems (< 100 PE) treating septic tank effluent can be calculated using Eq. (6.6):

$$A_1 = 3.6P^{0.35} + 0.6P \tag{6.6}$$

where A_1 is the area of the first VF bed (m^2), and P the population equivalent. This requires two VF beds to be used in series, with the area of the second bed (A_2) 50% of A_1. This gives an A_1 value ranging from 2 m^2 PE^{-1} at 4 PE to 0.78 m^2 PE^{-1} at 100 PE.

A VF RBTS was used to treat the domestic wastewater from an isolated farmhouse in Schillhuber, Austria (Kadlec *et al.* 2000). After settlement, the wastewater was pulsed four times a day onto a 40 m^2 VF bed designed at 5 m^2 PE^{-1}. The bed was 6.5 m × 6.5 m in area and 0.8 m deep, made water tight with 2 mm polyethylene liner and filled with gravel topped with sharp sand. It was planted with *Phragmites australis* and the wastewater distributed over the surface via a fixed distribution network of PVC pipes with 8 mm holes and splash plates. Removal efficiencies were BOD 97%, COD 91%, NH4 90% and total phosphorus 54% (Table 6.13). Phosphorus removal decreased from 72–< 50% over the five year study due to the limited adsorption potential of the medium.

The progressive loss in phosphorus removal is commonly seen in all constructed wetlands. As the net plant storage capacity becomes stabilised and the sorption capacity of the media is saturated, retention of phosphorus decreases. Continuing retention and accumulation within the bed becomes

Table 6.13. Mean annual performance of a vertical flow reed bed treatment system treating the wastewater from a farmhouse in Wolfern Schillhuber, Upper Austria (Kadlec *et al.* 2000).

	Hydraulic load	BOD_5			COD			NH_4-N			TP		
	(mm d^{-1})	In (mg l^{-1})	Out	Elim. (%)	In (mg l^{-1})	Out	Elim. (%)	In (mg l^{-1})	Out	Elim. (%)	In (mg l^{-1})	Out	Elim. (%)
1992	24	143	11	92	378	54	86	48	8.6	82	10.8	3.0	72
1993	32	186	9	95	533	47	91	88	16.4	81	12.5	3.6	71
1994	27	139	3	98	366	36	90	71	1.3	98	11.5	6.1	47
1995	30	120	3	98	383	30	92	63	4.9	92	14.0	7.0	51
1996	37	157	< 3	99	436	30	93	49	1.5	97	9.4	6.2	34
1997	30	278	< 3	99.6	549	25	95	59	5.5	91	9.6	4.9	49
Average	30	171	5	97	441	37	91	63	6.4	90	11.3	5.1	54

increasingly linked to the retention of organic and inorganic matter from the wastewater, or accumulation of organic matter produced by the macrophytes. This provides new sorption sites for phosphorus. Therefore, future removal is largely determined by the ability of the system to retain organic matter.

Hybrid reed bed systems combine the use of VF and HF beds (Cooper and Maesseneer 1996; Copper 1999). Vertical flow beds are primarily used to provide nitrification and are used in series to provide a high level of BOD and COD removal followed by nitrification. A HF bed normally follows as a final step to remove suspended solids and pathogens. Although VF beds are not efficient at retaining fine solids, they are employed primarily for denitrification, and extra BOD, and phosphorus removal. An example of a multistage system using both VF and HF RBTS is at Oaklands in Gloucestershire, (UK) (Burka and Lawrence 1990). Serving a population

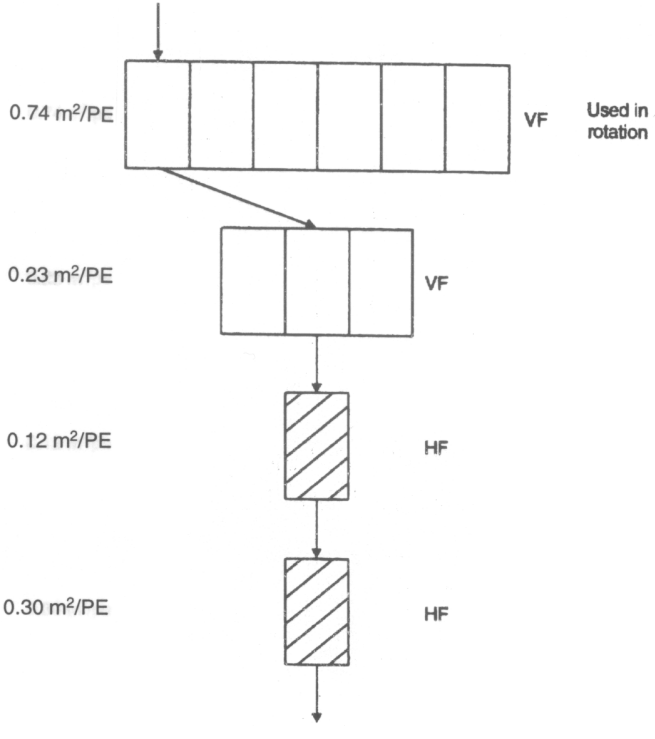


Fig. 6.17. Layout and design loadings of the reed bed treatment system used at Oaklands Park, UK (Burka and Lawrence 1990).

Table 6.14. Mean performance data from the secondary treatment reed bed treatment system at Oaklands Park, Gloucestershire, UK, from August 1989 to September 1991 (Cooper *et al.* 1996).

	Concentration (mg l^{-1})					
		Effluents				
	Influent	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
BOD_5	285	57	14	15	7	11
TSS	169	53	17	11	9	21
NH_4-N	50.5	29.2	14.0	15.4	11.1	8.1
TON	1.7	10.2	22.5	10.0	7.2	2.3
PO_4-P	22.7	18.3	16.9	14.5	11.9	11.2

equivalent of 65, the flow rate was approximately 9.8 m^3d^{-1}. After settlement in a septic tank, the wastewater flows through two VF stages in series followed by two HF stages and finally a pond (Fig. 6.17). Stage 1 consists of 6 parallel VF beds (each 8 m^2) planted with *Phragmites australis*, followed by stage 2 comprising of 3 parallel VF beds (each 5 m^2) planted with a mixture of *Phragmites*, *Iris* and *Schoenoplectus*. The beds are rotated with only one bed in operation in each stage, the others being rested. The flow then passes through a single HF bed of 8 m^2 (stage 3) planted with *Iris* and then another HF bed of 20 m^2 (stage 4) planted with *Accorus*, *Carex*, *Schoenoplectus* and *Sparganium*. The final stage is a pond. Gravel media is used on all the beds with the VF beds graded from the top as 8 cm sand, 15 cm of 6 mm washed gravel, 10 cm of 12 mm round washed gravel and finally 15 cm of 30–60 mm round wash gravel overlying drainage pipes. Details of performance are give in Table 6.14. The VF stages achieve good BOD removal and partial nitrification, the HF stages significant denitrification reducing the total oxidised nitrogen from 22.0 to 7.2 mg l^{-1} (Cooper *et al.* 1996).

6.3. Stabilisation Ponds

Ponds have been widely used as a method of sewage disposal since ancient times. It is possible that castle moats, which received excrement directly from garderobes built into the walls, as well as night soil thrown from the battlements, although primarily defensive devices were also effective oxidation ponds (Porges and Mackenthun 1963). Because such ponds were highly productive, they were often utilised for fish culture.

The development of waste stabilisation ponds (WSP) as a secondary treatment process has been largely accidental, with ponds initially constructed as simple sedimentation basins or as emergency holding tanks at treatment plants. It is only relatively recently that the design and operational criteria needed to successfully operate ponds has been established (US Environmental Protection Agency 1983). They are now accepted as a major treatment process and are used throughout the world, serving populations ranging from $< 1,000$ to $> 100,000$ (Gloyna 1971; Mara and Pearson 1998; Pearson *et al.* 2000). Ponds are a popular alternative to other biological treatment systems in countries where there is plenty of sunshine, and land is both cheap and readily available. They are particularly favoured in Australia (Parker *et al.* 1950, 1959); Africa (Stander and Meiring 1965; Marais 1970a; Mara and Pearson 1998); India (Arceivala *et al.* 1970); Israel (Watson 1962); the USA (Oswald 1963a; Porges and Mackenthun 1963), and Canada (Townshend and Knoll 1987). In the past decade, they have become widely employed in Europe where they are used primarily for treating domestic wastewater from small communities ($<$ 2000 PE), whereas larger sized plants are used for tourist areas where there is a large increase in population during the summer (Vuillot and Boutin 1987; Gomes de Sousa 1987). In France, there are currently 2,500 systems, 1,925 serving populations $<$ 1000 (Racault *et al.* 1995). The largest facility in France covering an area of 40 ha is at Rochefort sur Mer which serves a summer population of 50,000. In the south of France, it is not unusual for WSP systems to serve populations 2–20 times greater in July and August than that served in the winter (Drakides and Caligon 1983). Over twenty WSP systems have been constructed to serve tourist areas in the Algarve region of Portugal (Rodrigues 1997). They are especially popular in rural areas of the USA with 90% of the communities served having a population equivalent of less than 10,000 (Hammer 1977). In Canada there are over 1,000 stabilisation ponds in operation representing approximately half of the wastewater treatment systems in the country. Dependence on ponds increases to over 70% in Alberta, Manitoba, Saskatchewan, the Northwest Territories and the Yukon Territory (Townshend and Knoll 1987).

The terminology and classification of ponds used in the literature is somewhat confused. Hawkes (1983a) classifies a waste stabilisation pond as: "any natural, or more commonly, artificial lentic body of water in which organic wastes (either crude sewage, settled sewage, organic and oxidizable industrial effluents or oxidised sewage effluents) are treated by natural biological, biochemical and physical processes commonly referred to as 'self-purification' or 'stabilisation'". Waste stabilisation ponds are most

conveniently classified by the type of biological activity that occurs, for example whether breakdown is primarily anaerobic or aerobic, and the importance of algae in the process (Fig. 6.18). With the exception of anaerobic lagoons, they are similar to the activated sludge process but differ in that ponds: (i) have a much longer retention period; (ii) have a lower loading rate; (iii) have a less active microbial biomass; (iv) require less aeration due to a lower oxygen demand from the biomass; and (v) the lower aeration results in less mixing and agitation with the result that particulate solids settle and form a sludge layer in which anaerobic breakdown occurs.

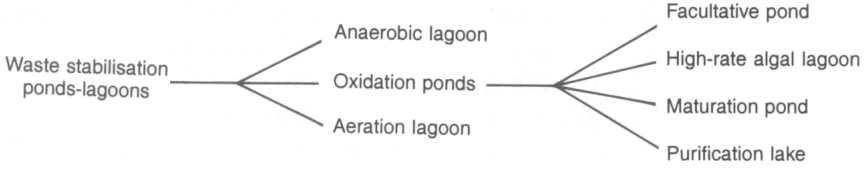


Fig. 6.18. The major types of waste stabilisation ponds.

Table 6.15. Capital and annual operation and maintenance costs, and land area requirement, for a range of conventional and natural treatment systems to treat a rural domestic population of 500 in Germany (Mara and Pearson, 1998).

Treatment proces	Capital costs[a] (DM/person)	O & M costs[b] (DM/M^3)	Land area (m^2/person)
Activated sludge	2,000	2.00	0.3–1 plus 500%[c]
Trickling filter	1,500	1.70	0.4–1 plus 500%[c]
Aerated lagoon	1,200	1.70	4–10 plus 100%[c]
Vertical-flow reedbed	1,200	1.50	1.5–4 plus 100%[c]
Horizontal-flow reedbed	1,500	1.30	6–8 plus 100%[c]
WSP	700	1.20	10–15 plus 50%[c]

[a] 1996 exchange rate: DM1 = 0.52 ecu.
[b] DM per m^3 of wastewater treated.
[c] Additional working area.

Where appropriate, modern WSP have significant advantages over conventional systems. They are simple to construct, operate and maintain, and are extremely robust. Due to long hydraulic retention times, WSP are able to withstand both organic and hydraulic shock loadings better than any other secondary wastewater treatment processes. Their simplicity is reflected by low capital costs, and with normally no energy required for either aeration or allied unit processes, running costs are also low (Burka 1996) (Table 6.15). Land requirements for WSP are, however, significantly higher than more conventional systems. Well designed WSP can produce high quality effluents with particularly good removal of all pathogens including viruses, bacteria, protozoan cysts and helminth eggs (Sec. 9.5.2) (Feachem *et al.* 1983). They are rarely used on their own, rather in a series of anaerobic, facultative and maturation ponds. Anaerobic ponds are used primarily for BOD removal and are so efficient that they reduce the area of other ponds required (Sec. 6.3.1). Facultative ponds also remove BOD as well as nutrients (Sec. 6.3.2). Maturation ponds are used mainly for pathogen removal, although they have a polishing effect on the treated wastewater removing residual BOD, nutrients and suspended solids (Sec 6.3.3).

6.3.1. *Anaerobic ponds and lagoons*

Treatment in lagoons, which are designed to be predominantly anaerobic, relies on the development of a biologically active sludge layer. The sludge takes several months to build up before maximum biological activity is reached. The design criteria for anaerobic lagoons are different, compared with other stabilisation pond systems; the main difference being depth. Oxygen transfer through the air–water interface is not important in anaerobic ponds and is, in fact, undesirable. Therefore, deep basins are used 2 to 5 m in depth to reduce the surface area to volume ratio minimising reaeration and heat loss. Three identifiable zones are observed in lagoons, the scum layer, the supernatant layer which contains about 0.1% volatile solids, and the sludge layer with 3–4% volatile solids (Fig. 6.19).

The scum or grease layer, which forms on the surface of the lagoon can become 40–60 mm thick and has a number of important functions. It insulates the pond, thus preventing heat loss, suppresses odours, and maintains anaerobic conditions in the supernatant by eliminating oxygen transfer between the air–water interface. Although the inlet pipe can be at the surface (Hawkes 1983c), anaerobic lagoons function most efficiently if the influent wastewater enters near the bottom of the basin. This prevents short-circuiting of the liquid and ensures that it mixes with the microbial

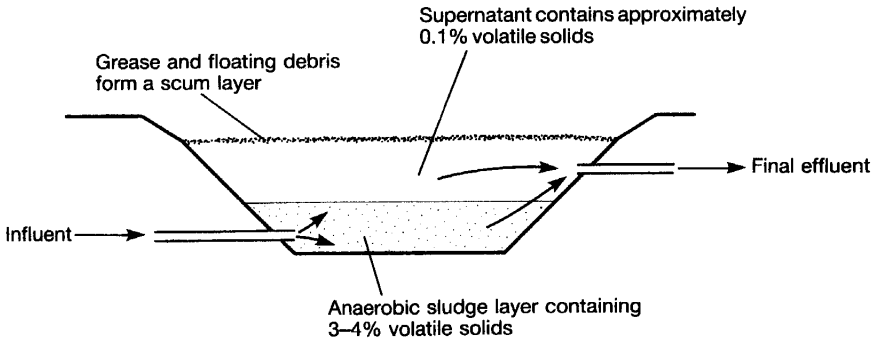


Fig. 6.19. Basic design of an anaerobic lagoon used for treating organically strong wastewaters.

solids in the active anaerobic sludge layer. The flow pattern of the liquid and the constant release of methane and carbon dioxide resulting from degradation of organic matter ensures that some sludge particles remain in suspension with the wastewater. In this way, decomposition of organic matter continues in the liquid phase as well as in the sludge blanket, thus a high mixing efficiency results in a high rate of BOD removal (Parker *et al.* 1950). The discharge pipe is located just below the scum layer at the opposite end of the basin to ensure an upward flow pattern. This encourages the bacterial flocs to settle out of suspension, ensuring a clarified effluent, and also eliminating the need for sludge return by retaining the microbial biomass in the lagoon.

Anaerobic lagoons are generally used as a preliminary treatment for strong organic wastes. This means that the lagoon only partially stabilises the wastewater and secondary aerobic treatment is required before discharge to natural waters, either in the form of facultative ponds in series or activated sludge. However, anaerobic treatment can considerably reduce the organic loading to secondary treatment units, thus reducing the secondary treatment capacity required. If the subsequent aerobic treatment process is a facultative pond, then the sludge will be less likely to rise to the surface in warm weather as the sludge has already undergone anaerobic breakdown. Most industrial ponds operate as anaerobic lagoons and the process is widely used for the treatment of slaughter house waste, sugar, pulp and food processing wastewater (Porges and Brackney 1962; Barnes *et al.* 1984; Vuillot and Boutin 1987). Wastewaters most amenable to anaerobic breakdown in lagoons have a high organic strength, are rich in fats and protein, have a relatively high temperature, are free from toxic materials, especially heavy metals, and contain sufficient biological nutrients.

Slaughterhouse and meat processing wastewaters are particularly suitable for treatment by anaerobic lagoons as they have a high BOD ($\sim 1,400$ mg l^{-1}), grease content (~ 500 mg l^{-1}), temperature ($\sim 28°C$), and a neutral pH. Minimum pretreatment is required prior to discharge to an anaerobic lagoon, which should normally be limited to blood recovery, screening to remove coarse solids, and dissolved air flotation or skimming to remove excessive grease. If pretreatment is too extensive, then insufficient grease may remain in the wastewater to form a scum when discharged to the lagoon. Domestic sewage is not suitable for treatment by this method due to a relatively low temperature, BOD concentration and grease content. When sewage has a high industrial content, anaerobic treatment often leads to serious odour problems due to lagoons becoming facultative instead of strictly anaerobic.

The design loadings for stabilisation ponds are normally measured in BOD per unit area (BOD m^{-2}), but as light is not an important factor in anaerobic lagoons, loading is expressed as BOD per unit volume (BOD m^{-3}) or as a liquid retention time. Hammer (1977) gives mean loading values of 320 g BOD $m^{-3}d^{-1}$ at a minimum temperature of 25°C to achieve 75% removal at a minimum retention time of 4 days. Similar figures are given by Gloyna (1971) who cites examples where longer retention times of up to 19 days are needed for strong organic industrial wastewaters. Using the volumetric loading (A_v), the size of anaerobic lagoons can be estimated using Eq. (6.7):

$$A_v = L_i Q / V_a \text{ gm}^{-3}\text{d}^{-1} \tag{6.7}$$

where L_i is the influent BOD (mg l^{-1} of g m^{-3}), Q the influent flow rate (m^2d^{-1}) and V_a the volume (m^3) of the lagoon (Mara and Pearson 1998). The value A_v increases with temperature but lies between 100 to 350 g $m^{-3}d^{-1}$, the former to maintain anaerobic conditions, the latter to avoid odour release (Table 6.16). Once A_v is selected, the lagoon volume (V_a) is calculated from Eq. (6.7). The mean hydraulic retention time (θ_a) is calculated as:

$$\theta_a = V_a / Q\text{d}\,. \tag{6.8}$$

The major advantages of an anaerobic system are that less sludge is produced compared with an aerobic system and no aeration equipment or power supply is required. The main operating limitations of the process are the temperature and strength of the wastewater. The temperature of the lagoon controls the BOD removal, and below 15°C purification will be due

Table 6.16. Design values of permissible volumetric BOD loadings and expected BOD removal rates for anaerobic ponds at various temperatures (Mara and Pearson 1998).

Temperature (°C)	Volumetric loading (g/m^3d)	BOD removal (%)
< 10	100	40
10–20	$20T - 100$	$2T + 20$
20–25	$10T + 100$	$2T + 20$
> 25	350	70

T = temperature, °C.

to physical settlement only, with no anaerobic breakdown of organic matter occurring. At higher temperatures, anaerobic digestion proceeds rapidly with biogas (70% CH_4, 30% CO2) bubbling to the surface. The same bacterial groups are involved as with other anaerobic systems. Thus, the process is inhibited by the same conditions (e.g. pH < 6.2). In temperate climates, anaerobic lagoons act as settling tanks during the winter months and the accumulated sludge is subsequently degraded during the warmer summer months. This has been demonstrated by Parker *et al.* (1950), who recorded a 65–80% BOD removal in lagoons during the summer with a retention period of 1.2 days which fell to 45–65% removal in the winter, even after 5 days retention. Therefore, special attention should be paid to those factors affecting the temperature of the lagoon such as ambient and wastewater temperature and insufficient scum development for insulation. A well designed anaerobic lagoon will achieve on average 40% BOD removal at 10°C rising to 60% at 20°C. The required hydraulic retention time is a function of temperature and wastewater strength. Low BOD and grease content of wastewater will also reduce scum development; where no scum formation occurs, then a thin surface film of *Chlamydomonas* often develops. Protection from wind is important in preventing mixing and the resultant break-up of surface scum.

Under normal operating conditions, no odours are released due to complete anaerobiosis and adequate scum cover. However, problems of odour because of sulphide formation at low temperatures or high sulphate in the supply water can occur. Sulphate can also be a problem in some papermill wastes that use alum as a sizing agent. With better design, it is now not always critical to have a surface scum as long as sufficiently high organic loading can be maintained to ensure anaerobic conditions.

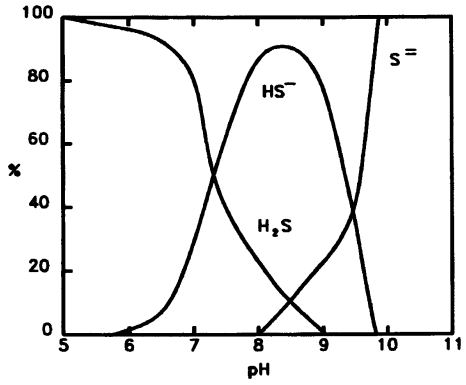


Fig. 6.20. The effect of pH on the equilibrium of hydrogen sulphide-bisulphide-sulphide.

Hydrogen sulphide is present in aqueous solution as hydrogen sulphide gas (H_2S), the bisulphide ion (HS^-), or the sulphide ion (S^{2-}). Thus, for any given total sulphide concentration, the amount present as odorous H_2S is pH dependent (Sayer *et al.* 1964) (Fig. 6.20). Mara and Pearson (1998) explain that designers have been reluctant to use anaerobic lagoons at treatment plants in case of odour formation. They stress that odour can be controlled by not exceeding the design loadings (Table 6.16), and ensuring that influent wastewater sulphate concentrations are < 500 mg SO_4 l^{-1}. Lagoons are now being covered, not only to guard against odour release, but to recover methane (Green *et al.* 1995; Hodgson and Paspaliaris 1996). Sulphide reacts with heavy metals to form insoluble metal sulphides, but if concentrations exceed < 50 mg l^{-1} there is a progressive inhibition of methanogenesis (Pfeffer 1970).

No odour should arise from a lagoon if the liquid temperature is above 20°C. The scum layer can become a breeding ground for a variety of flies and in warmer climates, this can become a serious nuisance. The operation of anaerobic lagoons in series is not recommended as it is difficult to maintain an adequate scum cover on the second lagoon. The reduced loading results in the surface layer of the lagoon becoming aerobic, thus the system becomes facultative rather than strictly anaerobic. Under-loaded lagoons will also tend to become facultative ponds.

6.3.2. *Oxidation ponds*

The oxidation pond is designed to provide aerobic breakdown of organic matter in wastewater, with a significant proportion of the dissolved oxygen

provided by photosynthetic algae. Three types of stabilisation ponds fall into this category: facultative ponds; high-rate aerobic lagoons; and maturation ponds. All three are different from each other and are treated separately below. A fourth type of oxidation pond, the purification lake, is also considered.

Facultative ponds

Facultative stabilisation ponds are characterised by having an upper aerobic and lower anaerobic zone, with active purification occurring in both. They are the commonest type of stabilisation pond in use being able to completely treat both crude and settled sewage, as well as a wide range of organic industrial wastewaters. Facultative ponds are often categorised as either primary or secondary ponds, treating raw or settled wastewaters respectively. As organic matter enters the basin, the settleable and flocculated colloidal matter settles to the bottom to form a sludge layer where organic matter is decomposed anaerobically. The remainder of the organic matter, which is either soluble or suspended, passes into the body of the water where decomposition is mainly aerobic or facultative, although it is occasionally anaerobic. The principles of the pond are summarised in Fig. 6.9. Organic matter in solution or suspension is broken down principally by aerobic and facultative bacteria, which releases nitrogen and phosphorus compounds and carbon dioxide. Algae use these inorganic compounds for growth using sunlight for energy and releasing oxygen into solution. The dissolved oxygen is then utilised by the aerobic bacteria, thus completing the symbiotic cycle. Oxygen is also introduced by natural oxygen transfer, the rate of which is increased by turbulence caused by wind action.

In the sludge layer, the settled solids are anaerobically broken down, with methane, nitrogen and carbon dioxide being released along with a variety of soluble degradation products. The gases escape to the atmosphere, and in a facultative pond up to 30% of the BOD load can be dissipated as gas production. The soluble degradation products, such as ammonia, organic acids and inorganic nutrients are also released, and subsequently oxidised aerobically in the water layer. The hydrogen sulphide released from the deposited sludge is also oxidised in the water zone, thus preventing odours from being released. Anaerobic degradation is temperature-dependent and no significant activity occurs in the sludge below a water temperature of 17°C. A four-fold increase in activity occurs in the sludge with every 5°C rise in the temperature, over the range of 4°–22°C (Gloyna 1971). Digestion rate of the sludge, expressed as the rate of gas production

(K_s), at a particular temperature (T) can be estimated by:

$$K_s = 0.002(1.35)^{-(20-T)}$$

Aerobic and facultative bacteria are the primary decomposers in the pond system. Although fungi are also present in ponds (Cooke 1963), they are not important and are thought to be restricted due to the high pH caused by the photosynthetic activity of the algae (Arceivala *et al.* 1970). The dominant bacteria found are similar to those isolated from other aerobic treatment systems, and belong to the genera *Pseudomonas*, *Achromobacter* and *Flavobacterium*. Apart from obligate aerobes, other types of bacteria such as coliform bacteria, methane bacteria, sulphate reducers and the purple sulphur bacteria such as *Thiocapsa floridana* and *Chromatium vinosum*, are all common (Holm and Vennes 1970; Pike 1975). Laboratory-based studies using experimental ponds have demonstrated that the degree of illumination has no effect on bacterial activity and that BOD removal is related to bacterial density. According to Uhlmann (1969), the amount of solids synthesised, however, which was largely algal biomass, was proportional to the degree of illumination and an increase in algal biomass resulted in an increase in nitrogen and phosphorus incorporated into the sludge. In these experimental ponds, oxygen was never limiting, even in complete darkness, whereas oxygen is normally a limiting factor in full size ponds. Therefore, the full importance of algae in the euphotic zone was not demonstrated. The oxygen for aerobic degradation is mostly supplied by the photosynthetic activity of the phototrophs.

Although there is a succession of dominant algal species during the year, generally only one or two species will be dominant at any one time in a facultative pond. Porges and Mackenthun (1963) found a striking similarity between the algal species present in facultative ponds throughout the US and concluded that geographical location had little effect on speciation. The most commonly recorded genera are: *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Micractinium, Euglena*, *Ankistrodesmus*, *Oscillatoria*, and *Microcystis* (Table 6.17). Motile genera such as *Chlamydomonas*, *Pyrobotrys*, and *Euglena* can alter their vertical position in the water column to take maximum advantage of light intensity and temperature, unlike non-motile genera such as *Chlorella*. The dominant algal species is determined by the organic loading with those algae able to tolerate anaerobic conditions being recorded in ponds receiving heavy organic loads e.g. *Chlamydomonas* spp. and *Euglena* spp. However, photosynthesis of many of the algae commonly found in ponds is inhibited by high ammonia conditions. The oxygenation

Table 6.17. Presence and absence of key algal genera in different waste stabilisation ponds (Mara and Pearson 1998).

Algae	Facultative Ponds	Maturation Ponds
Euglenophyta		
Euglena	+	+
Phacus	+	+
Chlorophyta		
Chlamydomonas	+	+
Chlorogonium	+	+
Eudorina	+	+
Pandorina	+	+
Pyrobotrys	+	+
Ankistrodesmus	⊗	+
Chlorella	+	+
Micractinium	⊗	+
Scenedesmus	⊗	+
Selenastrum	⊗	+
Carteria	+	+
Coelastrum	⊗	+
Dictyosphaerium	⊗	+
Oocystis	⊗	+
Rhodomonas	⊗	+
Volvox	+	⊗
Chrysophyta		
Navicula	+	+
Cyclotella	⊗	+
Cyanophyta		
Ocsillatoria	+	+
Anabaena	+	+

+ = present; ⊗ = absent

capacity of different algae varies appreciably. For example, blue green algae, e.g. *Anabaena variabilis*, *A. cylindrica*, *Phormidium faveolarum*, and *Calothrix membranacea*, are less efficient than green algae in ponds, with *Chlorella* spp., and *Scenedesmus* spp. being desirable because of superior oxygenating capability. There is also some evidence that certain algae are capable of growth by photo-assimilation of organic compounds. Utilisation

of organic matter as a supplementary source of carbon normally only occurs when light or carbon dioxide are limiting (Hawkes 1983c).

However, the main function of algae is as phototrophs, producing oxygen to maintain the aerobic condition of the pond. A supplementary role, but a very important one, is the removal of plant nutrients such as nitrogen and phosphorus. The facultative pond is the most effective of all the conventional biological treatment processes in removing these nutrients, and thus reducing eutrophication in receiving waters, without tertiary treatment. Apart from nutrient uptake to satisfy the normal nutrition requirements of algae, some species are able to take up nutrients, especially phosphorus, far in excess of their own requirements, which is known as 'luxury uptake'. Nutrients are also precipitated out of solution as a consequence of the pH change brought about by photosynthesis, which reduces the concentration of carbon dioxide in the water. Above pH 8, phosphates are precipitated out as calcium phosphate and at higher pH values, nitrogen can be lost as ammonia. However, above pH 9, the conditions are no longer optimal for normal aerobic and facultative bacterial activity. Maximum dissolved oxygen concentrations reach a peak in mid afternoon, falling to a minimum during the night as photosynthesis ceases but respiration continues. During periods of rapid photosynthesis, algal demand for carbon dioxide exceeds that produced by bacterial respiration. At this point, carbonate and bicarbonate ions dissociate to produce carbon dioxide, which is used by the algae, and hydroxyl ions, which accumulate, raising the pH even further to 10 and above.

$$2HCO_3^{2-} \longrightarrow CO_3^{2-} + H_2O + CO_2$$
$$CO_3^{2-} + 2H_2O \longrightarrow 2OH^- + CO_2$$

Once photosynthesis declines, free carbon dioxide accumulates, reversing the above reactions and returning the pH to normal. This is a major mechanism for the destruction of faecal bacteria in ponds.

Due to the absence of suitable surfaces for the attachment of nitrifying bacteria, nitrification is largely absent from facultative ponds. Unless there is nitrate in the influent wastewater, denitrification is also negligible. However, removal rates of 80% for total nitrogen and 95% for ammonia are possible. Where BOD removal is 90%, then removal efficiencies of total phosphorus can be as much as 45% (Huang and Gloyna 1984). Although no models are available to predict phosphorus removal, Pano and Middlebrooks (1982) and Reed (1985) have produced equations to predict the removal of ammoniacal nitrogen (Eqs. (6.9) and (6.10)) and total nitrogen (Eq. (6.11)) respectively in both facultative and maturation ponds.

Removal of ammoniacal nitrogen at temperatures below 20°C:

$$C_e = C_i/\{1 + [(A/Q)(0.0038 + 0.000134T) \times \exp((1.041 + 0.044T)(pH - 6.6))]\} \quad (6.9)$$

Removal of ammoniacal nitrogen at temperatures above 20°C:

$$C_e = C_i/\{1 + [5.035 \times 10^{-3}(A/Q)][\exp(1.540 \times (\text{pH} - 6.6))]\} \quad (6.10)$$

where C_e is the ammoniacal nitrogen concentration in pond effluent (mg N l^{-1}), C_i is the ammoniacal nitrogen concentration in pond influent (mg N l^{-1}), A the pond area (m^2), T the temperature (°C), and Q the influent flow rate ($m^3 d^{-1}$).

Removal of total nitrogen:

$$C_e = C_i \exp\{-[0.0064(1.039)^{T-20}][\theta + 60.6(\text{pH} - 6.6)]\} \quad (6.11)$$

where C_e is the total nitrogen concentration in pond effluent (mg N l^{-1}), C_i is the total nitrogen concentration in pond influent (mg N l^{-1}), T the temperature (°C) (range 1–28°C), and θ the hydraulic retention time (d^{-1}) (range 5–231 d).

The pH in Eqs. (6.9)–(6.11) can be estimated using Eq. (6.12):

$$\text{pH} = 7.3 \exp(0.0005\mathbf{A}) \quad (6.12)$$

where $\mathbf{A}$ is the influent alkalinity (mg $CaCO_3$ l^{-1}).

The purple photosynthetic sulphur bacteria of the family *Thiorhodaceae* can occur in significant numbers in facultative ponds. They oxidize sulphides using carbon dioxide as a hydrogen acceptor and so no oxygen is liberated. Unlike other bacteria, they do not contribute to the breakdown of organic matter, they synthesize it, depositing sulphur granules in the cell. However, by reducing the sulphide concentration in the pond, they do alleviate odours. As the group requires light, anaerobic conditions and the presence of sulphides, which are produced from protein degradation in the anaerobic sludge layer, they occur at the lowest depth in the pond where light can penetrate and as near the anaerobic sludge blanket as possible. The photosynthetic bacteria generally utilise longer wavelengths than the green algae and so are found at depths below the algal zone. However, as the population density of the photosynthetic bacteria increases, they extend to the upper layers excluding the algae due to the increased turbidity. It is at this stage that the pond will change from the normal green to brown colour to pink, red or brown (Pike 1975).

Unlike the other aerobic biological treatment processes, protozoans, except the phototrophic flagellates, do not appear to play a significant role in the process (Curds *et al.* 1968). Protozoa are unable to significantly reduce the number of algae in the final effluent (Taub 1968) and are out-competed for the dispersed bacteria by the filter-feeding Cladocera which also feed on the protozoans. Ponds have a range of zooplankton organisms including Rotifera, Copepoda and Cladocera. However, Cladocera are by far the most abundant group found in facultative ponds although Rotifera and Copepoda dominate in maturation ponds.

Cladocera are found in ponds with a retention period in excess of 10 days, and under suitable conditions can develop large populations, which can significantly affect the operation of the pond, not always beneficially (Tschortner 1967). The cladoceran population affects the pond by means of three activities: (i) by feeding off the bacteria; (ii) by feeding off the algae; and (iii) by the formation of boluses. By feeding on bacteria and suspended particulate matter, Cladocera contribute directly to the stabilisation process as well as clarifying the effluent by reducing the density of dispersed bacteria (Uhlmann 1969). Although they may help to maintain the bacterial population in an actively dividing phase, thus enhancing the rate of carbon metabolism in the pond, Cladocera do not appear to significantly reduce bacteria numbers overall (Loedolff 1965). The rejection of food in the form of settleable boluses is probably more important in the clarification process than direct predation, especially when turbidity is high. Most cladocerans in oxidation ponds are filter feeders. Water is forced, by the rhythmic beating of numerous flat limbs, to pass between barbs on the setae fringing the limbs, which act as a filtering apparatus. Particulate suspended matter, including bacteria and even colloidal solids, are filtered out of the water and collect in a food groove where it is concentrated before ingestion. If excess food is collected, which is normally the case when the pond is turbid, it is ejected from the food grooves as a bolus and rapidly settles because of its dense nature. Differences in the distance between the setae and barbs determine the size of particles collected by each species. For example, *Moina dubia*, which is a bacterial feeder, is more efficient at straining the bacteria *Escherichia coli* than *Daphnia magna*, an algal feeder, due to the difference in the coarseness of filtering setae (Loedolff 1965).

Although some Cladocera readily utilise small unicellular algae such as *Chlorella*, it is doubtful whether any species can feed on the larger algae such as diatoms, blue-green algae, or the filamentoids to any great extent. Unlike the filter feeding Cladocera, copepods seize and bite their food, being capable of utilising even filamentoids. Effects of algal grazing can be beneficial

by reducing the density of algal cells in the effluent and by reducing the biomass. Alternatively it can be detrimental, as reduced algal density will reduce the rate of reaeration of water, especially at higher temperatures when bacterial oxygen demand is greatest and solubility of oxygen is at its lowest. In India, blooms of Daphnids feeding on *Chlorella* have resulted in low dissolved oxygen concentrations due to reduced photosynthesis (Lakshminarayana 1965; Bodpardikar 1969). However, the daphnid population was controlled by the addition of lime. The role of Cladocera and the other zooplankton in controlling algae is unclear. Seasonal fluctuations in population and species dominance have been identified, and a general decrease in cladoceran numbers observed in the winter. It has been proposed that seasonal fluctuations in solar radiation and temperature, which affects algal activity, subsequently affects the cladocerans by altering the oxygen balance, pH and food supply. A more detailed summary of the microbial ecology and the major biochemical interactions that occur in facultative ponds is given in Fig. 6.21.

Suggestions have been made to utilise the zooplankton either by harvesting the cladocerans for sale as fish food (Dinges and Rust 1972) or by incorporating fish into the pond to feed directly on Cladocera, thus reducing the biomass and providing a more readily harvested end-product. The fish *Tilapia mossambica* is particularly successful in facultative ponds in warm climates as it feeds not only on the zooplankton and detritus, but also controls mosquitoes and other flies. However, it has been shown that the mortality constant for faecal coliforms decreases proportionally as the fish population increases in both facultative and maturation ponds. This is due to the reduction in the algal biomass by fish grazing and the potential elevation of the pH during the day (Rivas Hernandez 1997).

Several groups of invertebrates are present. In the sludge, benthic nematode worms, tubificid worms and chironomid larvae are all common (Gloyna 1971). The worms contribute to the stabilisation of the organic sludge, and both the worms and midge larvae increase the rate of exchange of materials between the sludge and water at the solids–water interface. The dominance and abundance of species is related to the dissolved oxygen conditions of the sludge (Kimerle and Anderson 1971) and the organic loading (Kimerle and Enns 1968).

It is important to prevent the development of surface mats of filamentous algae, or thin scum of encysted unicellular or blue-green algae (Hartley and Weiss 1970). Such growths seriously reduce the light penetration and interfere with the mass transfer of oxygen into the pond from the atmosphere. Infestations of surface dwelling macrophytes such as *Lemna* have

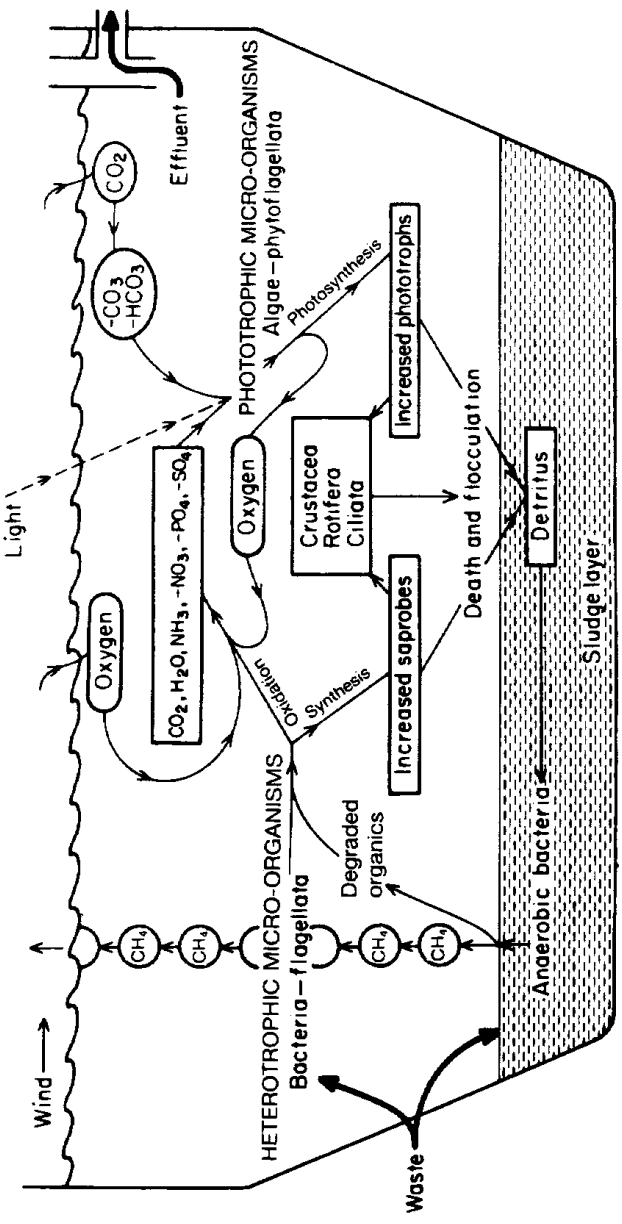


Fig. 6.21. Summary of the heterotrophic and phototrophic activities in a facultative pond that result in the complete stabilisation of organic waste (Hawkes 1983a).

the same adverse effects (Hawkes 1983c). Surface material will eventually collect either on the banks or in the corners of ponds due to wind action and unpleasant odours are produced as it decomposes.

Apart from nutrient and carbon dioxide availability, temperature and solar radiation are the major factors affecting algal photosynthetic activity. Most algae grow over a wide temperature range 4–40°C (Arceivala *et al.* 1970) with optimum growth for the green algae (Chlorophyceae) about 20°C but decreasing at 35°C. Above this temperature, other algae become dominant, such as the blue-green algae (Cyanophyceae) and euglenoids. Although different algae have different light intensity optima, a high light intensity is not necessary for the successful operation of a facultative pond. For example, ponds operate successfully throughout the Indian subcontinent from latitudes 8°N to 34°N although different organic loading rates are required for ponds from different latitudes to allow for the variability in temperature and solar radiation, both of which affect the rate of microbial action (Arceivala *et al.* 1970). Light penetration can be restricted by high densities of phytoplankton ($> 1 \times 10^6$ ml) in the surface layer of the pond. In many ponds, optimum light intensity for the algae occurs at < 30 cm, although effective photosynthesis may not exceed 45 cm under such conditions. Therefore most of the pond is in darkness, and while heterotrophic activity is independent of light, mixing is required to ensure that the rest of the pond depth is kept aerobic. Mixing, which can occur naturally in shallow ponds by wind or in deeper ponds by currents induced by temperature differences, also ensures the transfer of materials between the photosynthetic and bio-oxidation phases, which ultimately governs the rate of stabilisation. In dense algal layers, the water quickly becomes supersaturated during the day with the excess dissolved oxygen being lost by transfer to the atmosphere unless mixed with the deeper unsaturated water. The circulation of algae in and out of the light ensures that a larger biomass of algae is supported and that more oxygen is made available to the bacteria. This is because algal cells fix a higher proportion of light energy under intermittent exposure to light compared to algae under constant exposure.

Aerobic bacterial breakdown is less sensitive to temperature than anaerobiosis, and can proceed effectively over a range of 10–35°C. However, if the water temperature is only a few degrees above freezing, then the organic matter entering the pond will rapidly accumulate. Microbial activity will be reduced even further by ice and snow cover, which reduces light penetration and also effectively stops phototrophic production of oxygen, and also prevents oxygen transfer from the atmosphere normally helped by wind action.

The major design function of facultative ponds is to maintain dissolved oxygen in the basin, provided by photosynthetic activity of the algae, to allow heterotrophic activity to proceed at an optimum rate. The site for such a pond should be at least 0.5–1 km from residential areas and, because of the production of odours during the spring, it should be sited preferably downwind. It should be an exposed site to ensure maximum wind sweep over the surface of the pond, which is a function of wind speed and distance, to encourage mixing. Thus, planting trees or landscaping the site to shield the pond from view will severely reduce mixing. Leaf fall will also reduce light penetration. The shape of the pond is not important as long as the influent mixes rapidly with the pond supernatant (Pearson *et al.* 1995). Uniform flow throughout the pond is vital, therefore peninsulas and inlets should be avoided and corners of ponds should be rounded. If a rectangular pond is used, then the length should not be greater than 4 times its breadth. The pond should be located to ensure that its longest section lies in the direction of the prevailing wind to ensure maximum wind induced mixing of the surface layer of the pond. If variable seasonally, then the wind direction in the hot season should be used to minimise thermal stratification. Depth is a critical factor. Ponds range from between 0.7–1.5 m in depth with a freeboard of 0.9–1.5 m above the high water level to accommodate wave action. A minimum depth of 0.7 m has to be maintained to prevent the growth of rooted aquatic weeds, which not only damages the lining of the pond and affects circulation, but also encourages mosquitoes and other flies. The recommended depth is between 1.0–1.5 m. Depths in excess of 1.5 m may create odour problems due to excessive anaerobiosis. The sides should slope gently, a maximum slope of 1 in 3 is recommended to prevent earth movement, and should be covered with paving slabs to prevent erosion by wave action and the growth of marginal vegetation. If the soil is not impervious, then it must be lined with bentonite clay or plastic to prevent ground water pollution. The influent enters ponds horizontally at about 0.5 m above the bottom to induce a mixing current. The outlet is positioned to maximise mixing and avoid short circuiting. By ensuring that the wastewater flow is against the wind, short-circuiting is minimised. Simple baffles can also be used to enhance the plug flow nature of the pond and to minimise short-circuiting; although care must be taken in their precise location to avoid excessively high BOD loading in the inlet zone resulting in odour production. Grit and screenings are generally removed from the wastewater before entry into the pond which ensures that the sludge accumulates slowly over the years. Accumulation rates range from 10 to 90 mm per year, and depending on the rate, ponds will only require desludging

after long intervals. If ponds are operated in series, then the majority of sludge will accumulate in the primary pond, especially near the inlet (Nelson and Jiménez 2000). As accumulation will reduce the capacity of the pond over the years, the rate of accumulation must be taken into account when designing the pond depth and retention period.

Photosynthetic activity is determined by the light entering the basin which in turn is a function of the surface area of the pond. Organic loading is therefore expressed as kg BOD per hectare per day (kg BOD $ha^{-1}d^{-1}$) or g BOD per square metre per day (g BOD $m^{-2}d^{-1}$). The loading is limited by specific environmental factors such as insolation, temperature, and wind, that vary according to latitude. Therefore, different loading rates are recommended in temperate zones compared with tropical zones (Table 6.18). The maximum loading rate is limited by the need to maintain aerobic conditions in the pond during the most difficult season of the year when insolation and temperature are at their lowest and in temperate zones ice formation occurs. Although ice formation reduces the radiation reaching the algae, snow accumulation will rapidly eliminate all light until photosynthesis is inhibited and the pond becomes anaerobic. Severe odours are produced as the ice breaks up due to anaerobic activity and although the odours become less severe they continue to be given off until the algae are re-established and the pond becomes aerobic once more. This may take up to 4 weeks depending on temperatures, solar radiation and the amount of accumulated organic matter (Porges and Mackenthun 1963). The duration of odour production is a function of BOD loading and duration of ice cover (Svore 1968). To minimise this period, the organic loading can be reduced

Table 6.18. Generalised loading and design criteria for facultative ponds constructed in different climatic zones (Gloyna 1971).

Surface Loading (kg BOD $ha^{-1}d^{-1}$)	Population per ha	Detention (days)	Environmental conditions
< 10	< 200	> 200	Frigid zones, with seasonal ice cover, uniformly low water temperatures and variable cloud cover
10–50	200–1000	200–100	Cold seasonal climate, with seasonal ice cover and temperate summer temperatures for short season
50–150	1000–3000	100–33	Temperate to semi-tropical, occasional ice cover, no prolonged cloud cover
150–350	3000–7000	33–17	Tropical, uniformly distributed, sunshine and temperature, and no seasonal cloud cover

and the retention period increased, which of course results in the pond being underloaded for the rest of the year (Marais 1970). This problem, as well as a reduction in BOD removal as the temperature falls due to reduced bacterial activity, can be partially overcome by slowly reducing the pond level in the autumn and early winter when there is adequate dilution flow in the receiving waters. This allows the discharge from the pond to be minimal or stopped completely during winter so that the influent is stored until spring when discharge from the pond can be recommenced, as the aerobic activity is able to reduce the BOD of the effluent to an acceptable level. Little anaerobic activity occurs in the sludge during the winter, so no soluble degradation products are released to enhance the overall BOD of the stored wastewater. However, the sludge gradually accumulates as sewage is continued to be discharged to the pond, and it is not until the summer at the enhanced temperatures that there is a net reduction in sludge volume due to the increased rate of degradation. The increase in soluble degradation products released from the sludge layer results in a rise in the BOD of the supernatant, although no fluctuation in effluent BOD is recorded due to the enhanced heterotrophic activity at the warmer temperatures, which utilises the increase in organic load. The BOD of the stored wastewater in the pond remains almost constant throughout the year, whereas the actual oxygen demand on the pond varies considerably as it is a product of both the BOD and the rate of degradation. Therefore, at higher temperatures the oxygen demand can increase several fold due to the higher degradation rate although the BOD remains constant. This provides a problem to the operator, because at temperatures in excess of 20°C the oxygen requirement of the pond cannot be determined by the standard BOD_5, nor can BOD_5 be used as an indicator of the onset of anaerobiosis (Hawkes 1983a). The use of stabilisation ponds in cold climates has been the subject of a workshop organised by the Environment Protection Directorate of Environment Canada (Townshend and Knoll 1987).

In the northern states of America, where the climatic conditions are similar to those of Western Europe, the maximum loading permissible is 2.2 g BOD $m^{-2}d^{-1}$ if an odour nuisance is to be avoided in the spring. Where ice coverage of the pond does not occur, this loading can be increased, and in the southern and south-western states of America, loadings of up to 5.6 g BOD $m^{-2}d^{-1}$ are used. However, this is only equivalent to a volumetric loading of 0.0015–0.0037 g $m^{-3}d^{-1}$ for a 1.5 m deep pond, which is a much lower organic loading compared to the activated sludge or percolating filtration process. Retention time depends on the load, depth of the pond, evaporation rate, and loss by seepage, but periods of 3–6 months are

common for ponds in non-tropical locations. Ponds normally achieve a 90% reduction in BOD, which is less efficient compared with other biological treatment processes. Larger ponds are more effective because of the greater wind sweep over the surface, which enhances mixing. Generally, ponds are operated in series to prevent short-circuiting, to provide a greater reduction in pathogens (Sec. 9.5) and an increased BOD reduction. The first pond in series has a higher organic load and sludge accumulation compared with the other ponds, which may lead to excessive odour production. If ponds are operated in parallel, odours are prevented as the BOD load is distributed more evenly, although such an arrangement does allow short-circuiting. Facultative ponds rarely achieve a final effluent suspended solids concentration of 30 mg l^{-1}, even when in series. The suspended material is predominantly algae and zooplankton, which may be present at concentrations of between 50–70 mg l^{-1} in the final effluent. Tertiary treatment is required to remove the plankton biomass and any other colloidal or particulate matter if the receiving water is unable to assimilate the material. The three most popular options are chemical flocculation followed by a gravity filter, upflow filter, or by spray irrigation onto land (Sec. 2.4).

In practice, facultative ponds are designed on the basis of surface BOD loading (A_s) (kg $ha^{-1}d^{-1}$) (Eq. (6.13)):

$$A_s = 10L_iQ/A_f \tag{6.13}$$

where L_i is the influent BOD (mg l^{-1}), Q the flow (m^3d^{-1}), and A_f is the area (m^2) of the facultative pond. The temperature (T) and A_s are related, allowing the maximum permissible surface BOD loading rate to be calculated using Eq. (6.14) developed by McGarry and Pescod (1970):

$$A_s = 60(1.099)T \tag{6.14}$$

If this figure is exceeded, then the entire pond becomes anaerobic and fails.

Mara (1978) has suggested a more conservative design equation:

$$A_s = 350(1.107 - 0.002T)^{T-25} \tag{6.15}$$

Table 6.19 gives permissible surface BOD loadings based on Eq. (6.15). It is important that the temperature selected for design purposes should be the lowest mean monthly temperature. Once A_s has been selected, the pond area (A_f) calculated using Eq. (6.13). The HRT (θ_f) is calculated as:

$$\theta_f = A_fD/Q_md\,. \tag{6.16}$$

Table 6.19. Values of the permissible surface BOD loading A_S on facultative ponds at various temperatures calculated using Eq. (6.15).

$T(°C)$	A_S (kg/ha d)	$T(°C)$	A_S (kg/ha d)
≤ 8	80	17	199
9	89	18	217
10	100	19	235
11	112	20	253
12	124	21	272
13	137	22	291
14	152	23	311
15	167	24	331
16	183	25	350

where D is the depth (m) and Q_m the mean flow (m^3d^{-1}). As there is often considerable evaporation and infiltration from ponds, the mean flow is calculated by taking the mean of the influent and effluent flows. At temperatures below 20°C, θ_f should be a minimum of 5 d, whereas above 20°C then 4 d is sufficient. Sufficient HRT is important not only to prevent hydraulic short circuiting, but to allow algae sufficient time to multiply to counter balance loss of algae via the final effluent.

The depth of the aerobic zone fluctuates according to the photosynthetic activity and the degree of mixing, therefore, the depth of the aerobic zone will always be less during the hours of darkness. As the organic loading increases, the depth of the aerobic zone tends to decrease, with a greater portion of the organic matter being degraded anaerobically (Fig. 6.22). Apart from currents induced by the influent wastewater entering the pond, circulation is also caused by wind action on the surface or currents caused by temperature differences within the pond. In shallow ponds, wind action will normally be sufficient to induce mixing as long as the sides of the pond are not too steep or trees have been planted which may interfere with wind sweep. In deeper ponds, and under calm hot conditions, ponds can become stratified. This is due to the surface water being warmed, which reduces its density so that it forms a distinct layer (the epilimnion) which is isolated from the lower mass of cooler denser water (the hyperlimnion) by a thin layer of water called the thermoclime through which the temperature gradient falls rapidly. Once stratification has occurred, then mixing becomes restricted to the epilimnion only. As the density change per degree

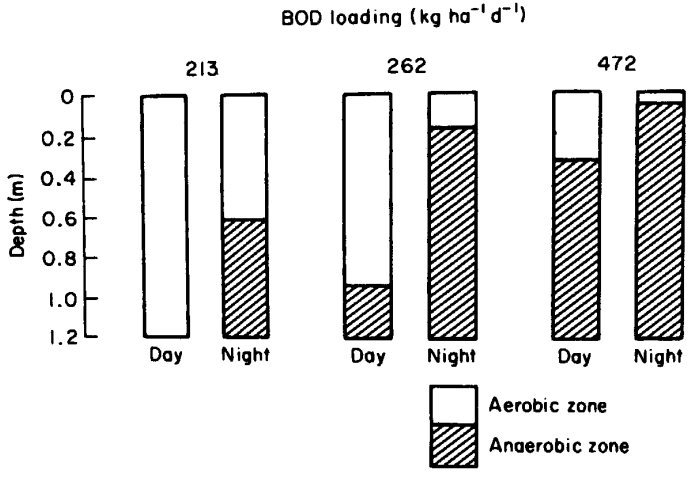


Fig. 6.22. The effect of organic loading on the depth of the aerobic zone during the day and night in facultative ponds at Nagpur, India (adapted by Hawkes 1983a from Arceivala *et al.* 1970).

is greater at higher temperatures, the resistance to mixing will increase as the temperature rises. The hyperlimnion rapidly becomes anaerobic, even though the epilimnion may be supersaturated with oxygen. Although the non-motile algae tend to sink in the less dense epilimnion and to subsequently die because of a lack of light in the hyperlimnion; motile forms, such as *Euglena* are able to maintain a position in the pond where the light conditions are most favourable and eventually become dominant, which is characteristic of stratified ponds. Stratification can lead to short-circuiting of the wastewater through the epilimnion with a resultant reduction in BOD and pathogen removal. Under certain climatic conditions where hot days are followed by cold nights, day-time stratification will be followed by night-time mixing. However, in most cases, mechanical mixing is required using compressed air to break up the stratification, although the use of weirs to induce an under and over pattern of flow has been successful (Hartley and Weiss 1970). Mechanical mixing should not be continuous as the increase in suspended solids from the sludge layer will reduce the light penetration and reduce photosynthesis. Therefore, mixing should be confined to short periods at night.

Facultative ponds are best suited to isolated communities where land is cheap and no industrial expansion is planned. In Europe, waste stabilisation ponds are increasingly used for sewage treatment for small rural communities, being robust, low maintenance, low energy systems. Capital costs are

about 30% that of a conventional system and 50% of a reed bed system (Table 6.15). When treating sewage, ponds are capable of producing odour free effluents throughout the year, except if ice formation is a problem in the spring. Industrial or strong organic wastes normally require pretreatment before discharge to a facultative pond to reduce the organic content, preventing overloading and permanent odours. The use of an anaerobic pond initially to maximise BOD removal followed by the facultative pond may be adequate. Incremental feeding, where some of the influent to the first pond in series is transferred to the second pond, or recirculation from the second pond to the primary pond may also improve efficiency and prevent odours (Marais 1970a). Facultative ponds are most popular in areas where there is sufficient solar radiation throughout the year. Where the climatic conditions are suitable, oxidation ponds are the cheapest form of sewage treatment in constructional and operational terms. The advantage of the system is the ease of operation, no skilled labour is required and no mechanical parts or power are required. However, among the disadvantages are a number of operational problems which include poor assimilative capacity for industrial wastes, production of odours and meeting minimum standards for discharge.

New ponds are easily commissioned once construction is complete and the basin cleared of any loose debris, soil, and vegetation. Obviously, the pond cannot be operated at its full design load until a balanced community of bacteria and algae has been established. This is most effectively achieved by allowing raw wastewater into the centre of the pond to a depth of about 200 mm. Where the area of the basin is large, then a smaller area should be partitioned off by constructing an embankment at one corner or in the centre and filled with wastewater. Bacteria become established first, followed more slowly by the algae. Colonisation occurs naturally, although the speed of colonisation can be increased by the addition of a few bucket loads of algal rich water from a pond or tank. Additional wastewater must be added at intervals to maintain the level in the pond, due to evaporation. Once an algal bloom has become established, the pond can be gradually filled with wastewater, trying to maintain the rich algal bloom until the pond is full. The pond should be allowed to stand for a further 3–4 days before full continuous-flow operation is commenced. In temperate climates, this process will take up to 6 weeks even in the summer months. Care must be taken to prevent weed growth developing during the start-up process, and to this end, it may be necessary to increase the initial depth to 1 m if it becomes a problem. Full details of commissioning all types of stabilisation ponds are given by Arceivala *et al.* (1970) and Gloyna (1971).

Table 6.20. Mean performance of 178 waste stabilisation pond systems in France (Racault *et al.* 1995).

Parameter	Ray wastewater (mg/l)	WSP effluent (mg/l)
BOD	277	23[a]
COD	657	99[a]
Suspended solids	256	60
Total Kjeldahl nitrogen	70	22
Ammoniacal nitrogen	48	14
Total phosphorus	21	8.5

[a]Filtered. Unfiltered values: BOD, 43; COD, 162.

Throughout Mediterranean Europe, WSP are widely used, especially at coastal holiday resorts where the population may increase by a factor of 20 during the summer (Mara and Pearson 1998). Of the 2,500 current WSP in France, nearly 80% serve small rural communities. The standard design uses three ponds in series, a primary facultative pond 1.0–1.5 m deep with an area equivalent to 6 m^2 per person followed by two maturation ponds, in series and of similar depth, each with an area equivalent to 2.5 m^2 per person. This is an organic loading of 83 kg $ha^{-1}d^{-1}$ employing a total pond area of 11 m^2 per person (CEMAGREF *et al.* 1997). Typical performance data is given in Table 6.20. In the UK, there are only 19 waste stabilisation pond systems treating raw sewage, in contrast to many more used solely for tertiary treatment (Table 6.21). Mara *et al.* (1998) describe a three pond system at Burwarton Estate in Shropshire (UK). It comprises a primary facultative pond and two maturation ponds in series (Fig. 6.23). The facultative pond is 1.5 m deep with an area of 1380 m^2, followed by two maturation ponds in series, both 1.5 m deep and 1057 m^2 in area. The surface BOD loading rate for the facultative pond is 55 kg $ha^{-1}d^{-1}$. The final effluent is discharged from the final pond into two constructed wetland ditches before entering a small stream. The system achieves the maximum discharge limits of 25 mg l^{-1} unfiltered BOD, 45 mg l^{-1} suspended solids and 10 mg l^{-1} ammonia. The Bertix Wastewater Treatment Plant in Belgium treats primarily domestic wastewater from a rural community of 7,500 PE. After preliminary treatment (bar screens, grit separation, rotary drum screen), the wastewater is treated by a series of two anaerobic lagoons, four facultative ponds and one maturation pond. Although unfiltered effluent values are within EU guidelines for BOD, COD and suspended solids, the removal of

Table 6.21. Waste stabilisation ponds in the UK treating raw sewage (Mara *et al.* 1998).

Location	Year commissioned	No. of ponds in series	Design flow (m^3/d)	Effluent quality requirements (mg/l)
Sturts Farm, Dorset	1989	3[a]	11	BOD 20; SS 30; amm. N 20
Tigh Mor Trossachs, Perthshire	1992	3	100	BOD 20; SS 30; total P 3
Larchfield/1, Teesside	1993	3	15	BOD 40, SS 60
Hapstead House, Devon	1993	3[a]	20	BOD 40; SS 60
Dulo Manor, Cornwall	1994	3[b]	50	Winter: BOD 10; SS 10; amm. N 5 Summer: BOD 5; SS 5; amm. N 2
Scolton Manor, Pembrokeshire	1994	3	10	None (discharge to groundwater)
Nature's World, Teesside	1994	3	4	None (volume consent)
Acklam Grange School, Teesside	1994	3	2[c]	None (volume consent)
Larchfield/2, Teesside	1995	3[a]	11	BOD 40; SS 60
Combermere, Shropshire	1995	3	10	None (discharge to planted leachfield)
Corfe Castle, Dorset	1996	3	6	None (discharge to groundwater)
Drummuir Castle, Banffshire	1995	3	35	BOD 10; SS 10
Barnardiston School Essex	1995	2	50	BOD 20; SS 30
Gordonstoun School, Morayshire	1995	2[d]	100[e]	BOD 10; SS 10; amm. N 10
Burwarton Estate, Shropshire	1995	3	27	BOD 25; SS 45; amm. N 10
Botton Village, North Yorkshire	1997	3	40	BOD 40; SS 30
Thornage Hall, Norfolk	1997	3	13	BOD 40; SS 60; amm. N 10
Earth Balance, Northumberland	1997	3	19	BOD 40; SS 60
Newnham, Gloucestershire	1997	2	10	BOD 40; SS 60

[a]Pretreatment in a septic tank.
[b]The WSPs are followed by a soakaway. No effluent has been discharged into the designated receiving watercourse.
[c]Grey water only.
[d]The WSPs are preceded by a balancing pond.
[e]Plus surface water and storm sewage up to 12 DWF.

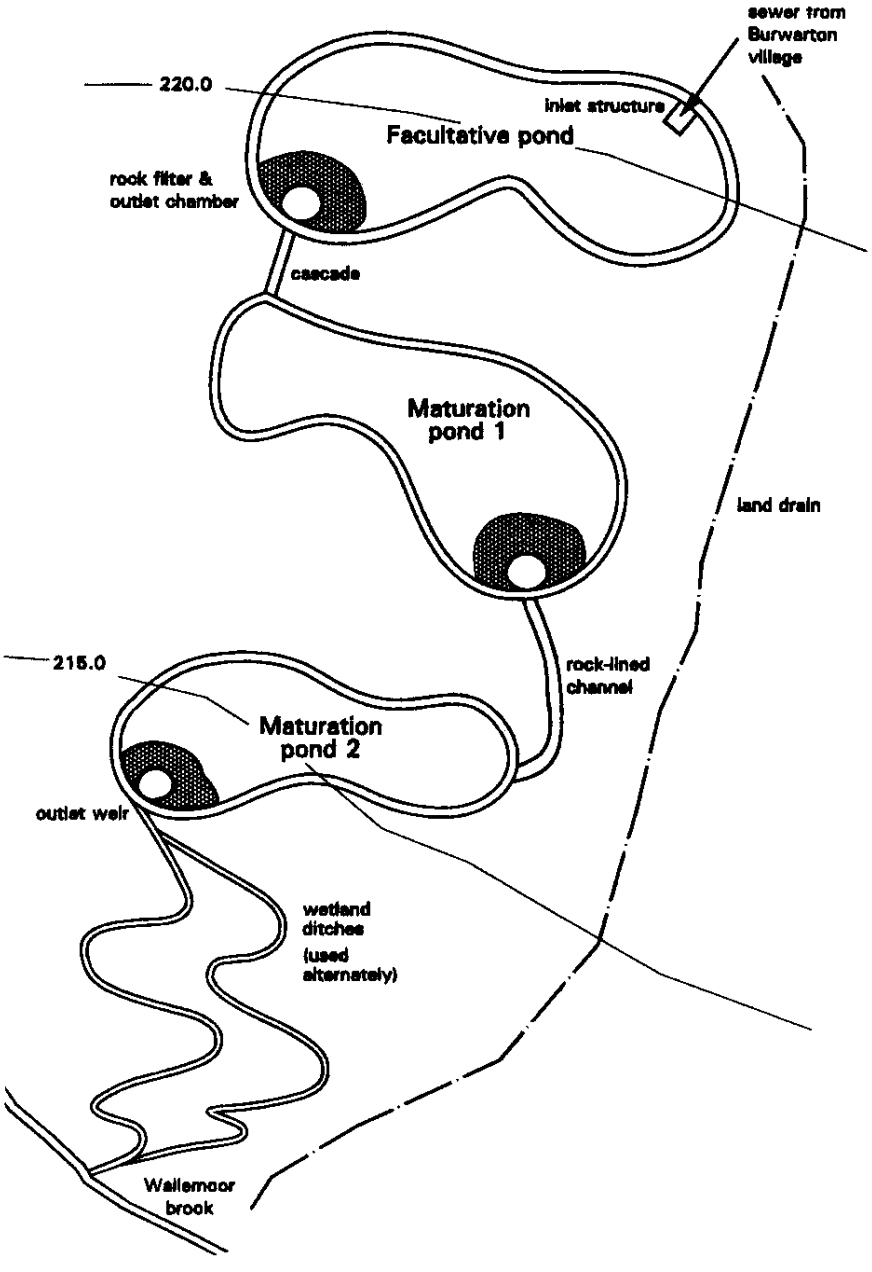


Fig. 6.23. Layout of the waste stabilisation ponds used at Burwarton Estate, UK (Mara *et al.* 1998).

nitrogen is similar to a conventional non-nitrifying system with the average removal of phosphorus approaching zero (Nameche *et al.* 2000).

The biologist plays a major role in the successful operation of facultative ponds by conducting regular microscopic examinations of the algae in order to give a clear quantitative assessment of the community structure within the pond (Arceivala *et al.* 1970). The overall photosynthetic activity of the pond can be determined either by measuring the biomass of the algae or the chlorophyll *a* concentration (American Public Health Association *et al.* 1985). Changes in the colour of the pond are also a useful guide to changes in condition. Healthy ponds are green or brownish due to the presence of green algae. However, a change to blue-green indicates a change in algal dominance from green to blue-green algae and as the latter are less efficient than the green algae, this indicates a deterioration in conditions. Changes in colour to pink, red or reddy-brown in the summer or autumn are caused by blooms of photosynthetic sulphur bacteria because of an increase in sulphate or sulphide concentration resulting from increased anaerobiosis. This is generally associated with overloading, thermal stratification or another operational problem. The commonest forms associated with sewage are *Chromatium* and *Thiospirillum*, whereas *Thiopeda rosea* and smaller spiral and rod forms are associated with industrial wastes (Gloyna 1971). A blue-green algae *Merismopedia tenuissina*, which is also associated with anaerobic conditions where sulphides are abundant, also imparts a pink colour to the pond. When the pond has broken down and gone completely anaerobic, then it takes on a grey or black colour. This is normally accompanied by rising sludge due to excessive gas production in the sludge layer. Such a condition is normally due to a rapid change in temperature, severe overloading, change in wastewater characteristics or an input of a toxic waste.

High-rate algal ponds

These ponds are not designed for optimum purification efficiency but for maximum algal production (Mara and Pearson 1998). The algae is harvested for a variety of uses, principally high quality algal protein (Oswald 1995). The ponds are shallow lagoons 20–50 cm deep, with a retention period of 1–3 days. The whole pond is kept aerobic by maintaining a high algal concentration and using some form of mechanical mixing. Mixing, which is normally carried out for short periods at night, prevents the formation of a sludge layer. Mixing may be required for short periods during the day to prevent a rise in pH in the surface water due to photosynthesis.

The hydrodynamics and oxygen balance in high-rate algal ponds (HRAP) is discussed by Ouarghi *et al.* (2000). The pond is commissioned in the same way as a facultative pond except that continuous loading should not be permitted until an algal bloom has developed. Loading depends on insolation, and in California, the average loading throughout the year is 134 kg BOD $ha^{-1}d^{-1}$ reaching an optimum summer loading of 366 kg BOD $ha^{-1}d^{-1}$ (Oswald *et al.* 1964). Strong organic sewages inhibit the photosynthetic action due to high ammonia concentrations, which results in the pond becoming anaerobic. Although currently used in California and Israel, no large scale system has been developed and they remain largely experimental. Algal productivity, harvesting methods and uses of the algal crop are considered further in Sec. 10.3.2.

Maturation ponds

Maturation ponds are widely used throughout the world as a tertiary treatment process for improving the effluent quality from secondary biological processes, including facultative ponds. Effluent quality is improved by removing suspended solids, reducing ammonia, nitrate and phosphate concentration, and by reducing the number of enteric micro-organisms. The primary function of such ponds in most parts of the world is the removal of pathogenic organisms. (Maynard *et al.* 1999). Their design, size, and number, in series, is determined by the required bacteriological quality of the final effluent (Marais 1974; Mara and Pearson 1998). They are of the same depth as facultative ponds, 1.0–1.5 m, although shallower ponds are used where insolation is high to achieve maximum light penetration. Unlike high-rate aeration ponds, maturation or tertiary lagoons are specially designed for maximum purification and not algal production (Hawkes 1983a). The retention period is normally 10–15 days, although shorter periods can be used for suspended solids (4 days) or phosphate removal (7–10 days) (Gloyna 1971; Toms *et al.* 1975). Biochemical oxgyen demand removal efficiency remains low due to the initial low organic loading (Toms *et al.* 1975). By acting as a buffer between the secondary biological phase and the receiving water, maturation ponds can reduce the effects of toxic loads and fluctuations in the performance of the secondary biological phase by dilution (Potten 1972). They differ from facultative ponds in showing less vertical and physico-chemical stratification, and remain fully oxygenated during the day. They have a more diverse algal population than facultative ponds (Table 6.17), the diversity increasing with each pond in series. Non-motile genera tend to dominate. Pathogenic bacteria are removed by

a number of mechanisms including settlement, natural die off, which is a function of time and temperature, elevated pH (> 9), UV radiation, and predation (Sec. 9.5.2).

Little of the residual BOD is removed in maturation ponds. However, due to the development of algae, 70 to 90% of the final effluent BOD can be attributed to its algal content (Mara *et al.* 1992). As algal cells are predated by zooplankton, or eaten by fish such as carp, there is normally an overall reduction in final effluent BOD. The filtered BOD of the final effluent can be estimated by assuming a 90% cumulative removal in the anaerobic and facultative ponds, and a further 25% reduction for each maturation pond in series (Mara and Pearson 1987). The BOD removal that does occur in maturation ponds is due to oxidation by the common genera of heterotrophic bacteria found in other secondary biological treatment processes, in particular *Pseudomonas*, *Flavobacterium*, *Archromobacter*, and *Alcaligenes* spp. In Europe, the EU Urban Wastewater Treatment Directive permits higher concentrations of BOD and suspended solids in the effluent from lagoons than other systems. Therefore, BOD and suspended solids analysis is carried out on filtered samples, whereas all other analyses must be unfiltered. Unfiltered suspended solids concentrations of up to 150 mg l^{-1} are permitted (European Community, 1991). The algal biomass is a function of the nutrient concentration. Therefore, if the availability of nutrients declines, so does the density of algal in the final effluent. Different planktonic organisms vary in their efficiency in removing nitrogen and phosphorus. For example, the algae *Scenedesmus obliquus* is particularly efficient in utilising organic and ammoniacal nitrogen, whereas *S. quadricauda* var. *alterans* is more efficient at using nitrate (Kalisz and Suchecka 1966). *Daphnia* reduces turbidity and enhances nitrogen removal by ingesting particulate matter and algae.

Removal of nitrate is not linked with the algal biomass and although small amounts are utilised by the algae, the majority is lost by denitrification at the sludge–water interface (Toms *et al.* 1975). Optimum nitrate removal is achieved by ensuring the development of a sludge layer and by using shallow ponds to give the maximum sludge–water surface contact. In this way, up to 0.8 g N $m^{-2}d^{-1}$ can be removed. It has been reported that nitrate levels tend to be much higher in the effluent than in the feed and that nitrate concentrations can build up during the autumn and winter (Van der Post and Englebrecht 1973). However, volatisation of ammonia and sedimentation of organic nitrogen via biological uptake are thought to be the principle mechanisms of nitrogen removal (Maynard *et al.* 1999).

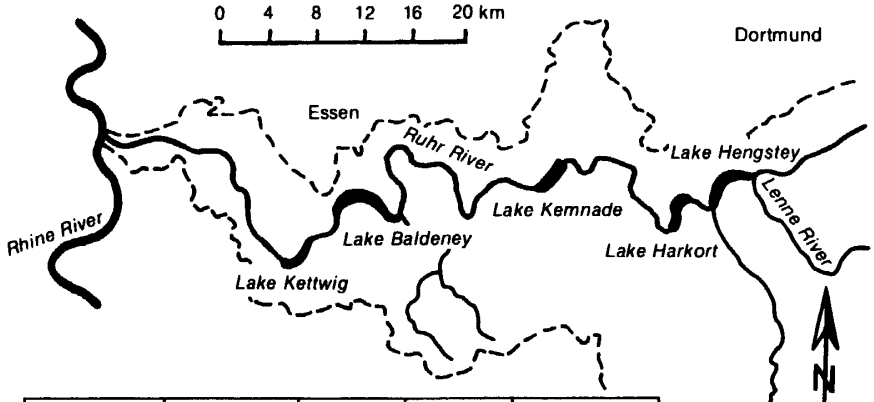
Work on the maturation ponds at Rye Meads sewage treatment works, which discharges to the River Lee in the UK, has shown that phosphate

removal is directly correlated with the algal biomass, reaching a maximum removal of 73% in May and a minimum of 2% in January (Toms *et al.* 1975). However, only a small proportion (< 20%) of the phosphate removal can be attributed to nutritional uptake by the phytoplankton, as the majority is removed by precipitation at enhanced pH values (>pH 8.2), which is due to photosynthetic activity. For every unit increase in pH, the concentration of phosphate remaining decreased by a factor of 10. In the temperate climate of the UK, it is only possible to maintain sufficient algal biomass to remove a reasonable proportion of the phosphate from the pond during the spring and autumn by having a long retention time. In winter, this is not feasible although the higher dilution factor, reduced temperature, and insolation all significantly reduce the risk of eutrophication in receiving waters. It has been reported that phosphorus can be regenerated during the night or during the winter at lower pH values (Hemens and Mason 1968). However, this was not observed at Rye Meads by Toms and his co-workers. In practice, phosphorus removal in maturation ponds is poor (Maynard *et al.* 1999).

In conclusion, although algae are beneficial in terms of phosphate removal and to a lesser extent nitrogen removal, their presence in the final effluent from a maturation pond increases the suspended solids and turbidity. Where strict effluent standards have to be met, simple algal removal systems can be added. Rock filters are most widely used for this purpose and consist of submerged beds, 1.5–2.0 m deep of crushed rock 75–100 mm in diameter (Middlebrooks 1988). The algae is retained within the filter and decomposes, releasing nutrients that are utilised by attached bacteria. The effluent is added below the surface of the medium to prevent odour production from cyanobacterial films that can develop over exposed wet surfaces. Significant nitrification occurs as nitrifying bacteria colonise the filter. It has also been suggested that algae can reduce the mortality of faecal bacteria (Potten 1971, 1972).

River purification lakes

The development of large impounded lakes to remove and degrade the residual pollution from a river is becoming increasingly popular. Such lakes are very similar to maturation ponds. Five lakes have been constructed in the Ruhr area (Germany) with Lake Baldeney, south of Essen, the largest with a retention period of 60 hours at low flows (Imhoff 1982, 1984) (Fig. 6.24). Together they provide an area of 4,488 km^2, with Lake Baldeney alone providing a purification capacity equivalent to the BOD produced by a population of 100,000 per day. Lake Baldeney is also able to remove dissolved



Impounded lake	Year of impounding	Average depth (m)	Surface area (km^2)	Volume the year of impounding (10^6 m^3)
Hengstey	1929	1.90	1.70	3.3
Harkort	1930	2.30	1.40	3.2
Kemnade	1979	2.40	1.25	3.0
Baldeney	1932	3.40	2.45	8.3
Kettwig	1949	2.60	0.55	1.4

Fig. 6.24. Impounded lakes on the River Ruhr (Germany) operated as a purification lakes system (Imhoff 1984).

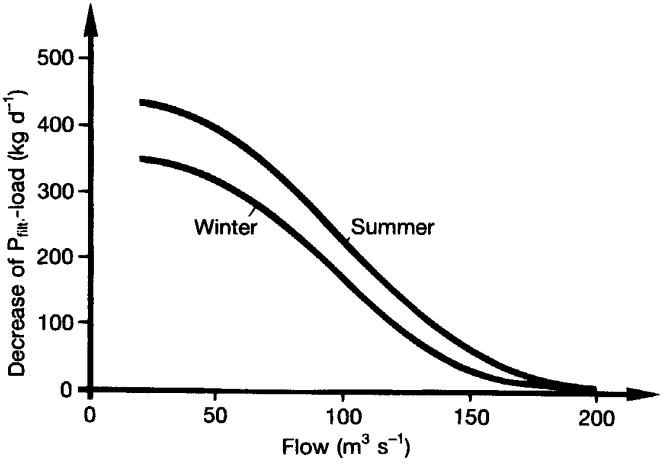


Fig. 6.25. The effect of flow rate (m^3s^{-1}) and season on the removal of phosphorus in the impounded Lake Baldeney (Imhoff 1984).

phosphorus from the River Ruhr (Fig. 6.25). The phosphorus is removed by algal growth and subsequent algal settlement. Up to 170 tonnes of phosphorus per annum, together with heavy metals, is removed by sludge settlement in the lake (Table 6.22). It was estimated that by 1984, Lake Hengstey, constructed in 1929, had accumulated some 400,000 m^3 of sludge, whereas in Lake Harkort, which was impounded in 1930, some 350,000 m^3. In Lake Baldeney, some 60,000 m^3 of sludge accumulates each year, but at flows 10 times greater than the average flow rate, the sludge becomes mobilised. The sludge is subsequently flushed out of the lake when the river is in flood and flows are in excess of 15 times the average flow rate (Imhoff 1982). Because of the reduction in the depth of these lakes, desludging was begun in 1988. Some 750,000 m^3 of sludge is to be removed from Lake Baldeney alone by hydraulic dredging at a cost of 15×10^6 DM at 1983 prices. However, even with these large desludging costs, it has been estimated that treatment by purification lakes is 30% cheaper than conventional treatment. The purification lakes form an important part of the water quality management strategy for the 4,488 km^2 Ruhr catchment, that includes 92 wastewater treatment plants and 473 storm water treatment plants. The total phosphorus concentration in the Ruhr at Essen has shown a steady decline over the past three decades from 0.8 m g l^{-1} in the 1970s to 0.15 mg l^{-1} in 2000 (Bode and Klopp 2001).

On the River Tame, UK, a polluted tributary of the River Trent in the West Midlands, a purification lake has also been built. The River Trent receives vast quantities of sewage and industrial effluent reaching in excess of 80% of the total volume of the river at low flows. Lea Marston purification lake was built at a disused gravel works after the last major conurbation and sewage input. It was opened in 1980 and removes settleable solids from the river which are especially a problem during storms. The solids are removed from the main body of water by settlement in the lake and subsequently by dredging. In contrast to the Ruhr lakes, the Lea Marston lake is continuously desludged although it takes about 12 months to cover the whole flow area of the lake. The lake also reduces the need for tertiary treatment at Coleshill and Minworth sewage treatment plants and acts as a buffer in case of accidental spillages. The results have been very encouraging with the water quality in the Rivers Tame and Trent significantly improved. A coarse fishery has been established and fish are being caught in the River Tame for the first time in 100 years. The river enters the system and passes through large booms that remove floating debris (Fig. 6.26). Grit traps and grit channels remove $>$ 2,000 tonnes of dry matter per annum from the river with the lake itself removing a further 15,000 tonnes of sludge each year.

Table 6.22. Comparison between sediments collected from the purification lakes at Lea Marston (UK) and the Ruhr (Germany) catchment (Woods *et al.* 1984).

Lake Site	Period	Dry solids (%)	Volatile solids (%)	Metal concentrations (mg kg^{-1} dry solids)					
				Ni	Cu	Cr	Zn	Cd	Pb
Lea Marston (sed. tank)	1969–1970	10.5	40.5	554	1743	1275	8000	44	617
Lea Marston (exp. lake 1)	1972–1974	11.4	35.1	1000	2350	1340	5160	70	950
Lea Marston (exp. lake 2)	1972–1974	7.5	32.7	970	2230	1650	5400	80	950
Lea Marston (dredged sludge)	1981–1982	4.5	24.9	410	1335	510	3570	39	780
Hengstey	1981–1982	39.4	12.3	261	1240	440	4030	18	450
Harkort	1981–1982	45.8	11.0	250	830	280	3120	29	480
Baldeney	1981–1982	45.6	14.0	312	730	400	3540	36	525

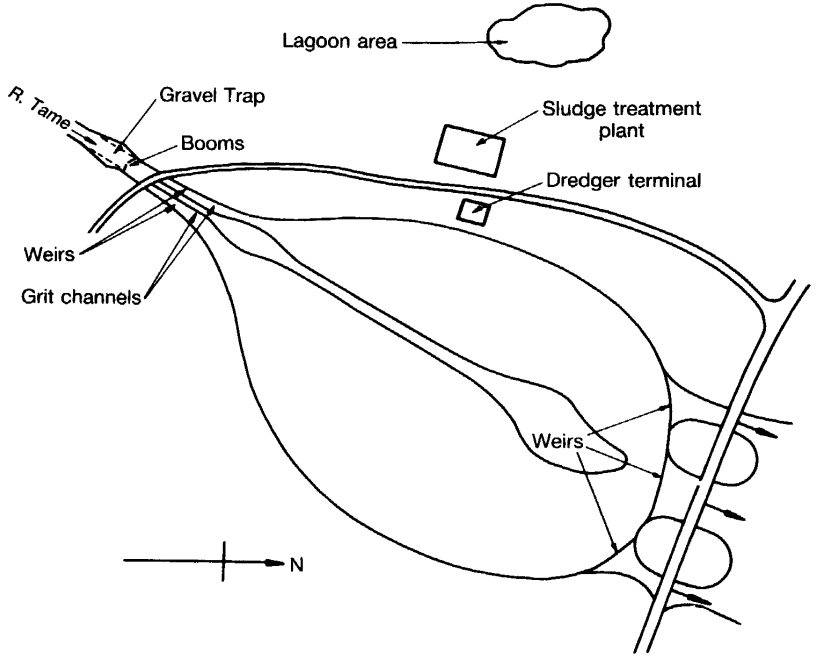


Fig. 6.26. Schematic plan of the Lea Marston purification lake (Woods *et al.* 1984).

The lake provides a retention time of 12 hours at the daily average flow, falling to 1–2 hours at the design flood flow. The effect on water quality is quite dramatic with the suspended solids reduced by 56% and the BOD by 34% overall (Woods *et al.* 1984). The quality of the sludge is compared with that removed from the Ruhr lakes in Table 6.22.

6.3.3. *Aeration lagoons*

Aeration lagoons can be used for the first-stage treatment of sewage or the pretreatment of industrial wastewater before secondary treatment, such as a facultative pond or activated sludge. The basins are deeper than other types of stabilisation ponds, 3.0–3.7 m, with oxygen being provided mechanically and not by the photosynthetic activity of algae. Oxygen is normally provided by mechanical pier-mounted or floating surface aerators, which ensures that the microbial biomass is kept in suspension and that sufficient dissolved oxygen is provided for maximum aerobic activity. Bubble aeration, provided by compressed air pumped through plastic tubing laid across the bottom of the pond is also used.

A predominantly bacterial microbial biomass develops and as there is no provision for settlement or sludge return, the process relies on sufficient mixed liquor developing in the basin. Retention times vary between 3–8 days depending on the degree of treatment required, the temperature and strength of the influent wastewater. A retention time of 5 days at 20°C provides an 85% reduction in the BOD of domestic sewage, although a fall in temperature by 10°C results in a reduction in BOD removal to 65% (Hammer 1977). Bubble aeration is most successful in keeping ponds aerobic in locations where pond surfaces are frozen for several months in the winter. Under these conditions, surface aerators cannot operate due to ice formation. In this type of climate, unaerated facultative ponds are generally unsuccessful and tend to become anaerobic.

As a unit process, they fall between the activated sludge process and facultative oxidation ponds. If the degree of agitation is sufficient, then all the solids may be kept in suspension under aerobic conditions corresponding to the activated sludge process. In systems with less efficient agitation, the solids may tend to settle and form an anaerobic sludge layer similar to facultative oxidation ponds. Where the aeration is inadequate, then enhanced deposition of particulate solids and the reduced dissolved oxygen concentration in the basin will lead to anaerobic conditions, a loss of efficiency and the production of odours. Therefore, sufficient agitation and mixing coupled with a strict loading regime are required for the successful operation of aeration lagoons. Unlike activated sludge systems, aerated lagoons develop diverse and abundant populations of zooplankton (Cauchie *et al.* 2000).

Aeration lagoons are susceptible to large inputs of biodegradable or toxic waste which can severely reduce efficiency. High infiltration and storm water result in reduced retention times and flush the microbial biomass out of the basin, thus reducing the MLSS and MCRT. Where this is a potential problem, provision should be made to divert a portion of the enhanced flow directly to the next stage, thereby maintaining optimum hydraulic loading to the aeration lagoon.

Further reading

Al-Nozaily *et al.* 2000a,b; Huang *et al.* 2000; Zimmo *et al.*; Manios *et al.* 2000.

Natural Treatment systems: Reed *et al.* 1988; WPCF 1990; Kruzic 1977.

Land Treatment: US Environmental Protection Agency 1981; Tare and Bokil 1982; Eikum and Seabloom 1982; Canter and Knox 1985;

Oliveira and Almeida 1987; Metcalf and Eddy 1991; Mottier *et al.* 2000; Nasser *et al.* 2002.

Macrophyte-based systems: Dinges 1982; US Environmental Protection Agency 1988; Brix 1994a, b, 1999; Cooper *et al.* 1996; Kadlec and Knight 1996; Etnier and Guterstam 1997; Mulamoottil *et al.* 1998; Vymazal *et al.* 1998.

Stabilisation ponds: Middlebrooks *et al.* 1982; Environmental Protection Agency 1983; Townshend and Knoll 1987; Mara and Pearson 1998; Maynard *et al.* 1999; Pearson *et al.* 2000.

7

Anaerobic Unit Processes

7.1. Introduction

Anaerobic processes are used to treat strong organic wastewaters (BOD > 500 mg l^{-1}) and for further treatment of primary and secondary sludges from conventional wastewater treatment. Strong organic wastes generated by the agricultural and food industries, often in large quantities, provide a particularly difficult wastewater treatment problem. These wastes contain large quantities of biodegradable organic matter and conventional aerobic treatment is beset with numerous operational difficulties. For example, the difficulty of maintaining aerobic conditions, especially if the wastewater has a high concentration of suspended solids, sludge bulking, inability to take high BOD or COD loadings, high operational and energy costs, and a high production of biomass as wasted sludge that requires subsequent disposal. Anaerobic treatment, although slow, offers a number of attractive advantages in the treatment of strong organic wastes: a high degree of purification; ability to treat high organic loads; production of a small quantity of excess sludge, which is normally very stable; and the production of an inert combustible gas (methane) as an end-product (Steritt and Lester 1988). The final effluent produced by anaerobic treatment contains solubilised organic matter that is amenable to quick aerobic treatment, which indicates the potential of combined anaerobic and aerobic units in series (Mathur *et al.* 1986). Unlike aerobic systems, complete stabilisation of organic matter is not possible anaerobically and subsequent aerobic treatment of anaerobic effluents is normally necessary. The advantages and disadvantages of anaerobic treatment are summarised in Table 7.1. Typical carbon balance in aerobic and anaerobic systems is compared in Fig. 7.1.

Table 7.1. The advantages and disadvantages of anaerobic treatment compared to aerobic treatment.

Advantages	Disadvantages
Low operational costs	High capital costs Generally require heating
Low sludge production	Long retention times required (> 24 h)
Reactors sealed giving no odour or aerosols	Corrosive and malodorous compounds produced during anaerobiosis
Sludge is highly stabilized	Not as effective as aerobic stabilization for pathogen destruction
Methane gas produced as end product	Hydrogen sulphide also produced
Low nutrient requirement due to lower growth rate of anaerobes	Reactor may require additional alkalinity
Can be operated seasonally	Slow growth rate of anaerobes can result in long initial start up of reactors and recovery periods.
Rapid start-up possible after acclimation	Only used as pretreatment for liquid wastes

There is a complex consortium of micro-organisms involved in the anaerobic degredation of high molecular weight organic compounds to methane. Predominately bacterial, although protozoa and fungi have been reported (Finlay and Fenchel 1991), anaerobic breakdown of organic matter is complex involving synergistic reactions between different groups (Zehnder 1988; Archer and Kirsop 1991). There are four groups involved in this synergistic relationship: (1) Hydrolytic bacteria; (2) Fermentative acidogenic bacteria; (3) Acetogenic bacteria; and (4) Methanogens (Fig. 7.2). These have been reviewed in Sec. 3.4.3.

Anaerobic degradation occurs in the absence of oxygen with methanogens adversely affected by even trace levels of oxygen (Oremland 1988). Anaerobic biomass can form a granular sludge under certain circumstances (e.g. UASB) and when this occurs the methanogens are protected from the effect of oxygen inside the granules (Katio *et al.* 1993) (Sec. 7.3.2). The basic difference between aerobic and anaerobic oxidation is that in the aerobic system, oxygen is the ultimate hydrogen acceptor with a large release of energy, but in anaerobic systems the ultimate hydrogen acceptor may be nitrate, sulphate or an organic compound with a much lower

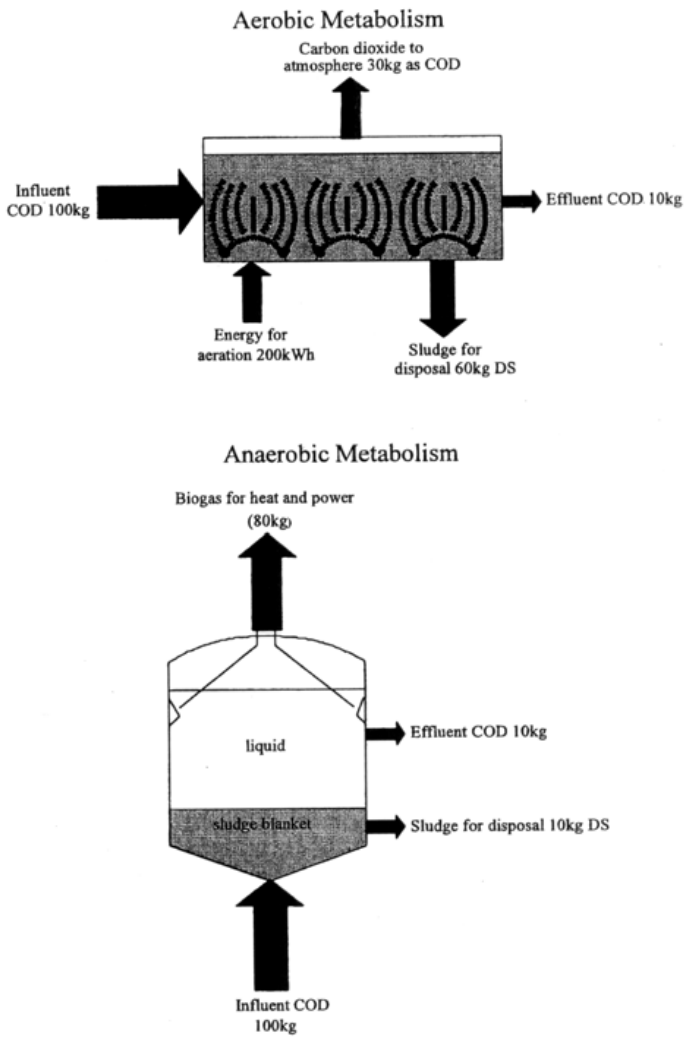


Fig. 7.1. Comparison of mass balance of carbon utilization in aerobic and anaerobic processes (Wheatley *et al.* 1997).

release of energy. Briefly, the process of anaerobic decomposition involves four discrete stages (Fig. 7.2). The first stage is the hydrolysis of high molecular weight carbohydrates, fats, and proteins that are often insoluble, by enzymatic action into soluble polymers. The second stage involves the acid-forming bacteria which convert the soluble polymers into a range of organic acids (acetic, butyric, and propionic acids), alcohols, hydrogen, and

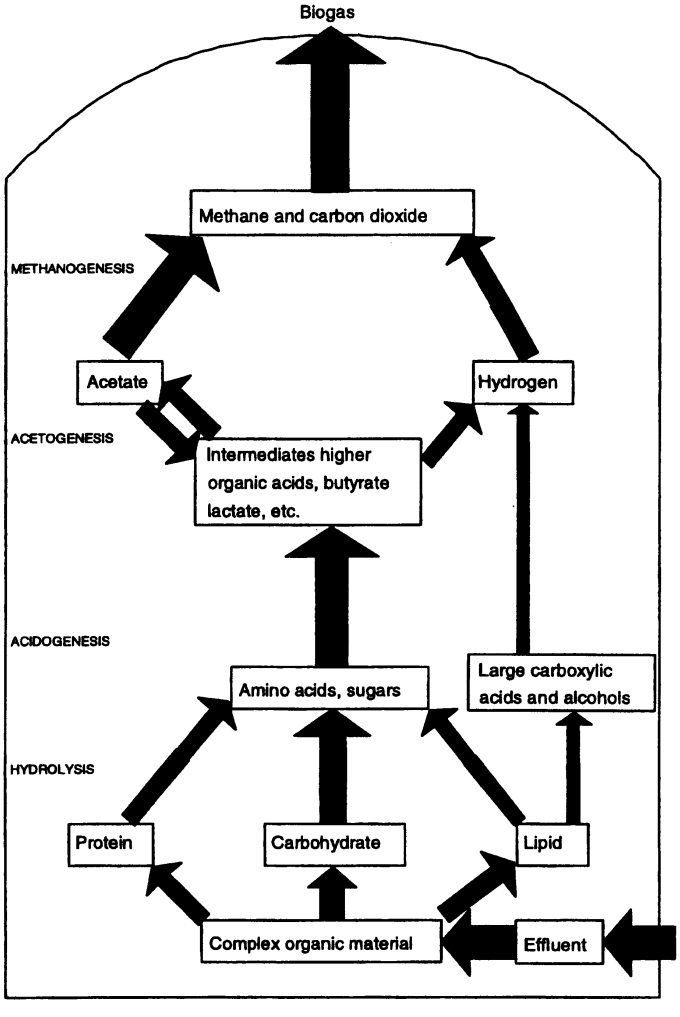


Fig. 7.2. Biochemical steps in anaerobic digestion (Wheatley *et al.* 1997).

carbon dioxide. Acetic acid, hydrogen, and carbon dioxide are the only end-products of the acid production that can be converted directly into methane by methanogenic bacteria. A third stage is present when the organic acids and alcohols are converted to acetic acid by acetogenic bacteria. It is in the fourth and final stage, which is perhaps the most sensitive to inhibition, when methanogenic bacteria convert the acetic acid to methane. Although methane is also produced from hydrogen and carbon dioxide, in practice about 70% of the methane produced is from acetic acid. Obviously, the

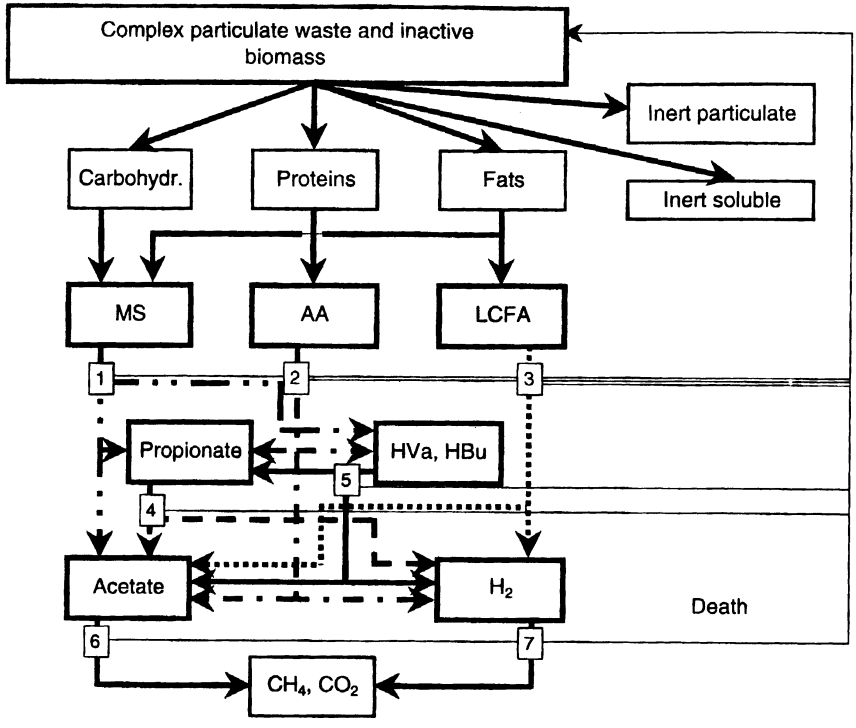


Fig. 7.3. The IWA anaerobic digestion Model No 1 (ADM1) (Batstone *et al.* 2002b). (1) acidogenesis from monosaccharides (MS), (2) acidogenesis from amino acids (AA), (3) acetogenesis from long chain fatty acids (LCFA), (4) acetogenesis from propionate, (5) acetogenesis from butyrate (HBu) and valerate (HVa), (6) aceticlastic methanogenesis, and (7) hydrogenotrophic methanogenesis.

methanogenic stage is totally dependent on the production of acetic acid and so it is the third stage, the acetogenic phase, that is the rate-limiting step in any anaerobic process (Fig. 7.3). The biochemistry of anaerobic decomposition has been fully explored in Sec. 3.4.

In Europe there were over 2,570 anaerobic digesters in 1994 treating 60% of the sewage sludge produced by member states before final disposal. There are a further 400 digesters treating industrial effluents, 400 treating farm slurry, and 200 treating landfill leachate (Nyns 1994). So anaerobic digestion is processing almost one billion tonnes of European waste each year. A large number of anaerobic processes are available, including anaerobic lagoons, digesters, and filters. Present anaerobic technology can be divided into two broad categories. *Flow-through systems* for the digestion of concentrated wastes, such as animal manures or sewage sludges that have

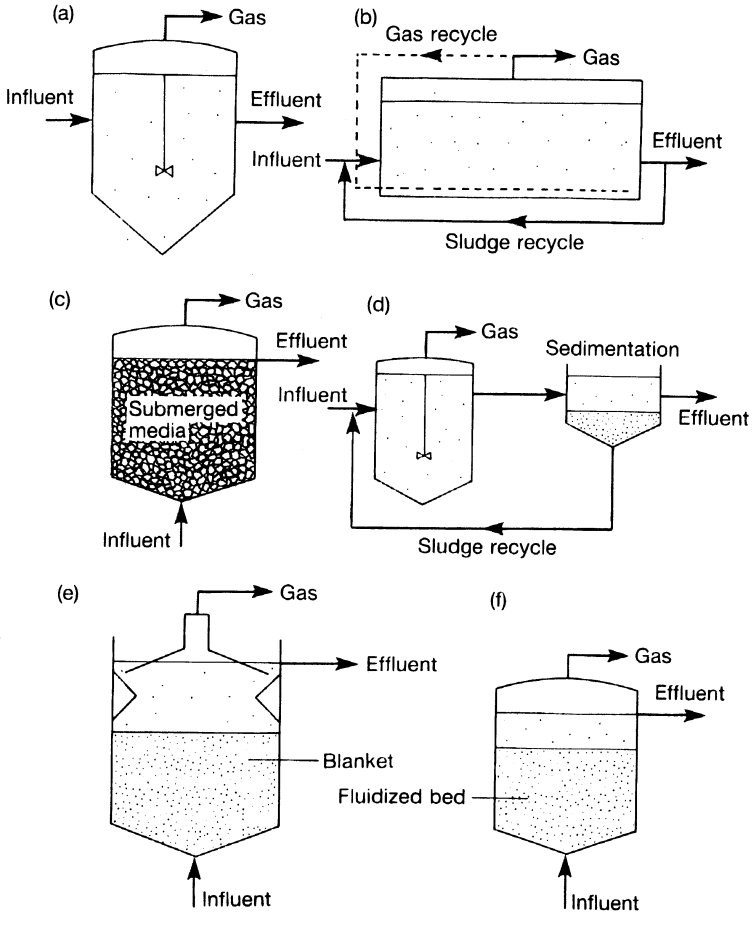


Fig. 7.4. Schematic representation of digester types. Flow through digesters (a-b) and contact systems (c-f) (adapted from Casey 1981)

a solids concentration in the range of 2–10%. These include conventional completely mixed reactors (Fig. 7.4a), which are used primarily for sewage sludges, and plug flow reactors used to a limited extent for the digestion of animal manures (Fig. 7.4b) (Hayes *et al.* 1979). Wastewaters with a lower solids concentration are treated anaerobically by *contact systems* in which the wastewater is brought into contact with an active microbial biomass that is retained within the system. Contact systems are primarily used for treating industrial effluents and are widespread in France, The Netherlands and Germany (Table 7.2). Of the 400 European anaerobic systems treating industrial wastewaters 278 are employed in treating food, drink

Table 7.2. Anaerobic digesters treating industrial effluents in the European Union (Wheatley *et al.* 1997).

	Number of digesters (by country)	Number of digesters according to industrial activity[(a)]	Number of digesters by origin of contractors
Germany	100	5	50
Holland	80	23	143
France	55	4	60
Italy	51	3	40
Belgium	25	10	45
Spain	22	3	2
UK	20	2	5
Denmark	18	16	25

(a) Numbers adjusted to digesters per 1000 large industries defined as with turnover greater than 1 million European currency units (ECUs).

and fermentation wastes, and 48 paper wastes. In flow-through systems the residence time of the waste (hydraulic retention time, HRT) and of the microbial biomass (mean cell retention time, MCRT) in the unit are the same, whereas in a contact system the MCRT is far greater than the HRT of the wastewater. The biomass is retained within the reactor in a number of ways. For example, by allowing the anaerobic micro-organisms to develop as an attached film on a static medium, or to develop as flocs maintained in suspension either by mechanical mixing or by the upward flow of effluent through the reactor. By using a system similar to the activated sludge process (contact (continuously) stirred tank reactors-CSTRs), the anaerobic biomass is present as suspended flocs which are recovered in a separate settlement chamber and recycled back to the main reactor (Fig. 7.4d). This process can also take place within a single reactor where the depth of the sludge blanket is controlled by the upflow rate of the influent wastewater (Fig. 7.4e). Attached anaerobic films can be on a static filter medium of natural stone or plastic, similar to a percolating filter (Fig. 7.4c); whereas fluidised or expanded beds incorporate a fine grained medium such as sand (Fig. 7.4f). Media reactors are completely flooded to ensure anaerobic conditions, and like the sludge blanket process are operated in the upflow mode (Casey 1981).

The basic kinetics of anaerobic treatment are fully discussed by Mosey (1983) and Hill (1983) (Sec. 3.1.2). Because so many different groups of bacteria are involved, the digestion process is not easily modelled, especially

as the methanogenic bacteria have a much lower growth rate than the acid-producing bacteria. However, as the conversion of volatile acids to biogas is generally considered to be the rate-limiting step of the overall reaction, the methanogenic phase is usually used for modelling purposes. The majority of models are applicable to the continuously stirred tank reactor (CSTR) as they are the most widely used and operate closest to the steady-state, which is easier to model. However, the development of dynamic models has demonstrated the need for more accurate ways of examining operational problems of digesters. Steady-state models are usually derived from one or two differential equations that treat the digester contents as a single substrate and the bacteria as a single population. Among the best known models of this type are those using Monod kinetics (Lawrence and McCarty 1970; Grady *et al.* 1972; Pfeffer 1974). Of particular interest are models based on contois kinetics developed by Chen and Hashimoto (1978, 1980) (Vavilin *et al.* 2001). The dynamic models available all use Monod kinetics and normally consider the interactions between several substrates and bacterial populations. This results in a system of differential equations that can only be solved by computer. Andrews and Graef (1971) considered that relating substrate concentration to specific growth rate (the Monod function) was invalid for anaerobic reactors, as the volatile acids not only acted as substrate for methanogenic bacteria but were also inhibitory at higher concentrations. They replaced the Monod function with an inhibition function (Haldane 1930) so that:

$$\mu = \mu_m/[1 + K_s/s + s/K_i]$$

where K_i is the inhibition function. This is fully expanded and discussed by Andrews (1971, 1983). Whereas this early dynamic model only included a single substrate and organism, more recent models of this type consider more than one substrate (Hill 1982; Lavagno *et al.* 1983) or more than one bacterial population (Hill and Barth 1977; Hill and Nordstedt 1980; Lavagno *et al.* 1983).

The International Water Association (IWA) has produced an excellent generalised anaerobic digestion model (Batstone *et al.* 2002b). The model includes multiple steps that describe both biochemical processes (e.g. disintegration from homogeneous particles to carbohydrates, proteins, and lipids; extracellular hydrolysis of these particulate substrates to sugars, amino acids, and long chain fatty acids (LCFA) respectively; acidogenesis from sugars and amino acids to volatile fatty acids (VFAs) and hydrogen; acetogenesis of LCFA and VFAs to acetate; and separate methanogenesis

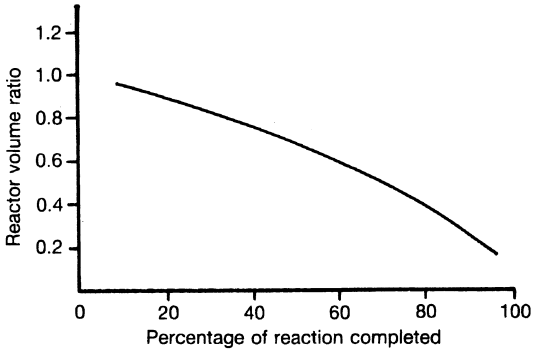


Fig. 7.5. Ratio of plug-flow reactor volume to completely mixed flow reactor volume for a first-order reactor process.

steps from acetate and hydrogen/carbon dioxide), as well as physico-chemical processes (e.g. ion association and dissociation, and gas-liquid transfer) (Fig. 7.3). The model comprises a set of differential and algebraic equations using 26 dynamic state concentration variables and 8 implicit algebraic variables per reactor vessel or element. It can also be implemented using differential equations only when 32 dynamic state concentration variables are used (Batstone *et al.* 2002a). The use of models in anaerobic digestion is reviewed by Parsons (1984), Pavlostathis and Gossett (1986), Pavlostathis and Giraldo-Gomez (1991) and Batstone *et al.* (2002a).

In plug-flow systems there is a substrate concentration gradient between the inflow and outflow ends of the reactor. As the rate of reaction is dependent on the substrate, the average rate of reaction in a plug-flow system is theoretically higher than for a CSTR. Casey (1981) illustrates the significance of this difference in terms of the reactor volume required for a first-order reaction process (Fig. 7.5). This shows that the advantage of the plug-flow reactor over the CSTR increases with the required degree of reaction completion. Although a number of contact systems can be considered as plug-flow processes, those treating domestic sewage sludge are largely of the completely mixed type.

7.2. Flow-Through Systems (Digestion)

Anaerobic digestion is a biological process in which the organic fraction of a wastewater sludge, proteins, carbohydrates, and lipids, are degraded in the absence of oxygen to methane and carbon dioxide by a variety of micro-organisms, principally bacteria. The process occurs quite widely in

natural environments such as lake sediments, marshes, peat bogs, and even in the rumen of certain herbivorous animals.

The first direct application of anaerobic digestion to sewage sludge is attributed to Louis H. Mouras of Vesoul in France who developed a sealed cesspool (c.1860) in which he claimed 'sewage solids were liquified'. By 1895, digester gas, also known as biogas or methane, was being collected and used as fuel. Donald Cameron constructed a large septic tank in Exeter (UK) and was able to use the gas for lighting the area around the treatment plant. By the turn of the century it had become common practice to incorporate a digestion chamber within sedimentation tanks, with the Travis hydraulic tank and the Imhoff tank the most widely adopted. The Imhoff tank, in particular, was installed at most small to moderate sized treatment plants and are still in wide use today in Ireland, although few have been constructed since 1950. The development of specific digesters for the anaerobic breakdown of sludges began in the early 1920s, with their use restricted to large cities. There has been a resurgence of interest in anaerobic digestion in the past 20–25 years, with much fundamental research and development work being done, which has led to the development of new digester and anaerobic reactor designs. The basic flow-through system can be separated into two categories, systems which combine settlement with digestion, and separate systems built for digestion only.

7.2.1. *Combined systems*

Combined systems of settlement and digestion are restricted to situations where there is only a small volume of wastewater to be treated. The combined system provides primary settlement and then sludge stabilisation by digestion, all within a single chamber. By reducing the volume of sludge produced, and storing it in such a way as not to impede the settlement process, such systems are ideal for single houses or small communities. Anaerobic lagoons can also be categorised as combined systems, although their use in Europe is largely restricted to strong organic wastes from the food-processing and agricultural industries (Sec. 6.3).

Septic tanks

Septic tanks are often confused with cesspools, the latter being an underground chamber constructed solely for the reception and storage of wastewater with no treatment taking place. It is important to differentiate between the two as in many areas these two terms are used interchangeably.

A cesspool is a storage tank which requires periodic emptying and is not intended as a septic tank in which decomposition of the settled material occurs.

Cesspools can be constructed out of concrete, plastic or fibreglass and according to the British Code of Practice (British Standards Institution 1972, 1983), they must be: (a) impervious; (b) not able to overflow; (c) have a minimum capacity of 18 m^3 or 45 days retention for two people assuming a per capita water usage of 180 l d^{-1}; (d) be constructed so that it can be completely emptied; (e) adequately ventilated; and, most importantly, (f) adequately covered to ensure safety. Clearly, cesspools are only used where no other form of treatment is possible, and the need to have them regularly emptied means that they are the most expensive form of treatment for domestic dwellings in terms of both capital and operational costs. The only improvement in this system can be achieved by adopting water saving improvements that reduce the volume of wastewater discharged. There are advantages, however, such as no power requirement, no quality control required, no mechanism that can go wrong, the process is not injured by intermittent use, and as there is no effluent discharge, there is no immediate environmental impact. The major limitation on the use of cesspools is the cost of emptying, although the construction of large underground storage tanks can be both difficult and expensive. The advantages and disadvantages of cesspools have been examined by Mann (1979). One area where storage tanks are still widely used is on farms. Storage of farm effluents is expensive and, ideally, raw effluent should be spread immediately on to the land. However, this is impossible because of seasonal crop cycles, and at certain times of the year because of the risk of surface runoff causing pollution. The use of slurry tanks, both of underground and above ground construction, is widespread and the design of such systems has been reviewed by Weller and Willetts (1977).

Septic tanks are essentially a chamber in which the settleable solids settle out of suspension to form a sludge which undergoes anaerobic breakdown. The process provides partial treatment only and cannot produce an effluent of Royal Commission standard, and further treatment is required either by a percolating filter or by the provision of a percolation area in which the sewage percolates into the soil via a system of underground distribution pipes. If the effluent from a septic tank is discharged directly to surface waters then a minimum dilution factor of 300–400 is required. Although most commonly used for individual houses they can be used to serve small communities of up to 500, and are frequently used in combination with a percolating filter for small rural villages. Septic tanks require

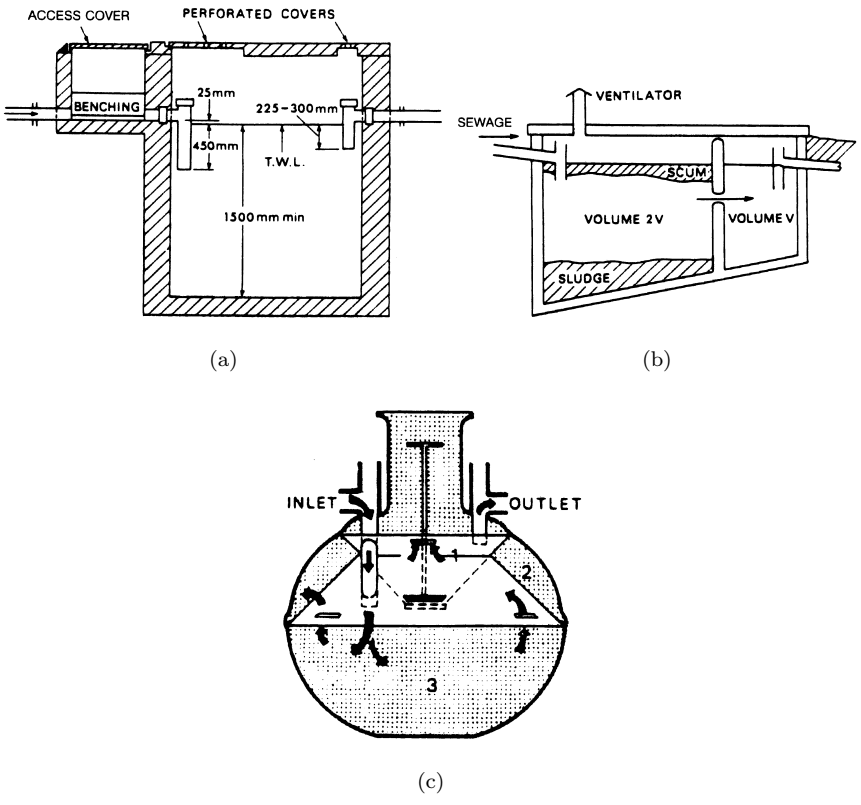


Fig. 7.6. Examples of manufactured septic tanks: (a) single-chamber system; (b) two-chamber system; (c) GRP septic tank (Mann 1979).

no power and where subsurface drainage is used quality control can be confined to suspended solids removal only. They are not adversely affected by intermittent use, have a very small head loss and can achieve a 40–50% BOD and 80% suspended solids removal. In its simplest form the septic tank consists of a single chamber (Fig. 7.6) with a single input and output. They can be of any shape although they are traditionally rectangular and made of concrete. New prefabricated units in plastic or fibreglass come in a variety of shapes, designs, and even colours. The tank consists of three separate zones, a scum layer on top of the clarified liquid, with a sludge layer in the base. Wastewater enters and leaves the tank via T-shaped pipes that prevents disturbing the scum layer or allowing solids to be carried out of the tank. As the wastewater moves through the chamber, settleable solids gradually settle to form a sludge, and any fats or buoyant material floats

to the surface where they are retained by the baffle edge of the T-pipe and form the scum layer. The scum layer, although not vital to the successful operation of the tank, helps its operation in three ways: it prevents oxygen transfer through the air-water interface; insulates the anaerobic chamber by preventing heat loss; by attracting and retaining fats, floating material, and raised solids from the sludge layer. It is in the sludge layer where anaerobic decomposition takes place, with the organic fraction slowly being degraded. Often, decomposition is incomplete and the settled material is only hydrolysed and not broken down to methane. This results in intermediate products of anaerobic digestion, such as short-chain fatty acids, being produced and slowly being diffused back into the clarified liquid to be discharged from the tank. This incomplete anaerobic activity results in unpleasant odours. Methane production is inhibited by a number of factors in septic tanks, most notably low temperatures and insufficient sludge storage capacity. Methane production in septic tanks is low even in the summer, whereas in winter it may cease completely resulting in an overall increase in sludge accumulation. However, gas formation occurs even in the absence of methane formation, which can carry solids back into the liquid phase and cause an increase in the rate of scum accumulation. Most wastewaters contain a significant proportion of non-degradable solids, and even if anaerobic digestion is highly efficient there will be a gradual increase in solids in the tank. As these build up, the volume of the liquid zone in the chamber is reduced, thus reducing the retention time of the wastewater and the degree of settlement that is possible. If discharge of solids to the next treatment phase is to be minimised, then the septic tank must be regularly desludged. The floor of the chamber is sloped towards the inlet end above which a manhole is situated into which a suction pipe can be lowered (Fig. 7.6). Ideally, septic tanks should be desludged every 12 months, with only the sludge removed leaving about 5–10% of the sludge to re-seed the new sludge layer to ensure rapid anaerobic activity. In practice, the sludge is pumped out until it turns into water and then 5–10 litres are returned. Many operators feel that enough sludge will be left behind in the corners of the chamber and adhering to the walls to ensure seeding (Shetty 1971; Mann 1974). The scum should be disturbed as little as possible and the tank, which is designed to operate full, should be refilled with water as soon as possible after desludging. When commissioning septic tanks, they should be filled with water before use and seeded with a few litres of sludge. Although it is possible to purchase a commercial seed, the former is cheaper and probably better. There are a number of materials advertised for septic tank problems. A common claim is ‘use chemical X and never again will you

have to clean out your septic tank'. Schwab *et al.* (1975) examined a number of these chemicals and found that none had any effect on septic tank action and a few even had adverse effects on the percolation area. There is no short-cut to effective tank care and the addition of yeasts, enzymes, and bacteria are not necessary for digestion within the tank. It is a common fallacy that odours produced by septic tanks can be reduced by cleaning the chamber using a proprietary cleaner. Under no circumstances should a tank be disinfected, as this will only result in even worse odours being produced on recovery and inhibiting the degradation processes already taking place in the chamber. If odours are a problem then the tank should be desludged.

The design and constructional details of septic tanks are given by the British Standards Institution (1972, 1983). The two most important design criteria for septic tanks are the suitability of land and the capacity of the chamber. Many by-laws insist on minimum distances for the siting of tanks and percolation areas from houses, wells, streams, and boundaries. The capacity and hence the retention time for settlement and sludge digestion is calculated according to the number of people discharging. The minimum capacity of a septic tank must not be less than 2720 1 with the actual capacity calculated as:

$$C = (180P + 2000) \text{ litres}$$

where C is the capacity in litres and P the number of people discharging into the system. It is quite common for people to purchase a country cottage in which an elderly couple had lived for many years only to find that once modernised, with mains water, a new bathroom, and a kitchen full of labour-saving devices, that the septic tank system can no longer cope. For example, a disposal unit for household kitchen waste (garbage grinder) results in a 30% increase in the BOD and a 60% increase in suspended solids. Where these are installed the capacity of the septic tank must be calculated as:

$$C = (250P + 2000) \text{ litres}$$

Therefore, in the design of a septic tank the future size of the family, number of bathrooms, visitors, and other potential developments must be included in the design. It is unacceptable to use half values for children in such calculations as their water demands are just as great as adults. Some wastewaters are not suitable for discharge to septic tanks. One lady who started a pottery at her country retreat discharged the wash water from her wheel directly to the septic tank. Within a year the excess clay had

compacted so hard in the base of the chamber it had to be drained and manually dug out.

Septic tanks are easy to up-rate by adding extra chambers in series. This reduces the effects of surge flows and excessive sludge accumulation in the first chamber. The commonest faults associated with septic tanks include: (i) Leaking joints when tanks have been constructed from concrete panels or concrete rings. It is important that septic tanks should be watertight to prevent contamination of the groundwater and should be constructed, if possible, without joints; (ii) Non-desludging is the commonest fault, resulting in a reduced retention time and a stronger effluent due to less settlement. The loss of sludge under these circumstances can block pipes, percolation areas or filters. Blocked outlet pipes are frequently a problem because of the scum layer becoming too thick or sludge physically blocking the outlet pipe, which causes the tank to overflow. In a review of Irish septic tanks, Keenan (1982) found that all the systems he visited had inadequate percolation areas and only a few had access to the tanks for the sludge tanker, with pipes often having to pass through peoples houses to reach the tank itself. The problems associated with soil percolation are examined in Sec. 6.1 and have been reviewed by Laak (1986).

Little information is available on the operation of septic tank systems, but on the whole they are very robust. However, care should be taken when using the following: *Disinfectants* should be used moderately as their bactericidal properties kill off the anaerobic bacteria, which can result in awful odours being produced during recovery. It is best to use disinfectants having free chlorine, as it reacts with the organic matter in the sewage rendering it harmless by the time it reaches the tank. *Caustic soda*, which is often used to remove grease from drains, can cause the sludge to flocculate and rise. It can result in sludge passing out of the tank and blocking the percolation area. Small amounts of acidic or alkaline cleaners do no lasting harm and should be used in preference. Some strong cleaners can upset the pH of the tank, which should be as near to neutral as possible. Kleeck (1956) suggests that the pH of the tank should be controlled by the addition of hydrated lime, but this should only be used as a last resort. Under good operational practice the tank will buffer itself. *High sodium* concentrations in the water does not affect the septic tank system directly but can impair the drainage properties of the soil. Those who have water softeners and use soil percolation as a secondary treatment process of septic tank effluents should take advice. *Detergents*, especially alkyl benzene sulphonate, are known to inhibit the digestion process, although providing the tank capacity is sufficient, if normal concentrations of detergents are

used the performance of the system will not be impaired. Enzyme-based washing powders have no effect on septic tank systems. *Large flushes of water* to the tank should be avoided if possible, to prevent scouring of the sludge, unless the tank is large enough to withstand them or a dual chamber system has been installed. Wherever legally permissible, bathwater should be diverted to a soakaway, as should rainwater and melted snow from roofs and paved areas. *Solid material* such as disposable nappies, tampons, sanitary towels, coffee grinds, bones, cigarette ends, and cat litter will not degrade in the tank and should not be discharged to the septic tank as they will reduce the volume of chamber very quickly and can be difficult to remove. Excessive amounts of fats, oils, and greases should also be disposed of separately whenever possible or a grease trap installed before the septic tank.

Maintenance of the septic tank, like all sewage treatment units, is important in maximising treatment efficiency and preventing pollution. The scum and sludge accumulation should be inspected twice a year, depending on its volume. The depth of the sludge and the thickness of the scum should be measured in the vicinity of the outlet pipe. Records should be kept so that desludging frequency can be accurately predicted. The tank should be cleaned whenever the bottom of the scum layer is within 7.5 cm of the bottom of the outlet pipe or the sludge level is within 25–30 cm of the bottom of the outlet pipe (Public Health Service 1969). Scum thickness is measured using a hinged flap device, which is a weighted flap attached to long rod (Fig. 7.7). Any device can be used which allows the bottom of the scum mat to be felt. The measuring device is pushed through the scum layer until the hinged flap falls into the horizontal position. It is then gently pulled upwards until the flap engages against the bottom of the scum layer. The handle is marked to correspond to a reference point on top of the tank. The same procedure is used to locate the lower end of the outlet pipe. The difference in height on the handle corresponds to the distance the scum is from the outlet. The depth of sludge is measured by wrapping a long stick in rough white towelling which is tied securely. The stick is slowly lowered into the tank through the vertical piece of the outlet pipe to the bottom of the tank to avoid the scum. It is left for a few minutes and then slowly removed. The depth of the sludge can be distinguished on the towelling by black particles clinging to it. Sludge level detectors, using light sensitive cells, are now widely used by professionals to monitor sludge levels. If the depth of the sludge is more than one-third of the total liquid and sludge depth at this point, desludging should be arranged.

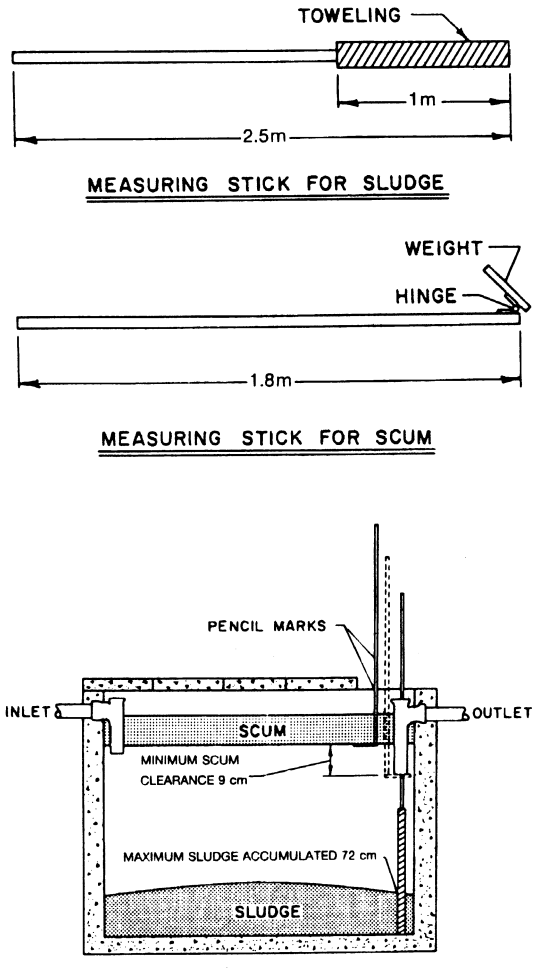


Fig. 7.7. Equipment and procedures for measuring the accumulation of sludge and scum in a septic tank (Schwarb *et al.* 1975).

lmhoff tanks

These combined settlement and digestion units are similar to septic tanks except they are specifically designed for sewage treatment plants rather than individual houses. They were widely adopted throughout the world and can still be seen in many small- and medium-sized sewage treatment plants in Ireland and the UK, although they have been superseded by separate sedimentation and digestion units in recent years. Imhoff tanks

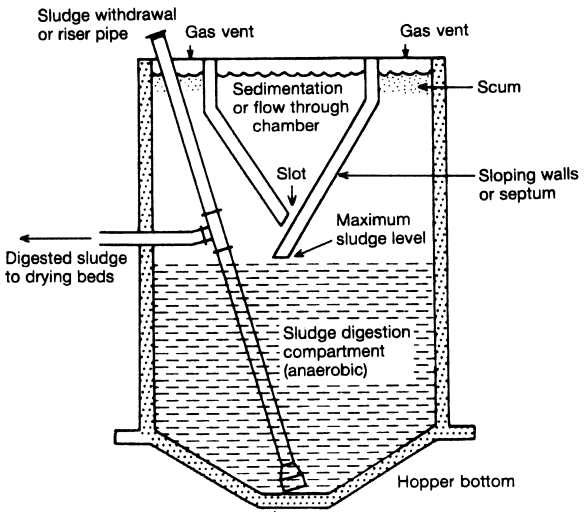


Fig. 7.8. Schematic diagram of an Imhoff tank (Dabney 1971).

are rectangular in plan and comprise of a settlement tank with a digestion chamber below. Single or double units were made in a variety of sizes serving populations ranging from 300 to 10,000 (Fig. 7.8). Screened sewage enters the settlement chamber with a retention time of about 4 hours and as the solids settle they fall down the steep sides of the settlement unit and pass through the wide longitudinal slot that runs along the length of the settlement chamber and collects as a sludge in the digestion compartment. The overlapping lip of the base of the settlement chamber prevents solids buoyed up by gas or gas bubbles themselves entering the settlement chamber and interfering with the sedimentation process. The digestion compartment generally has a large storage capacity and sludge only needs to be removed at long intervals. The long sludge retention time allows digestion to take place with any gas produced vented to the atmosphere (Fig. 7.8). In some warmer climates, digestion proceeds at a more rapid rate allowing the gas to be collected. Imhoff tanks are operated in the same way as a primary sedimentation tank with 50–70% reduction in suspended solids and a 30–50% reduction in BOD being possible. They are compact treatment units that require little maintenance, making them ideal for small communities. However, as digestion is psychrophilic there is incomplete breakdown of the sludge during winter, which produces odours and poor gas production. In the summer, gas production can become excessive and interfere with settlement causing foaming and bubbling. Although simple

in design they are not easy to construct in concrete, and soil stability is especially important as Imhoff tanks are fairly deep. The main problems associated with their operation in Ireland are: infrequent desludging, so that sludge is retained within the settlement chamber reducing the time for settlement; hydraulic overloading again reducing the retention time; the longitudinal slot at the base of the settlement chamber becoming blocked with large solids and in the summer gas accumulation under the harden crust of scum. Apart from proper operational management, the more interesting attempts to up-rate Imhoff tanks have included converting them into RBC units (Sec. 4.2).

Lagoons

The use of anaerobic lagoons has been fully reviewed in Sec. 6.3.1. They are used for strong organic wastes with digestion usually associated with the accumulated sludge in the bottom of the lagoon. Shallower lagoons are also used in some vegetable processing industries combining settlement of soil and waste vegetable matter with partial treatment. Such lagoons are commonly used in the sugar beet industry and although a 80–90% removal of suspended solids and a 60–70% reduction in BOD is possible, the final effluent produced will still be much stronger than a domestic wastewater (> 500 mg l^{-1} BOD, > 200 mg l^{-1} suspended solids), and requires further treatment. A number of problems are associated with lagoons. Vegetable-processing is generally seasonal and the operational period of the lagoons will be short, < 100 days per annum, leaving little time for an active anaerobic biomass to build up. Retention time in the lagoons is too short to ensure complete anaerobic breakdown and in plug-flow systems, it is not possible for a sufficient MCRT to develop. In the treatment of sugar beet wastewater, incomplete anaerobic breakdown occurs with the carbohydrate waste rapidly hydrolysed to volatile acids. However, the volume of process water used in the industry is so great that the acids are present in only a low concentration making recovery or conversion to biogas via separate digestion very difficult. Complete degradation to methane is often inhibited in shallow lagoons because of partial reaeration of the water, and low temperatures. With vegetable wastes, inhibition is also often due to a high C:N ratio.

Anaerobic lagoons are particularly effective for stronger wastes and are widely used on farms for the storage and treatment of animal slurry. Distinct layers form in such lagoons with a bottom layer of settled solids forming a sludge, a liquid layer, and a floating crust. Cattle slurry rapidly forms a

crust, excluding air and producing ideal anaerobic conditions, due to the high fibre content of cattle faeces. Pig slurry, in contrast, does not readily crust over thus allowing oxygen to diffuse into the lagoon so that the top 100–150 mm of the liquid is aerobic. Crust formation is quicker in summer and in a well-constructed anaerobic lagoon the BOD and suspended solids of the slurry will be reduced by 80–90%. During the summer, methane production can be high and large pockets of gas will be seen breaking through the crust. Desludging of farm lagoons should be done every 3–5 years depending on depth, total capacity, and degree of anaerobic degradation achieved, with the sludge spread directly on to the land without any of the associated pathogen problems. As most of these lagoons are between 2–3 m deep, they are potentially dangerous and the crust rapidly becomes covered with grass, weeds, and even small bushes giving it a false appearance of stability. Although in certain circumstances they may be strong enough for an animal or even a child or a man to walk on, they cannot support the weight of a small vehicle. So for safety they should be securely fenced. The construction of a lagoon of this depth is not straightforward and advice will be required as they may need to be lined with welded butyl rubber or PVC sheeting in order to prevent seepage, which could cause groundwater contamination. Emptying a deep lagoon will require extremely stable banks from which heavy plant can operate, therefore earth banks may not always be suitable.

7.2.2. *Digestion*

Modern sewage treatment practice separates primary sedimentation from digestion. Digestion is carried out either in large open tanks or lagoons at ambient temperature (psychrophilic digestion), or more rapidly in covered tanks heated to between 30–35°C (mesophilic digestion). The former digesters are usually unmixed and as they are uncovered any gas produced is dispersed to the atmosphere. In winter, the rate of digestion will be extremely low or even zero, whereas in the summer digestion will be rapid. Therefore, long retention times are required of between 6–12 months to stabilise sludges under these conditions and to balance between sludge accumulation and sludge degradation. Psychrophilic digesters are restricted to smaller works where the output of sludge is low and land is readily available. Both septic tanks and Imhoff tanks fall within the psychrophilic range that operate from 5–25°C.

Heated digesters are much more cost-effective at larger treatment plants where there is sufficient sludge available to ensure a continuous operation, with gas collected and used to either directly or indirectly heat the digesters.

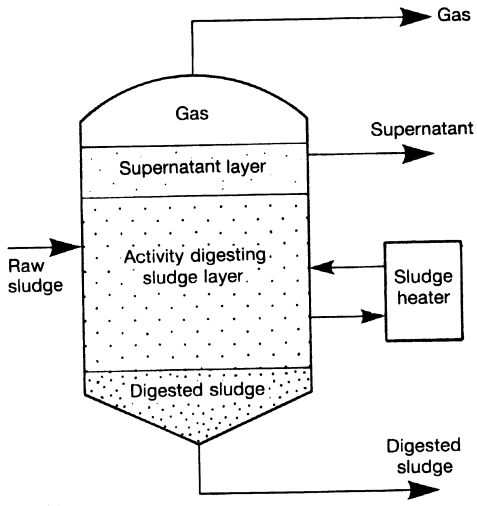


Fig. 7.9. Single-stage anaerobic sludge digester.

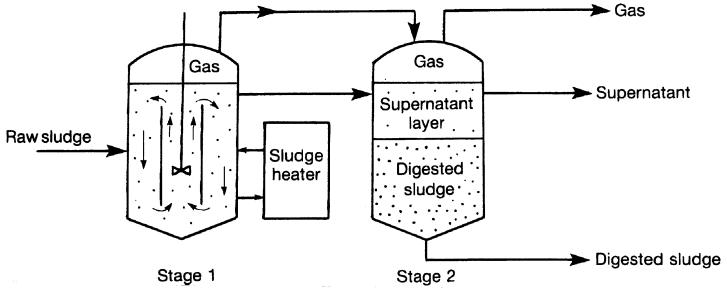


Fig. 7.10. Two stage anaerobic digester.

In their simplest form, digesters are intermittently fed with no recycle. They can be a single-stage reactor (Fig. 7.9), but in the UK and Ireland, conventional digestion is usually a two-stage process with the primary digester heated to the desired temperature in order to allow optimum anaerobic activity, with acid formation and gas production occurring simultaneously. The primary reactor is continuously stirred, unlike the earlier stratified digesters, which reduces the retention time from 30–60 d to < 15 d. The secondary digester is unheated and can be used for two functions. Either to continue digestion under psychrophilic conditions in which case it will be stirred to ensure complete mixing as in the primary digester, or it can be used for sludge separation (Fig. 7.10). However, as the evolution of gas

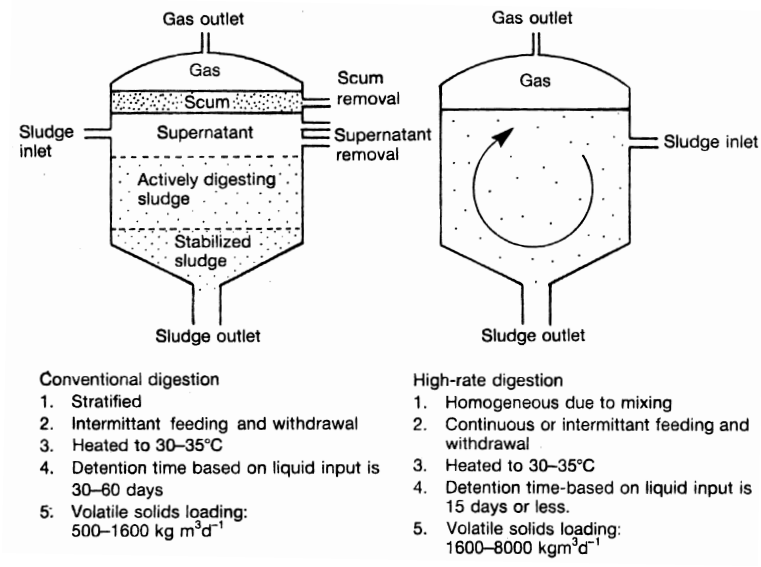


Fig. 7.11. Comparison of conventional and high-rate digestion of sludge.

interferes with settlement, the second reactor cannot be used for both functions. When used for sludge separation the secondary digester is not stirred and is generally uncovered. Under quiescent conditions, the solids separate leaving a strong supernatant liquor on top of the stabilised sludge. The liquor has a high soluble organic content with a high BOD which needs to be recirculated back to the inlet of the treatment plant. The settled solids are removed, dewatered, and disposed. When the secondary digester is used for digestion a third digester may be employed if thickening is required (Institute of Water Pollution Control 1979). This type of frequently fed reactor with no recycle of solids is used for the treatment of sludges with high solids content such as animal slurries and sewage sludge (Fig. 7.11) For example, the waste from a 300 pig unit can be treated in such a reactor with a HRT of 10 d and a gas yield of 0.3 m^3 kg total solids d^{-1} (Hobson 1984). Sludges and liquors with a lower solids concentration can only be digested in a conventional reactor where there is some form of solids recycle to prevent wash-out of bacteria. Generally, such systems have a short HRT of 0.5–5.0 d and an efficient secondary settlement system is vital to ensure an adequate SRT in the primary reactor (Sec. 7.3.1). The other type of flow through reactor is the plug-flow type (Fig. 7.4b), which is used mainly for animal manures, although still at the experimental stage they have also been used for primary sludge digestion in Dublin. However, adequate sludge

recycle is required for seeding purposes, while mixing can be achieved by gas recycle. The major advantage of this sytem is that it allows simple tank configurations to be used, resulting in significant savings on capital cost (Casey 1981).

Digestion is used to stabilise both primary and secondary sludges having a solids content of between 20,000–60,000 mg l^{-1} (2–6%). About 70% of the sludge is degradable and up to 80% of this will be digested reducing the solids content by about 50%. The remaining solids form a relatively stable sludge which has a characteristic tarry odour, although as discussed in Chapter 8, it can be difficult to dewater. The mixture of primary and secondary sludges fed into a digester is rich in carbohydrates, lipids, and proteins, which are ideal substrates for microbial degradation, and although the sludge is already rich in a variety of anaerobic bacteria, in order to obtain the correct balance of hydrolytic acid producers and methanogenic bacteria, the raw sludge needs to be seeded. This is particularly important as the main bacterial groups responsible for digestion are dependent on the end-product of the other. The particulate and high molecular weight organic matter is broken down to lower fatty acids, hydrogen, and carbon dioxide by facultative anaerobic bacteria (first stage) (Sec. 3.4.3.). The end-products are converted to acetic acid by acetogenic bacteria (second stage), which is subsequently converted in the final stage by methanogenic bacteria to methane and carbon dioxide. Obviously, anaerobic digestion will proceed most efficiently when the rates of reaction at each stage are equal. However, if the first stage is limited then the nutrient supply to the other stages will be reduced with the overall effect of suppressing the rate of digestion and biogas formation, but not inhibiting it. If either of the subsequent stages are restricted then the first-stage products will accumulate causing a gradual rise in the carbon dioxide fraction in the biogas ($> 30\%$) and a gradual fall in the pH as volatile acids accumulate until the pH falls below 7.0 and the whole process becomes stressed. All three stages proceed simultaneously within the same reactor with the bacterial groups in close proximity to each other. However, as you move along the anaerobic chain of reaction, the bacterial groups become progressively more sensitive to their environmental conditions. Hydrolysis and acid formation are carried out by a diverse and large group of facultative bacteria that can tolerate a wide variation in temperature, pH, and a range of inhibitory substances. In contrast, the acetogenic and methanogenic bacteria are far more sensitive micro-organisms that are highly specialised and severely inhibited by even minor changes in operating conditions. Thus, it is more common to have operational problems involving the second and third stages of digestion.

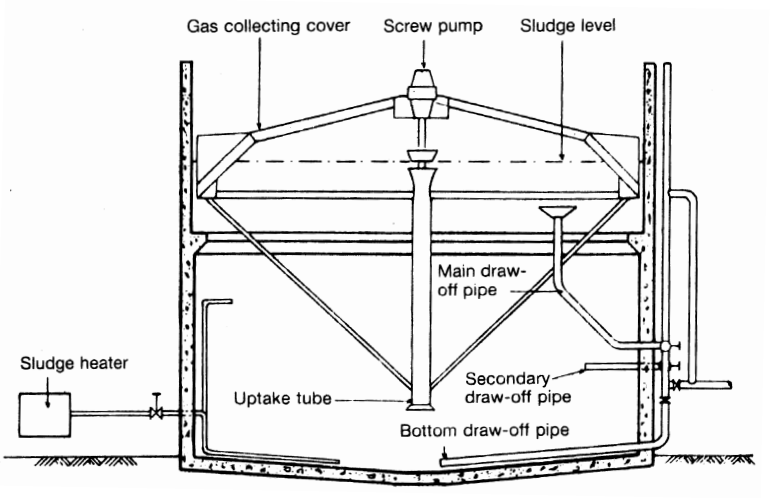


Fig. 7.12. Primary digestion tank with screw mixing pump and external heater (Institute of Water Pollution Control 1979).

Design

Primary digesters are normally covered with a fixed or floating top for gas collection (Fig. 7.12). Floating covers rise and fall according to the volume of gas and sludge, as they are the same design as conventional gas holders, with the weight of the floating cover providing the gas pressure. Fixed covers require a separate gas holder that allows the gas to move freely in both directions. This is particularly important if a vacuum is not to be produced when sludge is removed from the digester, which would not only affect operation but could affect the structural stability of a reactor with a fixed cover. Methane is biochemically inert and it can be stored above the sludge within the reactor without affecting or inhibiting the process. Digesters are usually circular in plan with maximum diameters of approximately 25 m, and to ensure the minimum depth, the ratios of reactor height to diameter is normally between 1:3 to 1:2, although smaller digesters tend towards a ratio of 1:1. The floor of the tank is sloped between 12–30° with facilities to withdraw heavier solids and grit from the base. Large tanks are built above ground, and smaller units can be constructed below ground level or surrounded by earth embankments to provide additional insulation. The entire contents of the tank needs to be turned over once every 3–4 hours, with the sludge warmed by heat exchangers using hot water from gas boilers or the cooling systems of gas engines. Heating and mixing of digester contents can be achieved by a number of techniques. The most common

being a central screw mixing pump with an external heat exchanger, a circulating pump, and exchanger housed in a projecting chamber or internal gas-lift pumps. All these methods are fully reviewed by the Institute of Water Pollution Control (1979). The system shown in Fig. 7.12 has a screw pump mounted in the top of a vertical uptake tube. Known as the 'Simplex System', it is designed and manufactured by Ames Crosta Ltd. The pump circulates the contents of the tank by drawing sludge from the bottom of the reactor and then spraying it out over the surface of the main body of sludge, providing surface scum control as well as efficient mixing. At regular intervals, the pump is reversed for a short period to prevent blockages and improve mixing. The heat-exchanger is external to the digester, the sludge being drawn off from near the base, heated and then returned towards the surface so that it is not immediately drawn up the central updraught tube, thus ensuring maximum utilisation of the heat within the reactor.

The amount of heat required to raise the temperature of the incoming sludge to the optimum temperature and maintain it is calculated as:

$$H = WC\,\Delta T + U A\,\Delta T$$

where H is the heat required to heat the incoming sludge and compensate for heat losses (kg cal h^{-1}), W the mass rate of influent sludge (kg h^{-1}), ΔT the difference between the digester temperature and influent sludge temperature, U the heat transfer through the digester walls (kg cal $m^{-2}h^{-1}$ °C), A the surface area of digester losing heat, and C the mean specific heat of the feed sludge (1 kg cal kg^{-1} °C).

The basic design criterion is the provision of sufficient capacity to ensure an adequate sludge retention time. It is common practice to use 25–30 d as the design retention period to allow for variation in daily sludge loading. However, as the theoretical retention time can be as low as 7–10 d, many feel that with the improvements in mixing and heating digesters now available, that 15 d allows an adequate safety margin to cover most operational difficulties. Digester capacity can also be calculated on population equivalent (FE). Assuming a per capita sludge production of 1.8 litres of raw sludge containing 4.5% dry solids, the capacity of a digester with a 25 d retention time is:

$$\frac{1.8 \times 25 \times \mathrm{PE}}{1000}\ \mathrm{m}^3$$

The volume of a batch digester can be more accurately calculated using the equation:

$$V_s = [V_1 - 0.66(V_1 - V_2)]t$$

where V_s is the digester volume (m^3), V_1 the initial sludge volume (m^3), V_2 the final sludge volume (m^3) and t the retention time (days).

For example, the digestion tank volume required to treat waste activated sludge from a treatment plant treating a population equivalent of 35,000 is calculated as follows where the sludge production rate is 0.1 kg (DS) ca^{-1} d^{-1}, the solids content is 3.5% with a volatile content of 78%, and a specific gravity of wet sludge of 1.017. After digestion, the sludge is 6% dry solids with a specific gravity of 1.025 and with 65% of the volatile solids destroyed.

Influent sludge production $= 35,000 \times 0.1 = 3500$ kg d^{-1}
Volatile suspended solids (VSS) $= 3500 \times 0.78 = 2730$ kg d^{-1}
Fixed suspended solids (FSS) $= 3500 \times 0.22 = 770$ kg d^{-1}
VSS destroyed $= 2730 \times 0.65 = 1774.5$ kg d^{-1}

Remaining VSS in sludge after digestion $= 2730 - 1774.5 = 955.5$ kg d^{-1}
Remaining FSS in sludge after digestion $= 770$ kg d^{-1}
Total solids in sludge after digestion $= 955.5 + 770 = 1725.5$ kg d^{-1}

Influent sludge volume $= 3500 \times (100/3.5) \times (1/1.1017) = 98.3$ m^3d^{-1}
Digested sludge volume $= 1725.5 \times (100/6) \times (1/1.025) = 28.1$ m^3d^{-1}

Using the equation above, the volume of digested sludge (V_s) is calculated using a sludge residence time of 25 days:

$$V_s = [98.3 - 0.66(98.3 - 28.1)]25 = 1299.2 \text{ m}^3$$

As the sludge in the digester only occupies two-thirds of the tank, the total tank volume required is:

$$1299.2 \times 1.5 = 1948.8 \text{ m}^3$$

Although often quoted, organic loadings expressed as kg organic or volatile matter m^{-3} are not appropriate parameters for digester design. With the standard 25 d retention time, the typical sludge feed of 4.5% total solids with an 85% organic (volatile) matter content results in an organic loading equivalent to 1.5 kg $m^{-3}d^{-1}$. However, in the UK, organic loading ranges from 0.27–2.76 kg $m^{-3}d^{-1}$ indicating the varying water contents of the sludges being digested. Thus, in order to achieve the optimum organic loading of 1.5 kg $m^{-3}d^{-1}$ with a thin sludge, a much shorter retention time will result with incomplete digestion occurring (Swanwick *et al.* 1969). For this reason sludges should be characterised and loadings specified as a concentration of total (dry) solids (%).

Secondary digestion tanks are generally used for storage and separation, although they can be used for the further digestion and gas collection. Sludge is passed as frequently as possible from the primary digester into the uncovered tank where it cools and allows the liquor to separate from the solids so that each can be withdrawn separately. Temperature differences between the cool and warm sludge can cause convection currents within the digester that will hinder settlement, as will gas production, so that it becomes difficult to obtain a solids-free liquor. To overcome this, secondary digesters are relatively shallow with a maximum depth of 3.5 m. The capacity of older tanks were between 50–70% of the primary digester providing 15–20 d retention. Newer tanks are approximately the same size as the primary tanks and provide similar retention times for the sludge. Tank volume can be estimated on a PE basis using the equation:

$$\text{Secondary digester volume} = 0.035 \times \text{PE m}^3$$

In order to ensure adequate operating conditions for digestion the pH value, the concentration of carbon dioxide in the biogas, and the volatile acid concentrations should be continuously monitored. The normal operating conditions should be a pH of 7.0–7.2, alkalinity (as $CaCO_3$) of 4000–5000 mg l^{-1}, and a concentration of volatile acids (as acetic acid) of < 1800 mg l^{-1}. The carbon dioxide content of the biogas should not exceed 30%, and once any of these values are exceeded then immediate remedial action is required.

Modern digester designs are discussed fully in Sec. 10.3.1.

Digester operational management

Successful operational management of anaerobic digesters depends on six major factors: composition of the raw sludge, method of addition of raw sludge to the digester, internal mixing and circulation, temperature, pH, and solids retention time.

(a) *Composition of raw sludge.* Like aerobic micro-organisms, those responsible for digestion require certain substrates, growth factors, trace elements, and nutrients for successful development. Mosey (1983) compares raw and digested sewage sludge and shows quite clearly that the major substrates such as lipids, cellulose, and some proteins are present as solids in suspension, and that a substantial fraction of the organic matter is either converted to microbial biomass or not metabolised at all as is the case with lignin (Table 7.3). Nitrogen and phosphorus are both vital for bacterial growth and are required at minimum concentrations of 2.5% and 0.5% of the dry

Table 7.3. Typical composition of sewage sludges before and after digestion (g per 100g total solids) (Mosey 1983).

Constituent or test	Raw sludge	Digested sludge
Suspended solids	95	97
COD	140	100
Organic carbon	40	31
Organic matter	60–80	45–60
Greases and fats	7–35	3.5–17
Cellulose	4	0.6
Hemicelluloses	3	1.6
Lignin	6	8
Protein	22–28	16–21
Anionic detergents	0.5–1.5	0.7–2.2
Zinc	0.09	0.14
Copper	0.035	0.055
Lead	0.016	0.026
Chromium	0.01	0.016
Nickel	0.0092	0.015
Cadmium	0.0022	0.0035

organic matter content of the sludge respectively. This is equivalent to a C:N ratio of between 10–16:1 and an N:P ratio of 7:1, although higher C:N ratios of up to 30:1 have been cited (McCarty and McKinney 1961). What is clear is that anaerobic processes are far less demanding in terms of N and P than aerobic systems. Huss (1977) found the optimum BOD:N:P ratio to be 100:0.5:0.1, whereas the COD:N:P ratio was found to range between 42:0.7:0.1 and 150:0.7:0.1 by Henze and Harremoes (1983). In practice, sewage sludges have excess N and P ensuring adequate digestion, although certain trade wastes, including sugar beet and other food-processing wastes, may be deficient in these nutrients making them less amenable to the digestion process (McCarty 1964; Mosey 1974; DoE 1974; Lettinga 1995). Sewage sludges are mixtures of complex organic materials and vary widely both in composition and strength from place to place. The average solids content in the UK for sewage sludge is often quoted as 4.5 g per 100 g wet sludge, although this will vary even from the same source depending on the operation of the settlement tank from which the sludge is removed.

Secondary sludges from activated sludge or percolating filter units are less amenable to digestion than primary sludges as they contain a lower

proportion of digestible matter per unit mass of solids as well as generally containing much more water than primary sludge. Only about 30% of the available organic and volatile matter is utilised in activated sludge compared with 50% in most primary sludges.

A wide variety of compounds normally present in sewage can inhibit the digestion process when present in excessive concentrations, most notably detergents, chlorinated hydrocarbons, heavy metals, and ammonia. The problem is that many toxic substances are separated at the primary settlement stage with organic compounds such as chloroform being heavier than water and thus collecting at the outlet hopper in the primary settlement tank, and many heavy metal salts being precipitated at this stage as hydroxides. All inhibitory susbstances will affect gas production as the methanogenic group of bacteria is made up of only a few sensitive species, unlike the diverse hydrolytic and acid-forming bacteria. Another important factor is that the hydrolytic and acid-forming bacteria are present in the raw sludge and are constantly replaced. In contrast, the methanogenic bacterial population is self-sustaining, and once the population has been reduced, it will take a long time for the population density to be restored and therefore re-seeding may be necessary.

Detergents. Non-ionic and cationic detergents have little effect on anaerobic digestion, even at high concentrations (Bruce *et al.* 1966), whereas anionic detergents inhibit the process. Household washing powders, which contain alkyl benzene sulphonates (ABS), both hard and soft, are non-degradable anaerobically (Little and Williams 1971; Klein and McGauhey 1965; Maurer *et al.* 1965; Punchiraman and Hassan 1986; Hernandez and Bloodgood 1960). As they are strongly adsorbed on to organic solids they are invariably present in sewage sludge. Anionic detergents at concentrations (expressed as Manoxol OT) in excess of 1.5% of the dry raw sludge solids inhibit anaerobic digestion even in heated digesters with relatively long retention times of up to 40 d, with gas production being particularly sensitive. Concentrations $< 1.5\%$ will reduce the tolerance of the process to other operational variables, such as temporary overloading. The effects of ABS on the bacterial population are numerous and include direct toxicity (Swanwick and Shurben 1969; Meynell 1976). Although degraded aerobically (Huddleston and Allred 1963; Linden and Thijsse 1965; McKenna and Kallio 1965), it is clear that these detergents are not utilised in the anaerobic process because the enzymes responsible for their degradation, such as mono-oxygenase (Cain 1981), are not able to function due to the lack of oxygen (Little and Williams 1971; Alexander 1965). These concentrations are quite common

Table 7.4. Concentration of chlorinated hydrocarbons in digesting sludge at which 20% inhibition occurs (Institute of Water Pollution Control 1979).

Chemical	Concentration (mg kg^{-1} dry solids)
Chloroform	15
Trichlorethane	20
1,1,2-Trichlorotrifluoroethane	200
Carbon tetrachloride	200
Trichloroethylene	1800
Tetrachloroethylene	1800

in domestic sewage sludges and although bacterial populations can become acclimatised, serious inhibition will occur if the concentration is allowed to rise to 2.0% of the dry sludge solids. As digestion proceeds, the concentration of anionic detergents will increase so that at 1.5% the concentration of anionic detergents will rise to 2.5% dry solids in the digested sludge. If inhibition occurs then the anionic detergents can be rapidly neutralised by the addition of long-chain fatty amines, such as stearine amine, to the sludge prior to digestion (Swanwick and Shurben 1969).

Chlorinated hydrocarbons. Although high concentrations of these solvents are unusual in sewage sludges, they can cause a problem in certain trade wastes or where domestic sewage contains trade discharges. The most frequent source of this group of inhibitors is from dry-cleaning operations that discharge chlorinated hydrocarbons to the sewer. Although their effect on the process depends on a number of factors, it is the concentration in the sludge that is most critical (Swanwick and Foulkes 1971; Department of the Environment 1971). It appears that many chlorinated hydrocarbons act selectively on the methane bacteria, which is why they are so toxic to anaerobic digestion compared to aerobic systems (Thiel 1969; Blum and Speece 1992). The concentration at which 20% inhibition occurs is summarised in Table 7.4 for the most widely used chlorinated hydrocarbons, although their inhibitory effect will be increased if other inhibitory substances are also present.

Chloroform is the most toxic of this group and although most will be lost by volatilisation in the sewer and during treatment, it can still cause detectable inhibition at concentrations as low as 10 mg kg^{-1} dry solids (Stickley 1970; Barrett 1972). Hickey *et al.* (1987) reported complete

inhibition of methane production and hydrogen accumulation at concentrations > 1 mg l^{-1}. Where chloroform is a problem it should be removed from the effluent by air stripping, prior to discharge to the sewer, although some acclimation to the compound is possible (Lumb *et al.* 1977).

Other organic compounds. Benzene ring compounds such as benzene, toluene, phenol, and pentachlorophenol all inhibit methanogens (Patel *et al.* 1991; Wang *et al.* 1991). Even when protected by the granulated sludge in UASB reactors (Sec. 7.3.2) organics such as aromatic compounds are still strong inhibitors of methanogens (Donlon *et al.* 1995; Fang *et al.* 1997).

Heavy metals. The most quoted cause of inhibition of sewage sludge digesters are heavy metals and, in particular, chromium, copper, nickel, cadmium, and zinc (Swanwick *et al.* 1969). However, particularly high concentrations of metals are required to have a significant effect (Institute of Water Pollution Control 1979) (Table 7.3). Toxicity of heavy metals to anaerobic digestion increases as the metal affinity for sludges decreases. Nickel has the lowest affinity for sludge and so is most toxic and vice versa for lead (i.e. toxicity to digestion is Ni > Cu > Cd > Cr > Pb). Synergistic effects have been noted with heavy metals and a number of other inhibitory substances, and under certain conditions even low concentrations of heavy metals may cause problems. Apart from the concentration of heavy metals in the raw sludge other factors such as solubility, pH, and the concentration of sulphide present will all affect their concentration in the digester. Anaerobic bacteria are able to withstand quite high concentrations of total heavy metals as a considerable percentage of the metal ions can be precipitated out of solution as either sulphides or carbonates. The concentrations of metals that can be present in sewage without adversely affecting anaerobic digestion of the resulting sludge have been summarised in Table 7.6 (Barth 1965). There is always more than one heavy metal present in a sewage sludge and the inhibitory effects are additive on an equivalent weight basis (Mosey 1976). Where the milligram equivalent weight (meq) per kg dry sludge solids (K) exceeds 400 meq kg^{-1} there is a 50% chance of digester failure which rises to 90% when K exceeds 800 meq kg^{-1}. To ensure a 90% probability that digestion will not be affected, the value of K should be < 170 meq kg^{-1}.

K is measured using the concentrations of the most abundant heavy metals (mg l^{-1}) in sewage sludge, excluding chromium, using the equation:

$$\mathrm{K} = \frac{(\mathrm{Zn}/32.7)+(\mathrm{Ni}/29.4)+(\mathrm{Pb}/103.6)+(\mathrm{Cd}/56.2)+(\mathrm{Cu}/47.4)}{\text{Sludge solids concentration (kg } l^{-1})} \text{ meq } kg^{-1}$$

Table 7.5. Concentrations of heavy metals in digesting sludge that cause a 20% reduction in gas production in laboratory experiments (Institute of Water Pollution Control 1979).

Metal	Batch digesters: concentration (mg kg^{-1} dry solids)	Typical concentration in digested sludges (mg kg^{-1} dry solids)
Nickel	2000	30–140
Cadmium	2200	7–50
Copper	2700	200–800
Zinc	3400	500–3000

Table 7.6. Highest metal concentrations in sewage that will allow satisfactory digestion of sewage sludge (Casey 1981).

Metal	Concentration in influent sewage (mg l^{-1})	
	Primary sludge digestion	Combined sludge digestion
Chromium (hexavalent)	> 50	$> 50^a$
Copper	10	10
Nickel	> 40	$> 10^a$
Zinc	10	10

[a] Higher dose not studied.

Some metals at trace concentrations (e.g. nickel, colbolt and molybdenum) stimulate methanogens (Murray and van der Berg 1981).

Ammonia. Ammonia or ammonium ions are essential nitrogen sources for anaerobic digestion, but can be inhibitory when present at concentrations of > 150 mg N l^{-1} and 3000 mg N l^{-1} respectively. These concentrations are only occasionally found in very thick sewage sludges and undiluted farm slurries. However, as Mosey (1983) clearly explains, the system is largely self-regulating in that inhibition causes an accumulation of volatile solids, which in turn depress the pH value, converting dissolved ammonia (NH_3) to the less toxic ammonium ionic form (NH_4^+), thus alleviating inhibition.

Other ions. Methane formation does not occur readily in the presence of electron acceptors, such as sulphate and nitrate. Sulphate can be a particular problem in the digestion process if present in sufficient quantities. The sulphate is reduced to sulphide by bacterial action (Sec. 3.4.4),

with hydrogen sulphide eventually being formed. Sulphate concentrations > 500 mg l^{-1} can reduce methane production and generate up to 4% hydrogen sulphide in the biogas. The hydrogen sulphide will form insoluble compounds with heavy metals. Therefore, as long as hydrogen sulphide and heavy metals are present in equivalent proportions, hydrogen sulphide production will cause no problems and, will in fact, be beneficial. However, two problems can occur: (i) hydrogen is consumed by sulphate reduction and is no-longer available for methane formation, which is inhibited; and (ii) hydrogen sulphide itself has a direct toxic effect on the methanogenic bacteria being toxic at concentrations > 200 mg l^{-1} (Mosey 1976). Remedial action involves precipitating the excess hydrogen sulphide out of solution as ferric sulphide by the addition of an iron salt, such as ferric chloride or ferric oxide, but not, of course, ferric sulphate. Nitrates can also cause problems because if denitrification occurs within the digester there will be a shift in the redox potential (Sec. 3.4.6), which will suppress methane production. Methanogens are strict anaerobes and can be completely inhibited by a dissolved oxygen concentrations as low as 0.01 mg l^{-1} (Wolfe 1971). They require a reduced environment with a redox potential within the range –200 to –420 mv. This has been a serious problem at a number of sewage treatment plants in the UK and has been remedied by allowing denitrification to occur before the sludge enters the digester, with the nitrate being converted to nitrogen gas under anoxic conditions (Sec. 3.4.5).

Inhibition. Pilot trials to test the biodegradability of wastewaters and assess the possible inhibitory effects of wastewaters and chemicals should always be carried out prior to anaerobic digestion (HMSO 1987, 1989).

(b) *Method of sludge addition to digester.* Digesters can be operated either as batch or continuous processes that may incorporate recycling of solids, gas, or both. For optimum performance, sludge should be added to the digester as frequently as possible in order to avoid fluctuations in gas production or problems with scouring the active bacteria from the primary digester. It is not generally possible to feed sludge directly from a sedimentation tank on a continuous basis, as it requires consolidation, or thickening, before it is suitable for use. Although the majority of digesters are fed with raw sludge at least once a day, two or three times a day is preferable (Swanwick *et al.* 1969).

Commissioning digesters is a topic of some controversy. The most commonly used method is to gradually fill the digester with raw sludge together with the seed sludge. However, it is not possible to commence circulation or

heating until the reactor is full, which can lead to problems in starting up due to compaction of solids at the base. Alternatively, the digester is filled with sewage so that circulation and heating can commence at once, with the raw and seed sludge added gradually. This method has the disadvantage that if the seed sludge is too diluted, such that the correct balance of micro-organisms is not maintained, incomplete digestion producing unpleasant odours will result. Although it is possible to use raw sludge which has been stored for several months as a seed, it is best to use sludge from another digester, preferably from the same plant. The ratio of seed sludge to raw sludge should be between 1:10–1:5. Once started, the percentage of seed can be increased but care must be taken to prevent excessive acid production from inhibiting the development of methanogenic bacteria. There will be little biogas production for the first 4–6 weeks and an alternative source of heating the reactor during commissioning will be necessary (Sambridge 1972).

(c) *Internal mixing and circulation.* Stratified and completely mixed digesters have already been discussed. The purpose of mixing and circulation within the digester is: (i) to promote close contact between the raw and digesting sludges; (ii) to maintain a uniform temperature and solids mixture throughout the tank, and prevent localised accumulation of inhibitory substances; (iii) to discourage scum formation and settlement of grit and dense solids; and (iv) most important of all, to encourage the release of gas from the sludge in the lower regions of the digester. Poor mixing will lead to stratification within the digester and will result in partially digested sludge being withdrawn (Institute of Water Pollution Control 1979). Efficient mixing turns the conventional plug-flow reactor into a high-rate digestion process by making use of the total reactor volume and ensuring a faster reaction rate due to the removal of mass transfer limitations of food and micro-organisms (Fig. 10.9). Mixing can be done either mechanically or by the recirculation of biogas. Mixing is enhanced by the circulation of sludge through the heat exchanger that operates continuously. The evolution of gas, which reaches a maximum 2–3 hours after the addition of raw sludge, will also supplement the mixing effect. The actual mixing mechanism may not be operated continuously at all plants.

Scum, which is composed of grease, oil, and soaps, with floating material entrained, will tend to form on the surface of the digestion tank if mixing is not adequate (Fig. 10.10). It causes a number of problems, such as reducing the effective capacity and thus the retention time of the reactor, interferes with mixing and the internal circulation of the sludge, interferes with the

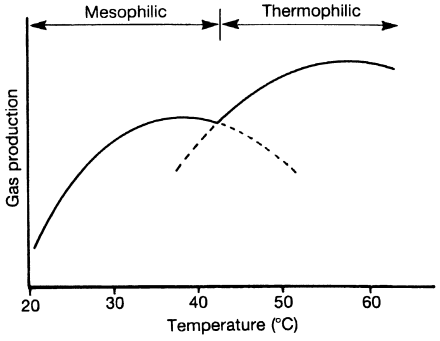


Fig. 7.13. Effect of temperature on gas production.

movement of the floating gas holder, and restricts the evolution of gas. Most mixing systems withdraw the sludge from the base of the tank and spray it over the surface preventing scum formation, and screw pumps can be reversed to carry any scum down into the body of the tank. Prevention is best, however, and the sludge should be adequately screened before entry to the digester and excessive grease and oil should be removed prior to discharge to the sewer. There are also a number of design features aimed at reducing scum formation including scum removal devices, which disperse sludge by discharging raw or digested sludge into the surface layer via jets or nozzles (Sec. 10.3.1).

(d) *Temperature.* Anaerobic digestion can occur over a wide temperature range which has generally been subdivided into three separate ranges, psychrophilic (5–25°C), mesophilic (25–38°C), and thermophilic (50–70°C) digestion. The rate of anaerobic digestion and gas production is temperature-dependent (Fig. 7.13), with optimum gas production at the higher temperature ranges. Therefore, the warmer the reactor the shorter the MCRT needs to be for complete digestion (Table 7.7).

Septic tanks, Imhoff tanks, sludge lagoons, and unheated sludge digesters, which are located mainly at smaller treatment plants fall into the psychrophilic range. At ambient temperatures in temperate climates, the rate of digestion is so low that the residence time of the bacteria needs to be of the order of 3–12 months. Suitable bacteria for seeding such systems, acclimatised to these low temperatures, can be obtained from marshlands. The majority of heated sewage sludge digesters operate in the mesophilic range, usually between 30–32°C (Swanwick *et al.* 1969), with residence times of 20–40 days. Thermophilic digestion is technically feasible and a

Table 7.7. Suggested mean cell residence times (MCRT) for the anaerobic digestion of sewage sludge at various temperatures.

Temperature (°C)	MCRT (days)
18	28
20	22
25	18
30	14
35	10
40	8–10

number of pilot plants have been built and operated successfully (Pickford 1984). However, the rate of gas production does not increase continuously with temperature but rapidly declines after reaching an optimum at 55°C (Fair and Moore 1934). A similar situation is found in the mesophilic range at 35°C (Fig. 7.13), indicating that different bacterial populations are responsible for thermophilic and mesophilic digestion, rather than thermotolerant mesophilic species. It appears that between these optima, erratic gas production will be encountered. Inhibition of methanogenic bacteria occurs at about 63°C (Pfeffer 1979). The effect of temperature is not great on the first stage of anaerobic digestion, as so many different species are involved that the operating temperature will always fall within the optimum range of some of the micro-organisms present. However, the acetogenic and methanogenic bacteria are particularly sensitive to temperature, with even a 2–3°C drop in a mesophilic digester adversely affecting biogas production.

Once operational, a heated digester will be adversely affected if the temperature is allowed to fluctuate by more than just a few degrees. It is general practice to ensure that the operating temperature is near the top of the preferred range before the onset of winter. However, a fall in temperature can be caused by a number of factors, including inadequate heating capacity, scaling of the heat exchanger surfaces, and the raw sludge having a low solids concentration. The temperature can be raised by reducing heat losses from the whole digestion unit by adequate insulation, increasing the heat input, reducing the water content of the raw sludge and thus reducing the total volume to be heated, and by descaling heat-exchanger surfaces.

(e) *pH.* Most anaerobic treatment systems have problems with pH control which arises from differences in the growth rate of the synergistic bacterial

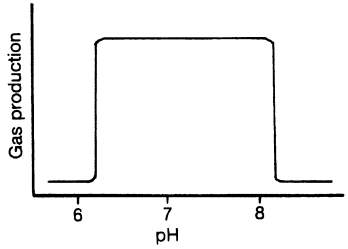


Fig. 7.14. The effect of pH on biogas production.

populations. The activity of the acid-producing bacteria tends to reduce the pH of digesting sludge from the optimal 7.0–7.5 range required by methanogenic bacteria. Under normal operating conditions, once a stable population of each of the groups has been established, an equilibrium is maintained by the buffering action of ammonium bicarbonate (the bicarbonate alkalinity), hence no external pH control is required. The bicarbonate ions are derived from carbon dioxide in the digester gas and the ammonium ions derive from the degradation of proteins in the raw sludge.

However, the digesting sludge does have a tendency to become acidic especially if the methanogenic bacteria are inhibited, or the digester is overloaded, which results in an excessive accumulation of volatile acids. Under these conditions, the buffering capacity may be exceeded with the pH rapidly decreasing to 6.0 causing the process to fail, resulting in a sudden decline in gas production (Fig. 7.14). Methanogenic bacteria exhibit a negative response when the pH shifts towards the acid region as they do when the temperature falls. Growth of methanogens is inhibited below pH 6.6, although the fermentative bacteria will continue to function until the pH has dropped to 4.5–5.0. It should be remembered that high concentrations of volatile acids that are likely to occur in digesters are not toxic to methanogenic bacteria in themselves, it is the pH that is inhibitory, with concentrations of acids up to 11,800 mg l^{-1} non-toxic to methanogens (Velsen and Lettinga 1979; Newell 1981). Therefore, any continuous downward trend in pH is an important warning sign requiring immediate attention. The measurement of pH must be done rapidly, as samples of digesting sludge once exposed to the atmosphere will rapidly lose carbon dioxide and cause erroneously high pH values. As long as the sludge has a fairly high alkalinity an increase in acid production will initially produce little effect on pH, and, in practice, the measurement of volatile acids is a better control factor of the buffering capacity within a digester. Any change in the

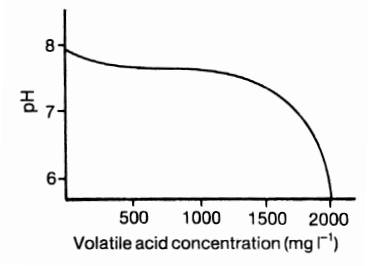


Fig. 7.15. Effect of volatile acid production on the pH in a digester.

loading of the digester must be gradual in order to ensure that the concentration of volatile acids does not exceed the normal buffering capacity of the system. Normal volatile acids concentrations in sewage sludge digesters are between 250–1,000 mg l^{-1}, but values in excess of 1,800–2,000 mg l^{-1} indicate problems (Fig. 7.15). Determination of individual volatile acids is also very useful, as a shift to higher volatile acids, such as acetic to butyric is a sign of instability and remedial action is required. If adequate buffering capacity is available, higher volatile acids concentrations may be tolerated, although this will lead to incomplete conversion of biodegradable material to gas with a subsequent increase of the BOD_5 of the digester effluent. Depending on the chemical nature of the sludge, it is possible to have rapid and effective digestion at all pH values between 6.2–7.8, although certain trade and sewage wastes restrict rapid digestion to much smaller ranges. In his excellent review on anaerobic processes, Mosey (1983) gives two interesting examples. Ferrous carbonate can become sufficiently soluble below pH 6.4 to release inhibitory concentrations of ferrous ions into solution, whereas above pH 7.5 an increasing percentage of ammonium ions will be converted to toxic dissolved ammonia gas. The latter example is particularly important in relation to strong sewage and farm wastes.

The pH value can be neutralised within the reactor by the addition of an alkali. The cheapest and most widely used alkali in full-scale digesters is probably calcium hydroxide (lime). Lime is extremely efficient at increasing the pH to about 6.4–6.5. However, further increases in pH can only be achieved by precipitating most of the dissolved carbonates as calcium carbonate. Therefore, at pH > 7.0 the lime reacts with carbon dioxide, which not only results in serious scale formation but also reduces the pressure of the gas phase above the sludge by removing the carbon dioxide, which could seriously affect the structural stability of the reactor. Bicarbonates or carbonates of either sodium or potassium are generally used to raise the pH from 6.5 to 7.0, although excessive use of these salts can result in partial

or severe inhibition at concentrations of 3,500–5,500 and > 8000 mg Na l^{-1} and 2,500–4,000 and > 12,000 mg K l^{-1} respectively. Other alkalis are less suitable. For example, sodium hydroxide also removes carbon dioxide from solution, and the use of ammonia or ammonium ions can inhibit the process. For laboratory-scale digestion units, the most useful reagents for adjusting the pH are hydrochloric acid and sodium bicarbonate.

If the sludge contains heavy metals in solution at concentrations likely to cause inhibition, the addition of alkali to raise the pH to 7.5–8.0 will precipitate most of the metal ions out of solution usually as carbonates, thus reducing the inhibitory effect. Sulphide can also be used to precipitate the heavy metal ions without causing a significant pH change.

Apart from chemical buffering, pH control can also be achieved by using other wastes or by increasing the sludge recycle rate where the reactor design allows, such as fluidised beds or downflow biofilters (Sec. 7.3) (Wheatley 1985).

(f) *Solids retention time.* The period that solids are retained in the digester is a crucial factor affecting performance. In flow-through systems the residence time of the waste (HRT) and of the microbial biomass (MCRT) will be the same. If complete digestion is to be achieved in heated mesophilic digesters then minimum retention times of more than 15 days are required to ensure that the methanogenic bacteria, which are slow growing, are able to accumulate to a sufficient population density. The design of digesters normally aims at a minimum retention period of 25–30 days, allowing for some loss in digester capacity due to accumulated non-biodegradable solids within the reactor. Clearly, any increase in the water content of the raw sludge will increase the HRT, and in flow-through systems this will cause an increased wash-out rate of the microbial biomass and an associated reduction in performance. As mentioned earlier, the minimum MCRT is dependent on the operating temperature of the reactor (Table 7.7).

Biogas and other products of digestion

There are three products of anaerobic digestion, the digested sludge, a waste liquor, and the sludge gas. Digested sludge is different from both primary or secondary sludges in a number of ways. It is pathogen free, stabilised, has a tarry smell which is far less offensive, and drying to an inert friable condition, thus making it ideal for disposal to agricultural land. The nature and utilisation of digested sludge is fully discussed in Chapter 8. The waste liquor from anaerobic digesters has a high suspended solids (500–1,000 mg l^{-1}) and BOD concentration (400–800 mg 1^{-1}), due to all the

soluble organics present. The BOD of the liquor can be very high indeed reaching up to 10,000 mg l^{-1}, although up to 60% of the BOD is due to the suspended solids fraction. Because of the degradation of organic nitrogen, the liquor may have high concentrations of soluble nitrogen present. The characteristics and strength of the liquor makes it difficult to dispose or treat separately, and it is returned to the works inlet where it is diluted by the incoming sewage and treated in admixture.

Sludge or digester gas is more commonly referred to as biogas. Gas production is the most direct and sensitive measure of the rate of anaerobic digestion, a decrease in production being the first indication that the process is unstable. Apart from trace amounts of water vapour, hydrogen sulphide, hydrogen, nitrogen, unsaturated hydrocarbons, and other gases, biogas is essentially a mixture of carbon dioxide and methane, the exact proportions of which determine its calorific value. A typical biogas contains between 65% and 70% methane by volume with the remaining 30–35% being carbon dioxide. Although hydrogen is an important precursor of methane formation in digestion, the concentration of hydrogen, which can be measured within the reactor, is usually very low, which suggests an immediate uptake by the bacteria (Zeikus 1979). When burnt, biogas produces water which complicates the determination of its calorific value. The net calorific value for combustion is where the water formed remains in the vapour phase, whereas the gross calorific value is where the water formed is condensed. The calorific values are calculated using the percentage of methane (M) present as the net calorific value = (334 × M) kJ m^{-3}, or the gross calorific value = (370 × M) kJ m^{-3} both at 15.5°C and at 1 atmosphere. For normal biogas, the gross calorific value is between 24,000–26,000 kJ m^{-3}, with the net value being some 10% less.

The production of biogas can be related to the amount and type of organic matter utilised. For example, the removal of 1 kg of COD yields 0.35 m^3 of biogas at standard temperature and pressure (STP), whereas 1 kg of organic carbon would yield 1.87 m^3 at STP. The exact gas yield per kg of volatile solids removed depends on the composition of the waste but is approximately 1.0 m^3. Some food-processing industries produce a pure substrate waste with 80–95% of the organic (volatile) matter removed, whereas only 40–50% of the organic matter in sewage sludge, and even slightly less in animal slurries, will be utilised with a proportionately lower gas yield in terms of m^3 of gas produced per kg of substrate supplied. Therefore, for a mixed primary and secondary sludge from a purely domestic sewage treatment works, the gas production will be of the order of 0.5 m^3kg^{-1} organic (volatile) matter, or about 0.375 m^3kg^{-1} of total (dry) solids, added

Table 7.8. Gas yield and composition of biogas produced by the digestion of carbohydrates, proteins, and lipids (Casey 1981).

Substrate	Gas yield	Composition	
Carbohydrates	0.8 m^3kg^{-1}	50% CH_4	50% CO_2
Proteins	0.7 m^3kg^{-1}	70% CH_4	30% CO_2
Lipids	1.2 m^3kg^{-1}	67% CH_4	33% CO_2

to the digester. The composition and quantity of gas produced by complete digestion can be theoretically determined using the equation:

$$\begin{aligned} &C_cH_hO_oN_nS_s + 1/4(4c - h - 2o + 3n + 2s)H_2O \\ &\quad = 1/8(4c - h + 2o + 3n + 2s)CO_2 \\ &\qquad + 1/8(4c + h - 2o - 3n - 2s)CH_4 + nNH_3 + sH_2S \end{aligned}$$

where c, h, o, n, and s are the number of atoms of carbon, hydrogen, oxygen, nitrogen and sulphur respectively. This equation replaces the earlier equation developed by Buswell and Mueller (1952), which ignored the nitrogen and sulphur component of the waste that are generally utilised within the digester.

Thus, by neglecting the very small volume of other gases formed, and using the simplified formula (Sec. 3.4.3), carbohydrate will produce 73% CO_2 and 27% CH_4, lipid 52% CO_2 and 48% CH_4, and protein 73% CO_2 and 27% CH_4. The total volume of biogas produced from each of these basic substrates is 0.75, 1.44, and 0.98 m^3kg^{-1} of dry matter respectively (Buswell and Mueller 1952). Actual values of gas yield and composition from the basic substrates have been given by Konstandt (1976) (Table 7.8). Lipids are only slowly degraded anaerobically and the measurement of their removal provides a very useful quality control parameter for the process.

A more accurate estimation of methane production per unit time can be obtained by using the equation:

$$G = 0.35(L - 1.42S_t)$$

where G is the volume of methane produced (m^3d^{-1}), L the mass of ultimate BOD removed (kg d^{-1}), and S_t the mass of volatile solids accumulated (kg d^{-1}). S_t can be estimated as:

$$S_t = aL/[1 + bt_s]$$

where a is the mass of volatile solids synthesised per kg of ultimate BOD removed, b is the endogenous respiration constant, and t_s is the solids retention time (Speece and McCarty 1964). Methane is the most valuable by-product of anaerobic digesters and it is useful to express the yield as m^3 CH_4 kg^{-1} organic matter removed, or m^3 CH_4 kg^{-1} COD removed. Typical methane yields from mesophilic digesters operated at 35°C are 0.86 m^3kg^{-1} organic matter removed for mixed sewage sludge, 0.55 m^3kg^{-1} for dairy manure, and 0.42 m^3kg^{-1} for pig manure. Under continuous reactor operation, a mesophilic digester will produce between 12–16 l of methane per capita per day for primary sludge, rising to about 20 l d^{-1} for combined primary and secondary sludges (Casey 1981).

Biogas is usually burned on-site either to produce heat directly for the digesters using gas boilers, or to generate electricity using modified diesel (dual fuel) engines to drive alternators, with the cooling water from these engines used to heat the digesters. Some plants now use biogas for vehicle fuel, which is discussed in Sec. 10.3.1. Smaller digestion units are used widely in the Third World for cooking and lighting, and there are numerous small digesters operated specifically to produce energy. The simplest method of utilising biogas, and the one requiring least capital investment, is using the gas to produce hot water in a boiler, giving the gas a value of 3.5p kWh^{-1}, compared to the cost of liquid petroleum gas (LPG). All the other methods involve the use of a generator to produce electricity (Parsons 1985). This is considered further in Sec. 10.3.1.

In recent years, there has been considerable interest shown by the agricultural industry in using anaerobic digestion, not only to reduce the problem of disposing of animal slurry faced by many intensive farmers, but also as an economic source of energy (Hobson *et al.* 1981). A detailed feasibility study carried out by Parsons in 1985 on the economics of anaerobic treatment of dairy cow waste in the UK found that it was not economic when considered only as a source of energy. The cost of anaerobic treatment, after deducting the value of the energy produced ranged from £9–70 per cow y^{-1} in 1985, with the lowest costs ($<$ £20 per cow y^{-1}) obtained only with herds of a minimum size of 200. Costs are reduced by reducing the water content of the sludge and by improving the gas yield at short SRTs. The system is only effective if there is a supply of slurry all the year round, and for animals only housed in the winter, slurry will have to be stored. At present, digestion is only economic for large intensive units where the disposal of slurry proves to be a particular and expensive problem. The economics of anaerobic digestion of farm animal wastes is reviewed by Parsons (1984). Other sources of biomass for the production of biogas include

Table 7.9. Typical digestion times and gas yields at 30°C for common agricultural waste materials (Mudrack and Kunst 1987).

Starting material	For complete digestion Gas production relative to:			CH_4 content (%)	Gas yield after time stated as % of ultimate (days)		
	Total solids (ml g^{-1})	Organic solids (ml g^{-1})	Digestion time (days)		10	15	20
Cattle dung	237	315	117	80	24	36	48
Pig excreta	257	415	115	81	40	57	69
Straw 30 mm	357	383	123	80	29	38	45
Straw 2 mm	393	423	80	81	51	67	77
Potato haulm	526	606	53	75	85	90	92
Sugar beet leaves	456	501	14	85	99	100	100
Grass	490	557	24	84	87	96	99

a wide range of fast-growing plants that are often grown specifically for biogas production (Sec. 6.2) (Table 7.9). However, there is a range of waste vegetable material that can also be used, such as sugar beet tops. One of the most effective substrates in terms of gas yield is waste silage.

7.3. Contact Anaerobic Systems

Because of the difficulties involved in degrading particulate organic matter and the slow growth of anaerobic bacteria, and of methanogens in particular, digester design has been traditionally based on flow-through stirred tanks with long retention times. Unlike sludge, the organic matter in wastewater is often in solution and is far more amendable to treatment. However, the primary problem with treating these wastes anaerobically has been how to retain sufficient biomass within the reactor and prevent wash-out of bacteria. Contact anaerobic systems are specifically designed to treat weaker sludges with low solids concentrations and strong effluents. Unlike the conventional continuously stirred digester, the HRT and MCRT are independent of each other, with the biomass either retained within the reactor or recycled after separation. Four major contact systems have been developed: anaerobic activated sludge; sludge blankets; static media filters; and fluidised media. Data on over 400 anaerobic plants, mainly treating vegetable-processing wastewaters, have been given by Demuynck *et al.* (1983), who give details on many contact systems having reactor

Table 7.10. Industrial applications of anaerobic digestion in Europe (Wheatley *et al.* 1997).

Food effluents		Non-food effluents	
Type	Number	Type	Number
Distillers	54	Paper	48
Sugar refiners	51	Pharmaceuticals	7
Potato processes	40	Chemicals	5
Breweries	32	Textiles	2
Starch refiners	25		
Milk processing	16		
Abattoirs	14		
Yeast processing	11		
Canneries	11		
Fruit processing	9		
Soft drinks	5		

Types of digester	% of total
UASB	41
Anaerobic filters	22
Contact digester	15
Stirred tank (no recycle)	12
Other types	10

volumes ranging from 2,000 to 20,000 m^3. Details of industrial applications of anaerobic contact systems are given by Wheatley *et al.* (1997) (Table 7.10).

While anaerobic treatment systems, especially up-flow anaerobic sludge blanket (UASB) reactors and anaerobic filters, are very efficient and cost effective at treating a wide variety of wastewaters, including sewage, the final effluent still has relatively high concentrations of COD, nutrients, and suspended solids. This requires further treatment before final discharge to receiving waters. Aerobic treatment is normally used as a post treatment process for both carbonaceous and nutrient removal to achieve modern discharge standards. All aerobic processes are employed including activated sludge (Nuñez and Martinez 2001); BAF (Lacelle *et al.* 2001); constructed wetlands (Denny 1997; Sousa *et al.* 2002); land treatment (Chericharo *et al.* 2001); maturation ponds (Dixo *et al.* 1995; Catunda and van Haandel 1996;

Cavalcanti *et al.* 2001); percolating filters (Chericharo and Nascimento 2001); SAF (Gonalvez *et al.* 1999) and SBR (Sousa and Foresti 1996; Zaloum and Abbott 1997).

7.3.1. *Anaerobic activated sludge process*

This method is similar in design to the conventional two-reactor digester described in Sec. 7.2.2 (Fig. 7.10), with the primary tank being the main anaerobic reactor and the secondary reactor used for solids separation (settlement), with solids recycled back to the primary digester (Fig. 7.4d). The settlement tanks are normally covered to prevent odour nuisance. Anaerobic activated sludge systems, also known as contact stirred tank reactors (CSTRs), operate at relatively short HRTs of between 0.5–5.0 d. Therefore, it is essential that the settlement is efficient in order to ensure that digestion in the primary tank carries a high solids concentration level to maintain final effluent quality. There are no internal fittings in these reactors so that high suspended solids wastewaters can be treated. This system is particularly suited to warm, dilute effluents, especially food-processing wastewaters that have a high suspended solids content (e.g. abattoir wastes, distillery slops, starch wastes, sugar beet processing wastes, and yeast fermentation) (Butcher 1988; Hobson and Wheatley 1993). The design ensures a long MCRT (or SRT) and a short HRT, resulting in high efficiency despite a comparatively small reactor volume. They have been used to treat a wide range of organic wastes and achieve high BOD_5 removal rates, for example, meat packing wastewater 91% removal (Steffen and Bedher 1961) and sugar beet wastewater 93–95% (Frostell 1981). There are two large anaerobic activated sludge systems operating in the UK. One is Ashford, Kent, which is operated by Tenstar Products and treats starch waste; it was commissioned in 1976 (Morgan 1981). The other is a plant that was opened in 1982 by the British Sugar Corporation, Bury St. Edmunds, to treat sugar beet process water (Shore *et al.* 1984). Examples of anaerobic digestion systems used in the sugar-processing industry are given in Fig. 7.16.

The major operational problem appears to be poor settlement in the secondary reactor because gas bubbles become attached to the solids. This is usually overcome by vacuum degassing (Fullen 1953), although other modifications to commercially available digesters have also been successful (Stander 1967; Cillie *et al.* 1969; Wheatley *et al.* 1997). However, the process has many advantages over conventional completely mixed digesters: including ease of startup; good process control; improved resistance to

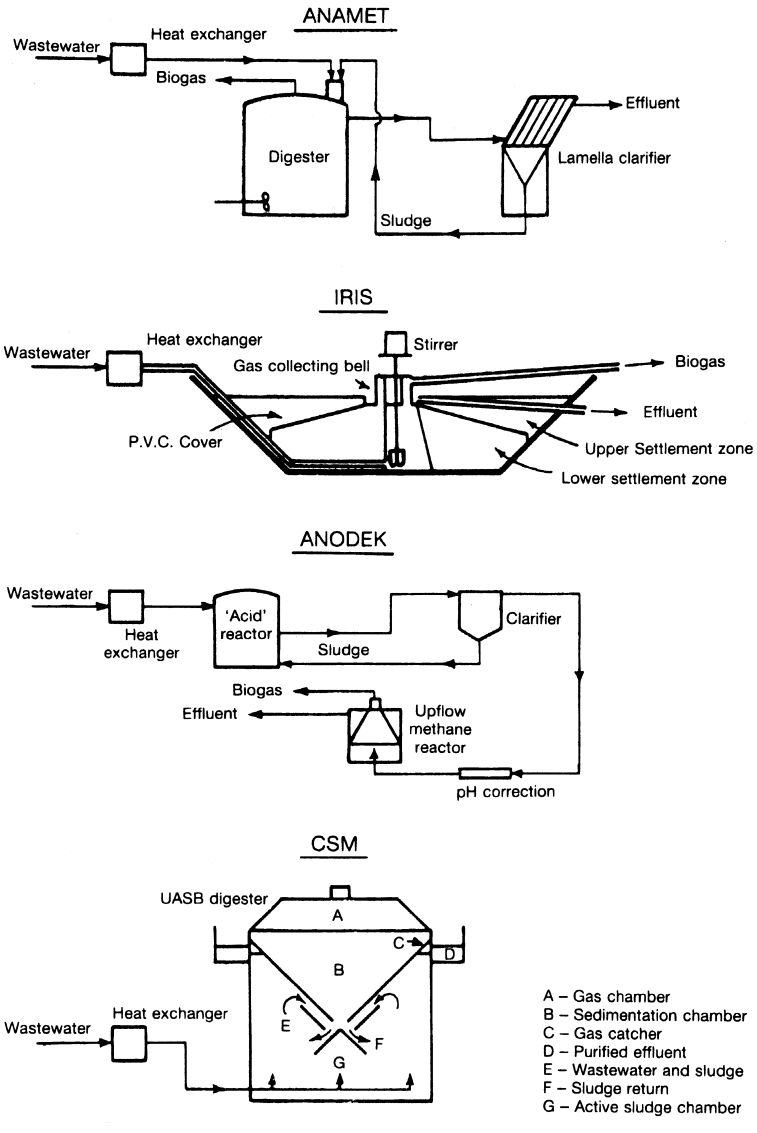


Fig. 7.16. Examples of anaerobic digestion systems used in the sugar-processing industry (Shore *et al.* 1984).

environmental shocks due to temperature; toxic or high loadings; and tolerance to a greater variation in influent quality. The main operational features of all the major anaerobic processes are compared in Table 7.11. Typical loading rates are given in Table 7.12.

Table 7.11. Comparison of features of various anaerobic reactors. ANFLOW, anaerobic flow-through digester without sludge recycle; ANCONT, contact digester with sludge recycle; ANBIOL, anaerobic biofilters; FANBIOF, fluidized anaerobic biofilter; UASB, upflow anaerobic sludge bed reactor. The more asterisks, the better the process (Oleskiewicz and Olthof 1982).

Feature	ANFLOW	ANCONT	ANBIOF	FANBIOF	UASB
Ease of start-up	*	*****	****	***	**
Ease of operation after proper acclimatization	**	**	*****	***	***
Good process control possible under transient influent conditions	*	*****	***	***	***
Resistance to shocks due to:					
temperature	*	***	*****	*****	*****
toxics	*	***	*****	*****	*****
high organic load	*	****	*****	*****	*****
Tolerance for influent quality variations	*	****	**	****	*****
Can tolerate high influent solids fluctuations	****	***	*	**	**
May incorporate sludges from pretreatment and aerobic polishing	*****	****	*	*	*

7.3.2. *Sludge blanket process*

In the up-flow anaerobic sludge blanket (UASB) system, the biomass is retained in the reactor by flocculation using similar process technology to that used in sludge blanket clarification in potable water production. Originally developed in The Netherlands (Lettinga *et al.* 1980), it is now the most popular type of anaerobic digester design for industrial wastewaters. A heavily flocculated sludge develops within the special reactor (Fig. 7.4e), which acts as a separate fluidised bed, able to withstand high mixing forces. It should be stressed that no support medium is added to the reactor and

Table 7.12. Examples of working CSTR reactors treating food wastes (Nahle 1991).

Type of effluent	Organic loading rate (kg COD/m^3. d)	F:M (kg COD: kg TS)	COD removed (%)
Sugar processing	0.6–13.0	1.3–3.0	90–95
Distillery	1.5–2.5	0.2–0.25	90–95
Citric acid	1.3–4.0	0.2–0.3	75–80
Yeast production	3.0–4.0	0.2–0.4	77–80
Sauerkraut	1.5	0.4	96
Vegetable cannery	2.0–4.2	0.1–0.3	90–95
Pectin factory	1.7–5.3	0.03–0.2	88–93
Starch factory	36	1.4	65
Meat processing	0.8–4.8	0.5–1.1	90–94
Cellulose condensate	1.3–1.8	0.1–0.2	95–98

that the biomass that is produced flocculates to produce dense granules with a diameter of up to several millimeters and a settling velocity of 10 m h^{-1} (Laguna *et al.* 1999). Granules are composed of methanogens (e.g. *Methanothrix*, *Methanobacterium*, *Methanobrevibacter*, *Methanosarcina*) as well as calcium precipitates (Wu *et al.* 1987; Vissier *et al.* 1991; Brammeler *et al.* 1985). Granules have three distinct layers with the exact microbial composition dependent on growth substrate (Grotenhuis *et al.* 1991: Harmsen *et al.* 1996). The external layer is a heterogenous layer of hydrogen-producing micro-organisms consisting of rods, cocci, and filaments. The middle layer contains predominantly bacterial rods including hydrogen-producing acetogens and hydrogen-consuming organisms. In the center is a homogenous bacterial core with a large number of cavities. It consists of *Methanothrix*-like cells that are thought to act as the nucleation centers for granule development (MacLeod *et al.* 1990).

Mixing is achieved by pumping the feed sludge through the base of the reactor up through the sludge blanket. Above the blanket, finer particles flocculate and in the upper settlement zones they settle as sludge back to the blanket, thus preventing wash-out of biomass. The settlement zones occur between the gas collection bowls that slope at an angle of 50°, which promotes the return of the sludge from the settlement areas. The process is characterised by very high MCRT, which is even higher than anaerobic filters (Sec. 7.3.3) and a low HRT (< 1 d). It takes up to six months for a suitable granular sludge to develop from a soluble wastewater, so a suitable inoculum is required for rapid start-up. The process

is dependent entirely on the ability of the biomass to form granules using particular wastewaters and is widely used for strong industrial wastewaters with a low suspended solids content (for example creamery wastes and the waste from the manufacture of soft drinks). Reactors are typically 3–5 m in height. A 200 m^3 digester of this type has successfully treated sugar beet waste at a rate of 16 kg COD $m^{-3}d^{-1}$, achieving a 90% removal at a HRT of only 4 h. USAB reactors have been used to treat a variety of complex wastewaters (Imai *et al.* 2000) including wastewaters from fish canning (Puñal and Lema 1999); fibre board manufacture (Fernández *et al.* 1995; Puñal *et al.* 1999); landfill leachate (Chang 1989; Barzacconi *et al.* 1999); agro-industries (Driessen and Yspeert 1999); polyethylene terephthalate (PET) (Fdz-Polanco *et al.* 1999); Malting and brewing (Fang *et al.* 1990; Yan and Tay 1996; Martínez *et al.* 2001); slaughterhouses (Manjunath *et al.* 2000; Nery *et al.* 2001); and papmills (van Lier *et al.* 2001). There are currently seven UASB reactors in the UK: Baymer (Citric acid waste), Goole; Caernarvon Creameries, Caernarvon; Coca Cola, Wakefield; Davidsons Papermill, Aberdeen; Everest Potato Foods, Kidderminister; General Foods, Banbury; and Smurfit Paper, Ashford. Compared to the other contact processes, it is difficult to operate and maintain the structure of the sludge blanket (Lettinga and Hulshoff-Pol 1991) (Table 7.11). Details of loading rates are summarised in Table 7.13. Sludge blanket reactors have been used as a separate methanogenic phase, with the first two stages of the digestion process being carried out in a separate reactor with increased biogas production (Morris and Burgess 1984).

A recent development of the UASB reactor is the expanded granular sludge bed (EGSB) reactor, which is designed to treat low strength wastewaters at high velocities at temperatures of 10°C and less (Kato *et al.* 1994; Rebac *et al.* 1995; Jeison and Chamy 1999; van Lier *et al.* 2001).

7.3.3. *Static media filter process*

Anaerobic reactors using fixed or static media to retain the active biomass are generally known as anaerobic biofilters (Fig. 7.4c). Although normally operated in the upflow mode, as all anaerobic contact reactors invariably are, anaerobic biofilters can also be operated in the downflow mode with distinct advantages and disadvantages to each (Berg and Lentz 1979; Kennedy and Berg 1982; Kennedy and Droste 1991; Young 1991; Hobson and Wheatley 1993). There is a counter movement of gas and liquid in downflow reactors resulting in intense mixing. These reactors are better suited to wastewaters with higher concentrations of suspended solids. In

Table 7.13. Comparison of loading rates used in anaerobic filters, UASB, and expanded beds (Hobson and Wheatley 1993).

Type of unit	Range
Anaerobic filter	
Organic load (kg COD/m^3. d)	2–10
Retention period (h)	10–15
COD removal (%)	70–80
Critical solids concentration in feed (mg/l)	450–1050
Upflow anaerobic sludge blanket reactor	
Load (kg COD/m^3. d)	2–15
Retention period (h)	10–50
COD removal (%)	70–90
Expanded bed	
Load (kg COD/m^3. d)	2–50
Retention period (h)	0.5–24
COD removal (%)	70–80

design, they are submerged percolating filters, full of wastewater so that no oxygen can enter the reactor and are operated using a wide variety of media, from mineral to random pack plastics filter media. The biomass comprises a thin bacterial film that is firmly attached to the support media. Apart from being operated as an anaerobic process, with some form of biogas collection device, submerged filters can be operated aerobically using aeration units or anoxically with a separate carbon input.

The design of anaerobic biofilters vary, although the shape and diameter of both the reactor and the media can have important effects on the stability of the attached film (Berg and Lentz 1980). Other factors, such as gas production, can also dislodge the film, although it may also reduce the chance of clogging within the filter medium. In cylindrical reactors, the most satisfactory diameter to height ratio is 1:4. The entire reactor can be filled with the medium, and most anaerobic filters use plastic media (Anderson *et al.* 1984), or it may be restricted to the upper part of the reactor only, with the detached biomass settling to the lower chamber, thus providing a dual mixed and fixed system. Various media have been used. For mineral media, porous stones, gravel, and pottery fragments appear to be the favourites, although these have a large bulk density and relatively low surface areas. In contrast, plastic media have a much greater specific

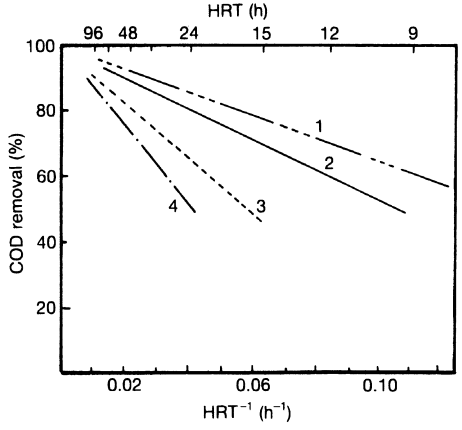


Fig. 7.17. COD removal efficiencies of anaerobic filters in relation to different types of media. 1. Large modular; 2. small modular; 3. pall rings; 4. spheres (Young and Dahab 1982).

surface area (90–350 m^2m^{-3}) and lower density (50–100 kg m^{-3}), with a voidage in excess of 90% (Characklis and Trlear 1982; Vijayalakshimi *et al.* 1990). Different media result in very different performance characteristics and unlike the traditional percolating filter, removal efficiency is not interrelated with specific surface areas of the media (Young and Dahab 1983; Wilkie *et al.* (1983) (Fig. 7.17). This is because in upflow filters, a significant portion of the active biomass will be present not as attached film, but as unattached dispersed growths in the interstices (voids) of the medium. The suspended solids flocculate as they travel up through the filter forming larger particles that finally settle back down the filter column. The movement of solids and gas bubbles that are released introduces an element of mixing into this essentially plug-flow system. Provision must be made for periodic desludging, which usually happens once a year (Young 1991). When operated in the downflow mode, all the dispersed solids are washed out of the reactor, leaving only the attached biomass. In these reactors, the media must ensure stable film development as well as prevent excessive solids accumulation. Therefore, channelled or ordered media, as opposed to random media, are the best kind of media (Wilkie *et al.* 1983; Bonastre and Paris 1989). As all the biomass is attached in downflow anaerobic biofilters, the performance is directly related to the specific surface area of the medium. In practice, filters employing porous media perform better than those using non-porous material due not only to higher specific surface areas, but to better surfaces for the adhesion and retention of biomass (Fox

et al. 1990; Picanço *et al.* 2001). Submerged rotating biological discs have also been developed (Laquidora *et al.* 1986).

Anaerobic biofilters are ideal for relatively cold and dilute wastes as they have an extremely high MCRT:HRT ratio. This gives the process a high degree of stability, an excellent resistance to inhibitory compounds, and a satisfactory performance even at low temperatures (Table 7.11). They can treat similar organic loadings as anaerobic activated sludge systems, even though the MCRT is much higher (Table 7.14). Successful applications include: treating sewage; starch; whey; cellulose; distillery; pharmaceutical; and fish-processing wastes (Young and McCarty 1969; Taylor and Burm 1973; Witt *et al.* 1979; Genung *et al.* 1982; Rittman *et al.* 1982, Sachs *et al.* 1982; Bedogni *et al.* 1983; Mathur *et al.* 1986; Elmitwalli *et al.* 2001), with an average COD removal rate between 80–90% at a typical organic loading of 3.5 kg $m^{-3}d^{-1}$. A downflow biofilter containing a 35 l volume of fired pottery clay medium with a surface area of 157 m^2m^{-3} was used to treat piggery waste at 35°C (Kennedy and Berg 1982). It was found to be able to treat higher loadings and have higher rates of methane production than partially or fully mixed reactors and also plug-flow reactors. The support medium was evenly coated with a thin microbial film 2–4 mm thick. Reactor performance, based on the amount of microbial film present, was between 1.1–1.4 g COD removed per g of film per day, which is similar to that reported by Berg and Lentz (1980) for other types of media. However, the biofilter system is limited to treating substrates with relatively low solids, as the interstices of the medium can become blocked easily. This is why they are often recommended to be operated in series, with an anaerobic activated sludge system, as the effluent from this process will be largely free from suspended solids, thus making it ideal for further anaerobic treatment using a biofilter. Loading rates are compared to other anaerobic systems in Table 7.13.

At present, the major use of anaerobic biofilters has been for denitrification, i.e. removing nitrates from sewage effluents (Sec. 3.4.5). There is growing interest in denitrification throughout Europe, especially in the UK. There has been a drastic increase in the concentration of nitrates in both surface and groundwaters due to modern farm practice, although the contribution from treated sewage effluents is significant in many areas (Greene 1980; Addiscott and Powlson 1989). In the past 20 years, the pressure on the farming industry to keep up yields has led to a continuing increase in the amount of nitrate leached from the soil. This has led to a steady increase in the concentration of nitrates in drinking water, and nitrates are thought to cause methaemoglobinaemia in babies, although the incidence of this is

Table 7.14. Features of various reactors compared with aerobic counterparts (Oleskiewicz and Olthof 1982). Key is given in Table 7.11.

Feature	Anaerobic processes					Aerobic processes	
	ANFLOW	ANCONT	ANBIOF	FANBIOF	UASB	Activated sludge	Trickling filter
Loads practised (kg COD m^{-3} d^{-1})	0.5–3	2–8	2–10	0.5–12	1–15	0.5–2	1–3 roughing
Loads used in experimental scale (kg $m^{-3}d^{-1}$)	0.5–10	0.5–100	0.5–25	1–40	1–60	1–10	2–15
HRT used (days)	8+	0.2–8	0.2–4	0.15–3	0.15–8	1–5	0.05–0.2
SRT resulting (days)	8+	15–80	20–300	20–100	30–300+	10–30	$\sim$ 30(+)
Temperatures used (C°)	35, 55	35, 55	15–35	35	5–35	15–25(+)	15–25(+)
COD removals attained at practised loads: similar waste assumed (%)	60	90+	90+	90+	90+	90+	60–80 in 1 stage. Multi-stages typical

extremely rare and no direct evidence of nitrate in potable supplies causing the condition has so far been found. It has been proposed that nitrates may also influence the incidence of cancer in humans by the formation of nitrosamines in the digestive system (Royal Society 1983; Gray 1994).

A wide range of facultative anaerobic bacteria can utilise nitrate as an alternative to oxygen for their terminal electron acceptor, releasing gaseous nitrogen. The stoichiometry of the process is outlined in Sec. 3.4.5. If a treated sewage effluent rich in nitrate was left to stand under anoxic conditions, denitrification would eventually occur as the population of denitrifying bacteria gradually increased. In order to make the process a part of the normal treatment operation, a more rapid and reliable method of denitrification is required. This is done by bringing the effluent into contact with a large and active population of denitrifying bacteria under ideal conditions. A source of readily degradable organic carbon is also required to act as a source of reducing agent. Some effluents will contain enough residual organic carbon to allow denitrification to proceed and, if not, untreated settled sewage can be used. However, the preferred source of organic carbon is methanol, which is also used for potable supplies, as the input of organic carbon into the reactor can be accurately controlled.

From the stoichiometric equations in Sec. 3.4.5, for denitrification, the amount of methanol, or organic carbon equivalent, can be estimated from:

$$C_{\mathrm{m}} = 2.47\ \mathrm{N_o} + 1.52\ \mathrm{N_i} + 0.87\ D_{\mathrm{o}}$$

where C_{m} is the concentration of methanol required (mg l^{-1}), $\mathrm{N_o}$ the initial nitrate concentration (mg N l^{-1}), $\mathrm{N_i}$ the initial nitrite concentration (mg N l^{-1}), and D_{o} is the initial dissolved oxygen concentration, as the reaction will take place under anoxic as well as anaerobic conditions. The biomass produced can be estimated as:

$$C_{\mathrm{b}} = 0.53\ \mathrm{N_o} + 0.32\ \mathrm{N_i} + 0.19\ D_{\mathrm{o}}$$

where C_{b} is the biomass production (mg l^{-1}). Thus, when $\mathrm{N_i}$ and D_{o} are zero, the methanol:nitrate ratio is 2.47 and the biomass production is 0.53 N (Barnes and Bliss 1983). In these calculations, only the amount of nitrogen present is used. Thus, for a sample containing 15 mg l^{-1} of nitrate ions at 0 mg l^{-1} nitrite ions and dissolved oxygen, the amount of methanol required will be $(2.47 \times 15 \times 14/62) = 8.4$ mg l^{-1}, with $(0.53 \times 15 \times 14/62) = 1.8$ mg l^{-1} of biomass produced. If, however, the sample contained 15 mg l^{-1} of nitrate-nitrogen, 37 mg l^{-1} of methanol would be required producing 8 mg l^{-1} of biomass.

It is important to provide the correct dose of methanol to the reactor as if it is under-estimated, denitrification will be incomplete and if over-estimated, methanol will remain in the final effluent and cause further pollution.

The major denitrification process is the denitifying filter which is a submerged filter containing an inert medium on which a film of denitrifying bacteria develop. This contact system provides the necessary MCRT for the development of these slow-growing bacteria and depending on the voidage of the medium the SRT can be very short, often less than one hour. In suspended growth systems, biological denitrification is a zero-order reaction, with respect to oxidised nitrogen and electron donor concentrations. There needs to be at least 2 mg l^{-1} of oxidised nitrogen and a C:N ratio of at least 3:1 for denitrification to occur. In fixed-film reactors, the rate of reaction is similar to suspended growth reactors except that the diffusion of both oxidised nitrogen and election donors within the biomass will modify the overall reaction. Where the film growth is thin, there will be no diffusion limitation on the rate of denitrification, but for thicker films a low dependence on these concentrations may be observed (up to half-order) (Riemer and Harremoes 1978). The overall effect of the denitrification reaction is to raise the pH by the formation of hydroxide ions. This replaces about 50% of the alkalinity consumed by the oxidation of ammonia during nitrification. For each mg of ammonia oxidised to nitrate, 7 mg of alkalinity are utilised, and 3 mg are produced during denitrification. The denitrification reaction is pH-sensitive, with an optimum range between 6.5–7.5, but falling to 70% efficiency at pH 6 or 8 (Environmental Protection Agency 1975; Moore and Schroeder 1970). Temperature is also important, with the reaction occurring between 0–50°C, but with the optimum reaction rate between 35–50°C. On the whole, the autotrophic nitrifying bacteria are more sensitive than the denitrifying heterotrophs and there will be little inhibition of denitrification of a particular wastewater if nitrification has already proceeded satisfactorily. Denitrifying filters can be operated in an upflow or downflow mode, although the former builds up considerably more biomass than the latter system (Polprasert and Park 1986). The most frequent operational problem is excessive film accumulation or entrapped gas bubbles, which alter the internal flow pattern, restricting the flow causing a significant head loss, or reducing the HRT by channelling the effluent through the filter. Nitrate removal efficiency increases with the accumulation of biological solids. However, removal efficiency sharply falls off as the flow is restricted and the pressure builds up until finally the liquid forces its way through the bed scouring away much of the film. Therefore, it is better to opt for

a larger medium (15–25 mm diameter), with larger voids and a slightly reduced surface area so that film accumulation is less of a problem. In this way, the filter will operate more consistently (Tamblyn and Sword 1969). Better film control has been achieved using fluidised bed systems, although this has been designed primarily for potable water treatment (Jewell 1982; Croll *et al.* 1985).

7.3.4. *Fluidised and expanded media*

A modification of the anaerobic biofilter is the anaerobic fluidised bed reactor (Fig. 7.4f) in which fine grained inert (sand) or reactive (activated carbon or zeolite) random media are used (Yoda *et al.* 1989). The medium, which is very light, becomes coated with bacteria and is fluidised by applying a high mixing velocity. However, it is difficult to maintain the optimum mixing velocity in order to ensure the medium remains in suspension without causing shear forces strong enough to strip off the accumulated biomass. Mixing is achieved within the reactor by applying high rates of gas or effluent recycle. The particles can be fully mixed (fluidised) which increases the volume of the reactor occupied by the medium by 20–25%, or expanded to increase the reactor volume by $< 10\%$ (Switzenbaun 1983; Iza 1991). Particle size ranges from 0.3 to 1.0 mm depending on the type of medium used. The decrease in media particle size has resulted in a significant increase in the specific surface area available for biological growth making such systems highly efficient. Smaller sizes are readily lost from the reactor while larger sizes are difficult to fluidise. Due to the difficulty in maintaining an optimum mixing velocity to ensure the medium remains in suspension without causing biomass stripping due to excessive shear, it may be that expanded bed reactors will be the more effective system. In expanded beds, the bacterial-coated medium remains on the floor of the reactor and is expanded by the movement of the liquid forced up through the medium. Fluidised beds are normally tall, with a height to diameter ratio of 5–6:1. Although difficult to start up, fluidised beds are highly resistant to temperature fluctuation, toxic compounds, and high organic loadings. They are also tolerant to wide fluctuations in influent quality and also a high solids content in the influent (Table 7.11). It has proved very difficult to scale up this system from pilot- to full-scale operation, resulting in very few full-scale plants worldwide. Details on the design and operation of fluidised bed reactors are given by Fernández-Polanco and Diez 1988; Iza 1991; Marín *et al.* 1999). Typical loading rates are given in Table 7.13.

In Cuba, fluidised beds are used to treat vinasse, the final waste from the alcohol distillation process from sugar cane molasses. With organic loading rates up to 10 kg COD $m^{-3}d^{-1}$ and COD removals > 70%, the waste produces methane rich biogas but with sulphide concentrations > 1% (Conde *et al.* 1993). However, sulphide concentrations can be controlled by using either granular activated carbon (GAC) or zeolites as support media (Fernández *et al.* 2001). Other workers have also observed simultaneous removal of sulphate and ammonia in anaerobic GAC fluidised beds treating vinasse (Fdz-Polanco *et al.* 2000, 2001). This resulted in high concentrations of molecular nitrogen in the biogas but low concentrations of hydrogen sulphide. The evidence to date indicates an unusal anaerobic nitrogen removal mechanism resulting in loss of N in the liquid phase and the presence of N in the biogas, with up to 55% of the total kjeldhal nitrogen removed by this process. The removal mechanism of sulphur is less clear with elemental sulphur found within the reactor and associated with the effluent suspended solids. The function of the GAC appears to be to increase the localised concentration of nitrogenous and sulphur species by adsorption close to the biofilm encouraging the biochemical reactions to occur. The overall reaction has been postulated as:

$$SO_4 + 2NH_4^+ \rightarrow S + N_2 + 4H_2O \ (\Delta G^o = -47.8 \text{ kJ mol}^{-1})$$

Fdz-Polanco *et al.* (2001) have suggested that this reaction comprises three separate reactions:

$$3SO_4^= + 4NH_4^+ \rightarrow 3S^= + 4NO_2^- + 4H_2O + 8H^+$$

$$3S^= + 2NO_2^- + 8H^+ \rightarrow 3S + N_2 + 4H_2O$$

$$2NO_2^- + 2NH_4^+ \rightarrow 2N_2 + 4H_2O$$

The last equation is the the Anammox reaction (Sec. 5.6).

Further reading

General: Oleszkiewicz and Olthof 1982; Statford *et al.* 1980; Hughes *et al.* 1981; Ferrero *et al.* 1984; Hawkes and Hawkes 1987; Malina and Pihland 1992; van Haanel and Lettinga 1994; Wheatley *et al.* 1997; Chamy *et al.* 1999; Field 2002.
Modelling: Batstone *et al.* 2002.
Biology: Zehnder 1988.

Septic tanks: Environmental Sanitation Information Centre 1982; Laak 1986; Payne and Butler 1993; EPA 2000a,b.

Imhoff tanks: Billings and Smallhurst 1971.

Digestion: Statford *et al.* 1980; Ferrero *et al.* 1984; Chernicharo *et al.* 2001; Schink 2002.

Biogas: Hobson *et al.* 1981; La Farge 1998.

Sludge blanket: Lettinga *et al.* 1983; Pette and Versprille 1982; Sousa 1986.

Fixed-film reactors: Henze and Harremoes 1983; Salkinoja-Salonen *et al.* 1983; Young 1991.

Denitrification: Barnes and Bliss 1983.

8

Sludge Treatment and Disposal

8.1. Sludge Characteristics and Treatment

The treatment of sewage is essentially a separation process, a method of concentrating and converting suspended and soluble nutrients into a settleable form that can be separated from the bulk of the liquid. The removal of the settleable fraction of raw sewage at the primary settlement stage, and of the settleable solids produced by biological conversion of dissolved nutrients into bacterial cells at the secondary stage, continuously produces a large quantity of concentrated sludge. Although the liquid fraction of the wastewater can be fully treated and disposed safely to surface waters, the accumulated sludge has to be transported from the wastewater treatment plant for disposal. Sludge separation, treatment, and disposal represents a major capital and operational cost in sewage treatment. Dewatering and disposal costs for a medium-sized activated sludge plant represents as much as 50% of the initial capital and 65% of the operating costs (Calcutt and Moss 1984). If one remembers that sludge is simply concentrated wastewater, then failure to provide adequate sludge treatment and disposal facilities can result in serious pollution.

Sludge is a complex material and is discussed in detail by Best (1980). At moisture contents greater than 90%, sludges behave as liquids, whereas below 90% they behave as non-newtonian fluids exhibiting a plastic rather than viscous flow (Fig. 8.1). The water held within the sludge is either free or bound. In sludges with a moisture content of more than 95%, some 70% of the water is in a free or readily drained form, and the remainder is bound to the sludge and more difficult to remove, with 20% present as floc or particle

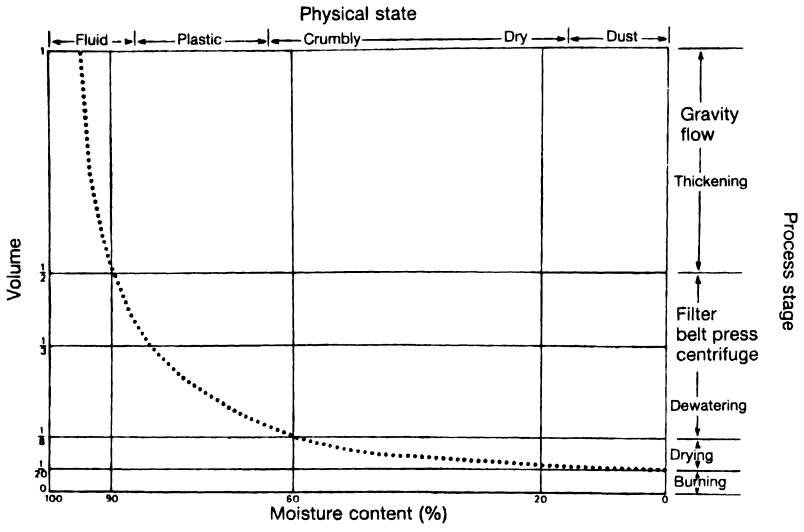


Fig. 8.1. Theoretical relationship between moisture content and the volume of sewage sludge as produced by various stages of sludge processing (Best 1980).

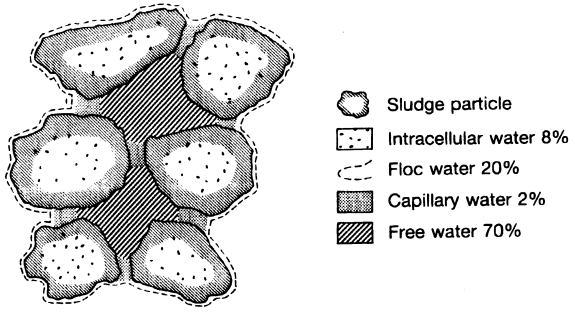


Fig. 8.2. Schematic diagram of a sludge floc showing the association of the sludge particles with the available water (Best 1980).

moisture, 8% chemically bound, and 2% as capillary water (Fig. 8.2). The more intimately the water is bound, the more energy is required to remove it (Kopp and Dichtl 2001). Although most of the free water can be removed by gravity settlement, some of the bound water must also be removed if the sludge is to be made handleable. This is normally done by coagulation, using chemicals and dewatering equipment which removes moisture by altering the particle formation of flocs and the cohesive forces that bind the particles together, thus releasing floc and capillary water. Mikkelson and

Keiding (2002) have studied sludge composition and structure in relation to dewaterability. They found that the fraction of extracellular polymeric substances (EPS) in sludges was the single most important parameter in regard to their structure. High EPS content gives sludges a lower shear sensitivity and lower degree of dispersion that gives better filterability. The type of sludge produced depends on a number of factors, such as the type of sludge separation and the treatment processes employed, which are really a function of the size of the treatment plant and wastewater characteristics.

Industrial wastewaters produce very different types of sludge that can be broadly categorised as either organic, such as those from slaughterhouse or fermentation effluents, or inorganic which may contain toxic materials, especially heavy metals. The presence of gross organic solids in sludge, such as offal from meat-processing wastes, pathogenic organisms or toxic compounds and heavy metals from pharmaceutical and metal industries may contaminate the sludge to such an extent that the disposal options for the sludge are restricted. Primary or raw sludge is the settleable fraction of raw or crude sewage and is a rather unpleasant smelling thick liquid, which is highly putrescible, with a moisture content of between 94–98%. Secondary sludges contain the solids washed out of the biological treatment units, and either comprise of wasted activated sludge or sloughed microbial film. The secondary sludges are more stabilised than primary sludge with percolating filter sludge (humus) far more stabilised than wasted activated sludge having a dry solids content of 6–8% compared with 1.5–2.5% for activated sludge (Table 8.1). The quantity, volume, and solids content of sludge produced per capita each day from the most common sewage treatment units is shown in Table 8.2. It is common practice to mix primary and secondary sludges together for treatment and disposal. In general, the mean flow of wet sludge produced in a treatment plant and requiring treatment will be between 1–2% of the flow of raw sewage. White (1978) gives a useful formula

Table 8.1. Normal volumes of sludge and their solids content from primary sedimentation and secondary treatment processes (Open University 1975).

Type of sludge component and source	Quantity (l cap^{-1} d^{-1})	Dry solids (kg cap^{-1} d^{-1})	Moisture content (%)
Primary sedimentation sludge	1.1	0.05 (4.5%)	95.5
Percolating filter sludge			
Low-rate filters	0.23	0.014 (6.1%)	93.9
High-rate filters	0.30	0.018 (6.0%)	94.0
Activated sludge, surplus sludge	2.4	0.036 (1.5%)	98.5

Table 8.2. Production of sludge by individual unit processes in sewage treatment (Casey and O'Connor 1980).

Process stage	Quantity (g cap^{-1} d^{-1})	Solids content (% by weight)	Volume (l cap^{-1} d^{-1})
Primary sedimentation	44–55	5–8	0.6–1.1
Biofiltration of settled sewage	13–20	5–7	0.2–0.4
Standard-rate activated sludge pre-settled sewage	20–35	0.75–1.5	1.3–4.7
Extended aeration raw sewage	22–50	0.75–1.5	1.7–6.7
Tertiary sand filtration	3–5	0.01–0.02	15.0–50.0
Phosphorus precipitation (Al or Fe)	8–12	1–2	0.4–1.2

from which the daily production of sludge can be estimated:

$$Q(K_1 S + (1 + K_2)YbK_3) \text{ kg solids d}^{-1}$$

where Q is the mean flow of sewage (m^3d^{-1}), S and b the mean concentration of suspended solids and BOD respectively (mg 1^{-1}), K_1 and K_2 the fraction of suspended solids (≈ 0.6) and BOD (≈ 0.3) removed at the primary sedimentation stage respectively, and K_3 the fraction of BOD removed at the biological oxidation stage (≈ 0.90–0.95), and Y is the sludge yield coefficient for the conversion of BOD to secondary sludge in the biological oxidation unit ($Y = 0.5$–1.0 for activated sludge and 0.3–0.5 for single pass percolating filter systems).

In 1991/2, some 6.48×10^6 t DS of sludge were disposed of annually by the then 12 Member States of the EU. This was equivalent to a mean daily per capita sludge production of 78 g DS $ca^{-1}d^{-1}$ (Table 8.3). The introduction of the Urban Wastewater Treatment Directive has set new standards for the treatment of organic liquid wastes that will lead to greater volumes of sludge being produced. This increase, along with the sewage sludge that was formally discharged to sea, is estimated to increase sludge disposal via the remaining routes in the EU by 35–40%, which is 3×10^6 tonnes y^{-1} (Table 8.4). As raw sludge is 94–98% water, the disposal problem can be significantly reduced by reducing the water content and so the volume of the sludge to be disposed. The putrescible nature of sludge is also a problem, especially if it has to be stored before final disposal. Therefore, although raw liquid sludge can be disposed directly either to land or sea, it is normal practice for the sludge to receive further treatment in the form of thickening and dewatering. This may include chemical conditioning, which reduces the

Table 8.3. Populations served and sludge production (as disposed) in the European Union 1991/2 (Matthews 1996).

Member state	Total population[a] (millions)	Population connected to sewer (%)	Population connected to STW (%)	Sludge disposed	
				(t DS per year)	(g DS per person per day)
Belgium	9.9	70	28	59,200	58
Denmark	5.1	93	92	170,300	99
France	56.9	65	50	852,000	82
Germany	79.7	89	83	2,681,200	111
Former West	62	92	90	2,449,200	119
Former East	17	77	58	232,000	64
Greece	10.2	45	34	48,200[b]	40[b]
Ireland	3.5	67	45	36,700	64
Italy	57.7	75	60	816,000	65
Luxembourg	0.4	97	87	7,900	62
Netherlands	15.0	97	88	322,900	67
Portugal	9.9	52	20	25,000[b]	35[b]
Spain	39.0	70	59	350,000	42
United Kingdom	57.5	96	85	1,107,000	62
Total (mean)[c]	344.8	(79)	(66)	6,476,400	(78)

[a] Population data for 1991.
[b] Upper estimate.
[c] Weighted means in parenthesis.

Table 8.4. The effect of the introduction of the Urban Wastewater Treatment Directive on EU sludge production.

	Quantity in dry solids/tonnes/per annum	
	Current (1994)	After UWWT Directive
Austria	180,000	250,000
Belgium	88,000	250,000
Denmark	170,000	170,000
Finland	150,000	150,000
France	800,000	1,300,000
Germany	3,200,000	3,850,000
Greece	70,000	180,000
Ireland	12,000	40,000
Italy	800,000	1,300,000
Luxembourg	7,500	15,000
Netherlands	340,000	450,000
(Norway)	(93,000)	(130,000)
Portugal	8,400	60,000
Spain	528,000	1,088,000
Sweden	240,000	240,000
UK	1,100,000	1,350,000
Total EU	7,693,900	10,693,000

volume of the sludge, and digestion or lime stabilisation to make the sludge more stable before final disposal (Fig. 8.3). Such treatment will alter the physical and chemical nature of the sludge, which is characterised by its water content and stability.

8.1.1. *Treatment options*

The treatment of sewage sludge follows a general sequence. The water content is reduced by some form of thickening that is either followed by a stabilisation process and secondary thickening, and/or chemical conditioning and a dewatering process to reduce the water content even further (Fig. 8.3). The sludge is then ready for disposal.

Thickening

Untreated sludges from the primary and secondary sedimentation tanks have high water contents (Table 8.1), and in order to reduce the volume

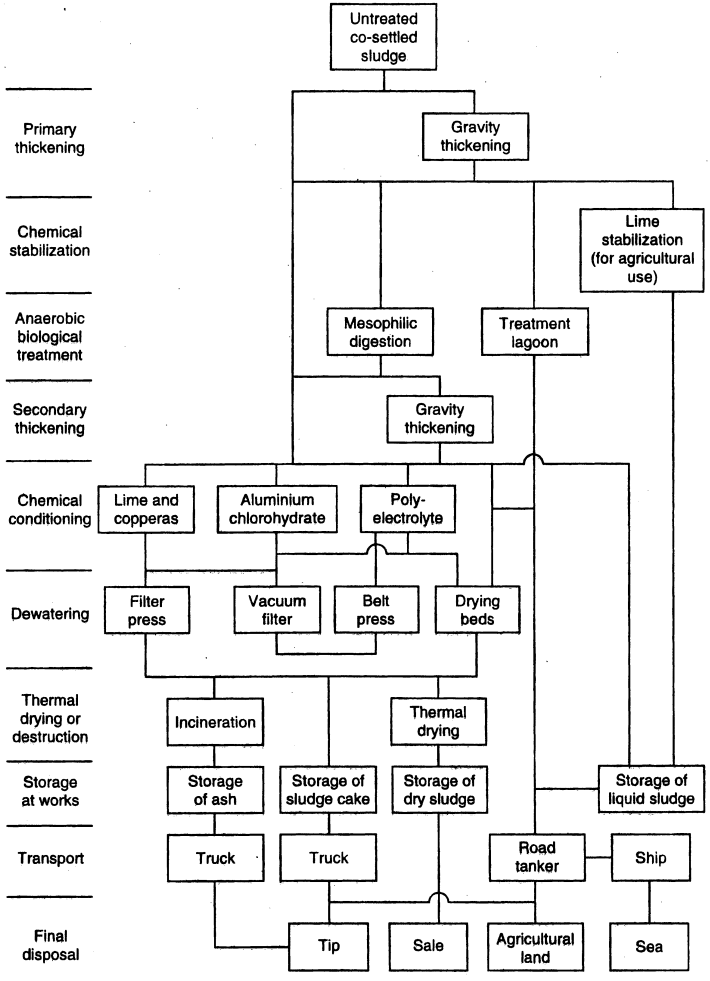


Fig. 8.3. Process selection for the disposal of sewage sludge (Hall and Davis 1983).

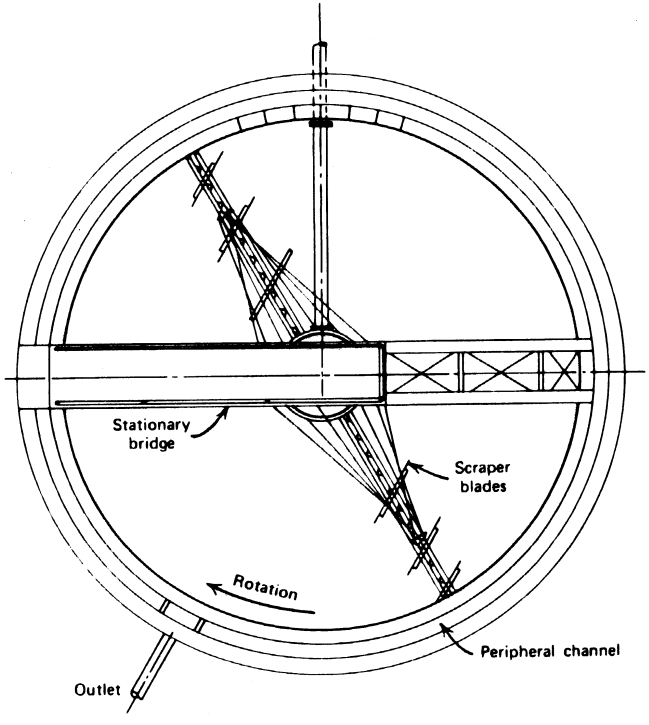
of sludge handled in the stabilisation or dewatering processes, the sludge needs to be concentrated or thickened. Thickening is achieved by physical means, such as flotation, centrifugation, and lagooning, but most usually by gravity settlement. Gravity thickeners can increase the sludge concentration in raw primary sludge from 2.5% to 8.0% resulting in a three-fold decrease in sludge volume, whereas a five-fold decrease in the volume of wasted activated sludge is not uncommon, with it being thickened from 0.8% to 4.0% solids (Table 8.5).

Table 8.5. Typical concentrations of unthickened and thickened sludges and solids loadings for gravity thickeners (Metcalf and Eddy 1984).

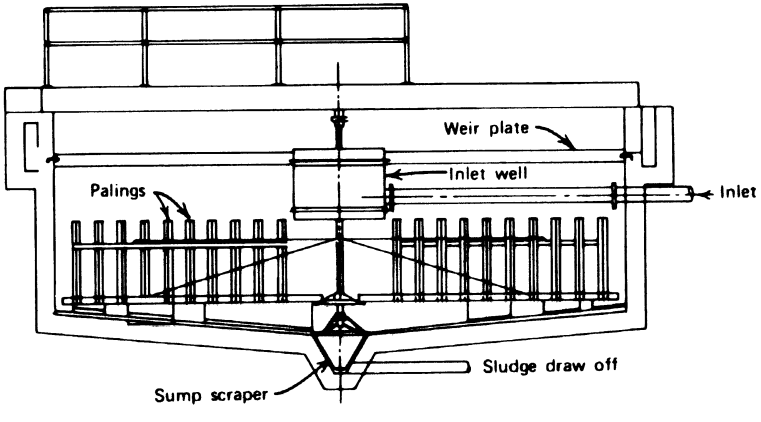
Type of sludge	Sludge concentration (%)		Solids loading for gravity thickeners (kg m^2 d^{-1})
	Unthickened	Thickened	
Separate			
Primary sludge	2.5–5.5	8–10	100–150
Percolating filter sludge	4–7	7–9	40–50
Activated sludge	0.5–1.2	2.5–3.3	20–40
Pure oxygen sludge	0.8–3.0	2.5–9.0	25–50
Combined			
Primary and percolating filter sludge	3–6	7–9	60–100
Primary and modified aeration sludge	3–4	8.3–11.6	60–100
Primary and air-activated sludge	2.6–4.8	4.6–9.0	40–80

Gravity thickening takes place in a circular tank that is somewhat similar in design to a primary sedimentation tank (Fig. 8.4). The dilute sludge is fed into the tank where the particles are allowed to settle over a long period, which may be several days. The concentrated sludge is withdrawn from the base of the tank and pumped to the digesters or dewatering equipment. Sewage sludge is very difficult to dewater because of the small size of the particles which results in small interstices that retain the water. Some of the water in the sludge exists as a gel, and combined with strong capillary and electrostatic forces, water separation is difficult. Settlement is enhanced by slowly stirring the sludge with a rotating set of vertical blades, or rods, that are spaced approximately 100 mm apart. The rate of rotation is fairly critical, with optimum thickening occurring between peripheral blade velocities of between 0.5–3.0 m min^{-1}. The stirrer, which looks like a picket fence, gives its name to the unit, which is universally known as *a picket fence thickener* (Fig. 8.4).

The sludge is thickened by the weight of the particles compressing the sludge at the base of the tank thus forcing the water out. The picket fence stirrer enhances particle settlement by encouraging particle size to increase by enhanced flocculation. It also encourages sludge consolidation in aiding water release by forming channels, preventing solids forming



(a)



(b)

Fig. 8.4. A picket fence thickener in (a) plan; and (b) cross-section (Hammer 1977).

bridges within the sludge, and by releasing gas bubbles formed by anaerobic microbial activity. Picket fence thickeners can be operated either as a batch or continuous process.

The liquid released from the sludge will contain more organic material than the incoming raw sewage and it must be returned to the inlet of the treatment plant and recycled. The strength and volume of this liquid must be taken into account for the organic loading to the plant.

Stabilisation

Once the sludge has been thickened, two options are available. It can be further dewatered to a solids content of between 30–40% or it can undergo stabilisation before the dewatering stage.

Sludges are stabilised to prevent anaerobic breakdown of the sludge on storage (i.e. putrefaction), which produces offensive odours. There are other advantages depending on the stabilisation process selected, including destruction of pathogens, partial destruction of sludge solids, increase in the concentration of soluble nitrogen, and improved flow characteristics. Specific sludge disinfection processes, specifically to kill pathogens, are becoming more widely practised (Bruce 1984). There are three categories of the stabilisation process: biological, chemical, and thermal. Each process prevents the utilisation of the volatile and organic fraction of the sludge during storage by different effects. Sewage sludge is only considered fully stabilised when it is humified, i.e. fully decomposed to humic substances that are non-putrescible, odourless, and degrade further only very slowly (Hartenstein 1981). Confusion exists with this definition as the EU Directive controlling sewage sludge disposal to agricultural land (86/287/EEC) (European Communities 1986) defines stabilised sludge as simply as one that has undergone biological or chemical treatment, or long-term storage. Clearly, there is no universally accepted definition of stability and as yet no standardised test of sludge stability has been developed. However, a number of possible methods have been proposed by Bruce and Fisher (1984) (Table 8.6). Sludge stability is normally measured by the specific oxygen utilisation rate (SOUR) (Sec. 3.6.3) which determines the residual biodegradable organic matter left in the sludge (Samson and Elama 2000).

Biological stabilisation processes are the most widely practised, resulting in the utilisation of the volatile and organic fraction of the sludge so that it will not undergo anaerobic breakdown on storage. The resultant sludge has a reduced volume, is odourless, and has a higher solids content. The most common stabilisation method for medium- to large-sized treatment plants

Table 8.6. Characteristics of sewage sludge stability (Bruce and Fisher 1984).

Basic parameter	Measurement	Possible applicability of each method of stabilisation				Comments
		Anaerobic digestion	Aerobic digestion	Lime stabilisation	Composting	
Odour emitted by the sludge	1. Odour intensity by dilution technique to give 'threshold dilution value'	✓	✓	✓	✓	Requires odour panel. Not related to odour 'quality'
	2. Gas chromatographic analysis (GCMS)	✓	✓	✓	✓	Expensive and difficult to interpret
Volatile solids	3. Volatile solids in the sludge as fraction of the total solids	✓	✓	×	?	Standard measurement
	4. Fraction of volatile solids destroyed (FVSD) by the stabilisation process	✓	✓	×	?	Standard measurement
Residual, readily biodegradable matter	5. BOD_5 of filtrate	×	✓	×	×	Standard measurement
	6. Rate of increase of COD of filtrate with storage	✓	✓	×	×	Tentative basis of a 'stability index'
	7. Specific oxygen uptake rate (SOUR)	×	✓	×	×	Temperature-dependent
	8. Gas production during anaerobic incubation at 35°C	✓	✓	×	?	Standard control sludge required to detect inhibition

Table 8.6. (*Continued*)

Basic parameter	Measurement	Possible applicability of each method of stabilisation				Comments
		Anaerobic digestion	Aerobic digestion	Lime stabilisation	Composting	
Chemical composition	9. Volatile fatty acids	✓	✓	×	×	Standard analysis
	10. pH and pH change during storage	✓	✓	✓	×	Standard test
	11. H_2S emission on storage	×	✓	×	×	Special test
	12. Nitrate concentration	×	✓	×	×	Standard analysis
Biological activity	13. ATP concentration	×	✓	×	×	Research application only
	14. Dehydrogenase concentration	×	✓	×	×	Research application only
Presence of putrescent matter	15. Attractiveness to house flies	✓	✓	✓	✓	Tentative. Under investigation

is heated anaerobic digesters, and at small works cold anaerobic digestion in tanks or lagoons is most common (Hall and Davis 1983). Anaerobic digestion utilises up to 40% of the organic matter present in raw sludge that results in an increased nitrogen concentration of up to 5% of the dry solids, of which 70% is in the form of ammonical nitrogen. The sludge entering digesters does not have to be pre-thickened, but if it is, then the resultant digested sludge will have a higher dry solids and nitrogen content and there will be a saving in digester capacity. Thickening is required after digestion and can be achieved by gravity settlement in a picket fence thickener or, alternatively, in lagoons over a period of several years with further digestion taking place. This results in a highly humified sludge with 8–10% dry solids (DS). The manurial value of lagooned sludge is reduced in terms of nitrogen and phosphorus content which is leached into the supernatant, with the proportion of total nitrogen present as ammonical nitrogen being reduced to $< 25\%$. Lagoons have a high potential storage capacity as the sludge volume is constantly being reduced by anaerobic digestion and water percolates into the underlying soil. Sludge is often stored in lagoons for many years resulting in a thick crust forming and becomes colonised by vegetation, with a dense sward of grass and even bushes developing. It is difficult to prevent this happening although it must be remembered that this gives a false impression of stability. The crust is not normally strong enough to prevent people, who inadvertently walk on to the lagoon, from falling in and most probably drowning. Therefore, a high level of maintenance is required to keep such sites securely fenced off. Both anaerobic and aerobic digestion processes are dealt with in earlier chapters. Another biological stabilisation process is composting, with or without a bulking agent or recycled material. Much interest has been shown in the use of various composting processes to convert sewage sludge into a useful soil conditioner and fertiliser. This is fully explored in Chapter 10.

Lime stabilisation is the most common form of chemical stabilisation and is particularly popular in Norway. Much interest is developing in other European countries, although few treatment plants are employing the process in the UK (Bruce and Fisher 1984). Unlike the biological digestion processes that utilise the organic fraction used by anaerobic micro-organisms, the addition of lime to untreated sludge until the pH is raised to > 11 creates an environment unsuitable for the survival of micro-organisms. Pathogens are readily killed by lime stabilisation at pH 12 for 3 h, giving a higher reduction in pathogens than anaerobic digestion. Lime stabilisation does not reduce the organic matter or provide permanent stabilisation, but prevents putrefaction as long as the high pH is maintained. However, although the

Table 8.7. Lime dosage required to maintain the pH > 11 for at least 14 days at 20°C (Paulstrad and Eikum 1984).

Type of sludge	Lime dosage (g $Ca(OH)_2$ kg DS^{-1})
Primary sludge	100–200
Septic tank sludge	100–300
Activated sludge	300–500
Mixed primary-chemical (Al, Fe) sludge	250–400
Mixed primary-chemical (Ca) sludge	None
Mixed activated-chemical (Al, Fe) sludge	300–500
Mixed activated-chemical (Ca) sludge	None

high pH suppresses the emission of volatile sulphides and fatty acids, the emission of amine and ammonia is increased making lime treated sludge less offensive than raw sludge but certainly not odourless. The elevated pH is normally maintained for several days or until incorporated into the soil. Obviously, lime addition has advantages to the farmer if sludge is disposed to agricultural land, and the addition of lime can help in the dewatering process. Two approaches to lime addition are used: the addition of hydrated lime to non-dewatered sludge and the addition of quicklime to dewatered sludge. The dosage is expressed as grams of lime per kg dry sludge solids (g kg DS^{-1}). The average dosage for a primary sludge is between 100–200 g $Ca(OH)_2$ kg DS^{-1}, and the mass of solids will increase after treatment. The pH of lime-treated sludges falls with time if the initial dosage is not sufficient, and it is important that sufficient lime is added to maintain the pH for the required period. The necessary lime dose required to maintain sludges at pH > 11 for at least 14 days is given in Table 8.7.

Thermal drying or heat treatment is a continuous process that both stabilises and conditions sludges by heating them for short periods (30 minutes) under pressure. This releases bound water, allowing the solids to coagulate, and proteinaceous material is hydrolysed, resulting in cell destruction and the release of soluble organic compounds and ammonical nitrogen. The design of driers used for the thermal treatment of sewage sludge is very varied (Fig. 8.5) and those currently in use are reviewed by CIWEM (1999). One such system, the Zimpro process, heats the sludge to 260°C in a reactor vessel at pressures up to 2.75 MN m^{-2}. The process is exothermic and results in the operating temperature rising. The solids and liquid separate rapidly on cooling with up to 65% of the organic matter being oxidised. The process sterilises the sludge, practically deodorises it

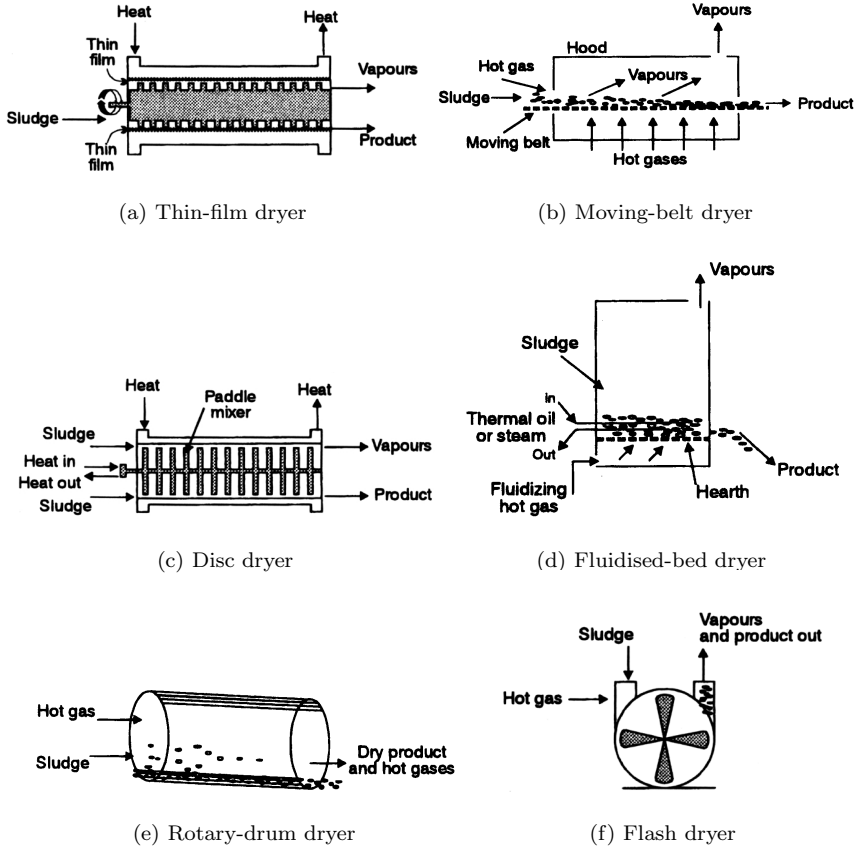


Fig. 8.5. The main types of thermal dryer used for sewage sludge treatment (CIWEM 1999).

and allows dewatering to be done mechanically without the use of chemicals. The Swiss Combi System® has been successfully installed at both the Avonmouth (England) and Dublin (Ireland) Sewage Treatment Plants. This is a rotary drum dryer with the dewatered cake mixed with previously dried cake to ensure a dry solids range of between 50–65% DS. The drum rotates passing and mixing the sludge through the drier exposing the material to hot gases. The product is a stable granulated material of various sizes. This is separated from exhaust gases using a cyclone and sized, with the granules that are either too large or small recycled back into the drum. The final product is stored in air cooled silos prior to bagging and delivery. The Swiss Combi System® has a closed-loop heating system that heats the air indirectly (Fig. 8.6). No odours can escape due to a slight negative

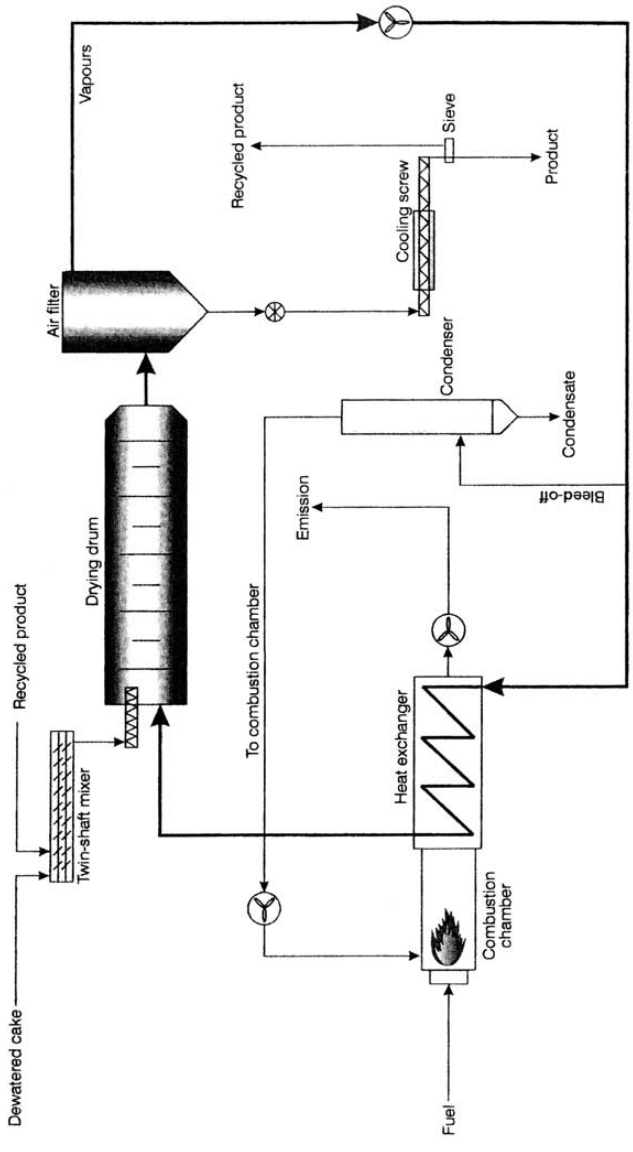


Fig. 8.6. Thermal dryer with a closed-loop heating system (CIWEM 1999).

Table 8.8. Main advantages of thermal drying of sewage sludge.

Significant reduction in sludge volume	Wide use: agriculture, forestry, land reclamation
Reduced storage costs	Suitable for landfill
Reduced transport costs	Can be used as fuel
Stable product	Suitable for long term storage
Easily stored, handled and transported	Optimum flexibility with regard to disposal options
Easily applied to agricultural land using standard farm equipment	Digested product is odourless
Almost pathogen free	
Good fertiliser properties	

operating pressure and all the non-condensable gases and odours generated as the sludge is dried are retained and transferred to the burner where they are fully oxidised at 800°C.

Due to the disadvantages of capital and operating costs, operational difficulties (e.g. the risk of combustion), and the significant volume (i.e. usually $< 1\%$ of the total plant flow) of strong waste liquor produced (e.g. high ammonia concentrations are also present when anaerobically digested sludges are dried), there are very few such plants operating in the UK or Ireland. However, there are currently over 110 thermal dryers in use in Europe (CIWEM 1999) and with the introduction of new legislation, especially the EU Urban Wastewater Treatment Directive (91/271/EEC), there is renewed interest in the process (Brown and Whipps 1995). The reason for this is that the process can be adapted to produce a variety of products that are suitable for use in agriculture, land reclamation, forestry, fertiliser blending and as an incinerator or boiler fuel. The main benefits are summarised in Table 8.8. The final product is a sterile and stable granule or pellet 3–6 mm in diameter. The product is very similar in nature to normal agricultural fertiliser and the granules can be spread on land using standard tractor-powered fertiliser spreaders. The granules are almost dust free ($< 1\%$), extremely durable and easily handled and transported. Once spread on the land, they absorb moisture rapidly and degrade releasing nutrients and trace elements. Typical characteristics of the granules are dry solid 95.3%, volatile solids 50.0%, nitrogen 3.3%, phosphorus 4.4%, and potassium 0.22%. Trace element content varies but in the UK is typically Zn 884 mg kg^{-1}, Cu 330 mg kg^{-1}, Ni 48 mg kg^{-1}, Cr 25 mg kg^{-1}, and Cd 5 mg kg^{-1}. Thermally dried sludge is also used as a fuel in all types of industrial furnaces, boilers and incinerators. It can also be used for power

generation to supplement coal or peat, waste incinerators, cement kilns and gasification plants. However, it remains uncertain if it is cost effective to dewater sludge beyond the autothermic stage (i.e. 35% DS) if it is to be used as a fuel supplement. Dried granules can spontaneously combust on storage. The auto ignition temperature is around 110°C and storage temperatures must be below 70°C in silos or < 50°C if stored in big bags. Recent developments in sludge drying have been reviewed by Lowe (1995) and Vaxelaire *et al.* (2000).

Dewatering

Dewatering is a mechanical unit operation used to reduce the water content of sludge in order to obtain a solids concentration of at least 15%, and usually much more. It is normally preceded by thickening and conditioning by the addition of chemicals, which aids flocculation and water separation, and may be followed by further treatment. This reduces the total volume of sludge even further and thus reduces the ultimate transportation cost of disposal. The resultant sludge is a solid, not a liquid, and can be easily handled by conveyers or JCB tractors, although experience has shown that the dried sludge, known as cake, is more easily handled at solids concentrations of $> 20\%$ (Institute of Water Pollution Control 1981). The resultant sludge is largely odourless and non-putrescible and can be stored without problems. Its solid nature makes it suitable for many more disposal options than liquid sludge.

The process is a physical one involving filtration, squeezing, capillary action, vacuum withdraw, centrifugal settling, and compaction. The most widely used European methods include drying beds, reed beds, lagoons, filter and belt presses, and vacuum filtration. Centrifuges are uncommon at present in the UK and are more widely used in Ireland.

Sludge drying beds. Drying beds are the cheapest and simplest form of dewatering. They are mainly restricted to smaller treatment plants because of the area of land required and the problems with odours and flies. Sludge drying beds are shallow tanks with a system of underdrains covered with layers of graded filter medium, normally a 100 mm of pea gravel covered with a 25 mm layer of sand (Fig. 8.7). The floor of the bed slopes towards the outlet at a minimum gradient of 1 in 60, and the sludge is pumped on to the bed to a depth of between 150–300 mm. Dewatering occurs mainly by drainage which is rapid for the first day or two and then progressively decreases until the solids have become so compacted on to the surface of the medium that drainage ceases. The surface liquid is decanted

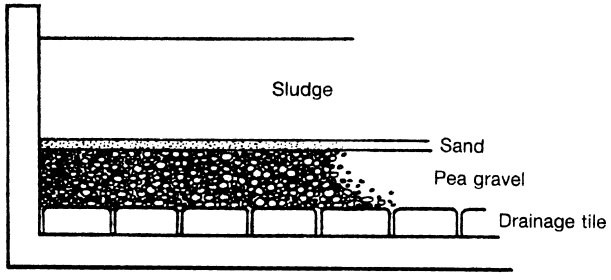


Fig. 8.7. The sludge drying bed (Institution of Water Pollution Control 1981).

off and returned to the inlet of the treatment plant, so that evaporation can take over as the major dewatering process. Evaporation is affected by the weather, in particular the wind, humidity, and solar radiation, and at this stage rainfall will dilute the solids concentration. As the sludge dries, it cracks, and this increases the surface area of the sludge, thus increasing the evaporation. When the cracks reach the support medium, rainfall is no longer a problem as it can drain through the sludge via the cracks to the medium below. At this stage, the sludge can be lifted either manually or by machine. The performance of drying beds depends on the type of sludge, the initial solids content, the period of drying, the porosity of the medium, loading rate, and weather conditions. Solids contents of up to 40% are possible, although 35% is a more commonly attained value. They can only be used when drying conditions are good, which in the UK limits their use to 6–7 months of the year. In hot weather, as in the summer of 1976, 40% solids can be obtained after 10–15 days storage, but periods of 30–40 days are usual.

Sludge is not usually conditioned by the addition of coagulants prior to application to drying beds, although aluminium chlorohydrate and polyelectrolytes do improve drainage characteristics, so that more water is able to escape via the underdrains during the first few days after application. The total surface area of bed required is related to the population, although local factors, such as average rainfall, the dry solids concentration of the sludge, and its filtrability are also important. As a rough guide, 0.12–0.37 m^2 of bed are required per head of population for primary sludge, with 50–60% less area required for digested sludge (White 1978b). Secondary sludges (humus and activated sludge) are more difficult to dewater, and conditioning is required before application to drying beds. The use of transparent plastic roofing for sludge drying beds allows them to be used for much longer periods and makes them more efficient by increasing evaporation

and protecting from rain. Full design and operational details are given in Institute of Water Pollution Control (1981) and Metcalf and Eddy (1984).

Sludge reed beds. Since the early 1990's, sludge drying beds have increasingly been converted into simple vertical flow wetlands (Sec. 6.2) for the dewatering and stabilisation of sludge. The common reed (*Phragmites australis*) is normally used and planted 30 cm apart in a layer of coarse sand overlying gravel with under drains. The bed must have sufficiently high freeboard to allow for 1.5 m of sludge accumulation. The roots penetrate the sand layer and become established, then sludge is added to the surface at very low rates to encourage the roots to spread over the entire bed area (Nielson, 1990; Hofman 1990; Linénard *et al.* 1990) (Fig. 8.8). Once the reeds are mature, sludge can be applied at between 1630 to 2444 l $m^{-2}y^{-2}$ depending on the type of sludge and solids concentration. Sludge is applied at regular intervals, 7–14 days during the summer and 14–28 days

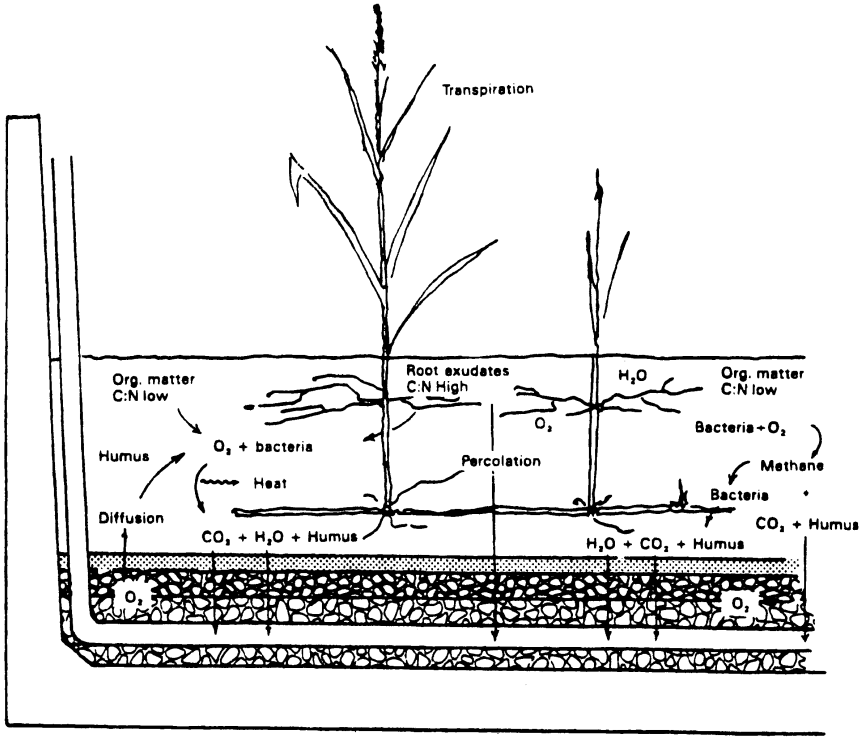


Fig. 8.8. Principal processes within a sludge reed bed (Klein 1988).

during the winter, with maximum loadings (100 l m^{-2}) applied during the growing season only. The reeds have been found to be inhibited if loading rates exceed 20–30 kg TSS $m^{-2}y^{-1}$, or if anaerobically digested sludge is used.

The reed's complex root system create zones of oxidation within the sludge layer enhancing decomposition rates as well as facilitating nitrification and denitrification. The roots, apart from transferring oxygen deep into the medium via their aerenchyme (i.e. spongy tissue that allows movement of air through it), maintain the porosity of the medium thereby maintaining drainage. The plants themselves utilise nutrients resulting in a rapid growth. Growth of up to 3 m can be achieved within three months to form luxuriant stands of reeds. Water loss is also very high during the growing season due to evapotranspiration. The continuous movement of stems in the wind and the equal distribution of reeds over the surface of the bed ensures that water quickly drains away and that water logging is prevented. Every spring, new shoots grow through the newly deposited sludge layer incorporating it into the root zone.

When sludge has accumulated to about 1 m, which should take between 3 to 10 years depending on the loading rate, the bed is taken out of service and allowed to stand for up to 4 months until maximum solids concentration of the deposited sludge is achieved. In practice this is about 40–50% DS. The sludge is removed mechanically along with the reeds, the medium is replaced and the bed replanted. The system does not require the addition of chemicals and can reduce the volume of sludge solids by up to 95% overall. The sludge can have an elevated metal concentration compared to normally processed sludge but can normally be safely landfilled. Although the process does not generate any unpleasant odour, in very cold climates odour can be problematic during the spring thaw. The percolation water from these systems is similar to that from mechanical dewatering units, and although free of any polyelectrolytes or conditioners, it must be returned to the inlet for treatment. Purpose built sludge reed beds are now common in the USA, Scandinavia, and Central Europe, and offer a truly sustainable, cost-effective, and safe sludge treatment and stabilisation process. One of the largest constructed wetlands treating sludge is in Denmark which is an activated sludge plant serving a population of 120,000 pe, although the majority of plants employing this technology are serving populations of < 1000 pe (Kim and Smith 1997).

Two experimental sludge reed beds were studied over a four year period at Darzlubie and Swarzewo in Northern Poland. These were used to treat digested primary sludge from Imhoff tanks. The beds (12×20 m) were

loaded with 36 m^3 of sludge (4–10% DS) eight times a year, which is equivalent to a depth of 2.4 m of sludge applied onto the surface over three years. This volume was reduced by 93% leaving just a 140 mm layer of stabilised sludge on the surface. The solids concentration was 38–45% DS which is twice as high as other comparable systems. The authors explain this difference by the long loading intervals of 6 weeks compared to the 2-week intervals normally used. The organic matter content fell by 18% to 42% dry matter over this period, although other studies have achieved higher reductions (e.g. 51+81%) (Reed *et al.* 1995). Full details of the bed design is given by Obarska-Pempkowiak *et al.* (1997). Liénard *et al.* (1995) compared planted and unplanted sludge drying beds treating waste activated sludge. Edwards *et al.* (2001) carried out trials treating a mixture of treated and untreated pig slurry. They achieved volume reductions of 84–86%, although they found high loadings of raw slurry in the spring could seriously damage emerging shoots. Other interesting studies include Kim and Smith (1997), DeMaesener (1997) and Begg *et al.* (2001).

Filter presses. Filter presses are comprised of a series of metal plates, between 50–100 in number, suspended either from side bars (side-bar press) or an overhead beam (overhead beam press). Each plate is recessed on both sides and supported face-to-face so that a series of chambers are formed when the plates are closed. Filter cloths are fitted or hung over each plate which are then closed with sufficient force, using either hydraulic or powered screws, to form sealed chambers. The sludge is conditioned before being pumped into the chamber under pressure ($\approx$ 700 KN m^{-2}) via feedholes in each plate. The pressure builds up and filtration occurs with the filtrate passing through the filter cloths and leaving the chamber via ports in the plates (Fig. 8.9). Pumping of sludge into the chambers continues until the chamber is filled with cake and the flow of filtrate has virtually stopped. Filter presses are normally situated on the first floor of a specially constructed building that allows access for trailers. The pressure is released to the press and the plates separated allowing the cake to drop out into the trailer parked directly below the press. In this batch process, the operational cycle can vary from 3–14 h, with filling and pressure build up taking between 0.2–1.5 h, filtration under maximum pressure from 2–12 h, and cake discharge 0.1–0.5 h, although cycles normally take between 3–5 h to complete. Much work has been done to try and reduce the time taken for the second phase of the cycle, which is filtration under pressure. Important factors affecting the rate of filtration include the choice of filter cloth, pump pressure, filtrability of the dry solids content of the sludge, and condition of the filter cloth (Institute of Water Pollution Control 1981).

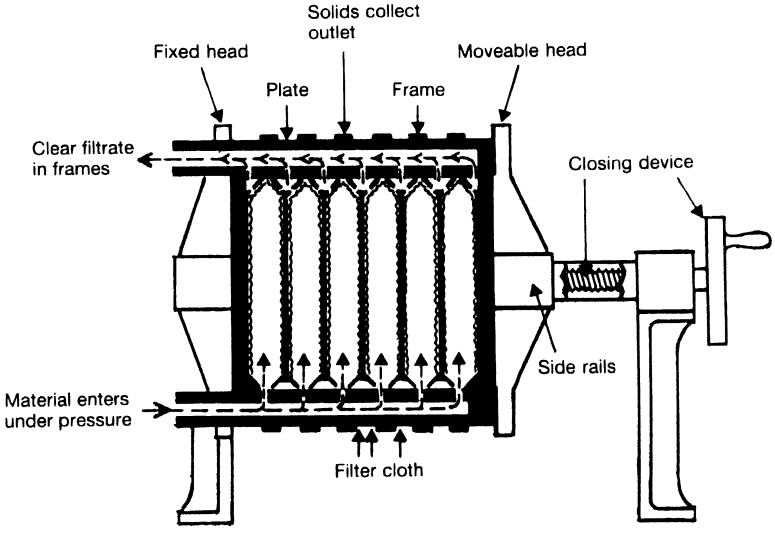


Fig. 8.9. Schematic outline of a sludge filter press (Casey and O'Connor 1980).

The process is primarily used for raw sludge, but is equally successful with digested, mixed, and thermally conditioned sludges. The final cake is quite thick, 25 or 38 mm, with a solids concentration of 30–40%. The advantage of the process is that it produces a drier cake than other dewatering devices, which results in a lower volume of sludge cake and reduced handling costs. The filtrate contains a relatively small concentration of suspended solids, although this is dependent on the type of sludge and filter cloth. Much has been written on the variation of filter cloths, including the material used, the weave, and the design. Gale (1975) found that cloths with an open weave had a higher concentration of solids in the filtrate than cloths with a tight weave, although the former were less susceptible to small particles packing into the spaces in the weave thus preventing the passage of filtrate. Filter cloths need to be washed occasionally between cycles using a high pressure water jet. The frequency depends on a number of factors, including the suitability of the cloth used and the chemical conditioner selected. For example, Pullen (1981) found sludge conditioned with lime and copperas gave 20 cycles between washing, whereas organic polyelectrolytes permitted 30 cycles before the filter cloths required washing. Ways of optimising the performance of filter presses are reviewed by Hoyland *et al.* (1981) and Bruce and Lockyer (1982).

Conditioners aid the dewatering process by improving the filtration characteristics of sludges by increasing the degree of flocculation of the

sludge particles so that the absorbed water can be more easily removed. It also prevents small particles from clogging the filter cloths in filter presses. Apart from heat treatment, sludge conditioning normally involves the addition of one or more chemicals. Although expensive, the use of chemical conditioners is cost-effective because the increased solids content of the sludges produced reduces the sludge volume that has to be disposed. There are numerous chemical conditioners available including lime, ferrous sulphate, aluminium chlorohydrate, organic polymers or polyelectrolytes, and ferric chloride. All are commonly used in the UK with the exception of ferric chloride ($FeCl_3$), which is widely used in the US.

Lime ($Ca(OH)_2$) is nearly always used in conjunction with copperas (ferrous sulphate $FeSO_4.7H_2O$) unless iron salts are present in the sludge when lime can be used on its own (Swanwick 1973). The combination is used almost exclusively in association with filter presses, not being suitable for belt presses or centrifuges which require a more rapid flocculation that can only be obtained from using polyelectrolytes. Copperas is kept in a crystalline form and is dissolved as required. It is an acidic and highly corrosive liquid and special precautions have to be taken when used. The lime is delivered by bulk tanker and is normally kept in a silo ready for use. Lime is usually added after the copperas with doses of 10% copperas and 20% lime (dry solids) used for raw sludges, and 40% copperas and 30% lime for digested sludges. The large weights of chemicals used, results in a significant increase in the weight of solid cake that has to be disposed of, and in the case of lime and copperas this can be as much as 50% of the solids in the final cake.

Aluminium chlorohydrate ($Al_2(OH)_4Cl_2$) is just one of a number of the aluminium salts used for conditioning sludge prior to dewatering, and is delivered to the site as a concentrated solution that needs diluting before use. It is only suitable for filter presses and drying beds and has the advantage over lime and copperas of not significantly adding to the mass of sludge cake produced. Lime addition (5–10%) is sometimes used to control the emission of odours, especially if the sludge has been stored for longer than 4–5 days.

The mode of action of these conditioners, i.e. the charges on the metallic ions neutralising the surface charges on the small sludge particles, is more controllable when using polyelectrolytes. These macromolecular organic polymers are water soluble, and like the other conditioners, are able to flocculate dispersed particles. They are available in a vast range of molecular weights and charge densities that allow the conditioner to be changed to cope with even subtle changes in sludge character. Polyelectrolytes are

available either as a liquid, granules or powder, with the dry forms requiring to be dispersed in water before use so that they contain between 0.10–0.25% of active ingredients. Dispersion can take up to 2 h and, as their ability to flocculate particles deteriorates with storage beyond this period, they are usually made up as required. Dosage is expressed as either volumetric (mg m^{-3} of sludge) or more commonly as the weight of active ingredient added to a unit weight of dry sludge (kg $tonne^{-1}$ DS). Normal doses for raw sludge are between 1–4 kg $tonne^{-1}$ for belt presses, so there is no significant addition to the mass of sludge. Although they can be used in conjunction with filter presses, polyelectrolytes are most efficient when used with belt presses and centrifuges. They are particularly useful for use with dewatering systems that utilise shear force to aid water release. As a general rule, the higher the shear force required, low in drying beds and filter presses but high in belt presses and centrifuges, then the greater the molecular weight of the polymer used. Lime can be used with polyelectrolytes to suppress odours, but hydrogen peroxide is more efficient for this purpose and is easier to inject into the sludge. The optimum dosage of conditioner for a specific sludge is calculated by measuring its filtrability after conditioner is added. Numerous methods of assessing the dosage are used, including visual observation by the beaker test, gravity drainage test, capillary suction time (CST), standard shear test or the Buchner funnel test. These methods are fully explained elsewhere (Institute of Water Pollution Control 1981; CIWEM 1999).

Belt presses. Unlike filter presses, belt presses are a continuous process. Instead of porous sheets of filter mesh being squashed between plates, the belt press comprises two continuous belts, one porous filter belt, and an impervious press belt, between which sludge is added, and are then driven by a series of rollers that also compress them, squeezing excess water from the sludge.

Conditioned sludge is fed evenly on to a continuously moving open meshed and endless filter belt that acts as a drainage medium. Dewatering then occurs in three distinct phases. First, before any pressure is added, water drains rapidly from the sludge due to the action of the polyelectrolyte. Sometimes a vacuum is used to increase water removal at this stage. The second phase is the initial compression of the sludge, as a low pressure is applied by a series of rollers as the belt press converges with the lower filter belt. Water is squeezed out of the sludge as the pressure increases.

In the final phase, the sludge is subjected to increased pressure and a shearing effect that rips open the sludge between the two belts producing new channels for more water to escape, while the increased pressure forces

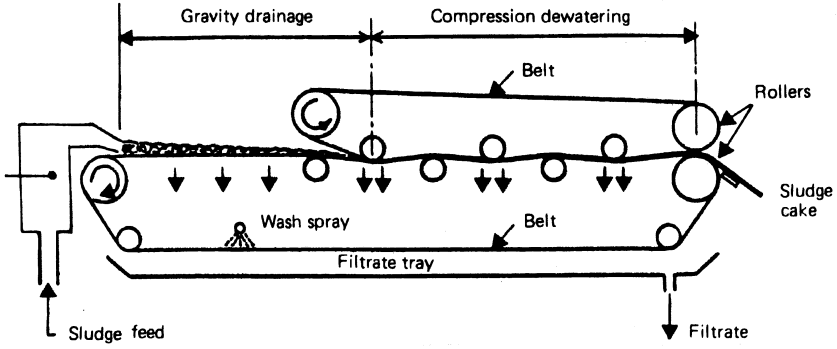


Fig. 8.10. Schematic outline of a sludge belt press (Casey and O'Connor 1980).

out the remaining free water. As the belts part, the sludge cake falls from the machine on to a conveyer belt or into a trailer ready for transportation to the disposal site. The filtrate from the process falls from the filter belt to drains under the machine and is pumped back to the inlet of the treatment plant (Fig. 8.10).

All belt presses are similar in principle, although the configuration of the rollers and the complexity of the machines can differ dramatically. The efficiency of these presses depend on the pressure applied to the sludge (i.e. clearance between rollers) and the retention time (i.e. length of compressed filter belt and belt speed). Other factors are also important, for example, with primary sludges fresh material dewaters more successfully than stored material or if insufficient polyelectrolyte is used then large conglomerate flocs are not formed so that complete free drainage does not occur (Institute of Water Pollution Control 1981). Organic polyelectrolytes are normally used with doses ranging between 1.3–7.7 kg tonne^{-1} (DS). Final solids concentrations of between 20–25% are normal, although surplus activated sludge and aerobically digested sludge are far less amenable to dewatering. New machine designs with vertical belts and spring-loaded rollers (e.g. multistage and incremental pressure type filter-belt presses) can produce cake with up to 35% solids (Department of the Environment 1975; Institute of Water Pollution Control 1981). Whereas filter presses are the most widely used mechanical dewatering device in the UK, belt filter presses are more frequently used in Ireland, with small mobile units often used at the smaller treatment plants (Takahashi 1997).

Other methods. Vacuum filtration and centrifugation are also used for dewatering sludges, although their use is largely restricted to industrial

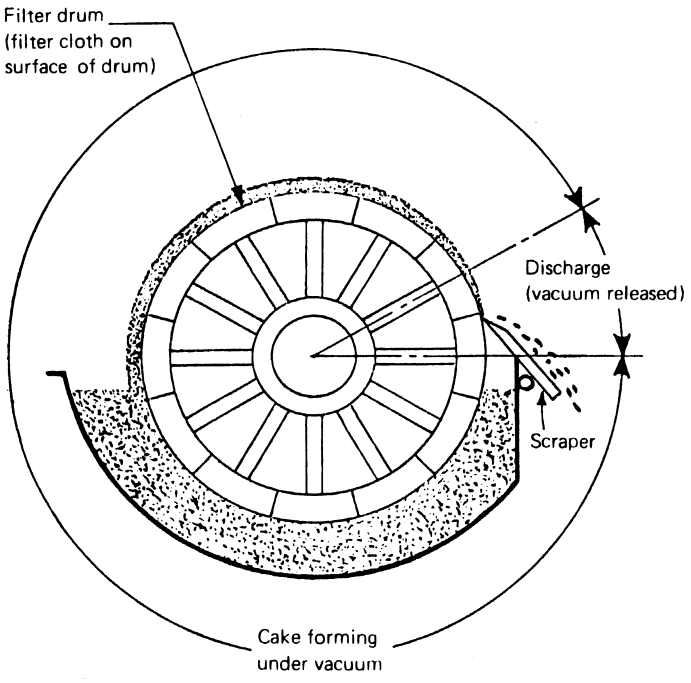


Fig. 8.11. Schematic diagram of vacuum filtration for dewatering sludge (Casey and O'Connor 1980).

wastewaters in the UK, and are not considered in detail here. With vacuum filtration, water is sucked from the sludge under vacuum through a filter cloth that is carried on a slowly revolving drum partly immersed in the sludge (Nelson and Tavery 1978) (Fig. 8.11). Centrifuges are now widely used in Ireland and consist of a rotating bowl into which sludge and polyelectrolytes are added. Centrifugal forces enhance the settling rates of the particles and cause the solids to separate out at the edge from where it is removed (Fig. 8.12) (Egglink 1975). An emerging technology is freeze-thawing which is used to condition and in some cases dewater sludges (Martel 1993; Chu *et al.* 1999; Parker and Collins 2000; Jean *et al.* 2001).

8.1.2. *Disposal options*

In many respects, the final disposal options available dictate whether sludge needs to be stabilised and dewatered, although the physical nature of the sludge limits the choice of disposal methods. Factors such as sludge quality, presence of toxic chemicals or pathogens, volume, location of treatment

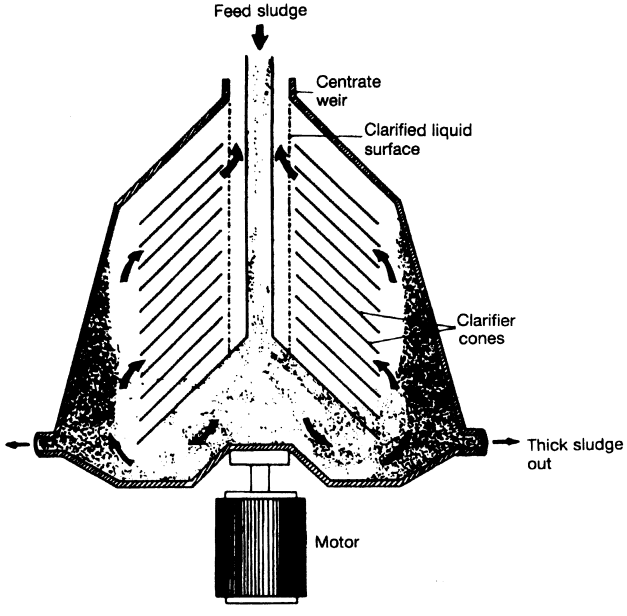


Fig. 8.12. Schematic diagram of a nozzle-bowl centrifuge for dewatering sewage sludge (Institute of Water Pollution Control 1981).

Table 8.9. Expected trends in sewage sludge disposal in the European Union.

Year	1995	2005
Volume (tonnes DS year^{-1})	6.5×10^6	10.1×10^6
Land disposal (%)	37	45
Sea (%)	6	0
Fuel/incineration (%)	11	38
Landfill (%)	40	17
Other (%)	6	Unknown

plant, and cost are all involved in the selection of disposal methods. However, overall cost and environmental factors are the foremost considerations. Disposal options, and these do not include stabilisation processes such as anaerobic digestion and composting, fall into five main categories: landfill, disposal to agricultural land, disposal to sea, incineration, and other processes (Table 8.9). In the UK during 1998/99, 1,058,000 tonnes DS of sewage sludge was produced, 504,000 tonnes DS went to farmland,

115,000 tonnes DS to landfill, 150,000 tonnes DS to the sea, 185,000 tonnes DS was incinerated, and 105,000 tonnes DS was used for land reclamation, forestry as well as soil and compost products (Water UK 2001). Other processes include sacrificial land use, forestry, land reclamation, use as building materials, and as a feedstuff. The other processes currently utilise a very small percentage of the total volume of sludge produced. The EU Directive on hazardous wastes (91/689/EEC) and its amendment (94/31/EEC) categories wastes as either hazardous or non-hazardous that then affects how it may be disposed of. It categorises wastewater sludge in the *European Waste Catalogue* (EWC) under the general code 19-08. Sludge from industrial wastewater (EWC code No. 19-08-04) and domestic wastewater treatment (19-08-05) are not included in the list of hazardous wastes. However, some types of water and wastewater treatment sludges are classed as hazardous (e.g. 19-08-03: fat and oil mixtures from oil separators; 19-08-06: saturated or used ion-exchange resins; 19-08-07: solutions and sludges from the regeneration of ion exchangers). Where sludges contain metals or metal compounds, making them unsuitable for use in agriculture, or contain listed toxic substances, then these sludges are automatically classified as hazardous and cannot be mixed with other non-hazardous wastes.

The most popular method of disposal is dumping on land. This encompasses a number of options, such as using sludge for landfill, dumping as cake on to municipal tips, allowing it to dry in shallow lagoons, or burying it in trenches. The reuse of non-toxic sewage sludge as either fertiliser or a soil conditioner is becoming increasingly popular in most European countries (Sec. 8.2). Spreading sludge on agricultural land is an ancient practice and it is used on grazing, arable, horticultural, and forestry lands, with digested sludge also used on allotments. It is used in land reclamation and as a soil conditioner by composting with either refuse or straw (Sec. 10.3.3). Dumping at sea has been adopted by most major cities located on the coast with the liquid sludge transported in purpose-built vessels and dumped well out to sea, or the screened wastewater discharged directly to coastal wasters via long sea outfalls (Sec. 9.4.1). The capital investment is extremely large and this disposal method is restricted only to major centres of population. Among its many advantages is its independence of seasonal factors including the weather, as well as being economical and final (Sec. 8.3). The dumping of sludge at sea was banned within the EU in 1998 under the Urban Wastewater Treatment Directive (91/271/EEC). This primarily affected the UK who disposed of 332,000 t DS y^{-1} to sea, some 30% of the country's total sludge production. Other countries affected included Ireland (35%; 12.8 t DS y^{-1}), Spain (10%; 35.0 t DS y^{-1}) and Portugal

(2%; 0.5 t DS y^{-1}). However, the practice of dumping sludge at sea or of disposing of screened sewage via outfall to coastal waters is still widely practiced worldwide.

The EU has advocated the use of the term biosolids to describe sewage sludge where solids from wastewater treatment contain a significant proportion of organic matter (> 5% by weight). The term solids is used to describe sludges of other industrial effluent treatment processes. Biosolids is seen as a more positive term for the marketing of sewage sludge for reuse.

Sludge contains more volatile combustible matter and less fixed carbon than coal, and once dried it will burn and generate considerable heat. Because of high capital costs, incineration has never been a widely adopted disposal route for sewage sludge except in Germany and France where 375,000 and 130,000 t DS respectively is incinerated annually (Table 8.10). Some smaller countries are more dependent on incineration as a disposal route for example, Austria (34%), Switzerland (25%), and Denmark (24%). Incineration has been selected primarily for industrial cities where heavy metal contamination of the sludge makes it unsuitable for other disposal routes, particularly disposal to agricultural land, where land for dumping is scarce, or the sea disposal option is not available. With the phasing out of sludge disposal to sea and the improvements in the provision of sewage treatment plants in the EU Member States under the Urban Wastewater Treatment Directive, significantly more sludge is being produced that must now be disposed of to land. Incineration has been selected as the preferred disposal option by most large cities. For example, Thames Water has installed two fluidised bed incinerators to dispose of the sludge produced at the Crossness and Beckton Treatment Plants treating the wastewater from London. Operating at 860°C, the dewatered sludge is instantly vaporised. Incineration destroys the organic and volatile components of the sludge, including toxic organic compounds, leaving a sterile ash in which all the toxic metals are concentrated. In comparison to the weight of the sludge cake incinerated, only a small weight of ash is left which is disposed at tips. However, because of the high concentration of heavy metals present, careful selection of disposal sites must be made to avoid leaching. The ash from the incinerator at Birmingham comprised 33.5% SiO_2, 25.0% Al_2O_3, 11.9% P_2O_5, 9.3% CaO, 8.8% Fe_2O_3, 1.2% K_2O, 1.0% Zn, 0.94% TiO_2, 0.85% MgO, 0.84% Na_2O, 0.69% Cu, 0.18% MnO, and 0.18% Pb (Institute of Water Pollution Control 1978). Details of emissions from sludge incinerators in France is discussed by Seban and Chabrier (1996). The EU legislation relating to sewage sludge and incineration is reviewed by Spinosa (2001).

Table 8.10. Quantity and disposal routes of European sewage sludge in 1992. Units are 10^3 tonnes DS y^{-1} and (%) (Matthews 1996).

Member state	Quantity		Agriculture		Dump		Incineration		Sea		Other (e.g. recultivation, forestry)	
Austria	170	(2.3)	30.6	(18)	59.5	(35)	57.8	(34)	—		221	(13)
Belgium	59.2	(0.8)	17.2	(29)	32.5	(55)	8.9	(15)	—		0.6	(1)
Denmark	170.3	(2.3)	92	(54)	34	(54)	40.9	(24)	—		3.4	(2)
Finland	150	(2.0)	37.5	(25)	112.5	(75)	—		—		—	
France	865.4	(12.0)	502	(58)	233.5	(27)	130	(15)	—		—	
Germany	2,681.2	(2.3)	724	(27)	1,448	(54)	375.2	(14)	—		134	(5)
Great Britain	1,107	(15.0)	488	(44)	88.6	(8)	77.4	(7)	322	(30)	121	(11)
Greece	48.21[a]	(0.6)	4.8	(10)	43.4	(90)	—		—		—	
Ireland	36.7	(0.5)	4.4	(12)	16.6	(45)	—		12.8	(35)	2.9	(8)
Italy	816	(11.0)	269.2	(33)	449	(55)	16.2	(2)	—		81.6	(10)
Luxembourg	8	(0.1)	1	(12)	7	(88)	—		—		—	
Netherlands	335	(4.5)	87	(26)	171	(51)	10	(3)	—		67	(20)
Norway	95	(1.3)	53.2	(58)	41.8	(44)	—		—		—	
Portugal	25	(0.3)	2.7	(11)	7.3	(29)	—		0.5	(2)	14.5	(58)[b]
Spain	350	(4.7)	175	(50)	122.5	(35)	17.5	(5)	35	(10)	—	
Sweden	200	(2.7)	80	(40)	120	(60)	—		—		—	
Switzerland	270	(3.6)	121.5	(45)	81	(30)	67.5	(25)	—		—	
Total	7,387	(100.0)	2,690.1	(36.4)	3,066.2	(41.6)	801.4	(10.9)	380.3	(5.19)	447.1	(6)

[a] Other authors give 200,000 tonnes dry matter per year.

[b] Surface waters.

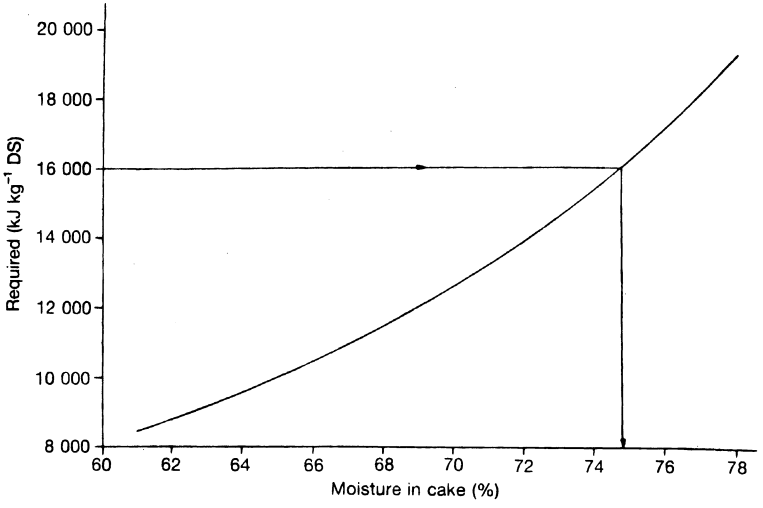


Fig. 8.13. Heat required to burn sludge cake with a specific moisture content (Grieve 1978).

The two most widely used types of incinerators for disposing sewage sludge are the multiple hearth incinerator and the fluidised bed incinerator. The amount of energy required to incinerate the sludge at the correct temperature in order to avoid odours and any possible harmful residues being formed, depends on the water content of the sludge cake. The amount of heat required per kg DS to burn sludge of various moisture contents is given in Fig. 8.13. If moisture is below 70% (> 30% DS), little additional fuel is used as the energy released during combustion is greater than that required to evaporate the water present (i.e. autothermic). Raw sludge contains more energy than digested sludge as much of its potential energy is removed during the digestion process because raw sludges having a higher calorific value (16,270–23,875 kJ kg^{-1} DS) compared with digested sludge (11,560–13,870 kJ kg^{-1} DS) (Grieve 1978). Where a town has a refuse incinerator then sewage sludge is often included. Conversely, refuse is occasionally mixed with sludge cake at treatment plant incinerators to increase the combustability of the sludge. New methods of sludge incineration, have been developed, especially in Japan, Germany, and the US, and these are reviewed by Smith (1986).

With the growing awareness of air pollution, the incineration of refuse and sewage sludge have become less popular, although the flue gases from burning sewage sludge are mainly water vapour with small amounts of particulate solids that may contain metals. These contaminants, however,

Table 8.11. Air emission limits for incineration plants (mean daily values) (referred to 11% O_2 concentration) (Spinosa 2001).

Parameter	Limit value (mg/m^3)
Total dust	10
TOC	10
HCI	10
HF	1
SO_2	50
NO+NO_2 as NO_2	
(existing plants > 3 t/h and new plants)	200
NO+NO_2 as NO_2	
(existing plants ≤ 3 t/h)	400
* Cd+Tl	0.05
* Hg	0.05
* As+Pb+Cr+Co+Cu+Mn+Ni+V+Sb	0.5
	(ng/m^3)
** PCDD+PCDF (TE)	0.1

* mean values measured in a sampling period (min. 30 minutes, max. 8 h)

** mean values measured in a sampling period (min. 6 h, max. 8 h)

are easily removed by conventional scrubbing processes (Table 8.11). The price of supplementary fuel for incinerators, such as oil and LPG has also made operating incinerators much more expensive in recent years. Incineration of sewage sludge is re-evaluated economically as a viable disposal method by Lowe (1988) and reviewed by CIWEM (1999). Risk assessment of waste incineration is discussed by Harrop and Pollard (1998).

Pyrolysis is less polluting than incineration as heavy metals are concentrated in a solid carbonaceous residue so that leaching is less probable than from incineration ash. It also produces hydrocarbon oils and gases that can be used as fuels (Sec. 10.3.1). Sludge melting is a relatively new thermal process where sludge is subject to temperatures of 1,500°C that is beyond the melting point of the inorganic materials present. These are then removed by cooling the molten slag while the organic fraction is thermally oxidised (Abu-Kaddourah *et al.* 2000). The slag is very stable and the metals are not leached under normal conditions making the residual waste safe for landfill or reuse with no adverse environmental impact (Idris and Saed 2002).

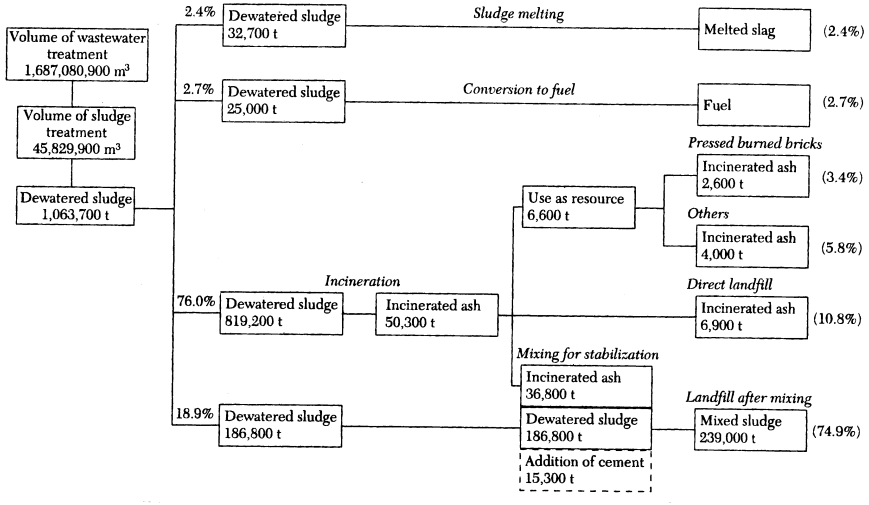


Fig. 8.14. Sludge treatment and disposal in the Ward District of Tokyo in the year 1994 (Watanabe and Maeda 1996).

Japan has led the world in its innovative treatment and disposal of sewage sludge. Watanabe and Maeda (1996) have described the way in which Tokyo disposes of its sewage sludge. Up to the mid 1940's it was disposed to sea, then used primarily for agriculture until the 1960's. However, increased urbanisation and sludge production led to an emphasis on volume reduction, followed by stabilisation and landfill (Fig. 8.14). Since then, incineration has been adopted to further reduce sludge volume using multiple hearth furnaces (52%) and fluidised bed combustors (48%) (Table 8.12). About 80% of the 29,000 tonnes of sewage sludge produced each day in the Ward District of Tokyo, which serves a population of 8,060,227, is incinerated. The ash is then mixed with the remaining dewatered sludge and a special cement for stabilisation. Approximately 240,000 m^3 of this mixture is landfilled annually. A further 420 tonnes of dedwatered sludge is reused each day (Fig. 8.15). In Singapore, sewage sludge is also used in the production of lightweight aggregate concrete materials, cement, and bricks (Tay and Shaw 1991, 1992; Tay *et al.* 1991, 2001; Hwa and Jeyaseelan 1996). A similar situation exists in Taiwan where due to a lack of landfill space it is proposed to incinerate sewage sludge. This will produce some 2,800 tonnes of sewage sludge ash daily and in order to minimise the potential for environmental damage this ash is to be reused rather than disposed. Pan and Tseng (2001) have looked in detail

Table 8.12. Incinerator capacity, standards and measured values of exhaust gases from some sewage sludge incinerators in Tokyo (Watanabe and Maeda 1996).

Treatment plant	Facilities' capacity (t/day)	SO_x (Nm^3/h)		NO_x (p.p.m.)		NO_x (Nm^3/h)		Smoke and dust (Nm^3/h)		Hydrogen chloride (Nm^3/h)	
		Standard	Measured	Standard	Measured	Standard	Measured	Standard	Measured	Standard	Measured
Sunamachi	300×2	22.77	0.81	300	19.5	52.63	1.93	0.10	0.0065	700	14.45
	300×3			250				0.10			
	250×1			250				0.08			
Odai	200×3	9.85	0.03	250	15.0	14.27	0.60	0.15	< 0.0005	700	< 1
Shingasi	300×1	25.24	0.072	300	21.8	27.90	1.06	0.10	0.0023	700	5.26
	250×1			250				0.15			
	250×1			250				0.08			
Kosuge	50×1	2.94	0.001	300	33.0	6.61	0.30	0.20	0.002	700	0.002
	50×1			250				0.15			
Kasai	100×1	18.88	0.238	250	10.4	22.08	0.81	0.20	0.0043	700	6.355
	150×1							0.15			
	250×1							0.08			
	350×1							0.08			
Nanbu	300×2	14.82	0.36	250	25.39	25.39	2.42	0.10	0.0026	700	57.4
sludge	250[a]		0.156	350			1.62	0.20	0.0683	—	—
plant	160[b]		0.078	250			1.26	0.15	0.0018	700	3.0
	10[c]		0.44	180			0.09	0.15	0.0021	—	—
		99.50	**1.034**		**145.88**	**145.88**	**5.39**				

[a] Capacity of sludge fuel plant.

[b] Capacity of sludge melting plant.

[c] Capacity of METRO renga plant; capacity is in terms of incinerated ash.

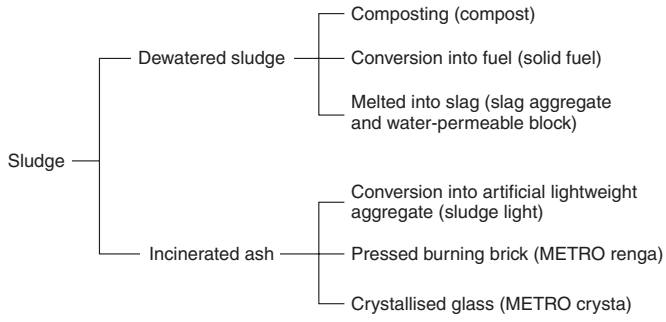


Fig. 8.15. Alternative uses of sewage sludge by Tokyo Metropolitan Government (Watanabe and Maeda 1996).

at the chemical and physical characteristics of Taiwan sewage sludge ash, including its pozzolanic activity, and have proposed various end uses.

The stabilisation/solidification process is used to treat hazardous sludges. The process improves the handling and physical characterisation of the sludge, decreases the surface area across which transfer or loss of contaminants can occur, and limits solubility or detoxification of any hazardous constituents in the sludge. Sludges are treated either with fly ash and cement or zeolite and cement binder mixtures. The zeolite achieves stabilisation, by ensuring the hazardous materials are maintained in their non-soluble or non-toxic form. The cement achieves solidification, which is normally a large block with high structural integrity (Conner 1990; USEPA 1986; Savvides *et al.* 1997, 2001).

In February 1993, the US EPA published standards for the use or disposal of wastewater solids in the Federal Register. Know as the 503 Regulations, they became effective on 22 March 1993. They consist of five sub-parts: (A) General provisions; (B) Land application; (C) Surface disposal; (D) Pathogen and vector-attraction reduction; and (E) Incineration. The Regulations are very comprehensive and lengthy and should always be consulted when designing disposal strategies. These are discussed further later in this chapter.

Further reading

General: Casey and O'Connor 1980; Dirkzwager and L'Hermite 1989; L'Hermite 1991; Tang *et al.* 1997; CIWEM 1999.

Legislation: Spinosa 2001

Thickening: Institute of Water Pollution Control 1981; CIWEM 1999; Dentel 2001.

Stabilisation: Bruce 1984; Nielsen 1993; Müller 2001.
Dewatering: Department of the Environment 1975; Egglink 1976; Nelson and Tavery 1978.
Disposal options: Institute of Water Pollution Control 1978; Calcutt and Moss 1984.
Thermal drying and incineration: Smith 1986; Frost 1988; Lowe, 1988.Cross 1993; Lowe 1995; Werther and Ogada 1999; Stolarek and Ledakowicz 2001.

8.2. Land Disposal

8.2.1. *Sludge disposal to land sites*

Apart from the utilisation of sewage sludge on agricultural land as a fertiliser and soil conditioner, there are five other widely used methods of land disposal: (1) irrigation; (2) shallow lagooning; (3) trenching; (4) disposal to refuse tips, (5) land reclamation.

The land used for these disposal methods can be categorised as either sacrificial or reclaimed. Irrigation, shallow lagooning, and continuous trenching are carried out permanently and intensively on a small area of land. Therefore, because of the level of contamination that inevitably results from such high loading rates of sludge, the land is unsuitable for agricultural purposes, and probably never will be again. Such sites can only be used for further sludge disposal, hence the term sacrificial land. Sludge is also used for land reclamation, for bringing marginal land to its full agricultural potential, and reclaiming derelict land, including spoil heaps and refuse tips. Reclamation provides a major use of sludge cake, although trenching using liquid sludge is also employed. The disposal of sludge to tips can fall into either category. Where sludge is disposed intensively to landfill, it is likely that the metal concentrations in the soil and herbage will always be too high for that land to be used for agricultural purposes, although it may have a function for landscape or amenity. However, where sludge is incorporated into a refuse tip or used to cover an existing tip then it has a reclamation function. In a survey of 193 sewage treatment plants in the UK, only 37 did not dispose of sludge to agricultural land. The most frequent reason given was due to the unsuitable quality of sludge, normally in terms of heavy metal concentrations (Table 8.13) (National Water Council 1981). Heavy metal contamination in Irish sludge is currently well below the European average due to less industrial development (Table 8.14). Throughout Europe, metal concentrations in sewage sludges have been steadily falling over the past thirty years. This has been due to improved industrial effluent

Table 8.13. Levels of metals in sewage sludges disposed to agricultural land in the UK (National Water Council 1981).

Metal	(mg kg^{-1} DS)				No. of works	No. of samples
	Min	Median	Mean	Max		
Zinc	199	1,270	1,820	19,000	193	2,386
Copper	36	546	613	2,889	193	2,379
Nickel	5	94	188	3,036	192	2,343
Zinc equivalent	507	3,440	4,550	40,502	192	2,343
Chromium	7	335	744	10,356	188	2,310
Cadmium	0.4	17	29	183	180	2,319
Lead	19	324	550	3,538	164	2,189

Note: Medians and means are weighted according to population served by works.

Table 8.14. Range of key metals in Irish sewage sludges (mg kg^{-1} dry weight). (a) 68 different plants surveyed in 1988; and (b) 27 different plants measured in 2000 (Gray 2001).

	Cd	Zn	Cu	Pb	Ni
Mean	2.7	1,319	631	228	852
Median	< 1	740	515	159	30
Minimum	< 1	< 1	39	7	< 1
Maximum	130	8,533	3,970	2,555	46,800

(a)

	Cd	Zn	Cu	Pb	Ni	Zn eqiv
Mean	0.93	488	365	67	28	1,432
Median	0.9	436	288	53	16	1,404
Minimum	0.5	265	136	18	6	665
Maximum	2.7	825	1,054	234	177	2,866

(b)

control, changes in the nature of traditional manufacturing industries and the adoption of cleaner manufacturing technologies. For example, records from Nottingham Wastewater Treatment Plant in England has shown a large reduction in sludge metal levels between 1962 to 1992 (e.g. 98% Cd, 80% Zn) (Rowlands 1992). Median concentrations in British sludges declined by 20–30% for Zn, Cu and Cr; 40% for Ni; 50% Pb and 70% for Cd

between 1982/3 to 1990/1 (DETR 1999). Similar reductions have also been recorded in Ireland (Table 8.14) and elsewhere (Balmér 2001).

Sacrificial land use

Irrigation is based on the established sewage farm principle except that liquid sludge is utilised and raw sewage is not. Areas of ground are ploughed along the contours and then, using a ridging plough, deep furrows are made across the ploughed field down the slope at 9 m intervals. The sludge is allowed to flow down the ridge furrows and by using earth stops, it overflows and floods different sections of the ploughed field. Usually, areas close to the treatment plant are kept specially for this purpose and are not used for agriculture. The sludge is allowed to dry periodically and is then ploughed into the soil. The land is generally reseeded and then left for several months, or even years, before being reused. The system is a particularly cheap method of disposal as there are no transport costs and the sludge need not be thickened, conditioned or dewatered. However, care must be taken to avoid runoff, contamination of groundwater, the production of odours, and in summer, the production of flies.

In their mode of action, shallow lagoons are very similar to irrigation, with exactly the same advantages and disadvantages. The lagoons are quite small in area and are formed by making parallel earth ridges, using a ridging plough, 9–15 m apart. These banks run along the contour of the land and similar ridges are then made at right-angles to them at similar intervals, thus forming square lagoons. The banks and comers are finished off by hand to ensure that they are continuous and stable, and the lagoons flooded with sludge to a depth of about 300 mm. This is allowed to dry, although the process may be repeated before the banks are levelled and the ground deeply ploughed.

Deeper and larger lagoons of a permanent structure are also used for sludge storage and treatment, but not for disposal. Here, anaerobic stabilisation takes place that reduces the volume of dry solids in the sludge by 40–60%. The sludge becomes highly stabilised, with digested sludges also undergoing further anaerobic digestion. Once the lagoon has become full, the sludge is allowed to dry and the highly humified sludge is dug out for disposal (Sec. 7.2.1).

Trenching also relies on soil percolation to reduce the water content of the sludge, which is then decomposed by the soil micro-organisms. Long trenches are dug 1.0 m wide and 0.6 m deep at intervals of between 0.6–2.0 m. Two approaches are then used. When trenching is used just once to

bring marginal land back into full production, the trenches are filled with sludge at a rate of 860–1000 tonnes DS ha^{-1}, back-filled and left for many months before being used for agriculture. The problem with this method is that the sludge can be displaced out of the trench during back filling and the land remains very unstable for some time afterwards, especially on heavy soils. In some extreme cases, vehicles are not able to get back on to the land for up to 12 months after treatment. The trenches are generally close together, about 0.6–1.0 m apart, so that maximum application of sludge can be achieved. At permanent disposal sites, trenching is a continuous process. The trenches are spaced 2 m apart and after being filled with sludge, are not immediately back-filled. Instead, the water is allowed to separate from the sludge solids and permitted to percolate into the soil so that more sludge can be added to each trench. This is repeated until the trenches are full of semi-dry sludge, when the area is taken out of service. After the sludge has dried, new trenches are constructed between the old ones, using the excess soil to fill in the old trenches, and the process is then repeated. The next set of trenches are constructed at right-angles to the original trenches, and so on. Permanent trenching sites require at least three areas of land for successful and continuous operation. The land is used in strict rotation with one area receiving sludge, another out of use while the sludge is drying, and a third area that is being retrenched. Within the EU, the use of sacrificial land for sludge disposal is rapidly declining, although it still remains popular in non-EU countries such as South Africa (Sayman 2000).

The majority of dewatered and dried sludge is disposed of to selected landfill sites. This is a safe method of disposal and is unlikely to add to the problems already arising from the presence of refuse. It is the single most popular disposal option in all the European countries except for the UK (8%), Portugal (29%), Switzerland (30%), and Spain (35%). Forty-two per cent of all European sewage sludge is disposed of in this way, which is equivalent to 3.07×10^6 tonnes DS per annum (Matthews and Lindner 1996) (Table 8.10). Some European countries are very dependent on landfill for example Greece 90%, Luxembourg 88%, Finland 75% and Sweden 60%. When mixed with refuse, there is no problem with sludge disposal, although if the sludge is disposed on its own to special tips then care must be taken. The sludge cake can rapidly revert back to a semiviscous state and will undergo decomposition, especially during the warmer months, producing very strong odours. Industrial sludge may contain very high concentrations of heavy metals that may be taken up by the vegetation once a tip is covered, therefore, these sites could be unsuitable for grazing livestock. If the sludge

is stored at depth, then it will heat up due to bacterial activity, making it easy for vandals to set it alight, although spontaneous combustion can also occur. If this happens, the smoke and odours are very unpleasant, and such fires have proved extremely difficult to extinguish.

Use of sludge in land reclamation

The aim of land reclamation is to restore vegetation to areas without an established soil cover. Such areas include colliery waste, shale tips, mine waste, china clay waste tips, sand dunes, sides of embankments and cuttings, pits filled with overburden from open cast mining, and refuse covered with a layer of soil; in fact, any land that has suffered extensive disturbance so that the original soil structure has been destroyed. Most of these materials are deficient in soil nutrients and, more importantly, organic matter which results in a high soil bulk density (Sopper 1993). This means that the soil has a poor structure and lacks water holding capacity (Khaleel *et al.* 1981). The restoration procedure requires deep cultivation and mole drainage followed by applications of lime and fertiliser. Grass is then grown for several years to allow the turf to provide enough organic matter for a top soil to be eventually established (Bradshaw and Chadwick 1980). However, this takes a very long time, the resultant soil is unstable and is easily damaged, and the nutrients and lime are easily leached, making their restorative effects short-term. Sludge is particularly valuable for land reclamation as the concentrations of plant nutrients and organic matter exactly complement the deficiencies of derelict soils. Also, the nature of the nitrogen and phosphorus, which are bound up with the organic matter, ensures that there is a slow release of nutrients over several years. In this way remedial treatment will have a permanent effect. By the time the nitrogen release has been severely reduced, the topsoil will already be established and natural clovers will be supplying nitrogen. The use of sludge in reclaiming derelict sites is almost totally restricted to dewatered sludge cake, due to the high proportion of dry solids, especially those which have been conditioned using lime or a mixture of lime and copperas. It has been used successfully to restore a variety of sites including colliery waste (Williams 1975; Rrooker and Farnell 1979; Toffey 1996), mine tailings (Johnson *et al.* 1976), chromate smelter waste (Gemmell 1974), and motorway verges and banks (Matthews 1980). Coker *et al.* (1982) have shown that a single application of dewatered sludge can permanently restore fertility to derelict land at an application rate of 100 tonnes DS ha^{-1}. This is due mainly to the reduction in bulk density by the organic matter in the sludge that encourages root development.

Lime-treated sludge helps to correct the low pH of acid soils, although very acidic substrata, such as colliery shale, will still require extra lime. The level of heavy metals in a single application of sludge (100 tonnes DS ha^{-1}) will not cause any contamination of the soil. However, slightly elevated but quite safe concentrations will be recorded in the herbage in the first year but will decline over subsequent years. Municipal sewage sludge has been successfully used to stabilise Pb, Zn, and Cd in a soil heavily contaminated by mining activities in Sardinia, Italy. Optimum application rates were 10% w/w sewage sludge addition (Xenidis *et al.* 2001).

In 1975, only 7% of the sewage sludge produced in the UK (equivalent to 60,000 tonnes DS per annum) was used for land reclamation. However, it is estimated that some 33,000 ha^{-1} of land in the UK would justify restoration, and at the same time provide a potential outlet of 3.3×10^6 tonnes of sludge at the application rate of 100 tonnes DS ha^{-1}. Of the 70×10^6 tonnes of refuse produced in the UK each year, about 80% is buried in the ground. The conventional restoration method used for refuse tips involves covering them with clay and topsoil. Sewage sludge has been shown to be an excellent replacement for topsoil, which is becoming increasingly scarce and expensive, and has been extensively used for restoring such tips back to agricultural use. In 1980, the cost of conventional restoration of a refuse tip was between £3000–£4000 ha^{-1}. Thus, the use of sludge as a partial or complete replacement for top soil will increase (Rennet 1981).

Dosages of sludge for agricultural use are in the range of 5–20 tonnes total solids ha^{-1} compared to up to 100 tonnes total solids ha^{-1} for reclamation and landscaping. High quality growth media can be produced by mixing sludges with soil (Panter and Hawkins 1991). Such material, called black soil, was widely used during the 1980's to establish green belts in London (Sopper 1993). Such soils can not be used immediately on mixing and must be left to mature and stabilise before it is acceptable for use (Andreasen *et al.* 2000).

8.2.2. *Sludge utilisation to farmland*

The manurial value of sewage has been recognised since the last century when raw sewage was disposed at sewage farms. However, it was during the Second World War that the high cost of artificial fertilisers and the need for increased crop production led to the widespread use of sewage sludge from drying beds on farmland. During the early-1950s, the demand for sludge exceeded the output from drying beds and road tankers were used to transport and spread liquid sludge. Since then, land disposal has

become a major disposal method of sewage sludge, with many plants relying totally on the utilisation of sludge by farmers as cheap manure as their sole method of sludge disposal. Most European countries dispose a significant proportion of their sewage sludge to land, with the majority of sludge in Denmark (54%), France (58%), Norway (58%) and Spain (50%) utilised in this way. In most other European countries, including the UK, land disposal is a major disposal method, accounting for 45% of sludge output. Only in Ireland and Greece, where sludge output is low, is only 10% of sludge used for agricultural purposes (Table 8.10). However, the utilisation of sewage sludge on agricultural land is expected to increase in all European countries, although the concern about pathogens and trace organic contaminants has reduced its use in some countries.

In spite of the costs of processing and transport, disposal of sewage sludge on to agricultural land is an extremely economical method of disposal as well as providing the farmer with low-cost fertiliser. It contains plant nutrients, trace elements, and organic matter that have the potential for improving soil structure, crop yields, and the grazing value of the land. Unfortunately, sewage sludges also contain heavy metals, trace organics, and pathogenic organisms. Furthermore, the toxic metal content of some sludges makes them unsuitable for agricultural use and even

Table 8.15. Major metal and nutrient content of 13 Irish sludges (Murphy *et al.* 1978).

Treatment plant	(mg kg^{-1}DS)							DS (%)		
	Type	DS (%)	Mn	Pb	Cu	Zn	Cd	N	P	K
Kildare	D	16.4	550	350	3,129	14,850	3.0	1.90	0.82	0.54
Mitchelstown	D	13.4	206	80	140	862	2.7	7.32	1.82	0.58
Macroom	EA	10.3	858	349	802	982	6.2	5.10	1.59	1.19
Newbridge	D	17.8	119	92	236	1,100	3.3	2.78	0.52	0.22
Athy	D	17.2	131	486	455	8,552	3.5	2.87	0.47	0.20
Mullingar	EA	8.1	232	345	774	475	4.2	7.15	1.61	0.50
Kilcoole	EA	6.8	441	250	558	605	2.8	5.28	1.52	0.72
Carrickmacross	EA	10.3	681	260	574	595	2.4	4.78	1.54	0.70
Letterkenny	EA	9.3	559	247	240	620	3.1	5.19	1.33	0.63
Leixlip	EA	2.9	422	178	370	1,325	3.6	3.56	1.51	0.67
Killarney	EA	1.5	471	315	618	562	2.7	4.59	1.08	0.37
Ballincollig	D	15.1	612	175	432	690	4.4	6.78	1.43	1.20
Mountmellick	EA	1.7	540	228	330	440	3.8	3.78	0.45	0.73

EA, Extended aeration — activated sludge; D, anaerobic digestion — digested sludge.

domestic sewage contains heavy metals that become concentrated in the sludge (Table 8.15). Once a harmful concentration of metals has built up in the soil, the damage to subsequent crops can be long lasting or even permanent. The potential toxicological and health hazards of using sewage sludge on agricultural land are reviewed below.

Manurial value of sewage sludge

Approximately 45% of the sewage sludge produced in the UK is utilised on agricultural land, which is equivalent to approximately 488,000 tonnes DS per annum. The general policy of the UK Water Companies is to expand this form of disposal as it appears to be the most economical and convenient method of sludge disposal for most treatment plants. Disposal to agricultural land is also seen to be the most environmental and sustainable disposal option. Sewage sludge is rich in organic matter and nutrients, especially nitrogen (N), phosphorus (P), and potassium (K), and farmers are able to utilise it as a cheap soil conditioner and fertiliser (Williams 1979). The proportion of organic matter, the average nutrient content of sludges, and the availability of nutrients to crops depends on the type of sludge used and whether it has been dewatered and, more importantly, stabilised. Therefore, the agricultural value of sludge depends very much on the treatment it receives (Table 8.16). There is considerable variation in the fertiliser value of sludge produced at different sewage treatment plants, although individual plants produce a sludge of reasonably consistent quality as the inputs to a particular plant varies little (Table 8.15).

Many of the soluble nutrients in sewage, mainly potassium salts and ammonical nitrogen derived from urine, are lost in the final effluent. However,

Table 8.16. Typical nutrient concentrations of UK sewage sludge (Institute of Water pollution Control 1978).

Element	Type of sludge (% DS)				
	Liquid primary	Liquid secondary**	Liquid, primary and secondary	Liquid digested	Air dried*
Nitrogen	2.1–7.6	3.8–7.6	1.0–6.5	0.9–6.8	1.5–2.5
Phosphorus	0.6–3.0	1.4–3.2	0.6–2.5	0.5–3.0	0.5–1.8
Potassium	0.1–0.7	Trace	0.1–0.7	0.1–0.5	0.1–0.3
Calcium	1.4–2.1	0.5–0.8	up to 2.0	1.5–7.6	1.6–2.5
Magnesium	0.6–0.8	0.5–0.8	up to 0.8	0.3–1.6	0.1–0.5

* Mainly digested sludges.
** Activated sludge.

some of this source of nitrogen is recaptured when it is utilised in the biological treatment process and becomes eventually stored in the sludge. The organic matter and nitrogen in the primary sludge is largely contained in bacteria and excreta, whereas the secondary sludges contain a greater proportion of organic matter, a higher nutrient content, and usually less metals on a dry weight basis than primary sludges. The organic matter and nitrogen is present as bacterial flocs in surplus activated sludge and as the remains of grazers and sloughed biological film in humus sludges from percolating filters. Because the nitrogen is present in non-soluble forms bound up with the organic matter, dewatering has little effect on the nitrogen content of undigested sludges, with only 5–10% of the total nitrogen present in solution as ammonical nitrogen. Only soluble nitrogen, either ammonical nitrogen or nitrate, is available for uptake by plants. All sewage sludges are generally low in potassium as it is mainly soluble, and when sludge is used as a fertiliser, a supplementary source of potash may be required. Although most arable crops and grass will require a potassium supplement, low quantities are desirable for dairy farmers, because a high concentration in herbage can cause hypomagnesaemia in cows. A single application of 100 m^3ha^{-1} of sludge with 5% dry solids content will provide 10–20 kg K ha^{-1}. The phosphorus content of sludges is not significantly affected by treatment because it is generally present in insoluble forms and is retained in the dry solids. The concentration of phosphorus remains constant at between 1.0–1.8% of dry solids, making the ratio of N:P low compared with artificial fertilisers. Sewage sludges are, therefore, phosphorus-rich manures, with 50–60% of the phosphorus readily available as in a super-phosphate fertiliser. Phosphorus as a plant nutrient appears less important than nitrogen as most soils have adequate reserves, and because the extent to which phosphorus is used is limited to the N:P ratio. It is difficult to separate the effect of phosphorus on plant growth, which is generally controlled by nitrogen availability. The introduction of nutrient removal at sewage treatment plants has increased the concentration of phosphorus in sewage sludges to as much as 8%. Farmers are already under increasing pressure to control phosphorus losses from their land to surface waters that can result in eutrophication (Tunney 2000). Although the quantities lost from fertilised agricultural land is only in the order of 1 kg P ha^{-1} y^{-1}, this is usually adequate to increase plant production (e.g. algae and macrophytes) in rivers and lakes where P is often the limiting nutrient. So for many farmers, such high levels of phosphorus may reduce the attractiveness of using sewage sludge on their land. Bossche *et al.* (2000) have examined the movement and loss of phosphorus from soils amended with sewage sludge.

Table 8.17. The effect of digested sewage sludge application on a free-draining loam (Hall and Davis 1983).

Sludge applied tonnes (DS) ha^{-1}	Soil bulk density (g cm^{-3})	Water at field capacity (%)	Fresh yields of lettuce (tonnes ha^{-1})
0	1.13	30.1	4.5
19	1.10	32.2	8.5
38	1.11	32.4	9.6
76	1.09	35.2	12.8
152	0.99	39.4	15.1
250	0.87	43.1	n.d.
500	0.72	47.3	n.d.

Note: n.d. = not determined.

A major problem with modern arable farming is that little organic matter is returned to the soil. Although high yields are possible with low organic matter contents, these soils are difficult to cultivate, suffer water logging, and are particularly susceptible to drought. Organic matter helps to bind the soil particles thus forming aggregates, which create more air spaces so that water and air can penetrate. This reduces bulk density and increases the water holding capacity. Although 50–70% of the dry solids in sludge is organic, the amount disposed on land in liquid sludge is very small, between 1–5 tonnes ha^{-1}, so that improvement in soil quality can only be achieved after many applications. Dewatered sludge allows much higher rates of organic matter to be spread to land and can significantly improve the quality of sandy and free-draining loams (Table 8.17). The ability to increase the water holding capacity of such soils is of particular use in improving drought susceptible soils (Institute of Water Pollution Control 1978; Hall and Davis 1983). Sludge is also a rich source of trace elements. Zinc and copper are both essential elements for crops and animals, but in a survey of grassland used for grazing, 95% of samples were found to be deficient in zinc (< 50 mg kg^{-1} DM) and 81% deficient in copper (< 10 mg kg^{-1} DM). Clearly, a light dressing of sludge will not only supply useful nitrogen, phosphorus, and potassium, but also trace elements to deficient grassland. Secondary sludges are usually mixed with the primary sludge for disposal as surplus activated sludge is not suitable for land disposal because it has a very low dry solids concentration.

A significant proportion of sludge that is utilised on agricultural land is undigested primary and secondary liquid sludges. However, if sludge receives further treatment, such as chemical conditioning or stabilisation,

then its manurial value to the farmer, especially its nitrogen content, is significantly altered. About 50% of the sludge spread on agricultural land receives some form of stabilisation, usually either heated anaerobic digestion at medium to large treatment plants or cold digestion at smaller plants. Very little sludge receives either aerobic digestion or lime stabilisation, although both these methods are becoming increasingly popular (Bruce and Fisher 1984).

Anaerobic digestion removes up to 40% of the organic matter in sewage sludge, converting it to gaseous methane. Therefore, on a dry weight basis, the nitrogen content of digested sludges increases by about 5%, with up to 70% of the total nitrogen biologically transformed to ammonical nitrogen. The sludge is far more stable than undigested sludge, far less offensive and has a dry solids content of 2–4%. Whereas the nitrogen in undigested sludge is slowly released for plant growth over a period of several years as the organic constituents are degraded by soil micro-organisms, digested sludge provides a more instant supply of nitrogen for plants.

Crop response to liquid undigested sludge is related to the total nitrogen in the sludge of which only 10% is in the ammoniacal form. An important factor in the response of plants to fertilisers is the texture of the soil and their ability to retain applied nitrogen. For example, soluble nitrogen is more readily leached from sandy soils than those containing silt or clay. In undigested sludge, the nitrogen is in a form that is not easily leached as it requires further mineralisation. Therefore it is less likely to be leached from soils, thus making it ideal for use on sandy soils. Unlike artificial fertilisers, this type of sludge can be applied at any time over the winter period, with little loss of fertiliser value, as long as surface runoff is controlled. Therefore, the value of sewage sludge as a fertiliser depends more on soil type than on time of application. This has a particular practical advantage for the farmer. Because of the six-month non-grazing rule following the application of undigested sludge to grassland, early winter treatment allows the farmer to graze treated grassland much sooner than if applied in the spring, when a hay or silage crop would have to be taken first. Crops respond differently to undigested sludge application. When it is applied to arable land and ploughed in, the nitrogen is released throughout the year and as mineralisation proceeds faster at warmer soil temperatures nitrogen availability reaches a maximum in summer and autumn. However, unlike grass, cereals absorb most of their nitrogen requirement by the end of June and so use only a small proportion of the available nitrogen from the sludge. Although undigested sludge is about 30–40% as effective as artificial fertilisers on grassland, it is only 15–20% as effective with cereals because of the

short growth periods. Thus, cereal crops are unable to take full advantage of the slow continuous mineralisation of the organic nitrogen in the sludge.

Two problems are associated with liquid sludge utilisation on farmland. If applied at a high rate on grass under dry conditions, a papery mat can be formed, which smothers the grass and inhibits growth. Ideally, rain is required following sludge application in order to help wash solids through the turf onto the soil surface. Nitrogen immobilisation can also occur if the C:N ratio in the soil exceeds 13.5:1, where C is the concentration of readily metabolisable carbon, which results in a poor crop response. However, this is only significant if the soil is very deficient in nitrogen with the effect usually disappearing after 1–2 months (Hall and Davis 1983).

Stabilisation of undigested sludge with hydrated lime (pH > 11) inhibits bacterial action and its subsequent decomposition in the soil. Therefore, mineralisation in the soil will be delayed until the soil pH has fallen to 8.5, which may take several weeks. Although liming reduces the nitrogen value to about half that of unlimed sludge, it has great potential for use on acid soils. The treated sludge contains about 20% (dry solids) of lime and an application of 100 $m^3 ha^{-1}$ of lime-stabilised sludge will provide in excess of 800 kg CaO ha^{-1}, and trials by the Water Research Centre have shown that long-term application will increase soil pH (Table 8.18). A pot and field study, using lime treated sewage sludge, is described by Akrivos *et al.* (2000). Plant yields increased significantly with sludge addition with no significant increase in the metal content in plant tissue.

Many rural areas in Britain and especially those in Ireland are not on main sewerage and still rely on septic tanks, and appreciable quantities of septic tank sludge needs to be disposed. In the South-West Water area in England, 10% of their sludge comes from this source. The dry solids of these sludges are variable, with mean nitrogen, phosphorus, and potassium concentrations in the order of 4.2%, 0.8%, and 0.7% dry solids respectively. Septic tank sludges have a higher ammoniacal nitrogen content at 18% of

Table 8.18. Effect of spreading lime and undigested sludge on the pH of soil (Hall and Davis 1983).

Rate (m^3 ha^{-1})	Total limed applied (tonnes CaO ha^{-1})	Soil pH after 2 years	Increase in pH
0	0	5.8	
70	1.16	5.9	+0.1
140	2.32	6.0	+0.2
280	4.64	6.3	+0.5

total nitrogen, and a higher potassium content than undigested primary sludges (3.5% N, 1.3% P, and 0.2% K). It can be used on grassland, although such sludges can produce very unpleasant odours (Carlton-Smith and Coker 1982).

Surplus activated sludge contains between 1.5–2.5% of dry solids, with 4–7% of nitrogen, mainly present as bacterial floc. The ammoniacal nitrogen concentration is very low, much lower than in primary undigested sludges, with the C:N ratio closer than for other sludge types. When applied to land, it decomposes very rapidly, resulting in odours, with up to 50% of the total nitrogen becoming available within the first 12 months. Because of its lower dry solids content and the difficulty in dewatering such sludges, it is generally mixed with primary sludge before treatment and/or disposal.

Liquid digested sludge contains more nitrogen on a dry solids basis than any other type of sewage sludge, with the major portion, up to 85% of total nitrogen, readily available for plant growth (Williams 1979). Little metabolisable carbon is left in sludge after digestion and the remaining organically bound nitrogen is mineralised much more slowly in the soil than undigested sludge, with 15% of the nitrogen being released in the first year, although the remainder may take many years to become available for plant growth. Nitrogen in sludge is not prone to leaching as it needs to be nitrified to nitrate before loss occurs. However, volatilisation of ammonia can be high, resulting in a significant loss of nitrogen if sludge is applied to bare, dry soil on hot windy days. This can be avoided if sludge is applied to land with a moist soil, a full crop cover or if cultivated immediately after treatment. If the dry solids content of sludge is high then it sticks to the blades of grass, which increases volatilisation. Therefore, it is best to apply thick sludges on wet days, whereas thin sludges can penetrate the turf mat areas on dry days. The nitrogen availability of liquid digested sludges can be estimated by:

$$\text{(Ammoniacal nitrogen)} + 15\% \text{ (organic nitrogen)}$$

The fertiliser value of liquid digested sludge can be estimated by multiplying the nitrogen availability by a factor F which is the proportion of ammoniacal nitrogen lost:

$$F(\text{NH}_4\text{–N}) + 15\% \text{ (organic nitrogen)}$$

When the formulae is applied to grassland in the British Isles, $F = 1.0$, but if applied under adverse conditions then F can be 0.5 or less (Coker

1982). Fertiliser efficiency of liquid digested sludge can be 35–100% that of artificial fertiliser, as measured on a ammoniacal nitrogen basis, and is related to the proportion of ammoniacal nitrogen to total nitrogen which rises as the percentage of ammoniacal nitrogen increases. Applications of digested liquid sludge are most effective for both grass and cereals if a applied during January and February, being 70–100% as effective as spring applied fertiliser on an ammoniacal nitrogen basis. Details of the amounts of available nutrients supplied at various rates of sludge application are given elsewhere (Institute of Water Pollution Control 1978).

Lagoon storage of digested sludge results in a more stabilised sludge with a lower proportion of ammoniacal nitrogen to total nitrogen, at 25%, although the total nitrogen increases with the dry solids concentration. The annual mineralisation rate of organic nitrogen for this type of sludge is about half that for liquid sludge, at 9%.

The proportion of sludge cake being spread on land is declining with liquid sludge being far more economical to use as it can be pumped, does not require dewatering, has a reduced volume of dry solids, and is easier to apply. However, 37% of all mechanically dewatered sludge and 21% of cake from drying beds are used on agricultural land annually in Britain. The dry solids content of sludge cake is between 20–35%, although most of the ammoniacal nitrogen has been lost, therefore the main value of cake to the farmer is as a bulk organic manure or as a slow release nitrogenous fertiliser. Sludge cake contains significantly less nitrogen than non-dewatered sludges and the release of nitrogen from sludge cake due to mineralisation of the organic nitrogen component in the soil decreases by about 50% each year. It is difficult to spread evenly, although spreading qualities can be improved by storage for several months before use to allow further stabilisation and mineralisation. This produces a more friable material with an improved nitrogen value. Sludge that has been chemically conditioned with lime and copperas can be of benefit to the farmer as it can increase the soil pH, but the volume of dry solids is increased by 20% due to the addition of chemicals. Aluminium chlorohydrate is still used, although polyelectrolytes are rapidly taking over.

Sludge cake from drying beds is more expensive to produce than mechanically dewatered sludge because of the large areas of drying beds required. The final cake is much drier and harder than the other types, with a dry solids content of between 50–70%. The sludge is much easier to handle due to its low moisture content and is used mainly as a soil conditioner for very poor soils.

Contamination by heavy metals

Metals originate from both domestic and industrial sources, with primary and secondary sludges containing between 44–96% of the total metal load of crude sewage (Davis 1980). All sewage sludges contain metals to a greater or lesser extent and certainly in higher concentrations than found in soil. Therefore, when sludge is disposed to land, heavy metals will accumulate in the soil.

The effects of heavy metal accumulation can be long lasting or even permanent with phytotoxic effects being the major problem, although there is a danger that metals may be introduced via domestic livestock or directly into the human food chain. For man, the main source of these elements is in meat and plants, with recommended maximum daily intake of the common sewage-associated metals of 30 μg d^{-1} for Cd, 170 μg d^{-1} for Pb, 5,000 μg d^{-1} for Cu, and 17,000 μg d^{-1} for Zn (WHO 1971, 1984). The quantity of metals found in sludge depends on the source of wastewater, although Zn, Cu, Ni, and Pb are present in relatively large concentrations even in domestic sewage. The most toxic metal in sewage is probably cadmium. In a survey of UK sludges, a mean value of 7.15 mg Cd kg^{-1}DS was obtained for domestic sewage with no industrial inputs, whereas levels of > 10 mg Cd kg^{-1} DS indicated industrial inputs. Even very small concentrations in crude sewage can result in high concentrations in the sludge. For example, cadmium is normally present in crude sewage at concentrations between 0.008–0.01 mg Cd l^{-1}. Assuming an 85% removal efficiency of the metal during treatment and that 350 mg of primary and secondary sludge solids are generated per litre of sewage treated, then the sludge will contain 20 mg Cd kg^{-1}DS (Hall and Davis 1983). The normal range of metals found in agricultural soils is compared with the levels found in sewage sludge in Table 8.19, although a more comprehensive table is given by Davis (1980).

There is a general lack of agreement as to what constitutes acceptable levels of metals in soils for plant growth, and at what levels accumulated metals in plants present a danger either to man or animals. This is complicated by the fact that availability of metals to plants varies with soil and plant type, the element concerned, and the environmental factors that affect plant growth. Leeper (1978) has reviewed the effect of soil properties on plant uptake of heavy metals, with pH and cation-exchange capacity the most important factors. Some elements, such as cadmium and zinc are rapidly absorbed and translocated to aerial plant pests, whereas others, e.g. lead, are largely unavailable. If the organic content and pH of the

soil are high, then the availability of most metals is reduced. As most of the contaminants from sludge are adsorbed to soil or organic matter, or form relatively insoluble precipitates at pH values normally associated with agricultural soils, heavy metals appear to be largely immobile and are not readily leached from the soil into the groundwater (Edworthy *et al.* 1978). Therefore, it would appear that lime-treated sludges help to reduce the availability of metals; however, care must be taken at high pH levels as molybdenum becomes more available and is accumulated by crops.

Table 8.19. The range (R) and common values (CV) of the major elemental contaminants in sewage sludge and soil (mg kg^{-1} DW) (Davis 1980).

	Sludge		Soil	
Contaminant	R	CV	R	CV
Ag	5–200	25	1–3	1
As	3–30	20	1–50	6
B	15–1,000	30	2–100	10
Be	1–30	—	0.1–40[a]	3[a]
Cd	2–1,500	20	0.01–2.4	1
Co	2–260	15	1–40	10
Cr	40–14,000	400	5–1,000	100
Cu	200–8,000	650	2–100	20
F	60–40,000	250	30–300	150
Hg	0.2–18	5	0.01–0.3	0.03
Mo	1–40	6	0.2–5.0	2
Ni	20–5,300	100	10–1,000	50
Pb	50–3,600	400	2–200	20
Sb	3–50	12	2–10[a]	—
Se	1–10	3	0.01–2	0.2
Sn	40–700	100	2–200	10
Tl	—	1[a]	—	0.1[a]
V	—	15[a]	—	100
W	1–100	20	—	1[a]
Zn	600–20,000	1,500	10–300	50
Dieldrin	< 0.03–300[a]	0.4[a]	—	—
PCB (Aroclor 1254)[b]	< 0.01–20[a]	3[a]	—	—

[a] Tentative data.

[b] A WRc survey of 11 UK sludges found PCB (Aroclor 1260) concentrations of 0.06–2.4 mg kg^{-1} dry solids.

Although high molybdenum levels are not toxic to plants it can result in copper deficiency symptoms in livestock. Lime-treated sludge can also cause trace element deficiency in plants under certain soil conditions. A single application of sludge with the common values (CV) shown in Table 8.19 at a rate of 5 tonnes ha^{-1} and cultivated into the top 20 cm of soil (density 1.0 g ml^{-1}) will produce very small percentage increases in soil metal concentrations, except for zinc and copper (Table 8.20). The concentration of metals at which toxic effects in soils and plants are recorded are given in Table 8.21. Davis and Beckett (1978) suggest that as the metal concentration as measured in soil is so variable, because it depends on so many environmental factors, the metal accumulated in plant tissue is a more convenient and accurate indicator of metal levels in soils and that phytotoxic levels have been reached. They observed that critical concentrations of metals in the

Table 8.20. Effect on soil concentration of potentially toxic metals of a single application (5 tonnes ha^{-1} of sewage sludge (Hall and Davis 1983).

Element	Concentration (mg kg^{-1})		Increase in soil concentration following dressing of sludge	
	Sludge	Soil	mg kg^{-1}	%
Cd	20	1	0.05	5
Pb	400	50	1	2
Cu	600	20	1.5	5.5
Ni	100	50	0.25	0.5
Zn	1,500	50	3.75	7.5

Table 8.21. Normal and toxic concentrations of common metals in soil and plants (Dodd 1980).

Element	Concentration in soil (mg l^{-1})		Concentration in plants (mg kg^{-1})	
	Normal	Toxic	Normal	Toxic
Zn	1–50	> 200	20–100	> 200
Cu	0.5–5.0	> 100	5–15	> 25
Ni	1–5	> 25	1–10	> 50
Pb	0.5–5.0	> 200	0.1–2.0	> 10
Cd	0.2–2.0	20–50	0.2–0.5	> 10
Hg	0.05–0.5	> 50	0.02	> 2

tissue of young barley plants occurred at between 18.2–20.3 mg kg^{-1} for Cu (median 19.1 mg kg^{-1}), 10.8–13.0 mg kg^{-1} for Ni (median 11.8 mg kg^{-1}), 124–220 mg kg^{-1} for Zn (median 199 mg kg^{-1}), and 6.0–10.0 mg kg^{-1} for Cd (median 8.0 mg kg^{-1}). Metals may be described as either phytotoxic or zootoxic depending on whether the concentration toxic to plants is lower or higher than the concentration toxic to animals that may eat them. Although uptake efficiency varies between plant species, and even varieties, the effect of contaminants, once in the tissue of the plant or the animal eating it, is relatively constant for most crop plants (Davis 1980;

Table 8.22. Background and upper critical concentrations of elemental contaminants in plant tissue (mg kg^{-1} DW) (Davis 1980).

Contaminant	Background concentrations	Upper critical concentrations	
		Phytotoxic threshold	Zootoxic threshold[a]
Ag	0.06	4	—
As	< 1	20	—
B	30	80	—
Be	< 0.1	0.6	—
Cd	< 0.5	10	—
Co	0.5	6	50
Cr	< 1	10	50
Cu	8	20	30
F	8	> 2,000[b]	30
Hg	0.05	3	1
Mo	1	135	10
Ni	2	11	50
Pb	3	35	15
Sb	< 0.1	—	—
Se	0.2	30	5
Sn	< 0.3	60	—
Tl	< 1[a]	20	—
V	1	2	—
W	0.07	—	—
W	< 0.07[a]	—	—
Zn	40	200	500

[a] Tentative data: it is particularly difficult to assign zootoxic thresholds to Cd, Pb, and Hg.

[b] Applies only to F taken up from soil.

Davis and Carlton-Smith 1984). Maisonnave *et al.* 2001 have studied the mobility of metals from sewage sludge into soils and then plants. Using ryegrass they found that only the roots absorbed large quantities of metals that did not travel to the leaves. Metal uptake by herbage will, they conclude, be minimal if recommended application rates are followed. They also observed that sludge addition reduced water consumption by the grass, which may have important ramifications for irrigation.

The background and upper critical concentrations of metals in plant tissue to give phytotoxic and zootoxic effects are summarised in Table 8.22, although no account has been taken of possible interactions between elements. Metals also reach soil from other sources and although only 2% of the UK agricultural land receives sludge in anyone year, all receive inputs from aerial deposition and phosphate fertilisers. The cadmium level in phosphate fertilisers ranges from 1–160 mg Cd kg^{-1}, depending on the origin of the rock phosphate used (Davis and Coker 1980). Button (1982) estimates that away from areas of localised contamination, about 8 g Cd ha^{-1} are deposited annually, 5 g originating from fertilisers and 3 g from atmospheric deposition. Interestingly, the contribution from sewage sludge application is too small to be included either on a national or regional basis.

The UK Ministry of Agriculture, Fisheries and Food introduced the concept of zinc equivalent to characterise the phytotoxicity of the metals in sludge and to calculate tolerable rates of application. Copper is assumed to be twice as phytotoxic as zinc, and nickel eight times more phytotoxic. As the toxic effect of the three elements is also assumed to be additive, the amount of toxic metal in the sludge can be expressed as a single figure (the zinc equivalent) by adding the metal concentrations in the sludge in the form of:

$$\textit{Zinc equivalent} = \mathrm{Zn} + (2 \times \mathrm{Cu}) + (8 \times \mathrm{Ni}).$$

where there is no previous contamination of the soil it is assumed that an addition of sewage sludge to the soil of 250 mg of zinc equivalent per kg DS of sludge over a 30-year period is quite safe, provided that the soil pH value is close to 6.5. The concept is extremely useful as Zn, Cu, and Ni are common in sewage sludge and are useful indicators of the overall toxicity of the sludge (Chumbley 1971). However, concern that the toxicity of these three metals is synergistic has been expressed, so that the use of zinc equivalents may be greatly under-estimating the amount of metals in sludge that could be safely applied to land at pH $\leq$ 6.5 (Beckett and Davis 1982).

It is now more common to calculate the application rate for all individual metals so that the maximum application rate of sludge can be controlled by the concentration and toxicity of the individual metals present. The application rate is calculated as:

$$(\text{A}-\text{B/C}) \times (1000/\text{D}) \text{ tonnes DS ha}^{-1}\text{yr}^{-1}$$

where A is the recommended limit for the addition of specific metals (kg ha^{-1}) (Table 8.19), B is the concentration of available metal already in the soil (kg ha^{-1} or 2.2 × mg kg^{-1} DS), C is the total concentration of the metal in the sludge (mg kg-1 DS), and D is the application period, which is generally over 30 years. By using the formula, the application rate is calculated for all the major metals present and the lowest application rate indicates which metal is limiting and gives the recommended annual loading (Tables 8.23 and 8.24). Incremental loadings can be in excess of the recommended annual loading as long as the maximum loading in anyone year does not exceed 20% of the total permissible loading, and that the total permissible loading is not exceeded during the 30-year period (National Water Council 1981).

The disposal of sewage sludge to agricultural land is now controlled by an EU Directive (86/278/EEC). Controls have been based on the concentration of metals in soil or sludge. So soil quality controls are in

Table 8.23. Example of elemental application rates for various metal concentrations in sludge and soil (Forster 1985).

	Concentration in sludge (mg kg^{-1})	Concentration in soil (mg kg^{-1})	Elemental application rate (tonnes DS ha^{-1} $year^{-1}$)
Zinc	1,650	40	$\frac{560-(2.2\times 40)}{1650}\times\frac{1000}{30}=9.5$
Cadmium	34	1	$\frac{5-(2.2\times 1)}{34}\times\frac{1000}{30}=2.7$
Lead	150	30	$\frac{1000-(2.2\times 30)}{150}\times\frac{1000}{30}=207.6$
Copper	400	15	$\frac{280-(2.2\times 15)}{400}\times\frac{100}{30}=20.6$
Nickel	30	10	$\frac{70-(2.2\times 10)}{30}\times\frac{1000}{30}=53.3$
Zinc equivalent	2,690	150	$\frac{560-(2.2\times 150)}{2690}\times\frac{1000}{30}=2.9$

Table 8.24. Recommended limits of toxic metals added to the soil from the spreading of sewage sludge used prior to the adoption of the EU Directive on the Agricultural Use of Sewage Sludge (Hudson and Fennel 1980).

Element	Recommended limit in the soil after 30 yr or more (mg kg^{-1})	Normal range in soils (mg kg^{-1}DS)	Reason for control**	Possible source of sludge contamination
Zinc	250*	10–300	P	Food, cosmetics, galvanizing, rayon
Copper (× 2 to give ZnE)	125*	2–100	P (Toxic to sheep)	Pig wastes, cable, and tube manufacture
Nickel (× 8 to give ZnE)	30*	5–500	P	Plating, chemical and steel industries
Chromium	450	5–500	Low toxicity	Plating, tinning, steel industry
Cadmium	2.3	1	P & T	Plating, plastics, electronics
Lead	450	2–200	T	Leaded petrol, metal processing and finishing
Mercury	0.9	0.01–0.3	T	Pharmaceuticals, dye products
Molybdenum	2.3	2	T	Metallurgy, electronics
Arsenic	4.5	0.1–40	T	Pesticides, electronics
Selenium	2.3	0.2–0.5	T	Electronics
Annual limits				
Boron *Pasture*		2–100	P	Detergents, glass
1st year	3			
subsequent years	2.3			
Arable				
1st year	2			
subsequent years	1.6			

* Limits may be increased by a factor up to 2 for permanent grassland. If more than one of these metals is present, use the *Zinc equivalent*.
** P, potentially phytotoxic; T, potentially toxic to animals/humans.

Table 8.25. EC maximum limit values of heavy metals in sludge and soil, and maximum permitted application rates as a 10 year average, based on the EU Sewage Sludge Directive (86/278/EEC).

Parameter	Sludge (mg kg^{-1})	Soil (mg kg^{-1})	Max. application (kg ha^{-1} $year^{-1}$)
Cadmium	40	3	0.15
Copper	1,750	140	12
Nickel	400	75	3
Lead	1,200	300	15
Zinc	4,000	300	30
Mercury	25	1.5	0.1

effect environmental quality standards, while sludge quality controls are in effect heavy metal emission standards. Because of the variable nature of sludge, guidelines based on soil are thought to give a more reliable estimate of heavy metal loadings. The EC Directive incorporates both standards. It defines: (1) limit values (G and I) for metals in soil; (2) limit values (G and I) for metals in sludge; and (3) limit values on application rates (Table 8.25). There are other requirements: (1) sludge must be treated (biological, chemical or heat treatment), otherwise no-grazing limits must be set to protect animal and public health. Also, untreated sludge must be injected or worked into soil; (2) sludge must be analysed every 6–12 months depending on variability; (3) soil must be analysed before and during application; and (4) records must be kept. The suitability of sludges for agricultural use depends on factors other than metal concentrations such as the presence of animal-processing waste (e.g. transmission of tapeworms), contamination by tannery wastes (e.g. if foreign hides are processed then there is a danger of anthrax transmission), and contamination from vegetable processing (e.g. cyst eelworm transfer to agricultural land).

Rates of addition can be calculated using simple formulae, as can the calculation of the amount of sludge required to satisfy the N and P requirement of plants or crops.

(i) The capacity C (mg l^{-1}) of a given soil to receive a potentially toxic compound is calculated as:

$$C = \text{EU guide limit (mg } l^{-1}\text{)} - \text{initial soil concentration (mg } l^{-1}\text{)}$$

(ii) The permissible load L (kg ha^{-1}) of a potentially toxic compound is calculated as:

$$L = (C \times 2500)/1000$$

where one hectare at a plough depth of 25 cm has a volume of 2,500 m^3.

(iii) The permissible rate of addition, R, in tonnes of dry solids (tonnes DS) ha^{-1} y^{-1} is calculated below using a 10 year application period:

$$R\,(\text{tonnes DS ha}^{-1}\text{y}^{-1}) = (L \times 1000)/(\text{Sludge quality } Q\,(\text{mg kg}^{-1}) \times 10)$$

(iv) In order to calculate the weight of sludge dry solids that must be applied to satisfy crop requirements for N, P (kg ha^{-1}y^{-1}), then:

$$P = 0.51 \times U$$

where a crop requires a certain number of units of nitrogen per hectare per annum (U) which is converted to the crop

The application rate T (tonnes DS ha^{-1}y^{-1}) is calculated as:

$$T = (P \times 1000)/\text{available N in sludge (N mg kg}^{-1})$$

(v) If the concentration of a potentially toxic compound is high, then T will be greater than R and, to avoid contamination of the soil, the application rate determined by R will have to be used and the crop requirement for N will not be satisfied. To meet the crop requirement the concentration of the toxic compound must be reduced in the sludge so that sludge quality Q (mg kg^{-1}) is now:

$$Q = (L \times 1000)/(T \times 10)$$

The Sewage Sludge Directive is currently under review with new limits being consideration (Spinosa 2001). These include revised sludge metal limits for cadmium and mercury as well as short term and long term reduction targets for all listed metals (Table 8.26). It is also proposed that metal concentrations be linked to phosphorus. Other changes proposed are: (1) review of metal standards every six years; (2) limits for key organic compounds (e.g. the proposed EU dioxin standard in sewage sludge is 100 ng TEQ kg^{-1}) (Table 8.27). The dioxin levels in 14 British sludges had a mean concentration of 65 and a maximum of 225 ng TEQ kg^{-1} where TEQ kg^{-1} is the toxic equivalent of the most toxic dioxin 2,3,7,8-TCDD per kg dry matter. The US EPA proposed new dioxin limits in sewage sludge in December 1999 of 300 ng TEQ kg^{-1}; (3) specifies a three tier system of

Table 8.26. Existing G and I values of heavy metals in sewage sludge for use on agricultural land within the EU, proposed limit values and proposed limit values based on phosphorus loading (Spinosa 2001).

Metal	Limit values (mg/kg-dry matter)		Limit values (mg/kg P)
	Directive 86/278	Proposed	Proposed
Cd	20–40	10	250
Cr	—	1,000	25,000
Cu	1,000–1,750	1,000	25,000
Hg	16–25	10	250
Ni	300–400	300	7,500
Pb	750–1,200	750	18,750
Zn	2,500–4,000	2,500	62,500

Note: The sludge producer may choose to observe either the dry matter related or the phosphorus related limit values.

Table 8.27. Proposed limit values of organic compounds in the revised EU Sewage Sludge Directive (Spinosa 2001).

Compound	Limit values (mg/kg-dry matter)
AOX (sum of halogenated organic compounds)	500
LAS (linear alkylbenzene sulphonates)	2,600
DEHP (di(2-ethylhexyl)phthalate)	100
NPE (nonylphenol and nonylphenolethoxylates with 1 or 2 ethoxy groups)	50
PAH (sum of various polycyclic aromatic hydrocarbons)	6
PCB (sum of some polychlorinated biphenyls)	0.8
PCDD/F (polychlorinated dibenzodiox./dibenzofur.)	(ng-TE/kg-dry matter) 100

Table 8.28. Characteristics of liquid sewage sludge from daily analysis at three sewage treatment plants serving Cairo, Egypt, during 1996 (Hall and Smith 1997).

Determinand	EU Directive 86/278/EEC	US EPA Rule 503	Abu Rawash[1]			Berka[1]			Helwan[1]		
			Mean	Median	90%ile	Mean	Mean	90%ile	Mean	Median	90%ile
(*a*) *Chemical analysis* (mg kg^{-1})											
Zinc	2,500–4,000	2,800–7,500	1,862	1,912	3,276	2,030	1,880	3,614	3,142	3,125	5,340
Copper	1,000–1,750	1,500–4,300	240	234	404	603	371	669	821	940	1508
Nickel	300–400	420	107	26	250	645	675	1,340	44	44	63
Cadmium	20–40	39–85	25	7	18	13	6.7	11.9	10.6	11.3	17.5
Lead	1,000–1,500	300–840	224	140	518	384	286	787	108	88	200
Mercury	16–25	17–57	5[2]	—	—	—	—	—	—	—	—
Chromium	no standard	1,200–3,000	174	168	288	215	97	435	402	360	605
Nitrogen	—	—	3.7	3.3	6.6	1.8	1.3	3.0	2.1	2.0	3.6
Phosphorus	—	—	0.4	0.4	0.7	0.2	0.2	0.4	0.1	0.1	0.2
Potassium	—	—	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2	0.7
(*b*) *Microbial analysis*			Percent of samples containing			Percent of samples containing			Percent of samples containing		
Parasite eggs		$<$ 1 per 4 g DS[3]	none	1–25	26–50	none	1–25	26–50	none	1–25	26–50
Eimeria sp.			43	39	9	64	18	18	50	30	20
Ascaris lumbricoides			86	14	0	93	7	0	—	—	—
Trichostrongylus sp.			84	14	2	86	14	0	83	17	0
Fasciola sp.			89	17	0	82	11	7	90	10	0

[1] n = 44, 28 and 20 for Abu Rawash, Berka and Helwan, respectively
[2] Limited data available from 1995 only
[3] Standard set for domestic garden use

treatment standards ranging from stabilisation to sterilisation; (4) sampling and analysis are only to be carried out by certified laboratories; (5) sludges from the food sector are to be included; (6) septic tank and cesspool wastes will no longer be permitted to be spread on land but will have to be disposed of at a treatment plant.

Fears have been expressed that the implementation of a single set of standards for all EU countries, as with the bathing water directive, will be extremely difficult because there is such a wide range of sewage treatment, and farming practices, climate, and soil conditions within the community. However, most countries have produced *Codes of Practice for Agricultural Use of Sewage Sludge*. The "Safe Sludge Matrix" is the new set of regulations used in the UK (ADAS 2002) (Sec. 11.3.3).

The term potentially toxic elements (PTE's), which are mainly heavy metals, have been widely adopted in describing the contaminants of sewage sludge. An example of using this approach in the characterisation of sludge has been done by Hall (1997) who has examined the sludge disposal strategy for the City of Cairo in Egypt (Table 8.28). Potentially toxic elements in sewage sludge are reviewed by Smith (1998). The determination of suitable land, risk assessment and water pollution risk is discussed by Towers and Horne (1997).

In the US, sludges are classified using pathogen reduction criteria. Rule 503 of the USEPA standards for the use and disposal of sewage sludge specifies pathogen limits and acceptable treatment procedures. Class A sludges have a highly reduced pathogen transfer risk due to thermal drying or lime stabilisation largely eliminating all pathogens. In contrast, Class B sludges are untreated and so have a high pathogen transfer risk. Class A have unrestricted use (including use on gardens and lawns) and have led to the widespread adoption of Class A process technologies, while Class B sludges are subject to permitting and extensive regulation (Water Environment Federation 1993; Lang *et al.* 1996).

Outside the EU and US, most countries have set their own guidelines. For example, in South Africa sewage sludges are classified into four groups according to their source and treatment (Table 8.29) (Water Research Commission 1997; Snyman *et al.* 2000; Du Preez 2000). Type D sludges are unrestricted in their use and are primarily recycled to agriculture. Metal limits in sewage sludge have been reduced since 1991 based on risk factor calculations to minimise water pollution (Table 8.30) (DWAF 1998). Typical characteristics for South African sludges are summarised in Table 8.31 (Smith and Vasilondis 1989).

Table 8.29. Classification of South African sewage sludges (Synman *et al.* 2000).

Type of sewage sludge	Origin/treatment (examples)	Characteristics-quality of sewage sludge
Type A sludge	Raw sludge Cold digested sludge Septic tank sludge Oxidation pond sludge	• Usually unstable and can cause odour nuisances and fly-breeding • Contains pathogenic organisms • Variable metal and inorganic content
Type B sludge	Anaerobic digested sludge (heated digester) Surplus activated sludge Humus tank sludge	• Fully or partially stabilised — should not cause significant odour nuisance or fly-breeding • Contains pathogenic organisms • Variable metal and inorganic content
Type C sludge	Pasteurised sludge Heat-treated sludge Lime-stabilised sludge Composted sludge Irradiated sludge	• Certified to comply with the following quality requirements (If not certified this sludge is considered a Type B sludge): — Stabilised — should not cause odour nuisances or fly-breeding — Contains no viable *Ascaris* ova per 10g dry sludge — Maximum 0 *Salmonella* organisms per 10g dry sludge — Maximum 1000 Faecal coliform per 10g dry sludge, immediately after treatment (disinfection/sterilisation) • Variable metal and inorganic content
Type D sludge A sludge product produced for unrestricted use on land with or without addition of plant nutrients or other materials	Pasteurised sludge Heat-treated sludge Lime-stabilised sludge Composted sludge Irradiated sludge	• Certified to comply with the following quality regiments: — Stabilised — should not cause odour nuisances or fly-breeding — Contains no viable *Ascaris* ova per 10g dry sludge — Maximum 0 *Salmonella* organisms per 10g dry sludge — Maximum 1000 Faecal coliform per 10g dry sludge, immediately after treatment (disinfection/sterilisation) • Maximum metal and inorganic content in mg/kg dry sludge (Table 8.30) • User must be informed about the moisture and N P K content. • User must be warned that not more than 8 t/ha.yr (dry sludge) may be applied to soil and that the pH of the soil should preferably be higher than 6.5.

Table 8.30. Changes in metal content of sewage sludge aimed for unrestricted use (Type D) (Table 8.29) in 1991 and 1997 South African Sludge Guidelines (Synman *et al.* 2000).

Metal	1991 Limit (mg/kg dry sludge)	1997 Limit (mg/kg dry sludge)
Cd	20	15.7
Co	100	100
Cr	1,750	1,750
Cu	750	50.5
Hg	10	10
Mo	25	25
Ni	200	200
Pb	400	50.5
Zn	2,750	353.5
As	15	15
Se	15	15
B	80	80
F	400	400

Table 8.31. Metal and nutrient characteristics of South African sewage sludge based on data form 77 different wastewater treatment plants (Smith and Vasiloudis 1989).

Parameter	Concentration ($mg/kg_{dry\ sludge}$)	Standard deviation ($mg/kg_{dry\ sludge}$)
Total Kjeldahl nitrogen	31,070	9,780
Total phosphorus	15,570	7,960
Potassium	2,550	1,990
Cadmium	13	25
Chromium	551	1,206
Copper	655	1,945
Lead	455	1,151
Nickel	155	342
Zinc	2,054	2,176
Mercury	5	4
Arsenic	7	5
Selenium	3	12
Molybdenum	7	4
Boron	30	14
Fluoride	128	152

Contamination by pathogens

Pathogens are common in sewage and are concentrated in the sludge. Although there is a range of animal and plant pathogens that can be transmitted via sewage sludge utilisation on farmland, two are of particular significance: *Salmonella* bacteria and the beef tapeworm *Taenia saginata*. However, the normal level of contamination by these pathogens in sewage sludge poses little risk of infection to livestock (Argent *et al.* 1977). The health hazards associated with land disposal of sewage sludge are fully reviewed in Sec. 9.4.2. In February 1998, the House of Commons Select Committee on Environment, Transport, and Regional Affairs (UK) issued a report on Sewage Treatment and Disposal, recommending that by the year 2022 all sludges that are recycled to land should be subjected to stabilisation and pasturisation (CIWEM 1999b) in order to reduce or eliminate pathogens (Bujoczek *et al.* 2001) (Sec. 11.3.3).

Application of sludge to land

The production of sewage sludge at a treatment plant is constant and continuous, whereas the requirement for sludge by farmers will fluctuate and is seasonal. It is important, therefore, that provision is made for the storage of sludge either at the treatment plant or at farms. However, other factors, such as bad weather, mechanical breakdown, industrial action, restricted movement into farms due to disease, will all slow up delivery and make storage at the treatment plant inevitable. Storage of dewatered sludge in the form of cake is relatively straightforward as it can be stacked until required. Liquid sludge must be kept in special tanks, lagoons or old drying beds. Storage can be advantageous as the separated liquor can be decanted before final transportation from the site, thereby increasing the dry solids content and reducing the volume of sludge to be transported. But care must be taken to ensure that valuable nutrients are not lost in the liquor, thus reducing its value as a fertiliser. This is less likely with undigested sludge as most of the nutrients are present in an insoluble form. However, after digestion, much of the remaining nutrients are soluble and will be lost if the liquor is removed.

The procedures adopted by the UK water companies for the disposal of sludge to agriculture land are generally similar. The sludge is usually supplied and delivered free in most areas to the farmer if within a reasonable distance, although farmers often collect the sludge themselves using their own equipment. Transportation charges depend on the demand for sludge because in some areas suitable land is at a premium. Although most sludge

is still spread by the Water Companies themselves, there is a growing trend to employ contractors to dispose of the sludge, and in some areas small firms have sprung up, marketing the sludge as vigorously as the major fertiliser companies. The disposal of sludge follows the Code of Practice for Agricultural use of Sewage Sludge produced by the Department of the Environment, Transport, and the Regions, although many of the water companies have their own guidelines, and contractors are monitored to ensure that they are followed. The guidelines are fully explained in the sections on contamination by heavy metals (Sec. 8.2) and pathogens (Sec. 9.4.2). Land and sludge are analysed to calculate annual application rates over a 10–30 year period and details are generally supplied to the farmer. Water companies are required to keep detailed records of where the sludge is spread, monitor the soil and sludge, and reassess the application rates on a regular basis. In order to prevent the transmission of pathogenic organisms present in the sludge to livestock, a minimum of 90 days should elapse between the application of unstabilised sludge to grassland and the return of livestock. However, this can be reduced to 21 days in the case of stabilised sludge. The Directive does not permit the use of sewage sludge to private gardeners or for use in horticulture (Sec. 11.3.3).

Application of sludge to land inevitably involves transportation costs that can be reduced considerably if the sludge is thickened or dewatered to form cake. However, three options are available for transporting liquid sludge, the selection of which depends on the location of the treatment plant in relation to the farmland requiring the sludge. The least common is the use of temporary or permanent pipelines that transport sludge directly from the plant to the farmland. This system is only suitable for small- to medium-sized treatment plants that are located in rural areas and are surrounded by suitable farmland. A network of pipes are laid and the sludge pumped to fields when and where required. The disadvantage of this system is that a network of pipes is very restrictive, which can result in over-application of sludge. Tractor-hauled trailer tanks are generally employed at small- to medium-sized treatment plants where only small volumes of sludge are produced or sludge production is intermittent. These units are limited to short distances and the tanker capacity restricted to between 3.5–5.5 m^3 (normally 3.6 m^3). Road tankers (4.0–22.5 m^3) are used for transporting larger volumes over longer distances. Four-wheel drive tankers (4.0–5.5 m^3) are the most economical as they can also be used for spreading the sludge, whereas over distances of 15 km, larger articulated tankers (> 20 m^3) are used, with the sludge transferred to storage tanks on the farm or smaller units for spreading. The farmer requires the sludge spread

Table 8.32. Summary of the recommendations by the UK regarding potentially toxic elements and the use of sewage sludge on used prior to the adoption of the EU Directive on the Agricultural Use of Sewage Sludge (National Water Council 1981).

Element	Maximum permissible addition (kg ha^{-1})	Provisional maximum soil concentration (mg l^{-1})
Cd	5	3.5
Cu[a]	280	140[b]
Ni[a]	70	35[b]
Zn[a]	560	280[b]
Pb	1,000	550
Hg	2	1
Cr	1,000	600
Mo	4	4
As	10	10
Se	5	3
B	3.5	3.25[a]
F	600	500

[a] These elements are assumed to have additive toxic effectsin the ratio Zn + 2(Cu) + 8(Ni) = *zinc equivalent*. The limits for the *zinc equivalent* are as for zinc.

[b] Extractable.

evenly over the soil, without damaging the soil structure, causing pollution or having unpleasant odours produced. The control of these factors is dependent on the method of application selected, although essentially they have four principle objectives: (1) to prevent unacceptable contamination of agricultural land with potentially toxic elements (Table 8.32); (2) to prevent dissemination of human, animal, and plant diseases (Table 8.33); (3) to avoid public nuisance; and (4) to avoid water pollution.

There are three widely used methods of applying sewage sludge to land; surface spreading; irrigation; and injection. Sludge cake is transported by lorry and is then distributed on to the land directly by conventional tractor-towed manure spreaders. Liquid sludge or slurry is spread on to land either from slurry tanks with heavy-duty flotation-type tyres enabling them to operate even in wet weather, without damaging the soil, or from small road tankers that can carry up to 2 m^3 more sludge than the tractor-hauled slurry tanks. The small road tankers also have wide tyres to minimise vehicle pressure, reducing damage to soil and improving tractability. Due to the weight of these vehicles, they cannot be used during wet conditions

Table 8.33. Recommendations on the application of sewage sludge to agricultural land to avoid potential pathogen problems used prior to the adoption of the EU Directive on the Agricultural Use of Sewage Sludge (National Water Council 1981).

Sludge	General arable including forestry, land reclamation, conservation, crops	Seed potatoes and export nursery stock	Grazed crops	Salad and crops consumed raw	Park flower beds	Orchards, turf
1. Liquid raw	✓	×	C	×	×	×
2. Liquid raw stored 2 weeks	✓	×	C	×	×	D
3. Cold anaerobic digested	✓	×	AB	E	✓	✓
4. Lagooned	✓	×	AB	E	✓	✓
5. Mesophilic anaerobic digested	✓	✓	AB	E	✓	✓
6. Heat processed	✓	✓	AB	E	✓	✓
7. Non-limed cake	✓	×	C	×	×	D
8. Non-limed cake stored	✓	×	AB	E	✓	✓
9. Limed, cake or stabilised	✓	×	C	E	✓	✓
10. Full treatment biological (inc. aerobic digested and extended aeration of whole sewage)	✓	×	C	E	✓	D
11. Partial treatment biological	✓	×	C	×	×	D

✓ Acceptable. × Unacceptable.

A 3 weeks grazing interval, but 5 weeks for cattle which produce milk which is consumed unpasteurized.

B Where 40% organic matter destroyed in digestion or storage period less than that recommended, the grazing interval should be increased accordingly from 3 weeks to 6 months for pigs or cattle.

C Grazing interval for pigs, cattle, 6 months, other animals as A.

D No fruit or turf harvested for 3 months after application.

E None to be sown until 12 months after application.

as the soil structure can be damaged or the tanker could become stuck. The tractor-drawn tanker is relatively expensive due to its smaller size and also completes less runs per day than a road tanker. However, it is often the only available option where the ground is not stable enough for the weight of a road tanker. The liquid sludge is discharged by gravity on to the land via fish-tail nozzles that ensure even distribution over a 3.0–4.5 m wide strip, with the rate of application being controlled by the speed of the vehicle. Other methods of spreading sludge from tankers involves a rotating disc distributor or a pair of contra-rotating discs. Sludge is first fed into a hopper from which it falls on to the discs that distribute the sludge over a much wider area. Liquid sludge can be also pumped, under pressure, through arms or sprayers so that lighter applications of sludge can be made over a wider area, up to 18 m or even 45 m wide strips, which is particularly useful for treating existing crops (Institute of Water Pollution Control 1978). Because of the weight of the tanker and sludge, access to the land must be restricted to the period of April to November, although this depends on the weather and condition of the land. Failure to restrict access will result in severe compaction and rutting of the soil, which may take several years to recover. After application, grassland should be chain harrowed, especially if raw sludge is used. When arable land is treated, this should be ploughed and cultivated to incorporate the sludge into the soil, and if raw sludge is used this should be done at once to avoid odours.

Liquid sludge can be sprayed on to agricultural land under pressure using rain or manure guns. The sludge is pumped via a pipeline to one or more rain guns with 15–50 mm openings. The guns are normally directional and able to cover areas of between 0.1–0.4 ha each. Coverage is not as even as the other methods, with blockages or wind effecting evenness of distribution. Care must be taken to ensure that slurry is not sprayed into local streams or onto roads or footpaths. The wind can take the slurry considerable distances and the operator must ensure that it is not allowed to drift into neighbouring fields or on to buildings. Aerosols which can be formed by these irrigation techniques have received much attention because of the risk of disease transmission, and precautions have to be taken (Sec. 9.4.3). This type of sludge application is labour-intensive, with the guns requiring frequent relocation that involves the operator walking over ground which has been already sprayed with sludge and moving the pipework, usually sectional aluminium pipes in 6 or 9 m lengths, and the guns that are both fouled by sludge. A method that is less labour-intensive and much less unpleasant for the operator to use is a sledge-mounted rain gun attached to flexible pipework wound on a tractor-drawn bobbin, known as a travelling

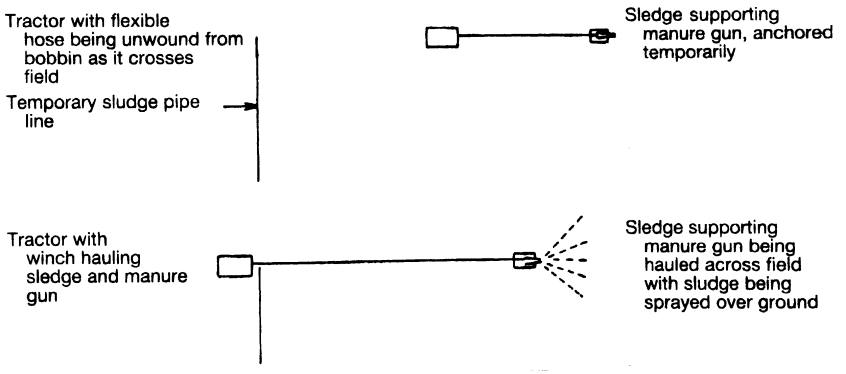


Fig. 8.16. The distribution of liquid sludge from a stationary and moving sledge-supported manure gun (Institute of Water Pollution Control 1981).

irrigator. The sledge supporting the gun is anchored temporarily at the far side of the field and the pipework fully unwound from the bobbin. The end of the flexible pipework is attached to a single temporary sludge pipe running along the near-side of the field and secured. The sludge is released, and the tractor very slowly rewinds the flexible pipework, slowly hauling the gun over the field as sludge is sprayed over the ground (Fig. 8.16). The operator is not required to handle the dirty pipe or gun and does not have to walk over treated land, with the sequence repeated on the next section of fresh field. The use of travelling irrigators is reasonably cheap and because it involves little movement of vehicles on the soil and no movement of heavy tankers, there is no risk of soil damage, and this allows operations to continue even when the soil is wet.

The final and least objectionable method of land application of sludge is direct sub-surface injection that uses a chisel plough to inject sludge 25–50 mm below the surface of the soil. The equipment can be fitted to most road tankers and tractor-hauled units, although custom-built vehicles are far more efficient and flexible in the depth of injection and rates of application that can be achieved. This method effectively eliminates odours and the risk of disease transmission, so that the long period between treatment and allowing animals back to the land to graze can be reduced considerably. High application rates are possible and up to 180 tonnes ha^{-1} can be achieved at a single pass, but at these levels of application the soil is too wet and unstable to allow animals or vehicles back on to the land for 4–6 weeks.

Table 8.34. Comparison of the various methods employed to spread liquid sewage sludge on to agricultural soil in terms of operational performance and cost (Hall and Davis 1983).

Application method	Prevention of odours and disease transmission	Effective on wet soil and for many land uses	Evenness of spread*	Economy	General comments
Tanker direct	+	+	++	+++	Widely used. Economic. Allows operational flexibility
Field tanker	+	++	++	+	High capital cost. Should be kept continuously supplied with sludge
Tractor-drawn tanker	+	++	++	+	Widely used
Movable rain gun	+	+++	+	++	Uneven application, problems of moving dirty pipes
Travelling irrigator	+	+++	+++	++	No risk of damage of soil
Sub-soil injection using field tanker or tractor-drawn	+++	++	+++	+	Avoids odour and disease-transmission problems

* Evenness of spread does depend considerably on the type of ancillary equipment used and driver skill.
+, fair; ++, good; +++, excellent.

The major application methods are compared in Table 8.34 and are reviewed more fully by Critchley *et al.* (1982). Caution must be exercised when applying sewage sludge to agricultural land. Groundwater contamination can occur if liquid sludge is applied directly to dry clay soils, which have cracked, as this allows the sludge to drain rapidly deep into the soil. Surface waters are also at risk if sludge is over-applied to wet land and surface runoff

occurs. Soil damage is a major concern when tankers of sludge are being driven over land, even though extra-wide tyres may be used. As a general rule, no vehicles should be driven on land immediately after rain, other than on sandy soils that drain readily. The soil should be allowed to drain until it regains sufficient bearing strength to support vehicles of high weights. The use of sewage sludge in agriculture is reviewed by Smith (1998).

Further reading

General: Best 1980; Vincent and Critchley 1983; Davis 1987; Water Environment Federation 1993; Cheremisinoff 1994.
Sludge disposal to land sites, general: USEPA 1977; Metcalf and Eddy 1984; Dirkzwager and L'Hermite 1989.
Sacrificial land use: Institute of Water Pollution Control 1978.
Use of sludge in land reclamation: Coker *et al.* 1982; Davis 1982; Younas 1987; Sopper 1993.
Sludge utilisation to farmland, general: Hudson and Fennel 1980; Hall and Davis 1983; Institute of Water Pollution Control 1986; O'Connor *et al.* 1986; Evans 1998; Smith 1998.
Manurial value of sewage sludge: Sommers 1977; Davis 1980; Smith 1998.
Contamination by heavy metals: Davis 1980; Davis and Carlton-Smith 1984; McGrath 1984; Elliott *et al.* 1986; McBride 1995: Smith 1998.
Contamination by pathogens: Watson *et al.* 1983; Lewis-Jones and Winkler 1991.
Application of sludge to land: Institute of Water Pollution Control 1978; Hall and Davis 1983; Towers and Horne 1997.
Legislative control: Matthews 1983, 1996; USEPA 1989.

8.3. Sea Disposal

8.3.1. *Introduction*

Since 31 December 1998, the disposal of sewage sludge to sea by EU Member States has been banned (Sec. 8.1.2). However, sea disposal of sludge is still widely practice world-wide. In the US, 40% of the sludge goes to landfill sites and some 15% is disposed to sea, including the sewage sludge from New York. Of the remainder, 25% is incinerated and 20% is applied to farmland (Rothman and Barlett 1977). Sea disposal is often the preferred disposal method in environmental, economic, and logistical terms, and where

geographical position permits, it is the cheapest method of sludge disposal (Collinge and Bruce 1981). The UK was particularly well suited to this disposal method as nowhere in the country is further than 120 km from the sea, and the coastline is the longest of all EU countries being, 9,840 km in length of which 3,790 km borders the Irish Sea. The tidal currents are fast (100–200 cm s^{-1}) and the tidal ranges moderate to high, reaching maxima of approximately 6 m off the east coast, 5 m in the English Channel, 7 m in the Irish Sea, and 10 m in the Bristol Channel. Therefore, the fast currents and high tidal ranges combined to provide excellent dispersal and dilution characteristics (Oslo and Paris Commissions 1984). Sludge disposal to the sea had a long history in the UK, being used to dispose of London's sludge to the outer Thames estuary as early as 1889. Until it was banned in 1998, dumping at sea accounted for 29% of all the sludge produced in the UK, a total of 10.5×10^6 wet tonnes from 30 locations, which is equivalent to 300,000 tonnes DS per annum. The dependence of

Table 8.35. Amount of sewage sludge dumped at sea at licensed sites in 1981 by UK water authorities, see also Fig. 8.17, (Oslo and Paris Commissions 1984).

Location	Amount dumped (net tonnes)	% of UK total (nearest 1%)
England and Wales		
Northumbrian Water Authority	271,000	3
Yorkshire Water Authority	92,000	1
Severn Trent Water Authority	1,000	< 1
Anglian Water Authority	225,000	2
Thames Water Authority	4,920,000	49
Southern Water Authority	267,000	3
South West Water Authority	148,000	1
Wessex Water Authority	266,000	3
Welsh National Water Authority	62,000	< 1
North West Water Authority	1,693,000	17
Total	7,945,000	79
Scotland		
Strathclyde Regional Council	1,512,000	15
Lothian Regional Council	287,000	3
Total	1,799,000	18
Northern Ireland		
Total	279,000	3
United Kingdom		
Total	10,023,000	100

Table 8.36. Amount of sewage sludge dumped at sea by the UK between 1976–81 (to nearest 10^3 tonnes) (Oslo and Paris Commissions 1984).

Year	England and Wales	Scotland	N. Ireland
1976	7,011,000	n.a.	213,000
1977	7,572,000	n.a.	218,000
1978	7,844,000	n.a.	242,000
1979	7,625,000	1,965,000	262,000
1980	8,242,000	2,016,000	296,000
1981	7,945,000	1,799,000	279,000

water companies on this disposal methods varied, with 9 of the 10 major companies in England and Wales disposing of some sludge by dumping at sea. Only Severn and Trent Water did not use this method. Strathclyde Regional Council, North West Water, and Thames Water disposed of 80%, 49%, and 30% of their sludge by this route respectively (Table 8.35). All of London's sludge was disposed to sea at the Barrow Deep by Thames Water, which represented 49% of all the sludge disposed to the sea in the UK (Oslo and Paris Commissions 1984). The quantities of sludge disposed of at sea increased by 10% between 1976–81 (Table 8.36), with dumping from Edinburgh commencing in 1978 (350,000 wet tonnes per annum) and from Newcastle-upon-Tyne in 1980 (500,000 wet tonnes per annum) (Fig. 8.17). Clearly, sea disposal was recognised as a major disposal route by many water companies and the level of investment during the 1980's and 1990's indicated that it would have continued to be exploited if not for the EU Urban Wastewater Treatment Directive.

Sea disposal is mainly carried out by dumping liquid sludge into estuaries and coastal waters from specially designed ships, with $< 5\%$ being discharged from pipes (Fish 1983). This should not be confused with sewage outfalls.

8.3.2. *Legislative control*

The Oslo and London International Conventions regulate dumping to the marine environment. Under these conventions, sludges can only be dumped if substances listed in Annex I of the Oslo Convention, which are considered extremely toxic (e.g. mercury, cadmium, and organochlorine compounds) (Table 8.37), are not present other than as "trace contaminants". This is currently interpretated as not substantially in excess of levels in domestic or light industrial sludges, Similarly, for Annex II substances, which

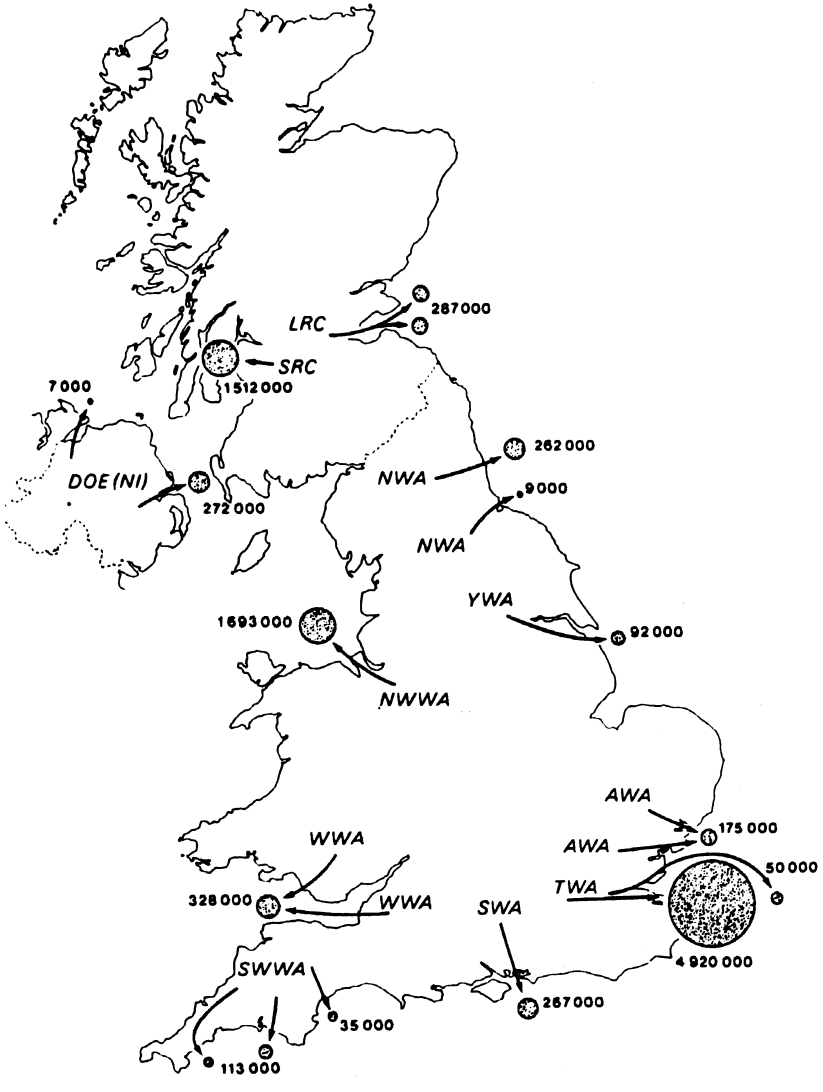


Fig. 8.17. Licensed UK sludge dumping sites in 1982 and the amount dumped each year in tonnes (see Table 8.35 for key to authorities) (Oslo and Paris Commissions 1984). Abbreviations are the relevant water authorities

Table 8.37. Summary of the substances listed in Annex I of the Oslo and London Conventions that may not be dumped at sea.

Prohibited under Oslo and London Conventions	Prohibited under Oslo Convention only	Prohibited under London Convention only
Organohalogen compound[a,c]	Organosilicon compounds[a]	Oils taken on board for dumping[c]
Mercury and its compounds	Carcinogenic substances[b,c]	High level radioactive wastes
Cadmium and its compounds[c]		Materials of biological and chemical warfare
Persistent plastics and other persistent synthetic materials		

[a] Excluding those which are non-toxic or rapidly converted in the sea into substances which are biologically harmless.
[b] As agreed by the contracting parties.
[c] Does not apply to those wastes containing these substances in trace contaminants.

include potentially bio-accumulative and toxic substances (e.g. arsenic, lead, copper, and zinc) and toxic substances (e.g. cyanides, fluorides, and those pesticides excluded from Annex I) (Table 8.38), "special care" is to be taken where sludges contain "significant" quantities of these substances, which is currently defined as 0.1% of the weight of the wet waste (Institute of Water Pollution Control 1978; Norton 1980; Collinge and Bruce 1981; Oslo and Paris Commissions 1984). Licences issued under individual countries legislation to conform with the Oslo and London Conventions must have regard to the factors listed in Annex III (Table 8.39), which include the composition and properties of the waste, the characteristics of the proposed dumping area, and the method of disposal. In all cases, the decision to license dumping operations is solely a matter for the national licencing authority. This has led to disagreements about licensing policy between countries who share common seas, for example, the dumping of radioactive waste in the Irish Sea by the UK.

The Oslo Convention dealt with the prevention of marine pullution by dumping from ships and aircraft, while the Paris Convention covered the prevention of marine pollution from land-based sources. The latter also dealt with areas affected by high levels of nutrients that have been linked to the occurrence of abnormal algal blooms. In 1992 it was agreed that the Oslo and Paris Coventions, and the Commissions that manage them, should be replaced by the introduction of a single new convention entitled

Table 8.38. Summary of the substances listed in Annex II of the Oslo and London Conventions that may not be dumped at sea[a].

Included in both Oslo and London Conventions	Included in Oslo Convention only	Included in London Convention only
Arsenic, Lead, Copper, Zinc, Cyanides (and their compounds)	Non-toxic substances which may be harmful because of large quantities in which they had dumped	Organosilicon compounds
Fluorides		Beryllium, Chromium, Nickel, Vanadium (and their compounds)
Pesticides not listed in Annex I		Radioactive matter not included in Annex I
Containers, scrap metal and other bulky wastes[b]		

[a] For the purposes of the London Convention all wastes other than those listed in Annex I and Annex II require a prior general permit. For the purposes of the Oslo Convention, the provisions of Annex II apply only to wastes containing significant quantities of the substances shown.

[b] For the purposes of the Oslo Convention, such substances may be dumped only in waters where the depth is greater than 2000 m and the distance from land is not less than 150 nautical miles.

Table 8.39. Annex III of the Oslo Convention gives the provisions governing the issue of permits and approvals for the dumping of wastes at sea.

1. *Characteristics of the waste*
   (a) Amount and composition.
   (b) Amount of substances and materials to be deposited per day (per week, per month).
   (c) Form in which it is presented for dumping, i.e. whether as a solid, sludge or liquid.
   (d) Physical (especially solubility and specific gravity), chemical, biochemical (oxygen demand, nutrient production) and biological properties (presence of viruses, bacteria, yeasts, parasites, etc.).
   (e) Toxicity
   (f) Persistence.
   (g) Accumulation in biological materials or sediments.
   (h) Chemical and physical changes in the waste after release, including possible formation of new compounds.
   (i) Probability of production of taints reducing marketability of resources (fish, shellfish, etc.).
2. *Characteristics of dumping site and method of deposit*
   (a) Geographical position, depth, and distance from coast.
   (b) Location in relation to living resources in adult or juvenile phases.
   (c) Location in relation to amenity areas.
   (d) Methods of packing, if any.
   (e) Initial dilution achieved by proposed method of release.
   (f) Dispersal, horizontal transport and vertical mixing characteristics.
   (g) Existence and effects of current and previous discharges and dumping in the area (including accumulative effects).
3. *General consideration and conditions*
   (a) Interference with shipping, fishing, recreation, mineral extraction, desalination, fish and shellfish culture, areas of special scientific importance, and other legitimate use of the sea.
   (b) In applying these principles the practical availability of alternative means of disposal or elimination will be taken into consideration.

'The Protection of the Marine Environment of the North-East Atlantic' (i.e. the OSPAR Convention). Since 1998 the Oslo and Paris Commissions have worked as a single entity, the OSPAR Commission. While the original standards remain largely unchanged, they are being currently being reviewed and, where necessary, modernised.

There are at present five specific areas covered by the OSPAR Convention, which are listed as Annexes. These are: Annex I: Prevention and elimination of pollution from land based sources; Annex II: Prevention and

elimination of pollution by dumping or incineration; Annex III: Prevention and elimination of pollution from offshore sources; Annex IV: Assessment of the quality of the marine environment; and Annex V: Protection and conservation of the ecosystems and biological diversity of the maritime area. The Commission has adopted specific strategies to deal with (i) Protection and conservation of ecosystems and biological diversity; (ii) Hazardous substances; (iii) Radioactive substances; (iv) Eutrophication; and (v) Environmental goals and management mechanisms for offshore activities.

8.3.3. *Dumping sites*

The careful selection of dumping sites can minimise any detrimental effects sewage sludge may have on the environment and minimise the interference with other legitimate uses of the sea. Sites should either ensure a high degree of dilution and dispersion, thus preventing the build up of sludge or harmful concentrations of substances in sludge, or ensure sludge dispersion is contained so that any effects are restricted to as small an area as possible. Thus, sites are characterised by their different hydrographic qualities as either dispersive or accumulative. At dispersive sites, dumping occurs in generally shallow well-mixed waters in areas with an open coastal aspect, so the site is swept by high tidal currents to give rapid dispersion. Dispersal is enhanced by the release of sludge, at mid-tide, into the wake of the moving vessel that increases the area over which particles will settle. Most sites are of this type, although a few, such as the former dumping site at Garroch Head on the Clyde are containment or accumulating sites. There is little dispersion at such sites which are characterised by low tidal velocities that are usually more physically restricted and are in deeper waters. Settlement is encouraged by rapid release of sludge from the bottom of the vessel while it is stationary above the site. Dumping is most effective in accumulative sites on or just before slack water, ensuring minimum dispersion.

Similar criteria have to be met for both types of site and these are listed in Annex III of the Oslo Convention (Table 8.39). This states that the potential effects of sludge dumping on all the other legitimate uses of the sea should be fully evaluated before a licence is issued. Norton (1978) has listed the most important uses that may be affected by dumping activities. These are:

Amenity: avoidance of contamination of bathing waters and beaches with sewage-associated solids and debris, and, in particular, with pathogenic

micro-organisms. To achieve this, dumping sites should be located several miles from the nearest shore.

Shipping: sites should be away from busy shipping lanes, especially if the sludge vessel has to sail within the defined area for an extended period to dispose of its load.

Commercial fishing: dumping sites must avoid areas that are extensively fished whether by trawling, seining, lining or potting.

Spawning and nursery grounds: areas where eggs, larval, and juvenile stages of fish and shellfish abound should be avoided. The developmental stages are generally more sensitive to environmental changes than adults.

Mineral extraction: offshore mineral extraction is now relatively common and dumping should avoid areas that may be suitable for future exploitation, especially for sand and gravel extraction. Unless there are very strong dispersive currents, settlement of sludge would make any future exploitation of the minerals very difficult.

Existing discharges: although sludge dumping is insignificant in terms of the amount of nutrients and certain metals that are discharged, combined with land based and atmospheric inputs, local environmental problems could result if the local dispersive capacity is exceeded.

Clearly, site selection will require extensive field evaluation in order to define an area suitable for sludge disposal, with specialist studies on the hydrography to measure current strengths and direction, direction of residual movement, vertical stratification of the water column, and water quality. Specialist studies are also required to locate the fisheries, and to characterise the physical and chemical nature of the existing sediment, and the biological nature of the sediment in order to establish the susceptibility of the existing fauna to sludge. Once selection has taken place and dumping has commenced, then a permanent monitoring programme is required to determine the chemical, physical, and biological effects on the site. What is of most interest is the short- and long-term fate of the sludge, nutrients, and metals in the area, and the overall effect on fish and shellfish quality (Sec. 9.4.1).

8.3.4. *Environmental impact*

Former dumping sites in the UK were chosen to minimise environmental impact, although the major criterion for controlling sea discharges remains the protection of seafood for human consumption. Before December 1998,

the Ministry of Agriculture, Fisheries and Food, and the Department of Fisheries for Scotland monitored all major licensed sites. They employed a system of intermittent intensive studies at approximately five-yearly intervals, supported by less intensive routine monitoring. Disposal sites were also chosen to preclude contamination of commercial fish and shellfish, and some monitoring of fish for pathogens and metal content was also undertaken. Traditionally, the major impact of sludge dumping is assessed in terms of abundance and diversity of species plus the concentration of metals in the sediment. From their data, it would appear that sludge dumping had very little impact on the marine environment. At sites where the sludge was highly dispersed, such as the Thames Estuary and Liverpool Bay dumping sites, environmental damage was not detectable above normal background variations. However, at sites where sludge accumulated, such as Garroch Head, some localised effects were detected (Davis *et al.* 1985).

High turbidity and low dissolved oxygen concentrations are rarely serious problems at sludge dumping sites. The dissolved oxygen concentration is not significantly affected at dispersed sites, as (in open coastal situations) any oxygen depletion in overlying waters is rapidly removed by dilution and dispersion, and only localised and transient reductions occur. For example, in the Thames Estuary and Liverpool Bay, the dissolved oxygen concentration rarely fell below 99% saturation with localised concentrations only falling as low as 97% in the surface waters at former dumping sites (Norton 1978). The sediment oxygen demand at accumulative sites can often be considerable, resulting in a reduced oxygen concentration in the overlying water, with subsequent reduction or elimination of sensitive species. Therefore, at sites where there is less mixing, reductions in dissolved oxygen will be larger and longer in duration. Density stratification of the water inhibits vertical mixing that can result in critically low oxygen concentrations.

Changes in environmental conditions will disturb the ecological balance of dumping sites resulting in the reduction of species diversity. Under extreme conditions, only those species tolerant of the extremes of the particular stress factors survive, although they will generally proliferate, resulting in large population densities because of reduced competition. Where sludge does not accumulate, the benthic fauna is unaffected in terms of species diversity (Domenowske and Matsuda 1969). However, where deposition takes place, species diversity can be reduced over a wide area (Pearce 1969; Pearson and Rosenberg 1978). On the boundaries of dumping sites where the level of organic enrichment is low, a number of species will preferentially feed off the organic material with a subsequent

increase in their numbers. At high levels of organic enrichment, a reduction in species diversity is recorded as sensitive species are eliminated and only tolerant species are left. High biomasses of tolerant species are recorded at the centre of dumping sites and are dominated by the detritiophagous polychaetes such as *Capitella capitata* and also several species of Nereids. These sites are characterised by low-diversity polychaete communities and are well documented (Bellan 1967; Kitamori 1971; Reish 1973). At Garroch Head, where the organic carbon concentration reached 8% in the central area of the dumping site, the species diversity was severely restricted, although the animal biomass was very high (Topping and McIntyre 1972). In the surrounding areas, where the organic carbon was $< 3\%$, a normal mixed fauna was present. However, in the Thames and Liverpool Bay dumping areas, it was the mobility of the bottom sediments that appeared to be the main factor controlling the benthos, with few adverse effects attributable to sludge dumping (Gould 1976). The stability of the sediment is an important factor affecting the burrowing forms of molluscs and crustaceans, and sludge dumping tends to eliminate many of these species. For example, at the Garroch Head dumping site, the sludge made the sediment unsuitable for the Norway lobster (*Nephrops*) over a 10 km^2 area, so that the species is severely reduced in numbers (McIntyre and Johnston 1975). In localised areas in dumping sites where excessive accumulation has occurred and there is little mixing, low dissolved oxygen conditions can persist, eliminating even tolerant species. The response of organisms depends very much on the physical and chemical nature of the site as well as the level of dumping that occurs, and individual sites tend to be unique in terms of the response by the flora and fauna, making generalisations difficult.

So while the areas affected are relatively small, some impact is discernible with the major environmental effects of sludge dumping are caused by nutrient enrichment, metal and pesticide accumulation, and the persistence of pathogens (Chen and Orlob 1972; Eppley *et al.* 1972; Jenkinson 1972; Halcrow *et al.* 1973; Watling *et al.* 1974; Caspers 1976; Eagle *et al.* 1979).

The nutrients released during the degradation of sewage can lead to an enormous increase in algal productivity when discharged to the marine environment. The subsequent death of the biomass formed adds, and can even exceed, the load of organic material derived directly from the outfall or discharge, releasing further nutrients as the algal mass degrades. In extreme cases, this secondary degradation of organic matter can lead to anaerobic decomposition, with the release of hydrogen sulphide gas and severe stress

on the existing biota. This is a particularly serious problem in the larger enclosed estuaries that receive large effluent. The water quality at dumping sites changes because of the presence of organic matter, nutrients, trace metals, and suspended particulates in the sludge. Although changes in water quality are generally minimal because of dilution and dispersion, the input of nutrients is so significant that elevated nutrient concentrations occur on a local basis. However, at dumping sites located near major estuaries, such as the Mersey or the Clyde estuaries, the nutrient enrichment in the water at the dumping site is usually less than the adjacent inshore areas that receive nitrogen and phosphorus from the rivers and coastal sewage outfalls. Therefore, nutrient enrichment arising from sludge dumping may not in itself be important, but it can make a significant contribution to the total nutrient levels in some coastal waters.

Primary productivity of phytoplankton in marine waters appears limited by nutrient concentrations rather than temperature or light, and any increase in nitrogen and phosphorus could result in increases in algal biomass as measured by chlorophyll *a*. However, no elevated primary productivity has yet been recorded at a sludge dumping site, even accumulative ones, and the problem of algal blooms and coastal eutrophication is most likely due to sewage outfalls and nutrients originating from riverine sources. Blooms generally appear in restricted waters where poor dilution and dispersion leads to locally high nutrient concentrations. Algal growth is enhanced in shallow waters where the water temperature is high and light penetration is at a maximum. These conditions cause an explosion in the growth of algae, which results in a very dense, low diversity or even mono-specific algal crop. If this bloom is composed of toxin-producing species, further damage may result from the concentration of toxins in the marine food web. Some blooms are promoted by organic enrichment as well as nutrients, such as the algae *Phaeocystes* and dinoflagellates, and sludge dumping may well encourage the formation of these blooms in existing nutrient-rich waters. These particular blooms are generally toxic to both fisheries and man via contaminated shellfish. Although the effects of nutrient enrichment in open coastal waters may be rather subtle and difficult to detect, phytoplankton will become concentrated at the shoreline as they are washed ashore. Luxuriant growths of *Ulva* and *Enteromorpha* in shallow enriched waters are particularly common and like the phytoplankton blooms, will cause secondary pollution upon decay, resulting in odour and discolouration of the water (Gould 1976). It has been suggested that sludge dumping acts as a marine fertiliser (Segar *et al.* 1985), as it does on land. There is,

however, little evidence to support this and many authors feel that sea disposal represents a waste of a useful resource (Rothman and Barlett 1977; Norton 1978).

The disposal of sewage sludge from ships accounts for only a small proportion of the total quantity of pollutants reaching the marine environment. Metals, for example, come from three other sources: via rivers; direct discharge of sewage, and industrial wastes; and from atmospheric pollution. Of the total quantity of cadmium in the North Sea prior to 1998, for example, 63% came from aerial deposition, 34% via rivers, 2% from direct

Table 8.40. Annual discharge of heavy metals to five major UK estuaries and the percentage input from sludge dumping prior to the EU ban on dumping sewage sludge at sea (Collinge and Bruce 1981).

Estuary	Cd	Cr	Zn	Ni	Cu	Pb
Clyde						
Total discharge (tonnes yr^{-1})	6	205	716	49	158	185
Percentage as sludge	16	59	18	13	30	23
Firth of Forth						
Total discharge (tonnes yr^{-1})	20	160	823	110	338	453
Percentage as sludge	2	4	2	2	3	2
Liverpool Bay						
Total discharge (tonnes yr^{-1})	53	324	1,907	188	238	—
Percentage as sludge	—	28	14	8	46	—
Severn						
Total discharge (tonnes yr^{-1})	40	492	1,652	107	294	458
Percentage as sludge	1	4	2	4	9	4
Thames						
Total discharge (tonnes yr^{-1})	42	142	1,126	243	242	189
Percentage as sludge	12	36	40	21	42	54

discharges, and only 1% from sludge dumping. Similar figures are available for mercury with 14%, 68%, and 4% respectively (Oslo and Paris Commissions 1984). Sixty percent of sludge dumped from vessels in the UK was dumped in the North Sea. However, sewage sludge dumping in this area represented only 5% of the total BOD load and 1% of both the nitrogen and phosphorus loads. However, the southern North Sea, which is closest to the UK but is only 7.5% of the total area of the North Sea, received over half the nutrient load and between a third and a half of all the metals. Therefore, if the southern North Sea is considered separately, then the proportion of nutrients supplied from sludge dumping rises to 12%, 2%, and 3% for BOD, nitrogen, and phosphorus respectively (Norton 1982; Hill *et al.* 1984). In some areas, however, such as the Clyde, Liverpool Bay, and the outer Thames estuary, sludge disposal accounted for 30–50% of the input of certain metals, whereas in other areas, such as the Severn and the Firth of Forth, the contribution from sludge rarely exceeded 5% (Table 8.40) (Collinge and Bruce 1981). Considerable efforts have been made by the water companies to reduce the concentrations of Annex I substances and other less toxic substances listed in Annex II reaching treatment plants, and eventually being concentrated in the sludge and dumped at sea. Thames Water Authority was able to reduce the average concentration of mercury and cadmium in its sludge disposed at sea by 78.3% and 41.0% respectively, by setting new limits for metals discharged to sewers by the 800 firms discharging metals to the Beckton, Crossness, Riverside, and Deephams treatment plants that served London during the late 1970's and early 1980's (Fish 1983). This is reflected in the total quantities of metals disposed by the UK over that period (Table 8.41). Most recent records covering the period 1985 to 1996 show that the quantity of metals discharged continued

Table 8.41. Quantities of heavy metals dumped annually (tonnes) at sea as sewage sludge. 1974–84 by the UK (DoE 1987).

Heavy metal	1978	1979	1980	1981	1982	1983	1984
Mercury	2.8	2.8	3.6	2.4	1.4	1.1	0.9
Cadmium	8.6	8.6	9.3	6.5	5.1	4.0	4.0
Copper	235.7	236.6	220.7	199.8	203.9	158.6	156.8
Lead	207.6	197.6	182.3	159.8	164.8	160.0	158.0
Zinc	867.3	939.6	684.8	640.8	442.0	439.2	500.3
Total amount of sludge ($\times 10^6$)	8.3	8.5	8.9	8.5	8.1	7.3	7.5

Table 8.42. Dumping of sewage sludge, industrial waste and dredgings at sea[1] 1985–96 from the United Kingdom (DETR 1998).

United Kingdom	Tonnes, unless otherwise stated							
	1985	1990	1991	1992	1993	1994	1995	1996
Sewage sludge:								
Total amount dumped (thousand wet tonnes)	9,216	9,335	9,735	9,985	9,923	9,657	9,731	9,910
Total amount dumped (thousand dry tonnes)	262	274	287	292	274	289	296	277
Zinc	353	288	325	323	260	270	293	285
Lead	182	129	125	114	95	99	103	91
Copper	162	147	165	163	141	156	152	130
Chromium	102	104	104	93	69	77	75	71
Nickel	25.2	20.7	20.0	19.5	18.2	17.0	17.7	15.0
Cadmium	3.83	2.06	2.15	1.95	1.31	1.10	1.09	0.85
Mercury	1.37	1.07	1.06	0.97	0.75	0.83	0.66	0.59
Solid industrial waste: [2,3]								
Total amount dumped (thousand wet tonnes)	3,550	4,652	4,230	3,418	2,206	164	188	0
Total amount dumped (thousand dry tonnes)	1,567	3,602	3,326	2,554	1,572	157	169	0
Zinc	389	435	408	314	241	—	—	0
Lead	191	212	200	154	117	—	—	0
Copper	152	176	166	128	98	—	—	0
Chromium	12.9	21.0	19.5	15.7	12.1	—	—	0
Nickel	51	64	60	46	36	—	—	0
Cadmium	0.129	0.158	0.148	0.115	0.088	—	—	0
Mercury	0.129	0.235	0.218	0.176	0.136	—	—	0

Table 8.42. (*Continued*)

United Kingdom	Tonnes, unless otherwise stated							
	1985	1990	1991	1992	1993	1994	1995	1996
Dredged material:								
Total amount dumped (thousand wet tonnes)	41,622	36,155	41,746	28,976	31,505	35,963	40,248	51,254
Total amount dumped (thousand wet tonnes)	20,600	16,259	22,411	14,596	18,571	19,649	20,254	23,249
Zinc	6,533	3,939	3,391	2,527	2,663	3,471	3,518	4,149
Lead	2,909	1,537	1,286	990	1,090	1,423	1,535	1,814
Copper	1,455	900	792	597	676	764	746	835
Chromium	1,580	1,093	1,201	923	937	1,328	1,455	1,613
Nickel	1,131	518	523	332	491	604	616	702
Cadmium	29.81	13.16	7.91	7.22	11.47	8.82	7.19	9.25
Mercury	19.02	7.6	7.54	6.05	7.06	6.38	8.69	7.61

[1] All UK waters.

[2] This table now covers mainly colliery waste, i.e. minestone and tailings. It also includes some small amounts of dumping of explosives and of sediment processed in recovering coal. It excludes fish waste. The dumping of colliery waste ceased in 1995.

[3] Flyash dumping ceased at the end of 1992. Figures of previous years are shown exclusive of flyash to provide consistency with post-1992 data.

to decrease even though the volumes of sludge disposed to sea had significantly increased reaching nearly 1×10^7 tonnes per annum (Table 8.42). Compared to 1978, reductions in metals discharged via sludge in 1996 had decreased by 90% for Cd, 79% Hg, 67% Zn, 56% Pb and 45% Cu (DEFR 1999).

At dispersive sites, there is very little accumulation of organic matter and contaminants, whereas at accumulative sites the reverse is true. The concentration of metals and other contaminants is linked to the quantity of organic matter present. At two accumulative sites, the New York Bight and Garroch Head dumping grounds, the organic carbon levels reached 5% and 8% respectively, with correspondingly elevated levels of contaminants. After dumping, metals and organic compounds are solublised either from the sediment or from the suspended particulate matter, resulting in enhanced metal levels in the water. At the former Liverpool Bay dumping site, high concentrations of mercury (200 μg l^{-1}) and polychlorinated biphenyls (PCBs) (1.5 μg l^{-1}) were recorded (Gardner and Riley 1973; Dawson and Riley 1977).

It is difficult to isolate the effect of sludge dumping from the effects of other dumping operations and other sources of metals (Table 8.42). However, there is evidence of elevated heavy metal concentrations in sludge dumping grounds and also increased levels in some commercially fished species of fish and shellfish in some coastal areas. In the Clyde estuary, where there are no significant dispersion effects, the changes in the sediment and benthos was clearly attributable to sludge dumping, and when compared with the Liverpool Bay dumping site, where dispersion is good, significantly higher concentrations of metals were recorded (Gould 1976).

Heavy metals, PCBs, pesticides, and pathogenic micro-organisms can all be accumulated by fish and shellfish, with some materials being passed through the food chain. Many of the species most at risk such as inshore fish and shellfish, are commercially fished for human consumption. Accumulation can be either direct by feeding on contaminated organic matter or indirect due to species feeding on organisms which already contain high concentrations of contaminants. Sub-lethal effects of heavy metals on the marine biota have been reviewed by Jones (1978). Primary productivity can be suppressed by quite low levels of heavy metals (Bernhard and Zattera 1975), although larval stages of marine animals are generally more vulnerable than the adults (Reish 1973). Work done by McIntyre (1975) has demonstrated that copper can be passed through the marine food chain with adverse effects noted at metal concentrations of just 2 or 3 times above the normal background levels, which is two orders of magnitude less than

the LD_{50} values for copper on the species involved. The concentration of toxic substances, including metals and pesticides accumulated in fish, were monitored annually. Fish and shellfish around the UK shoreline have shown elevated levels of metals, especially mercury, from areas used for sludge disposal (Ministry of Agriculture, Fisheries and Food 1971). However, the level of mercury in cod caught in the Thames estuary declined significantly, in line with the reduction of the quantity of the metal dumped at this site.

Table 8.43. Breakdown of the major constituents (mg kg^{-1}DS) of sewage sludge from Manchester and Salford (Gould 1976).

Total P	16,000
Total N	30,000
Anionic surfactant	9,500
Mineral oil	38,000
Metals	
Zn	2,900
Cu	1,900
Pb	530
Cd	56
Hg	37
Ni	500
Cr	930
Fe	22,000
Mn	650
Chlorinated solvents	
1,2-Dichlorobenzene	78
1,2-Trichlorobenzene	600
Chloroform	2.6
1,1,1-Trichloroethane	0.5
Carbon tetrachloride	0.3
Trichloroethylene	2.7
Tetrachloroethylene	2.2
PCBs as Arochlor 1254	5.1
Organochlorine pesticides	
BHC	0.1
DDE	< 0.3
TDE	< 0.3
DDT	< 0.3

The persistent organic contaminants in sludge constitute a much greater potential threat to fish and human health than the other contaminants found in sludge, because their high persistence and toxicity. Although they are not abundant in sludge they are present in measurable concentrations in sewage sludge (Table 8.43), providing another route for these highly dangerous compounds to enter the environment. Levels in fish remain low enough not to be a hazard, although the concentration of both total DDT, which includes DDT, DDE, and DDD, and PCBs in cod caught in the Thames, Liverpool Bay, and South Bight dumping areas, were all significantly higher than concentrations found in deep sea fish. Dioxins are common in sewage sludge (Rappe *et al.* 1996; Eljarrat *et al.* 1999) and a survey of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in the sediments from former sludge dumping grounds off the Catalonia coast (northeast Spain) found them to be elevated in these sediments, often exceeding the European quality objective (Ever *et al.* 1996) of 20 pg I-TEQ g^{-1} d.w. (Eljarrat *et al.* 2001).

The fate of pathogenic micro-organisms associated with sewage and sewage sludge in the marine environment is examined in Sec. 9.4.1. The contamination of bathing beaches is largely due to sewage outfalls with no evidence of such contamination arising from sludge dumping from vessels. However, the contamination of commercial fish and, in particular, shellfish, has been closely associated with sludge dumping operations (Sec. 9.4.1). As long as good cleansing and heat treatment is practised, then all the potentially hazardous bacteria can be removed from shellfish prior to consumption. However, the fate of viruses in the sea from dumping and other discharges is still relatively unknown and occasional food poisoning outbreaks still occur with the causative agents being viruses associated with contaminated seafoods (Anon 1985b). A major study carried out at two eastern American dumping sites in the mid-Atlantic region, the New York Bight, and the Philadelphia dump site, showed that viruses can survive for up to 17 months in the sediments. Also, that viruses could be isolated in the absence of faecal indicator bacteria, reinforcing the inadequacy of such bacteria in predicting the virological quality of water. Human enteric viruses were not only detected in the water and sediments at these sites but also in crabs (Goyal *et al.* 1984).

Monitoring the dispersal of organic material and contaminants is difficult at sludge dumping sites because of sea area and the rapid dilution and dispersion that normally occur. Monitoring the dispersion of sludge can only be effectively done using radioactive tracers, although other techniques have been proposed, including characterisation of the origin of organic material

using the carbohydrate to organic ratio (Hatcher and Keister 1976), the use of faecal bacteria (Ayres 1977), and the use of tomato pips as an indicator of sludge-contaminated sediments (Shelton 1973). Many biological and biochemical methods are used to investigate the effects of pollutants on the fauna at dumping sites. Davis *et al.* (1985) used mussels in cages suspended from buoys at dumping sites and examined the digestive glands after exposure, for cellular damage caused by elevated metal levels. This method, and a range of other cellular and physiological indices, such as the lysosomal test (Moore 1980, 1985) and the "scope for growth" test (Widdows 1985), is compared in an assessment of the biological effects of sludge dumping at the Plymouth sites by Lack and Johnson (1985).

As nearly all sludge is dispersed, it is important to consider total inputs into marine waters, not only from dumping, but from the other sources as well. However, increasing pressure to restrict disposal of wastes to sea from EU countries has now extended to sewage sludge dumping, with the introduction of the Urban Wastewater Treatment Directive which prohibits dumping of sludge at sea from vessels or outfalls from December 1998. While these are minor routes for entry of contaminants into the marine environment, they are the easiest to control. The UK water industry regards the phasing out of the disposal of sewage sludge to sea as essentially a political decision; although while emphasising that at sites where there is strong dispersal (e.g. North Sea) there is no adverse environmental effects from the practice, it acknowledges that it is undesirable at other sites, such as the Baltic, where exchange is slow with consequential eutrophication (CIWEM 1999b). Costello and Read (1994) concluded that there is in fact very little evidence to show that sewage sludge dumping is detrimental to marine organisms, including fish embryos and larvae. Mesocosm and field trials have shown that acute toxicity is unlikely to occur at ecologically significant or detectable levels at dump sites. The main impact is organic enrichment leading to changes in species richness, relative abundance and the biomass of macro-invertebrates.

Further reading

General: Calcutt and Moss 1984; McGlashan 1983.
Legislative control: Norton 1980; Oslo and Paris Commissions 1984.
Dumping sites: Norton 1978; Whitelaw and Andrews 1988.
Environmental impact: Head 1980; Watling *et al.* 1974; Wilson 1988; Whitelaw and Andrews 1988; Costello and Read 1994.

9

Public Health

9.1. Disease and Water

The term pathogenic is applied to those organisms that either produce or are involved in the production of a disease. This direct and indirect action of pathogens allows water-related diseases to be classified into one of the following four categories (Bradley 1974).

Water-borne diseases are enteric diseases caused by organisms that are excreted in large numbers by infected persons and the route of infection is normally by oral ingestion. The diseases are usually transmitted when pathogens in water are drunk by an animal or a human which may then become infected. The classical water-borne diseases are mainly low-infective dose infections, such as cholera, typhoid, and leptospirosis. All the remaining diseases are high-infective dose infections and include infectious hepatitis and bacillary dysentery. All water-borne diseases can also be transmitted by other routes that permit faecal material to be ingested. For example, by faecal-oral contact via contaminated food, the most notorious case being that of the Irish cook who worked in North America in the late-nineteenth century and was known affectionately as 'Typhoid Mary' (Table 9.1).

Water-washed diseases are caused by a lack of personal hygiene because of water scarcity. The incidence of all these diseases will fall if adequate supplies of washing water, regardless of microbial quality, are provided. These are diseases of mainly tropical areas and include infections of the intestinal tract, the skin, and the eyes. The intestinal infections are all faecal in origin and include all the water-borne diseases that are contracted because of poor personal hygiene. There is growing evidence that Shigellosis is linked more closely to personal hygiene than water quality (Feachem

Table 9.1. The waterborne pathogens associated with wastewater important in European temperate areas.

Group	Genus or species	Disease or effects on human health	Notes on distribution if restrictive
Viruses	Poliovirus	Fever, poliomyelitus enteritus	
	Coxsackievirus A	Headache, muscular pain	
	B	Nausea, meningituis	
	Echovirus	Diarrhoea, hepatitis	
	Adenovirus	Fever, respiratory infection, enteritis, conjunctivitis, involvement of the central nervous system	
	Reovirus	Common cold, respiratory tract infections, diarrhoea, hepatitis	
	Hepatitis A	Infectious, hepatitus (fever, nausea, jaundice)	
Bacteria	*Salmonella* spp.	Typhoid fever, parahtyphoid fever, bacterial enteritis, Salmonellosis, food poisoning	
	Campylobacter spp.	Campylobacter enteritis, gastro-enteritis, acute diarrhoea, food poisoning	
	Shigella spp.	Bacillary dysentry	
	Escherichia spp.	Enteritis (pathogenic strains)	
	Vibrio cholera	Cholera, enteritis, food poisoning	Not established in Australia, New Zealand, Pacific Islands or Americas
	Leptospira spp.	Leptospirosis	
	Mycobacterium spp.	Tuberculosis, skin granuloma	
	Clostridium spp.	Gas gangrene, tetanus, botulism, food poisoning	
	Brucella tularensis	Tularaemia	Mainly in N. America, Europe, former USSR, and Japan
Protozoa	*Entamoeba histolytica*	Amoebic dysentry	
	Giardia intestinalis	Giardiasis	
	Cryptosporidium parvum	Cryptosporidiosis	
	Balantidium coli	Balantidiasis	Epidemics reported in Brazil, Georgia, and former USSR

Table 9.1. (*Continued*)

Group	Genus or species	Disease or effects on human health	Notes on distribution if restrictive
Nematodes	*Ascaris lumbricoides*	Ascariasis (roundworm infestation)	
	Anchylostomum duodenale	Hookworm infestation	
Cestode	*Taenia* spp.	Tapeworm infestation	

1977). Most of the intestinal infections are diarrhoeal diseases responsible for the high mortality rates among infants in the developing world. The infections of the skin and mucous membranes are non-faecal in origin and include bacterial skin sepsis, scabies, and cutaneous fungal infections (such as ringworm). Diseases spread by fleas, ticks, and lice are also included in this category, such as epidemic typhus, rickettsial typhus, and louse-borne fever.

Water-based diseases are caused by pathogens that have a complex life-cycle and which require an intermediate aquatic host. All these diseases are caused by parasitic worms with the severity of the infection depending on the number of worms infesting the host. The two commonest water-based diseases are Schistosomiasis carried by the trematode *Schistosoma* spp. and Guinea worm which is the nematode *Dracunculus medimensis*. *Schistosoma* worms use aquatic snails as intermediate hosts and are estimated as infecting as many as 200 million people, and the Guinea worm uses the small crustacean *Cyclops* spp. as its intermediate host.

Water-related diseases are caused by pathogens carried by insects that act as mechanical vectors and which live near water. All these diseases are very severe and control of the insect vectors is extremely difficult. The most important water-related diseases include two viral diseases, yellow fever, transmitted by the mosquito *Aedes* spp. and dengue, which is carried by the mosquito *Aedes aegypti* which breeds in water. Gambian sleeping sickness, trypanosomiasis, is caused by a protozoan transmitted by the riverine tsetse fly (*Glossina* spp.) which bites near water, and malaria is caused by another protozoan, (*Plasmodium* sp.) and is transmitted by the mosquito *Anopheles* spp. which breeds in water.

This classification has been further extended by Mara and Feachem (1999) who have proposed seven categories of water- and excreta-related communicable diseases: (i) Faeco-oral waterborne and water-washed

diseases; (ii) Non-faeco-oral water-washed (skin and eye) diseases; (iii) Geohelminthiasis; (iv) Taeniasis; (v) Water-based diseases (bacterial and fungal, as well as helminthic); (vi) Insect-vector diseases; (vii) Rodent-vector diseases.

This chapter deals exclusively with water-borne diseases, caused by pathogens present in sewage from temperate regions of Western Europe, which, in terms of wastewater treatment, is the most important category of water-associated diseases. However, water-washed diseases are also important to those involved with the treatment, disposal, and re-use of wastewater (Sec. 9.4.1), including farm wastes, treated effluents, and sewage sludge. A number of diseases of the skin or respiratory tract are directly related to contact with, or close-proximity to, contaminated wastes (Secs. 9.2.2 and 9.2.4).

As contamination of water by faeces from people and animals suffering from enteric diseases is the cause of water-borne disease, the introduction of wastewater treatment to remove these pathogens and the disinfection of water supplies has led to the virtual elimination of all the classical diseases. The development of the public health authorities with specialist inspectors and laboratories, linked with better sanitary conditions and a greater awareness of personal hygiene, has resulted in all the water-borne and water-associated diseases being brought under control throughout the developed world. However, the situation in developing countries is very different with many water and excreta related diseases preventing social and economic development. The global burden of these diseases has been reviewed by Mara and Feachem (1999) who estimate that they were responsible for 2.7×10^6 deaths in 1990 which represents 5.3% of all deaths that year. Such diseases are also responsible for the loss of 9.32×10^7 disability-adjusted life years (DALYs) which represents 6.8% of all DALYs; 99.9% of deaths and 99.8% of DALYs caused by waterborne diseases occurred in developing countries.

9.2. Water-Borne Diseases

9.2.1. *Introduction*

Industrial wastewaters rarely contain pathogens, whereas pathogens are common in food-processing wastes. The pathogens found are directly related to the original plant or animal materials being processed. For example, in the case of potato processing, the effluent contains plant pathogens mainly specific to potatoes, such as the potato cyst eelworm (*Heterodera rostochiensis* or *H. pallida*) and a variety of other viruses, fungi, and bacteria

all pathogenic to potatoes. In sewage, it is the diseases excreted by man via faeces and urine that are of primary importance to public health. The numbers and diversity of potential pathogens in sewage reflects the standards and the socio-economic levels of the community. For example, fewer pathogens are present in sewage from industrialised countries having high standards of living compared with those countries with little industrialisation and a correspondingly lower standard of living.

Pathogens in sewage are able to infect man and animals by oral ingestion, via the skin or by respiratory routes. The commonest infection route for pathogens is oral ingestion, which generally causes gastro-enteric disorders. The pathogen multiplies and is excreted with the faeces and subsequently ingested by the next host via sewage-contaminated water or food (Table 9.1).

The most important water-borne pathogens associated with wastewater treatment are summarised in Table 9.1. Of these, the most important in terms of frequency of isolation in sewage-contaminated water, in sewage or sludge are strains of *Salmonella*, *Shigella*, *Leptospira*, enteropathogenic *Escherichia coli*, *Francisella*, *Vibrio*, *Mycobacterium*, human enteric viruses, *Cryptosporidium parvum* oocysts, and cysts of *Giardia* spp., *Entamoeba histolytica*, or other pathogenic protozoans, and larvae of various pathogenic worms. All these organisms are well characterised and have been fully reviewed elsewhere (Bitton 1999). Therefore, only a brief summary of the major organisms is given below. (Details of potential public health risks from pathogenic organisms in wastewater are discussed in Sec. 9.4.)

9.2.2. *Bacteria*

Salmonellosis

The genus *Salmonella* is now probably the most important group of bacteria affecting the public health of humans and farm animals in Western Europe. For humans, this is undoubtedly due to the elimination of the other classical bacterial diseases through better sanitation, higher living standards, and the widespread availability of antibiotic treatment. The greater awareness of animal hygiene linked with new drug thereapy has also largely eradicated many of the formally important diseases, such as bovine tuberculosis and brucellosis. *Salmonella* species are extremely widespread in nature being recorded as pathogens not only of man but of nearly all known animals. Salmonella conform in general to the Enterobactericeae but can be further differentiated biochemically into four sub-genera (I to IV). Only

Table 9.2. Number of incidents of *Salmonella* infections in animals caused by various serotypes in England and Wales during 1968–1974 (Carrington 1980b).

Serotype	Cattle	Poutry and other birds	Sheep	Pigs	Other species	Total
S. dublin	15,446	48	302	78	55	15,929
S. typhimurium	3785	732	59	97	169	4842
S. abortus-ovis	0	0	243	0	0	243
S. cholerae-suis	1	0	3	309	1	314
S. gallinarum	0	44	0	0	0	44
S. pullorum	0	65	0	0	0	65
Other serotypes	1094	855	68	74	81	2172
Total incidents	20,326	1744	675	558	306	23,609

species of sub-genus I are known to be human pathogens and these are all, characteristically, β-galactosidase-negative. By reacting a *Salmonella* strain in the agglutination reaction with the antibodies produced in the serum of animals inoculated with standard strains of *Salmonella*, over 2000 subtypes or serotypes can be identified (Carrington 1980b). Some serotypes are largely specific to a single host, and these include the typhoid organism *S. typhi*, specific to man; *S. dublin*, specific for cattle; *S. abortus-ovis*, specific for sheep; and *S. cholerae-suis*, specific for pigs. Other serotypes are not host-specific, for example, *S. typhimurium* which can infect a wide range of animals, including man (Table 9.2).

The pathogen produces an endotoxin causing the typical symptoms of salmonellosis such as acute gastro-enteritis with diarrhoea, and is often associated with abdominal cramps, fever, nausea, vomiting, headache, and in severe cases, even collapse and possible death. In pregnant animals, abortion may occur. In comparison with farm animals, the incidence of salmonellosis in humans is low and, interestingly, shows distinct seasonal variation. A large number of serotypes are pathogenic to man and their frequency of occurrence varies annually from country to country (Table 9.3). Low-level contamination rarely results in the disease developing, because between 10^5–10^7 organisms have to be ingested before development. Once infection has taken place, then large numbers of the organisms are excreted in the faeces ($> 10^8$ g^{-1}) resulting in up to 2000 organisms per 100 ml in contaminated wastewaters (Feachem *et al.* 1983). Infection can also result in a symptomless carrier-state, in which the organism rapidly develops at localised sites of chronic infection, such as the gall bladder or uterus, and

Table 9.3. The most common *Salmonella* serotypes pathogenic to humans isolated from the UK in 1965 compared to those isolated in Denmark during 1960–1968 (Dart and Stretton 1977).

UK	Denmark
S. typhimurium	*S. typhimurium*
S. heidelberg	*S. paratyphi* B
S. newport	*S. enteritidis*
S. infantis	*S. newport*
S. enteritidis	*S. typhi*
S. saint-paul	*S. infantis*
S. typhi	*S. indiana*
S. derby	*S. montevideo*
S. oranienberg	*S. blockley*
S. thompson	*S. muenchen*

is excreted in the faeces or other secretions. Carriers comprise of between 1–4% of the human population depending on the country of residence, although the number of persons excreting *Salmonella* at anyone time is never exactly known. Estimates for the USA and UK put the number of carriers at $< 1\%$ whereas in Sri Lanka it is nearly 4% (Dart and Stretton 1977). In the UK, the number of reported human cases varies annually with 5564 being reported in 1971 (Lee 1974).

The main source of infection for man is by eating infected food, particularly meat and milk, which has been contaminated during production and subsequently carelessly prepared and stored. The species most commonly implicated in food contamination are *S. enteriditis* and *S. typhimurium*, both of which can readily grow in contaminated foods. *Salmonella enteriditis* has become particularly common since the mid-1980s through the consumption of contaminated poultry and in particular eggs (Shimshony 1997). The infection pathways of *Salmonella* are more fully explored in Sec. 9.4. Since the last major outbreak of typhoid in Britain, which occurred in Croydon during the autumn of 1937 when 341 cases were reported resulting in over 40 deaths, there have been five minor outbreaks of typhoid and three of paratyphoid fever. The number of reported cases of typhoid fever in the UK has fallen to less than 200 per annum, with 85% of cases contracted abroad. Of the remainder, few are the result of contaminated

drinking water (National Water Council 1975). Although *Salmonella typhi* has been recorded from surface waters from around the British Isles (Public Health Laboratory Service 1978). There are less than 100 reported cases of paratyphoid fever reported annually (Galbraith *et al.* 1987). Typhoid has also largely been eliminated from the USA, although in 1973 there was an outbreak in Dade County in which 225 people contracted the disease from contaminated well water (Craun 1986). However, typhoid fever is still common in countries where there is neither a safe water supply nor adequate sewage treatment (Hornick 1985; Ohasi 1988). Salmonellosis carries a significant mortality amongst those with Acquired Immune Deficiency Syndrome (AIDS) and poses significant problems in its management (Wong *et al.* 1994).

The incidence of *Salmonella* infection is much higher in farm animals than in humans. Table 9.2 summarises the frequency of infection by the main serotypes involved in animal salmonellosis. Cattle (86%) and poultry (7.4%) are the most 'at-risk' groups of animals, with *S. dublin* responsible for 67%, and *S. typhimurium* for 21% of all reported cases. *Salmonella* can be isolated from perfectly healthy farm animals with 13.4% of pigs, 13.0% of cattle, and 15.0% of sheep being symptomless carriers (Grunnet and Nielsen 1969; Prost and Riemann 1967). Other commonly isolated serotypes of cattle include, *S. derby*, *S. oranienburg*, *S. java*, *S. anatum*, *S. infantis*, *S. abony*, *S. neurington*, *S. stanley*, *S. meleangridis*, and *S. chester* (Richardson 1975; Dart and Stretton 1977). Manufactured animal feed that has not been subject to pasteurization has been widely implicated in the transmission of animal salmonellosis (Skovgaard and Nielsen 1972; Williams 1975). Associated human salmonellosis has also been reported (Richardson 1975), the most recent case being the contamination of eggs by *S. enteritidis* in 1988. Due to the importation of contaminated protein used for animal feed, there has been a steady increase in the incidence of 'exotic' serotypes of *Salmonella*.

Campylobacter

Campylobacter are Gram-negative spirally shaped bacteria 2000–5000 nm in length comprising of 2–6 coils. They have a single polar flagellum giving them a characteristic darting motility. The bacteria are oxidase-positive, reduce nitrates but are unable to produce acid in the presence of carbohydrates. Although discovered in the late nineteenth century, they were not isolated from diarrhoetic stool specimens until 1972. It is only since the development of a highly selective solid growth medium, allowing culture

of the bacterium, in 1977 that its nature has been revealed (Bolton *et al.* 1982; Kist 1985). *Campylobacter* species have been isolated from both fresh and estuarine waters (Pearson *et al.* 1985) with counts ranging from 10–230 campylobacters per 100 ml in rivers in North-west England (Bolton *et al.* 1982, 1985). Although the epidemiology of human campylobacter infections remains to be fully elucidated, certain sources of infection are well established (Skirrow 1982). The pathogen causes acute gastro-enteritis (i.e. fever, nausea, adominal pains, diarrhoea and vomiting), and infection may lead to Guillan-Barré syndrome, which is an acute paralytic illness (Altekruse *et al.* 1997). There were 27,000 reported outbreaks of campylobacter enteritis in the UK during 1987 rising to over 30,000 in 1990 and it is now thought that *Campylobacter* is the major cause of gastro-enteritis in Europe, the US and other parts of the world, being more common than *Salmonella* (Andersson and Stenstrom 1986; Blaser *et al.* 1986). In the USA, the annual incidence of this organism is between 30–60 per 100,000 of the population (Skirrow and Blaser 1992). In developing countries, outbreaks of campylobacter enteritis are a major cause of morbidity and mortality in the first two years of life. Although campylobacter enteritis is essentially a food borne disease, with the most important reservoirs of the bacterium being meat, in particular poultry, and unpasturized milk, waterborne transmission has been implicated in several large outbreaks (Mentzing 1981; Vogt *et al.* 1982; Taylor *et al.* 1983; Bates *et al.* 1984). Waterborne transmission of *Campylobacter* occurs in untreated, contaminated waters, in situations where faulty disinfection has occurred or where waters have been contaminated by birds and animals (Tauxe 1992). For example, 3000 of a total population of 10,000 developed campylobacter enteritis from an inadequately chlorinated mains supply in Bennington, Vermont (Vogt *et al.* 1982), while 2000 people who drank unchlorinated mains water contaminated with faecally polluted river water in Sweden also contracted the disease (Mentzing 1981). Water is either contaminated directly by sewage which is rich in *Campylobacter* (Marcola *et al.* 1981) or indirectly from animal faeces. Household pets, farm animals and birds are all known to be carriers of the disease (Hill and Grimes 1984; Fox *et al.* 1981, 1983; Sticht-Groh 1982). There is a definite seasonal variation in the numbers of campylobacters in river water, with the greatest numbers occurring in the autumn and winter (Bolton *et al.* 1985). This is opposite to the seasonal variation of infection in the community with number of infections rising dramatically during May and June (Jones and Telford 1991). The genus comprises two distinct groups, one that grows at 42°C and are considered thermophilic which include both the important human pathogens *C. jejuni* and *C. coli*, and the other which grow only

at 25°C but not at 42°C. Serotyping of isolates confirmed that *C. jejuni* serotypes common in human infections were especially common downstream of a sewage effluent sites, confirming sewage effluents as important sources of *C. jejuni* in the aquatic environment. Gulls are known carriers of protozoan and bacterial pathogens contaminating water supply reservoirs while they roost (Graczyk *et al.* 1998) (Sec. 9.4.1). Dog faeces, in particular, are rich in the bacterium (Svedhem and Norkans 1980). In a UK study, *C. Jejuni* was isolated from 4.6% of 260 specimens of dog faeces sampled whereas *Salmonella* spp. were isolated from only 1.2% (Wright 1982a). Other studies have shown the incidence of *C. jejuni* amongst dogs to range from 7–49% (Bruce *et al.* 1980; Holt 1980). So dog faeces can cause contamination of surface waters during storms as surface runoff removes contaminated material from paved areas and roads. An outbreak affecting 50% of a rural community based in Northern Norway was identified to contaminated faecal deposits from sheep grazing the banks of a small lake which were washed into the water during a heavy storm which melted the snow on the banks. The water supply for the village came directly from the lake without chlorination (Gondrosen *et al.* 1985). Natural aquatic systems in temperate areas are generally cool and research has shown that campylobacters can remain viable for extended periods in streams and groundwaters. Survival of the bacterium decreases with increasing temperature but that at 4°C survival in excess of 12 months was possible. The incidence of campylobacter in water can be estimated by an MPN technique (Skirrow and Blaser 1992), although it does not show any correlation with counts of indicator bacteria such as faecal and total coliforms, faecal streptococci, or heterotrophic plate counts (Bitton 1999). Isolations of this organism from properly treated and disinfected waters have not been reported, thus implying that current water treatment practices are adequate for elimination of *Campylobacter* spp. (Blaser *et al.* 1982).

Shigellosis

Shigella causes bacterial dysentery or shigellosis and is one of the most frequently diagnosed cause of diarrhoea in the USA (Blasser *et al.* 1984). Bloody stools are produced as a result of inflammation and ulceration of the intestinal mucosa. Shigellosis is a problem of both developed and developing countries with the Eastern Mediterranean countries considered as an endemic region for the disease (Samonis *et al.* 1994). The bacteria of the genus are Gram-negative non-motile rods which are oxidase-negative. With the exception of *S. dysenteriae* type 1, the genus is catalase positive. The

bacterial genus is rather similar in their epidemiology to *Salmonella* except they rarely infect animals and do not survive quite as well in the environment. When the disease is present as an epidemic, it appears to be spread mainly by person to person contact, especially between children, shigellosis being a typical institutional disease occurring in over-crowded conditions. There has been a significant increase in the number of outbreaks arising from poor quality drinking water contaminated by sewage with infected individuals excreting up to 109 shigellae per gram of faeces (Samonis *et al.* 1994). It may also be carried asymptomatically in the intestinal tract. Of the large number of species (> 40), only *S. dysenteriae*, *S. sonnei*, *S. flexneri* and *S. boydii* are able to cause gastrointestinal disease. *Shigella sonnei* and *S. flexneri* account for $> 90\%$ of isolates, although it is *S. dysenteriae* type 1 which causes the most severe symptoms due to the production of the shiga toxin. The number of people excreting *Shigella* are estimated as 0.46% of the population in the USA, 0.33% in Britain and 2.4% in Sri Lanka (Dart and Stretton 1977). In England and Wales, notifications of the disease rose to between 30,000 to 50,000 per year, falling to < 3000 per annum in the 1970s. However in the 1980s, notifications have doubled to nearly 7000 per annum (Galbraith *et al.* 1987). Shigella survives for shorter periods in the environment than faecal coliforms, but due to difficulties in cultivating the organism, there is very little data on its occurrence and removal in either water or wastewater (Bitton 1999).

Leptospirosis

All the species of the genus *Leptospira* are pathogenic to man except for *L. biflexa*, which is a heterotroph found in rivers and lakes. Although there are over 100 serotypes known, it is *L. icterohaemorrhagia* that causes *Leptospira* jaundice or Weils disease. This is probably the most widely known form of the disease as it is transmitted to man via infected rats. The bacteria are characterised by being motile and comprised of very fine spirals wound so tightly that they are barely distinguishable under the microscope (Dart and Stretton 1977). Entry to the body is via abrasions of the skin or the mucous membranes where they enter the blood stream and affect the kidneys, liver, and central nervous system.

Infection is widespread in domestic and wild animals, especially rats, and the bacteria can be transferred to man either directly or indirectly via water into which infected animals have urinated. In Israel, 2.3% of cattle and 27% of dogs are carriers, although the level of infection in the human population is only 0.37% (Torten *et al.* 1970). The disease occurs throughout

Europe, North and South America, the Middle and Far East. However, in general, the incidence of leptospirosis reported is low (< 1%) except for those in high-risk occupations. Those at high-risk include persons handling animals, involved with meat-processing, or who are in contact with sewage or polluted waters; including fish farms, where the contamination occurs from the urine of infected rats. In these groups, the annual rate of infection can be as high as 3%, and up to 20% have a positive antibody reponse to the organism. *Leptospira* survives best in cold waters with low levels of organic contamination and it is from these situations that the low-risk category people are infected. It would appear, from a number of studies, that the majority of low-risk category people become infected when swimming in water heavily contaminated by the excreta of infected animals. For exam-

Table 9.4. Most frequently reported diseasese organism responsible, and incidence of infection, from the recreational use of water in the UK from 1937–1986 (Galbraith *et al.* 1987).

Disease	Organism	Incidence
Skin infections	*Pseudomonas aeruginosa*	Outbreaks associated with swimming pools and whirlpools, probably common
	Mycobacterium marinum	Probably about 100 cases since the 1960s
Conjunctivitis	Various	Not known, probably common
Gastro-intestinal infections	*S. typhi*	
	(i) contact with sewage polluted river water	12 + cases
	(ii) drinking polluted river water	61 + cases
	(iii) sea bathing	2 cases
	S paratyphi	
	(i) contact with sewage polluted river water	12 cases
	(ii) sea bathing	7 cases
	Campylobacter	One outbreak of 4 cases
	Non-specific	One outbreak of 21 cases
Respiratory infections	Adenovirus	Not known
	Mycobacteria	5 reported infections
	Legionella	One outbreak, 26 cases of legionnaires' disease and 7 of Pontiac fever
Primary amoebic meningoencephalitis	*Naeglaria fowleri*	6 cases
Leptospirosis	*Leptospira icterohaemorrhagia*	45 cases, 1978–1983 About 200, 1937–1986

ple, in The Netherlands, people who had swum in a canal contaminated by animal effluent became infected. During 1933–1948, 5% of the 983 reported cases of leptospirosis in the UK were associated with bathing, accidential immersion in water, or water sports. During the six years from 1978–1983, the proportion of cases associated with the recreational use of water or accidental immersion increased to 25%, out of a total of 177 reported cases. These were mainly bathers in freshwater ponds and streams and those engaged in water sports, such as canoeing (Waitkins 1985) (Table 9.4). Since 1986, there has been a four-fold increase in reported cases of Leptospirosis in the UK, reaching 130 cases in 1988. Nineteen of these died, 13 having contracted the disease during water sports. The increase in the incidence has been linked to (a) the explosion in the population density of rats and (b) to the increasing popularity of all water sports and of windsurfing, on both inland and coastal waters, in particular.

Escherichia coli

Most strains of the bacterium *Escherichia coli* are part of the normal microbial flora of the gastro-intestinal tract of warm-blooded animals, including man. A number of strains of *E. coli* are pathogenic and cause characteristic gastro-enteritis. Pathogenic *E. coli* are classified into four main groups based on virulence properties, clinical syndrome, epidemiology, and O:H serogrouping. These are listed below with examples of common serotypes causing gastrointestinal illness given in parentheses:

(i) Enterpathogenic *E. coli* (EPEC) (18, 26, 44, 86, 111, 114, 119, 125, 126)
(ii) Enteroinvasive *E. coli* (EIEC) (28ac, 112ac, 136, 143, 144, 152, 164)
(iii) Enterotoxigenic *E. coli* (ETEC) (6, 8, 15, 25, 27, 63, 78, 115, 148, 153, 154)
(iv) Enterohaemorrhagic *E. coli* (EHEC) (157)

A fifth category, Enteroaggregative *E. coli* (EAggEC), has also been proposed (Guerrant and Theilman 1995).

Enteropathogenic *Escherichia coli* include the serotypes that cause gastro-enteritis in man and animals, being especially serious in the newborn and in children under five years of age. Although many are harmless, it is common throughout Europe and is also thought to be the cause of 'traveller's tummy', the bout of diarrhoea that affects so many tourists who visit the warmer areas of Europe. The symptoms are profuse watery diarrhoea, with little mucus, nausea, and dehydration. The disease does

Table 9.5. The level of *E. coli* found in various animals and birds, compared with sewage and sewage effluents (Jones and White 1984).

	Faecal production g d^{-1}	Average number of *E. coli* per g faeces	Daily load *E. coli*
Man	150	13×10^6	1.9×10^9
Cow	23,600	0.23×10^6	5.4×10^9
Pig	2700	3.3×10^6	8.9×10^9
Sheep	1130	16×10^6	18.1×10^9
Duck	336	33×10^6	11.1×10^9
Turkey	448	0.3×10^6	0.13×10^9
Chicken	182	1.3×10^6	0.24×10^9
Gull	15.3	131.2×10^6	2.0×10^9
		E. coli concentration 100 ml^{-1}	
Sewage		$3.4 \times 10^5 - 2.8 \times 10^7$	
Sewage effluent		$1 \times 10^3 - 10^7$	

E. coli survival in freshwater = mean T_{90} 62.3 h

E. coli survival in seawater = mean T_{90} 2.3 h

not cause any fever and is rarely serious in adults. Up to 2.4% of children in England and Wales are thought to be carriers, although much higher percentages are found in people engaged in high-risk occupations, such as food handling. Enteropathogenic *E. coli* is commonly isolated from sewage making up between 2–8% of the total coliforms present in polluted waters (Table 9.5). However, only 100 organisms are required to cause illness. Survival of the organism is the same as for other serotypes of *E. coli* and in warm nurient-rich conditions, they are able to multiply in water. The outbreak of gastro-enteritis in Worcester in the winter of 1965–1966 affected 30,000 people, was thought to be due to contamination of the water supply because of flooding. An incident in north-east Leeds in July 1980, caused by a leaking sewer contaminating a borehole in a limestone aquifer, affected 3000 people. The failure of the chlorination system to deal with this pollution was traced to complaints from consumers close to the borehole who found the water too chlorinous to the taste, so the chlorine dose had been kept too low to deal with the resultant pollution (Short 1988). Also the water tanks on a cruise liner became contaminated by sewage resulting in an outbreak of the disease that affected 251 passengers and 51 crew (O'Mahony *et al.* 1986).

Escherichia coli 0157:H7 belongs to group (iv), and is an atypical faecal coliform which causes haemorrhagic colitis, haemolytic-uraemic syndrome and is a leading cause of kidney disease in children. In the USA, approximately 20,000 cases due to this organism are reported each year (Finelli *et al.* 1995), while 650 cases were reported in the UK during 1994 (Maule 1995). Like *Campylobacter jejuni*, this organism is generally associated with food, in particular beef and milk (Hancock *et al.* 1994; Neill 1994), but in recent years has been implicated in a number of waterborne outbreaks (Dev *et al.* 1991; Mc Gowan *et al.* 1989). However, the organism is still restricted in its distribution. For example, a survey of 1267 samples of drinking and recreational waters in Northern Greece failed to isolate the serotype and it appears not to be present in potential animal reservoirs of the disease (Arvanitidou *et al.* 1996; Kansouzidou *et al.* 1991). The number of organisms required to initiate infection is thought to be < 100 and at present, there is no specific treatment for the disease making the emergence of this new strain of *E. coli* particularly worrying (Maule 1996). In Cabool, Missouri (US) *E. coli* 0157:H7 contaminated water in the winter of 1990 after the water distribution network had been disturbed, this resulted in 240 confirmed cases of diarrhoea and 4 deaths (Geldreich *et al.* 1992). In all these outbreaks, infection was the result of faecally contaminated surface water or where inadequate disinfection had occurred.

Detection of *E. coli* 0157:H7 cannot be readily done using standard faecal coliform methodology as the organism does not grow well at 44°C in non-selective media and will not grow above 41°C in selective media. However, as it is known not to be able to ferment sorbitol at 37°C, total coliforms with a sorbitol negative result are presumptive *E. coli* O157: H7. These organisms can also be differentiated from other *E. coli* strains using phase typing or pulsed field gel electrophoreses (Reilly 1995). Final identification is done by confirming for H7 antigens (Arvanitidou *et al.* 1996; Reasoner 1992), although other techniques such as pyroysis mass spectrometry are also under investigation (Freeman *et al.* 1995). The revised standard methods for microbial analysis of drinking water (Department of the Environment 1994a) proposes a tentative method for the isolation of *E. coli* 0157:H7 based on an enrichment in modified peptone water followed by sub-culture to modified sorbitol MacConkey Agar, and selection of non-sorbitol fermenting colonies. The method was originally developed for screening milk samples and has yet to be fully evaluated for water.

Tularaemia

This disease is only endemic in North-west America, although cases have also been reported in the former USSR, and Czechoslovakia (Dart and Stretton 1977). The bacterium *Franciscella tularensis* enters the body via abrasions and mucous membranes, leaving an ulcer at the site of contact. Infection results in progressively worsening symptoms: chills and fever, swollen lymph nodes, and general malaise. Without treatment, patients suffer from delirium coma and possibly death. Most cases occur in people who have handled infected wild animals, especially rodents and rabbits, and in the USA ground squirrels are the natural vector, although contact with wood ticks can also result in infection (Bow and Brown 1946). The disease cannot be transmitted from person-to-person and multiple infection, such as the case in the Soviet Army reported by Schmidt (1948), is generally caused by contact with, or drinking water contaminated by, urine, faeces, or the corpses of infected animals.

Cholera

Cholera is thought to have originated in the Far East where it has been endemic in India for many centuries. In the nineteenth century, the disease spread throughout Europe where it was eventually eliminated by the development of uncontaminated water supplies, water treatment, and better sanitation. It is still endemic in many areas of the world especially those which do not have adequate sanitation and, in particular, situations where the water supplies are continuously contaminated by sewage. This is the cause of a major epidemic that raged through out South America which started in Peru and at the beginning of 1992. However, over the past ten to fifteen years the incidence and spread of the disease has been causing concern which has been linked to the increasing mobility of travellers and the speed of travel. Healthy symptomless carriers of *Vibrio cholerae* are estimated to range from 1.9 to 9.0% of the population (Pollitzer 1959). However, this estimate is now thought to be rather low with a haemolytic strain of the disease reported as being present in up to 25% of the population. The holiday exodus of Europeans to the Far East, which has steadily been increasing since the mid-1960s, will have led to an increase in the number of carriers in their home countries and an increased risk of contamination and spread of the disease. There were 50 reported cases of cholera in the UK between 1970–1986, although no known cases were waterborne (Galbraith *et al.* 1987). Although the disease is now extremely rare in the developed world, major waterborne outbreaks occur in developing countries, war zones

and disaster areas. For example, there were over 500,000 cases of cholera in Peru during the period 1991–1994.

Up to 10^6–10^7 organisms are required to cause the illness, so cholera is not normally spread by person-to-person contact. It is readily transmitted by drinking contaminated water either by eating food handled by a carrier or which has been washed with contaminated water. It is a widespread organism and is regularly isolated from surface waters in the UK (Lee *et al.* 1982). An infected person or symptomless carrier of the disease excretes upto 10^{13} bacteria daily, enough to theoretically infect 10^7 people! It is an intestinal disease with characteristic symptoms, that is sudden diarrhoea with copious watery faeces, vomiting, suppression of urine, rapid dehydration, lowered temperature and blood pressure, and complete collapse. Without therapy the disease has a 60% mortality rate, the patient dying within a few hours of first showing the symptoms, although with suitable treatment the mortality rate can be reduced to less than one percent.

The bacteria of the genus are Gram-negative curved or comma shaped rods that are actively motile. They are aerobic, facultatively anaerobic, oxidase-positive and able to grow at pH 8.6. *Vibrio cholerae* has been subdivided into over 80 O-serovars, although epidemic cholera is caused by toxin-producing strains of the O1 serovar. Recent evidence shows that epidemic cholera is caused by two different somatic serotypes O1 and O139 (Bhattacharya *et al.* 1993). O139 *V. cholerae* was first reported in Madras in 1992 and appears to be as virulent as the O1 serovar (Swedlow and Ries 1993). It is now widely distributed and threatens to become the eighth world-wide cholera epidemic (pandemic) (Bodhidatta *et al.* 1995; Mukhopadhyay *et al.* 1995). Other species of the genus can also cause diarrhoea although normally less severe than O1 *V. cholerae* (e.g. *V. parahaemolyticus*, *V. fluvialis* and *V. mimicus*). The bacteria are natural inhabitants of brackish and saline waters and are rapidly inactivated under unfavourable conditions such as high acidity or high organic matter content of the water, although in cool unpolluted waters *Vibrio cholerae* will survive for up to two weeks. Survival is even greater in estuarine and coastal waters.

Mycobacteria

Mycobacterium tuberculosis, *Mycobacteria balnei* (*marinum*), and *M. bovis* all cause pulmonary tuberculosis. Infection is by inhalation or ingestion of bacilli released in the sputum, milk or other discharges, including the faeces, of infected animals. The source of infection is difficult to identify as

Table 9.6. Opportunistic mycobacteria that commonly infect humans (Jenkins 1991).

Mycobacterium avium	*Mycobacterium malmoeuse*
Mycobacterium chelonae	*Mycobacterium marinum*
Mycobacterium fortuitum	*Mycobacterium scrotulaceum*
Mycobacterium intracellulare	*Mycobacterium szulgai*
Mycobacterium kansaii	*Mycobacterium xenopi*

the disease has a very long incubation period before clinical tuberculosis is diagnosed, which in some cases may be many years. However, *M. tuberculosis* is frequently isolated in wastewater from hospitals and meat-processing plants. Like *Leptospira*, the bacilli are able to survive for several weeks at low temperatures in water contaminated with organic matter. Clearly, drinking contaminated water must be a source of infection and there is considerable circumstantial evidence to support this.

Traditionally, non-tubercular mycobacteria were regarded as environmental contaminants or as transient colonizers in humans. However, they are now recognised as being opportunistic pathogens of considerable significance. Table 9.6 lists the species of opportunistic mycobacteria that commonly cause disease in man, including pulmonary disease, cervical lymphaclemopathy as well as localised and soft tissue infections (Jenkins 1991). Disease associated with these bacteria is steadily rising, particularly among patients with AIDS. Disseminated mycobacterial disease is now the third most common opportunistic terminal infection for patients with AIDS (Du Moulin and Stottmeier 1987). Newborn babies and the elderly are also particularly at risk.

It is well established that mycobacteria are commonplace in all types of aquatic environments, including estuaries, ocean water, groundwater, surface waters and distribution systems (Jenkins 1991). The majority of waterborne mycobacterial outbreaks are attributable to treatment deficiencies such as inadequate or interrupted chlorination, but other factors may also influence the growth of this organism in water supplies, such as pitting and encrustations found inside old water pipes which protect bacteria from exposure to free chlorine (Du Moulin and Stottmeier 1987). Mycobacteria can also colonize areas where water is moving slowly, as in water distribution systems in large buildings such as blocks of flats, offices and hospitals, thus continuously seeding the system (Du Moulin and Stottmeier 1987).

Detection and enumeration of mycobacteria from water samples involves the use of membrane filtration and selective and inhibitory media. The major problem in examining these organisms is the extended incubation period of up to 30 days that is required (Reasoner 1992).

Brucellosis

Brucellosis, or undulant fever, is rare in man but is common in cattle where it is known as contagious abortion. It is an extremely serious disease in cattle and can result in economic ruin for farmers whose herds become infected. The bacteria are excreted from cattle but not by humans. Therefore, *Brucella* is rarely found in sewage but is common in wastewaters from milk parlours and yards where infected cattle are kept.

Aeromonads

Aeromonas spp. have been isolated from a number of water sources, both raw and treated (Burke *et al.* 1984a,b). This organism has been implicated as the causative agent in a number of waterborne outbreaks and is now recognised as an opportunistic pathogen (Schbert 1991). Aeromonads are considered to be an important, and often fatal, cause of non-gastrointestinal illness in immuno-compromised individuals (Schbert 1991).

Aeromonads have been isolated from both chlorinated and unchlorinated drinking water supplies (Burke *et al.* 1984a,b) occurring in greatest numbers during the summer months. They have also been isolated in waters containing no *E. coli* and few total coliforms (Schbert 1991) which raises the question as to how adequate the coliform test is for evaluating water quality. Studies by Versteagh *et al.* (1989) have shown that the addition of copper to drinking water considerably reduces the number of Aeromonads present in a sample.

In addition to these organisms, which are considered as emerging problems for water quality, Reasoner (1992) has also identified a number of other organisms which may become significant as waterborne pathogens in the future (Table 9.7). Present standards relating to the microbiological quality of water do not take into account the potential pathogenicity of these organisms. Although many opportunistic organisms present no threat to healthy individuals, they pose a high health risk to the more vulnerable members of the community i.e. the elderly, the very young and the immuno-compromised. As these groups within the population are steadily increasing, it is likely that in the future, greater emphasis will have to be placed on opportunistic bacterial populations. Reasoner (1992) argues that

Table 9.7. Emerging and potential waterborne opportunistic pathogens (Reasoner 1992).

Organism	Old/Emerging	Potential candidates
Anaerobiospirillum succiniciproducens		X
Aeromonas spp.	X	
Campylobacter spp.	X	
Escherichia coli 0157:H7	X	
Helicombacter pylori		X
Mycobacterium spp.	X	
Plesimonas shigelloides		X
Vibrio fluvialis		X

there is a tendency to look for a single causative agent for a waterborne outbreak. In the future, he concludes, it is likely that 'the collective impact of simultaneous and repeated exposures to variable levels of several opportunistic bacteria will be considered, rather than the effects of an individual organism'.

Perhaps of greatest concern in relation to these organisms is that the coliform count does not reflect their incidence in water. In addition to the public health significance, the presence of these emerging organisms indicates an inadequacy in the barriers in place to protect public health, leading to the exposure of the population to potentially polluted waters.

Helicombacter pylori

In recent years a bacterium, *Helicombacter pylori*, has been identified as causing peptic ulcers, chronic type B gastro-enteritis, and as a risk factor for gastric cancer, gastric lymphoma, and coronary heart disease. The bacteria has been isolated in a wide range of natural waters as well as drinking water and wastewater using PCR. There is evidence that it can be transferred via drinking water and food (Hopkins *et al.* 1993). While its natural niche is the human stomach, it needs to survive in the natural environment in order to be a life-long infection. However, nothing is known of its survival or ecology in the natural environment (Hegarty *et al.* 1999). Yet it is estimated that half of the world's population suffers from *H. pylori* infection (Malaty *et al.* 1996). It has been widely reported in surface waters throughout the world including Mexico (Mazari *et al.* 2001), Sweden (Hulten *et al.* 1998), and the USA (Hegarty *et al.* 1999). The organism is readily eliminated

Table 9.8. Opportunistic bacterial pathogens isolated from drinking water (Reasoner 1992).

Acinetobacter spp.
Achromobacter xylosoxidans
Aeromonas hydrophila
Bacillus spp.
Campylobacter spp.*
Citrobacter spp.
Enterobacter aerogenes
E. agglomerans
E. cloacae
Flavobacterium meningusepticum
Hafnia alvei
*Klebsiella pneumoniae**
*Legionella pneumopila**
Moraxella spp.
Mycobacterium spp.
Pseudomonas spp. (non-aeruginosa)*
Serratia fonticola
S. liquefaciens
S. marcescens
Staphylococcus spp.
*Vibrio fluvialis**

*Indicates that the organism may be a primary pathogen.

from drinking water by correct chlorination (Johnson *et al.* 1997) or other disinfection procedures.

Opportunistic bacterial pathogens

These opportunistic bacteria are usually found as part of the normal heterotrophic bacterial flora of aquatic systems (Reasoner 1992) and may also exist as part of the normal body microflora (Table 9.8) (Sec. 9.3.8). These organisms are normally not a threat to healthy individuals. However, under certain circumstances they can lead to infection in certain segments of the community, particularly newborn babies, the elderly and the immunocompromised (Bitton 1994). It is thought that numerous hospital-acquired

infections are attributable to such organisms (De Zuane 1990). Payment *et al.* (1993) carried out an eighteen-month epidemiological study of gastrointestinal illness on a number of families. The drinking water consumed met current bacteriological and physico-chemical quality standards, but a significant level of gastrointestinal illness was reported. A weak association between the level of illness and heterotrophic bacterial numbers was observed. Further analysis revealed that bacteria growing at 35°C were responsible for the observed effects. Observations such as these suggest that bacteria generally considered harmless may in fact be disease causing which raises concerns about the safety of bacterial growth within the distribution system, particularly as HPC counts in excess of 500 cfu/ml tend to mask coliform occurrences. It is difficult to assess the health implications of these organisms for a number of reasons:

(i) The lack of data on the occurrence and densities of these organisms in water.
(ii) The lack of data on infectious doses required to establish infection.
(iii) The lack of data on the incidence of human disease caused by waterborne exposure to such organisms.
(iv) The interactive effects of exposure to mixed types and densities of these organisms.
(v) The range of susceptible individuals in the exposed population.
(vi) The effectiveness of treatment procedures and post-disinfection for control of these agents.
(vii) The need for good detection methodologies that would allow adequate surveillance and monitoring for such organisms.

9.2.3. *Viruses*

There are over 140 distinct known types of human pathogenic viruses. Of most concern are those which cause gastrointestinal illness known as the enteric viruses which includes enteroviruses, rotaviruses, astroviruses, calciviruses, hepatitis A virus, Norwalk virus and other 'small round' viruses (West 1991). As enteric viruses are usually gut-associated, the illnesses they cause are primarily gastro-intestinal in nature (Sellwood and Dadswell 1991). However, the health risks presented by these viruses are not just restricted to gastro-enteritis (Table 9.9). Many of the entroviruses such as reovirus, coxsackievirus and echovirus, cause respiratory infections and are present in the faeces of infected people. Poliomyelitis in particular is common in sewage due to the vaccination programme within communities, but

Table 9.9. Common waterborne enteric viruses and the disease they cause (Bitton 1994).

Virus group	Serotypes	Some diseases caused
Enteroviruses		
Polioviruses	3	Paralysis, aseptic meningitis
Coxsackievirus		
A	23	Herpangia, aseptic meningitis, respiratory illness, paralysis, fever
B	6	Pleurodynia, aseptic meningitis, pericarditis, congenital heart disease anomalies, nephritis, fever
Echovirus	34	Respiratory infection, aseptic meningitis, diarrhoea, pericarditis, myocarditis, fever and rash
Enteroviruses (68–71)	4	Meningitis, respiratory illness
Hepatitis A virus (HAV)		Infectious hepatitis
Reoviruses	3	Respiratory disease
Rotaviruses	4	Gastro-enteritis
Adenoviruses	41	Respiratory disease, acute conjunctivitis, gastro-enteritis
Norwalk agent (calcivirus)	1	Gastro-enteritis
Astroviruses	5	Gastro-enteritis

does not indicate actual infection. In fact virological examination of sewage has been used to document the effect of vaccination campaigns as those who are vaccinated with the live attenuated oral poliovirus vaccine shed faecal virus for a considerable time afterwards (Pyry *et al.* 1988). However, during outbreaks, wild poliovirus will also be identified in sewage and in waters receiving treated and untreated sewage (Avoort *et al.* 1995). Adenovirus 3 is commonly associated with swimming pools and can also cause pharyngo-conjunctival fever. In addition to those listed in Table 9.9, coxsackie B virus has been associated with Myalic Encephalomyelitis (ME), acute myopericarditis and dilated cardiomyopathy, a chronic cardiac disease which is the second most common reason for cardiac transplants in the UK (Watkins and Cameron 1991).

Human viruses present in sewage are almost entirely derived from faecal matter. Viral contamination arises when sewage containing pathogenic viruses contaminates surface and ground waters which are subsequently used as sources of drinking waters (West 1991). Large outbreaks of viral disease occur when massive sewage contamination takes place overwhelming existing water treatment mechanisms. Infectious hepatitis, entroviruses, reovirus and adenovirus are all thought to be transmitted via water. Of most concern in Europe is viral hepatitis. There are three subgroups: Hepatitis

A Virus (HAV) which is transmitted by water; hepatitis B which is spread by infected blood or sexual contact is endemic in certain countries such as Greece; and hepatitis C which is a non- A or B type hepatitis virus (Moe 1997).

Hepatitis A is a 27 nm RNA entrovirus that is spread by faecal contamination of food, drinking water, and areas that are used for bathing and swimming (Jehl-Pietri 1992). Epidemics have been linked to all these sources, and it appears that swimming pools and coastal areas used for bathing which receive large quantities of sewage are particular sources of infection. The virus can be replicated using tissue culture techniques although gene probes including PCR (polymerase chain reaction) are increasingly used (Altmar *et al.* 1995). Hepatitis A outbreaks usually occur in a cyclic pattern within the community, as 'once infected' the population is immune to further infection by the virus. So no new cases occur for 5 to 10 years until there is a new generation (mainly of children) which has not been previously exposed. There is no treatment for hepatitis A, with the only effective protection good personal hygiene, and the proper protection and treatment of drinking water. Immune globulin is often given to prevent the illness developing in possible contacts, although it is not always successful. Symptoms develop 15 to 45 days after exposure and include nausea, vomiting, muscle ache, jaundice, and liver damage. Hepatitis A virus accounts for 87% of all viral waterborne disease outbreaks in the USA (Craun 1986). In June 1979, a large waterborne outbreak of gastro-enteritis and hepatitis occurred in Georgetown, Texas, affecting approximately 79% of individuals supplied by the contaminated water following a period of heavy rainfall that washed sewage into the ground water supply. The best documented outbreak of waterborne viral disease occurred in New Delhi, India in 1955/56, when 35,000 cases of infectious hepatitis were reported following gross contamination of the water supply by sewage (Dennis 1959). Brugha *et al.* (1998) reported that those who are regularly exposed to sewage have a significant risk factor for HAV infection and should be vaccinated. However, using a saliva test to detect antibodies to HAV (antiHAV), Trout *et al.* (2001) failed to identify a significant occupational risk in wastewater treatment workers for antiHAV.

Warm-blooded animals appear able to carry viruses pathogenic to man. For example, 10% of beagles have been shown to carry human enteric viruses; therefore, there appears a danger of infection from waters not contaminated by sewage but by other sources of pollution, especially storm water from paved areas. Most viruses are able to remain viable for several weeks in water at low temperatures, so long as there is some organic matter

Table 9.10. Examples of waterborne outbreaks due to the Norwalk virus (Bitton 1994).

Year	Location	No. reported ill	Remarks
1978	Tacoma, WA	600	Contaminated well
1979	Arcota, CA	30	Contaminated sprinkler system
1980	Maryland	126	Contaminated well
1980	Rome, GA	1500	Contaminated community water supply
1982	Tate, GA	500	Contaminated well and spring

present. Viruses are found in both surface and groundwater sources. In the USA as many as 20% of all wells and boreholes have been found to be contaminated with viruses. Two viruses which have caused recent outbreaks of illness due to drinking water contamination are Norwalk virus and rotavirus (Cubitt 1991; Craun 1991).

Norwalk virus results in severe diarrhoea and vomiting. It is of particular worry to the water industry in that it appears not to be affected by normal chlorination levels. Also it seems that infection by the virus only gives rise to short-term immunity, while lifelong immunity is conferred by most other enteric viruses. In 1986 some 7000 people became infected with a Norwalk-like virus who stayed at a skiing resort in Scotland. The private water supply, which was untreated, came from a stream subject to contamination from a septic tank. Table 9.10 lists some examples of Norwalk associated waterborne outbreaks. The largest of these outbreaks occurred in Rome, Georgia (USA) in 1980, when contaminated water from a textile factory came into contact with a community water supply. The largest viral associated outbreak of gastro-enteritis in Britain occurred in Branham in 1980 when over 3000 cases were reported. This incident occurred when source boreholes became contaminated by sewage (Short 1988). Norwalk virus is a very small 27 nm virus originally discovered in Norfolk, Ohio. Most detection methods are not sensitive for environmental monitoring, although it can be detected in stools and shellfish using reverse transcriptase-polymerase chain reaction (RT-PCR) (DeLeon *et al.* 1993; Altmar *et al.* 1995).

Rotavirus is a major contributor to child diarrhoea syndrome. This causes the death of some six million children in developing countries each year. This is not, thankfully, a serious problem in Europe due to better hygiene, nutrition, and health care. Outbreaks do occur occasionally in hospitals, and although associated with child diarrhoea, can be much more serious if contracted by an adult. A large outbreak of gastro-enteritis affecting 900 people due to contamination of a water supply by sewage containing

rotaviruses was reported in Arizona in 1991. Other major outbreaks include 11,600 infected in East Germany in 1981–1982 when flood water contaminated wells, and a year later in China when 13,311 were infected with rotavirus due to a contaminated water supply (Williams and Akin 1986). Details of detection methods are given by Gajardo *et al.* (1995). The virus is a 70 nm particle containing double-stranded RNA that is surrounded with a double-shelled capsid. The enteric adenovirus serotypes 40 and 41 are almost as important as rotavirus as aetiological agents of viral gastroenteritis in children (Cruz *et al.* 1990). These viruses are 80 nm particles comprising of a non-enveloped double-stranded DNA. Although they have not been confirmed as waterborne pathogens, they are more stable in water than either poliovirus 1 and hepatitis A virus, and are able to survive conventional sewage treatment more effectively than other enteroviruses. They are widespread in water and so it is likely that adenoviruses are indeed waterborne pathogens (Enriques and Gerba 1995). Astroviruses are another common cause of gastro-enteritis in young children, with 75% of those aged between 5–10 years shown to have the astrovirus antibody in the UK (Kurtz and Lee 1978), with astrovirus 1 the major infection causing serotype (Lee and Kurtz 1994). As with bacterial infections, many incidents of viral disease associated with drinking water have been attributable to untreated or inadequately treated water or to defects within the distribution system (Craun 1988). Gerba and Rose (1990) have produced an excellent review on viruses in source and drinking waters.

Outbreaks of waterborne viral disease, other than infectious hepatitis, are difficult to recognise because viruses tend to cause non-apparent or latent infections (Tyler 1985). Each year a large percentage of reported cases of waterborne disease are of unknown aetiology (Herwaldt *et al.* 1992; Galbraith *et al.* 1987). One possible explanation for such defects in the data is that epidemiological methods are not adequate enough to detect low level transmission of viral diseases *via* water. This is because a single viral type may produce a wide variety of symptoms which may not be attributable to a single aetiologic agent, also different viruses can produce similar symptoms (Tyler 1985). Therefore it can be almost impossible to establish the original cause of the outbreak.

Viruses are usually excreted in numbers several orders of magnitude lower than that of coliforms (APHA 1992). Because they only multiply within living susceptible cells, their numbers cannot increase once excreted. Once in a cell free state, their survival and infectivity in the aquatic environment depends on a variety of biotic (i.e. type of virus, bacterial and algal activity and predation by protozoa) and abiotic factors (i.e.

temperature, suspended matter, pH, salinity, ultra-violet light penetration, organic compounds, adsorption to suspended matter and aggregation) (Geldenhuys and Pretorius 1989). Temperature is considered to be the most important factor influencing viral destruction outside the host cell. They are rapidly inactivated once exposed to temperatures in excess of 50°C (Bitton 1978). Suspended solids provide a certain degree of protection for viruses. Absorption in organic matter can prevent inactivation by U.V. light. Once adsorbed the viruses can settle from suspension and survive for long periods in sediments to become re-suspended if the water becomes turbulent (Watkins and Cameron 1991).

Sewage treatment, virus dilution, natural inactivation, water treatment, and other factors combine to reduce viral numbers to a few survivors in large volumes of water (Metcalf 1978). In reality viruses generally pass unaffected through wastewater treatment plants and so will be found in surface waters receiving both treated and untreated sewage. In the more developed regions of the world, the possibility of viral transmission of waterborne disease depends on the ability of minimum quantities of virus causing infections (APHA 1992). The minimum infectious dose for many infectious viruses is unknown but is thought to be as low as 1–10 infectious units (Watkins and Cameron 1991). It is critical, therefore, that small numbers of virus be detected from relatively large volumes of water. In addition, viral detection methods should satisfy the following criteria: (i) They should have a high virus concentration factor (1000–10,000) with a high efficiency of recovery for very low concentrations of viruses. (ii) They should be able to concentrate and detect all enteric virus types with equally high efficiency. (iii) The method should be relatively inexpensive (Metcalf 1972).

There are a number of methods available for the detection of viruses; however, none satisfy all of the above criteria. Even the most recent methods are continually being modified and changed. None of the available techniques have been adequately tested with all viral types of public health importance. Recovery rates may vary depending on water quality. In addition, some methods require large pieces of equipment for sample processing. Identification procedures require cell cultures and related laboratory facilities — such requirements are generally beyond the financial and technical capabilities of most laboratories (APHA 1992). Detecting viruses in water through the recovery of infectious virus involves three steps: (i) Collecting of representative samples. (ii) Concentrating the viruses in the sample. (iii) Identifying and estimating quantities of the concentrated virus (APHA 1992).

Viral numbers are generally so low that their detection is virtually impossible unless they are first concentrated. Many of the concentration techniques available are based on one of two principles; either the filter adsorption-elution systems or ultra filtration systems (Sobsey 1975).

Filter adsorption-elution systems are based on the fact that viruses present in water containing little or no organic matter will adsorb the surfaces of microporous cellulose ester or fibreglass filters under conditions of low pH or in the presence of polyvalent cation salts or both (Sobsey 1975). Filters may be electropositive or electronegative. Adsorbed viruses are then eluted from the filter surface by pressure-filtering a small volume of eluent fluid through the filter in-situ (APHA 1992). Despite certain limitations, this technique is considered to be the most promising and is the most widely-used technique for virus concentration. The basis for virus concentration by ultrafiltration is that water and microsolutes can be driven through a microporous membrane by pressure while microsolutes such as viruses and other high molecular weight materials are retained and thereby concentrated (Sobsey 1975). The ultrafiltration process described in Standard Methods involves placing the sample in a cellulose dialysis bag and exposing it to Polyethylene Glycol (PEG), a hygroscopic material. The viruses retained in the dialysis bag are recovered by opening the bag, collecting the remaining sample and eluting any viruses that may have been adsorbed onto the inner walls of the bag. The collected concentrate and eluate are combined and assayed for viruses (APHA 1992).

After removal of any sample toxicity, viruses may be isolated using tissue culture assays (West 1991). At present there is no single host system that can be used for all enteric viruses and some, notably the hepatitis A virus, the human rotavirus and Norwalk-like viruses, cannot be assayed using routine tissue cultures (APHA 1992). Advances in immunochemistry, tissue culture and genetic engineering have seen the development of more rapid techniques for the identification of viruses. Such techniques include immunofluorescence, radio-immunoassay (RIA), and enzyme-linked-immunesorbent-assay (Table 9.11).

As with bacteria, the development of gene probes for specific viruses has introduced the potential for fast and sensitive assays. Specific probes already exist for rotaviruses, enteroviruses and adenoviruses (Watkins and Cameron 1991). Gene probes have been found to be equally, if not more sensitive than, tissue culture techniques. They have the added advantage of not relying on tissue culture and therefore offer a cheaper alternative. At present the main limitations associated with using gene probes for virus detection is that there is no distinction made between viable and non-viable

Table 9.11. Rapid detection techniques for enteric viruses (Gerba *et al.* 1989).

Technique	Assay time (hrs)	Detection limit (pfu)	Max. vol. which can be assayed (mL)	Comment
Immuno-electron microscopy (IEM)	2–4	10^5–10^6	0.05	Requires specific antibody against each virus type
Direct immuno-fluorescence (IF)	2–4	10^5–10^6	0.1	
IF of infected cell culture	24–48	1	0.5–1.0	
Enyzme linked immuno-sorbent assay (ELISA)	2–4	10^3–10^5	0.2	
Radio-immuno-assay (RIA)	2–4	10^4–10^5	0.2	
Gene probe	24–48	1	30–100	One probe can detect related viruses

cells. Also, they cannot determine the infectivity of the viruses. However, it is important to remember that the use of gene probes for viruses is relatively recent and initial problems are to be expected.

For many years chlorination was considered to be effective in preventing contamination of water supplies by viruses. This conclusion was drawn from the results of epidemiological studies, where it has been repeatedly shown that outbreaks due to viral contamination occurred largely in situations where there was inadequate or no chlorination (Galbraith *et al.* 1987; Herwaldt *et al.* 1992). However, in recent years there has been a noticeable change in this situation. Enteric viruses have been isolated from drinking waters which have been treated by chlorination or other processes such as ozonation and chemical coagulation. Such drinking waters contain chlorine levels originally thought to be virucidal (0.1–0.3 mg l^{-1}) (West 1991). Initially the ability to survive chlorination was thought to be due to a lack of contact time with chlorine (Melnick and Gerba 1980). It is now well established that some enteric viruses are more resistant to chlorination than coliforms (Shaffer *et al.* 1980). Peterson *et al.* (1983) showed that exposure to over 2 mg l^{-1} of chlorine for 30 minutes was required to inactivate the infectivity of the hepatitis A virus, while exposure to as much as 5–6 mg l^{-1} chlorine for 30 minutes may be required to destroy the infectivity of the Norwalk virus (Keswick *et al.* 1985). It has been suggested that such

resistance may be due to the protective effect of viral aggregation (West 1991).

Enteroviruses have been recovered from waters which are free from indicator organisms. There has been considerable debate over whether or not there is continual low-level viral contamination of drinking water which subsequently results in sporadic viral infections among consumers (Sellwood and Dadswell 1991). Such outbreaks largely go undetected due to the large proportion of asymptomatic infections and varied symptomatology in those individuals experiencing such infections (Feachem *et al.* 1983). This view, while popular in the USA has not afforded much attention in Europe where it is considered that there is no evidence to substantiate the existence of low level transmission (Feachem *et al.* 1983; Sellwood and Dadswell 1991). The possibility of low level transmission has considerable significance in terms of determining the level of viruses allowable in drinking waters and more especially the costs involved in implementing stricter viral standards, particularly in developing countries where there is a severe shortage of financial and technical resources (Feachem *et al.* 1983).

9.2.4. *Protozoa*

Protozoan pathogens of man are almost exclusively confined to tropical and sub-tropical areas, which is why the increased occurrence of *Cryptosporidium* and *Giardia* cysts in temperate areas is causing so much concern. However, with the increase in travel, carriers of all diseases are now found worldwide, and cysts of all the major protozoan pathogens occur in European sewages from time to time. Two other protozoan parasites that occur occasionally in the UK are *Entamoeba histolytica* which causes amoebic dysentery and *Naegteria fowleri* which causes the fatal disease amoebic meningo-encephalitis.

Giardiasis

Giardia lamblia is a flagellated protozoan belonging to the class Zomastigophorasida, order Diplomonadonda and family Hexamitidae. It was first recorded in 1681 by Leeuwenhoek who discovered the organism in his own stools. It was later named *Giardia lamblia* after Giard who studied the parasite and Lamb who first described it. For more than a century after its initial discovery, the pathogenic potential of *Giardia* was not fully appreciated. Indeed up to the 1960s, it was widely believed to be a commensal parasite of doubtful pathogenicity. It is now recognised that this organism

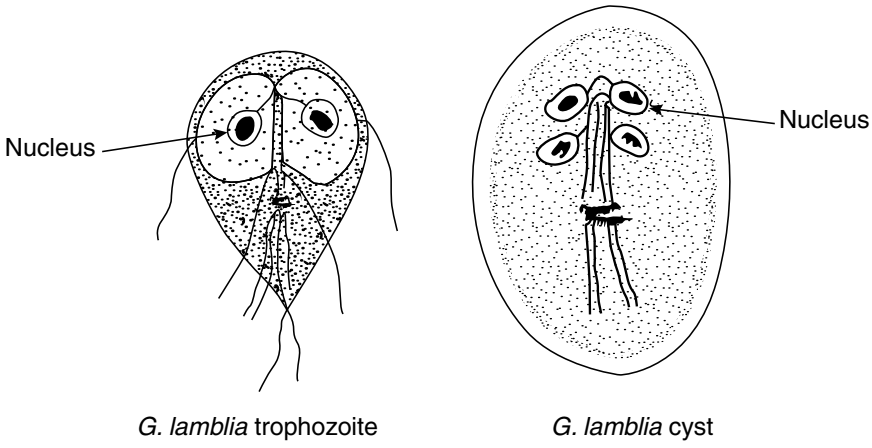


Fig. 9.1. Typical trophozite and cyst of *Giardia lamblia* (Lin 1985).

is a significant cause of gastro-enteritis ranging from mild to severe and disabilitating disease with a worldwide distribution (Akin and Jakubowski 1986).

Giardia lamblia exists in a trophozoite and cyst form. Trophozoites are easily recognised. Their bodies are pear or kite shaped, approximately 9–21 μm long by 6 μm wide with an anterior sucking disc on the flattened ventral surface (Fig. 9.1). There are four pairs of flagella and the organism is binucleate (Feachem *et al.* 1983). Cysts are ovoid, 14–16 μm long and 6–12 μm wide and are quadrinucleate. *Giardia* cysts are relatively resistant to environmental conditions and are capable of survival once excreted for long periods, especially in winter.

The life cycle of *G. lamblia* is discussed in considerable detail by Lin (1985). In brief, the life cycle begins when an ingested cyst passes into the stomach of an exposed individual. Excystation follows with the resulting trophozoite attaching itself to the epithelium of the small intestine by an adhesive disk. The trophozoite multiplies by binary fission to large numbers which move down the intestinal tract transforming into cysts in the ileum. The cysts are passed from the body in the stools (Fig. 9.2) (Akin and Jakubowski 1986). Between $1–5 \times 10^6$ cysts may be shed by an infected person per gram of faeces (Lin 1985).

The symptoms of giardiasis, or backpackers' disease as it is commonly known in the USA due to its high incidence amongst those who drink unfiltered and non-disinfected water from mountain streams, develops between 1–4 weeks after infection. The predominant clinical feature associated with giardiasis is severe diarrhoea, occurring in 50% of those infected by the

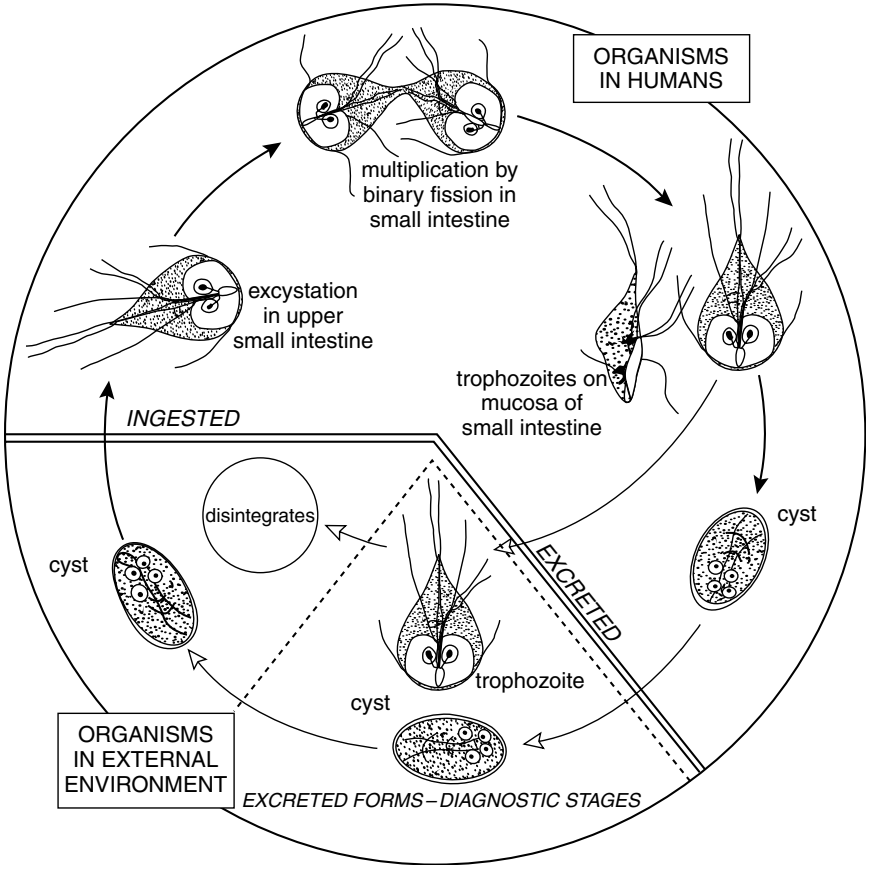


Fig. 9.2. Life cycle of *Giardia lamblia* (Lin 1985).

pathogen. Other symptoms range from malaise, weakness, fatigue, dehydration, weight loss, distension, flatulence, anorexia, cramp like abdominal pain, and epigastric tenderness to steatorrhea and malabsorption (Lin 1985). Generally giardiasis occurs only as a mild disease but it can develop into a serious illness. It has a world-wide distribution with a prevalence of about 7% and is three times more common in children than in adults (Ellis 1989). Asymptomatic giardiasis may also occur (Akin and Jakubowski 1986). Usually only the symptomatic patient is treated and there are a number of drugs available for this (Lin 1985).

The exact infectious dose necessary for infection is not exactly known but it is thought to be somewhere between 25 and 100 cysts (Feacham *et al.* 1983; Ellis 1989).

Transmission of *Giardia* cysts may be by faecal contamination of either hands, food or water supplies (Feacham *et al.* 1983). Originally cysts were thought to be host specific. However, it is now well established that the disease is a zoonosis. Cysts from a human source can be infective to about 40 different species of animal including guinea-pigs, dogs, beavers, racoons and big horn montflou sheep and *vice versa* (Faubert 1988). Cysts are therefore widely distributed in the environment entering waterways through sewage or storm water discharges or via the droppings of infected animals. A study of raw water supplies in the USA by Le Chevallier *et al.* (1991a) found that *Giardia* cysts are present in as many as 81% of raw water supplies largely due to the introduction of sewage effluents and in 17% of finished water supplies (Le Chevallier *et al.* 1991b). A similar survey in Scotland found that 48% of raw waters and 23% of treated water supplies sampled contained cysts (Gray 1994). Waterborne transmission of giardiasis is well documented (Lin 1985; Akin and Jakubowski 1986) and is now established to be one of the most common causes of waterborne diseases in the developed world. It is particularly common in the USA where it is now considered to be endemic with a carrier rate of 15–20% of the population, depending on their socio-economic status, age and location. *Giardia* is the most common animal parasite of humans in the developed world, although water is probably not the most common mode of transmission. However, *Giardia* remains one of the most common causes of waterborne diseases. In the US, *Giardia lamblia* causes more waterborne disease than any other identifiable aetiologic agent with more than 697 outbreaks reported between 1989 and 1990 (Herwaldt *et al.* 1992) The first major documented outbreak of giardiasis associated with water supply occurred in Rome, New York (USA), with approximately 50,000 people affected. The outbreak was a result of consumption of water that had been chlorinated but not filtered. The number of reported cases in England and Wales has risen from 1000 per annum in the late 1960s to over 5000 per annum by the late 1980s, although these were largely associated with people travelling overseas. The number of outbreaks in the UK associated with drinking water contamination is steadily increasing with the most significant outbreak of giardiasis occurring in South Bristol (UK) in the summer of 1985 when 108 cases were reported (Jephcote *et al.* 1986; Browning and Ives 1987). It is thought that contamination of the supply occurred in the distribution system and was not due to any failure of the water treatment process. Worldwide, Giardia is estimated to be responsible for one million severe cases a year and ten million mild cases of giardiasis per annum (Smith 1996).

Craun (1979) indicated that most *Giardia* outbreaks occur in waters where chlorination is the only form of water treatment. This resistance to disinfection levels typically used in water treatment indicates the need for additional treatment barriers. In recognition of this, recent amendments to the USA Safe Drinking Water Act now requires that all surface waters intended for human consumption must undergo filtration to specifically

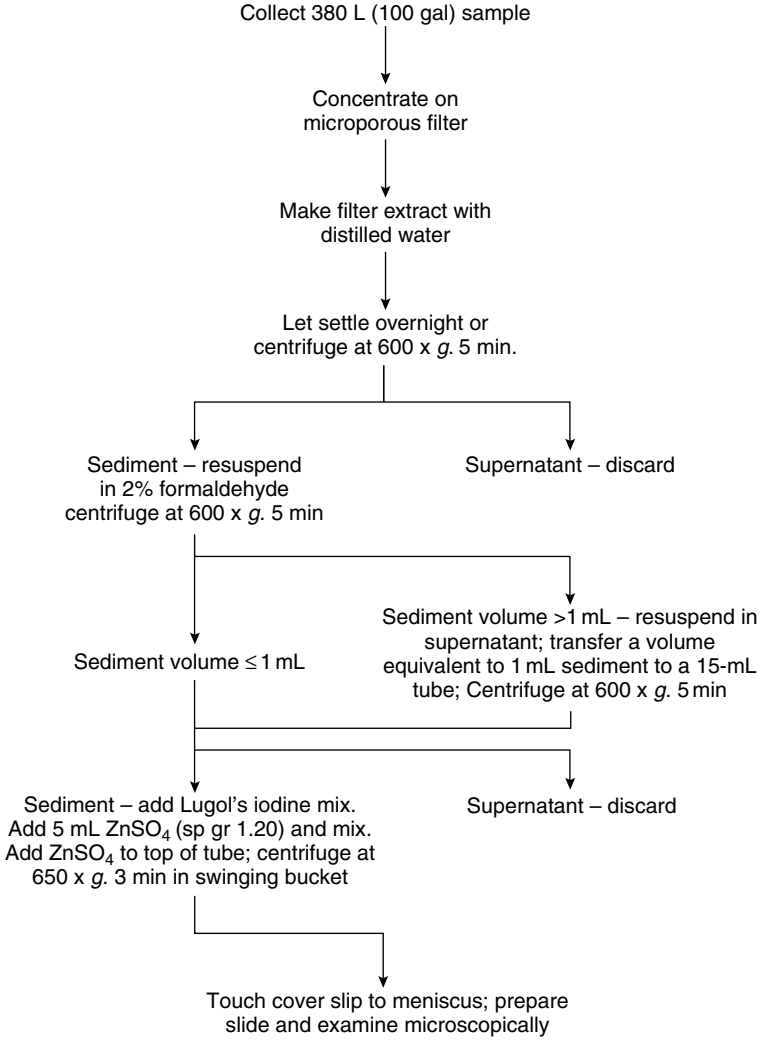


Fig. 9.3. Method for isolating *Giardia lamblia* (APHA 1992).

remove cysts and sufficient disinfection to destroy *Giardia* and prevent disease transmission. To date, no such provisions exist in European or British legislation. There is no way of preventing infection except by adequate water treatment and resource protection. Current disinfection practices are generally inadequate as the sole barrier to prevent outbreaks. Boiling water for 20 minutes will kill cysts, while the use of 1 μm pore cartridge filters to treat drinking water at the point of use are also effective.

Methods for detecting *Giardia* cysts have been available since about 1975; however, none to date have been especially successful. At present, the method most commonly used is that outlined in Standard Methods (Fig. 9.3) (APHA 1992). A similar method is recommended in Britain (Standing Committee of Analysts 1989). *Giardia* is not free living and therefore is unlikely to reproduce outside the host animal. Because of this, cysts are generally present in very low densities and so samples must be concentrated first. This is achieved by using ultra filtration cassettes or finely wound polypropylene cartridges (APHA 1992). As cyst numbers cannot be amplified by in-vitro cultivation, they are generally detected by immunofluorescence with poly- or monoclonal antibodies or by direct phase contrast microscopy (Sauch 1986; Standing Committee of Analysts 1989). More recently a cDNA probe has been constructed for the detection of *Giardia* cysts in water and wastewater (Abbazadegan *et al.* 1991). The main problem with these is that they do not allow for differentiation between viable and non-viable cells. Mahbubani *et al.*, (1991) have now developed a technique using PCR which allows some distinction between dead and viable cells, and Abbazadegan *et al.* (1997) have used a PCR amplification technique. Detection of *Giardia* and *Cryptosporidium* is reviewed by Smith (1996).

Cryptosporidiosis

Cryptosporidium spp. are coccidian protozoan parasites which belong to the phylum Apicomplexa (Barer and Wright 1990). Of the ten species that are recognised, four are of particular importance, two in mammals (*C. parvum* and *C. muris*) and two in birds (*C. baileyi* and *C. meleagridis*). *Cryptosporidium parvum* is the species primarily responsible for clinical illness in humans and animals (Rose 1988; Fayer *et al.* 2000). Despite its discovery early in 1912, the first case of human infection was not recorded until 1976 (Gray 1994). *Cryptosporidium* is now recognised as being a significant cause of gastro-intestinal disease in human beings, particularly in children and immuno-compromised individuals.

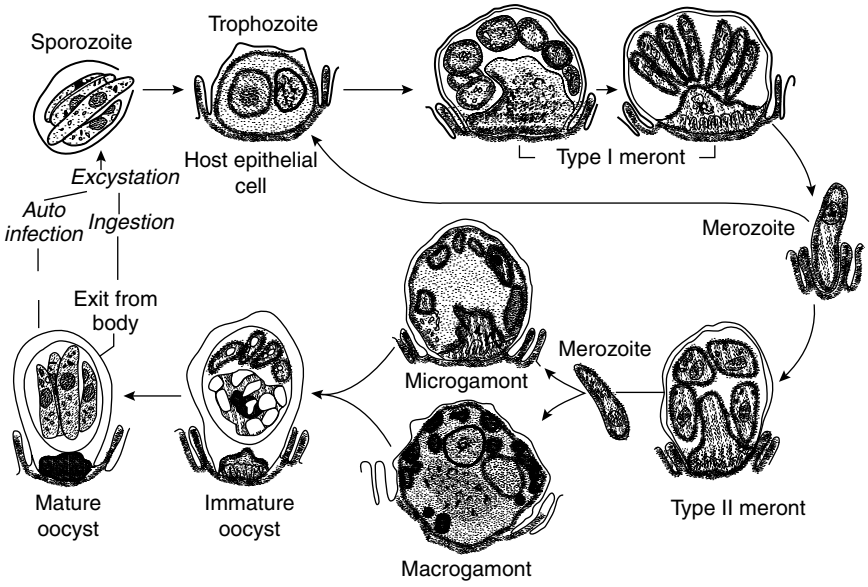


Fig. 9.4. Life cycle of *Cryptosporidium parvum* (Fayer and Ungar 1986).

Detailed reviews of the biology and life cycle of *Cryptosporidium parvum* can be found in Rose (1988) and in Fayer and Ungar (1986). In brief, the parasite has a complex life cycle involving both sexual and asexual stages (Fig. 9.4). Following ingestion, the infectious stage, i.e. the oocyst, releases 4 sporozoites after excystation. The sporozoite differentiates into the trophozoite which undergoes asexual multiplication to form Type 1 meronts and then merozoites. It is during asexual reproduction that recycling of the merozoite stage occurs. The sexual stage occurs when merozoites from Type 2 meronts produce microgametes and macrogametes, which fuse and form the zygote which is then excreted in the faeces (Rose 1988). About 80% of zygotes produce are thick-walled oocysts which are immediately infectious when excreted. These oocysts are very resistant and are capable of survival outside the intestine for considerable lengths of time (Smith and Rose 1990). The remaining 20% are thin walled and are responsible for auto-infection. This permits *Cryptosporidium* to complete its life cycle within a single host, so once infected the host becomes a life-time carrier and subjected to relapses ensuring that high number of oocysts are continually being produced (Fayer and Ungar 1986).

Cryptosporidiosis is acquired by ingesting viable oocysts (Barer and Wright 1990). In immuno-competent individuals cryptosporidiosis is a

common cause of acute self-limiting gastro-enteritis with symptoms commencing on average 3–4 days after infection and lasting for up to two weeks. Clinical symptoms include an influenza-like illness, diarrhoea, malaise, abdominal pain, anorexia, nausea, flatulence, malabsorbtion, vomiting, mild fever and weight loss. Generally this disease is not fatal among healthy individuals. However, in young malnourished children, it can cause severe dehydration and sometimes death. Infection in the immuno-competant has been described world wide with a prevalence of 0.6–20% reported in Western countries and 4–20% in developing countries (Smith and Rose 1990).

In the immuno-compromised, including those with AIDS, other acquired abnormalities of T lymphocytes, congenital hypogammaglobulinaemiam, severe combined immunodeficiency syndrome, those receiving immunosuppressive drugs, and those with severe malnutrition, cryptosporidiosis can become a life-threatening condition causing profuse intractable diarrhoea with severe dehydration, malabsorption and wasting. Sometimes the disease spreads to other organs. These symptoms can persist unabated until the patient eventually dies. Among AIDS patients cryptosporidiosis has a prevalence of 3–4% in the USA and greater than 50% in Africa and Haiti (Smith and Rose 1990). A study on the filtration of drinking water in California (USA) has shown that AIDS sufferers are no more at risk of contracting cryptosporidiosis than the rest of the population from the presence of the pathogen in drinking water, the higher incidence being linked to sexual transmission of the disease between homosexual and bisexual men (Sorvillo *et al.* 1994). However, the benefit of filtration of drinking water to prevent outbreaks of the disease is well established, especially during periods of heavy *Cryptosporidium* contamination. In the UK, cryptosporidiosis is currently the fourth most commonly identified cause of diarrhoea in which a parasitic, bacterial or viral cause was established. Approximately 2% of all current cases of diarrhoea in the UK are due to *Cryptosporidium*, with children more at risk than adults (Department of the Environment and Department of Health 1990).

Little is known of the exact infectious dose size, but it is thought to be small, probably less than 10 oocysts (Casemore 1990). More recent studies indicate that a single oocyst may be enough to cause infection (Blewett *et al.* 1993), although outbreaks of cryptospiriodiosis have been associated with gross contamination (Wilkins 1993). Messener *et al.* (2001) have carried out dose-response studies to calculate the risk of infection for a range of different *C. parvum* isolates.

At first cryptosporidiosis was thought to be a zoonosis contracted by direct contact with animals especially young farm animals and their faeces.

Table 9.12. Factors that favour the development of waterborne cryptosporidiosis.

Lack of species specificity
Close association between animal and human hosts
Low infective dose
Monoxenous development with autoinfective cycle
Large numbers of oocysts excreted
Fully sporulated oocysts excreted
Oocysts are environmentally robust
Oocysts are chlorine and UV insensitive
Small size of oocysts

The organism is not host specific and is capable of infecting many species of mammal, bird and reptile (Packham 1990). To date, *C. parvum* has been isolated from 152 different species of mammals (Fayer *et al.* 2000). The disease is now known to readily spread from person to person, especially among young children (Department of the Environment and Department of Health 1990). Oocysts from humans are infective for numerous mammals (Fayer and Ungar 1986). Both foodborne and airborne routes have been documented, but further evidence is required to clarify the significance of both these transmission routes. Water has increasingly been recognised as an important indirect route of transmission (Table 9.12).

Waterborne transmission of cryptosporidiosis was first suggested because of its association with travellers diarrhoea and with Giardia infections, both of which are well-documented cases of waterborne diarrhoea (Smith and Rose 1990). A number of surveys of the incidence of cryptosporidiosis were carried out in the 1980s. During this period, a number of outbreaks were reported. In some of these incidents oocysts were detected in the water supply of those affected, while in others, there was strong circumstantial evidence that water was the vehicle of transmission (Barer and Wright 1990). Studies of water resources in the UK and the USA found that oocysts commonly occurred in all types of surface water (i.e. lakes, reservoirs, streams, and rivers) including pristine waters with densities ranging from 0.006–2.5 oocysts/litre (Badenoch 1990; Madore *et al.* 1987 and Le Chevallier *et al.* 1991a). Significantly higher numbers of oocysts are found in water resources receiving untreated or treated wastewaters, while oocysts to occur much less frequently in groundwaters. Rose (1997) gives ranges for the occurrence of oocysts in sewage (1–120 oocysts l^{-1}), filtered treated

Table 9.13. Major contamination routes for waterborne cryptosporidiosis.

Slurry tank leakage
Leaching from solid manure storage
Runoff from farmyards
Road and street runoff
Runoff from agricultural land used for grazing
Direct contamination from animals drinking from streams
Percolation through soil to field drainage system and ultimately to watercourse
Disposal of contaminated sewage sludge or sewage effluents
Disposal of water works sludge

wastewater (0.01–0.13 oocysts l^{-1}), surface waters (0.001–107 oocysts l^{-1}), groundwater (0.004–0.922 oocysts l^{-1}), and treated drinking water (0.001–0.72 oocysts l^{-1}). These findings suggest that a background level of oocysts exists in many waters which may suddenly become increased by accidental pollution or by heavy pollution, especially if such situations follow shortly after solid or liquid manure has been added to land adjacent to watercourses (Department of the Environment and Department of Health 1990). The Badenoch Report (Department of the Environment and Department of Health 1990) also found that it is probable that most of the oocysts found in both surface and groundwaters are derived form agricultural sources. Oocyst contamination can occur via several routes (Table 9.13).

Oocysts are ovoid, between 4–6 μm in diameter, and tend to occur in low numbers in water (Barer and Wright 1991). Their detection in water samples relies on filtration of large volumes of water to remove oocysts and examination of the concentrate by microscopy. Most methods available for oocyst detection are adaptations of those used for *Giardia* detection (Ongerth and Stibbs 1987; Musial *et al.* 1987; Standing Committee of Analysts 1989). Current standard recovery methods involve the passage of large volumes of water (100–500 litres), at rates of approximately 1.5 litres per minute, through wound propylene fibres with a 1 μm pore size (Musial *et al.* 1987; Standing Committee of Analysts 1989). Polycarbonate filters are also commonly used (Ongerth and Stibbs 1987). The resulting concentrate is eluted and the oocysts are counted. Varying recovery rates have been reported.

Identification of *Cryptosporidium* oocysts depends on determination of their size, shape and staining characteristics. Two approaches are available: direct staining (such as modified Ziehl-Neelsen) or the use of fluorescent

labelled antibodies (IFAT) which bind specifically to the surface of the oocyst (USEPA 1999; Myoda and Huang 2001) However, none of the available identification methods distinguish between *Cryptosporidium parvum* oocysts and the oocysts of other species of *Cryptosporidium*, nor do they indicate whether the oocysts are viable (Hayes and Cooper 1994). However, Call *et al.* (2001) have proposed a quantitative assay that can detect as few as 50 viable oocysts in a 1 ml assay, even in high turbidity waters (< 200 NTU). Using monoclonal and polyclonal antibodies, specific for *C. parvum*, developed against a sporozite antigen released only during excystation, the antibodies are captured on a magnetic head and then detected by ruthenium-labelled polyclonal antibodies via electrochemiluminescence.

Two genotypes of *Cryptosporidium* have been identified using PCR-restricted fragment length polymorphism of the oocyst wall protein. Genotype I exclusively infects humans while genotype II infects a broad host range. McLaughlin *et al.* (2000) used this technique to examine the origin of oocysts in 1,705 and 105 infected faecal samples collected from humans and livestock animals respectively. Genotype I was detected in 37.8% of human samples, while genotype II was recorded in 61.5%. Both genotypes were recovered in the remainder of the samples. All the livestock animals tested yielded genotype II. The technique helps to identify the source of outbreaks. For example, in 8 drinking water related outbreaks five were due to genotype I and three to genotype II. Isolated and family cases are usually of the same genotype, although farmers and those involved with animals are generally infected by genotype II. McLaughlin *et al.* found that the spring peak in cases was due to genotype II, but those recorded in the summer and autumn were due to genotype I contracted normally while on holiday.

A survey of a number of surface water filter plants by Le Chevalier *et al.* (1991a) demonstrated that *Cryptosporidium* oocysts are frequently isolated from filtered drinking waters (approximately 27%). Finding oocysts in water does not necessarily mean that the population is at risk. However, because the minimum dose for cryptosporidiosis is thought to be so low and the fact that oocysts can withstand considerable environmental pressures, remaining viable for long periods of time at low temperatures, low-level contamination of a potable water supply has the potential to result in large-scale infection of the population (Smith 1992). Table 9.14 shows that many outbreaks have occurred in waters which have undergone water treatment. However, it is often difficult to identify the source of cryptosporidiosis contamination of water supplies (Maguire *et al.* 1995). Since 1988 a number major outbreaks of waterborne cryptosporidiosis have been recorded in the

Table 9.14. Examples of outbreaks of cryptosporidiosis.

Year	Country/Location	Nos. affected	Suspected cause
1983	Surrey (UK)	16	Source contamination
1984	Texas (USA)	2000	Sewerage contamination of well
1985	Surrey (UK)	50	Source contamination
1987	Georgia (USA)	13,000	Operation irregularities
1988	Ayrshire (UK)	27	Post-treatement contamination
1989	Swindon/Oxfordshire (UK)	500	Source contamination
1989	L. Lomond, Scotland (UK)	442	Source contamination
1990	Humberside (UK)	140	Source contamination
1991	South London (UK)	44	Source contamination
1993	Milwaukee (USA)	370,000	Post-treatment contamination

UK (Table 9.14). The first in 1988 occurred in Ayreshire when 27 cases were confirmed, although many more people were thought to have been infected. Of those infected, 63% were less than 8 years of age. The cause was most likely due to the finished water being contaminated from runoff from surrounding fields on which slurry had been spread. The oocysts were found in the chlorinated water in the absence of faecal indicator bacteria. The second outbreak was far more serious and affected over 400 people in the Oxford and Swindon areas early in 1989 (Richardson *et al.* 1991), although, according to Rose (1990), as many as 5000 people may have been affected. The outbreak was quickly traced to the Farmoor Water Treatment Plant near Oxford, which takes its water from the River Thames. On investigation oocysts were found in the filters, in the filter backwash water, and in the treated water even though it had been chlorinated and the microbiological tests had shown the water to be of excellent quality. The oocysts were found in the Farmoor Reservoir and also in a tributary of the River Thames, upstream of the treatment plant. The seasonal presence of the organisms was particularly associated with the grazing of lambs and with the scouring that often occurs in their early lives. Although the rapid sand filters at Farmoor were removing 79% of the oocysts after coagulation, the recycling of the backwash water had given rise to exceptionally high concentrations of oocysts (10,000 per litre) with resultant break through. Disinfection with chlorine was not effective, and the first action by Thames Water was to stop recycling the backwash water (up till then a normal practice) which brought the problem under control. There was a decrease in the number of reported cases as the number of oocysts in the water decreased. These oocysts can survive for up to 18 months depending on the temperature.

There have been further outbreaks since then, but on a much smaller scale (Table 9.14). One in North-West Surrey at about the same time as the Oxford outbreak. Again water was taken from the River Thames down stream of Oxford. In April 1989, an outbreak occurred in Great Yarmouth following a change in the water supply, while in Hull there were 140 cases reported in January 1990. There was also an outbreak in the Loch Lomand area in 1989. In this case livestock grazing around the reservoir or in the catchment of rivers was the source of the organism. Cattle and infected humans can excrete up to 10^{10} oocysts during the course of infection, so that cattle slurry, wastewater from marts, and sewage should all be considered potential sources of the pathogen. In the USA there have been a number of reported outbreaks (Table 9.14). The most important outbreak of *Cryptosporidia* in the USA in recent years occurred in April 1993 in Milwaukee. The water distribution system serving 800,000 people was contaminated by raw water from a river swollen by spring run off. In all 370,000 people became ill, 4400 were admitted to hospital and approximately 40 died (Jones 1994).

These outbreaks suggest that present methods of water treatment are not adequate to deal with the parasite. Procedures of water treatment vary from place to place but all ultimately terminate with chlorination. It has been established that chlorination at levels used in water treatment is ineffective against oocysts, with concentrations as high as 16,000 mg l^{-1} required to prevent excystation (Korich *et al.* 1990). Alternative disinfectants for water treatment and inactivation of *Cryptosporidium* oocysts have been investigated including chlorine dioxide, monochloramine, hydrogen peroxide and ozone (Peeters *et al.* 1989; Korich *et al.* 1990; corona-Vasquez *et al.* 2002). Of these, ozone is considered to have the most potential. Korich *et al.* (1990) found more than 90% inactivation after treating oocysts with 1 ppm of ozone for 5 minutes (Fig. 9.5). Similar results were also observed by Parker *et al.* (1993) (Fig. 9.6). The use of ozone is not without its disadvantages in particular its organic by-products (Department of the Environment and Department of Health 1990). A more recent report by the WRc (1994) does not consider ozone alone to be a practical or cost effective method of protection against *Cryptosporidium* oocysts as very high doses would be required to achieve a high degree of oocyst inactivation. The Ct values (i.e. the product of average disinfectant concentration and contact time) (Sec. 9.5.3) for these disinfectants is 20–50 times higher for *C. parvum* oocysts than is required for the same level of inactivation of *Giardia lamblia* cysts. This is exacerbated during the winter as the required Ct increases for the inactivation of *C. parvum* with both oozone and chlorine dioxide

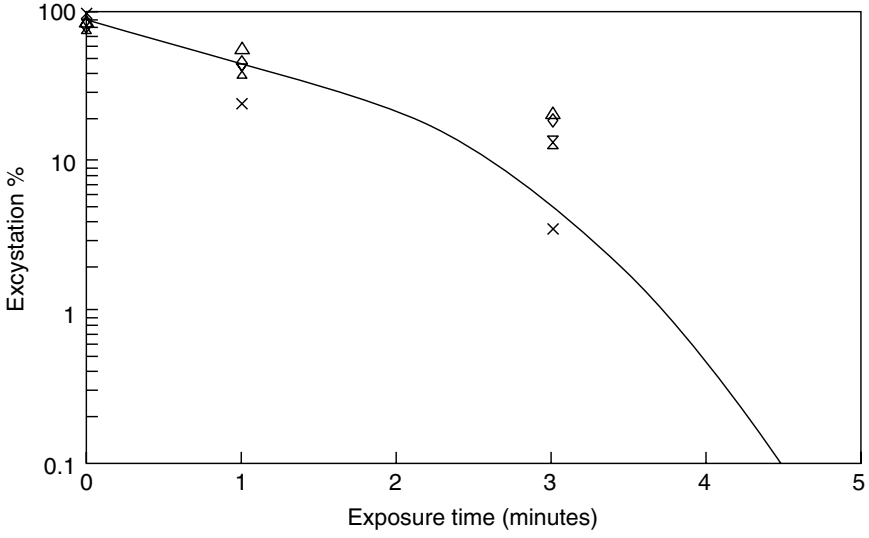


Fig. 9.5. Decline in the mean percent excystation of *Cryptosporidium* oocysts exposed to 1 ppm of ozone at 25°C (Korich *et al.* 1990).

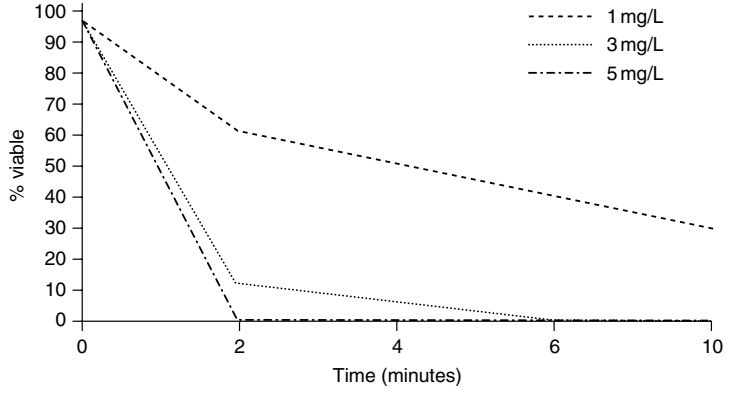


Fig. 9.6. The effect of residual ozone concentrations on the viability of *Cryptosporidium parvum* oocysts at 20°C (Parker *et al.* 1993).

at low temperatures (Driedge *et al.* 2000; Ruffell *et al.* 2000). This is considered further in Sec. 9.5.3. In any case it is accepted that well-operated treatment processes with proper filtration and disposal or re-use of filter backwash water should be capable of achieving 99% reduction in oocyst concentration (West 1991; WRc 1994).

At present little can be inferred about the safety or otherwise to the public if oocysts are detected in water samples. This is because of the many gaps in our knowledge of this organism including their occurrence in natural waters, the minimum infective dose, and their survival capabilities. The main problem with *Cryptosporidium* is the inability to accurately detect viable oocysts, and therefore at present oocyst numbers are largely underestimated (Smith 1992). Further details on the transmission, detection, and identification of *Cryptosporidium* is given in an extensive review by Fayer *et al.* (2000).

Other protozoans

Entamoeba histolytica is carried by about 10% of the population in Europe and 12% in the USA. In countries where the disease is widespread the highest rate of incidence occurs in those groups with unprotected water supplies, inadequate waste disposal facilities, and poor personal hygiene. In Europe the number of cysts (10–15 μm in diameter) in sewage is generally low < 5 cysts l^{-1} in untreated raw sewage; however, concentrations up to 5000 cysts l^{-1} have been recorded (Bitton 1999). Because of their poor settleability, like all protozoan cysts, they tend to pass through the sewage treatment plant and reach surface waters. Where surface waters are reused for supply purposes, any cysts present will be taken up with the water. The disease, amoebic dysentery, is very disabilitating, with the infection centred in the large intestine where the amoebae multiply and adhere to enterocytes causing ulceration. The symptoms include mid-abdominal pain, diarrhoea alternating with constipation, or chronic watery diarrhoea with a discharge of mucus and blood.

Naegteria are free living protozoans widely found in variety of surface and waste waters, especially surface waters that are heated (Marciano-Cabaral *et al.* 1988). *Naegteria fowleri* is the main human pathogenic species which was first reported in 1965 in Australia. It normally enters the body through the nasal cavities while swimming in infected water. Although it is thought infection is possible through drinking contaminated water. Once in the body the protozoa rapidly migrate to the brain, cerebrospinal fluid, and bloodstream. Cases are very rare but generally fatal, with death occurring within 4–5 days of first exposure. The disease is exclusively associated with swimming and diving in warm contaminated waters, especially hot springs and spa waters. *Naegteria fowleri* can be distinguished from other species of the genus using rapid detection techniques DNA probes (Kilvington and Beeching 1995).

Human enteric viruses and protozoan parasites possess certain traits which aid waterborne transmission and which have contributed to their increase in recent years (West 1991). These include: (i) An ability to be excreted in faeces in large numbers during illness; (ii) Failure of conventional sewage treatment to remove them; (iii) They can survive as an environmentally robust form or they demonstrate resilience to inactivation whilst in an aquatic environment; (iv) They are largely resistant to common disinfectants used in drinking water treatment; (v) Most importantly, they only require low numbers to elicit infection in hosts consuming or exposed to water.

These factors are compounded by the difficulty of isolating and accurately detecting these pathogens in both treated and untreated effluents as well as in both surface and ground water resources. The identification of several viral agents is problematic because some of these agents cannot be propagated in the laboratory. For both viruses and protozoa, large sample volumes must be examined in order to detect small numbers of organisms. Propagation and identification may take several days. In addition, by the time the outbreak is eventually recognised, it is usually long after the initial contamination event and that water is no longer available for examination. This is particularly the case with protozoan pathogens when a minor operational error during backwashing sand filters may result in the breakthrough of cysts and oocysts in to the treated water. There is also the question of determining the viability of such organisms particularly *Cryptosporidium* and *Giardia* as present detection methods do not allow for such distinction. Recent developments in PCR and gene probe technology show considerable promise particularly in relation to determining viability. However, as with the detection of bacterial pathogens, the use of PCR is very much hampered by the presence of substances such as humic acids, commonly found in water originating from upland reservoirs and acidic rivers.

9.2.5. *Parasitic worms*

The incidence of worm infection in European countries is generally low and is limited to *Taenia* and *Ascaris*. Infection is normally contracted from animals reared for food, although many incidents of infection from contaminated water have been reported. Ova of both genera are commonly isolated from sewage.

Taenia saginata, the beef tapeworm, is generally disseminated by polluted waters. Infected persons can excrete up to 10^6 ova per day, and in raw sewage 20 ova 1^{-1} is common. *Taenia* ova are abundant in meat-processing

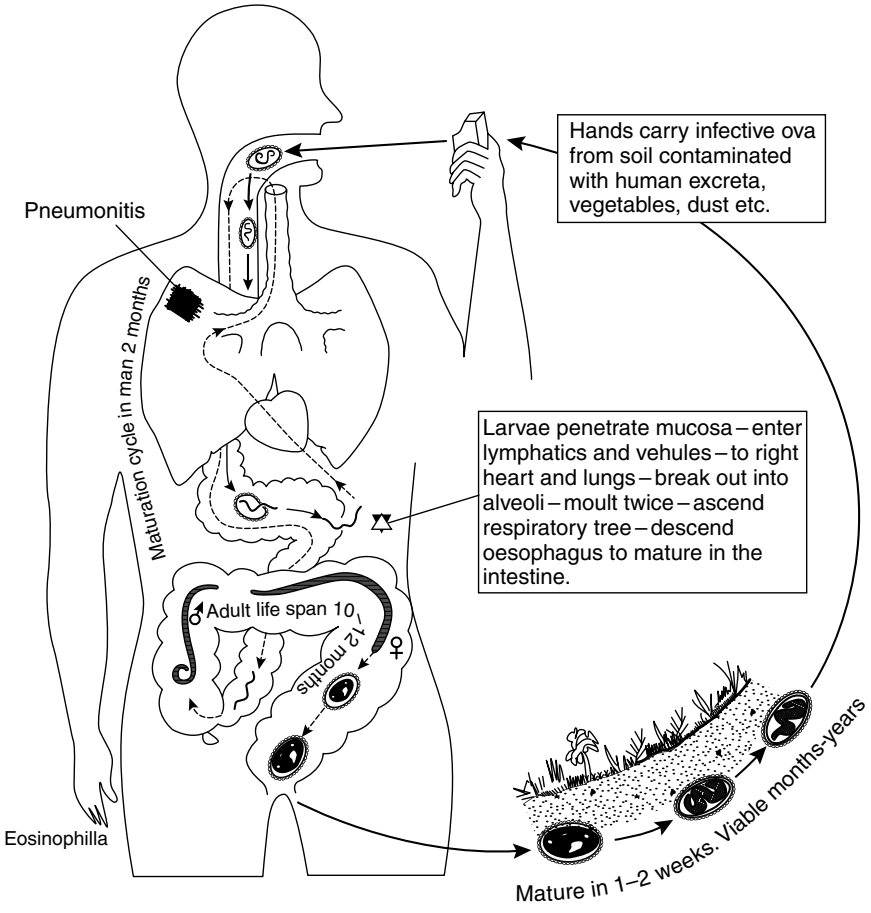


Fig. 9.7. Life cycle of *Ascaris Lumbricoides* (roundworm). Adapted from Jeffery and Leach (1972) by Bitton (1999).

effluents and extreme caution should be taken if handling the effluent or sampling a river receiving this type of wastewater. Ascaris or roundworms also produce large numbers of ova and infected people can excrete $> 200{,}000$ ova d^{-1} (Fig. 9.7). Ascariasis is especially associated with young children, although the ova of *A. lumbricoides* may also enter water from soil and vegetables, as well as from faeces of infected people (Dart and Stretton 1977).

There are a number of other parasitic worms that can also be transmitted by faecal contamination of water supplies. These include several hookworms, schistossomes, and a number of tapeworms, such as the

fish tapeworm *Diphyllobothrium latum*. Infection by the dog tapeworm (*Echinococcus* and *Canidae* spp.) is normally by direct contact with infected dogs. However, contamination of water supplies by dog faeces and by stormwater from paved areas has contributed to the significant increase in the incidence of this parasite in man. The frequency of occurrence of helminth ova in wastewater, sludge, and soil has been reviewed by Theis and Storm (1978) (Table 9.15).

9.3. Indicator Organisms

The wide diversity of pathogenic micro-organisms found in wastewaters are normally present in low numbers compared with other non-pathogenic organisms that are also present. The isolation and identification of individual pathogenic organisms is often complex, being different for each species and extremely time consuming. It is, therefore, impossible to examine all water samples on a routine basis for the presence and absence of all pathogens. Also, the selection of individual pathogens may be misleading as each species can tolerate different environmental conditions and the presence of one may not indicate the presence of another. In order to routinely examine water supplies a rapid and, preferably, a single test is required. It is far more effective to examine a water supply frequently by a simple test, as most cases of contamination of water supplies occur infrequently, than occasionally by a series of more complicated tests (HMSO 1977, 1983b). This has led to the development of the use of indicator organisms to indicate the likelihood of contamination by faeces. The main criteria for selection of an indicator organism are: (i) They should be a member of the normal intestinal flora of healthy people; (ii) They should be exclusively intestinal in habit and therefore exclusively faecal in origin if found outside the intestine; (iii) Ideally they should only be found in humans; (iv) They should be present when faecal pathogens are present and only when faecal pathogens are expected to be present; (v) They should be present in greater numbers than the pathogen they are intended to indicate; (vi) They should be unable to grow outside the intestine with a die-off rate slightly less than the pathogenic organisms; (vii) They should be resistant to natural environmental conditions and to water and wastewater treatment processes in a manner equal to or greater than the pathogens of interest; (viii) They should be easy to isolate, identify and enumerate; (ix) They should be non-pathogenic (Cabelli 1979; Lynch and Poole 1979). The non-pathogenic organisms that are always present in the intestine of man and

Table 9.15. Summary of helminth life-cycles (Thesis and Storm 1978).

Species	Free-living stage	Infective stage	Larval worm migrates through body of host	Adult worms (location)	Intermediate host(s) required	Normal definitive vertebrate host	Human infestation possible	Able to complete life-cycle in US
Ascaris lumbricoides	No	Ova with larvae *in situ*	Yes	Small intestine	No	Humans	—	Yes
Toxocara	No	Ova with larvae *in situ*	Yes	Small intestime	No	Dogs or cats	Yes	Yes
Toxascaris	No	Ova with larvae *in situ*	No	Small intestine	No	Dogs or cats	No	Yes
Oxyuris equi	No	Ova with larvae *in situ*	No	Colon	No	Horses	No	Yes
Trichuris trichiura	No	Ova with larvae *in situ*	No	Colon Ceacum				
No	Humans	—	Yes					
Necator	Yes	Larvae that burrows through skin	Yes	Small intestine	No	Humans	—	Yes warm, moist, areas
Strongyloides larvae	Yes, may live as a nonparasite	Larvae that burrows through skin	Yes	Small intestine	No	Human, dogs, sheeps	—	Yes warm, moist, areas
Strongylus ova	No	Larvae that climb up grass blades and are eaten	Yes	Small intestine	No	Horses	No	Yes

Table 9.15. (*Continued*)

Species	Free-living stage	Infective stage	Larval worm migrates through body of host	Adult worms (location)	Intermediate host(s) required	Normal definitive vertebrate host	Human infestation possible	Able to complete life-cycle in US
Taenia	No	Cyst in meat of cow or pig	Yes in cow or pig	Small intestine	Yes cow or pig	Humans	—	Yes
Hymenolepis nana	No	Ova with embryo	No	Small intestine	No	Humans	—	Yes
H. diminuta	No	Cyst in tissue of insect	No	Intestine of insect	Yes	Rodents	Yes rare	Yes
Clonorchis	No	Cyst in flesh of fish	Yes in part	Liver	Yes snail & fish	Humans	—	No
Fasciola	No	Cyst on grass stems	Yes in part	Liver	Yes snail	Sheep	Yes uncommon	Yes cool, moist areas

animals are excreted along with the pathogens, but in far greater numbers. Several of these are easily isolated and are ideal for use as indicators of faecal contamination. The most widely used are the non-pathogenic bacteria, in particular coliforms, faecal streptococci, and *Clostridium perfringens*.

The EC Drinking Water Directive (80/778/EEC) specifies numerical standards for total and faecal coliforms, faecal streptococci, sulphide-reducing clostridia and descriptive standards for total viable counts of heterotrophic bacteria at 22° and 37°C. Routine monitoring is restricted to coliforms and heterotrophic bacteria only. The following maximum admissible concentrations (MAC) apply to finished waters: (i) Zero total coliorms per 100 ml sample. Where a sufficient number of samples are examined, then a 95% consistent result is acceptable; (ii) Zero faecal coliforms (*E. coli*) per 100 ml sample; (iii) Zero faecal streptococci per 100 ml sample; (iv) Less than one for sulphate reducing clostridia per 20 ml sample; (v) There should be no significant increase in the total bacteria colony counts above background levels, although a guide value (G) of < 10 per ml at 22°C and < 100 per ml at 37°C has been set. Analysis is to be done by membrane filtration for total coliforms, faecal coliforms and faecal streptococci, and by the multiple tube method for sulphate reducing clostridia. The EU Directive does not require either viruses or protozoan pathogens (cysts or oocysts) to be routinely measured, although it makes it clear that water intended for human consumption should not contain any pathogenic organisms. However, in terms of regulated parameters it relies totally on the indicator organisms listed above. The Directive does contain the rather ambiguous statement: *If it is necessary to supplement the microbiological analysis of water intended for human consumption, the samples should be examined not only for the bacteria listed but also for pathogens including salmonella, pathogenic staphylococci, faecal bacteriophages, and enteroviruses; nor should such water contain parasites, algas or other organisms such as animalcules.*

In December 1998 the newly revised Drinking Water Directive was published (98/83/EEC). As well as complying with new fixed parameters, water supplied under the directive will have to be free of pathogenic microorganisms and parasites in numbers constituting a danger to public health (Table 9.16). Although as before there are no guide values given for specific viruses, protozoans, or bacterial pathogens. The new directive gives separate maximum permissible concentrations for the microbial parameters for both tap and bottled waters, except natural mineral waters that have their own directive. For tap water maximum permissible concentrations are given for *E. coli* (0 per 100 ml) and enterococci (0 per 100 ml). For bottled waters the maximum permissible concentrations are stricter,

Table 9.16. The quality parameters listed in the new EC Drinking Water Directive (98/83/EEC).

Part A: Microbiological parameters	
Parameter	Parametric value
Escherichia coli	0/100 ml
Enterococci	0/100 ml
In water offered for sale in bottles or containers	
Escherichia coli	0/250 ml
Enterococci	0/250 ml
Pseudomonas aeruginosa	0/250 ml
Colony count at 22°C	100/ml
Colony count at 37°C	20/ml

Part B: Chemical parameters	
Parameter	Parametric value
Acrylamide	0.1 μg l^{-1}
Antimony	5.0 μg l^{-1}
Arsenic	10 μg l^{-1}
Benzene	1.0 μg l^{-1}
Benzo(a)pyrene	0.01 μg l^{-1}
Boron	1.0 mg l^{-1}
Bromate	10 μg l^{-1}
Cadmium	5.0 μg l^{-1}
Chromium	50 μg l^{-1}
Copper	2.0 mg l^{-1}
Cyanide	50 μg l^{-1}
1, 2-Dichloroethane	3.0 μg l^{-1}
Epichlorohydrin	0.1 μg l^{-1}
Fluoride	1.5 mg l^{-1}
Lead	10 μg l^{-1}
Mercury	1.0 μg l^{-1}
Nickel	20 μg l^{-1}
Nitrate	50 mg l^{-1}
Nitrite	0.5 mg l^{-1}
Pesticides[a,b]	0.1 μg l^{-1}
Pesticides (total)[a]	0.5 μg l^{-1}
Polycyclic aromatic hydrocarbons[a]	0.1 μg l^{-1}
Selenium	10 μg l^{-1}
Tetrachloroethene plus trichloroethane	10 μg l^{-1}
Trihalomethanes (total)[a]	100 μg l^{-1}
Vinyl chloride	0.5 μg l^{-1}

[a]Relates to specified compounds in Directive 98/83/EEC.

[b]For aldrin, dieldrin, heptachlor and heptachlor epoxide the parametric value is 0.03 μg l^{-1}.

Table 9.16. (*Continued*)

Part C: Indicator parameters Parameter	Parametric value	Notes
Physico-chemical		
Aluminium	200 μg l^{-1}	
Ammonium	0.5 mg l^{-1}	
Chloride	250 mg l^{-1}	Water should not be aggressive
Clostridium perfringens	0/100 ml	From, or affected by, surface water only
Colour		Acceptable to consumers and no abnormal change
Conductivity	2500 μS cm^{-1}	Water should not be aggressive
Hydrogen ion concentration	pH $\geqslant$ 6.5, $\leqslant$ 9.5	Water should not be aggressive. Minimum values for bottled waters $\leqslant$ pH 4.5
Iron	200 μg l^{-1}	
Manganese	50 μg l^{-1}	
Odour		Acceptable to consumers and no abnormal change
Oxidisability	5.0 mg $O_2 l^{-1}$	Not required if TOC used
Sulphate	250 mg l^{-1}	Water should not be aggressive
Sodium	200 mg l^{-1}	
Taste		Acceptable to consumers and no abnormal change
Colony count at 22°C	0/100 ml	
Coliform bacteria	0/100 ml	For bottled waters 0/250 ml
Total organic carbon (TOC)		No abnormal change. Only for flows $> 10,000$ m^3 day^{-1}
Turbidity		Acceptable to consumers and no abnormal change. Normally < 1.0 NTU
Radioactivity		
Tritium	100 Bq l^{-1}	
Total indicative dose	0.1 mSv $year^{-1}$	

i.e. *E. coli* (0 per 250 ml) and enterococci (0 per 250 ml). *Pseudomonas aeruginosa* has also been included for the first time with a maximum permissible concentration of 0 per 250 ml. Maximum colony counts at 22 and 37°C are 100 and 20 per ml, respectively. The microbial parameters are listed in a new section, Part A, of the directive. Part B contains chemical

parameters, and Part C indicator parameters. Included in Part C are total coliforms with a maximum permissible concentration of 0 per 100 ml for tap waters and 0 per 250 ml for bottled waters; and also included are total bacterial counts (22°C) which must not show any abnormal change with a maximum permissible value of 0 per ml in tap waters and sulphate reducing clostridia at 0 per 100 ml. Specific and detailed notes are given on analysis, including the composition of all recommended media (Table 9.17.).

In the USA, the USEPA set a maximum contaminant level (MCL) based on the presence or absence (P-A) concept for coliforms. This revised MCL came into force on 31st December 1990. The recommended sampling frequency of water supplies is dependent on the population served. For systems requiring more than 40 samples per month, $< 5\%$ samples must be total coliform positive. For systems where the frequency of analysis is less than 40 samples per month, then no more than 1 sample per month may be total coliform positive. If a sample is found to be total coliform positive then repeat samples must be taken within 24 hours. Repeat samples must be taken at the same tap and also at adjacent taps within 5 service connections both up and down stream of the original sample point. If these repeated samples are also total coliform positive, then the samples must be immediately tested for

Table 9.17. The recommended microbial methods in the new EC Drinking Water Directive (98/83/EEC).

Total coliforms
Membrane filtration followed by incubation on membrane lauryl broth for 4 h at 30°C followed by 14 h at 37°C. All yellow colonies are counted regardless of size.
E. coli
Membrane filtration followed by incubation on membrane lauryl broth for 4 h at 30°C followed by 14 h at 44°C. All yellow colonies are counted regardless of size.
Faecal streptococci
Membrane filtration followed by incubation on membrane enterococcus agar for 48 h at 37°C. All pink, red or maroon colonies which are smooth and convex are counted.
Suphite-reducing clostridia
Maintain the sample at 75°C for 10 min prior to membrane filtration. Incubate on tryptose–sulphite–cycloserine agar at 37°C under anaerobic conditions. Count all black colonies after 24 and 48 h incubation.
Pseudomonas aeruginosa
Membrane filtration followed by incubation in a closed container at 37°C on modified Kings A broth for 48 h. Count all colonies which contain green, blue or reddish-brown pigment and those that fluoresce.
Total bacteria counts
Incubation in a yeast extract agar for 72 h at 22°C and for 24 h at 37°C. All colonies to be counted.

faecal coliforms or *E. coli*. If these prove positive then the public must be informed. Full details of the revised coliform rule is given by Berger (1992). A 100 ml sample bottle must be used in analysing total coliforms using one of the following techniques: 10-tube multiple tube fermentation technique, the membrane filtration technique, the P-A (presence-absence) coliform test, or the minimal-media ONPG-MUG test. This Presence-Absence concept has a number of potential advantages: (i) Sensitivity is improved because it is more accurate to detect coliform presence than to make quantitative determinations. (ii) The concept is not affected by changes in coliform density during storage. (iii) Data manipulation is much improved. The introduction of new and rapid testing systems will most likely see a similar P-A coliform test introduced throughout Europe.

The World Health Organization guidelines for drinking water are used universally and are the basis for both the EU and USA legislation. The original guidelines were published in two volumes in 1984. Volume 1 provide the guidelines while volume 2 contains scientific evidence on which the recommendations in volume 1 are based. The existing guidelines were based on the available toxicological evidence up to 1981 and so are now very much out-of-date. A revision began in 1987 with new guidelines finally agreed in Geneva in September 1992. The new guidelines include microbiological, chemical, and radiological parameters. The new microbiological standards are shown in Table 9.18, although there are no guideline values for either viruses or parasites. This is because it is considered that the analytical methods for these organisms are too costly, complex, and time consuming for routine laboratory use. Instead, guideline criteria are outlined based on the likely viral content of source waters and the degree of treatment necessary to ensure that even large volumes of water have a negligible risk of containing viruses. It is considered that *the attainment of the bacteriological criteria and the application of treatment for virological reduction should ensure the water presents a negligible health risk* (WHO 1993). The WHO guidelines are expected to be reviewed in 2003.

There is now much interest in other indicator organisms and the ability to rapidly detect minute concentrations of faecal-associated compounds, which are unaffected by environmental factors. This has put the whole use of indicator organisms to test water for faecal contamination under review. There is considerable interest in the development of chemical indicators of faecal pollution and sterols are particularly promising (Murtaugh and Bunch 1967). For example, the faecal sterol coprostanol which is produced in the intestine by the microbial reduction of cholesterol has been shown to be an accurate indicator of faecal pollution (Dutka *et al.* 1974; Hatcher

Table 9.18. World Health Organization drinking water guide values for bacteriological quality of drinking water (WHO 1993).

Organisms	Guideline
All water intended for drinking *E. coli* or thermotolerant coliform bacteria*†	Must not be detectable in any 100 mL sample
Treated water entering the distribution system *E. coli* or thermotolerant coliform bacteria*	Must not be detectable in any 100 mL sample
Total coliform bacteria	Must not be detectable in any 100 mL sample
Treated water in the distribution system *E. coli* or thermotolerant coliform bacteria*	Must not be detectable in any 100 mL sample
Total coliform bacteria	Must not be detectable in any 100 mL sample. In the case of large supplies where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period

Immediate investigative action must be taken if either *E. coli* or total coliform bacteria are detected. The minimal action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause must be determinded by immediate further investigation.

*Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

†It is recognized that in most rural water supplies in developing countries faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies.

and McGillivary 1979; McCalley 1980; McCalley *et al.* 1981). Coprostanol has been successfully used to trace faecal pollution in the Clyde estuary, where it closely paralleled the faecal coliform counts (Goodfellow *et al.* 1977) (Sec. 9.3.10).

Three groups are normally used to indicate faecal contamination, *Escherichia coli* or faecal coliforms, faecal streptococci, and *Clostridium perfringens*. The three groups are able to survive for different periods of time in the aquatic environment. Faecal streptococci die fairly quickly outside the host and their presence is an indication of recent pollution. *Escherichia coli* can survive for several weeks under ideal conditions and are far more easily detected than the other indicator bacteria. Because of this, it is the most widely-used test although the others are often used to confirm faecal pollution if *E. coli* are not detected. *Clostridium perfringens* is a sporulating

anaerobe that can exist indefinitely in water. When *E. coli* and faecal streptococci are absent, its presence indicates remote or intermittent pollution. It is especially useful for testing lake water, although the spores eventually settle out of suspension. The spores are more resistant to industrial pollutants than the other indicators and it is especially useful in waters receiving both domestic sewage and industrial wastewaters. It is assumed that these organisms do not grow outside the host and, in general, this is true. However, in tropical regions *E. coli*, in particular, is known to multiply in warm waters and there is increasing evidence that *E. coli* is able to reproduce in enriched waters generally, thus falsely indicating an elevated health risk (Eliasson 1967; Henricks 1972; Dutka 1973 1979). Therefore, great care must be taken in the interpretation of results from tropical areas, and the use of bacteriological standards designed for temperate climates are inappropriate for those areas (Mara 1974).

To successfully protect water supplies, especially those in rural areas, it is necessary to be able to trace the source of faecal pollution. Standard bacteriological techniques are traditionally used but fail to distinguish between human and animal faecal pollution. There are a number of modifications to traditional methods available such as estimating the ratios of faecal coliforms to faecal streptococci or the ratios of different types of streptococci, identification of faecal coliforms from humans and animals by DNA fingerprinting or antibiotic resistance profiles, or the use of specific indicator bacteria are perhaps the most promising.

The use of indicator organisms is the barometer by which the safety of water for human consumption is judged. Although still routinely used and found effective (Schaffter and Parriaux 2002), there has been an increase in the numbers of incidents where waterborne outbreaks have occurred in waters that met the required standards for indicator organisms, in particular the coliform test. Many of these outbreaks have been caused by viral and protozoan agents, particularly the enteric viruses, and the protozoa *Giardia* and *Cryptosporidium* (Gleeson and Gray 1997). Consequently, methods to directly detect microbial pathogens in water and wastewaters are actively being investigated. The main detection method being evaluated is polymerase chain reaction (PCR) which is rapid, highly sensitive, and accurate for viruses, bacteria, protozoa, and helminths. Current limitations include inhibition by environmental contaminants, difficulty in quantification, and the generation of false positives through the detection of naked nucleic acids, non-viable micro-organisms, or laboratory contamination (Toze 1999).

9.3.1. *Escherichia coli and coliforms*

The coliform group of bacteria comprises of several genera belonging to the family Enterobacteriaceae. Among the common genera of the group are *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*. Bond (1977) considered these to be type genera of the group. The group is heterogeneous and includes non-faecal lactose fermenting bacteria as well as other species that are rarely found in faeces but are capable of multiplication in water (WHO 1993).

Historically, the definition of the coliform group has been based on methods used for its detection rather than on the tenets of systematic bacteriology (APHA 1992). Accordingly, when the multiple tube method is used, the American Public Health Association defines coliforms as '*all aerobic and facultatively anaerobic*, *Gram negative*, *non spore-forming*, *rod shaped bacteria that ferment lactose with acid and gas production*'. Where the membrane filtration technique is used, the coliform group is normally defined as comprising of all aerobic and facultatively anaerobic, Gram negative, non spore-forming, rod shaped bacteria that develop a red colony with a metallic sheen within 24 hours at 35°C on an Endo type medium containing lactose (APHA 1992). The WHO definition is broader and refers to Gram negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface active agents with similar growth inhibiting properties, able to ferment lactose at 35–37°C with the production of acid, gas and aldehyde within 27–48 hours. They are also oxidase negative, non spore-forming and display β-galactosidase activity. With the development of methods for detecting coliforms which do not rely on characteristics such as the production of acid and gas from lactose, the use of method-related definitions become largely obsolete and a more scientific definition is required.

The fermentation of lactose into glucose and galactose requires the presence of the enzyme β-galactosidase. Thus the possession of the gene coding for the production of the enzyme is the most fundamental characteristic of the Enterobacteriaceae (coliforms). However, the production of gas from lactose has been found to be extremely variable. The expression of this gene can be affected by many factors including time, temperature and the growth medium used, so that the same organism may or may not ferment lactose sufficiently to register as a lactose fermenter under different test conditions (Department of the Environment 1994a). Yet it is now widely accepted that any new definition of a coliform must be based on the possession of the β-galactosidase gene.

The coliform group also includes the thermotolerant faecal coliforms. These are defined as being able to ferment lactose at 44°C (WHO 1993), and not only include *E. coli* but also species of the *Klebsiella*, *Enterobacter*, and *Citrobacter* genera. *Escherichia coli* is considered to be the only true faecal coliform as other thermotolerant coliforms can be derived from non-faecally contaminated waters. McLellan *et al.* (2001) found that β-glucuronidase activity was critical in distinguishing *E. coli* from other faecal coliforms. Therefore, *E. coli* is a better indicator of faecal pollution than faecal coliforms that may replicate in the environment and falsely elevate indicator organism concentrations.

The coliform test initially involves examining the sample for total coliforms. Total coliforms are largely faecal in origin, but also include species which are commonly found in unpolluted soils and vegetation and therefore do not present a public health problem. Subsequently, total coliform results are interpreted as presumptive results. The sample is then examined for thermotolerant coliforms (Department of the Environment *et al.* 1983). Although this group is often termed faecal coliforms, this is not entirely correct, as some non-faecal organisms (such as non-faecal *Klebsiella* spp) are also capable of growth at 44°C. The growth of such organisms may result in high counts that may be interpreted as faecal coliforms (Bayley and Seidler 1977). In the EU Drinking Water Directive the term faecal coliform is used specifically to indicate coliforms of faecal origin which it defines as those that are thermotolerant, i.e. capable of growth at 44°C. As not all thermotolerant coliforms are faecal in origin, they must be regarded as presumptive faecal coliforms (Department of the Environment 1994). Therefore the presence of *E. coli*, which is known to be exclusively faecal in origin, is usually also determined. *Escherichia coli* consists of up to 95% of the enterobacteria found in faeces (Waite 1985). In addition to the production of acid and gas at 44°C, *E. coli* is also able to produce indole from tryptophan and most strains produce β-glucuronidase (Department of the Environment *et al.* 1983; Department of the Environment 1994). Generally for assessment of the microbiological quality of surface waters, it is the faecal coliform (*E. coli*) count which is primarily determined, due to its public health implications. However, for treated drinking waters enumeration of total coliforms is generally sufficient since it is assumed that waters designated for human consumption should not contain any micro-organisms (Cabelli 1978).

Enumeration techniques

The exact methods employed to enumerate indicator bacteria are specified by the legal standards used. Two techniques are principally used, membrane filtration and the multiple tube methods. The EC Drinking Water Directive specifies that total and faecal coliforms, and faecal streptococci must be isolated using the membrane filtration method, while the multiple tube method is used for clostridia. It should be noted that different methods and materials are used in the USA for nearly all the standard bacteriological tests compared with Europe, so care should be taken when selecting methods to check for the preferred methods in the bacteriological standards being followed.

Coliforms do not only occur in faeces, they are normal inhabitants of water and soil as well. So the presence of coliforms in a water sample does not necessarily indicate faecal contamination, although in practice it must be assumed that they are of faecal origin unless proved otherwise. The total coliform count measures all the coliforms present in the sample. However only *E. coli* is exclusively faecal in origin with numbers in excess of 10^8 per gram of fresh faeces. So it is important to confirm *E. coli* is present. Routine coliform testing comprises of two tests giving the total coliform count and faecal coliform (*E. coli*) count.

Membrane filtration technique

This technique, originally developed in the 1940s (Waite 1985), is now almost exclusively used in preference to other methods. The use of the multiple tube method has declined because the preparation of large quantities of tubes with media and inverted Durham tubes is very time consuming. The membrane filtration method is far more rapid, essentially disposable, has a smaller percentage error than other methods, is simpler to use, and does not require specialist training, but it can be comparatively expensive if pre-prepared plates are purchased (Table 9.19).

Known volumes of water are passed through a membrane filter (pore size 0.45 μm) that retains the bacteria present. The filter is placed on Membrane-Enriched Teepol medium, which contains the detergent Teepol to inhibit non-intestinal bacteria, and is then incubated (Geldreich *et al.* 1965; APHA 1992) (Fig. 9.8). The technique assumes that each bacterium retained by the filter will grow and form a small visible colony. During the incubation period, the nutrients diffuse from the medium through the membrane and the coliforms are able to multiply and form recognisable colonies. Membranes are incubated at 30°C for 4 h, followed by 14 h at 37°C for total

Table 9.19. Comparison of the advantages and disadvantages of the multiple tube (MPN) and membrane filtration (MF) methods of coliform analysis (Hutton 1983).

Multiple tube MPN	Membrane filtration
Costs	*Costs*
Large quantities of culture media and glassware and large autoclave	Smaller quantities of media Membrane expensive. Disposables (petri dishes, pipettes) expensive
Capital costs fairly high	Capital costs of proprietary equipment high
Accuracy	*Accuracy*
Statistically based estimate Possibly large errors especially at low levels	More accurate especially to low levels (less than 100 colonies per 100 ml)
Field use	*Field use*
Needs static base	May be operated in the field and in transport if portable incubator used. Transport media can be used prior to incubation
Suspended matter	*Suspended matter*
May be used for turbid samples	Not suitable for turbid waters due to membrane clogging
Convenience	*Convenience*
Large amount of material to be prepared prior to analysis and disposed of after incubation	Less manipulation and hence lower chance of contamination Disposable, pre-sterilized equipment can be purchased
Incubation times	*Incubation times*
Up to 48 h or 72 h	24 h (or even 7 h in some special cases)

coliforms, or 14 h at 44°C for *E. coli*, the colonies of which are a distinctive yellow colour. The volume of water filtered should yield between 10 and 100 colonies, and a series of water volumes should be filtered. Problems have been reported using this media with chlorinated waters, and Mara (1974) describes an alternative isolation technique that should be used to prevent false results. Different media are used in the USA; Membrane-Endo Medium (commonly abbreviated to ME Medium) is used for total coliforms, whereas, Membrane-Faecal Coliform Medium (or MF Medium) is used for *E. coli*. No preliminary incubation is recommended, just 24 h at either 37°C (total coliforms) or 44°C (faecal coliforms).

The actual number of coliforms is determined by counting the number of colonies and is expressed as the number per 100 ml of water. The filters have a grid printed on the surface to aid counting. Like all the bacteriological tests good sterile practice is essential. With the membrane filtration

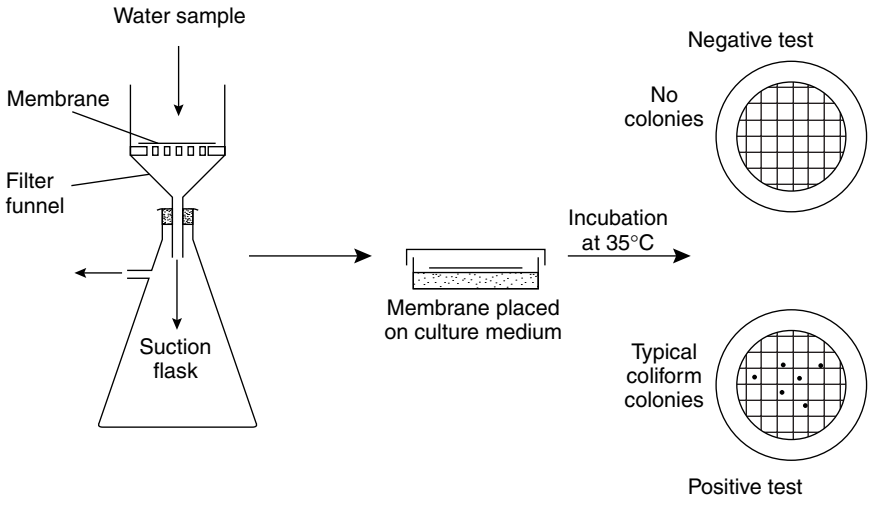


Fig. 9.8. Major steps in coliform testing by the membrane filtration technique.

technique, both the forceps used to pick up the filters and the filter unit have to be sterilized between successive filtrations. The most rapid system is a UV sterilizer and a second unit is required if a large number of samples are routinely processed. The method is not suitable for turbid effluents that are difficult to filter. Smaller volumes can be used but it is wise to increase the number of replicates; prefiltration through a series of coarser filters overcomes the problem of filterability but the estimation of coliforms will always be greatly reduced. Full details of the method are given in many standard reference texts including Mara (1974), HMSO (1983b), American Public Health Association *et al.* (1983), and Viessman and Hammer (1985). A useful working guide for those not familiar with the multiple tube and membrane filtration techniques has been produced by Button (1983) who explains how they can be used under field conditions. The brand of membrane and the pore size of membrane filters may have significant effect on the counts obtained (Tobin and Dutka 1977; Tobin *et al.* 1980). The effect of temperature on this test has also been under scrutiny and those who are undertaking comparative studies on the group are advised to read the excellent review in Dutka (1973).

There are many emerging new technologies for microbial water testing, primarily to detect coliforms. These are focused on complex biochemical techniques such as hybridisation and polymerase chain reaction (PCR), gene probe technology and monoclonal antibody methods, which allow

single bacterial cells to be detected (Gleeson and Gray 1997). Enzyme detection methods are now widely in use, especially in field situations. Total coliform detection is based on the presence of β-galactosidase, an enzyme that catalyses the breakdown of lactose into galactose and glucose. While *E. coli* is based on the detection of β-glucuronidase activity (McLellan *et al.* 2001). These tests are known as ONPG and MUG methods respectively after the substrates used in the tests and are now accepted standard monitoring methods in the USA. These ONPG-MUG tests can be used to give an MPN value or simply indicate the presence or absence (P-A) of coliforms or *E. coli*. These tests will become increasingly important as there is a general swing away from standards based on microbial density to those simply based on presence or absence of coliforms in a sample. The best known commercial ONPG-MUG preparations are currently Colilert® (Access Analytical, Branford, CT), Coliquick® (Hach Co., Loveland, CO) and Colisure® (Millipore Co., Bedford, MA). The ingredients for these new tests come in powder form (in test tubes for the quantitative MPN method and in containers for P-A analysis). A measured amount of water is added to each tube or container and the powder dissolves into a colourless solution. The tubes are placed in an incubator for 24 hours at 35°C. The solution in tubes with total coliforms will be yellow which are then exposed to a hand-held fluorescent light. If the tube contains *E. coli* the solution will fluoresce brightly. The specificity of this method eliminates the need for confirmatory and completed tests.

The coliform test is still widely considered the most reliable indicator for potable water. However, in recent years there has been growing dissatisfaction with the use of coliforms as indicator organisms. Recent years have seen increasing reports of waterborne outbreaks largely as a result of protozoan and viral agents in waters considered safe to drink under current legislation which relies largely on the coliform test. The major deficiencies identified with the use of coliforms as indicators for drinking water quality assessment are: (i) The regrowth of coliforms in aquatic environments; (ii) The regrowth of coliforms in distribution networks; (iii) Suppression by high background bacterial growth; (iv) They are not directly indicative of a health threat; (v) A lack of correlation between coliforms and pathogen numbers; (vi) No relationship between either protozoan or viral numbers; (vii) The occurrence of false positive and false negative results. This has been reviewed by Gleeson and Gray (1997).

The multiple tube method

Although far more time consuming than the membrane filtration technique, the multiple tube method is still used by many public health laboratories. The principle of the test is that various volumes of the sample water are inoculated into a series of tubes containing a medium which is selective for coliform bacteria. From the pattern of positive and negative growth responses an estimate of the number of coliforms and subsequently *E. coli* can be made. The technique is carried out in two discrete stages. The first estimates the number of coliforms present on the assumption that all the tubes which show acid and gas production contain coliform organisms. Because this assumption is made, this is known widely as the presumptive coliform count. The second stage tests for the presence of *E. coli*. The first stage is completed over 48 hours, and the second takes a further 24 hours, with the whole test taking three days to complete.

A range of small volumes of the water sample are added to tubes containing the selective medium. A range of volumes are used to ensure that the optimum numbers of coliforms are inoculated into the medium. For example, with good quality waters one 50 ml and five 10 ml volumes should be inoculated into the medium. If water of doubtful quality is tested then one 50 ml, five 10 ml, and five 1 ml volumes should be used making 11 tubes for the one sample. If effluents are tested then the water sample will have to be diluted using 0.25% Ringer's solution. With heavily polluted waters, such as raw sewage, dilution of 10^{-2} 10^{-3}, or even higher may be required to give some negative reactions and thus obtain a finite figure for the most probable number (HMSO 1983b). Minerals Modified Glutamate Medium is now used in Britain and has superseded MacConkeys Broth, which was made from peptone and bile salts that were complex compounds which varied from batch to batch in their nutrient and inhibitory properties respectively (Mara 1974). The media used for the multiple tube test contains lactose as the principle carbon source and a pH indicator to show if acids are produced from the fermentation of the lactose by coliforms. Minerals Modified Glutamate Medium is sufficiently selective for the coliform group and, unlike MacConkeys Broth, does not include a compound specifically to inhibit non-coliform bacteria. An inverted Durham tube (a small vial) is used to detect the formation of gas, and the inoculated tubes are incubated for 48 hours at 37°C. At this temperature, coliforms can ferment lactose producing acid and gas, and any tubes showing a pH reaction and with a bubble of gas trapped in the inverted Durham tube, are considered as positive (Fig. 9.9). The results are expressed as the most probable

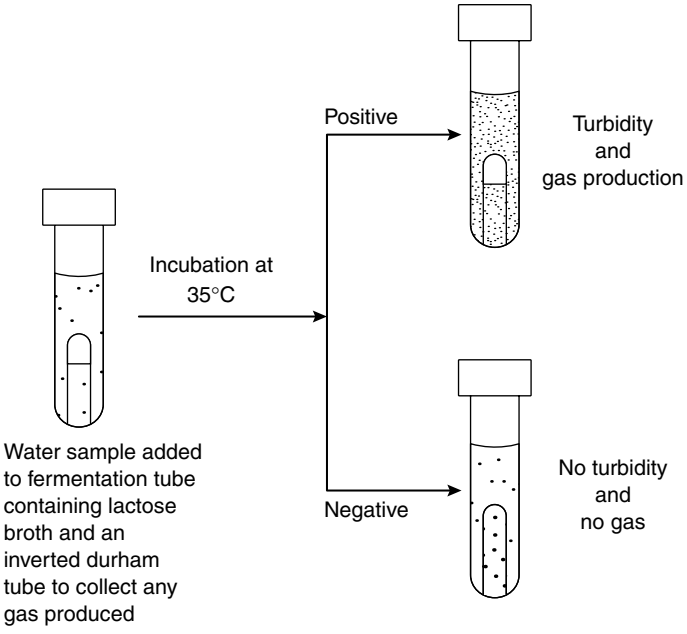


Fig. 9.9. The production of gas and turbidity are the positive signs in the presumptive coliform test using the multiple tube technique. Either lactose or lauryl trypose broth can be used in the fermentation tubes (Fig. 9.10).

number (MPN) of presumptive coliforms per 100 ml of water. The pattern of positive and negative tubes in decreasing volume, or increasing dilution, are used to obtain an estimate of the presumptive coliforms using special probability tables with 95% confidence limits (Table 9.20). This allows for the lower and upper limits between which the real density of coliforms in a particular water sample can be expected to occur. A worked example is given in Table 9.21.

The second stage of the multiple tube method is to test the positive tubes in the presumptive coliform test for the presence of *E. coli* (faecal coliforms). A small subsample (< 0.01 ml) is taken from each tube that showed a positive reaction in the first stage presumptive coliform test, using a sterile wire loop to inoculate each of two tubes, one containing Tryptone water the other containing either Lactose Ricinoleate Broth or Brilliant Green Bile Lactose Broth. Both tubes are incubated at 44°C, but anaerobic lactose fermenting bacteria can also grow in it at this temperature and cause false results. Therefore, the sodium ricinoleate or brilliant green dye inhibits the growth of these spore-forming anaerobic bacteria, which might

Table 9.20. Tables for the calculation of the most probable number (MPN) of coliform bacteria in 100 ml of original water sample for various combinations of positive and negative results, with 95% confidence limits. *Table A* is used when five 10 ml, five 1 ml, and five 0.1 ml subsample are used. *Table B* when one 50 ml and five 10 ml subsamples are used, and *Table C* when one 50 ml, five 10 ml, and five 1 ml subsample are used (HMSO 1983b).

Table A

No. of tubes giving positive reaction out of			MPN per 100 ml	95% confidence limits	
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower limit	Upper limit
0	0	1	2	< 0.5	7
0	1	0	2	< 0.5	7
0	2	0	4	< 0.5	11
1	0	0	2	< 0.5	7
1	0	1	4	< 0.5	11
1	1	0	4	< 0.5	11
1	1	1	6	< 0.5	15
1	2	0	6	< 0.5	15
2	0	0	5	< 0.5	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	96
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	114
5	1	0	33	11	93

Table 9.20. (*Continued*)

Table A

No. of tubes giving positive reaction out of			MPN per 100 ml	95% confidence limits	
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower limit	Upper limit
5	1	1	46	16	120
5	1	2	63	21	154
5	2	0	49	17	126
5	2	1	70	23	168
5	2	2	94	28	219
5	3	0	79	25	187
5	3	1	109	31	253
5	3	2	141	37	343
5	3	3	175	44	503
5	4	0	130	35	302
5	4	1	172	43	436
5	4	2	221	57	698
5	4	3	278	90	849
5	4	4	345	117	999
5	5	0	240	68	754
5	5	1	348	118	1005
5	5	2	542	180	1405
5	5	3	918	303	3222
5	5	4	1609	635	5805

Table B

No. of tubes giving positive reaction out of		MPN per 100 ml	95% confidence limits	
1 of 50 ml	5 of 10 ml each		Lower limit	Upper limit
0	1	1	< 0.5	4
0	2	2	< 0.5	6
0	3	4	< 0.5	11
0	4	5	1	13
1	0	2	< 0.5	6
1	1	3	< 0.5	9
1	2	6	1	15
1	3	9	2	21
1	4	16	4	40

Table 9.20. (*Continued*)

Table C

No. of tubes giving positive reaction out of			MPN per 100 ml	95% confidence limits	
1 of 50 ml each	5 of 10 ml each	5 of 1 ml each		Lower limit	Upper limit
0	0	1	1	< 0.5	4
0	0	2	2	< 0.5	6
0	1	0	1	< 0.5	4
0	1	1	2	< 0.5	6
0	1	2	3	< 0.5	8
0	2	0	2	< 0.5	6
0	2	1	3	< 0.5	8
0	2	2	4	< 0.5	11
0	3	0	3	< 0.5	8
0	3	1	5	< 0.5	13
0	4	0	5	< 0.5	13
1	0	0	1	< 0.5	4
1	0	1	3	< 0.5	8
1	0	2	4	< 0.5	11
1	0	3	6	< 0.5	15
1	1	0	3	< 0.5	8
1	1	1	5	< 0.5	13
1	1	2	7	1	17
1	1	3	9	2	21
1	2	0	5	< 0.5	13
1	2	1	7	1	17
1	2	2	10	3	23
1	2	3	12	3	28
1	3	0	8	2	19
1	3	1	11	3	26
1	3	2	14	4	34
1	3	3	18	5	53
1	3	4	21	6	66
1	4	0	13	4	31
1	4	1	17	5	47
1	4	2	22	7	69
1	4	3	28	9	85
1	4	4	35	12	101
1	4	5	43	15	117
1	5	0	24	8	75
1	5	1	35	12	101
1	5	2	54	18	138
1	5	3	92	27	217
1	5	4	161	39	> 450

have been responsible for false positive reactions in the presumptive test. Only *E. coli* can reduce indole and ferment lactose at this temperature and so *both* tubes must show a positive reaction to confirm its presence. From the pattern of positive and negative tubes the number of *E. coli* or faecal coliforms can be estimated from the MPN tables. The test used in the USA is slightly different and is summarised in Fig. 9.10.

The membrane filtration technique is considered to have many advantages over the multiple tube method for water testing. These include: (i) Presumptive coliform counts are available in a shorter time (18–14 hours); (ii) It is a simpler test with less steps; (iii) There is considerable saving in the laboratory in the amounts of culture media, labour and glassware required; (iv) Larger volumes of sample may be processed. (v) It is possible to carry out filtration in the field; (vi) False negative results due to the development of aerobic and anaerobic spore-bearing organisms are unlikely to occur (Table 9.19). Details of all microbial methods are given in *Standard Methods* for the USA (APHA 1992) and for the UK in *Report on Public Health and Medical Subjects No. 71* (Department of the Environment 1994).

Pour plate method

This technique is often used when counting coliforms in very polluted waters. Small subsamples of water are inoculated directly onto Lactose Teepol Agar.

The plates are incubated at 30°C for 4 h followed by 20 h at either 37°C or 44°C to yield coliform or *E. coli* counts respectively. When coliforms are present the green agar is changed to a translucent yellow colour due to acid production. The colonies of coliforms are counted directly from the plate. It is always advisable to do a series of dilutions to ensure that at least one plate contains a countable number of distinct colonies.

In addition to the development of improved techniques for the isolation and enumeration of total coliforms and faecal coliforms, the realization that there are inherent problems with their use as indicator organisms has also resulted in the search for an alternative indicator system which unequivocally denotes the presence of faecal material and the existence of a potential health hazard. Over the years, several groups of organisms have been suggested as tentative alternatives to coliforms and *E. coli*. Some of these, most notably, the faecal streptococci and *Clostridium perfringens* have a history of use as long as the faecal coliforms, while the use of other groups is much more recent.

Table 9.21. Calculation of number of coliform bacteria (MPN) from samples collected in the field.

(i) The Coliform count for a sample of river water taken below an outfall from a sewage treatment plant at Osberstown, Co. Kildare on the River Liffey was calculated using the most probable number technique (MPN). Five replicate tubes were prepared using 10 ml, 1 ml, and 0.1 ml of sample.

Sub sample used (ml)	Replicates	Positive tubes
10	5	3
1	5	1
0.1	5	0

Using Table 9.20(A) the combination of 3–1–0 gives an MPN value of 11 coliforms per 100 ml of sample.

(ii) Stronger samples such as wastewaters and effluents will need to be diluted before setting up the tubes. When calculating the coliform count this dilution factor must be taken into account. For example the final effluent from Carlow sewage treatment plant was examined and the results were:

Sub sample used (ml)	Replicates	Positive tubes
0.1	5	4
0.01	5	3
0.001	5	1

Using Table 9.20(A) the combination of 4–3–1 gives an MPN value of 33, this is multiplied up by the dilution factor of 100 to give a coliform count of 3300 coliforms per 100 ml of sample.

9.3.2. *Faecal streptococci*

The faecal streptococci are a group of Gram positive cocci, occurring in chains of varying length. They are both non-sporulating and non-motile and all give a positive reaction with Lancefield's Group-D antisera. They grow in the presence of bile salts at 44°C, in concentrations of sodium azide that is inhibitory to most Gram-negative bacteria including coliforms (Mara 1974). Taxonomically the faecal streptococci belong to the genera *Enterococcus* and *Streptococcus* (Devriese *et al.* 1992; Holt *et al.* 1993). The genus *Enterococcus* includes all streptococci that share certain biochemical properties and have a wide tolerance of adverse growth conditions (WHO 1993).

They are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6 and at 10°C and 45°C (APHA 1992) and include the species *Ent. avium, Ent. faecium*, *Ent. durans, Ent. faecalis* and *Ent. gallinarium*. The term enterococcus is rarely used in the field of water pollution control (Ellis 1989). Of the genus Streptococcus, only *Str. bovis* and *Str. equinus* are considered to be true faecal streptococci as these are the only members of this genus to possess the Group-D antigen (WHO 1993). These species are predominately found in animal faeces (Geldreich and Kenner 1969; Wheater *et al.* 1979; Devriese *et al.* 1993) while *Ent. faecalis* and *Ent. faecium* are considered to be more specific to the human gut. Other streptococcal species have also been isolated from the human gut, albeit in less numbers (Wantabe *et al.* 1981). The group also includes other biotypes, which are ubiquitous in nature, such as *Ent. casseloflavus, Ent. faecalis* var *liquefaciens*, *Ent. malodoratus* and *Ent. solidarius* (Sinton *et al.* 1993a; WHO 1993).

Interest in the use of faecal streptococci as a pollution indicator dates back as far as 1900, when they were found to be consistently present in the faeces of warm blooded animals and in waters associated with discharges from such animals. However, the literature shows little evidence of their application as potential indicators of human pathogens until improved methods for their enumeration and detection were developed in the 1950s (Geldreich and Kenner 1969). The new EU Drinking Water Directive (98/83/EEC) gives maximum permissible values for faecal streptococci in finished waters and bottled waters (EU 1995). Their use as pollution indicators has been reviewed by Sinton *et al.* (1993b).

Numerically faecal streptococci can be equally as abundant as coliforms, especially in stormwaters and specific effluents (e.g. intensive animal rearing units). But under normal circumstances they are slightly less abundant. Faecal streptococci are considered to have certain advantages over coliforms as pollution indicators: (i) They rarely multiply in water (Geldreich 1970; Feacham *et al.* 1983; WHO 1993); (ii) They are more resistant to environmental stress and chlorination than coliforms (Pipes 1982a); (iii) They generally persist longer in the environment (McFeters *et al.* 1974; Vascoucelos *et al.* 1976), with the exception of *S. bovis* and *S. equinus* which die off rapidly once outside the animal intestinal tract (Geldreich and Kenner 1969). In contrast to other studies Dutka and Kwan (1980) have also observed *Ent. faecalis* to have a faster die off rate than *E. coli*. However, this observation should be treated with some caution. As Feacham *et al.* (1983) note, '*there are probably considerable inter- and intra-species variation in survival ability. Studies on mixed populations of faecal Streptococci*

and E. coli cannot be compared directly with studies on the survival of single laboratory maintained strains.'

The primary value of faecal streptococci in water quality examination is in situations where the coliform test is of limited value. In Britain, faecal streptococci are used to assess the significance of doubtful results from other organisms. For example, in the event of a large occurrence of coliforms in the absence of *E. coli*, the presence of faecal streptococci is used to confirm faecal contamination (Department of the Environment *et al.* 1983). The World Health Organization (1993) recommends the use of faecal streptococci as additional indicators of treatment efficiency. As these organisms are resistant to drying, they may be valuable for routine control after laying new mains or repairs in distribution systems or for detecting pollution by surface run-off to ground or surface waters.

As *Str. bovis* and *Str. equinus* are predominately found in warm-blooded animal faeces, which suggests that they may serve as specific indicators of non-human (warm blooded animal) pollution (Geldreich 1970). *Streptococcus bovis* is widely associated with cattle and other farm animals and is a relatively uncommon member of the human gut flora (Mara and Oragui 1983). *Enterococcus faecalis* and *Ent. faecium* have both been used to indicate pollution due to faeces, although the latter is far more specific. The absence of *Ent. faecalis* should not exclude the possibility of pollution by human excreta. It is now currently felt that it is not possible to differentiate the source of faecal contamination based on speciation of faecal streptococci (APHA 1993). However, because all the species are indicative of faecal pollution, it is not necessary to identify individual species, as a single test for the whole group is sufficient. This is particularly appropriate as many of the so-called emerging pathogens are zoonoses.

Despite its advantages as an indicator organism, there are a number of characteristics which detract from the value of faecal streptococci: (i) They are less numerous than coliforms in human faeces which makes them a less sensitive indicator of human faecal contamination (Table 9.22) (Pipes 1982a); (ii) Secondly is the limitation presented by the sub-group *Ent. faecalis* var *liquefaciens*. This biotype is ubiquitous in nature. Geldreich and Kenner (1969) found it to be highly variable persisting longer in water than similarly exposed faecal coliforms (Fig. 9.11) (Geldreich 1970); (iii) The lack of standard methodology for their selective enumeration, with over 70 different media proposed (Pavlova *et al.* 1972; Yoshpe-Purer 1989). (iv) Taxonomic and ecological heterogeneity of faecal streptococci (Audicana *et al.* 1995); (v) Species of the group having different levels of sanitary significance (Gauci 1991); (vi) Their selective enumeration is very time-consuming.

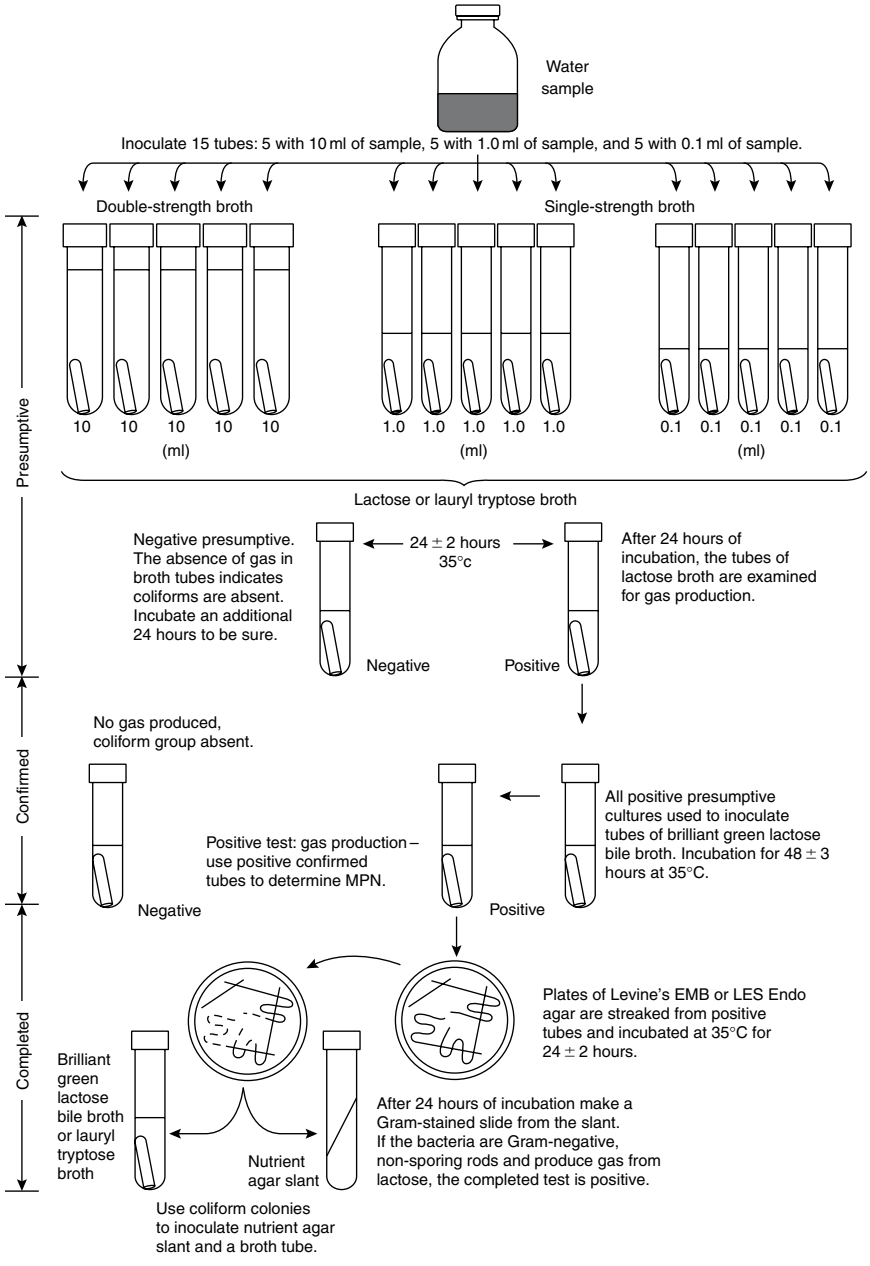


Fig. 9.10. Major steps in the multiple tube fermentation test for coliforms (Prescott *et al.* 1983).

Table 9.22. The number of indicator bacteria commonly found in human faeces expressed as cells per gram of faeces (wet weight) (Feacham *et al.* 1983).

Indicator	Cells/g faeces (w/w)
Bacteroides spp.	10^7–10^{11}
Bifidobacterium spp.	10^7–10^{11}
Clostridium perfringens	10^3–10^{10}
Coliforms	
Faecal	10^6–10^9
Non-faecal	10^7–10^9
Faecal streptococci	10^5–10^8

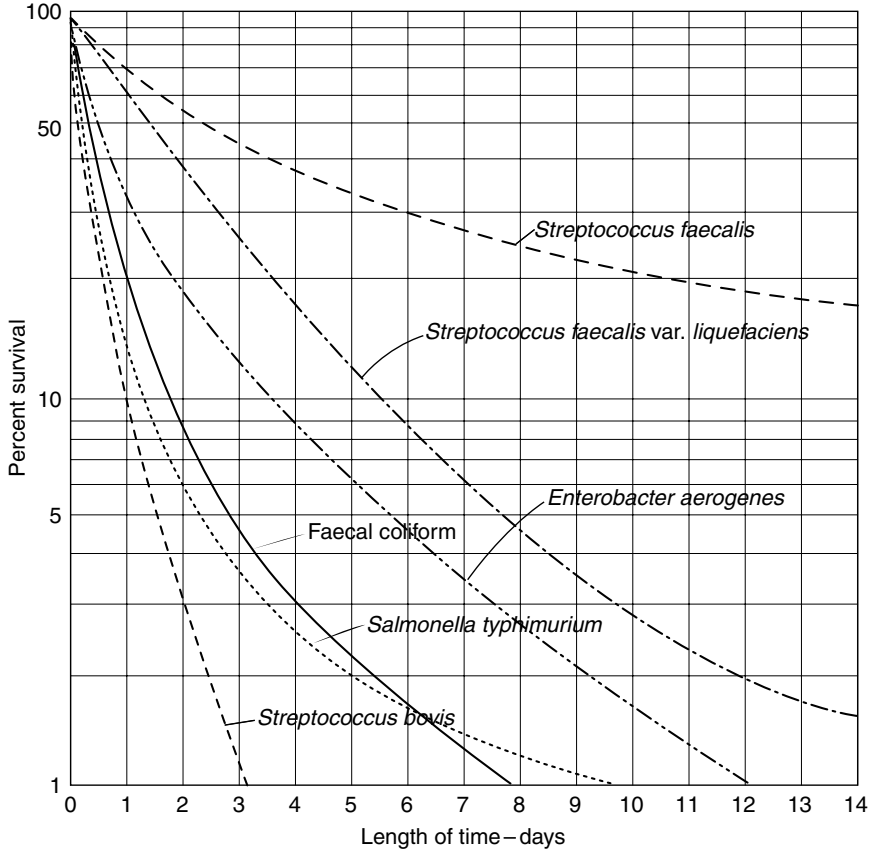


Fig. 9.11. Persistence of selected enteric bacteria in storm water stored at 20°C (Geldreich 1970).

Further studies by Geldreich (1970) showed that *Ent. faecalis* var *liquefaciens* frequently forms a substantial portion of the low faecal streptococci densities common to good quality waters. In these waters this organism may reach significant proportions. This causes concern when interpreting faecal streptococcal numbers below 100 organisms 100 ml^{-1}. Finally, as it is impossible to differentiate between faecal streptococci of faecal and non-faecal origin using standard methodologies the use of faecal streptococci is perhaps of limited value. Pourcher *et al.* (1991) have suggested that the identification of various faecal streptococci is a more viable solution as opposed to their enumeration in polluted waters. Wiggins (1996) has used antibiotic resistance patterns in faecal streptococci as a method to differentiate human and animal sources of faecal pollution in natural waters.

The use of faecal streptococci as pollution indicators is fully reviewed by Sinton *et al.* (1993a,b).

Enumeration techniques

Both the membrane filtration and most-probable-number techniques may be used for the isolation of faecal streptococci. As *Ent. faecalis* and closely related species are able to reduce 2,4,5-triphenyltetrazolium chloride (TTC) to formazan, a red dye, isolation methods use agar containing TTC with faecal streptococci colonies appearing red, maroon or pink due to formazan formation.

Membrane filtration. The method used is similar to that used for coliforms except that the medium used is Membrane-Enterococcus Agar (MEA) with incubation at 37°C for 48 hours for treated potable waters and for untreated water 37°C for 4 hours followed by 44°C for 44 hours. The more temperature sensitive faecal streptococci can be inhibited if incubated at 45°C even though it is more selective. In fact inhibition of temperature sensitive species may occur with a temperature rise of less than 0.5°C. All red, maroon, and pink colonies which are smooth and convex in shape are counted as presumptive faecal streptococci. Colourless colonies may be produced by some faecal streptococci. Confirmation of faecal streptococci is by sub-culture onto Bile Aesulin Agar and incubation for 18 hours at 44°C. Faecal streptococci form discrete colonies being formed surrounded by a brown or black halo due to aesculin hydrolysis (APHA 1992; Department of the Environment 1994). This method is specified for use in the EU Drinking Water Directive (EU 1995).

The most probable number technique. The technique employed is similar to that previously described for coliforms (MPN) except that Azide

Dextrose Broth (APHA 1992) or Glucose Azide Broth (GAB) is used (Mara 1974; Department of the Environment 1994). Tubes containing Azide Dextrose broth are incubated at 35°C and examined for turbidity after 24 and 48 hours. All turbid tubes are then subjected to the confirmation test. While tubes containing GAB are incubated at 37°C and examined after 24 and 48 hours. Growth and acid production is a positive result indicating the presence of presumptive faecal streptococci, although subculture into Bile Aesculin Agar is required for confirmation.

Other techniques. Clark's P-A technique may also be used for detection of faecal streptococci. This technique is, however, very time consuming and awkward for faecal streptococci enumeration (Clark 1968).

Enumeration techniques for faecal streptococci have been revised by a number of workers (Audicana *et al.* 1995; Dionisio and Burrego 1995). Dionisio and Burrego (1995) found membrane filtration using MEA to give the best performance characteristics of the enumeration media they evaluated in terms of recovery efficiency, precision, accuracy and specificity.

9.3.3. *Faecal coliform/faecal streptococci (FC/FS) ratio*

The FC/FS ratio has been used to determine whether pollution is of animal or human origins. The basis for using the ratio can be found in the early literature. Geldreich and Kenner (1969) reported faecal coliforms to be more numerous than faecal streptococci in human faeces with a FC/FS ratio always greater than 4. Conversely, faecal streptococci were found to be more numerous than faecal coliforms in animal faeces with the FC/FS ratio always less than 0.7. This large difference is exploited in the following way (Mara 1974):

FC/FS ratio	*Source of pollution*
> 4.0	Strong evidence that pollution is of human origin.
$> 2.0 < 4.0$	Good evidence of the predominance of human wastes in mixed pollution.
$> 0.7 < 2.0$	Good evidence of the predominance of domestic animal wastes in mixed pollution.
< 0.7	Strong evidence that pollution is of animal origin.

Expected densities of faecal coliforms and faecal streptococci in human and various animal faeces and the resultant FC/FS ratios are given in Table 9.23. According to Geldreich and Kenner (1969), this ratio is only really valid in the first 24 hours after the bacteria have been discharged into the water. However, Poucher *et al.* (1991) have shown that even in the

Table 9.23. Densities of faecal coliforms (FC) and faecal streptococci (FS) in animal faeces and the resultant FC:FS ratios (Lynch and Poole 1979).

Faecal source	Densities g^{-1} of faeces (median values)		Ratio FC:FS
	Faecal coliform	Faecal streptococci	
Man (USA)	13,000,000	30,000,00	4.33
Cow	230,000	13,000,00	0.177
Pig	3,300,000	84,000,000	0.039
Sheep	16,000,000	38,000,000	0.421
Horse	12,600	6,300,000	0.002
Duck	33,000,000	54,000,000	0.611
Chicken	1,300,000	3,400,000	0.382
Turkey	290,000	2,800,000	0.104
Cat	7,900,000	27,000,000	0.293
Dog	23,000,000	980,000,000	0.024
Field mouse	330,000	7,700,000	0.043
Rabbit	20	47,000	0.0004
Rat	180,000	78,900,000	0.0023
Chipmunk	148,000	6,000,000	0.002
Elk	5100	760,000	0.007
Robin	25,000	11,700,000	0.002
English sparrow	25,000	1,000,000	0.025
Starling	10,000	11,800,000	0.0009
Red-winged blackbird	9000	11,250,000	0.0008
Pigeon	10,000	11,500,000	0.0009

first few hours after collection, the FC/FS ratio is not constant, even for samples of the same origin. The validity of the FC/FS ratio has been questioned considerably. McFeters *et al.* (1974) pointed out that the ratios are dependent on the differential die-away rates of faecal coliform and faecal streptococci. As a result of these die-away rates, the ratios will change once the faeces is excreted (Feacham *et al.* 1983). The enterococci (*Ent. faecalis* and *Ent. faecium*) survive much longer than faecal coliforms which survive longer than either *Str. equinus* or *Str. bovis*, both of which die-off rapidly when exposed to aquatic environments (McFeters *et al.* 1974). Dutka and Kwan (1980) observed that *Ent. faecalis* had a faster die-off rate than *E. coli*. This observation, if valid, would suggest that *E. coli* is more resistant to environmental stresses and therefore such results would bias the FC/FS ratio towards human faecal pollution. Wheater *et al.* (1979) observed that

not all animals maintain a FC/FS ratio < 1. Hussong *et al.* (1979) found the FC/FS ratio seemed to be dependent on diet and that it would not be sufficient to separate avian from human faecal contamination. In addition, disinfection of wastewaters appears to have a significant effect on the indicator ratio which may lead to inaccurate conclusions. In their studies, Poucher *et al.* (1991) concluded that it is not possible to determine the source of water contamination on the basis of the FC/FS ratio.

The ratio is also almost always affected by the methods for enumerating faecal streptococci (Wheater *et al.* 1979; APHA. 1992). So in general, it would appear that there are too many factors which influence the FC/FS ratio for it to reliably differentiate between human and animal faecal contamination of water. In addition to those problems already mentioned, other variables such as time, temperature, pH, type of membrane used and the possibility of multiple sources of pollution may also affect the validity of the results (Department of the Environment *et al.* 1983). Consequently, the use of the FC/FS ratio is generally not recommended as a means of differentiating between different pollution sources (Pourcher *et al.* 1991; Rutkowski and Sjøgren 1987). The FC/FS ratio is now being replaced by other techniques to separate human and animal faecal contamination (Sec. 9.3).

Duncan Mara and John Oragui (1981,1983) of the University of Leeds have suggested that *Rhodococcus coprophilus* and *Streptococcus bovis* should be used for detecting animal faecal pollution, while sorbitol-fermenting bifidobacteria should be used for human faeces (Sec. 9.3.7). Faecal streptococci and *E. coli* are excreted by both animals and humans, and so cannot be used as a specific indicator. Also, the ratio of faecal coliforms to faecal streptococci is not always reliable. Therefore specific indicators are best. *Streptococcus bovis* is often present in significant numbers in faeces from people living in tropical areas including India and parts of Africa, while *R. coprophilus* is only found in animal faeces. Bifidobacteria have also been isolated in animal dung in low numbers, however the sorbitol-fermenting strains isolated using YN-17 and Human Bifid Sorbitol Agar media appear restricted to humans.

New methods to determine the source of waterborne faecal contamination fall into two categories, DNA fingerprinting and antibiotic resistance profiles. Databases are created from a broad selection of known human and animal faecal bacteria. Bacteria yielding similar DNA banding patterns (Parveen *et al.* 1999; Dombeck *et al.* 2000; Carson *et al.* 2001) or antibiotic resistance profiles (Hagedorn *et al.* 1999; Haywood *et al.* 2000) can be identified to give the source of faecal contamination. At the Department of Soil, Water, and Climate at the University of Minnesota over 2400 isolates

of *E. coli* have been DNA fingerprinted using a rep-PCR technique (Rademaker *et al.* 1997) to produce a unique genetic database that is currently used to discriminate between human and animal faecal pollution in four Minnesota watershed areas. Of the 2466 fingerprints obtained from 12 different animal groups as well as humans, 1616 were unique to specific source animals. From 197 human faecal sample tested 307 different *E. coli* DNA fingerprint patterns were obtained, 226 of which are specific to humans (Dombek *et al.* 2000).

9.3.4. *Clostridium perfringens*

Sulphite-reducing clostridia are anaerobic spore-forming non-motile, Gram positive rods which are exclusively faecal in origin. Spores are very resistant and are able to withstand heating at 75°C for 15 minutes, they can reduce sulphite to sulphide, ferment lactose and produce gas, and along with *Clostridium perfringens*, the most important member of the group, form a stormy clot in Litmus Milk Medium (Mara 1974). They are also pathogenic, causing gas gangrene and food poisoning.

The use of *Clostridium perfringens* as an indicator organism was first proposed in 1899 by Klein and Houston (Bisson and Cabelli 1979). Clostridial spores can survive in water much longer than either coliforms or streptococci. This persistence can indicate occasional or intermittent pollution, which then implies the need for greater frequency of sampling (Department of the Environment *et al.* 1983). Clostridial spores are also resistant to disinfection and therefore are not reduced appreciably by treatment (Table 9.24). Subsequently, they are of little value in assessing the efficiency of water treatment (Fujioka and Shizumura 1985); however, they are of use in assessing the efficiency of filtration and the susceptibility of water resources to intermittent pollution (WHO 1993).

Although *Clostridium perfringens* is consistently found in faecal wastes, it is not used as a faecal indicator in the USA and is only used to provide supplementary information in Europe (Bisson and Cabelli 1980). There are a number of reasons for this. Firstly, the spores are too resistant to chlorination to be of value in assessing drinking water quality (Pipes 1982a; Department of the Environment *et al.* 1983). Secondly, they are not suitable as indicator organisms in recreational waters as sedimented spores can be re-suspended by bather activity, surf action, or in areas where there is significant land run-off. Spores can survive for very long periods and in such cases spores may be detected long after a pollution incident giving rise to false alarms (Cabelli 1978; Bisson and Cabelli 1980). Lastly, there is the

Table 9.24. The occurrence of *Clostridium perferingens* vegetative cells and heat sensitive spores in treated wastewaters before and after chlorination (Bisson and Cabelli 1980).

Treatment Plant	% recovery from wastewater			
	Pre-chlorination		Post-chlorination	
	Vegetative cells	Heat sensitive spores	Vegetative cells	Heat sensitive spores
1	6.5	50	0	14.0
2	0	12.2	0	34.7
3	13.4	—	—	—
Average	6.6	31.0	0	24.4

problem of finding a reliable method for enumerating *Cl. perfringens* from water. There are several methods for recovering *Cl. Perfringens*, the MPN, pour plate, and membrane filtration methods. All of these methods use sulphite reduction as the differential characteristic with the use of stormy-fermentation of milk for specific identification (Mara 1974; Bisson and Cabelli 1980). A two-stage MPN technique is used to isolate and enumerate *Cl. perfringens*. First the water sample is heated to 75°C for 10 minutes and after cooling is inoculated directly into Differential Reinforced Clostridial Broth and incubated at 37°C for 48 hours. The inocula are placed in screw topped bottles partially filled with medium, which are then filled to the top with excess medium and tightly closed to ensure anaerobic conditions. The medium contains sulphite and if clostridia are present then it will be reduced and precipitated to ferrous sulphide which turns all the medium black. Confirmation of *Cl. perfringens* requires an innoculum for each positive bottle to be transferred to freshly prepared tubes of Litmus Milk which are incubated for 48 hours at 37°C. To encourage anaerobic growth the redox potential of the medium is reduced by adding a small length of iron wire, often a small nail, that is sterilized immediately before use by heating until red hot. If *Cl. perfringens* is present a stormy clot is formed in the tube (Mara 1974). Membrane filtration is also widely used. After preliminary heat treatment to destroy the vegetative bacteria present, a volume of the sample is filtered through a membrane that is incubated anaerobically on a sulphite-containing agar medium. Black colonies are formed by the presence of sulphite-reducing clostridia. *Clostridium perfringens* is confirmed by subculturing the colonies into a tube of Litmus Milk as described previously. There are currently a number of media available, but no method

has been officially adopted by the Department of the Environment (1994). Fujioka and Shizumura (1985) have assessed a more practical membrane filtration method for recovery of *Cl. perfringens* from water samples using Modified *Clostridium perfringens* Agar (m-CP) Medium. This procedure is outlined in Fig. 9.12. After incubation at 45°C on m-CP Medium, typical colonies are approximately 1–3 mm in diameter, are somewhat opaque, slightly butryous in consistency and have a pale yellow colour. Upon exposure to ammonia vapours, these colonies turn from a pink to red (but not purple) colour. This procedure was found to be very reliable with low numbers of false positive results. However, it is generally considered that while these methods are adequate for research and special investigations, they are time consuming and largely impractical for use on a routine basis.

9.3.5. *Bacteriophage*

Because of their constant presence in sewage, faeces, and polluted waters, several authors have proposed the use of bacteriophage (or bacterial viruses) as appropriate indicators of faecal pollution (Dhillon *et al.* 1976; Borrego *et al.* 1987; Ratto *et al.* 1989; Borrego *et al.* 1990). These organisms have also been suggested as indicators of viral pollution. This is because their structure, morphology and size, as well as their behaviour in the aquatic environment closely resembles that of enteric viruses (Kott *et al.* 1974; Simkova and Cervenka 1981; Stetler 1984; Geldenhuys and Pretorious 1989).

The use of bacteriophage as indicators of faecal pollution is based on the assumption that their presence in water samples denotes the presence of bacteria capable of supporting the replication of the phage. Two groups of phage in particular have been studied: the somatic coliphage, which infect *E. coli* host strains through cell wall receptors; and the F-specific RNA bacteriophage, which infect strains of *E. coli* and related bacteria through the F- or sex pili (WHO 1992). A significant advantage of using bacteriophage is that they can be detected by simple and inexpensive techniques which yield results in 8–18 hours (Grabow 1986). A proposed method for coliphage detection is outlined in the 18th edition of Standard Methods (APHA 1992).

To date, most attention has been directed to the use of coliphage (Cornax *et al.* 1991; Morinigo *et al.* 1992). Most research has been done on the use of coliphages to mimic viruses during water treatment, and indeed coliphages and enteroviruses appeared to be removed or inactivated at similar rates during treatment processes, including chlorination (Havelaar and

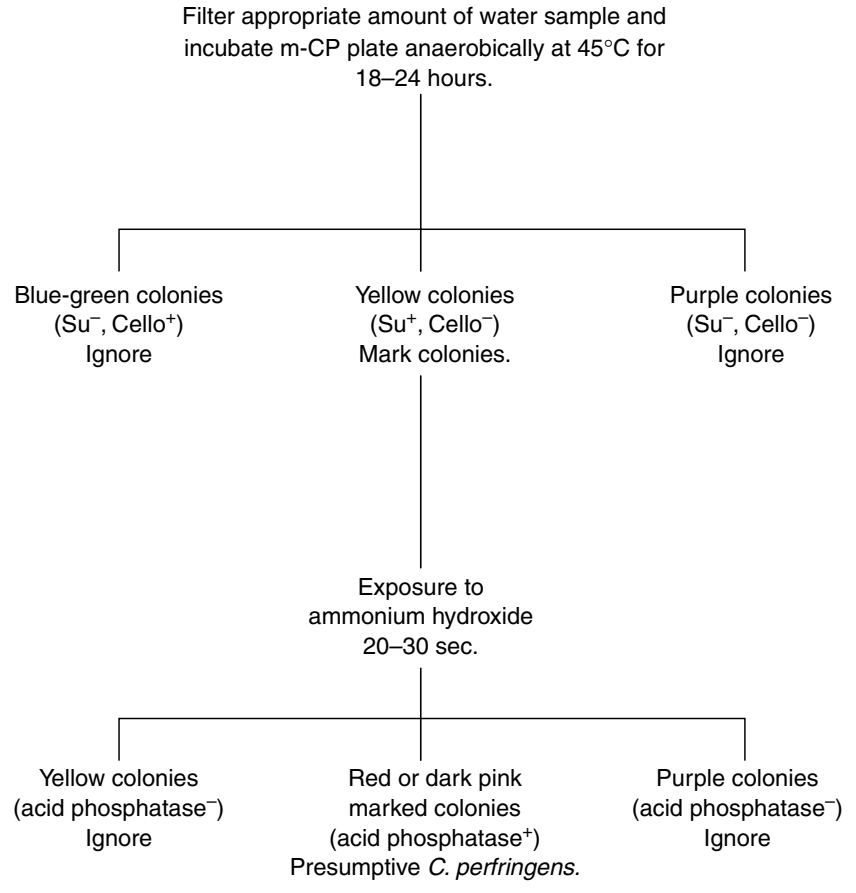


Fig. 9.12. Flow scheme for the m-CP procedure (Bisson and Cabelli 1979).

Hogeboom 1984; Payment 1991). However, their use may be limited as indicators of faecal pollution as they are not widely carried by a significant portion of the human population (Havelaar *et al.* 1986, 1990). Certain limitations associated with the use of coliphages have been reported. These include: (i) Enteric viruses have been detected in their absence; (ii) They may replicate in natural waters under certain conditions; (iii) Autochthonous coliphage have been detected in unpolluted waters (Morinigo *et al.* 1992). These limitations have prompted research into the suitability of other phage such as the F-specific RNA bacteriophage as indicators of faecal pollution. Studies by Havelaar *et al.*, (1990) show that these phage occur rarely in faeces and show no direct relationship with faecal pollution. Although Cornax

et al., (1991) observed that high concentrations of F-specific bacteriophage were found in sewage, despite low concentrations in faeces. While these phage cannot be recommended as indicators of faecal pollution, their presence in high numbers in waste waters and their relatively high resistance to chlorination makes them suitable as indicators of sewage pollution.

Bacteriophage of *Bacteroides fragilis* have also been suggested as potential indicators of human viruses in the environment (Tartera and Jofre 1985). *Bacteroides* spp. are strict anaerobes and are a major component of human faeces (Table 9.22) and therefore bacteriophage active against these organisms have the potential to be suitable indicators of viral contamination.

Table 9.25. Bacteriophage active against *B. fragilis* HSP40 in faeces of various animals (Tartera and Jofre 1987).

Animal spp.	No. tested	No (%) of positive tests
Human	40	4 (10)
Cow	40	0 (0)
Pig	50	0 (0)
Rabbit	21	0 (0)
Mouse	28	0 (0)
Hen	20	0 (0)
Quail	10	0 (0)

Table 9.26. Presence of bacteriophage active against *B. fragilis* HSP40 in different environmental samples (Jofre *et al.* 1987).

Sample type	Range of faecal coliforms/100 mL	No. of samples	% of positive samples
Water from lagoons with abundant wildlife	10^3–10^4	1	0
	10^2–10^3	6	0
	10–10^2	8	0
Surface water near urban areas	$> 10^4$	50	100
	10^3–10^4	13	70
	10^2–10^3	3	66
	10–10^2	2	50
Sediments from lagoons with abundant wildlife	10^2–10^3	7	0

There are a number of factors which would support this suggestion:

(i) Bacteriophage infected *Bacteroides fragilis* appear to be exclusively human in origin (Table 9.25) (Tartera and Jofre 1987) and appear only to be present in environmental samples contaminated with human faecal pollution (Table 9.26) (Jofre *et al.* 1987). This may help to differentiate human from animal contamination;
(ii) They are absent from natural habitats which is a considerable advantage over coliphage which are found in habitats other than the human gut;
(iii) They are unable to multiply in the environment (Tartera and Jofre 1989);
(iv) Their decay rate is very similar to that of human enteric viruses.

The above table illustrates that these bacteriophage behave in many ways similar to human enteric viruses and thus would seem to be ideal as indicators of their presence in drinking waters. However, as with the *Bifidobacteria*, bacteriophage of B. fragilis are anaerobic, which involves complicated and tedious methodology which limits their suitability as routine indicator organisms (Tartera and Jofre 1989).

9.3.6. *Bifidobacteria*

Bifidobacteria are Gram positive, non-sporulating, obligately anaerobic catalase-negative, rod-shaped bacteria. They are common in the faeces of humans, pigs, cattle and sheep, but not in horses, poultry or household pets such as cats and dogs. They were first described by Tissier in 1912. Over 17 species of *Bifidobacteria* are now recognised (Resnick and Levin 1981) with the sorbitol-fermenting strains with *B. adolescentis* and *B. breve* only found in humans.

Bifidobacteria were first proposed as a possible indicator of faecal pollution by Mosel in 1958 because: (i) They are anaerobic and cannot multiply outside the intestine (Dutka 1979; Levin and Resnick 1981). (ii) *Bifidobacteria* are exclusively faecal in origin, with some species occurring in humans in proportions exceeding that of *E. coli* (Resnick and Levin 1981) (Table 9.26); (iii) They are considered ideal indicator organisms for tropical samples as such samples may contain organisms which can multiply, ferment lactose and produce indole at 44°C, but are not faecal in origin (Evison and James 1973; Feacham *et al.* 1982; Carrillo *et al.* 1985).

The main drawback in using of *Bifidobacteria* as an indicator organism is the lack of a suitable selective media and the difficulty with anaerobic

methodology (Levin and Resnick 1981). Various media have been developed in an attempt to overcome these restrictions (Evison and Morgan 1978; Levin and Resnick 1981; Mara and Oragui 1983; Munoa and Panes 1988), but for various reasons, including a lack of selectivity, and the tendency of some of these media to inhibit certain *Bifidobacterium* species, these have proved relatively unsatisfactory. More recently, species specific gene probes for Bifidobacteria species have been designed (Yamamato 1992), but no further evaluation has been made of these.

In addition to problems of isolation there are also certain characteristics of Bifidiobacterium which may limit its usefulness as an indicator organism. For example, it has a short survival time in the environment. The time lapse between when samples are collected and examined must be kept to a minimum and even then only 60–70% of samples can be recovered (Levin and Resnick 1981). Also, Bifidiobacterium are sensitive to chlorine which renders this organism ineffective for examining chlorinated waters (Levin and Resnick 1981).

9.3.7. *Rhodococcus spp.*

Rhodococcus coprophilus is an aerobic, *Nocardia*-like actinomycete commonly found on herbivore dung and in aquatic environments (Rowbotham and Cross 1977). As this organism is only excreted by farm animals, it has been suggested as a specific indicator organism of farm animal contamination. In a survey of a wide range of animals and birds, Mara and Oragui (1981) found that this organism was not recovered from faecal specimens of humans, cats, rabbits, rats, mice, or turkeys. The frequency of isolation was 100% in cattle, sheep, pig and horse faeces. *Rhodococcus coprophilus* survives longer and better in aquatic environments than other indicator organisms such as faecal streptococci and *E. coli* (up to 100 days in polluted waters) and has been suggested as a useful indicator of the presence of remote faecal pollution of farm animal origin. The presence of both *Str. bovis* and *R. coprophilus* would indicate animal pollution of recent origin whereas the presence of *R. coprophilus* alone is suggestive of remote animal pollution. The use of this organism as an indicator organism is somewhat limited because of the long incubation period it requires (17–18 days) and the difficult isolation technique required (Mara and Oragui 1981), factors that make it currently impractical for routine monitoring. It is however useful in the assessment of the suitability of surface and ground water resources for supply purposes in rural areas that are at risk from farm animals (Sec. 9.3.3).

9.3.8. *Heterotrophic plate count bacteria*

Heterotrophic plate count (HPC) represent the aerobic and facultatively anaerobic bacteria that derive their carbon and energy from organic compounds. This group includes Gram negative bacteria belonging to the following genera: *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Serratia*, *Acinetobacter*, *Proteus*, *Alcaligenes*, and *Moraxella* (Bitton 1994). Certain HPC organisms are considered to be opportunistic pathogens (Sec. 9.2.2) and have been implemented in gastrointestinal illness (Geldreich and Rice 1987).

Heterotrophic bacteria such as those listed in Table 9.27 are commonly isolated from raw waters and are widespread in soil and vegetation. They can survive for long periods in water and rapidly multiply, especially at summer temperatures. In reality the counts themselves have little health significance, although there is evidence that excessive numbers can cause gastrointestinal illness in the very young and other high risk groups. There is also concern that these organisms can rapidly multiple in bottled waters, especially if not stored properly once opened. The EU Drinking Water Directive (80/778/EEC) requires that there is no significant increase from background levels of HPC bacteria in either tap or bottled waters (EU 1995). While HPC bacteria are not a direct indicator of faecal contamination, they do indicate variation in water quality and the potential for pathogen survival and regrowth.

The HPC organisms typically found in raw waters and within water supply distribution systems are listed in Table 9.27. Heterotrophic plate counts are done normally using the spread plate method using Yeast Extract Agar (YEA) and incubated at 22°C for 72 hours and 37°C for 24 hours. Results are expressed as colony forming units (cfu). Counts at 37°C are especially useful as they can provide rapid information of possible contamination of water supplies. The low nutrient medium R2A Agar (Reasoner and Geldrich 1985) is recommended instead of YEA for the recovery of disinfectant-damaged bacteria (Department of the Environment 1994).

Heterotrophic plate counts have long been employed to evaluate water quality. Miguel, in 1891, provided standards, based on HPCs, to evaluate water quality (Pipes 1982a) (Table 9.28). In Britain not much importance is placed on HPC counts for assessing the potability of drinking water. It is considered that their value lies mainly in indicating the efficiency of various water treatment processes including disinfection, as well as the cleanliness and integrity of the distribution system (Department of the Environment *et al.* 1983). Changes in the pattern of colony counts of samples taken from a

Table 9.27. Examples of HPC bacteria isolated from distribution and raw waters (Bitton 1984).

Organism	Distribution water		Raw water	
	Total	% of total	Total	% of total
Actinomycete	37	10.7	0	0
Arthrobacter spp.	8	2.3	2	1.3
Bacillus spp.	17	4.9	1	0.6
Corynebacterium spp.	31	8.9	3	1.9
Micrococcus luteus	12	3.5	5	3.2
Staphylococcus aureus	2	0.6	0	0
S. epidermidis	18	5.2	8	5.1
Acinetobacter spp.	19	5.5	17	10.8
Alcaligenes spp.	13	3.7	1	0.6
F. meningosepticum	7	2.0	0	0
Group IVe	4	1.2	0	0
Group M5	9	2.6	2	1.3
Gropu M4	8	2.3	2	1.3
Moraxella spp.	1	0.3	1	0.6
Pseudomonas alcaligenes	24	6.9	4	2.5
P. cepacia	4	1.2	0	0
P. fluorescens	2	0.6	0	0
P. mallei	5	1.4	0	0
P. maltophilia	4	1.2	9	5.7
Pseudomonas spp.	10	2.9	0	0
Aeromonas spp.	33	9.5	25	15.9
Citrobacter freundii	6	1.7	8	5.1
Enterobacter agglomerans	4	1.2	18	11.5
Escherichia coli	1	0.3	0	0
Yersinia enterocolitica	3	0.9	10	6.4
Group IIK biotype I	0	0	1	0.6
Hafnia alvei	0	0	9	5.7
Enterobacter aerogenes	0	0	1	0.6
Enterobacter cloacae	0	0	1	0.6
Klebsiella pneumoniae	0	0	0	0
Serratia liquefaciens	0	0	1	0.6
Unidentified	65	18.7	28	17.8
Total	347	100	157	99.7

Table 9.28. Sanitary quality of water according to Miguel.*

Quality	No. of bacteria/mL
Excessively pure	< 10
Very pure	10–100
Pure	100–1000
Mediocre	1000–10,000
Impure	10,000–100,000
Very impure	$> 100,000$

*Miguel, P. (1891) *Manuel Pratique d'Analyse Bacteriologique des Eaux*, Paris.

given supply have more significance than a single numerical count. A sudden marked change in the colony count of water in a supply may indicate more serious pollution, where as deviations in the expected seasonal trend may suggest longer-term changes in the water supply. In contrast, considerably more emphasis has been put on the importance of sampling and analysing HPC bacteria in the USA by the US EPA, so much so that the National Primary Drinking Water Regulations now include maximum containment levels of no more than 500 cfu/ml for HPCs (US EPA 1990b). This is to reduce possible interference with the detection of coliforms. While many HPC bacteria are recognised as opportunistic pathogens, the full public health significance of such organisms to the general public is not yet known. Subsequently, it is likely that more emphasis will be placed on the value of such counts in the future.

9.3.9. *Other indicator organisms*

There are a number of other organisms which have also been considered as having potential as alternative indicator organisms including *Pseudomonas* spp., *Bacteroides* spp., and *Candida albicans*.

Pseudomonas aeriginosa is a Gram-negative, non-sporulating opportunistic pathogen which causes infection in wounds, as well as ear and urinary tract infections, meningitis, and respiratory infections (Feacham *et al.* 1983). It has interesting growth properties, forming both oxidase and catalase, growing at 42°C but not at 4°C. It is also able to reduce nitrates and nitrites, produce ammonia from the breakdown of acetamide, and is able to hydrolyse casein but not starch. An important characteristic of the

pseudomonad is that it can produce the blue-green pigment pyocyanin or the fluorescent pigment fluorescein, or both. *Pseudomonas aeriginosa* is particularly associated with disease among swimmers. Numerous cases of folliculitis, dermatitis, ear, and urinary tract infections due to *P. aeriginosa*, contracted after swimming in contaminated waters have been reported (Yoshpe-Purer and Golderman 1987). Because of this association, and its consistent presence in high numbers in sewage, *P. aeriginosa* was thought to have potential as an indicator of water quality, particularly recreational waters (Cabelli 1978). However, as this organism is known to be ubiquitous in nature and can multiply under natural conditions, it is in practice of little use in faecal contamination studies. While it should not be used as an indicator organism, bottled waters are required to be free of the organism and so must be monitored at bottling plants (EU 1995). Full details of the isolation of *P. aeriginosa* are given by the Department of the Environment (1994) and the American Public Health Association (APHA 1999).

Bacteroides spp. are Gram-negative, non-motile, non-sporing, obligately anaerobic bacteria which form a major component of human and animal faeces (Table 9.22). In fact they are more numerous than *E. coli* in human faeces. There are currently five species *B. diastonis*, *B. fragilis*, *B. ovatus*, *B. thetaiotaomicron* and *B. vulgatus*. *Bacteroides fragilis* is the species of the genus most commonly associated with human faeces. They are of limited value as indicator organisms, mainly because of the problems associated with the isolation and enumeration of anaerobes. Allsop and Stickler (1984) report that in practice they appear to have little advantage over *E. coli*, especially as they rapidly die off in water.

Candida albicans is the most exciting potential indicator to be evaluated in recent years (Grabow *et al.*, 1980). It is an extremely widespread yeast found in the populations of all developed countries, with up to 80% of the adults having low levels of infection and detectable levels of the yeast in their faeces. Although it lives at low levels of activity in the rectum of most people, it can result in mouth, vaginal, groin, and general skin infections. The latter are especially common in swimmers (Buckley 1971), in whom there has been a steady increase in the incidence of serious infections (Briscou 1975). For most people infection becomes problematic in the intestinal tract after a course of antibiotic treatment. The yeast can not exist for prolonged periods without a natural host and does not exist or grow independently in water, therefore the presence of *C. albicans* is a direct result of faecal contamination and makes it ideal for monitoring water, sewage, and estuarine waters. The yeast is effectively removed by

sewage treatment (Buck 1977), although concentrations of up to 100 cells of *C. albicans* per 100 ml have been reported in treated effluents (Dutka 1979).

The coliform test has been used for many years to determine the safety of swimming pool water, yet the contamination was not always faecal in origin with infections primarily of the respiratory tract, skin, and eyes (Esterman *et al.* 1984; Robinton and Mood 1966). For this reason *Staphylococcus aureus* and *C. albicans* have been proposed as better indicators of the types of infection associated with swimming (Sato *et al.* 1995). There are no universally recognised standard enumeration procedures for *C. albicans*, although Dutka (1978) has evaluated a membrane filtration system and medium originally developed by Buck (1977) that he found to be both rapid and extremely reproducible. For example, typical counts for bathing beaches with high faecal coliform counts can be up to 25 *C. albicans* per litre, falling to 0–2 *C. albicans* per litre for relatively unpolluted beaches. As an indicator of faecal contamination of drinking waters it has yet to be fully evaluated. Sato *et al.* (1995) have compared several methodologies and selective culture media for the isolation and enumeration of *C. albicans*. They found that the membrane filtration method using m-CA Agar and incubated at 35°C for 2–4 days (Buck and Bubucis 1978) was most effective. Colonies are chocolate brown and approximately 1 mm in diameter. Confirmation of colonies was done by germ tube and chlamydospore production and sugar assimilation (Dutka 1978).

There is a much higher incidence of staphylococci infections in swimmers than non-swimmers. Much concern has been expressed that the normal enteric indicators do not provide an index for these infections as contamination is via the mouth, nose, throat, and skin of bathers, rather than urine and faeces. *Staphylococcus aureus* is the major pathogen of concern. It is commensal in the nostril, armpits, and groin in about 40% of healthy adults, but can cause a variety of diseases of the upper respiratory tract, eyes, and ears. Among the commonest diseases are osteomyelitus, impetigo, minor boils, and carbuncles. *Staphylococcus aureus* forms characteristic clusters of Gram-positive cocci, 1 μm in diameter, which are non-motile, non-sporing, and non-capsulated. Colonies appear as yellow colonies when isolated by the membrane filtration technique and grown on a mannitol salt medium, such as Membrane Staphylococcus Medium.

There appears to be an urgent need to measure the incidence of staphylococci in swimming pools and natural waters that are used for swimming or other recreational pursuits. As the organisms are more resistant to chlorination and other environmental factors than *E. coli*, staphylococci would

appear ideal indicators, although enumeration and isolation techniques *for Staph. aureus* are not fully effective. The techniques used for the group are described elsewhere (Mara 1974; Alico and Palenchar 1975; American Public Health Association *et al.* 1983).

9.3.10. *Chemical indicators*

Faecal sterols

Dutka, Chan and Coburn (1974) have proposed using faecal sterols such as coprostanol and cholesterol to indicate human contamination of water systems. Both of these sterols are present in mammalian faeces and have been found in domestic sewage and receiving wastes. Unlike biological indicators, faecal sterols do not seem to be affected by chemical disinfectants or toxic wastes discharges. Thus, for chlorinated effluents, faecal sterol levels would appear to be ideal indicators of human faecal contamination and possibly of health hazards associated with non-activated viruses. The use of faecal sterols may be of particular benefit in situations where the use of conventional bacterial indicators is difficult such as industrial effluents or in situations where it can not be established whether the bacterial indicators are of faecal origin or are land washed (Dutka 1979; Grimalt *et al.* 1990).

Humans and higher animals excrete high concentrations of cholesterol, a precursor to coprostanol, in their faeces. It is also found in some non-faecal sources (Waite 1984). This lack of specificity means that this particular sterol is not given serious consideration as a potential indicator. On the other hand, coprostanol is stable and non-pathogenic. It is easily degraded by sewage treatment and therefore its presence in water is indicative of recent sewage contamination (Waite 1984). Sewage effluents contain on average 33 μg/l of coprostanol of which between 80–95% is associated with particulate matter and quickly settles out of solution to become assimilated into the aquatic sediment (Brown and Wade 1984). For this reason it is accepted as a measure recent or intermittent faecal pollution of surface and coastal waters by the examination of coprostanol in the sediment (Hasset and Lee 1977; Walker *et al.* 1982; Writer *et al.* 1995). The faecal stanol 5β-coprostanol is a product of biohydrogenation of cholesterol by enteric bateria of higher animals, including man. It is an ideal molecular marker for sewage and sewage sludge as it is relatively resistant to microbial alteration in the marine environment (venkatesan and Kaplan 1990). Once incorporated into anoxic sediments 5β-coprostanol and cholestanol, the 5α isomer of coprostanol, remain stable for long periods (Nishimura and Koyama

1977). Although the faecal stanols can provide quantitative measurements, the ratio of faecal stanol/sterol ratio are generally used for comparison and trend analysis. The commonest ratios used are: 5β-coprostanol to total sterol content in sediment, 5β-coprostanol to 5β-coprostanol + cholestanol content in sediment, 5β-coprostanol to cholestanol + cholesterol content in sediment, 5β-coprostanol to cholesterol ratio (Cop/Chol ratio) in sediment (Grimalt *et al.* 1990; Leenheer *et al.* 1995; Seguel *et al.* 2001).

Dutka and El-Shaarawi (1975) found that there is no consistent relationship between bacterial parameters and faecal sterols but that they could still be considered unequivocal indicators of the presence of faecal contamination. Problems associated with their use as faecal contamination indicators include the laborious time required to process samples. Monitoring coprostanol requires an elaborate extraction process with chromatography which is not feasible on a routine basis. Also, there is, at present, a lack of information regarding background levels of faecal sterols in the natural environment.

Urobilins

The most recent suggestion for an alternative indicator of faecal pollution are urobilins. These compounds are formed from conjugated bilirubin by hydrolysis and reduction by intestinal microflora and thus originate only from mammalian faeces and urine (Miyabara *et al.* 1994). To date, these compounds have been used to detect hepatic function and have only recently been suggested as a potential indicator. Studies in Japan indicate that these compounds remain relatively stable in river water (Miyabara *et al.* 1994). Table 9.29 shows how urobilins correlates with other routinely-used indicator organisms.

Urobilins are detected using high performance liquid chromatography (HPLC) with fluorometry based on the Jaffe Schlesinger reaction. At

Table 9.29. Correlation coefficients (r) and significant (p) between *i*-urobilin and other indicators of faecal pollution (Miyabara *et al.* 1994).

COD	0.02 $p > 0.05$
Ammonia nitrogen	0.72 $p < 0.01$
Total coliform	0.83 $p < 0.001$
Faecal coliform	0.81 $p < 0.001$
n	15

present, due to the limited research into these compounds, it is difficult to assess their potential as indicator organisms.

9.4. Hazards Associated with Wastewater and Sludge

9.4.1. *Water pollution*

A wide variety of enteric pathogens can be detected in sewage (Table 9.1), the frequency and population density of which is a reflection of the degree of infection within the community. Therefore, special care should be taken at treatment works when there are outbreaks of diseases within the community, and precautions should be taken when treating wastewaters from hospitals. Occasionally, wastewaters from isolation hospitals are sterilized or disinfected before being discharged into the main sewer in order to minimize the chance of infection spreading. Other wastes are also potentially hazardous. There is a wide range of pathogens, especially *Salmonella* and intestinal parasites, that are associated with meat-processing. Thus, care should be taken when coming into contact with wastewaters, even after large dilution, from abattoirs, meat and poultry processing, and intensive animal and poultry units.

Diseases that are commonly associated with faecal contamination of water in temperate regions include *Salmonella* (typhoid, paratyphoid, food poisoning), *Shigella* (bacterial dysentry), *Mycobacterium* (tuberculosis), and *Leptospira icterohaemorrhagia* (Leptospira jaundice). Viruses are excreted in large numbers in faeces, for example, poliovirus, coxsackievirus groups A and B, echovirus, infectious hepatitis A virus, reovirus, and adenovirus (Pike 1975). The most serious of these, in terms of human infection in temperate climates, is hepatitis A and there is a direct link between faecal contamination of water supplies and bathing water, and infection (Brugha *et al.* 1998). After mass vaccination of children at schools with live attenuated vaccine, large increases in the numbers of poliovirus can be recorded in the sewage from the community. Children are often symptomless excreters of poliovirus as well as other enteric viruses, although the number varies according to socio-economic as well as environmental factors (Kollins 1966). The level of infection will depend on a number of factors, such as the density of the pathogen in the water, the invasiveness and toxigenicity of the pathogen, the degree of contact between the pathogen and the host, and the innate and acquired immunity of individuals. Although sewage workers appear to be among the healthiest in the community, which suggests that they are immunized by regular exposure to low levels of pathogens, mainly

via aerosols, they still run a high risk of infection by certain water-borne diseases (Benarde 1973; Clark *et al.* 1984a,b), especially gastro-intestinal infections (Khudar *et al.* 1998). The incidence of Leptospira jaundice is particularly high amongst sewage workers as is the incidence of intestinal parasites, such as *Entamoeba histolytica*. The health of sewage workers has been extensively studied with respiratory symptoms, fatigue, and general flu-like symptoms most commonly reported. Other studies have suggested a link between such non-specific symptoms and exposure to endotoxins produced by Gram-negative bacteria (Thorn and Kerekes 2001). Employing a health questionnaire, 147 sewage workers were interviewed by Douwes *et al.* (2001). They found significant correlations between working with sewage and flu-like symptoms, although they recorded low endotoxin exposures at treatment plants of < 10 endotoxin units m^{-3}. A significant correlation was also recorded between neurological symptoms and chemical exposure. However, Khuder *et al.* (1998) found no significant difference in the incidence of respiratory disease in sewage workers when compared to other occupations. The hazards that sewage workers are exposed to, and possible health effects, are reviewed by Mulloy (2001).

Most pathogens of mammalian origin are highly specialized parasites which grow best at mammalian body temperatures (37°C) and in a suitable nutrient environment. Once excreted, pathogens are in a hostile environment and their numbers rapidly decline (Fennell 1975). However, the chance of infection decreases after treatment, after discharge and dilution in the receiving water, and with time, so the chance of infection from faecal contamination by treated effluents is extremely low.

However, certain areas are at particular risk of contamination: *bathing waters*, notably coastal waters that receive large volumes of untreated or, at best, settled sewage, where the dilution effect may be reduced by currents and wave action; *edible shellfish* growing in polluted coastal and estuarine waters also accumulate pathogens; *groundwater* contamination from septic tanks, leaking sewers or agricultural wastes; *reservoirs* from roosting gulls; and *water which is re-used* several times for supply purposes.

Bathing waters

The microbial quality of water is comparatively unimportant except where a risk of infection exists. Infection normally only occurs if there is intimate human contact with contaminated water or it is swallowed. Therefore, in water-based recreational activities that require participants to enter the water, such as swimming, wind surfing, or water ski-ing, a risk of infection does

exist and so these activities require water that is free from faecal contamination. There is only minimum contact with water with other water-based activities, such as boating and fishing, and because there is a much lower risk of infection, much higher levels of contamination can be tolerated. This is supported by studies that have shown a higher incidence of water-borne diseases among swimmers than non-swimmers, with skin irritation, gastro-intestinal infections, and infections of the eyes, ears, nose, and throat all associated with contaminated coastal waters (WHO 1975). A wide range of infections are known to be transmitted from person to person at swimming pools, especially skin infections caused by *Pseudomonas aeruginosea* and *Mycobacterium marinum*, conjunctivitis caused by *Chlamydia trachomatis*, and respiratory infections (Table 9.4). However gastro-intestinal infections have been reported in people bathing or becoming immersed in water either accidentally or during water sports. For example, a study of swimmers who took part in a snorkel swimming contest in Bristol during 1983 showed that 21% experienced gastro-intestinal symptoms within 48 h of entering the untreated water (Phillip *et al.* 1985). Other examples of more serious diseases contracted during the recreational use of both fresh and seawater including typhoid, paratyphoid, polio, and leptospirosis are cited by Galbraith *et al.* (1987). In fact, 25% of the reported cases of leptospirosis in the UK during 1978–1983 were associated with the recreational use of water or accidental immersion (Waitkins 1985) (Table 9.4). A study by the Environmental Protection Agency in the USA shows a strong link between bacterial counts in coastal waters at the time of bathing and the number of cases of severe gastro-intestinal disease among the swimmers. With an enterococcus bacterial concentration of 10 per 100 ml there were 8 cases per 1000 swimmers, at 100 per 100 ml there were 30 cases, which rose to 50 cases as the concentration of bacteria reached 1000 per 100 ml (Cabelli 1980; Pearce 1981). However, the risk of contracting such diseases from bathing waters is still extremely small, unless gross pollution is present, and under those conditions it is unlikely that it would be aesthetically acceptable to the bather for swimming (Moore 1977; Barrow 1981).

In England and Wales, there were 333 sewage outfalls discharging to coastal waters in 1972, and at that time only 49 of these (15%) outfalls extended further than 90 m below the low water mark at ordinary spring tides (Agg and Stanfield 1979). Since 1974 only long outfalls have been built in Europe. However, in 1981 some 190 beaches in England and Wales were still at risk from sewage pollution, including some of the major resorts. For example, Scarborough, with a summer population of 100,000, was discharging raw sewage at the low water mark (Pearce 1981). However, length

of outfall is not the only important criterion in the design of outfalls, with depth and currents also of importance (Grace 1978). In Ireland, the majority of the population live in coastal towns and settlements, all of which were discharging their sewage directly to the sea via short outfalls. Therefore, with so many coastal towns in the British Isles discharging either raw or primary treated sewage directly to the sea, often with very short outfalls, it is not surprising that so many bathing beaches were contaminated by faecal pathogens. Although beaches can show visible signs of such pollution in the form of plastic strips from disposable toiletries or actual faecal matter, normally, contamination can only be detected by microbial examination of the water. Sea outfalls are not the only source of faecal contamination on beaches and in coastal waters. There is a considerable pathogen load from rivers that have received effluents from treatment plants serving inland towns, with higher levels of contamination at estuaries (Wyer *et al.* amy 1994 1996 1998). Runoff from adjacent land areas used for livestock (Kay *et al.* 1999) as well as gull and wildfowl droppings (Gameson 1975b; Jones and Obiri-Danso 1999) are all sources of coliforms and *Salmonella*, with enhanced levels of these faecal bacteria often recorded at sites used for watering cattled or near to roosting colonies of gulls and wildfowl. However, there are so many factors affecting the level of contamination of beaches, such as dilution, currents and tide movements, environmental factors affecting die-off of bacteria, length of outfall, quality of effluent, and concentration of pathogens in sewage, that it is impossible to generalize. Microbial water quality at designated bathing areas will vary according to the magnitude of such inputs. However, the flux of such pathogens is a function of the hydrodynamics of the near-shore area, exposure to UV (Davis Colley *et al.* 1994), and the rate of deposition and entrainment (Obiri-Danso and Jones 2000).

The problem of sewage contaminated bathing waters has been realized for a long time, although many traditional holiday resorts, famous for their beaches and 'safe swimming-areas', are often those very towns that discharge their untreated sewage to coastal waters via short outfalls. In the USA, legal standards are in force with a maximum microbial standard of 200 faecal coliforms per 100 ml generally used for all direct-contact recreational waters (i.e. bathing waters), with public health authorities posting '*sewage-polluted beach*' warning signs advising against bathing in areas exceeding this limit (Waldichuk 1985). In 1976, the risk to those bathing in faecally contaminated water in Europe was recognised by the introduction of the EEC Directive on the Quality of Bathing Waters (76/160/EEC) (European Communities 1976a). The Directive cites mandatory (I) standards for total

coliforms, faecal coliforms, *Salmonella*, and enteroviruses in bathing waters. It states that samples of water must be taken from bathing areas at fortnightly intervals during the bathing season and that they must contain $< 10,000$ total coliforms and < 2000 faecal coliforms per 100 ml in 95% of cases. More stringent guideline (G) values are also given but these are not mandatory (Table 9.30). Only total and faecal coliforms are routinely monitored, whereas faecal streptococci, *Salmonella* and entroviruses are sampled only where an investigation shows, or where there are other grounds for believing, that water quality has deteriorated in respect to these parameters. There are also a number of physico-chemical parameters listed in the Directive (Table 9.31) including mineral oils, surface active substances that cause foaming, phenols, transparency, colour, and tarry residules. Compliance to the Directive is only required during the bathing season, which is from the 15 May to 30 September in England and Wales, and from the 1 June to 15 September in Scotland and Northern Ireland. A minimum of 20 samples are usually taken over this season, which is in excess of the minimum requirements of the Directives. The levels of compliance of member states with the mandatory values are summarised in Table 9.32. National compliance data is published annually by member states with a single Europe-wide report published each year by the European Commission (EPA 2001).

There are many difficulties with the interpretation of this Directive, for example, deciding which bathing areas are covered by the legislation, the choice of sampling sites on a particular beach, sampling frequency, and the time of sampling in relation to tide and currents (Moore 1977; Water Research Centre 1977). Originally, Britain and Ireland only designated coastal (marine) bathing areas. However, in the rest of Europe there is a strong tradition for bathing at inland (freshwater) sites and this resulted in inland bathing areas also being designated. In Germany, for example, only 417 coastal sites are designated as bathing areas compared to 1656 inland sites. In the Directive, bathing areas are defined as waters where (a) bathing is explicitly authorized, or (b) bathing is not prohibited and is traditionally practiced by a large number of bathers. It is the second definition that has been largely adopted by member states as the basis for determining areas to which the Directive applies. In the early years of the Directive this led to numeric values being adopted. For example, in the UK, beaches were only designated if a threshold of 1000 bathers per km was exceeded. This originally led to many of England's major holiday resorts, such as Blackpool, Brighton, and Eastborne, being excluded. At the time, Pearce (1981) asked the pertinent question 'if there are 2000 bathers on a 2 mile beach all congregated in a 0.5 mile stretch around the pier, is the bathing

Table 9.30. Microbial quality requirements specified for bathing waters within EEC countries. Percentage values in parentheses are the proportion of samples in which the numerical values stated must not be exceeded (European Communities 1976a).

Parameters		G	I	Minimum sampling frequency	Method of analysis and inspection
Microbiological:					
Total coliforms	100 ml^{-1}	500 (80%)	10000 (95%)	Fortnightly (1)	Fermentation in multiple tubes. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo agar, 0.4% Teepol broth, subculturing and identification of the suspect colonies The incubation temperature is variable according to whether total or faecal coliforms are being investigated
Faecal coliforms	100 ml^{-1}	100 (80%)	2000 (95%)	Fortnightly (1)	
Faecal streptococci	100 ml^{-1}	100 (90%)	—	(2)	Litsky method. Count according to MPN (most probable number) or filtration on membrane. Culture on an appropriate medium
Salmonella	1 l^{-1}	—	0 (95%)	(2)	Concentration by membrane filtration. Inoculation on a standard Medium. Enrichment — subculturing on isolating agar — identification
Enteroviruses	PEU* 10 l^{-1}	—	0	(2)	Concentrating by filtration, flocculation or centrifuging, and confirmation

G, recommended; I, mandatory.

*PFU = Plaque Forming Unit — a method of counting viruses; one PFU may be regarded as one infective virus particle.

(1) When a sampling taken in previous years produced results which are appreciably better than those in the Directive when no new factor likely to lower the quality of the water has appeared, the competent authorities may reduce the sampling frequency by a factor of 2.

(2) Concentration to be checked by the competent authorities when an inspection in the bathing area shows that the substance may be present or that the quality of the water has deteriorated.

Table 9.31. Physico-chemical water quality requirements for bathing waters as prescribed by the Bathing Water Directive (76/160/EEC).

Parameters	Directive 76/160/EEC	
	G (Guide)	I (Mandatory)
pH*	—	6 to 9
Colour	—	No abnormal change in colour
Mineral oils (mgl^{-1})	≤ 0.3	No film visible on the water surface and no odour
Surface active substances (mgl^{-1})	≤ 0.3	No lasting foam
Phenol (mgl^{-1} C_4H_3OH)	≤ 0.005	≤ 0.05 and no specific odour
Transparency (m)	≥ 2	≥ 1
Dissolved oxygen* (per cent saturation O_2)	80 to 120	—
Tarry residues and floating material	Absence	—
Ammonia (mgl^{-1} NH_4)**	—	—
Nitrogen Kjeldahl (mgl^{-1} N)**	—	—
Other Substances		
Pesticides (mgl^{-1})*	—	—
Heavy metals (mgl^{-1} Cd, Cr VI, Pb, Hg)*	—	—
Cyanides (mgl^{-1} Cn)*	—	—
Nitrates and phosphates (mgl^{-1} NO_3, PO_4)*	—	—

*to be sampled where an investigation shows or where there are other grounds for believing that water quality has deteriorated in respect of this parameter.
**to be sampled where there is a tendency towards eutrophication of bathing water.

density 1000 or 4000 per mile'? The fact that Blackpool has 8 miles of beaches may well provide the answer as to why it was not originally designated. The European Commission has since urged member states to adopt a more liberal interpretation of the *term large number of bathers* in the interest of ensuring that the objectives of the Directive are attained. This resulted in a rapid increase in the number of designated areas, especially in the British Isles. The criteria for selecting designated beaches are so broad that Ireland originally felt that none of the existing bathing beaches came within the Directive. However, seven beaches were originally designated, which was increased to 51 designated areas by 1988, and to 130 in 1998. Four of these designated areas lie within Dublin Bay that receives almost a third of the sewage from the country's total population of three million. In

Table 9.32. Bathing water compliance in the UK 1989–1998 (Environment Agency, 1999).

Number of identified bathing waters	1989		1990		1991		1992		1993		1994		1995		1996		1997		1998	
	pass	total	pass	total	pass	total	pass	total	pass	total	pass	total	pass	total	pass	total	pass	total	pass	total
Total UK	**336**	**440**	**345**	**446**	**343**	**453**	**358**	**455**	**365**	**457**	**376**	**457**	**413**	**464**	**416**	**472**	**429**	**486**	**440**	**496**
EA region																				
Anglian	23	28	27	29	29	33	31	33	28	33	27	33	30	34	34	35	35	35	36	36
Northumbrian	20	32	21	32	21	33	20	34	46	56	49	56	53	56	49	56	51	56	47	56
Yorkshire	18	22	17	22	19	22	20	22												
North West	11	33	10	33	10	33	11	33	13	33	24	33	15	33	13	33	17	34	21	34
Southern	45	65	48	66	45	67	51	67	58	67	53	67	62	67	62	69	67	75	75	77
South West	113	132	118	133	105	133	117	134	142	175	151	175	167	176	167	180	164	180	167	183*
Wessex	31	38	39	39	36	39	36	39												
Thames	3	3	3	3	2	3	3	3	3	3	2	3	3	3	2	3	3	3	3	3
Welsh	40	48	35	50	45	51	39	51	42	51	39	51	49	56	52	57	60	64	64	68
England & Wales	**304**	**401**	**318**	**407**	**312**	**414**	**328**	**416**	**332**	**418**	**345**	**418**	**379**	**425**	**379**	**433**	**397**	**447**	**413**	**457**
% compliance	76		78		75		79		79		83		89		88		89		90	
Scotland	**16**	**23**	**12**	**23**	**15**	**23**	**15**	**23**	**18**	**23**	**16**	**23**	**19**	**23**	**21**	**23**	**18**	**23**	**12**	**23**
∼ North of Scotland													7	7	7	7	4	8		
∼ East of Scotland													8	8	8	8	7	8		
∼ West of Scotland													4	8	6	8	3	8		
Northern Ireland	**16**	**16**	**15**	**16**	**16**	**16**	**15**	**16**	**15**	**16**	**15**	**16**	**15**	**16**	**16**	**16**	**14**	**16**	**15**	**16**

Note: the Scottish water authorities were formed on 1 April 1996, therefore no data pre- 1995 is available.
Severn Trent region has no identified bathing waters therefore is not included.
Yorkshire and Northumbrian have merged to form Northeast, South West and Wessex have merged to form South West.
*One bathing water, Redgate in the South West was closed during 1998 bathing season and is therefore not included in compliance assessment. for 1998.

Ireland, model criteria are now used to identify bathing areas. These are: (i) a long established tradition of bathing; (ii) an increased popularity of a location following improved treatment of sewage discharges; (iii) major beach amenity and environmental works completed; (iv) the area is entered under the Blue Flag Scheme; (v) facilities are provided for access to the beach; (vi) facilities such as toilets, parking spaces, life guards, shops, and first aid services are available; (vii) the bathing area is newly created and specially equipped (EPA 2001).

The EEC standards for total and faecal coliforms are generally felt to be too high; it is alleged that this is in order to make it politically acceptable to all the member states. However, some member states have set more severe limits and in the case of Ireland, is half the EEC values (Fegan 1983). The same standards apply to all European waters from the Atlantic to the Mediterranean, with an enormous variety of physical and meterological conditions. Bathing habits also vary in terms of duration and frequency. They are much higher in the Mediterranean than elsewhere, resulting in much higher water-skin contact times and therefore a higher risk of infection. Problems in the selection of techniques for faecal coliform analysis result in variability between counts, and even the risk that the results could be manipulated by the careful selection of specific techniques and sampling sites.

The Directive is based on coliforms that are only directly related to the incidence of bacterial gastro-intestinal infections. Although in practice, the ratio of this indicator to other pathogens is reasonably good (Bonde 1977), it is less effective in indicating risks of skin infections or those of the eyes, ears, nose, or throat, which are most frequently associated with swimmers. It may be that more specific and longer lived indicators, such as *Staphylococcus aureus* would be more effective.

In the long term, the answer to this problem and that of coastal eutrophication, resulting in algal blooms (Granéli 1999), is to limit the discharge of pathogens and nutrients by adequate treatment at coastal sites (Baalsrud 1975). However, the authorities responded by building longer (> 1000 m) and deeper outfalls with diffusers, thus ensuring maximum dilution and dispersion of the sewage entering the sea. For example, the outfall at Bray, County Wicklow in Ireland, is 1690 m in length. A useful case study of the 1000 m long Kirkcaldy sea outfall in the Forth estuary explains in detail the various environmental, biological, and hydrographical investigations that need to be carried out, as well as highlighting some of the design and constructional problems (Moore *et al.* 1987). This particular project was of special interest as the sea-bed did not shelve as steeply as in other areas

where such outfalls have been constructed. Therefore, the available water depth was marginal, resulting in very weak currents to disperse the discharged waste. The construction of long sea outfalls, usually referred to as submarine outfalls, is complex (Grace 1987; Roberts 1996). For example, Ludwig (1998) describes the Casablanca, Morocco, submarine outfall which is 3600 m in length. The first 900 m is constructed of 6 m long reinforced concrete pipes with an internal diameter of 2.1 m that have been placed in a trench excavated in the seabed rock. The remaining 2700 m is made up 81.5 m long sections of protected steel pipe of 2.04 m internal diameter. These are supported just above the sea bed by laying the pipes on concrete (Berceaux) supports held in place by metal straps attached to chains embedded in 27 tonne anchoring blocks (Masques) either side of the pipe, spaced approximately 10 m apart. The steel pipe is protected externally with anticorrosion coal tar/enamel and coated with a 170 mm layer of concrete. Internally the pipe is protected using an epoxy lining and cathode protection using sacrificial anodes. The wastewater is dispersed into the sea via 110, 16 cm round ports along the final 550 m section of the outfall. Although designed to take up to 10 m^3s^{-1} when all the pumps are in operation, the average flow at present is 3 m^3s^{-1} with peak flows of 4.5 m^3s^{-1}. The design, construction, and maintenance of long sea outfalls is examined in depth elsewhere (Institution of Public Health Engineers 1986).

Under the Urban Wastewater Treatment Directive (91/271/EEC) the disposal of sewage sludge from ships or via pipelines to coastal waters was phased out by 31 December 1998. Strict compliance dates have been introduced by the EC for the provision of minimum treatment for wastewaters entering estuaries and coastal waters (Sec. 1.1.2) (Fig. 1.2). Additional standards are set for receiving waters considered as sensitive under this directive, which has been taken to mean those waters sensitive to eutrophication. However, there is growing pressure to expand this definition to include any water that fails an EC Directive (e.g. Bathing Water, Shellfish etc.) as a sensitive classification will result in stricter discharge standards imposed on treatment plants.

Many treatment plants disinfect their effluents during the bathing season in order to reduce pathogen discharge and to comply with the Bathing Water Directive. However, the provision of sewage treatment, where none existed previously, has not always been sufficient to achieve compliance with the Directive. For example, a new waste water treatment plant and major improvements to existing plants completed in 1996 and costing £150 m failed to achieve the required microbial compliance for eight designated bathing areas along the Fylde Coast in England which includes the resorts

of Cleveleys, Bispham, Blackpool, and St. Annes. Interestingly, faecal streptococci concentrations have remained high and have not been improved by the new sewerage schemes (Crowther *et al.* 2001). From this study it was shown that prior to the completion of the sewerage schemes in this area higher bacterial concentrations were strongly associated with rainfall, and sewage sources were important sources of coliforms (both total and faecal), but less important for faecal streptococci which largely originate from diffuse catchment sources. On completion of the sewerage schemes, the catchment sources then became more important for all parameters with sunshine and onshore winds, that affect the survival and movement of bacteria that have already entered the coastal waters, becoming important survival variables.

Stricter target standards, than those in the Bathing Water Directive, have been proposed by the World Health Organization (1998). These are currently being considered by the European Commission, which published a policy document on bathing water on the 21 December 2000 (Com(00)860). This proposes a new Directive to complement the revised water legislation (i.e. Water Framework (2000/60/EEC), Urban Wastewater Treatment and the Nitrate (91/676/EEC) Directives) so that use can be made of existing provisions and powers. The new directive will include provisions for revised and legally binding water quality standards, extending the management of bathing water beyond the bathing area itself to include land use and upstream emissions, and to provide as near-real time information as possible so that the public can make informed choices about where to bathe. Specifically it will introduce: (i) A clear and unambiguous definition of *bathing* and *bathing area*; (ii) Suitable and prompt management actions, such as beach closure, whenever water quality standards are breached; (iii) The compulsory development of beach profiles that will identify and quantify all potential sources of pollution and facilitate the modelling of quality trends over long periods. This will be used to allow a more flexible approach to sampling; (iv) The adoption of improved health standards linked to levels of faecal contamination and based on the draft WHO guidelines for intestinal enterococci and *E. coli*. These measures will be complemented by indicators showing divergences from normal levels of acidity in freshwaters or salinity in coastal waters (WHO 1998). These instant indicators will quickly assess whether microbial quality may have been compromised. The development of a protocol for algae and macrophyte blooms has also been proposed. Existing physico-chemical parameters will be dropped as these are addressed in the Water Framework Directive; (v) Obligations to improve water quality within defined time-frames, as under the current Directive,

non-compliance does not automatically lead to a prescribed course of action; (vi) The development of modelling techniques to predict water quality in advance; (vii) Wide and rapid dissemination of updated information to potential users using all forms of media.

Shellfish

Enteropathogenic diseases are known to be transmitted by the consumption of contaminated shellfish, in particular mussels, oysters, clams, cockles, and to a lesser extent, scallops (James and Wilbert 1962; Ohara *et al.* 1983). This particular hazard has been known since the latter part of the nineteenth century when typhoid was shown to be transmitted via contaminated shellfish and since then the list of associated diseases has grown to include cholera, paratyphoid, and other gastro-enteric disorders (Pike and Gameson 1970). Viruses are a particular problem with hepatitis A and Norwalk virus both reported from shellfish (Altmar *et al.* 1995; Abad *et al.* 1997). In 1998 an epidemic of hepatitis A in Shanghai, China, infected 292,000 people who had eaten contaminated shellfish (Hu *et al.* 1989). In Valencia, Spain, 183 people also contacted hepatitis A after eating Coquina clams (*Donax* spp.) imported from Peru (Bosch *et al.* 2001). *Cryptosporidium* oocysts are also concentrated in shellfish, especially mussels and oysters (Grazcyk *et al.* 1997). Shellfish are filter feeders that ingest food indiscriminately and extract faecal bacteria along with the other debris. However, the faecal micro-organisms are not digested by the shellfish or even inactivated, instead they accumulate at gill surfaces and in the alimentary canal (Wood 1979). These shellfish are common in estuarine and coastal waters throughout Europe that receive organic pollution. Also, many species are commercially farmed in suitable coastal and estuarine locations.

The major hazard to health arises from eating shellfish raw. The risk can be minimized by either cooking them at a sufficiently high temperature for a suitable length of time or by immersion in tanks of clean seawater to allow self-cleansing to occur (Carrington 1980b). Water quality standards for growing shellfish that will be uncontaminated have been adopted in both Canada and the USA. There is a maximum permissible faecal coliform count < 14 per 100 ml with $< 10\%$ of samples exceeding 43 per 100 ml, and a maximum permissible faecal coliform count of 230 per 100 g of shellfish meat (Environmental Protection Agency 1976; Waldichuk 1985). In Europe, the suitability of shellfish for human consumption is controlled by two directives; the Shellfish Directive (79/923/EEC) deals with the quality of shellfish production waters, while the Shellfish Hygiene Directive (91/492/EEC)

controls the health conditions for the production and marketing live bivalve mussels. In England and Wales, the Department of the Environment, Transport and the Regions (DETR) have designated 119 shellfish harvesting areas, 93 in England and 26 in Wales, under the original Shellfish Directive. In many cases compliance with the Bathing Water Directive (76/160/EEC) or the Urban Wastewater Treatment Directive (91/271/EEC) have been sufficient to protect shellfish production areas. All molluscan shellfish harvesting areas are required by the Shellfish Hygiene Directive to be classified according to the extent to which shellfish samples from each area are contaminated with *E. coli*. The classification of areas ranges from clean, where the molluscs can be sold for direct human consumption, to areas where the molluscs need to be treated before consumption, or areas where the mollucs are prohibited for human consumption. A separate European Directive (93/51/EEC) now sets microbial standards for the production of cooked crustaceans and molluscan shellfish.

Groundwater

Chemical pollution can travel up to twice the distance of bacterial pollution in soil, while both viruses and bacteria are able to move through the soil and contaminate wells and groundwater (Allen and Geldreich 1985; Filip *et al.* 1988; Polprasert *et al.* 1982). The enteric organisms originate from sewage irrigation, grass-plot treatment of final effluents, septic tank percolation areas, and land disposal of sewage sludge (Yates 1985). The movement of these pathogens, which travel in both a vertical and horizontal direction, depends on factors such as the size and shape of the micro-organism, the soil, type, the surface characteristics of the soil, and the viscosity of the interstitial water. In fact, so many factors influence the survival and passage of viruses and bacteria in the soil that it is difficult to generalize for all soil types. However, bacteria can travel faster in highly permeable soil (> 3.0 m d^{-1}) than in soils with slow permeability (< 1.2 m d^{-1}). Faecal coliforms have been reported as travelling long distances horizontally through soils (> 100 m) (Dappert 1932) and at flow rates of up to 15 m h^{-1} (Bouma *et al.* 1974), with the distance travelled related to the soil properties and hydrological variables (Reneau 1978; McMurray *et al.* 1998) (Table 9.33). There is a reduction in the number of pathogenic organisms with depth and distance from the source of pollution, although this varies with the adsorptive properties of the soil (Carrington 1980a). For example a column of garden soil 0.9 m high reduced the *E. coli* concentration by 75% and Coxsackievirus by 50%. Reneau *et al.* (1975) have suggested that the removal of pathogens is a function of

Table 9.33. Examples of the distance travelled by faecal micro-organisms in soil (Polprasert and Rajput 1982).

	Distance transported (m)	
Type of organism	Vertical	Horizontal
E. coli	3.04–9.14	70.71
E. coli		24.38
E. coli		121.92
'Lactose fermenters'	0.76	0.61
Coliform bacteria	0.61–0.91	3.048–121.92
Coliform bacteria		54.86
Coliform bacteria	45.72	
Clostridium welchii	2.13–2.43	
Bacteria		609.6

the distance from the source of pollution, for that particular soil type. Vertical contamination is increased much more than horizontal contamination by rain. Viruses have been reported at depths of 2.6 m below the surface after heavy rain (Wellings *et al.* 1975). Viruses, because of their smaller size, are able to penetrate deeper and farther than bacteria, although they are less able to survive for long periods. The retention of viruses decreases proportionally to the increase in the flow rate. Adsorption is greater at lower pH values, with some metal complexes (e.g. magnetic iron oxide) rapidly adsorbing viruses. Cations can reduce the repulsive electrostatic potential between soil particles and viruses thus enhancing adsorption, and the high ion exchange capacity and large surface area of clay soils gives a high retention capacity. The presence of soluble organics has been shown to reduce adsorption capacity (Gerba *et al.* 1975; Goyal and Gerba 1979). This topic has been reviewed by numerous authors (Hutchinson 1974; Allen and Geldreich 1975; Carrington 1980a; Seppanen and Wihuri 1988; Rehmann *et al.* 1999). All pathogens have been isolated from water and biofilm samples from groundwater and boreholes, including *Legionella* (Riffard *et al.* 2001). Micro-organisms have been used as tracers in groundwater for many years (Harvey 1997), including bacteriophage and native bacteria labeled using genetic markers or stable isotopes (Daniell *et al.* 2000). Bacteriophages have been used at high concentrations from 10^5 to 10^{13} pfu ml^{-1} (McKay *et al.* 1993; Harvey 1997). *Escherichia coli*, *Bacillus subtilis* endospores as well as F-RNA bacteriophage MS2 are also frequently employed (Sinto *et al.* 2000).

Table 9.34. Microbial quality requirements specified for surface waters intended for the abstraction of drinking water within EEC countries (European Communities 1975).

Parameter		A1 G	A1 I	A2 G	A2 I	A3 G	A3 I
Total Coliforms, 37°C	100 ml^{-1}	50		5000		50000	
Faecal coliforms	100 ml^{-1}	20		2000		20000	
Faecal streptococci	100 ml^{-1}	20		1000		10000	
Salmonella		Not present in 500 ml		Not present in 1000 ml		—	

I, mandatory; G, recommended.
Category A1: waters treated by simple treatment and disinfection, e.g. rapid filtration and disinfection.
Category A2: normal physical treatment, chemical treatment, and disinfection, e.g. prechlorination, coagulation, flocculation, decantation, filtration, disinfection (final chlorination).
Category A3: intensive physical and chemical treatment, entended treatment, and disinfection, e.g. chlorination to breakpoint, coagulation, flocculation, decantation, filtration, adsorption (activated carbon), disinfection (ozone, final chlorination).

Reuse of wastewater for human consumption

Not all areas of a country will have access to upland reservoirs or groundwater sources of good quality water for human consumption. In order to meet the demand for water in highly populated areas alternative sources have to be utilized. The commonest source of water in such situations are rivers, and some major cities in Europe rely almost exclusively on rivers that have already received sewage from other towns upstream to supply all their water needs. Therefore, a consumer downstream of other towns could be drinking water that has already been used, treated, and returned to the river on several previous occasions.

The volume of sewage effluent discharged into a river may remain constant; however, the ratio of sewage effluent to river water (the dilution factor) will vary. It is possible at time of drought for the volume of effluent discharged to exceed the volume of clean water in the watercourse. The River Thames in the UK comprises up to 14% sewage effluent under normal conditions and the River Lea is even higher (Sec. 10.2.2). The only available surface water in Rotterdam is the River Rhine that receives over 70% of the total sewage of West Germany (Dart and Stretton 1977). During periods of drought in Agra (India), the water supply is comprised entirely of sewage effluent from New Delhi 190 km away (WHO 1973).

Although it is possible to purify almost any river water, no matter how polluted, to drinking water standard (Table 9.34), the costs can be prohibitive. The most effective method of re-use is to combine maximum sewage treatment of wastewaters before discharge to a river with adequate treatment of abstracted water. Although this is comparatively easy to ensure in a small catchment, such as the River Thames where there is a single controlling authority, it becomes increasingly difficult when more than one authority is involved and near impossible in the case of international rivers like the Rhine, which travel through several countries. The problem that normally occurs is that one authority or country may wish to discharge wastes to the river and interfere with another who needs to re-use the water. In the future this will be overcome in Europe by the introduction of the new EC Water Framework Directive (2000/60/EEC) that requires whole catchments to be managed in an integrated manner regardless of the national or international boundaries.

Provision of a clean supply of drinking water is the major cause of the decline in the incidence of water-borne diseases. Therefore, extreme care must be taken to ensure maximum removal of pathogens during sewage treatment so that only a small population of pathogens exist in the river. When water is re-used, full treatment is necessary no matter how clean it is bacteriologically. Water supplies taken from lowland rivers are normally stored for several days prior to treatment to enhance the die-off of pathogens. The water is then subjected to full treatment, i.e. microstraining, sand filtration, and, finally, disinfection. This ensures that the water is free from all pathogens. There is, of course, an increased risk of infection when water is used more than once, and routine and regular bacteriological monitoring is required (HMSO 1983b). Not all the chemical compounds that are added to water during consumption can be subsequently removed either at the sewage treatment or water treatment stages, resulting in them being supplied to the next consumer downstream. Some of these chemical compounds may be toxic or carcinogenic and particular concern is being expressed about a number of organic pollutants from domestic sewage including pesticides and oestrogen mimicking compounds (Sec. 1.2.2, 11.3.1) (Gray 1994; Dempsey 1998).

Numerous surveys have indicated that there may be long-term effects in re-using water after being consumed, with higher incidences of cancer among people drinking river water already used for supply purposes compared with those drinking groundwater (Diehl and Tromp 1953; Stocks 1973; Page *et al.* 1976; Kuzma *et al.* 1977). However, a study carried out in London over the period 1968–1974 showed that there was no significant

difference between the mortality rate between those drinking re-used River Thames water and groundwater (Beresford 1980). This work did, however, provide epidemiological evidence, consistent with the earlier studies, that a small risk to health existed from the re-use of drinking water. In this study, the percentage of domestic sewage effluent in the water was positively associated with the incidence of stomach cancer and bladder cancer in women (Beresford 1983; Beresford *et al.* 1984).

Pathogen concentrations in rivers increase with surface runoff and reuse. The percentage of bacteria, both pathogenic and natural, showing multiple antibiotic resistance also increases downstream, especially below sewage outfalls (Sec. 9.4.4). Current strategies for controlling health risks posed by microbes in drinking water are based on a barrier approach involving the treatment of both wastewaters and raw supply waters used for drinking (Fig. 9.13). The establishment of limits of specific microbial indicators of water quality is also an important mechanism in the protection of the public from waterborne pathogens (Sobsey *et al.* 1993). When water is reused, it must be subjected to very strict and regular microbial, toxicological, and chemical testing (Dewettinck *et al.* 2001). International water recycling guidelines have been proposed by Anderson *et al.* (2001). Reuse of effluents is considered further in Sec. 10.2.2.

Pollution by gulls

Although migratory waterfowl, which roost at reservoirs, have no serious deliterious effect on water quality (Brierley *et al.* 1975), gulls, which feed on contaminated and faecal material at refuse-tips and sewage works, have been shown to excrete pathogens. All five British species of *Larus* gulls continue to increase with the herring gull (*Larus argentatus*) doubling its population size every 5 to 6 years. This has resulted in a very large increase in the inland population of *Larus* gulls throughout the British Isles, both permanent and those over-wintering (Gould 1977). These birds are opportunist feeders and have taken advantage of the increase in both the human population and its standard of living, feeding on contaminated waste during the day and then roosting on inland waters, including reservoirs, at night (Hickling 1977; Gray 1979; Lévesque *et al.* 2000). Faecal bacteria, especially *Salmonellae* spp., have been traced from feeding sites such as domestic waste-tips to the reservoirs, showing that gulls are directly responsible for the dissemination of bacteria and other human pathogens (Williams *et al.* 1976; Riley *et al.* 1981; Benton *et al.* 1982; Hatch 1996). Many reservoirs and recreational waters have shown a serious deterioration

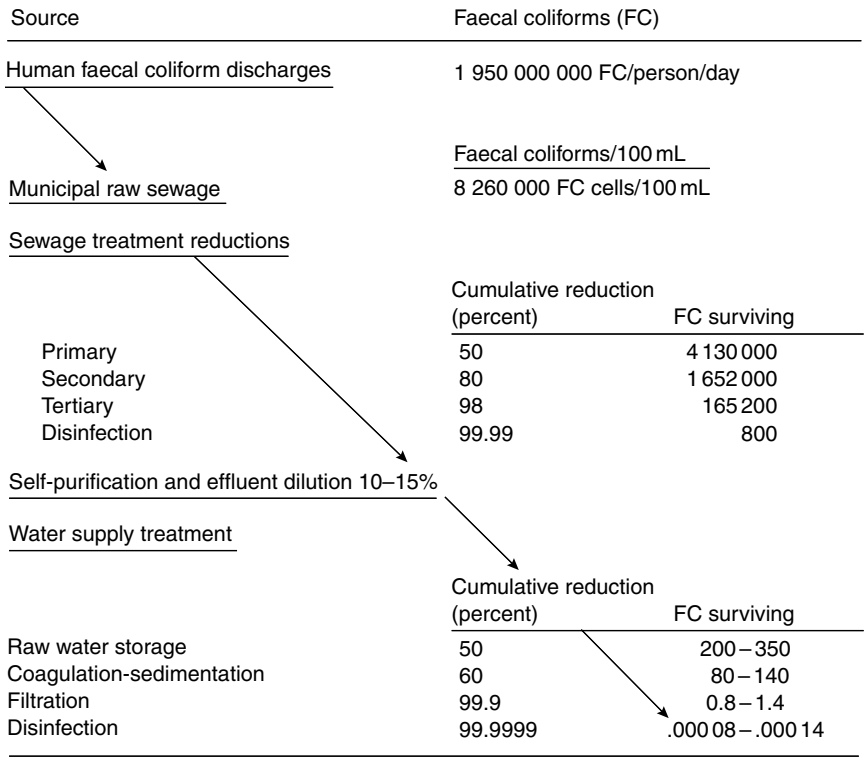


Fig. 9.13. The use of barriers is vital in the control of pathogens in water supplies (Geldrich 1991).

in bacterial quality because of contamination by roosting gulls (Fennell *et al.* 1974; Jones *et al.* 1978; Benton *et al.* 1983; Lévesque *et al.* 1993). Those situated in upland areas, where the water is of a high quality and treatment is minimal before being supplied to the consumer, are particularly at risk from contamination. Numerous *Salmonella* serotypes can be isolated from gull droppings (Fenlon 1981; Girwood *et al.* 1985) (Table 9.35), as well as faecal coliforms, faecal streptococci, *Campylobacter* spp., and spores of *Clostridium welchii* (Gould 1977; Hatch 1996). Densities of these indicator bacteria are given in Table 9.36

Lévesque *et al.* (2000) studied the bacterial content of ring-billed gulls (*Larus delawarensis*) by monitoring the droppings from three colonies for faecal coliforms, *Aeromonas* spp., *Campylobacter* spp., *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus*. They found no significant difference between colony, age groups, or sampling date. Geometric mean

Table 9.35. The most frequently isolated *Salmonella* serotypes obtained from gulls during 1982–1983 compared to the frequency and ranking from humans, cattle, and sheep. The number of isolates are given in parentheses (Girdwood *et al.* 1985).

Salmonella serotype	Seagull	Human	Cattle	Sheep
S. virchow	1 (271)	3 (571)	3 (78)	—
S. typhimurium	2 (175)	1 (2487)	1 (1002)	2 (83)
S. bredeney	3 (49)	9 (64)	—	—
S. infantis	4 (17)	—	—	—
S. hadar	5 (16)	—	—	—
S. meunchen	6 (15)	—	—	
S. stanley	6 (15)	—	6 (9)	—
S. mbandaka	8 (14)	—	—	—
S. montevideo	9 (13)	—	6 (9)	—
S. agona	10 (11)	8 (65)	4 (28)	—

bacterial concentrations from all the sites are: faecal coliforms 2.1×10^8 colony forming units (cfu) per gram of droppings, *Aeromonas* spp. 4.0×10^5 cfu g^{-1}, *Campylobacter* spp. 1.4×10^5 cfu g^{-1}, *P. aeruginosa* 1.2×10^6 cfu g^{-1}, *Salmonella* spp. 2.6×10^6 cfu g^{-1}, *S. aureus* 5.3×10^6 cfu g^{-1}.

Faecal coliform (FC) concentrations in the Kensico Reservoir in Westchester County, New York, became elevated during the autumn and winter due to increased roosting by ring billed gulls (*Larus delawarensis*) and Canada geese (*Branta canadiensis*). To examine this problem, faecal samples from 249 ring billed gulls and 236 Canada geese were analysed for a period of two years (Alderisio and Luca 1999). The gull faeces contained a larger average concentration of faecal coliforms (3.68×10^8 FC g^{-1}) than goose faeces (1.53×10^4 FC g^{-1}). However, the geese produced 15 times more faeces, on average, by weight than the gulls. The faecal droppings of migratory Canada geese have also been shown to contain cysts of *Giadia* spp. (Graczyk *et al.* 1998). The infection rates of *Cryptosporidium parvum* are on average 50%, regardless of the waterfowl species tested (Fallacara *et al.* 2001). Ova of parasitic worms have also been isolated from gull droppings (Crewe 1967; Fallacara *et al.* 2001); the birds are thought to be a major cause of the contamination of agricultural land with the eggs of the human beef tapeworm (*Taenia saginata*) (Crewe and Owen 1978).

There is a clear relationship between the number of roosting gulls on reservoirs and the concentration of all the indicator bacteria, including

Table 9.36. Number of droppings, total weight, and bacterial loads produced in a 24 h period by individual gulls of four different species. Total coliforms (TC), faecal coliforms (FC), faecal streptococci (FS), and spores of *Clostridium welchii* (CW) are given. The estimated 24 h nutrient loads for the four species of gull are also given (Gould 1977).

Larus species	Total no.	Total wt (g)	TC (10^8)	FC (10^8)	FS (10^6)	Ratio FC:FS	CW (10^3)	Org. C.	NH_3	Kj·N	Org. N. (mg^{-1})	Sol. P	Total P
L. argentatus	39	17.5	5.1	5.2	<5.9	87	< 0.98	1392	402	1819	1416	92	> 115
	34	24.9	17.6	17.7	<20		< 3.4						
L. fuscus	41	13.4	47	50	14.7	340	11.8	705	211	919	708	47	58
L. canus	62	11.8	7.2	6.2	1.1	585	< 0.63	698	134	829	689	42	50
L. ridibundus	52	11.2	2.9	3.0	2.2	135	< 0.20	502	113	608	495	30	38

E. coli, faecal streptococci, *C. welchii*, and *Salmonellae* spp. Fennell *et al.* (1974) reported maximum densities of gulls on a reservoir of 16,000 individuals that resulted in high levels of faecal contamination (*E. coli* 4000, faecal streptococci 600, and *C. perfringens* 16 per 100 ml of reservoir water). The results in Table 9.36 are particularly interesting as they show that the faecal coliform (FC) to faecal streptococci (FS) ratio for gulls is higher than the 4:1 ratio proposed by Geldreich (1972) to indicate contamination of human origin (Sec. 9.3.3). In a study by Gould (1977), 65% of all gull droppings had a FC:FS ratio in excess of 4:1, with the percentage of values exceeding this limit for the major *Larus* species being *L. argentatus* (94%), *L. fuscus* (100%), *L. canus* (33%), and *L. ridibundus* (35%). Gould went on to suggest that the FC:FS ratio could be replaced by the ratio of faecal coliforms to *Clostridium welchii* as an effective indicator of human pollution. Gull droppings can also contribute significant amounts of nitrogen and phosphorus to reservoirs which can lead to eutrophication (Table 9.36).

Although contamination of small water storage reservoirs has been eliminated by covering them, this is impracticable for large upland reservoirs serving major cities and towns. A considerable degree of success has been achieved by using bird-scarers and, more recently, by using techniques developed for the control of birds at airports (Bremond *et al.* 1968). Roosting can be discouraged by broadcasting species-specific distress calls of *Larus* gulls, which has led to dramatic reduction in bacterial contamination. Such a control option is far more cost-effective than installing and operating more powerful disinfection treatment systems (Benton *et al.* 1983).

9.4.2. *Land Pollution*

Sewage sludge disposal

Sewage sludge arises from the separation and concentration of the settleable fraction of wastewater as it passes through the treatment plant. Sludge is essentially of two types, raw and treated, with raw sludge from primary settlement tanks usually mixed with the less noxious secondary sludges, and treated sludge which has undergone some form of stabilization process, such as digestion, lime treatment, composting, or drying (Sec. 8.1.1). Sludge disposal is a major operational cost in the treatment of wastewaters and the water industry tends to dispose of sludge *at minimum cost consistent with acceptable environmental impact and with flexibility and security* (Davis 1980). Sewage sludge is rich in organic matter, nitrogen, phosphorus, and trace elements. Therefore, combined with the philosophy

of utilization of waste rather than disposal, spreading on agricultural land has steadily grown throughout Europe. However, the utilization of sewage sludge on agricultural land has been shown to affect the growth of plants and the health of animals because of a range of contaminants. Inorganic and organic contaminants are dealt with in Sec. 8.2.2, however, the third category of contaminants, the pathogens, are considered below.

The density and diversity of pathogens in sewage sludge depends on the nature of the wastewater, which is determined by the general health of the community and the presence of wastewater from hospitals, abattoirs, and meat-processing factories. Other factors, such as the diurnal and seasonal variation inflow and sewage constituents, and dilution by infiltration and stormwater are also important. The majority of the pathogen content of crude wastewater is concentrated in the primary sludge (Engelbrecht 1978). The effectiveness of the secondary treatment processes in removing pathogenic micro-organisms determines their concentration in secondary sludges (Table 9.37). However, as primary and secondary sludges are usually combined for disposal it is the choice of stabilization process that will ultimately control the pathogen load being spread on to the land. The efficiency of the various stabilization methods of sewage sludge are compared and fully discussed in Sec. 8.1. The traditional methods of treatment, such as digestion and drying, are unable to eliminate pathogens, and although

Table 9.37. The removal of pathogenic micro-organisms by sewage treatment (Carrington 1978).

Micro-organism or pathogen	Range of percentage removals of organism and number of reports reviewed (in parentheses)		
	Percolating filter	Activated sludge	Anaerobic digestion
Total bacteria	70–95 (4)	70–99 (3)	
Salmonella	84–99 (2)	70–99 (2)	84–92.4 (1)
Escherichia	82–97 (4)	91–98 (1)	
Mycobacterium tuberculosis	66–99 (2)	88 (1)	69–90 (2)
Poliovirus		Most (1)	
Coxsackievirus A	60 (1)		
Tapeworm ova	62–70, 18–26 (2)	Little effect (1)	97 (1)
Entamoeba histolytica	88–99.9 (1)	No reduction (2)	Removed (1)
Ascaris	70–99.8 (2)	93–98 (1)	45 (1)
Hookworm	100 (1)	81.5–96 (1)	

numbers are reduced, it appears that digestion, in particular, has little effect on the removal of pathogens such as *Salmonella* (Argent *et al.* 1977; Gantzer *et al.* 2001). Methods, such as composting and heat treatment, however, that involve heating the sludge to about 60°C, are able to totally eliminate or reduce pathogens to very low levels (Gantzer *et al.* 2001) (Table 9.38).

In the UK, and Europe generally, the major hazard is associated with *Salmonella* and *Taenia* infections (WHO 1981), although other bacterial pathogens commonly found in sludge include *Shigella*, *Campylobacter*, *Yersinia*, *Leptospira*, and of course enteropathogenic *Escherichia coli*. The bacterium *Brucella abortus* has also been identified as a potential hazard for brucellosis-free attested herds feeding on sludge-treated grassland. Infected

Table 9.38. Relative pathogen treatment efficiency of various sludge treatment processes (Carrington 1980; Smith 1996).

Process	Relative reduction		
	Poor	Moderate	Good
Raw sludge storage	*Ascaris* ova *Teania* ova *Cryptosporidium* oocysts	Viruses Bacteria	
Digestion	Ascaris ova[(1)]	Hookworm ova Bacteria *Taenia* ova	Viruses *Entamoeba* cysts *Heterodera* cysts *Cryptosporidium* oocysts
Composting			Viruses Bacteria Fungi Helminth ova
Lime treatment	*Ascaris* ova		Bacteria
Heat treatment[(2)]			Viruses Bacteria Helminth ova *Cryptoridium* oocysts
Irradiation		*Ascaris* ova	Bacteria Viruses

[(1)] Anaerobic digestion at temperatures $> 36°C$ will inactivate depending on exposure time.
[(2)] Includes thermal drying and lime treatment.

humans do not excrete Brucellosis and it is rarely found in sewage. However, it is encountered in farm and dairy wastes, although infection is normally transmitted by the aborted discharges of acute cases and by the milk of chronically infected animals. Sewage sludge can occasionally contain *Brucella* if it contains contaminated wastes from abattoirs or intensive farming units (Bell *et al.* 1978), although the bacterium cannot survive for long in the sludge. If an institutional source of infection exists in the community then *Mycobacterium tuberculosis* may enter and remain in the sewage treatment plant and even survive sludge digestion and drying. However, as there is such a low frequency of infection of the disease within the community it is unlikely to be normally present in sewage and sludge (Argent *et al.* 1977). Viruses are extremely host-specific and it is unlikely that any human enteroviruses in sewage sludge would present a hazard to animals or vice versa. In farming terms, the only viruses of importance are foot and mouth and also swine vesicular disease, both of which are controlled by veterinary inspection so that they are unlikely to find their way into sewage sludge (Hudson and Fennell 1980) (Sec. 11.3.3).

The utilization of sewage sludge on agricultural land is subject to strict controls in the form of guidelines (ADAS 2002) and an EEC Directive (European Communities 1982) for controlling the spread of diseases, with greater restrictions imposed on the use of raw than for treated sludges (Anon 1982a). In essence, the UK guidelines, the Safe Sludge Matrix (ADAS 2002), state that raw or untreated sludges can no longer be used on agricultural land. Sludges are now required to be treated for restricted use or fully stabilized (i.e. heat treatment) for wider use (Sec. 11.3.3). However, Watson (1980) found that *Salmonella* was no longer detectable on cabbages seven weeks after the application of contaminated sewage sludge, indicating that a ban on the consumption of all vegetables may be over-cautious. There does appear to be some evidence that contamination of vegetable salad crops occurs when the soil has been treated with sludge (Wright *et al.* 1976). However, as Watson points out, this must be weighed against the dosage required to produce clinical symptoms. It would appear that if accurate assessments of the possible health risks of sludge disposal to land are to be made, then the concentration of pathogens being applied must be known. The only restriction for treated (digested) sludge is that if used on grassland or pasture a minimum of three weeks must be allowed before grazing recommences in order to ensure that pathogen concentrations are below the minimum infective dose. The minimum infective dose is the number of pathogens that have to be ingested before the disease is contracted. This varies with the pathogen and the health of the animal (Pike

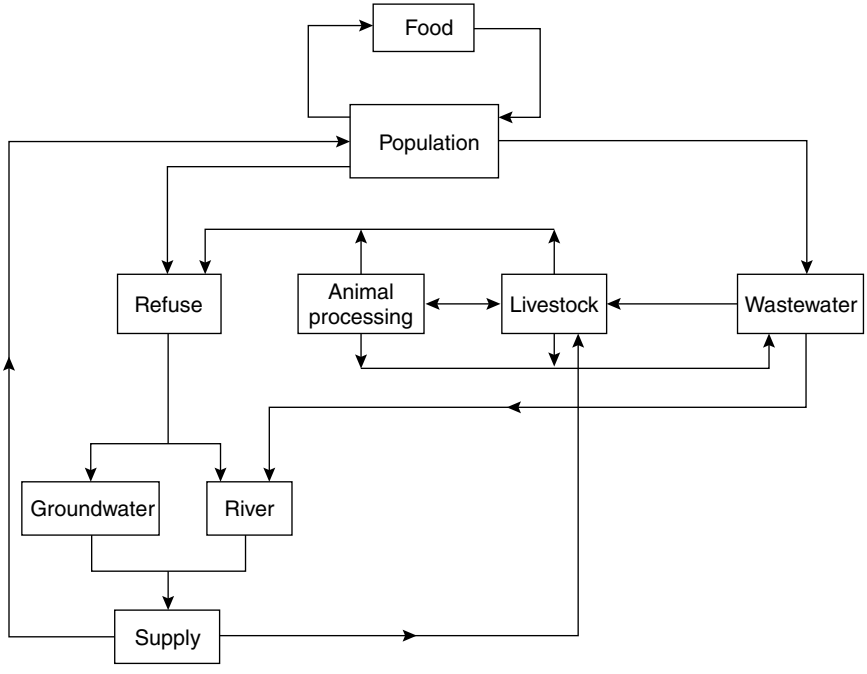


Fig. 9.14. The *Salmonella* infection cycle.

and Davis 1984). For example, in healthy cattle the minimum infective dose of *S. typhimurium* or *S. dublin* is between 10^7–10^8.

The overall concentration of *Salmonella* in sewage sludge applied to grassland is low in relation to the minimum infective dose for cattle, with between 3–24,000 *Salmonella* bacteria per 100 ml of wet raw sludge (mean 4500), compared with 3–210 per 100 ml (mean 60) for digested sludge (Gray 1979). The cycle of infection between man and grazing animals involving sludge is complex (Fig. 9.14) and has been reviewed by Pike (1981). No clear association between sludge utilization and salmonellosis in grazing animals has been established in the UK (Hall and Jones 1978; Williams 1978), although there have been a number of reported cases (Jack and Hepper 1969; Bicknell 1972; Burnett *et al.* 1980). However, there is some evidence from other European countries, such as The Netherlands, Germany, and Switzerland, that infection can occur via this route (Pike 1981). There are also several cases where contamination has been linked to contaminated water, leaking sewers, and septic tank discharges (Carrington 1980a). Over 50 serotypes of *Salmonella* that can infect either man or animals have been

isolated from sewage sludge, and if animals are unusually susceptible or if there is heavy contamination of grazing land by sludge, then infection could readily occur (Hall and Jones 1978; Haugaard 1984; Linklater *et al.* 1985). *Salmonella montevideo* was recorded as the most common serotype isolated from sewage sludge and abattoir effluents in a survey conducted in Scotland by Linklater *et al.* (1985). This serotype has caused frequent outbreaks in animals throughout eastern Scotland and causes abortion in sheep that has resulted in heavy losses in flocks from that part of the UK. This is of particular interest as the transfer of this serotype between flocks and herds by the gull *Larus argentatus* has also been demonstrated (Coulson *et al.* 1983). Methods of isolation and identification of *Salmonella* spp. from sewage sludge are summarised by Carrington (1980b) and Gantzer *et al.* (2001).

Apart from salmonellosis the dissemination of the human beef tapeworm, *Taenia saginata*, is the other major microbial hazard associated with the agricultural use of sewage sludge. The cestode worm infects both cattle (*T. saginata*) and pigs (*T. solium*), but it is the former that is of particular concern. The primary host is man where it grows in the intestine into an adult worm and continuously sheds ova which are passed out with the faeces. In the sewage works, the ova, that are denser than water (density = 1.1), readily settle out in the settlement stages and concentrate in the sludge. Once the sludge is spread on to grassland the secondary host, cattle, ingest the ova that hatch, forming cysticeri (*Cysticerus bovis*), which develop into muscle tissue. The cycle is completed if humans consume infected and inadequately cooked beef. The ova are very resistant and can remain viable for long periods, making the strict adherence to the six-month ban on grazing absolutely necessary. The frequency of infection in humans has decreased since the Second World War because of two main factors. First, meat is rigidly inspected at the abattoir and infected carcasses are either frozen to destroy the cysts or if infection is heavy the carcass is destroyed, and secondly, the greater awareness of adequately cooking meat, which destroys the cysticeri. There are less than 100 confirmed cases of infection in humans per year in England and Wales, with 50–65% of these being in foreign visitors from countries where infection is far more prevalent. The frequency of infection in cattle has remained constant for a long time at < 0.05–0.10% (Blamire *et al.* 1980), although infection in cattle can only occur by ingestion of ova shed by infected humans. However, there is growing evidence that birds feeding at sewage treatment works or on infected material at refuse tips may ingest tapeworm proglottides and disseminate eggs in their droppings (Crewe and Owen 1978). Other modes of

transmission, such as defecation by motorists near lay-bys and discharge of stormwater to watercourses used by cattle for drinking, may also be possible (Pike and Davis 1984). A study on the outbreaks of *Cysticerus bovis* in Scotland has shown that only 7% of the 218 outbreaks reported occurred on farms using sewage sludge. The geographical pattern of outbreaks, however, approximated to the major road routes suggesting that the sanitary habits of motorists may well be involved (Reilly *et al.* 1981). Many other parasites are isolated from sludge including *Ascaris* spp (e.g. *A. lumbricoides* the human intestinal roundworm and *A. suum* the pig roundworm), *Toxocara* (e.g. *T. canis* and *T. cati* the dog and cat roundworm respectively), and *Tichuris* (e.g. *T. trichiura* the human whipworm); however, due to their low density, protozoan cysts are much rarer (Little 1986). Barbier *et al.* (1990) measured the number of parasitic eggs in anaerobically digested (35°C) sludge from a digester with a 42 day retention time at a domestic sewage plant. They recorded 410–1200 eggs kg^{-1} for *Ascaris* spp., 2200–2400 eggs kg^{-1} for *Taniidae* spp., 350–410 eggs kg^{-1} for *Toxocara* spp., and 4910–7250 eggs kg^{-1} for *Trichuris* spp. The life-cycles and the factors that govern the survival of these parasites in sewage sludge are discussed by Watson *et al.* (1983) and also by Kiff and Lewis-Jones (1984). All the water and faecal associated viruses are found in sludge, with hepatitis A particularly common and widespread. Albert *et al.* (1990) found 97–1230 pfu of enteriviruses per litre of primary sludge in Nacy, France. Viruses are also common in digested sludges, although aerobic digestion is more effective in their destruction than anaerobic digestion.

The survival of pathogens on agricultural land depends on a number of environmental factors and will vary according to whether the herbage or soil is contaminated. Trevisan *et al.* (2002) have studied the survival and leaching of faecal coliforms after slurry spreading on mountain hay meadows and have identified those factors that cause mortality of the pathogens as well as transfer from the vegetation and through the soil (Fig. 9.15). Once sludge is spread there is a continuous die-off of pathogenic micro-organisms, the rate of which depends on the environmental conditions, although they can persist for a long time. The meterological factors are most important as pathogens are highly sensitive to sunlight and desiccation with the canopy provided by the vegetation offering protection (Knudsen 1991). However, rainfall will help in the dispersion of pathogens. Those micro-organisms on the herbage are most exposed and are short-lived in comparison with those that find their way into the soil (Table 9.39). Both the intensity and duration of daylight are important. For example, coliform bacteria on green plants are inactivated within 10 hour in summer daylight, whereas in wet

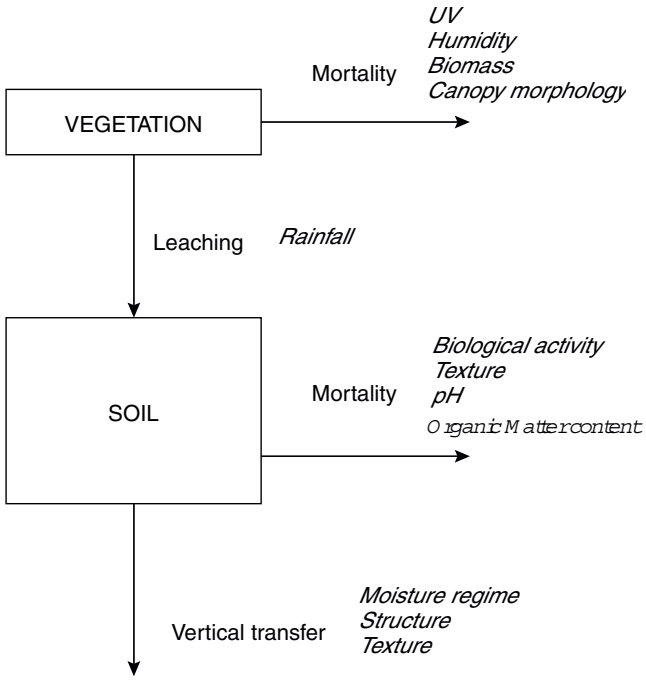


Fig. 9.15. Mortality and transfer of faecal coliforms after spreading of slurry. The main control factors identified during the study are shown in italics (Trevisan *et al.* 2002).

Table 9.39. Median bacterial counts (per gram) in four replicate samplings of a single grassland site at varying times after application of digested sludge (Carrington 1980a).

	Soil			Grass		
Days after application	*E. coli*	Total coliform bacteria	Faecal streptococci	*E. coli*	Total coliform bacteria	Faecal streptococci
15	250	770	17	689	4720	0.8
22	4602	1774	20	464	>1415	1.3
29	8550	950	23	12	144	1
36	1200	1225	250	32	175	<0.2

cold weather, this is extended to 28 hours. The protection offered by the vegetation (e.g. grass sward) is critical for the survival of pathogens, so survival is greater where biomass is higher. However, once the vegetation is cut, pathogens are exposed to desiccation and UV, and so are rapidly inactivated. Within the soil, bacteria will survive for shorter periods in more permeable drier soils, at higher temperatures, in acid conditions, at the soil surface exposed to sunlight, when the organic matter content is low and regrowth is prevented, and, finally, where there is an active soil microfauna that can predate on the pathogens (Thunegard 1975). The role of protozoans in the soil is crucial in the elimination of pathogenic bacteria (Marino *et al.* 1991; England *et al.* 1993). The availability of organic matter in the immediate vicinity of the bacterium is important for its survival as it can only be utilized as food for maintenance as well as for protection. The pH of the soil is also important as the nearer the pH is to neutrality the better the bacterial survival rate (Watson 1980). *Salmonella* survival is very variable, but reports of bacteria surviving on grass and soil for up to 27 weeks is not unusual. Field trials on arable land has indicated that although the survival of *Salmonella* is linked with the environmental conditions and the concentration of bacteria in the sludge, under summer conditions, *Salmonella* will only survive for about 6 weeks in the soil (Watson 198). When sludge is buried in trenches and covered with 0.3 m of soil, *Salmonella* can still be isolated 9 months later. The ova of *Ascaris* remain viable in the soil for up to 5 months, but if contaminated grass has to be used for silage then all the ova should be destroyed after 80–90 days storage. The persistence of pathogens in the soil has been reviewed by Carrington (1980a, b). Mavridou *et al.* (2001) have examined the microbial quality of sludge from 13 different treatment plants throughout Greece which is disposed to agricultural land.

Unlike the chemical contaminants of sludge, the hazards presented by the presence of pathogens are short-term, depending less on their numbers but rather on the method of application to the soil. Four methods of applying sewage sludge to land are widely used: surface spreading, trenching, irrigation using sprayers, and direct injection (Sec. 8.2.2). All contaminate the soil, although trenching and direct injection prevent the herbage becoming contaminated, thus reducing the risk of disease transmission to grazing animals. The problem of using irrigation via sprayer rain guns, is that the herbage is more effectively covered with sludge, thus increasing the distribution of pathogens. There is also a possible risk of disease transmission from the formation of aerosols (Sec. 9.4.3).

Table 9.40. Species of *Heterodera* associated with sewage sludge.

Species	Name	Host crops
H. avenae	Cereal cyst-nematode	Oats, barley, wheat
H. carotae	Carrot cyst-nematode	Carrot
H. cruciferae	Cabbage cyst-nematode	Brassicas and other plants
H. goeltingiana	Pea cyst-nematode	Peas and beans
H. pallida	Pale potato cyst-nematode	Potato, tomato
H. rostochiensis	Potato root eelworm	Potato, tomato
H. schachtii	Beet cyst-nematode	Sugar beet and fodder beet

Apart from human and animal pathogens, sewage also contains vegetable waste and soil that harbour a wide variety of plant pathogens, including viruses, fungi, bacteria, and nematodes. The use of sewage for irrigation, and especially the use of sewage sludge on agricultural land, helps the dissemination of plant pathogens back to agricultural soils. The most important plant pathogens are the cyst-forming nematodes belonging to the genus *Heterodera*. Seven species are regularly isolated from sewage sludge (Table 9.40), the most important being the potato cyst eelworms, *H. rostochiensis* and *H. pallida*, that are endemic in Europe. In the soil, the nematode is present as cysts that contain the eggs of the parasite. Even in the absence of the host plant these cysts can remain viable for many years and infection is particularly difficult to eradicate. The parasite is easily dispersed via contaminated soil, and in wet weather, when vegetables retain a greater proportion of soil on their surface, there is a particular risk of spreading the disease. Cysts accumulate in the soil washed from potatoes, sugar beet, and carrots during processing, the sludge from vegetable-processing factories must be carefully disposed of, if *Heterodera* contamination of cyst-free soil is to be avoided. Potato growing is excluded from heavily infected soils, but the danger that sludge could introduce the pathogen to cyst-free soils is a real one. The EEC Directive that concerns potato growing, stipulates that potatoes grown for export can only be produced on certified cyst-free soil. Several species of cyst nematodes, including *H. rostochiensis* and *H. pallida*, can also parasitize the roots of tomatoes. If tomato plants are allowed to grow on sludge beds or on sludge dumps, or even as weeds, after the application of sludge to land, this will greatly increase the number of cysts in the sludge or soil. Sludge can be examined for cysts, using a simple flotation method, and as long as the level of infection in the soil remains below 25 cysts per 100 g of dry soil then no significant losses in production will occur (Linfield 1977).

Sewage irrigation

Since the development of the sewage farm, when sewage was allowed to flow on to deeply ploughed land, sewage has been disposed of by land irrigation. This type of treatment has been almost completely replaced in the British Isles, although some European cities have retained part of their sewage farm system. For example, the sewage farm developed between 1890–1920 in Paris is still in use, although since then the city has grown dramatically, with an enormous increase in the total flow of sewage, the majority of the sewage is now treated by large biological units before being returned to the River Seine (Dean 1978). The 100-year-old sewage farm at Wroclaw (Poland) is also still in use. The sewage, after a 6-hour settling period, is used for the irrigation of grazing land for dairy cattle, fodder, and trees (Cebula and Kutera 1978) (Secs. 6.1 and 10.2).

Sewage irrigation of agricultural land is practised throughout the entire arid tropical and semi-tropical areas of the world, being particularly common in the Southern Mediterranean, South-west USA, South America, Australia, and India. Contamination of plants and soil by faecal microorganisms and pathogens readily occurs, allowing pathogens to complete life cycles by re-infecting animals. Sewage irrigation also introduces plant pathogens into the soil and spreads viruses and bacteria that will either infect grazing animals or find their way into surface or groundwater. Bacteria are fairly persistent in the soil with *Salmonella* surviving for up to 6 weeks after irrigation (Joshi *et al.* 1973). Plant pathogens are spread by the reuse of infected water or sewage, and plant-pathogenic bacteria, fungi, and nematodes can remain viable for many years in the soil (Steadman *et al.* 1975).

In crop irrigation the excreted pathogens of most concern are human intestinal nematodes and faecal bacteria. Irrigation with untreated wastewater is extremely hazardous to both field workers and consumers, the former are particularly susceptible to helminth infections and the latter to bacterial infections such as cholera and typhoid fever. Crops are categorized as suitable for either restricted or unrestricted irrigation. Restricted irrigation covers crops not grown for direct human consumption or food crops that are not eaten raw. Unrestricted irrigation is required for vegetables and salad crops that are eaten raw. The World Health Organization has set microbial quality guidelines for treated wastewaters used for irrigation (WHO 1989) (Table 9.41); although the quality guidelines of < 1 egg per litre required for both restricted and unrestricted irrigation is thought to be too high for restricted irrigation. Ayres *et al.* (1992) have proposed that nematode

Table 9.41. Microbial quality quidelines for treated wastewater used for irrigation (WHO 1989).

Reuse conditions	Exposed group	Intestinal nematodes[a] (arithmetic mean no. of eggs per litre)	Faecal coliforms (geometric mean no. per 100 ml)
Unrestricted irrigation (crops likely to be eaten uncooked, sports fields, public parks)	Workers, consumers, public	≤ 1[b]	≤ 1000[c]
Restricted irrigation (cereal crops, industrial crops, fodder crops, pasture and trees[d])	Workers	≤ 1	No guideline required

[a] *Ascaris lumbricoides, Trichuris trichiura* and the human hookworms.
[b] Not applicable in the case of localised (i.e. drip, trickle) irrigation (but see Teltsch *et al.*, 1991).
[c] A more stringent guideline ($\leq$ 200 faecal coliforms per 100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.
[d] In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

guidelines for restricted irrigation be relaxed to 10 eggs l^{-1}. Waste stabilization ponds are typically used in regions where effluents are reused. For that reason, pond design is normally based on required levels of helminth egg removal. Design examples for waste stabilization ponds, where effluents are to be used for restricted and unrestricted irrigation, are given by Mara and Pearson (1998). Where effluents are discharged to fish ponds then no trematode eggs are allowed due to the high sexual multiplication of *Schistoma* spp. in water snails, the intermediate host.

The greatest proportion of irrigation water is applied to pasture or to grain crops that need to be processed and cooked before being eaten. Cotton also responds favourably to irrigation with sewage (Dean and Lund 1981). The treatment requirements for sewage used for crop irrigation varies significantly and depends on the quality of the raw sewage and the type of crop to be irrigated. In California, for example, primary effluent may be used for fodder, fibre, and seed crops, and secondary treatment is the minimum treatment required before sewage can be used on food crops or on pasture used for dairy herds (Pettygrove and Asano 1984). The use of sewage for irrigation of vegetables and fruit, however, is discouraged (Sheikh *et al.* 1984).

But in desert areas, where water is the limiting factor for plant growth, sewage may have to be used for these types of crops, with the nutrients in sewage being an extra economic incentive. Fresh vegetables and fruit in arid areas are a rich source of income to farmers and it is inevitable that sewage will be used. However, the dangers are very real, for example, a cholera outbreak in Jerusalem was traced to lettuce grown by a farmer who had illegally diverted raw sewage to irrigate his crop (Shuval and Fattal 1980; Fattal *et al.* 1986b). Poliovirus was isolated from lettuce sprayed with sewage up to 4 weeks after application, although survival appeared to be weather-dependent (Larkin *et al.* 1976). The virus was shown to persist for between 93–123 days in the winter when sprayed on the soil used to grow vegetables, but this was reduced to 8–11 days in the spring (Tierney *et al.* 1977). The degree of contamination depends on the quality of the wastewater used. The USEPA (1989) calculate that the acceptable risk to consumers of contracting a waterborne disease from drinking water complying with current USA microbial standards is 10^{-4}, i.e. an individual has a 1-in-10,000 chance of contracting the disease in question. Shuval *et al.* (1997) have examined the health risks of consuming raw salad crops irrigated with wastewater effluents of varying microbial quality. Where WHO guidelines were met, i.e. < 1000 FC 100 ml^{-1}, the annual risk of contracting hepatitis A was $10^{-6} - 10^{-7}$ and rotovirus infection 10^{-5}–10^{-6}. In contrast, where irrigation water had 10^7 FC 100 ml^{-1} the risk of contracting hepatitis A increased to 10^{-2}. Thus, according to the authors, the consumption of salad crops irrigated with wastewaters treated to < 1000 FC 100 ml^{-1} is 1–3 orders of magnitude safer than drinking water conforming to the USA Safe Water Standards.

Chlorinated secondary wastewater effluents are widely used in the southern USA for the irrigation of golf courses, school grounds, parks, and cemeteries, although UV disinfection is also used (Bourrouet *et al.* 2001). More stringent guidelines are required for such areas of grass, as in these open spaces the public are more likely to come into direct contact with pathogens (DeBoer and Linstedt 1985). The World Health Organization (1989) has suggested effluent discharge limits of < 200 FC 100 ml^{-1}, while Krishnazwami (1971) has proposed that sewage effluents should be treated and disinfected before use, and contain < 100 coliforms or < 20 FC 100^{-1} (Sec. 10.2.2).

9.4.3. *Atmospheric pollution*

Aerosols are particles with a diameter of between 0.01–50 μm that are suspended in the air. Only particles < 5 μm are medically important and

these are readily released during the treatment of sewage, especially from the aeration unit of the activated sludge process and from the distributor nozzles on percolating filters (Adams and Spendlove 1970). The aerosols from sewage treatment plants can contain any of the pathogenic viruses, bacteria, or endotoxins associated with sewage (Dart and Stretton 1977), and can infect animals or humans by inhalation. Airborne endotoxins are of particular concern (Olenchock 1997). The degree of inhalation is dependent upon particle size, with particles $>$ 2–5 μm in diameter bring retained in the upper respiratory tract, but smaller ones able to enter the alveoli of the lungs (Sorber and Guter 1975). Such small droplets of water rapidly evaporate so that any micro-organisms in the aerosol would probably be dehydrated within seconds. Therefore only those people who inhale aerosols within close proximity of the treatment plant ($<$ 20 m) are at risk. At distances $>$ 20 m from units, the risk of contamination rapidly falls, so that there is negligible risk to people who live or work adjacent to treatment plants. However, aerosols that contain micro-organisms resistant to dehydration or contain organic matter which can delay the dehydration of micro-organisms, can increase the effective distance at which aerosols can be hazardous. This may be a particular problem when sewage sludge is sprayed on to agricultural land.

Aerosols are primarily a problem associated with activated sludge aeration basins (Raygor and Mackay 1975; Fannin *et al.* 1985; Carducci *et al.* 1995), although aerosols are also formed as sewage is poured on to percolating filters, being especially serious when spray nozzles are used in conjunction with plastic media. The concentration of bacteria in aerosols and the distance they can travel from source depends on a number of factors, the most important being wind velocity, temperature, solar radiation, and humidity. The spread of micro-organisms from treatment units follows the Gaussian-plume equation with counts of up to 2.4×10^6 *E. coli* per 50 m^3 recorded by Grunnet (1975) in the air next to an activated sludge aeration tank, and with *S. paratyphi* B also occasionally isolated. Goff *et al.* (1973) examined the air around two percolating filters and recorded coliforms extending 100 m from the filters in concentrations up to 90 coliforms per m^3. Airborne bacterial densities above aeration tanks are directly related to aeration rate, with aerator design also important (Fedorak and Westlake 1980). Carducci *et al.* (2000) found medium to high bacterial concentrations in the air around all moving mechanical equipment (e.g. primary and secondary screening, and screw conveyors) or machinery performing wastewater aeration (e.g. grit chambers). This is examined in detail by Bitton (1994). The problem of thermophilic actinomycetes

such as *Aspergillus fumigatus* produced during composting is dealt with in Sec. 10.3.3.

When sewage is used for irrigation, contaminated aerosols can be produced if the effluent is either poured from tankers or sprayed on to the land (Shuval *et al.* 1986c). This is particularly a problem in public areas where sewage is used for the irrigation of golf courses and parks. Crop spraying employing high-pressure jets produce visible aerosols that carry viable pathogenic bacteria up to 650 m downwind. Therefore, in order to minimize any risk to public health, a 1000 m zone around the sprayer is cleared of people and animals (Shtarkas and Krasil'Schchikov 1970). Camann *et al.* (1988) studied the effects of spray irrigation and also found that microbial levels in the atmosphere decreased with distance from source, although entroviruses, in particular polioviruses, were detected up to 60 m down wind of the irrigation site. Attempts have been made to reduce the production of aerosols by using low-pressure jets, but, surprisingly, research has indicated that such sprayers may increase aerosol production (King *et al.* 1973), although this has yet to be fully evaluated. There is some evidence that an irritant contact dermatitis from airborne contamination from spreading sewage sludge is also a problem for those engaged in this activity (Nethercott 1981). A three-year epidemiological study in Clermont-Ferrand, France, where 700 ha of land was irrigated using sprinklers with effluent from waste stabilization ponds showed that there were no associated outbreaks of disease (Devaux *et al.* 2001). The effects of exposure to pathogens at sewage treatment plants have been discussed in Sec. 9.4.1. Fannin *et al.* (1985) reported a non-specific illness, generally referred to sewage worker's syndrome, the symptoms of which are general malaise, weakness, acute rhinitis, and general fever, associated with aerosols produced at activated sludge plants. These general symptoms are also related to exposure to endotoxins. Endotoxins, highly reactive molecules that originate from the outer membrane proteins of Gram-negative bacteria, have been recorded at concentrations of between 0.6–310 ng m^{-3} in the air at a Finish wastewater treatment plant. An occupational exposure limit for airborne endotoxins of 30 ng m^{-3} over an 8-hour day has been proposed (Palchak *et al.* 1988). An interesting survey of the sources of aerosols, at several treatment plants around Livorno, Italy, has been published by Carducci *et al.* (2000). Measurement of the factors affecting biological aerosols is reviewed by Bitton (1999).

9.4.4. *Antibiotic resistance in enteric bacteria*

The widespread use of antibiotics in medical and veterinary treatment, and in the control of infection in intensively reared livestock by the medication of food and drinking water has led to the emergence of antibiotic-resistant strains of bacteria. Although resistant strains arise by genetic mutation within the cell, which then produce resistant bacteria by normal cell division, resistance to antibiotics is also transferable either *in vivo* or *in vitro* to other enterobacteria. Not all resistant bacteria have the ability to transfer antibiotic resistance, only those processing R-factors. The R-factors, and the genetic factors conferring resistance, are carried on extra-chromosomal elements of nucleic acids (plasmids) that can replicate autonomously during cel1 division. Genetic material conferring resistance can be transferred during conjugation between pairs of bacteria without R-factors. For example, an R^+ strain of *E. coli* could transfer antibiotic resistance to a R^- strain of *E. coli* or *Salmonella* spp. (Pike 1975; Carrington 1979; Davies 1997).

Antibiotic-resistant enteric bacteria are particularly hazardous as they are extremely difficult to treat medically, especially where strains have acquired multiple resistance to antibiotics, which is a feature of transferable resistance. This has been highlighted by the rapid spread of multi-resistant strains of *Staphylococcus aureus* in hospitals world wide, resulting in numerous deaths (Swatz 1997). Once established in a host, such bacteria have a distinct competitive advantage over similar sensitive strains when particular antibiotics are administered. Transmissable R-factors have been found in 61% of resistant strains (Linton *et al.* 1972), and a high proportion of the resistant isolates from rivers and coastal waters examined by Williams-Smith (1970; 1971) were able to transmit resistance to *Salmonella typhi* and *S. typhimurium*. It is estimated that about 50% of healthy adults in Britain excrete some antibiotic-resistant coliform bacteria (Linton *et al.* 1972), with higher percentages recorded in adults, having close contact with farm animals. The rapid increase in the use of antibiotics worldwide has resulted in antibiotic resistance becoming a major health problem with patients suffering from antibiotic resistant bacterial infections requiring hospitalization for considerable periods (Lee *et al.* 1994). Antibiotic resistance is also seen in natural aquatic and terrestrial environments (Boon 1992; Ashelford *et al.* 1997; Leff *et al.* 1993; Boon and Cattanach 1999).

Antibiotic resistant bacteria are found in large numbers in the intestinal tract of humans and animals receiving antibiotic treatment. This means that wastewaters are highly contaminated with such bacteria as well as the antibiotics themselves (Goni-Urriza *et al.* 2000). Antibiotic resistance in

bacteria in fish ponds, where antibiotics are used, is also recorded (Tendencia and Pena 2001). The biological stage of wastewater treatment is an ideal environment for gene transfer between micro-organisms (McClure *et al.* 1990). The transfer frequency of R plasmids among bacteria in wastewater has been investigated by Mach and Grimes (1982) who observed a maximum transfer frequency of 2.7×10^{-4} between *Salmonella enteritidis* and *E. coli*. *Pseudomonas putida* UWC8, a non-indigenous bacteria containing the conjugative Plasmid pQKH6, has been used to study gene transfer in percolating filters (Ashelford *et al.* 1995). The plasmid has a mean transfer frequency of up to 8.4×10^{-4} within pilot scale percolating filters. It was also noted that gene transfer also occurred within the guts of *Sylvicola fenestralis* larvae, an invertebrate grazer of the biofilm, at frequencies up to 1.6×10^{-2} indicating that the grazing fauna could participate in gene transfer (Ashelford *et al.* 2001). The proportion of bacteria carrying R-factors has been shown to increase after water and wastewater treatment. Armstrong *et al.* (1981 1982) found the occurrence of multiple resistance rose from 15.8% to 57.1% before and after water treatment, and from 18.6% to 67.8% before and after wastewater treatment. Like other bacteria, the overall numbers of antibiotic-resistant strains are reduced by sewage treatment with 90% removal achieved by full biological treatment. In the absence of the relevant antibiotic, resistant bacteria have no advantage over sensitive organisms in the aquatic environment, which is shown by the ratio of antibiotic-resistant to antibiotic-sensitive coliforms remaining constant before and after treatment. The release of antibiotic-resistant bacteria into the environment is best controlled by restricting the prescribing and use of antibiotics (HMSO 1969; Cohen 1992; Rollin 2001), especially in the selective use of antibiotics with a low resistance potential while restricting those with a high resistance potential (Cunha 2000). There is growing concern on the extent of antibiotic resistance seen in natural heterotrophic bacteria in surface waters (Boon 1992). Working in Australia, Boon found bacteria from rivers were more resistant to antibiotics than those isolated from billabongs (lakes). Boon and Cattanach (1999) found no significant difference in the incidence of resistance between native and faecal bacteria to tetracycline, with both groups almost totally resistant to penicillin. Chee-Sanford *et al.* (2001) were able to demonstrate that antibiotic resistance can pass from treatment lagoons into the indigenous soil microbiota and finally into ground water. Further information is given by Goni-Urriza *et al.* (2000). The distribution of antibiotic-resistant bacteria in sewage and the effect of sewage treatment is reviewed by Carrington (1979). Antibiotic resistance profiles are being used to determine the source of waterborne faecal contamination (Wiggins

1996; Harwood *et al.* 2000) (Sec. 9.3.2). Antibiotic resistance is also seen in anaerobes, especially the *Bacteroides fragilis* group with high levels of resistance to clindamcycin piperacillin, cefoxitin, and ceftizoxine. Resistance is much lower in non-*B. fragilis* anaerobes such as *Clostridium* spp. (e.g. clindamcycin and cephamycins) (Hecht *et al.* 1999). As little work has been done in this area, the true state of antibiotic resistance in anaerobic bacteria remains unknown.

9.5. Removal of Pathogenic Organisms

9.5.1. *Environmental factors and survival*

All pathogens are able to survive for at least a short period of time in natural waters, both fresh and saline. Usually, this period is extended when there are cooler temperatures and if organic pollution is present. The major environmental conditions affecting the survival and viability of pathogens are: sedimentation, solar radiation, predation, bacteriophages, nutrient deficiencies, algal and bacterial toxins, and physico-chemical factors. Adsorption and sedimentation can be significant factors in the removal of enteric bacteria from surface waters, depending on the nature of sewage, the degree of treatment, and the effect of vertical dispersion. In raw sewage, 50–75% of the coliforms are associated with particles with settling velocities > 0.05 cm s^{-1}. Therefore, in conventional primary sedimentation at a treatment plant, significant removals of enteric bacteria are expected (Sec. 9.5.2). If discharged to coastal waters other conditions, such as enhanced flocculation, adsorption to marine and estuarine sediments combined with sedimentation, may result in significant removals of bacteria. However, Irving (1977) demonstrated that adsorption of bacteria onto particulate matter was not significant in seawater. By comparing the mortality rate (i.e. T_{90} — the survival of enteric bacteria or virus expressed as time in hours or days for 90% or 1 $\log_{10}$ inactivation) at different concentrations of suspended solids (0–10,000 mg l^{-1}) under dark and light conditions he was able to show that although the value of T_{90} varied with turbidity in the light, under dark conditions it remained stable showing that solar radiation was the major factor controlling inactivation, whereas adsorption to particulate matter was insignificant (Table 9.42). These processes are hindered by vertical mixing because of wind and bottom scouring, which can maintain in suspension or re-suspend settleable bacterial (Mitchell and Chamberlin 1975). Normally, sewage receives some degree of treatment before being discharged and even those effluents that are discharged directly to coastal

Table 9.42. Effect of particulate matter on the mortality rate of coliform bacteria expressed as the time in hours required for 90% mortality to occur (T_{90}) in light (50 cal $cm^{-2}h^{-1}$) and dark conditions. The correlation coefficients for the regression lines calculated from the mortality data in each case are also given (Irving 1977).

Suspended-solids content (mg l^{-1})	Light		Dark	
	T_{90}	Correlation coefficient	T_{90}	Correlation coefficient
0	1.0	0.98	116	0.94
300	1.3	0.99	155	0.89
600	6.5	0.77	382	0.61
1000	12	0.86	123	0.91
5000	20	0.92	107	0.88
10000	37	0.64	108	0.96

waters generally receive primary settlement. Therefore, the effect of sedimentation in the natural environment is not generally significant.

The rate of *Escherichia coli* mortality in seawater has been shown to be proportional to the size of the marine microfauna (Mitchell *et al.* 1967). Mitchell (1971) implicated three groups of micro-organisms, cell-wall lytic marine bacteria, marine amoebae, and marine bacterial parasites. Although bacteria in the natural environment competes with the unadapted enteric bacteria, especially for nutrients (Jannasch 1968), the concentration of predators such as, protozoans and bacteriophages, is normally too low to have a significant effect on pathogen survival (Pike and Carrington 1979). In seawater, the diversity of predators is lower, and bacteriophages are inactivated. Toxins and antibiotics produced by algae and other bacteria can cause a significant increase in the death-rate of enteric bacteria in enclosed water bodies such as, ponds and lakes, but the dilution effect in rivers, estuaries, and seawater renders them harmless (Carlucci and Framer 1962; Foxworthy and Kneeling 1969). The concentration of organic matter in the form of carbon, nitrogen, and phosphorus can aid survival and, in some instances, encourage growth of enteric bacteria, although in seawater the concentration of the important nutrients is too low (Won and Ross 1973).

Some ideas of the effect of time on the survival of pathogenic micro-organisms can be obtained by examining the removal efficiency of storage lagoons that are used at some waterworks to improve water quality prior to treatment and supply. Up to 99.9% reduction of enteric

Table 9.43. half-life of enteric bacteria in well water (McFeters *et al.* 1974).

Species	Half-life (h)
Salmonella paratyphi B	2.4
Streptococcus bovis	4.3
Vibrio cholerae	7.2
Streptococcus equinus	10.0
Salmonella paratyphi A	16.0
Salmonella typhimurium	16.0
Coliform bacteria	17.0
Salmonella paratyphi D	19.2
Enterococci	22.0
Shigella dysenteriae	22.4
Shigella sonnei	24.5
Shigella flexneri	26.8
Aeromonas	72.0

bacteria can be obtained by storage, although this is dependent on temperature, retention time, and the level of pollution of the water (Kool 1979). For example, a 99.9% reduction in pathogenic viruses by storage requires a retention time of 20 days at 20–27°C, but up to 75 days at 4.8°C. The half-life or die-off rates of the commonest pathogenic bacteria have been assessed by McFeters *et al.* (1974) who suspended samples in well water enclosed in membrane chambers (Table 9.43). These results indicate that the half-life of pathogenic bacteria and coliforms are of the same order of magnitude, except for *Shigella* sp. Using a similar technique, the percentage survival of bacteria in flowing river water was calculated after 4 days: 78% for *E. coli*; 46% faecal streptococci; and 74% *Salmonella* sp. (Smith *et al.* 1973). However, pathogenic bacteria have been reported as surviving for much longer periods, especially *Salmonella* sp. (Geldreich *et al.* 1972; Leclerc *et al.* 1976). Viruses can survive longer than bacteria in natural waters, with some viruses surviving for up to twice as long as certain indicator bacteria (Table 9.44) (Kool 1979).

Temperature is an important factor in the survival of viruses, and at low temperatures (4–8°C) survival time will increase (Table 9.44). For example, echovirus type 6 is inactivated more readily in seawater at 22°C (8 days) than at 3–5°C (91 days) (Won and Ross 1973). Coliphages survive for only

Table 9.44. Effect of temperature on the inactivation of enteric micro-organisms during storage. Time taken for 99.9% reduction of micro-organisms (Kool 1979).

Species	Temperature (°C)	Time (d)	Temperature (°C)	Time (d)
Poliovirus	4	27	20	20
Echovirus	4	26	20	16
E. coli	4	10	20	7
Str. faecolis	4	17	20	8
Poliovirus type 1, 2, 3	4–8	27–75	20–27	4–20
Coxsackie virus type A2, A9	4–8	12–16	20–27	4–8

Table 9.45. Effect of temperature and salinity on the survival of pathogenic micro-organisms in seawater, expressed as the time for a 99.9% reduction of the original inoculation of 1.5×10^7 ml^{-1} (Jamieson *et al.* 1976).

		Period for a 99% reduction in concentration (d)					
Temp.	Salinity		*Myco.*			Shig.	
(°C)	(g kg^{-1})	*E. coli*	tuberculosis	*L. interrogans*	*S. typhi*	*dysenteriae*	*V. cholerae*
4	5	5	7	1	6	5	1
	20	5	7	1	6	4	1
	35	5	7	1	6	1	1
25	5	4	6	1	5	1	1
	20	3	6	1	5	1	1
	35	3	6	1	5	1	1
37	5	1	6	1	3	1	1
	20	1	6	1	1	1	1
	35	1	6	1	1	1	1

7 days in pond water at 20°C in the absence of hosts, but will survive for up to 14 day when bacteria are present (Scarpino 1975). Hepatitis A virus is more persistent in seawater at 5°C than at 25°C (Chung and Sobsey 1993a) with survival rates (T_{90}) in synthetic seawater of 92 days at 4°C falling to 11 days at 25°C (Crance *et al.* 1998). Many studies have demonstrated that the death-rate of enteric and indicator bacteria is accelerated at higher temperatures in natural waters (Table 9.45) (Jamieson *et al.* 1976). This is also illustrated by Evison and James (1973) who compared the survival of indicator bacteria over a 5-mile distance in two rivers with simi-

lar flow rates in East Kenya (mean temp. 18.5°C) and Britain (mean temp. 2°C). There was a 96.5% reduction of indicator bacteria in the warmer river compared with only a 56% reduction at the lower temperature. Wait and Sobsey (2001) examined the survival of a range of enteric viruses and bacteria in Atlantic seawater. They found that microbial survival was greater under laboratory conditions than in-situ. For the laboratory experiments, seawater (31.5–35.0 mg l^{-1} salinity, pH 7.9–8.3, turbidity 0.4–11 NTU) was collected seasonally and seeded with test bacteria and viruses and sampled over a 16-week period to determine survival rates (Table 9.46). The clear relationship between microbial activation rate and temperature observed in the laboratory tests was not consistently observed in the in-situ field experiments due to, Wait and Sobsey explain, other factors influencing microbial survival (Table 9.47).

Both UV radiation and short-wave visible light are lethal to bacteria, with the rate of death related to light intensity, clarity of the water, and depth. In the dark, the death-rate of coliforms follows first-order kinetics over the initial period; however, predation by protozoans causes a departure from the log-linear relationship (Morgan 1985). In the dark, at 20°C, the T_{90} for coliforms is 49 hours, whereas under midday sunlight, the T_{90} is reduced to 0.3 hour. *Salmonellae* sp. have mortality rates comparable with the faecal coliforms (Gameson 1984). The death-rate is also temperature-dependent, decreasing by a factor of 1.97 for each 10°C rise in temperature, and is proportional to the total radiation received, regardless if this is continuous or intermittent. The amount of radiation required to kill 90% (S_{90}) of coliforms is estimated as 23 cal cm^{-2} (11.3 cal cm^{-2} for *E. coli*). The S_{90} ratio of faecal streptococci to coliforms varied from 10–39, indicating that the die-off rate for faecal streptococci is appreciably slower than for total coliforms (Gameson and Gould 1974). The effect of temperature is far less marked under light conditions and although variations in salinity have no effect on the death-rate, it is substantially slower in fresh and brackish waters (Gameson 1985a). The daily solar radiation in southern Britain is between 50–660 cal cm^{-2}, and there appears to be ample radiation to inactivate all faecal coliforms. Wavelength is also important, with about 50% of the lethal effect of solar radiation attributable to wavelengths below 370 nm, 25% to near visible UV (370–400 nm), and 25% to the blue-green region of the visible spectrum (400–500 nm). The effect of wavelengths > 500 nm is negligible (Gameson and Gould 1974). The amount of radiation reaching the sea is dependent on a number of factors, such as solar elevation and weather conditions. The lethal radiation is rapidly absorbed by suspended and colloidal solids, which rapidly reduce its

Table 9.46. Time (days) for 90% reduction in microbes in seawater-laboratory study (Wait and Sobsey 2001).

Microbe	°C	Winter	Spring	Summer	Fall	Average
Escherichia coli B	6	2.0	8.5	6.9	4.7	5.5
	12	0.9	5.5	4.4	3.8	3.7
	20	1.0	7.3	2.1	2.7	3.3
	28	0.9	0.9	0.9	2.5	1.3
Salmonella typhi	6	12.6	18.5	21.2	14.5	16.7
	12	5.0	10.5	7.4	7.5	7.6
	20	2.9	4.5	4.7	3.1	3.8
	28	2.4	2.7	2.8	2.5	2.6
Shigella sonnei	6	4.4	24.8	20.5	11.8	15.4
	12	2.8	14.3	8.2	5.9	7.8
	20	1.4	4.5	4.0	3.8	3.4
	28	2.2	2.4	1.9	2.5	2.3
Poliovirus 1	6	10.0	7.5	1.8	10.5	7.5
	12	5.0	6.4	1.9	0.8	3.5
	20	2.0	3.1	1.0	1.0	1.8
	28	1.5	2.1	1.0	1.8	1.4
Minute Virus of Mice	6	No data	No data	8.0	11.4	9.7
	12	No data	No data	3.1	2.8	3.0
	20	No data	No data	2.1	1.5	1.8
	28	No data	No data	1.4	1.2	1.3

Salinity = 31.5–35 mgl^{-1}, pH = 7.9–8.3, turbidity = 0.4–11, Secchi disk depth = 1.6 to > 6.5 M.

effect, with depth being effective only up to 5 m in seawater (Pike 1975). In seawater, the mortality rate of bacteria rapidly decreases as the turbidity of the water increases (Fig. 9.16). The die-off rate is related to depth, with an effective attenuation coefficient of approximately 0.22 m^{-1}. As this die-off rate for coliforms is proportional to the light intensity and is therefore essentially a first-order relationship, it can be expressed as:

$$(dC/dt) = -kl_0\,\mathrm{e}^{-\alpha z}C$$

where C is the coliform concentration at time t at a depth Z, k is a proportionality coefficient, l_0 is the light intensity just below the water surface and α the effective attenuation-coefficient (Mitchell and Chamberlin 1975). The effect of UV is considered further in Secs. 9.5.2 and 9.5.3.

Table 9.47. Time (days) for 90% reduction in microbes in two depths of seawater-field study (Wait and Sobsey 2001).

	Winter (4–7.5°C)		Spring (19–22°C)		Summer (22–24°C)		Autumn (18–20°C)	
Test microbe	At 3 m	At 10 m	At 3 m	At 10 m	At 3 m	At 10 m	At 3 m	At 10 m
E. coli B	3.4	2.8	1.8	1.2	4.0	3.4	0.9	1.0
S. typhi	1.8	3.3	1.4	1.4	1.4	1.4	3.7	3.5
Sh. sonnei	2.5	1.9	1.2	1.4	2.0	2.4	1.0	2.0
Poliovirus 1	7.1	6.6	No data	No data	2.6	1.5	1.7	0.8
Minute virus	4.0	6.2	3.4	3.5	3.0	2.1	No data	No data

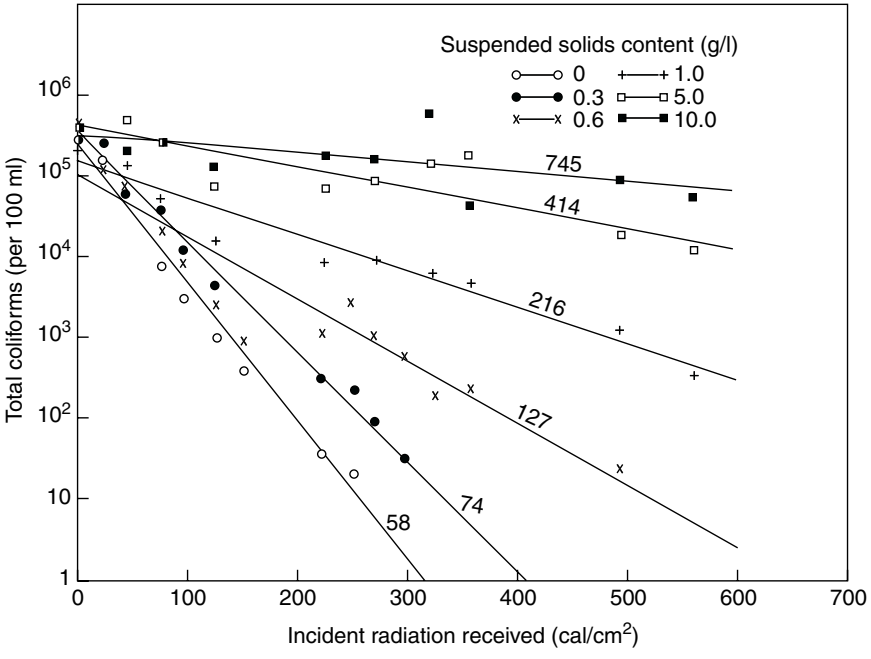


Fig. 9.16. The effect of suspended solids concentration on the amount of radiation required to inactivate coliform bacteria in 1 litre beakers. The amount of radiation required to inactivate 90% of the coliforms originally inoculated (S_{90}) in cal cm^{-2} is shown against the plotted regression (Irving 1977).

Since the introduction of the Bathing Water Directive (76/160/EEC) (European Communities 1976), there has been considerable interest and research into the survival of pathogens in estuarine and coastal waters. Seawater does not favour the survival of pathogens, and *E. coli* in particular, with osmotic effects, pH, UV, and specific ion toxicity, such as heavy metals (Jones 1964), all important factors effecting the survival of enteric bacteria (Mitchell and Chamberlin 1975; Solic and Krstulovic 1992). However, only a slight increase in the death-rate of pathogens is recorded at increased salinities (Table 9.45), although the mean T_{90} for coliforms in river water is double the value for seawater (Carrington 1980a). It is thought that genetically controlled osmoregulatory processes, induced by salts, can help enteric bacteria survive osmotic stress in saline environments (Munro *et al.* 1987). These processes involve K^+ uptake as well as the accumulation of compatible organic osmolytes such as glycine-betaine (also found in marine sediments), trehalose, and glutamate (Gauthier *et al.* 1991). Viruses also

Table 9.48. Effect of temperature and salinity on the survival of enteroviruses inoculated into seawater at a concentration of 10^5–10^6 ml^{-1}, expressed as the time for a 99% reduction in virus concentration and the time in weeks (wks) until no virus is recovered (Loo *et al.* 1976).

Incubation temp (°C)	Salinity (g kg^{-1})	Period for 99% reduction (wks)			Period until virus no longer recovered (wks)		
		Polio	Echo	Coxsackie	Polio	Echo	Coxsackie
4	10	18	18	32	46	46	> 53
	20	12	20	46	46	53	> 53
	34	10	18	53	53	53	> 53
15	10	40	12	46	53	24	> 53
	20	10	12	20	22	32	53
	34	6	14	32	22	32	46
25	10	4	6	6	8	12	12
	20	2	4	4	6	8	10
	34	4	4	6	8	6	10
37	10	< 2	< 2	< 2	< 2	< 2	< 2
	20	< 2	< 2	< 2	< 2	< 2	< 2
	34	< 2	< 2	< 2	< 2	< 2	< 2

show a slight increase in death-rate as the salinity increases (Table 9.48). This is examined further by Chung and Sobsey (1993b).

9.5.2. *Treatment processes*

The primary objective of wastewater treatment is to reduce the BOD_5 of the wastewater so that it can be safely discharged without causing deoxygenation in the receiving waters. A secondary objective, and in some respects more important than the first, is the removal of enteric bacteria and other pathogens. It is the introduction of the biological treatment of sewage, linked with the chlorination of domestic water supplies, that has eliminated the classical water-borne diseases from Western Europe. The effectiveness of the removal of pathogenic micro-organisms from wastewaters depends on a number of factors, such as the diversity and numbers of pathogens present, the type of treatment processes used (Tables 9.37 and 9.38), loading rates and plant efficiency, and seasonal factors, e.g. temperature and the chemical nature of the wastewater. During treatment of sewage the microbial flora changes from predominately faecal in character to that found more commonly in enriched fresh waters.

Methods of removal

The removal of pathogenic micro-organisms is affected by a combination of physical, chemical, and biological processes. Physically pathogens are removed by adsorption and settlement, and the overall concentration is reduced by dilution. The chemical nature of the wastewater will determine whether the environmental conditions are suitable for the survival or even the growth of pathogens. However, factors, such as hardness, pH, ammonia concentration, temperature, and the presence of toxic substances, can all increase the mortality rate of the micro-organisms. Biologically, death of pathogens can occur because of a number of reasons including starvation, although predation by other micro-organisms and grazing by macro-invertebrates are important removal mechanisms. Bacteriophages and the predatory bacterium *Bdellovibrio bacteriovirus* are common in sewage. However, if they were active during treatment then a rapid increase in their numbers would be expected as treatment progressed. Infected bacterial host cells will release hundreds of phage progeny some 20–30 minutes after infection, and host cells of *Bdellovibrio* result in a 3–5 fold increase of the organism 4–5 hours after infection. However, during sewage treatment this does not occur, indicating that they are inactivated by contact with sewage (Pike 1975). This has been confirmed by studies on rivers receiving sewage (Fry and Staples 1976) and activated sludge plants to which coli phages have been added (Balluz *et al.* 1978). Enteric viruses tend to survive longer than bacteria in wastewater as bacteria are more susceptible to environmental factors than viruses because of their cellular nature. Also, viruses are much smaller than bacteria and so behave as colloids in water, carrying a charge on their surface and thus are more readily adsorbed by solids than bacteria. There is also evidence to suggest that adsorbed viruses are protected from inactivation and will remain viable longer than unadsorbed viruses and bacteria (Funderburg and Sorber 1985).

It is convenient to look at the wastewater treatment plant as an enclosed system with inputs and outputs. It is a continuous system and, therefore, the outputs, in the form of sludge and a final effluent, will also be continuous. Although a comparison of the number of pathogens in the influent with that of the final effluent will provide an estimate of overall efficiency, it will not give any clues as to the mechanism of removal. Essentially, pathogens are either killed within the treatment unit, discharged in the final effluent, or concentrated in the sludge. The latter will result in secondary contamination problems if disposed either to agricultural land or into coastal water (Sec. 9.4). An estimation of the specific death-rate ($-\mu$) of a pathogen can be calculated by accurately measuring all the inputs and outputs of

the viable organism (Pike and Carrington 1979). All the individuals of the population of the pathogen (x), which are assumed to be randomly dispersed within the reactor, have the same chance of dying within a specific time interval ($t - t_0$). Under steady-state conditions, the causes of death can be assumed to remain constant in terms of concentration (in the case of a toxic substance) or number (of a predator), and the rate of death will be proportional to the number of survivors (x_t) of the original population (x_0). This can be represented by a first-order, exponential death equation:

$$x_t = x_0 e^{-\mu(t-t_0)}$$

The problem with using this equation in practice is obtaining accurate estimates of the viable micro-organism present in the outputs, especially if the cells have flocculated or are attached to film debris (Pike *et al.* 1972; Pike 1975). The type of reactor is also important in the estimation of the death-rate. In an ideal plug-flow system (e.g. a percolating filter) in which first-order kinetics apply, the fraction of pathogens surviving (x_t/x_0) can be related to the dilution rate or the reciprocal of the retention time (D) as:

$$(x_t/x_0) = (-\mu/D)$$

In a continuous-stirred tank reactor (CSTR), the specific death-rate is calculated by assuming the rate of change in pathogen concentration within the reactor (dx/dt) equals the input concentration (x_o) minus the output concentration (x_t) minus those dying within the reactor under steady-state conditions, then:

$$(dx/dt) = Dx_0 - Dx_t - \mu x$$

Primary settlement

Counts of indicator bacteria and viruses are not greatly reduced by primary settlement (Pike 1975), although some removal of pathogens must be occurring as primary settled sludge contains the whole range of pathogens found in raw sewage (Table 9.49). Some reports suggest that the concentration of bacteria that is particularly associated with particulate matter is reduced by settlement, with removals of 10% for *E. coli* and 60% for *C. perfringens* being recorded by Bonde (1977). Numbers of some indicator bacteria have been reported to increase during primary settlement (Harkness 1966), although viruses are generally not effectively removed at

Table 9.49. Typical concentration of *E. coli* and faecal streptococci in raw and treated sewage (Gross and Cook 1995).

Treatment stage	*E. coli* 100 ml^{-1}	Faecal streptococci 100 ml^{-1}
Raw sewage	1×10^7	1×10^6
Stormwater	1×10^5	1×10^4
Primary treated sewage	5×10^6	5×10^5
Secondary treated sewage	1×10^6	1×10^5
Disinfected final effluent*	5×10^2	5×10^1

*UV disinfection assuming a 99.9% kill.

this stage. Berg (1966) found that 33–67% removal of poliovirus type 1 occurred after 24 hours, whereas normal retention periods were ineffective. Rao *et al.* (1977) studied the removal of enteric viruses over a two-year period at the Dadat Sewage Treatment Works in Bombay, India. Removal by primary sedimentation varied seasonally, i.e. rainy season (June–July) 24.1–33.5%, autumn (September–November) 56.0–73.0%, winter (January–February) 41.4–83.4%, and summer (March–June) 57.0–74.7%.

Ova (eggs) and cysts of parasites are only significantly removed at this stage in wastewater treatment plants. Settlement efficiency is dependent on the size and density of the ova and cysts, and as their free-falling settling velocities are not much greater than the theoretical upflow velocity, near quiescent conditions are required for optimum removal (Sec. 2.2). The rate of removal of these pathogens is easily affected by currents, eddies, reduction in retention time, and any other factor that reduces settling efficiency. The larger, denser ova of *Ascaris lumbricoides* and *Taenia saginata* are more efficiently removed than the smaller cysts of *Entamoeba* spp. The settling velocity of *T. saginata* is about 0.6–0.9 m h^{-1}, although much less if detergents are present, resulting in 68% removal after 2 hours and 89% after a 3-hour settlement (Liebmann 1965). No significant settlement of *E. histolytica* occurred in a settlement tank 0.67 m deep after 3 hours, although the ova of *A. lumbricoides* settled out rapidly (Cram 1943). It has been estimated that up to 97% of the major genera of worms (*Ascaris*, *Trichuris*, *Enterobius*, *Diphyllobothrium*, and *Taenia*) are normally removed by primary settlement (Silverman and Griffiths 1955). However, ova of *Taenia* and *Ascaris*, and cysts of *Entamoeba* are regularly detected in settled sewage and occasionally in the final effluent.

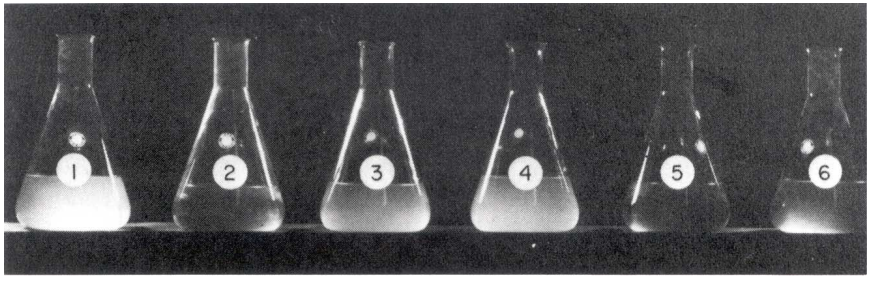


Fig. 9.17. Photographs of effluents from replicate pilot-scale activated sludge plants. Effluents from plants 2, 5, and 6 contained ciliate protozoans, whereas plants 1, 3, and 4 did not, resulting in more turbid effluents (Curds 1975).

Activated sludge

The activated sludge process is highly efficient in the removal of pathogenic bacteria and viruses, achieving a 90% removal efficiency or more. Among the many references to removal efficiency, Geldreich (1972) found a reduction of 88–99% for *Salmonella*, *Shigella*, and *M. turberculosis*. Bacteria are removed by grazing, inactivation, and absorption onto flocs. The major removal mechanism of bacteria in the activated sludge process is predation by a variety of amoebae, ciliate protozoans, and rotifers. In reactors without sludge return predator-prey oscillations are discernible. However, the return of wasted sludge, which is normally practised, tends to dampen out these oscillations so that under steady-state conditions the overall removal of bacteria by predation remains constant (Curds 1971a, b). The ciliate protozoans and rotifers feed only on the freely suspended bacteria and not on flocculated forms. The ability of ciliates to clarify turbid effluents caused by high concentrations of suspended bacteria was demonstrated by Curds *et al.* (1968) (Figs. 9.17). Amoebae occur in similar numbers to ciliates and have similar yield coefficient biomass and generation times. They also play a significant role in the removal of bacteria by predation and are able to feed on flocculated forms as well as the freely suspended bacteria. Clearly, the amoebae may have a particularly important role in grazing on the pathogens that have become adsorbed onto sludge flocs.

Protozoal feeding is a major process in the removal of *E. coli* cells (Curds and Fey 1969). Using the data of Curds and Fey, Pike and Carrington (1979) estimated the specific growth rate of *E. coli* to be $-7.9\ d^{-1}$ in the presence of protozoans compared with $+0.12\ d^{-1}$ in the absence of protozoans. This suggested that *E. coli* is capable of very slow growth in sewage when ciliates are absent. In general terms, the percentage removal of coliform bacteria is

directly related to the specific sludge wastage rate, with 90% removal at a sludge wastage rate of 0.65 d^{-1} that rapidly decreases as the wastage rate increases (Pike and Carrington 1979) (Sec. 5.5).

Experiments on predation have suggested that physical adsorption is not important in the removal of pathogenic bacteria. For example, Curds and Fey (1969) found that the half-life of *E. coli* introduced into a plant lacking protozoans was 16 hours compared with 1.8 hours in a parallel plant with protozoans, indicating that removal mechanisms other than protozoan feeding are insignificant in comparison. However, this may not be the case, as rapid adsorption of *E. coli* on to sludge flocs have been shown to occur within the first hour of sewage entering the aeration tank (Drift *et al.* 1977). After an hour, the removal of coliforms being primarily by predation, was no longer as rapid or constant. The adsorbed bacteria are retained in the solids fraction where mortality by predation, starvation, or other means continues as in the liquid phase. However, the wasted sludge is rich in pathogenic bacteria that have been concentrated mainly by adsorption.

The prime removal method of viruses is adsorption onto sludge flocs, predation having a negligible effect on concentration in the liquid phase. Both viruses and bacteria are adsorbed according to the empirical Freundlich adsorption isotherm, where the count of particles adsorbed per unit mass of sludge (Y/m) is proportional to a power (n) of the count of particles (x) in the liquor at equilibrium:

$$(Y/m) = kx^n$$

This adsorption model is for unreactive sites, whereas Michaelis-Menton or Monod kinetics are for reactive sites. However, as Pike and Carrington (1979) point out, the successful fitting of an adsorption model to data may not demonstrate that adsorption is the only factor operating because, for example, protozoans attached to the activated sludge flocs and feeding off freely suspended bacteria will quantitatively behave as a continual adsorption site.

Upwards of 90% of enteroviruses are removed by the activated sludge process (Pike 1975; Rao *et al.* 1977), with high removal efficiencies of poliovirus type 1 (90%) and coxsackievirus type A9 (98%) being recorded (Geldreich 1972; Varma *et al.* 1974), although removal efficiencies are erratic (Berg 1973). Apart from the indicator viruses, other viruses, such as coxsackievirus type B, echovirus, and adenovirus, have also been shown to be adsorbed onto sludge flocs (Lund and Rønne 1973), but are also present in the final effluent (Irving and Smith 1981). Adsorption has been shown

to be very rapid, for example poliovirus is adsorbed from solution within three minutes of being added to liquor from a waste stabilization pond (Sobsey and Cooper 1973). This rapid adsorption of viruses was confirmed by Malina *et al.* (1975) who used radioactivity counts of tritium-labelled poliovirus to monitor removal mechanisms. They found that the initial rapid adsorption was followed by a period of equilibrium which, they suggested, was due to the maturation of available adsorption sites on sludge flocs. There is a significantly higher concentration of viruses in secondary sludge than primary sludge (Lund and Rønne 1973). This is due to the mixing action of the aeration tank that allows contact between available adsorption sites and viruses. This is confirmed by the increase in the adsorption of viruses by increasing the mixing rate of sludge flocs after the initial uptake of viruses, by breaking up flocs and providing more adsorption sites. A lack of mixing is probably why percolating filters have so little effect on virus removal. Many workers have noted that the concentration of viruses in the sludge is directly related to the concentration of viruses in the influent. The addition of alum to aeration tanks to precipitate phosphates also enhances the removal of viruses and faecal bacteria (Davis and Unz 1973 1975). Other physico-chemical processes will also greatly reduce the virus and bacterial load by increasing flocculation (Berg 1973). For example, the addition of lime to sewage thus raising the pH > 11.5 results in 99.99% removal of poliovirus type 1 (Satter *et al.* 1976) and *E. coli* (Morrison *et al.* 1973).

Viruses in the activated sludge process are also inactivated, provided that the aeration of the mixed liquor continues (Glass and O'Brien 1980). Inactivation rates of poliovirus of $-1.47\ h^{-1}$ have been recorded. Inactivation also occurs in settlement tanks (Balluz *et al.* 1977 1978). They also recorded that poliovirus is mainly associated with the solids phase of mixed liquor (85%), whereas coliphages are associated mainly with the supernatant (83%).

Ova and cysts of parasites are not inactivated within the aeration tank. Although the majority of helminth ova are removed at primary sedimentation, residual ova are normally removed during secondary settlement. However, Schwartzbrod *et al.* (1989) reported that *Ascaris* and *Toxocara* ova were not totally eliminated by activated sludge. Cysts and oocysts of protozoan parasites generally pass through primary sedimentation due to their size and density. They become entrapped in the flocs and removed in the waste activated sludge stream to the sludge treatment stages. Removal rates are variable, being high for *Giardia* spp. $> 99\%$ (Mayer and Palmer 1996) and *Entamoeba histolytica* $> 98\%$ (Panicker and Krishnamoorthi 1978

1981), but variable for *Cryptosporidium* oocysts ranges 80–97% (Chauret *et al.* 1995; Mayer and Palmer 1996).

Fixed-film reactors

Percolating filters are extremely effective in removing coliform bacteria with normal removal efficiencies of $> 95\%$ (Pike 1975). Removal is achieved by similar mechanisms as in the activated sludge process, except that filters are plug-flow systems with a fixed and not a mixed microbial biomass, so that opportunities for contact between pathogens and adsorption sites in the biomass are probably reduced.

Removal of bacteria is directly related to the bacterial count of the sewage and at low-rate loadings to the surface area of the filter medium (Tomlinson *et al.* 1962). In a comparison between high- and low-rate filtration, Bruce *et al.* (1970) found that the removal of coliforms in high-rate filters with hydraulic loadings of 6 or 12 $m^3m^{-3}d^{-1}$ was only 6–74% compared with 90–99.7% in a control low-rate filter operated in parallel with a hydraulic loading of only 0.4 $m^3m^{-3}d^{-1}$. They also observed that removal of pathogenic bacteria was directly related to BOD removal, thus linking removal of pathogenic bacteria with adsorption. A similar situation was observed using faecal streptococci and *E. coli* as indicator organisms (Bruce and Merkens 1973). An earlier study had shown that removal efficiency fell off during winter (Allen *et al.* 1944), which suggests that maximum removal of pathogens occurs when the film is most actively growing and under maximum grazing pressure: this is when maximum availability of adsorption sites will occur.

This is further confirmed by the low efficiency of percolating filters in removing viruses and bacteriophages (Berg 1966; Malherbe and Strickland-Cholmley 1967; Sherman *et al.* 1975; Carrington 1980a), which are known to be removed by adsorption, which in the activated sludge process is more effective due to the mixing action in the aeration tank. In the percolating filter, there is far less chance of viruses being attached to available sites, especially under conditions of ponding and short-circuiting, when only a small portion of the available surface area of the medium is being used, or under high-rate loading when the retention time (or film contact time) is very short (Omura *et al.* 1989).

Ciliate protozoans can ingest pathogenic bacteria as can rotifers, nematodes, and annelid worms. However, in percolating filters there is a much larger range of macro-invertebrate grazers feeding directly on the film and thus indirectly feeding on the pathogens. Once pathogens have been

adsorbed onto the film they are essentially *removed* and their subsequent ingestion by a grazing organism may not be significant. The major limitation of percolating filtration in the removal of pathogens is their physical adsorption from suspension.

Percolating filtration is not very effective in the removal of parasite ova and cysts, although the nature of the film does allow some retention of ova and removals of up to 30% have been observed (Silverman and Griffiths 1955). Geldreich (1972), in a review on water-borne pathogens, quotes higher removal rates, 18–70% for tapeworm ova and 88–99% for cysts of *E. histolytica*. This is confirmed by Panicker and Krishamoorhi (1978) who found the removal rates for *E. histolytica* similar to *Giardia* cysts. Overall, the removal efficiency of percolating filters is lower than that of activated sludge (Casson *et al.* 1990).

Rotating biological contactors are, in comparison, also extremely efficient in the removal of pathogenic bacteria, with median removal of *E. coli* normally $> 99.5\%$. Although the numbers of bacteria in raw and settled sewage varies diurnally, the removal efficiency remains constant and unaffected by factors such as temperature, bacteria concentration in the influent, or the organic loading of disc surfaces over the range 1.59–9.47 g BOD $m^{-2}d^{-1}$ (Pike and Carrington 1979; Sagy and Kott 1990).

Constructed wetlands

There is an overall reduction in viruses, bacteria, helminth eggs, and protozoan cysts as screened or settled wastewater passes through wetland systems (Herskowitz 1986; Gersberg *et al.* 1989; Rivera *et al.* 1995). However, certain pathogens such as faecal coliforms, streptococci, and *Salmonella* are associated with warm-blooded animals other than man, especially birds, resulting in a negative treatment effect. Natural wetlands often have significant background concentrations of faecal coliforms in particular. For example, the wetland at Bryon Bay, Australia, has background faecal coliforms of up to 645 per 100 ml (Table 9.50) (Kadlec *et al.* 2000). While little research has been done on pathogen removal in the different types of wetlands in use, they provide excellent physical, chemical, and biological conditions for their removal. These include exposure to UV, sedimentation, mechanical filtration, chemical oxidation, adsorption to organic matter and the media, exposure to plant secreted biocides, predation by micro- and macro-fauna, attack by lytic bacteria, natural antibiotics, and normal die-off due to prolonged retention (Gersberg *et al.* 1989). Existing data has been reviewed by Kadlec and Knight (1996). The variability in design and construction of

Table 9.50. Reduction rate constants for faecal coliforms in surface flow wetlands (Kadlec *et al.* 2000).

Site	System	HLR (cm d^{-1})	FC in (cfu/100 ml)	FC out (cfu/100 ml)	k_1 ($C_\circ = 0$) (cm d^{-1})	k (cm d^{-1})	$C_\circ$ (cfu/100 ml)
Arcata, California, USA	Pilot 1	13.33	3183	416	27.1		
	Pilot 2	7.89	12,500	316	29.0		
	Transect	27.72	15,850	1608	39.0	45	118
	Woolgrass	4.72	4747	135	16.8	38	28
	Cattail	4.72	4747	458	11.0	34	151
Boggy Gut, South Carolina, USA		3.01	2	236			
Brookhaven, New York, USA		2.02	4175	378	4.8		
Byron Bay, Australia		5.53	28,918	667	20.8	21.4	646
Carolina Bays, South Carolina, USA		0.15	66,000	56	1.1		
Central Slough, South Carolina, USA		0.51	857	50	1.4		
Cobalt, Ontario, Canada		1.7	159,300	1087	8.5		
Denham Springs, Louisiana, USA	1	12.18	39,620	4115	27.6	66	2325
	2	12.18	42,030	3810	29.2	59	2080
	3	12.18	39,866	2854	32.1	66	2034
Harriman, Pennsylvania, USA	1	3.75	1,953,329	14,180	18.5		
	2	3.75	29,278	538	15.0		
Iron Bridge, Florida, USA	1990	2.97	1	33			
	1991	2.85	1	91			

Table 9.50. (*Continued*)

Site	System	HLR (cm d^{-1})	FC in (cfu/100 ml)	FC out (cfu/100 ml)	k_1 ($C_\circ = 0$) (cm d^{-1})	k (cm d^{-1})	$C_\circ$ (cfu/100 ml)
Lakeland, Florida, USA	1	4.37	25,536	55	26.9	124	26
Listowel, Ontario, Canada	1	2.80	1773	72	9.0	27	2
	2	2.92	1773	573	3.3	32	86
	3	2.10	1773	56	7.3	47	4
	4	1.95	228,292	141	14.0	17	4
	5	2.60	228,292	2251	11.0	12	98
Neshaminy, Pennsylvania, USA		5.28	1,290,600	5600	28.7		
Pembroke, Kentucky, USA		4.38	165,959	266	28.2	288	60
Richmond, NSW, Australia	Open water	6.40	1,698,244	50,119	22.5		
	Myriophyllum	7.35	1,698,244	56,234	25.0		
Waldo, Florida, USA	Pilot	17.64	7,700,000	270,000	59.1		
West Jackson Co., Mississippi, USA		3.18	239	674			
Whangarei, New Zealand	Trial	6.00	400,000	2300	31.0		
	Full-scale	7.50	1085	481	6.1		
Average					19.79 (72 m yr^{-1})		

Abbreviations: cfu, colony-forming units; FC, faecal coliforms; HLR, hydraulic loading rate.

constructed wetlands (Sec. 6.2.3) makes interpretation of existing studies difficult. However, while removal of faecal coliforms follows first order reaction kinetics in gravel beds, longer retention times are required in those planted with emergent vegetation (i.e. reeds, rushes) than unvegetated beds to achieve the same degree of removal (Bavor *et al.* 1989). Similar results are reported by Qunonez-Diaz *et al.* (2001). Kadlec and Knight (1996) have reported similar removal rate constants (k) for faecal coliforms in the USA as found elsewhere ranging from 50–300 m yr^{-1} with an average k value around 70 m yr^{-1}. Mandi *et al.* (1996) compared three horizontal flow-reed beds of differing lengths (30, 40 and 50 m) planted with *Phragmites australis*. They found excellent removal of helminth eggs ranging from 88% in the 30 m bed to 93% in the 50 m bed at even short retention times of 1 to 4 hours.

Waste stabilization ponds

In general, all stabilization ponds and lagoons are effective in removing bacteria, viruses, and other parasites. A variety of removal mechanisms has been reported, including settlement, predation, inactivation because of solar radiation which is also linked with temperature, increase in pH due to daytime assimilation of carbon dioxide by algae which can reach in excess of pH 9, and, finally, anti-bacterial toxins produced by algae (Pike 1975).

It is most appropriate to consider ponds as CSTRs, so that the survival of pathogens (x_t/x_0) can be calculated as:

$$(x_t/x_0) = 1/[1 - (\mu/D)]$$

Ponds are normally in series, and if they have similar dilution rates the survival of pathogens can be calculated as:

$$(x_n/x_0) = 1/[1 - (\mu/D)^n]$$

where n is the number of ponds. A problem arises if the number of ponds in series is large (i.e. > 5) because then the system behaves more like a plug-flow reactor. In this case the relationship becomes:

$$(x_n/x_0) = \exp -(\mu/D_n)$$

where D_n is the dilution rate of the complete system.

In temperate climates, only maturation ponds are commonly used in the treatment of domestic sewage. They are very effective in the removal of bacteria and viruses (Windle Taylor 1966; Adams *et al.* 1972; Toms *et al.* 1975). The removal efficiencies of *Streptococci* sp. and *Salmonella* sp. are

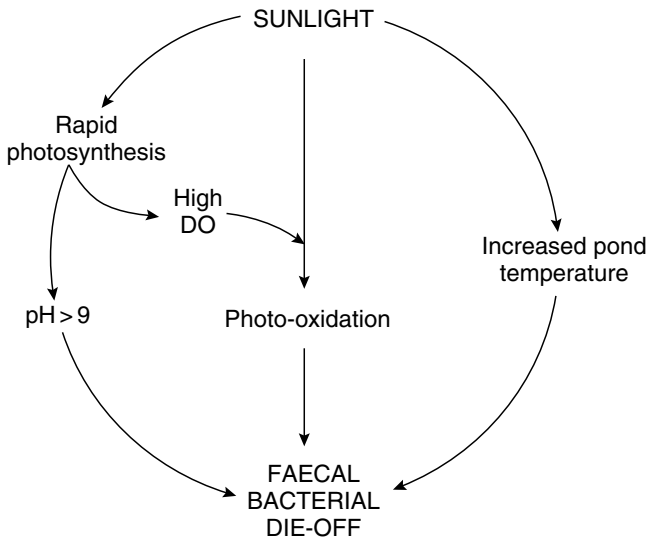


Fig. 9.18. Physico-chemical mechanisms for faecal coliform die-off in stabilization ponds (Mara and Pearson 1998).

equal to or better than the removal rate for *E. coli*, although *C. perfringens*, both as active cells and spores, are less effectively removed (Toms *et al.* 1975). Removal efficiency in maturation ponds is related to retention time, with removal rates falling rapidly as the retention time is reduced. For example, high removals of *E. coli* have been reported in Britain at retention times of 8.9 days (99.9%) and 17.0 day (99.5%) (Windle Taylor 1966; Adams *et al.* 1972), whereas at 3.5, 3.0, and 2.3 days retention, the removal rates were 94.0, 92.4, and 81.0% respectively (Fish 1966; Windle Taylor 1966). Retention time is critical and any short-circuiting within ponds will reduce removal efficiency.

Solar radiation is a major removal mechanism in maturation ponds. The sun promotes the die-off of faecal bacteria in waste stabilization ponds by increasing the pond temperature and providing the energy for algal photosynthesis that both increases the pH above 9 and promotes photo-oxidative damage (Mara and Pearson 1998) (Fig. 9.18). Faecal bacteria are damaged by light with wavelengths between 425–700 nm being absorbed by humic material in the wastewater that enters an excited state for a long enough period to damage cells. The rate of die off is dependent on the presence of oxygen, being significantly enhanced at high pH (Curtis *et al.* 1992). In South Africa the mean removal efficiency of *E. coli* during the summer was 99.6% compared with 96.9% in winter (Drews 1966). This

is more clearly illustrated in Britain where the difference between solar radiation in the summer and winter is more pronounced. At a retention time of 3.5 days, the removal rate of *E. coli* was > 90% in the summer but fell to 40% in the winter (60% for faecal streptococci) because of the seasonal difference in light intensity (Toms *et al.* 1975). In the British Isles, the algal density is never sufficient to significantly shift the pH so that the pathogens are killed. In fact, the mortality of bacteria and viruses can be reduced by algae because of the reduction of light intensity by shading. The depth is also an important factor with removal rates being reduced with depth. Under ideal conditions, pond algae can consume carbon dioxide faster than it can be replaced by bacterial respiration. This results in carbonate and bicarbonate ions dissociating to release carbon dioxide:

$$2HCO_3^- \rightarrow CO_3^{2-} + H_2O + CO_2$$

$$CO_3^{2-} + H_2O \rightarrow 2OH^- + CO_2$$

The carbon dioxide is fixed by the algae and the hydroxyl ions accumulate raising the pH as high as 10. At pH > 9 faecal bacteria die rapidly, except for *Vibrio cholerae* (Pearson *et al.* 1987).

Pond design is often based on faecal coliform or helminth egg reduction, especially where effluents are used for irrigation (Secs. 9.2.4 and 10.2.2). In tropical and sub-tropical countries, the removal of pathogens is as important as BOD removal is in temperate zones, and in many countries more so. Both facultative and maturation ponds are very efficient in the removal of pathogens (Arceivala *et al.* 1970; Gloyna 1971; Mara and Pearson 1998), with removal rates of coliform and streptococci bacteria > 99%, and viruses inactivated by light and removed by absorption onto settleable solids and settlement so that > 90% removals are achieved. The greater efficiency of facultative ponds compared with other systems is because of the much longer retention times (Coetzee and Fourie 1965), with ponds in series achieving greater removals than single ponds (Marais 1970). Major removal mechanisms are the high pH values created by photosynthesis and the higher zooplankton predation rate. However, the function of maturation ponds is primarily to improve the bacterial quality of the effluent from facultative ponds. The size and number of ponds is governed by the required removal of both faecal coliforms and helminth eggs. Maturation ponds only remove small quantities of BOD, but due to a diverse population of algae nutrient removal can be significant. Their shallow design (1–1.5 m depth) and long retention times ensures maximum pathogen destruction. Helminth eggs and protozoan cysts are primarily removed by

settlement. The settling velocities of eggs are quite high (e.g. *Ascaris lumbricoides* 3.4×10^{-4} m s^{-1}), thus pond systems appear extremely effective in the removal of parasites because long retention times allow maximum settlement. The average number of helminth eggs in wastewater in developing countries can be as high as 500 eggs per litre in poor, recently sewered, areas. Although this high incidence of eggs gradually declines as the opportunities for re-infection gradually falls due to the introduction of sewerage. The removal of eggs from pond effluents is extremely important if they are to be used for irrigation or fish pond fertilization. Cysts of the protozoans *Entamoeba histolytica* and *Giardia lamblia* are almost completely removed by well-designed pond systems, and the helminth parasites, such as *Schistosoma*, *Ascaris*, *Enterobius*, *Ancylostoma*, and *Trichuris* are also effectively removed (Gloyna 1971). Maximum removal occurs in the first pond, and as the nematode eggs in particular are highly resistant, extreme care must be taken with the disposal of the raw sludge from the pond if contamination is to be prevented. Removal efficiencies for three ponds in India have been given by Veerannan (1977). For helminth ova these were *Ascaris lumbricoides* (89–93%), hookworm (> 92%), *Trichuris* (> 68%), *Enterobius* (> 95%), *Hymenolepis* (> 83%), and for protozoan cysts removals were *Entamoeba coli* (39–77%), *Entamoeba histolytica* (> 62%), and *Giardia lamblia* (> 98%). The efficiency of a pond system in removing bacteria and viruses, comprising of an anaerobic pond (P1) with a mean retention time of 1 day, a facultative pond (P2) with a retention time of 5 days followed by three maturation ponds (P3-5) each with a retention time of 5 days, is shown in Table 9.51.

Sludge treatment

Unit processes do not generally inactivate pathogens but concentrate them in the waste sludges produced (Table 9.38). So in order to act as an effective barrier to the transfer and dissemination of pathogens into the environment, sludge treatment and disposal is required to further reduce pathogen numbers. All the pathogens found in wastewaters are also found in the sludges produced during treatment with particularly high bacterial concentrations of *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Leptospira*, and enteropathogenic *E. coli*. Viruses are also abundant. Albert *et al.* (1990) recorded 97–1230 pfu of enteroviruses per litre of primary sludge from Nancy, France, with hepatitis A virus particularly abundant. Barbier *et al.* (1990) recorded high numbers of parasitic ova in French sludges: 2200–2400 ova kg^{-1} of anaerobically digested sludge for Taniidae, 410–1200 ova kg^{-1}

Table 9.51. Geometric mean of bacterial and viral numbers[a] and percentage removals in raw wastewater (RW) and the effluents of five waste stabilization ponds in series (P1–P5[b]) at a mean mid-depth pond temperature of 26°C (Oragui *et al.* 1987).

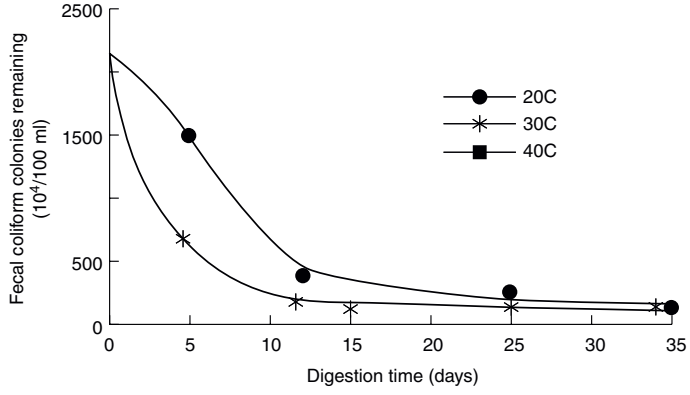
Organism	RW	P1	P2	P3	P4	P5	Percentage removal
Faecal coliforms	2×10^7	4×10^6	8×10^5	2×10^5	3×10^4	7×10^3	99.97
Faecal streptococci	3×10^6	9×10^5	1×10^5	1×10^4	2×10^3	300	99.99
Clostridium perfringens	5×10^4	2×10^4	6×10^3	2×10^3	1×10^3	300	99.40
Campylobacters	70	20	0.2	0	0	0	100.00
Salmonellae	20	8	0.1	0.02	0.01	0	100.00
Enteroviruses	1×10^4	6×10^3	1×10^3	400	50	9	99.91
Rotaviruses	800	200	70	30	10	3	99.63
BOD (mgl^{-1})	215	36	41	21	21	18	92

[a]Bacterial numbers per 100 ml, viral numbers per 10 litres.
[b]P1 was an anaerobic pond with a mean hydraulic retention time of 1 day: P2 and P3–P5 were secondary facultative and maturation ponds respectively, each with a retention time of 5 days.

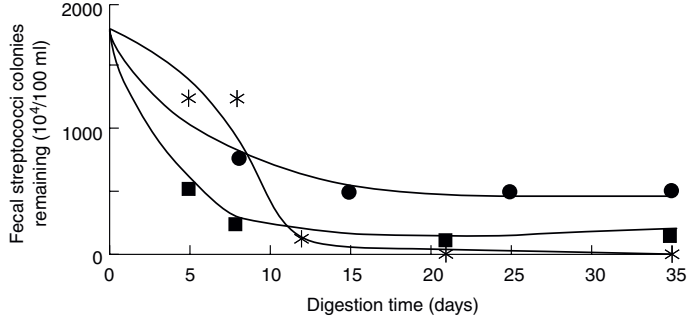
for *Ascaris* spp., 350–410 ova kg^{-1} for *Toxocara* spp., and 4910–7250 ova kg^{-1} for *Trichuridae*. Sludge treatment and disposal is discussed in detail in Sec. 8.1. The main processes that reduce pathogen concentrations are digestion (anaerobic and aerobic), pasteurization, heat treatment, composting, and lime stabilization.

Anaerobic digestion certainly reduces the numbers of pathogens considerably but not always completely (Bates 1972; Carrington 1978). Although many bacteria, fungi, and viruses are rapidly killed by air-drying, they can survive anaerobic digestion at 20°C or 30°C for long periods. For example, *S. typhi* can survive digestion for 12 days at 20°C or 10 days at 30°C (Smith *et al.* 1975). In practice 1 to 3 log reductions in bacterial pathogens can be expected, with two-phase anaerobic digesters able to achieve greater reductions in total coliforms, faecal streptococci, and faecal coliforms than conventional single-phase digesters. Entero-virus removal is the same (Lee *et al.* 1989). While temperature plays an important role in virus inactivation, neither mesophilic (30–35°C) or thermophilic (50°C) digestion completely eliminates viruses. Unionized ammonia also inactivates viruses by targeting nucleic acids (Ward and Ashley 1977), but when embedded within sludge flocs they are able to survive for long periods. Parasites can also survive digestion for significant periods, for example, Ascaris ova 90 days at 30°C, and hookworm ova 64 days at 20°C or 41 days at 30°C. Both the latter ova can also withstand air-drying. Some researchers have indicated that complete destruction of pathogens is only possible by heating the sludge to 55°C for 2 hours or treating with lime (Smith *et al.* 1975). Oropeza *et al.* (2001) compared the removal of faecal coliforms and helminth eggs from a municipal sludge by anaerobic mesophilic (35°C) and thermophilic (55°C) digestion. They found a much higher removal efficiency of pathogens at 55°C, with this sludge complying with the Class A biosolids classification of the US EPA, allowing it to be used in agriculture without restriction. Aerobic digestion is increasingly used at smaller treatment plants and can be highly effective in eliminating pathogens. Reduction efficiency depends on both retention time and temperature (Fig. 9.19), with temperature the single most important factor in the destruction of poliovirus 1, coxsachie B3, echovirus 1, and rotavirus SA-11, during aerobic digestion (Scheuerman *et al.* 1991). Removals are comparatively poor at mesophilic temperatures but at thermophilic temperatures aerobic digestion can virtually eliminate all bacterial, viral, and parasitic pathogens (Fig. 9.20) (Kabrick and Jewell 1982; Kuchenrither and Benefield 1983).

Heat treatment of sludge is widely practiced to inactivate pathogens. Pasteurization, raising the temperature of sludge to 70°C for a minimum of



(a)

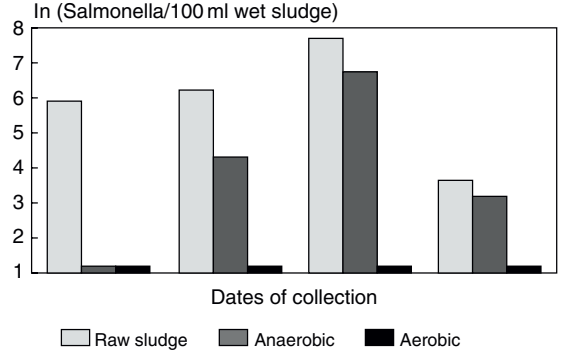


(b)

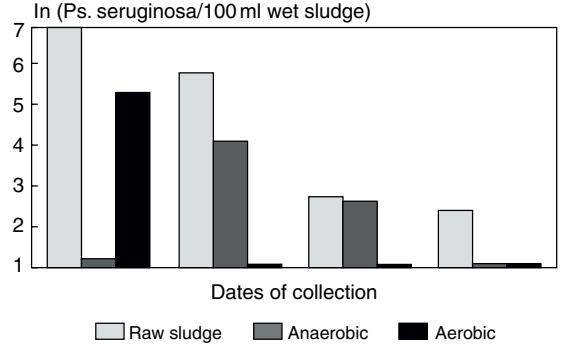
Fig. 9.19. Effect of temperature on inactivation of indicator bacteria during aerobic digestion of sludge: (a) Faceal coliforms, (b) Faecal streptococci (Kuchenrither and Benefield 1983).

30 minutes, destroys all bacterial and viral pathogens as well as helminth ova (Saier *et al.* 1985; Pike *et al.* 1988). Increasingly sludges are dewatered to high dry solids concentration under pressure at temperatures up to 260°C for 30 minutes. Under these conditions all pathogens are destroyed.

Composting uses elevated temperatures up to 55°C for long periods (3 days in static piles and 15 days in windrow systems) to destroy pathogens. However, it is difficult to expose all the compost biomass to such a temperature for sufficient time resulting in incomplete pathogen destruction. Regrowth of some pathogens (e.g. *Salmonella*) has been reported in composted sludge when the moisture content is $> 20\%$, the temperature 20–40°C and the C:N ratio is in excess of 15:1 (Russ and Yanko 1981), although the indigenous microflora limits pathogen regrowth to some extent



(a)



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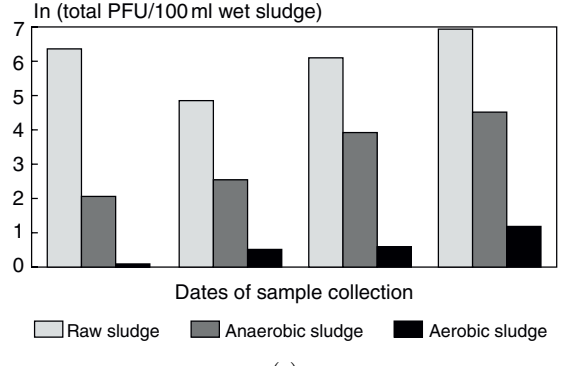


(c)

Fig. 9.20. Inactivation of pathogenic micro-organisms by thermophilic aerobic sludge digestion: (a) *Salmonella*, (b) *Pseudomonas aeruginosa*, (c) Viruses (Kabrick and Jewell 1982).

(Hussong *et al.* 1985). The minimum temperature for inactivation of helminth eggs and protozoan cysts is 55°C. Composting can also create pathogen problems as *Aspergillus fumigatus* grows readily in the outer layers of compost where the temperature is between 30–45°C. This opportunistic pathogenic fungus can cause serious lung damage and allergies to exposed workers (Sec. 10.3.3) (Clark *et al.* 1984c).

Lime is widely used in the water industry as either $Ca(OH)_2$ or CaO. In wastewater treatment it is used primarily as a flocculating agent or odour control. A slurry of lime is added to liquid sludge to raise the pH $\geq$ 12 for $\geq$ 2 hours. This process is known as lime stablilization and can achieve a 3 to 6 log reduction in total coliforms, faecal coliforms, and faecal streptococci (Venosa 1986). Sattar *et al.* (1976) report complete elimination of viruses after 12 hour contact time, although parasite elimination is not complete (Sec. 8.1.1).

The disposal of sludges both in Europe and the USA are controlled by legislation that includes standards on pathogen content. This is reviewed in Sec. 8.2.2.

9.5.3. *Sterilization and disinfection methods*

Sterilization and disinfection of final effluents to remove any disease-causing organisms remaining in effluents is not widely practised in Europe, but is common in the USA with up to 50% of the total treatment works employing chlorination (Thoman and Jenkins 1958). The increasing need to re-use water for supply after wastewater treatment will mean that the introduction of such methods to prevent the spread of diseases via the water supply is inevitable (Dean and Lund 1981). The two processes are distinct from each other. Sterilization is the destruction of all the organisms in the final effluent regardless of whether they are pathogenic or not, whereas disinfection is the selective destruction of disease-causing organisms. There are three target groups of organisms, viruses, bacteria, and amoebic cysts, and each is more susceptible to a particular disinfection process than the other. The main methods of sterilization and disinfection are either chemical or physical in action (Table 9.52).

Chemical

This group includes the most widely used methods of removing pathogens from wastewater effluents. The oxidizing chemicals are the commonest and safest chemicals to use, with chlorine, ozone, and hydrogen peroxide all

Table 9.52. Comparison of technical-economic characteristics of advanced disinfection technologies (Lazarova *et al.* 1999).

Characteristics/ Criteria	Chlorination/ Dechlorination	UV	Ozone	MF	UF
Safety	+	+++	++	+++	+++
Bacteria removal	++	++	++	+++	+++
Virus removal	+	+	++	+	+++
Protozoa removal[1]	–	–	++	+++	+++
Bacterial regrowth	+	+	+	–	–
Residual toxicity	+++	–	+	–	–
By-products	+++	–	+	–	–
Operating costs	+	+	++	+++	+++
Investment costs	++	++	+++	+++	+++

"–" none; "+" low; "++" middle; "+++" high

[1] *in vitro* analysis of *Cryptosporidium*.

widely used. There are a variety of other chemical methods used to disinfect wastewaters and these are fully reviewed elsewhere (Venosa 1983; Metcalf and Eddy 1984). The factors affecting the efficiency of chemical disinfectants are contact time, concentration and type of chemical agent, temperature, types and numbers of organisms to be removed, and the chemical nature of the wastewater. First-order kinetics are used to describe the effect of contact time on pathogen inactivation:

$$N_t/N_0 = e^{-kt}$$

where N_0 is the number of micro-organisms at time 0, N_t, the number of micro-organisms at time t, and k the decay constant (time^{-1}). However, in practice the inactivation rate slows with time due to numerous factors including the survival of resistant organisms, microbial aggregation, protection from adsorption onto particles, and ingestion by nematodes (Rubin *et al.* 1983; Hoff and Akin 1986). For example, Loge *et al.* (2002) used a 16S rRNA oligonucleotide probe, specific to the family *Enterobacteriaceae*, to determine the proportion of particles in activated sludge effluents that harboured coliforms. The fraction of particles with associated coliforms declined with increasing mean cell residence time (MCRT) (Fig. 9.21). They concluded that even small increases in MCRT would produce significant improvements in subsequent disinfection efficiency of such effluents.

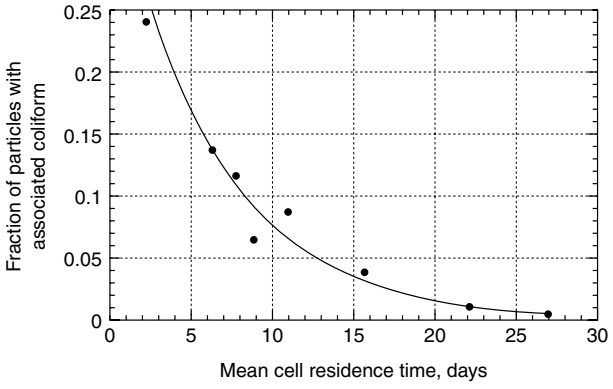


Fig. 9.21. Fraction of particles with associated coliform bacteria in the secondary effluent of eight activated sludge wastewater treatment plants operated at different mean cell residence times (Loge *et al.* 2002).

Efficiency of disinfectants is expressed as Ct where C is the concentration of the disinfectant (mg l^{-1}) and t the contact time required to kill a certain percentage of a pathogen population (min) at a specific temperature and pH (Table 9.53). The rate of inactivation of micro-organisms will increase (i.e. Ct decreases) as the temperature rises, although the effect of pH on Ct depends on the disinfectant used. The relationship between C and t is a constant (k) under specific conditions for any given pathogen and disinfectant:

$$k = C^n t$$

where n is the coefficient of dilution, which is also a constant. The slope of the straight line obtained by plotting t against C using log-log paper is n, and this determines whether micro-organism inactivation is more influenced by contact time ($n < 1$) or disinfectant concentration ($n > 1$) (Fig. 9.22) (Clark *et al.* 1989). In general, resistance to disinfectants can be summarised as: vegetative bacteria < enteric viruses < spore-forming bacteria < protozoan cysts (Bitton 1999).

The most widely used chemical disinfectant is chlorine. However, unlike drinking water, the disinfection of treated effluents or crude and settled sewage, if discharged to coastal waters, requires a high chlorine dose. Chlorine is normally used in its elemental form or as hypochlorite, and depending on the pH of the water and the presence of ammonia, the chlorine may take the form of HOCl, OCl^-, Cl_2 or chloramines when in solution. Chlorine gas (Cl_2) is hydrolyzed in wastewater effluents to form hypochlorous acid

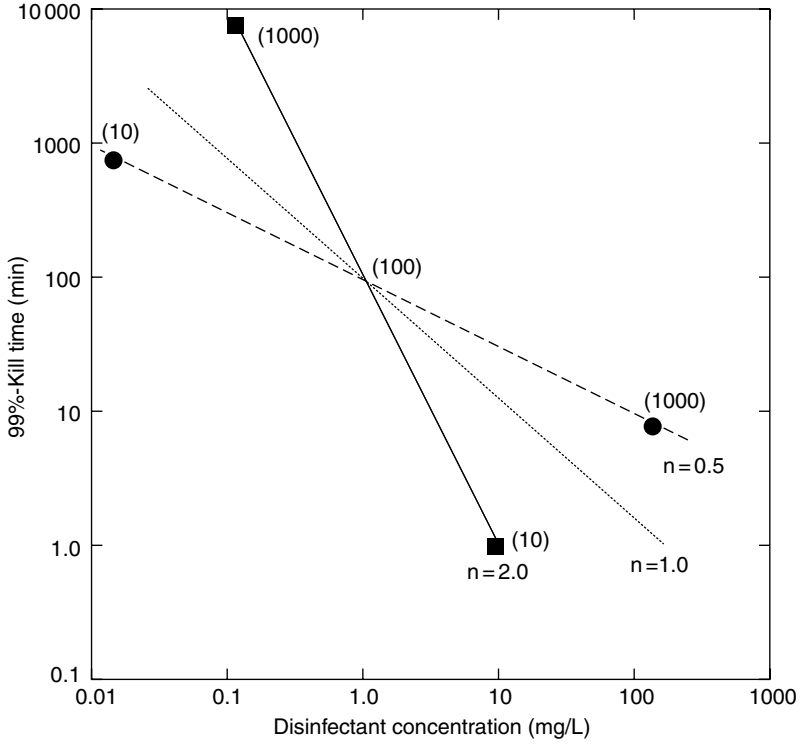


Fig. 9.22. Effect of n value on Ct at various disinfection concentrations. The Ct values are given in parentheses (Clark *et al.* 1989).

(HOCl) which dissociates to the hypochlorite (OCl^-) ion.

$$Cl_2 + H_2O \longleftrightarrow \underset{\substack{\text{Hypochlorous}\\ \text{acid}}}{HOCl} + H^+ + Cl^-$$

$$\underset{\substack{\text{Hypochlorous}\\ \text{acid}}}{HOCl} \longleftrightarrow H^+ + \underset{\substack{\text{Hypochlorite}\\ \text{ion}}}{OCl^-}$$

The degree of dissociation is pH dependent, being suppressed as the pH falls. Thus if the pH is > 9.0 then 100% of the Cl_2 is in the hypochlorite form falling to 50% at pH 7.5. Where the pH is < 5.0 then 100% of the Cl_2 is in the hypochlorous acid form (Fig. 9.23). Hypochlorous acid is about 80 times more effective than the hypochlorite ion, so disinfection is more effective under acidic conditions (i.e. Ct increases with pH for chlorine).

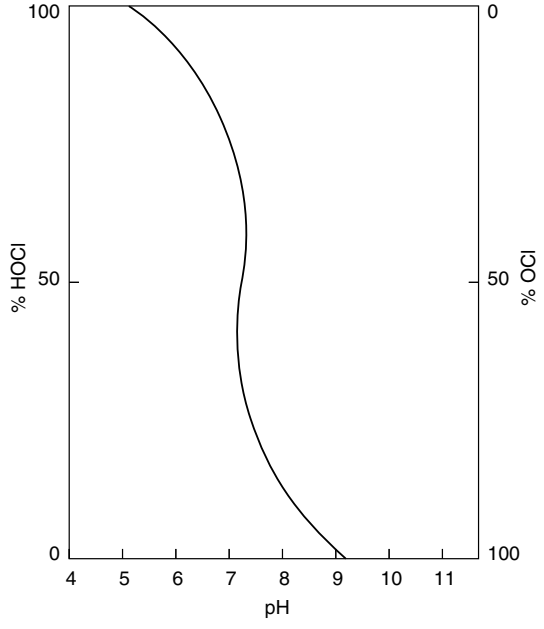


Fig. 9.23. Distribution of HOCl and OCl^- in water as a function of pH (Bitton 1980).

At a neutral pH, free chlorine is converted almost instantaneously in the presence of ammonia to monochloramine so that free chlorine is not found in sewage effluents, although some free chlorine residuals may exist in highly nitrified effluents (White 1978a). The reaction of chlorine with a compound containing a nitrogen atom will continue forming a range of N-chloro compounds:

$$NH_3 \longleftrightarrow \underset{\text{(monochloramine)}}{NH_2Cl} + H_2O$$

$$NH_2Cl + HOCl \longleftrightarrow \underset{\text{(dichloramine)}}{NHCl_2} + H_2O$$

$$NH_2Cl + HOCl \longleftrightarrow \underset{\text{(nitrogen trichloride)}}{NCl_3} + H_2O$$

At higher pH values chloramines can be slowly oxidized to nitrogen gas:

$$NH_2Cl + NHCl_2 \longleftrightarrow N_2 + 3H^+ + 3Cl^-$$

With time, inorganic chloramines formed in sewage will be converted to organic chloramines that are less reactive and thus less efficient in the

inactivation of bacteria or viruses. The chemistry of chlorination has been explained in most standard texts and will not be discussed here. A particularly good summary of the process is given by Irving and Solbe (1980). Normal dosage rates for treated effluents range from 2–15 mg Cl_2 l^{-1}, depending on the degree of treatment with a minimum contact time of 20–30 minutes. Bradley (1973) reported chlorine doses, using a contact time of 15 minutes, of 6–24 mg l^{-1} for raw sewage, 3–18 mg l^{-1} for settled sewage, and 3–9 mg l^{-1} for an activated sludge effluent.

There is a noticeable difference in the resistance of bacteria, viruses, and protozoan cysts to chlorine (Kool and Kranen 1977). Chlorine is a strong oxidizing agent and attacks the chemical constituents of bacterial cells and viruses (Kool 1979). The exact method of inactivation in bacteria is still not fully understood, although chlorine is suspected of interfering with the sulphydryl groups of the enzyme triose-phosphate-dehydrogenase. The main target sites for inhibition or destruction include the peptidoglycan layer, cytoplasmic membrane, outer membrane, structural proteins, thiol groups of enzymes, nucleic acids, viral envelopes, capsids, or nucleic acids, and bacterial spore coats (Russel *et al.* 1997). Although chlorination may not eliminate all potentially pathogenic bacteria, for example, lactose non-fermenters can survive normal chlorination, certain bacteria are extremely sensitive to the process. *Salmonella* spp., for example, are more sensitive than *E. coli*. Bacterial cells require longer contact times with chlorine as they age, and free residual chlorine is required to eliminate many important viruses. Poliomyelitus virus type 1, hepatitis A, and coxsackievirus type A2 both require much higher concentrations of chlorine, or longer contact times for their destruction than *E. coli* (Botha 1984) (Table 9.54). Viruses also have various sensitivities to disinfectants (Table 9.55). Protozoans are more resistant to chlorine than viruses with normal chlorine doses and contact times ineffective against *Cryptosporidium* oocysts and *Entamoeba histolytica* cysts (Table 9.53) (Korich *et al.* 1990; Gyürek *et al.* 1997). Ozone is normally used for oocyst inactivation (Rennecker *et al.* 1999) although consideration is being given to using ozone together with free or combined chlorine (Driedger *et al.* 2000; Rennecker *et al.* 2001).

In order to obtain sufficient free residual chlorine (breakpoint chlorination) higher concentrations of chlorine are required, resulting in free residual chlorine remaining in the final effluent. This residual chlorine is extremely toxic to organisms, especially fish, and can react with organic compounds in wastewater to form toxic compounds, such as chlorinated biphenyls, some of which are carcinogenic (Jolley 1975; Gaffney 1977; Irving and Solbe 1980). Fawell *et al.* (1987) have studied the reaction of Cl^- with various organic

chemicals produced during water treatment chlorination (Table 9.56). Many of these compounds have been shown to be potentially carcinogenic using a bioassay technique with *Salmonella typhimurium* strain TA100. Some regulatory agencies in the USA have specified a maximum residual chlorine concentration of 0.1–0.5 mg l^{-1} in undiluted effluents to prevent potential toxicity in receiving waters. Once discharged, the residual chlorine is too reactive to persist for long in most environments (both fresh and saline waters). This has allowed the permissible level of chlorine in natural waters to be set nearer to the lethal limits for most organisms than would otherwise be advisable. The European Inland Fisheries Advisory Committee (1973) have proposed a limit of 0.004 mg HOCl l^{-1} for European rivers, but even at this level, some freshwater and marine organisms will be susceptible. If chlorine exceeds these limits dechlorination is required to detoxify the discharge. This is normally achieved by adding sulphur dioxide or using activated carbon. The level of residual chlorine needed to destroy viruses in final effluents will mean that such effluents will always have to be dechlorinated before

Table 9.53. Microbial inactivation by chlorine: *Ct* values (temperature 5°C, pH 6.0) (Hoff and Akin 1986).

Micro-organism	Chlorine Concentration (mgl^{-1})	Inactivation Time (min)	*Ct*
Escherichia coli	0.1	0.4	0.04
Poliovirus I	1.0	1.7	1.7
Entamoeba histolytica cysts	5.0	18	90
Giardia lamblia cysts	1.0	50	50
	2.0	40	80
	2.5	100	250
Giardia muris cysts	2.5	100	250

Table 9.54. Comparison of the concentration (mg l^{-1}) of the most frequently used disinfectants required to inactivate the major microbial groups within 10 minutes (White 1978a).

Disinfectant	Enteric bacteria	Viruses	Bacterial spores
HOCl	0.02	0.40	10.0
OCl^-	2.0	>20.0	>1000
NH_2Cl	5.0	100.0	400
Free Cl_2 (pH 7.5)	0.04	0.8	20.2
O_3	0.001	0.10	0.2

Table 9.55. Time required for 99.99% inactivation of enteric viruses by 0.5 mg l^{-1} free chlorine residuals at pH 7.8 at 20°C in natural river water (White 1978a).

Species	Times for 99.99% inactivation (min)	Species	Time for 99.99% inactivation (min)
Polio type II	36.5	Coxsackie virus type B1	8.5
Coxsackie virus type B5	34.5	Adenovirus type 12	8.1
E. coli type 29	18.2	Coxsackie virus type A9	7.0
E. coli type 12	16.7	*E. coli* type 7	6.8
Polio type III	16.6	Adenovirus type 3	4.3
Coxsackie virus type B3	15.7	Reovirus type 2	4.2
Adenovirus type 7a	12.5	Reovirus type 3	4.0
Polio type I	12.0	Reovirus type I	2.7

being discharged to the natural environment. The toxicity of free chlorine and chloramines to freshwater and marine organisms is reviewed by Evins (1975) and Irving and Solbe (1980) respectively. Chlorination of crude and settled sewage discharged to coastal waters is not a practical substitute for efficient treatment nor the provision of a suitably long sea outfall. In problem areas the use of chlorination of effluents can protect bathing areas or shell-fisheries from bacteriological contamination. Turbidity in wastewaters reduces the disinfection efficiency of chlorination and other disinfection systems. Any organic particles will exert a chlorine demand in their own right, but any micro-organisms associated with faecal matter or cell debris will be protected from the action of the disinfectant (Berman *et al.* 1988). Therefore tertiary treatment to remove associated turbidity is normally required. Laboratory studies have indicated that although chlorination is effective in destroying bacteria, small numbers survive and this can result in regrowth of pathogens, especially if the water is stored before discharge (Irving 1980). However, regrowth is governed by the degree of dilution, so when the water is discharged to the sea it is assumed that regrowth will be minimal.

Ozone has similar bactericidal properties to chlorine and is generally considered more effective in destroying viruses (Venosa 1972, Katznelson and Biederman 1976). As with chlorine, enteric viruses are much more resistant to ozone treatment than coliform bacteria (Table 9.54) (Sproul *et al.* 1982). Ozone has a more powerful action than chlorine, by a factor of 10–100 depending on the form of chlorine used (Archer *et al.* 1997). For example,

Table 9.56. Organic compounds produced during water treatment chlorination (Fawell *et al.* 1987).

Benzaldehyde	Chloropicrin
Benzylcyanide	Dibromoacetonitrile
Bromoethane*	Dibromoiodomethane
Bromobutane*	Dibromomethane*
Bromochloroacetonitrile	Dichloroacetic acid
Bromochloroiodomethane	Dichloroacetonitrile*
Bromochloromethane*	Dichlorodibromomethane
Bromochloropropane isomers (4)	1,2-dichloroethane*
Bromodichloromethane*	Dichloroiodomethane
Bromoform*	Dichlorophenol
Bromopentachloroethane	Dichloropropene*
Bromopropane*	Hexachloroethane*
Bromotrichloroethylene	Hexachloropentadiene
Carbon tetrachloride	*p*-Hydroxybenzyleyanide
Chloral*	Iodoethance*
Chlorobutane	Methylbromodichloroacetate
Chlorodibromomethane*	1,1,1-Trichloroacetonitrile
Chlorodiiodomethane	Trichloroacetonitrile
Chloroform	Trichlorophenol

*Mutagenic to strain TA100 (without S9 activation).

ozone has a higher oxidation potential (−2.07 v) when compared with HOCl (−1.49 v), Cl_2 (−1.36 v), or NH_2Cl (−0.75 v) (Kinman 1972). The effectiveness of ozone is not significantly affected between normal wastewater pH values (pH 6.0–8.5), although it has a reduced mass transfer efficiency at higher water temperatures (White 1978a). Unlike chlorine, ozone does not interact with ammonia. Ozone has very low Ct values for 99% inactivation for *E. coli* (0.001–0.02) and enteric viruses (0.04–0.42) (Engelbrecht 1983; Hall and Sobsey 1993). Unlike chlorine, ozone is able to inactivate *Cryptosporidium parvum* oocysts in 6 minutes at concentrations of 1.1 mg O_3 l_{-1}, with *Giardia lamblia* cysts inactivated within a few minutes at < 0.5 mg O_3 l^{-1} (Wickramanayake *et al.* 1985; Peeters *et al.* 1989; Rennecker *et al.* 1999). Inactivation of *C. parvum* oocysts by ozonation increases with temperature rise, with the Ct required for a 2 $\log_{10}$ reduction in both infectivity and viability increased by an average factor of 4.2 for every 10°C decrease in water temperature (Hirata *et al.* 2001). Typical dosage rates are

> 50 mg O_3 l^{-1} for raw sewage and 15 mg O_3 l^{-1} for treated effluents, with 5 minutes contact for complete virus inactivation. However, although ozone is expensive, it is completely and rapidly converted to oxygen on addition to water:

$$2O_3 \rightarrow 3O_2$$

Therefore, there are no persistent toxic chemical residuals remaining in the final effluent. However, there is a potential to produce bromate (BrO_3^-) when bromide is present, as well as keto acids and aldehydes produced by ozone reacting with natural organic matter (Najm and Krasner 1995; Libert and Notarnicola 1995). Recent studies have shown that ozonation of wastewater effluents increases their overall toxicity as measured using toxicity bioassay (Blatchley *et al.* 1987). Suspended solids and particulate organic matter significantly impedes the inactivation ability of ozone, especially of viruses, and it is only suitable for use with good quality effluents (Kaneko 1989). All disinfection processes are more efficient and cost-effective if used after a physical tertiary treatment process, such as sand filtration, micro-straining, or treatment on grass plots, all of which help to reduce the organic as well as the bacterial load. Ozone has another advantage over chlorine in that it aerates the final effluent as well as removing phenols and chlorophenols from water. Ozonation of wastewater is widely practised in France, and because it is such a strong oxidizing agent it must be generated on site at the point of use. Hydrogen peroxide and the other oxidizing chemicals can also be used in the collection (for the control of odours, hydrogen sulphide production and subsequent corrosion of sewers, and in the control of sewer slime) and the treatment of wastewater (in the control of ponding, filter flies, sludge bulking, and in the removal of grease and for BOD_5 reduction) as well as for the disinfection of final effluents. There is considerable literature on the treatment processes for the removal of micro-organisms from public supplies (White 1978a; Kool 1979) and Carrington (1980a) has reviewed the effectiveness of the various disinfection procedures in removing pathogens from crude and treated wastewaters.

Physical

Sunlight is known to be a good disinfectant, and in wastewater stablization ponds it is sunlight that is largely responsible for reducing the concentration of pathogenic bacteria. A modification of this principle is the growing use of UV radiation to sterilize effluents. Ultraviolet radiation acts on the

Table 9.57. Approximate dosage for 90% inactivation of selected micro-organisms by UV (Bitton 1999).

Microorganism	Dosage (μW·s cm^{-2})
Bacteria	
Escherichia coli	3000
Salmonella typhi	2500
Pseudomonas aeruginosa	5500
Salmonella enteritis	4000
Shigella dysenteriae	2200
Shigella paradysenteriae	1700
Shigella flexneri	1700
Shigella sonnei	3000
Staphylococcus aureus	4500
Legionella pneumonphila	380
Vibrio cholerae	3400
Viruses	
Pollovirus I	5000
Coliphage	3600
Hepatitis A virus	3700
Rotavirus SA 11	8000
Protozoan cysts	
Giardia muris	82,000
Cryptosporidium parvum	80,000
Giardia lamblia	63,000
Acanthamoeba castellanii	35,000

cellular nucleic acids destroying bacteria, viruses, and any other organisms present. Although expensive in terms of energy, such systems can be highly effective. The greatest effect occurs at a wavelength of 265 nm, with low pressure mercury arc lamps (254 nm) most widely used. The approximate UV dose in μW.s cm^{-2} for 90% inactivation of micro-organisms is summarised in Table 9.57. Linden *et al.* (2001) examined the effectiveness of UV wavelengths from 210 to 295 nm in inactivating *Cryptosporidium parvum*, and found the optimum range to be 250–270 nm. The major operational problem is to obtain maximum penetration of the rays to ensure that even turbid effluents are fully sterilized. Numerous systems have been evaluated to obtain maximum exposure of wastewater to the radiation, but the most

effective system to date is the use of thin film irradiation (< 5 nm) (Venosa 1983; Acher *et al.* 1997). Working in Greece, Andreadakis *et al.* (1999) determined that the UV dose to achieve effluent faecal coliform concentrations < 2000 100 ml^{-1} in secondary treated effluents varied from 30–60 $\mu W{\cdot}s\ cm^{-2}$, depending on water quality, with high effluent suspended solids significantly increasing the dose required. Tertiary treated effluents only require a UV dose 10 $\mu W{\cdot}s\ cm^{-2}$ to meet the < 200 faecal coliform 100 ml^{-1} criterion increasing to 40–50 $\mu W{\cdot}s\ cm^{-2}$ to achieve < 10 faecal coliforms 100 ml^{-1}. Inactivation of coliforms in their experiments followed first order kinetics at low UV doses. Similar dose rates have been reported by Oppenheimer *et al.* (1997) and Bourouet *et al.* (2001). Bacteria damaged during UV treatment can repair themselves if exposed to visible light at wavelengths of 300–500 nm, a process known as photoreactivation (Mechsner 1990). The cell does this by excising the UV-damaged DNA segment and replacing it with a newly synthesized segment. This may pose a problem at wastewater treatment plants where total and faecal coliforms in particular can be photoreactivated, with faecal streptococci showing only slight photoreactivation (Baron 1997). To minimize reactivation after UV disinfection, wastewater effluents should not be exposed to visible light during storage prior to discharge (Knudson 1985). Ultraviolet irradiation is particularly useful in preventing contamination by pathogens of lakes and coastal waters that are popular for bathing. The introduction of the Bathing Water Directive (76/160/EEC) in Europe (European Communities 1976a) has made it very difficult for local authorities to meet the high bacterial standards required, therefore UV sterilization is increasingly being adopted (Whitby *et al.* 1984). This is particularly so as chlorination of sewage is not fully effective when used with primary treated effluents in the destruction of viruses and protozoal cysts. Furthermore, chlorinated discharges appear to have deleterious effects on the marine and estuarine environments near the outfalls (Irving and Solbe 1980). VonSonntag and Schuchmann (1992) have proposed that UV radiation has the potential to form potentially toxic by-products, although a number of studies have failed to find such products in UV disinfected wastewater effluents (Linden *et al.* 1998; Liberti and Notarnicola 1999). VonSonntag and Schuchmann suggest that a molecule known as a chemophore can be chemically modified by direct radiation absorption, alternatively, indirect photolysis may occur when UV radiation acts on a photosensitive molecule that is able to absorb the radiation to make it highly energetic so that it reacts with other molecules, producing chemical transformation. As amino- and phenolic-derivatives (chemophores) as well as nitrate and nitrite ions, and humus materials (photosensitizers) are

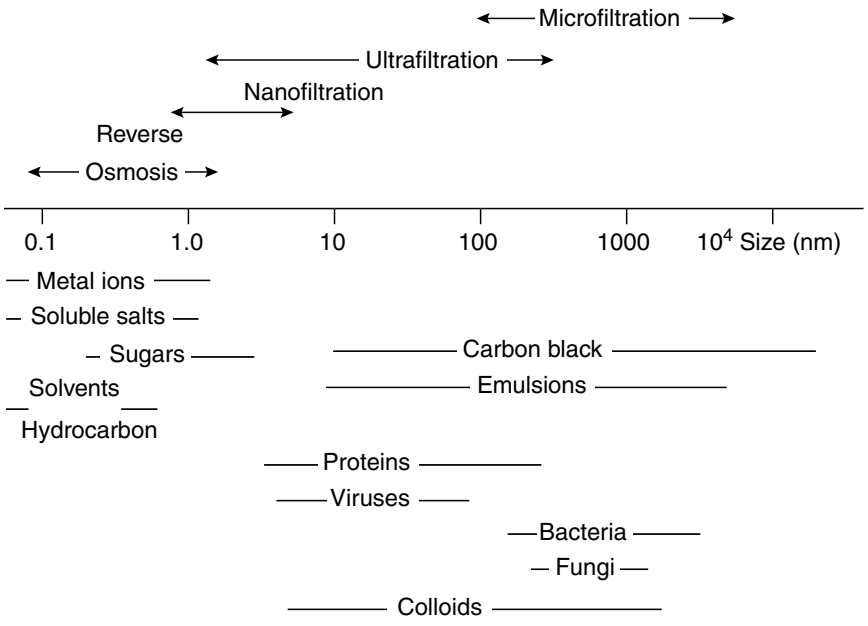


Fig. 9.24. Application size range of membrane filtration processes (Scott and Hughes 1996).

common in treated wastewaters, there is a possibility of N-nitroso-amines and nitro-phenols being formed (Liberti and Notarnicola 1999).

Gamma rays have also been used to sterilize wastewaters (Melmed 1976; Metcalf and Eddy 1984; Bitton 1999).

Filtration is the most practical treatment technology for *Giardia* cyst and *Cryptosporidium* oocyst removal (Swertfeger *et al.* 1999), either using sand filtration or membrane filtration. The surface properties of cysts and oocysts significantly influence their removal efficiency (Bush *et al.* 1998; Hsu *et al.* 2001).

Membrane filtration is a highly sophisticated process that employs primarily synthetic polymeric membranes to physically filter out of solution, under pressure, minute particles such as viruses. Conventional filtration can only deal effectively with particles larger than 10^{-2} mm so a range of synthetic membranes with very small pores are used to remove from water particles of any size down to 10^{-7} mm (Fig. 9.24). As with all filtration processes the size of the particles retained are approximately an order of magnitude smaller than the pore size of the filter. Membrane filtration is widely used for the advanced and tertiary treatment of potable and waste

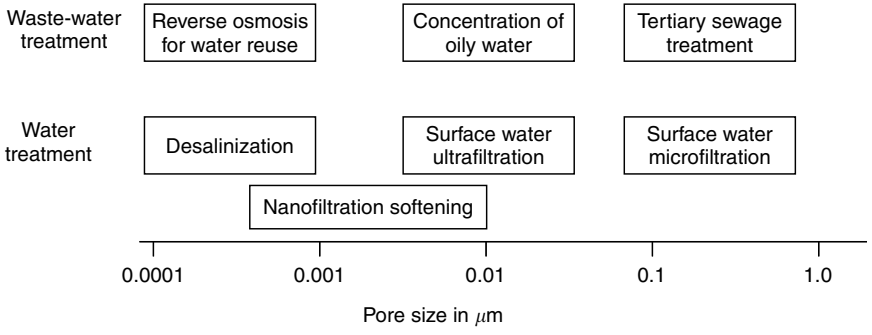


Fig. 9.25. Principal water and wastewater treatment membrane filtration applications by pore size (Gray 1999).

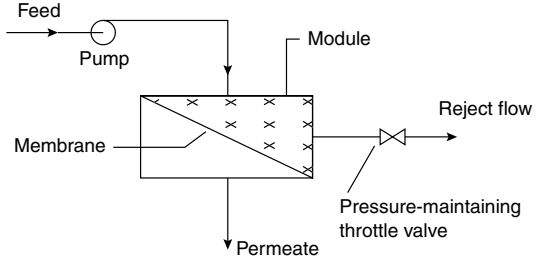


Fig. 9.26. Schematic layout of a membrane filtration module (Casey 1997).

waters (Fig. 9.25), and as the technology develops it will gradually replace many of the existing conventional treatment processes.

Microfiltration (MF) can remove particles between 0.05 to 5 μm in size with membranes in the form of tubular, capillary, hollow fibre, or spirally wound sheets. Treated effluents are pressurized to 100–400 kPa, forcing the permeate (clarified liquid) through the membrane with the particulate matter retained (Fig. 9.26). Membranes used in MF are generally made from a thin polymer film with a uniform pore size and a high pore density (75–80%). The high density of pores results in a low hydrodynamic resistance allowing high flow rates. Most MF systems are not continuous but batch processes with the permeate produced continuously during the period of operation but the concentration of the retained solids or solutes increasing over time. Therefore, membranes require periodic backwashing with either water or gas, under pressure, to remove trapped solids from the micropores. Cross flow is where the flow is introduced tangentially to the membrane surface which promotes self-cleansing ensuring longer operation periods between

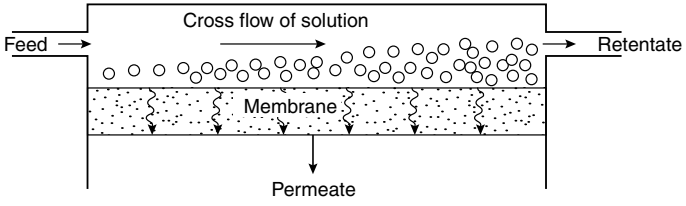


Fig. 9.27. Cross flow membrane separations (Scott and Hughes 1996).

replacement or backwashing (Fig. 9.27). The process can remove microbial cells and is widely used to remove chlorine resistant pathogens such as *Cryptospridium* oocysts and *Giardia* cysts. Bacteria are also removed depending on the particle cut-off limit, while viruses are only removed if they are associated with large particulate matter. Membrane filtration has broad industrial applications not only in the production of pure water for the electronic, chemical, pharmaceutical, and food industries, but for process solvent recovery, removing heavy metal precipitates, small inert particles from process waters (e.g. glass), and algae. It is used for the tertiary treatment of domestic wastewaters to reduce the suspended solids concentration and thereby reduce the BOD, COD, and the turbidity of the effluent.

Ultrafiltration (UF) is similar to MF, except membrane micropores are much smaller and the pressure required to force the untreated water through is much greater (up to 3000 kPa). Widely used polymers for UF membranes include polysulphone, polyacrlonitrite, polyimide, and cellulose acetate. Inorganic ceramic membranes are also used for certain industrial processes. The membranes are very thin (0.1–1.0 μm) and so must be supported on a stronger but highly porous layer (50–250 μm) that takes the form of either tubular, capillary, or spirally-wound units or modules. Each module is composed of a series of hollow membranes with the treated wastewater fed tangentially at high velocity to minimize blinding or fouling of the membrane surface. The permeate passes through the membrane and the concentrated particles are pumped to a waste tank. Owing to the smaller particles removed (0.001–0.02 μm) UF has different applications to MF being used primarily for advanced potable water treatment. In industry they are used to concentrate proteins, enzymes, and hormones. Macromolecules, with a molecular mass greater than 1000, such as dyes, can be recovered as can oil from oil emulsions. In wastewater treatment they can be used to remove bacteria, viruses, and cellular fragments (pyrogens) (Madaeni 1999).

Further reading

Pathogens: Dart and Stretton 1977; Carrington 1980; Andersson and Stenstrom 1986; Ford 1999; Mara and Feachem 1999.

Indicator organisms: Burman *et al.* 1969; Bonde 1977; Mara 1974; Hoadley and Dutka 1977; Dutka 1979; HMSO 1983b; WHO 1984; Ayres and Mara 1996; Fewtrell and Bartram 2001.

Hazards: Lundholm and Rylands 1983; Sridhar and Oyemade 1987; Clark 1987; Mulloy 2001; Thorn and Kerekes 2001.

Bathing waters: US EPA 1999; Haile *et al.* 1999; Mugglestone *et al.* 2000, 2001.

Shellfish: Wilson 1988; UNEP 1991; Bosch *et al.* 1994; Abad *et al.* 1997.

Groundwater: Allen and Oeldriech 1975; Seppanen and Wihuri 1988.

Reuse of wastewater: Mara and Cairncross 1989; WHO 1989; EPA 1992; Rowe *et al.* 1995; Shuval *et al.* 1997; Bonomo *et al.* 1999; Fox 2001.

Pollution by gulls: Gould 1977; Hatch 1996; Friend *et al.* 2001.

Sewage sludge: Pike 1981; Strauch *et al.* 1985; Sorber 1986; Schwartzbrod *et al.* 1987; Lewis-Jones and Winkler 1991.

Atmospheric pollution: Sorber and Guter 1975; Bitton 1994.

Antibiotic resistance: Carrington 1979; Mach and Grimes 1982; Neu 1992.

Environmental factors: Gameson 1975a; Mitchell and Chamberlin 1975; Carrington 1980a; Gameson 1985b; Berk and Gunderson 1993; Chung and Sobsey 1993b.

Treatment processes: Pike 1975; Pike and Carrington 1979; Yaziz and Lloyd 1979; Carrington 1980a; Bitton *et al.* 1980; Grabow 1986; Rao *et al.* 1986.

Sterilization and disinfection methods: White 1978a; Koo1 1979; Venosa 1983; Fawell *et al.* 1987; Lazarova *et al.* 1999.

10

Biotechnology and Wastewater Treatment

10.1. The Role of Biotechnology

A particularly clear definition of biotechnology is given by Rothman *et al.* (1981), as the "exploitation of living organisms, generally micro-organisms, or biological processes in an industrial or commercial situation to provide desired goods or services". Such a definition encompasses a large number of areas, including wastewater treatment. Biotechnology is not a distinct discipline but rather a result of four traditionally separate disciplines, biochemistry, microbiology, engineering, and chemistry, interacting with each other (Sikyta 1983). Although the term biotechnology only become widely studied as a subject in its own right in the mid-1970s, many biotechnological processes have been used by man for centuries, such as the exploitation of yeasts in baking and brewing, or bacteria to ripen cheese. The recent progress and upsurge in interest in biotechnology has been due to a number of incentives. First, the increased cost of energy led to a re-examination of the processes producing fuel from renewable sources, and the increasing scarcity of raw materials, especially minerals, has made them more expensive as the poorer resources are exploited. Micro-organisms can be used not only in the extraction of metals from low grade ores but also to reduce the pollution load on the environment. The recent advances in biotechnology are closely associated with developments in general and molecular genetics. New strains of micro-organisms can be developed that produce a desired substance in far greater quantities than the original parent strain. Although this can be achieved by careful culturing and selection, the most important advance is the development of genetic manipulation, known ubiquitously

as genetic engineering. This involves the transfer of genetic material from one cell to another in order to modify that cell's behaviour to benefit some industrial process. Turning the bacterial cell into a factory capable of producing enzymes, vaccines, amino acids, steroids or other cellular products, such as insulin and interferon, has revolutionised the biomedical and food industries (Old and Primrose 1980). The application of genetic manipulation to wastewater treatment is varied, ranging from the inoculation of specially cultured bacteria to enhance the performance of an existing conventional effluent treatment plants to the biodegradation of recalcitrant compounds.

Most of the investment in biotechnology to date has been in those areas with most capital return, which have been the food and biomedical sectors. While it was predicted that environmental biotechnology would be the market that would develop most in the 1990s (Anon 1982b) (Table 10.1), this proved not to be the case. The lack of financial return in comparison with other sectors, linked with the conservative outlook of the water technology industry itself, which is very slow to move away from the familiar treatment methods, is why biotechnology has so far had little real impact on wastewater treatment (Anon 1982c; Gray 1985b).

Effluent treatment is undoubtedly the largest controlled application of micro-organisms in the manufacturing and service industries. The size of this market is very large and with the installations constructed during the early expansion of wastewater treatment in the late-nineteenth and

Table 10.1. Markets for microbes and enzymes, where AGR is the average annual growth rate estimated for 1981–91 (Anon 1982c).

Application	1975	1977	1979	1981	1986	1991	(AGR)
Environmental clean-up	5	7	9	10	22	48	17
Biomass conversion	0.5	3.3	5.5	7.0	8.5	10.0	3.8
Sweeteners	7.8	28.0	29.5	30.5	42.5	60.0	7
Processed food beverages	35.7	39.6	42.4	45.0	52.9	64.0	3.6
Biomedical	15	18	29	31	45	67	8
Laundry products	7.0	5.0	6.0	6.0	6.3	6.6	1
Tanning and textiles	18.0	18.0	9.0	8.6	8.4	7.8	–1
Miscellaneous (insecticides, mining, feed, biosensors, etc.)	0.4	0.4	1.1	2.1	3.3	5.6	10
Total ($m)	89.4	119.3	131.5	140.2	188.9	269.0	

early-twentieth centuries now nearing the end of their useful lives. Therefore the opportunities for the biotechnologist to apply new technologies to pollution control, such as genetic manipulation combined with new reactor designs, are enormous. The fact that the total value of crude fats, proteins, and metals in wastewater disposed to sewers in the UK was estimated in 1980 as being worth about £150 million annually is an added incentive (Clapham 1980).

In the future, cheaper, more efficient and more compact processes will be developed, with the traditional aims of removing organic matter and pathogens to prevent water pollution and protect public health, replaced with a philosophy of environmental protection linked with conservation of resources and by-product recovery. Unlike the other sectors of biotechnology, development in environmental biotechnology is unlikely to be stimulated or supported by normal market forces. However, the introduction of the "polluter pays" principle for industrial wastewater (Deering and Gray 1986) and water charges for domestic wastewater, with the privatisation of the UK water industry, has gone some way towards this goal. Any economic deficit can be rectified by legislation on pollution and government subsidy for the conservation of vital resources. For example, in Italy, an effluent treatment plant which produces biogas from waste receives a 70% grant, as the country is short of indigenous energy (Wheatley *et al.* 1983). In the future, the operating costs of wastewater treatment plants will be offset by the various by-products or resources that are recovered. Although it is unlikely that this will normally result in a clear operating profit, taken with its service role of environmental protection then there is no reason why wastewater treatment should not become increasingly cost-effective. It is unrealistic to think that the by-products of environmental biotechnology, which are mainly of low to intermediate value, would ever recoup capital investment. It is likely, therefore, that the initial cost of wastewater treatment plants will always have to be funded centrally by governments. At present, this concept is more widely seen in relation to solid waste disposal, especially by incineration. Plants in the UK and Ireland are to be operated by private companies with the major income coming from the local authorities who not only pay to have their refuse disposed of, but also for the energy generated which is generally used for group heating schemes.

The major areas of biotechnology that have the greatest potential in wastewater treatment are resource recovery (Sec. 10.2), biological conversion (Sec. 10.3), and environmental protection (Sec. 10.4).

10.2. Resource Reuse

10.2.1. *Fertiliser value*

Sewage and agricultural sludges are rich in organic matter and the major plant nutrients, nitrogen, phosphorus and potassium, as well as all the important trace elements. Farmers are able to utilise these sludges as an effective but cheap soil conditioner and fertiliser. The exact manurial value of sewage sludge depends on the nature of the sludge, whether it is domestic, industrial or agricultural and whether it is primary or secondary sludge, and also whether it has been dewatered or stabilised (Table 10.2). Liquid digested sludge in the UK is mainly used on agricultural land. There are problems with contamination of the soil and vegetation by heavy metals and pathogens. This has led to the introduction of legislation to control the level of contamination as well as to prevent the transfer of human, animal, and plant diseases. The whole topic of sludge utilisation as a fertiliser is dealt with in Sec. 8.2.2.

The development of sustainable sludge disposal strategies is vital for the long-term safe exploitation of sewage sludge. Towers and Horne (1997) describe how soils and related environmental data can be interrogated and interpreted to assess (i) the sustainability of land for sludge utilisation; (ii) the risk of water pollution in relation to different soil conditions; (iii) the ability of soils to adsorb potentially toxic elements in the sludge to prevent transfer to water and plants; and (iv) the operational security and environmental sustainability of sludge recycling. Sludge management practice and legislation varies widely around the world (Leschber 2002). Stypka *et al.* (2002) insist that sewage sludge disposal can become more sustainable if two key elements of sludge management, i.e. regional planning and product recovery, are adopted. They demonstrate this by comparing sludge disposal practices in Poland and Sweden.

Table 10.2. The manurial value of sewage sludge in terms of nitrogen availability.

Sludge type	% N	% NH_4	% N as NH_4
Alkaline stabilised	3.25	0.10	0.03
Anaerobic digested	4.46	1.13	25.03
Aerobic digested	3.75	0.21	0.06

10.2.2. *Reuse of effluents*

Due to the relatively low costs of mains water and effluent disposal in Europe, it is rarely economical to treat wastewater for reuse within a particular production process. Recycling of water is only economic when the quality of the water required is unimportant, as with industries such as power generation, steel making, and coal washing. Thus, the water can be continuously recycled with only minimum treatment required to remove gross particles or to cool the water. However, two situations can make the reuse of effluent economic. Where local water capacity is insufficient to meet the needs of industry and the effluent is the only other source of water available, and where effluent treatment costs levied by water companies make it more economic for industries to treat their own waste before discharging effluent. In these circumstances it may be cheaper to reuse their own treated effluent rather than pay for mains water and even minimum disposal charges. Usually, water is reused several times within the factory before eventually being discharged, starting with processes that require clean water and finishing up being used for processes which only require low grades of water, such as vegetable washing (Shore *et al.* 1984). With the introduction of water supply and disposal charges, the conservation and the multiple use of water, industry has greatly reduced water usage and alleviated water pollution. An interesting example of water reuse can be seen in the sugar beet processing industry. The total water requirement for processing a tonne of sugar beet is between 9–19 m^3, and until recently, most beet factories operated on a once through basis with little or no recirculation of water which resulted in vast quantities of wastewater being generated. Wastewater from sugar beet is particularly polluting as it contains low molecular weight carbohydrates, usually sucrose or volatile fatty acids, in high concentrations (2,000–5,000 mg l^{-1} BOD). These can cause severe sewage fungus outbreaks and deoxygenation in receiving waters (Gray 1987, 1988). However, with careful water management, the surplus water can be reduced to as little as 0.5–1.0 m^3 t^{-1} (McNeil 1984). The Bury St. Edmonds factory operated by British Sugar processes more than 11,000 t of beets daily during the processing season. They have been able to reduce the quantity of effluent produced from > 100 t m^{-3} d^{-1} to only 4.8 t m^3 d^{-1} by introducing an extensive water re-use programme (Fig. 10.1). This has the added advantage of producing an effluent volume more amenable to treatment, normally by anaerobic processes. The high water content of sugar beets (78%) makes it impossible, however, for such systems to operate as a closed loop, with no excess water produced and so no discharge at all

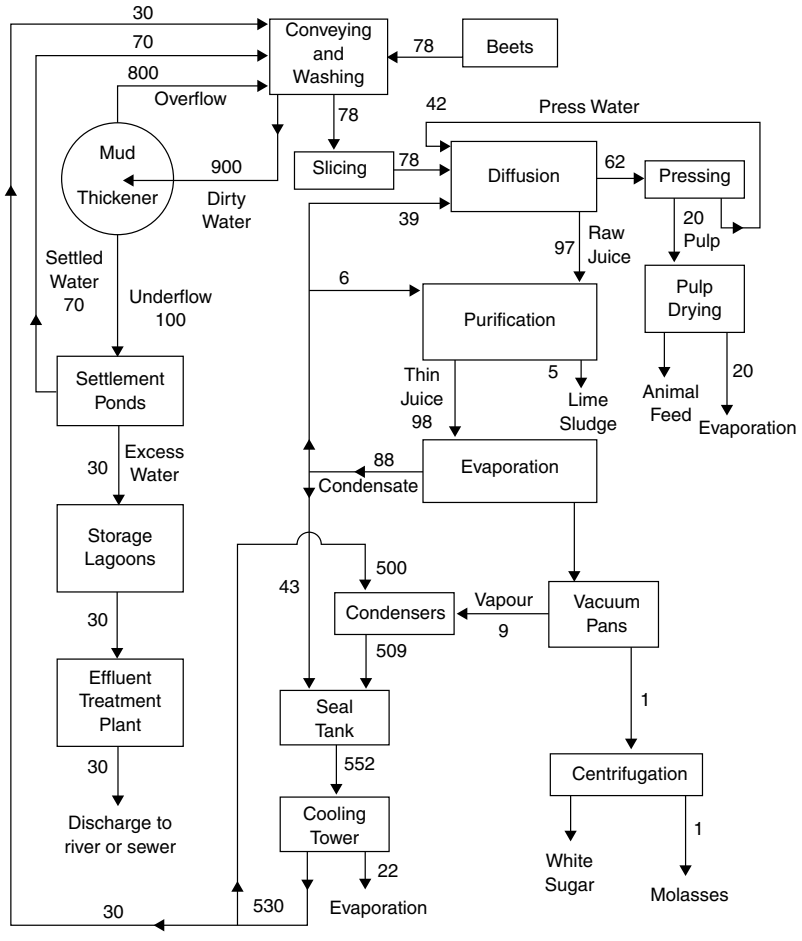


Fig. 10.1. The water cycle in a sugar beet processing factory operated with maximum recirculation. Figures are the nominal water % w/w on beets (Shore *et al.* 1984).

(Shore *et al.* 1984). Orhon *et al.* (2001) give a detailed analysis of wastewater recovery and reuse in the textile industry.

In areas where water is scarce, treated sewage effluent may be reused after sufficient disinfection for uses not associated with human or animal consumption. At one time, it was proposed to have a dual water supply system in UK towns, one with fully treated water and the other with a lower grade of water, usually untreated river water. The idea was to conserve water of the highest quality for consumption and household uses while also saving money by not having to treat all the water being supplied.

Although this system is in use in some other countries it was never feasible in the UK because of the high cost of providing two separate mains, having two separate plumbing systems in buildings, plus the danger of people mistakenly using the lower grade of water for consumption. However, in arid areas it is common to use sterilised treated effluents for non-consumable activities such as car washes, flushing toilets, and even washing clothes (Fewkes and Ferris 1982).

In the UK at present about 30% of the raw water used for public supply is obtained from recycled effluent (Water Data Unit 1979). This is a mean value for the whole of England and Wales, and in areas where supplies of upland water are very restricted, such as the South-East of England, this figure may be as high as 70%. Treated effluent is discharged from one consumer area into a lowland river and abstracted for reuse at the next urban area downstream. It is incredible to realise that the River Thames and Lee consist of 95% effluent during dry summers (Pacham 1983). With the major areas of population centred in the Midlands and the South-East, it is unlikely that new upland supplies will be made available in the future and any subsequent increase in demand will have to be met by using groundwater or reclaimed water. The Thames River Basin in England is an example of open cycle reuse, when the sewage from one community is converted to drinking water in another. Overall, the population of London is in excess of 10 million, with water being supplied from bore holes, the River Thames, and its tributaries, including 1.2×10^6 $m^3 d^{-1}$ of sewage. The recycle rate is, on average, 13% but during the 1975–76 drought it exceeded 100% (Blackburn 1978). The sewage receives full biological treatment followed by nitrification and denitrification when required (Cooper *et al.* 1977), whereas the water supply is stored for seven days prior to treatment, which normally involves slow sand filtration followed by chlorination. The re-use of the River Thames water is shown schematically in Fig. 10.2. It is interesting that one community, Walton Bridge, actually discharges its effluent upstream of its own intake (Eden *et al.* 1977).

All municipal wastewaters discharging to rivers used for public supply are fully treated biologically and the abstracted water is then subjected to full water treatment (which may also include treatment with activated carbon, membrane filtration or de-ionization if necessary). However, when water is recycled many times dissolved salts will accumulate in it, particularly the anions from biological oxidation. Nitrate, sulphate, phosphate, and chloride all accumulate in the water supply which causes unpleasant tastes and a fall in quality, corrosion and scaling in pipes, and even toxicity. If there is no alternative source of supply then these inorganic salts may have to be

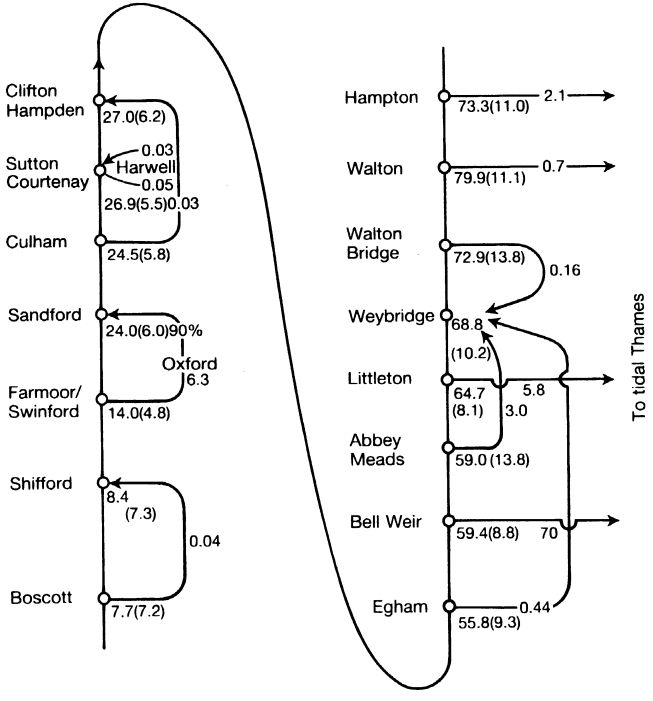


Fig. 10.2. An example of the reuse of water. The schematic diagrams shows the main abstractions and discharges on the lower River Thames. Average river flows are given in m^3s^{-1} and in parentheses the percentage of river flow under average conditions that comprises sewage effluent (Dean and Lund 1981).

removed by advanced water treatment techniques such as ultrafiltration, reverse osmosis or ion exchange, which makes the water expensive (Botto and Pawlowski 1983; Hedges and Pepper 1983). The final effluent from Chelmsford Sewage Treatment Works (UK) is currently discharged to the Blackwater Estuary. However, during the drought of 1995–98, the effluent was discharged directly into the Hanningford Water Supply Reservoir from July 1997 to December 1998 as an emergency measure to maintain supplies. During this period the effluent was disinfected using UV sterilisation. It is now proposed to reduce current phosphorus, ammonia, nitrate, BOD, pathogen and oestrogen levels using advanced treatment techniques, at a cost of eight million pounds sterling, and recycle the effluent by discharging it into the River Chelmer from which drinking water is abstracted (Walker 2000). Some pollutants are not easily removed and are very persistent. Although they are normally only present in trace amounts they are still toxic or in some cases carcinogenic. These compounds, known widely as

recalcitrants, can be degraded or scavenged by specialised micro-organisms (Sec. 10.4.1). In recent years pharmaceuticals have increasingly been reported in surface waters. Drugs used for the treatment of human and animal medical conditions result in the parent compounds and their metabolites being excreted. The removal of these compounds is incomplete in conventional sewage treatment systems so that they reach receiving waters (Halling-Sörensen *et al.* 1998; Heberer *et al.* 1998; Sacher *et al.* 1998; Ternes 1998; Buser *et al.* 1999) (Chapter 11). The short- and long-term health effects of consuming re-used water is examined further in Sec. 9.4.1. Oestrogens and other endocrine disrupting substances are common components of wastewater. Their effects on wildlife are well documented and there is increasing concern for human health. They have been implicated in testicular cancer, breast cancer, sex-organ malformation and decreased sperm counts (Environment Agency 1998). The health implications and guidelines for the reuse of domestic grey water (i.e. all the wastewater except that arising from the WC) are reviewed by Dixon *et al.* (1999) (Chapter 11).

Sewage contains a wide range of inorganic plant nutrients, principally N, P, and K salts, although they are in much lower concentrations than either liquid sewage sludge or synthetic plant feeds (Table 10.3). Even though sewage contains small quantities of nutrients its main potential is only realised if the effluent is also needed as a water supply. Therefore, irrigation with sewage is not widely practiced in Western Europe, as the nutrients are

Table 10.3. Comparison of inorganic nutrient concentrations (mg l^{-1}) in domestic sewage with synthetic nutrient solutions used to culture micro-organisms (Wheatley 1985).

Nutrient	Domestic sewage effluent	Synthetic nutrient solution
Nitrate (as N)	20–25	40–45
Ammoniacal nitrogen (as N)	0.1–5.0	0.1
Phosphorus (as P)	5–12	90–100
Potassium	10–18	90–100
Calcium	80–90	125
Magnesium	5–9	85
Iron	0.01–0.1	8–12
Manganese	0.1	1
Sodium	80–90	60
Chloride	60–100	50
Boron	1–2	—

so dilute, that large quantities of sewage would be required to meet plant needs, making it an expensive source of nutrients. However, in drier and more arid areas, sewage irrigation is common, especially in Israel, India, Australia, and Southern USA. For example, lint cotton yields in Cyprus increased 29% from 2,775 kg ha^{-1} using freshwater irrigation to 3,585 kg ha^{-1} using treated wastewater (Papudopulos and Stylianou 1988). However, as sewage contains pathogens and heavy metals, care must be taken to prevent contamination or accumulation of toxic materials in the crops. This is done by careful selection of crop species and restricting the use of certain effluents to particular categories of crops. This is fully discussed in Sec. 9.4.2. Sewage irrigation is widely used in South-Western USA where primary effluents are used as fodder, fibre, and seed crops, and secondary treated effluents on food crops and on pasture used for dairy herds (Pettygrove and Asano 1984). In China, sewage effluents have been successfully evaluated for irrigating rice paddy fields (Kwun *et al.* 2001). Much interest is being shown in using sewage effluents in soil-less nutrient-film hydroponics (Winfield and Bruce 1981). The use of sewage directly onto vegetables and fruit is discouraged, especially if eaten raw, because of the long survival of bacteria and viruses. The WHO (1989) recommended that irrigation with effluents should cease at least two weeks before fruit is picked, and that fruit that had fallen onto the ground should not be gathered. Chlorinated secondary effluents are widely used throughout Southern USA for the irrigation of all large areas of grass, such as golf courses and parks (United States Golf Association (1994) (Sec. 9.2.4.2). An interesting study is described by Murakami and Ray (2000) who used secondary treated, filtered and chlorinated effluents to irrigate a golf course on the island of Oahu, Hawaii. In the Bay area of San Francisco, California, up to 4% of the dry weather flow of the local wastewater treatment plant is reclaimed and reused by both residential and commercial users via a separate distribution system solely for landscape irrigation (Hermanocwicz *et al.* 2001). Wastewater reclamation and reuse has also been widely adopted in the major cities of Japan and is reviewed by Ogoshi *et al.* (2001).

Irrigation with sewage removes much of the nitrogen and phosphorus from the effluent, which prevents eutrophication, and so land treatment is widely used as a tertiary treatment method. Effluents from waste stabilisation ponds contain algae which, when used for irrigation, not only adds to the humus content of the soil, but also slowly releases plant nutrients as they slowly decompose. The effects of effluents on soil structure is examined in Sec. 6.1.1. Before use on agricultural land, wastewater effluent should be checked for heavy metals and other potential toxicants. Mara and Pearson

(1998) list five key parameters that should be checked before effluents from waste stabilisation ponds are used for irrigation on crops.

(1) The electrical conductivity in miilisiemens per metre at 25°C is a simple measure of total dissolved solids. This can be used to test the potential salinity hazard to crops.
(2) The sodium adsorption ratio (SAR) which is calculated using Eq. 6.1. The relationship between conductivity and SAR is shown in Fig. 6.1.
(3) The permissible pH range is 6.5–8.4.
(4) Excessive total nitrogen can lead to reduced crop yields even though there may be increased vegetative growth. Some sensitive crops can tolerate < 5 mg N l^{-1} while most are unaffected up to 30 mg N l^{-1}.
(5) Boron is found in synthetic detergents and can affect a wide range of fruits and nuts at concentrations > 0.5 mg l^{-1}, although most crops tolerate up to 2 mg l^{-1}.

More details of the effects of nutrients and trace elements is given by Ayres and Westcot (1985). Wastewater reclamation and reuse is reviewed by Asano (1998).

10.2.3. *Metal recovery*

The consumption and therefore the value of metals world-wide has risen dramatically over the past two decades. This is not only because of demand but also to scarcity, and increasing costs of production, as less metal-rich ores are worked. Also restrictions, both natural and international, on the discharge of metals into the environment, because of toxicity and the dangers associated with bioaccumulation and metals entering the food-chain, has led to a growing awareness of the importance of their recovery and possible reuse. Nearly all wastewaters contain some metals but industrial wastewaters and, in particular, mining wastewaters can be rich in metals. It is not generally wastewaters that contain high concentrations of metals that pose the greatest environmental risk, as such wastes can be economically treated by conventional physical and chemical recovery systems. The wastewaters that pose the greatest problems are those where the concentrations may be low enough to make conventional chemical recovery uneconomical but at the same time, high enough to cause environmental damage. Domestic wastewaters and acid mine drainage are two common sources of heavy metals (Gray 1997; Níc Aonghusa and Gray 2002). Certain micro-organisms are well known to be able to remove metals from solution by precipitation (*Sphaerotilus*, *Leptothrix*, and *Gallionella*),

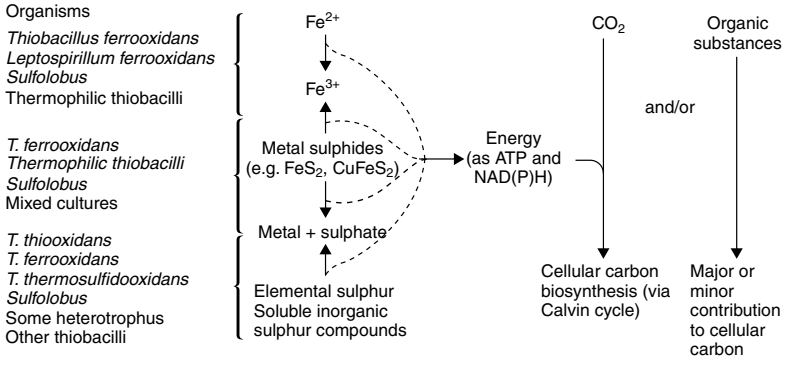


Fig. 10.3. Summary of acidophilic leaching bacteria and their basic metabolism (Kelly *et al.* 1979).

by precipitating soluble metals in the form of insoluble sulphides (many anaerobic bacteria) and by oxidation (*Thiobacillus*). Recovery of metals using micro-organisms would only be a fraction of the cost of physical or chemical recovery processes. Therefore, the active utilisation of suitable micro-organisms to remove metals from wastewaters appears very attractive both economically and environmentally.

Water exposed to sulphides or sulphur-rich coal will have associated with it several types of bacteria that derive their energy from the oxidation of inorganic substances, such as ferrous iron, sulphur, and soluble and insoluble sulphides. These bacteria can be used to extract these elements from process wastewaters (Fig. 10.3) (Kelly *et al.* 1979; Kleins and Lee 1979). Sulphur is a major constituent of many effluents, including gypsum wastewaters, excess sulphuric acid, coal sulphurisation by-products, acid mine waters, and general metallurgical effluents. Cork and Cusanovich (1978) studied the quantitative conversion of sulphate to elemental sulphur using *Desulfovibrio desulfuricans* to convert sulphate to sulphide and photosynthetic bacteria, such as *Chromatium thiosulfatophilium* and *C. vinosum* to convert sulphide to elemental sulphur, and concluded that a viable industrial process could be developed from such a system. In the laboratory, mixed cultures of *Thiobacillus ferrooxidans* and *T. thiooxidans* are effective in the removal of pyritic sulphur from 20% slurries of a commercial grade of pulverised coal, even though neither species is effective on its own (Dugan and Apel 1978). Although further work remains to be done on the use of micro-organisms to solubilise the organic sulphur fraction of coal, as these were not removed in the laboratory-scale trials, Dugan and Apel are confident that commercial-scale operation is feasible.

Bacterial sulphate reduction has been widely used to remove both metals and sulphate from acid mine drainage (AMD) (Dvorah *et al.* 1992; Christensen *et al.* 1996). Once the AMD has been neutralised, as it is too acidic for the successful growth of sulphate reducing bacteria, reduction of sulphate to sulphide (sulphidogensis) takes place under anaerobic conditions; the metal sulphides produced having low solubilities (Chang 1993; White and Gadd 1996). An organic supplement is required as an electron donor for the sulphate reducing bacteria. Hay, straw, and mushroom compost are all commonly employed and slowly degrade supplying the necessary carbon substrate and nutrients needed by the bacteria. Cellulose is not used directly but needs to be degraded by hydrolytic fermentative anaerobes to fatty acids and alcohols that can be used by the sulphate reducers. The reduction of a molecule of sulphate to sulphide results in 8 electrons being consumed:

$$2CH_2O + SO_4^{2-} \rightarrow 2CO_2 + 2H_2O + S_2^-$$

So 60 g of substrate is consumed to remove 96 g of sulphide. However, only 25% of the biomass will be utilised under anaerobic conditions so that the removal of 1 kg of sulphate from AMD requires approximately 2.5 kg of biomass (Seop *et al.* 2000).

The principle contaminants of coal mining wastes are ferrous (Fe^{2+}) iron and sulphuric acid. Wichlacz and Unz (1981) were able to remove $> 90\%$ of the ferrous iron using a rotating biological contactor, regardless of the ferrous iron concentrations or the hydraulic residence times tested. The biological film that developed on the discs was found to contain the chemolithotrophic iron oxidising bacterium *Thiobacillus ferrooxidans*, as expected, and also at the higher mass loading concentrations of ferrous iron, acidophilic heterotrophic bacteria. They concluded that the practice of treating soil and strip mine overburden with organic inhibitors to prevent oxidative weathering may be pointless, as a heterotrophic bacterial population may be induced to grow on the organic molecules.

The AMD produced as leachate or runoff from mine tailing dumps are acidic and rich in metals. This observation has led to the development of the microbial leaching process, which is the major method of recovering metals using micro-organisms. The process has been known since Roman times and the practice of percolating acidified water through heaps of low-grade ore to remove the metal sulphide formed by the bacterial activity within the heap, was carried out in Anglesey as early as the sixteenth century. However, it is only in the latter half of this century that bacterially assisted leaching

has been practiced on a large scale (Rothman *et al.* 1981). Ore deposits contain a wide range of metallic sulphides that are naturally oxidised and solubilised by a complex microbial community. Initially, the autotrophic oxidising bacteria, such as *Thiobacillus* spp. are able to oxidise both the sulphide ores and the lower oxidation states of the metals (Kelly *et al.* 1979). The leaching process is quite simple. Low-grade ore and other waste rock are heaped on to an impermeable base. Little preparation is required except the grading and shape of the ore particles should allow sufficient voidage to permit maximum recirculation within the heap ensuring long residence times, unimpaired drainage, and aeration. The leaching solution is sprayed on to the surface of the heap and percolates slowly through, dissolving metals released by microbial oxidation. The metal salts are extracted and concentrated by electroprecipitation, solvent extraction or conventional precipitation. The leaching solution is recycled after the metal salts have been extracted, although it needs to be replenished with acid and bacteria. Although copper and uranium are the principal metals extracted in this way commercially, the leachate contains a variety of other valuable metals. Iron, arsenic, antimony, cadmium, lead, silver, gold, and chromium have all been extracted by this method (Ferraiolo and Del Borghi 1987).

Between 10–15% of the annual primary copper production in the US is by microbial leaching. In Utah, a massive heap of 250,000 tonnes of mineral ore produces 150 tonnes of copper per day, with the liquid heavily charged with bacteria. The bacteria form copper sulphate from which the copper can be readily recovered with the acid solution returned to the leaching process. Microbial leaching operations run continuously and require only a small workforce; the plant described above in Utah only requires six people to keep it running. Similar units of equal size are recovering copper in Chile and Romania, and uranium is being extracted by microbial leaching in Canada (Rothman *et al.* 1981).

Using *Thiobacillus* spp. Ebner (1978) leached metals from sulphidic dust, acidic fly ash from a copper process, slag from a lead smelting process, and javosite from zinc electrolysis. He found that leaching efficiency depended on material, treatment, and bacterial species, with maximum outputs for zinc and copper of 95% and 70% respectively (Table 10.4). Ebner recorded high concentrations of other metals in the leachate, especially cadmium, arsenic, and lead. Torma *et al.* (1970) were able to leach zinc rapidly using *Thiobacillus ferrooxidans*, producing leachate concentrations of up to 72 g l^{-1} of zinc, concluding that at this level of recovery the process was commercially competitive with other forms of mining. Electron micrographs of bacteria from a mixed culture of *T. ferrooxidans*

Table 10.4. Maximum leaching efficiencies (%) from different wastes using either *Thiobacillus ferrooxidans* (F) or *Thiobacillus thiooxidans* (T) (Ebner 1978).

	Sulphidic dust concentrate *a*			Sulphidic dust concentrate *b*			Fly ash			Javosite		Lead slag	
	Cu	Zn	As	Cu	Zn	As	Cu	Zn	As	Cu	Zn	Cu	Zn
Bacterial leaching	12	38	3	35	94	14	42	85	81	69	52	33	50
Sterile control	0.1	16	—	0.4	10	0.2	10	16	—	5	10	0	0
Thiobacillus strain	F-E2 F-E21	F-E2	F-E2	F-E2	F-E2	F-E2	T-E2	T-E2	T-E2	T-E2	T-E2	T-E2	T-E2

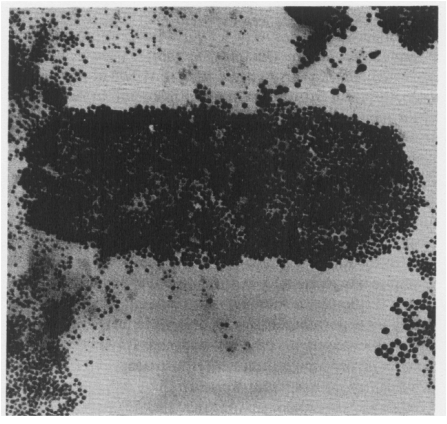


Fig. 10.4. Electron micrograph of a simple bacterium emptied of its cell contents and showing silver sulphide particles adsorbed on to its surface (Pooley 1982).

and *T. thiooxidans* revealed that silver was being concentrated by the bacteria without inhibition (Pooley 1982). Small silver sulphide granules growing on the surface of bacteria collected at different stages of a batch leaching process, which finally accumulated to form large lumps (Fig. 10.4). The cells containing the silver could be readily collected, enabling the viability of the bacterial leaching of precious sulphide minerals, such as silver and possibly gold, from previously uneconomic ores. More recent leaching methods employ stirred reactors with finely crushed ore kept in constant suspension (Lundgren and Malouf 1983). Much attention is being focused on developing a more efficient strain of *Thiobacillus ferrooxidans*, the bacteria mainly used in this process, by using genetic manipulation.

More than 50% of sewage sludges are unsuitable for disposal to agricultural land due to heavy metal contamination (Couillard and Merceir 1991) (Sec. 8.2.2). Much research has been undertaken in removing heavy metals from sewage sludge using micro-organisms of the thiobacilli group (Couillard *et al.* 1991; Blais *et al.* 1993; Sreekrishnan and Tyagi 1994; Tyagi *et al.* 1997; Lombardi and Garcia 1999). Bioleaching of insoluble metal sulphides in anaerobic sludges is carried out principally by *Thiobacillus ferrooxidans*. The bacterium mediates several acid producing reactions that culminate in local pH reduction and increase in the oxidation-reduction potential (ORP). The removal of metals is not 100% efficient with some metals more amenable to leaching than others. For example, Lombardi and Garcia (2002) reported solubilisation efficiencies for Mn, Zn, Cu, Al, and Ti from an anaerobic sludge using *T. ferrooxidans* as 80%, 80%, 24%, 10% and 0.2% respectively. Bioleaching rates can be improved by supplementing the

energy sources in the form of S° or Fe(II) (Lombardi *et al.* 2001). Metals can also be successfully removed chemically from sewage sludge by acid leaching, with the sludge mixed with sulphuric acid (20% v/v) for 60 minutes (Naoum *et al.* 2001).

A number of heterotrophic bacteria are capable of accumulating heavy metals in rivers and streams where the concentration of metals is extremely low (Friedman and Dugan 1968b; Patrick and Loutit 1976; Norris and Kelly 1980; Gray and Clarke 1984; Gray 1985a). Biological wastewater treatment units are also effective in removing metals from domestic and industrial wastewaters, with the metals being concentrated in the biomass and sludge. Activated sludge biomass is particularly effective at metal adsorption (Chang *et al.* 1975; Brown and Lester 1979; Çecon and Gürsoy 2001). The concentration of metals accumulated in the sludge can be so high that it restricts the disposal options for the sludge (Sec. 8.1). For example, the biomass produced from a plant treating acetate rayon wastewater which contained between 50–100 mg l^{-1} of zinc, accumulated 12% by weight of zinc without interfering with the BOD removal (Kiff and Brown 1981). The major removal mechanism of metals is adsorption which is controlled by physico-chemical factors, such as temperature and competing ions. The micro-organisms and their extracellular polymeric secretions are highly charged due to the presence of carboxyl, amine, and hydroxyl groups in their protein, carbohydrate, and phospholipids. Adsorption, which is extracellular binding of the metal ion on to the microbial cell, occurs because of the electrostatic attraction between positively charged metals and the negatively charged surfaces of micro-organisms or their secreted polymers. Wheatley (1981) has demonstrated with some excellent electron photo-micrographs that the active biomass in biological treatment units of wastewater treatment plants is largely composed of extracellular bacterial polymer rather than bacterial cells. This is not altogether surprising as the bacteria rely on the polymer to adsorb and transfer nutrients from solution to the parent cell. The area and structural nature of the polymer varies according to the substrate with carbohydrates, and especially oligosaccharides, generating most polymer under controlled conditions. To the process engineer, the very large active surface area supplied by microbial surfaces and microbial polymers is cheaper and more flexible for adsorption than chemical surfaces, such as activated carbon. Heavy metals appear to be taken up by specific uptake mechanisms by micro-organisms along with useful metals necessary for metabolism. This property and the mechanisms by which micro-organisms may resist metal toxicity, such as polymer traps, enzymatic oxidation, precipitation, and efflux pumps, may well provide the genetic engineer with the means to modify existing species to enhance metal

accumulating properties (Wood and Wang 1983). The ability of *Sphaerotilus natans*, in particular, to accumulate metals in the mucilaginous layer outside its sheath makes it ideal for concentrating and recovering metals that are present in low concentrations in wastewaters. Similarly, metals are bound in the extracellular slime of the zoogloeal growths that are a major component of the biomass of all aerobic biological treatment units. Hatch and Menawat (1979) found that *S. natans* grew well in the presence of sulphates of iron, magnesium, copper, cobalt, and cadmium, accumulating these metals in the outer layer of its sheath. However, growth was inhibited in the presence of nickel and chromium sulphate, and in chlorides of iron, magnesium, copper, and cobalt. So far, the ability of the *Sphaerotilus-Leptothrix* group of bacteria to recover metals has not been fully exploited, although the potential is enormous. The bacteria could be particularly useful in removing low-level metal contamination from wastewater or even reducing the metal load of rivers *in situ*. For example, Gray and Clarke (1984) found that *S. natans* was able to accumulate cadmium when grown at concentrations ranging from 0.001–0.1 mg l^{-1}, reaching maximum concentrations in the bacteria of between 12.5–19.6 μg g^{-1} dry weight. Another potential area is in the treatment of radioactive contaminated water used in the processing of nuclear fuel. A number of heavy metals are present in this wastewater, in particular, uranium. Although conventional methods are generally ineffective or very expensive, experiments using the yeast *Saccharomyces cerevisiae* and the bacterium *Pseudomonas aeruginosa* were both able to recover the metals from solution. The bacterium was more effective than the yeast with the uranium concentration in solution approaching the equilibrium value after a contact time of only 10 minutes (Schumate *et al.* 1979). Initially, uranium binds to an active site but then additional accumulation occurs by crystallisation on to the bound molecules (Beveridge and Murray 1976). The rate and degree of uranium recovered from solution by the bacterium suggests that such a system could be used to decontaminate process wastewaters from the nuclear fuel cycle and allow the uranium to be recycled. Like all metals bound to a microbial surface, uranium can be elutriated by acid hydrolysis. Both yeasts and fungi can also accumulate metals (Norris and Kelly 1980; Gray and Clarke 1984), with metal accumulation in the yeast *Saccharomyces* being particularly effective, reaching 10–15% of its cell weight. Two particular species have demonstrated tolerance to particularly toxic metals. The fungus *Chrysoposporium* is able to absorb mercury (Williams and Pugh 1975), and the bacterium *Citobacter* is able to accumulate cadmium (Macaskie and Dean 1984). Such species could be particularly useful for detoxifying wastewaters.

Biosorption is defined as the accumulation and concentration of pollutants from aqueous solution by the use of biological materials thus allowing the recovery and/or the environmentally acceptable disposal of the pollutants. Aksu and Dönmez (2001) have summarised the mechanisms involved with biosorption by micro-organisms. In natural situations, biosorption normally involves a combination of active and passive transport mechanisms. First the metal ion diffuses to the surface of the cell wall of the micro-organism. It then binds to sites on the cell wall surface that exhibit some chemical affinity for the metal. A number of passive accumulation processes are involved in this step that may include adsorption, ion-exchange, coordination, complexation, chelation, and microprecipitation. This step is generally considered as adsorption and is both fast and reversible. It may be followed by a slower phase where the metal becomes permanently bound. This may involve a number of different mechanisms such as covalent bonding, surface precipitation, redox reactions, crystallisation on the cell surface, diffusion into the cell and subsequent binding to proteins or other intracellular sites.

Biosorption is achieved using either non-living biomass or by bioaccumulation by living cells or biomass, including activated sludge or anaerobic biomass (Haytoglu *et al.* 2001). The use of dead biomass is advantageous, as it is not affected by high concentrations of metals found in some wastewaters, or inhibited when significant amounts are absorbed by the micro-organism. Also, dead biomass is not affected by toxicity nor requires nutrients or a complex growing environment. Dead cells or biomass can be stored for extended periods at room temperature without putrefaction occurring. Cells are killed by physical methods such as drying or heat treatment, or chemically by acidic, caustic or organic treatment. The preparation of the biomass can have significant effects on its biosorptive properties. Yeasts are the most widely employed biomass for biosorption of metals as it is readily available as a waste product of many fermentation processes and hence, relatively cheap (Huang *et al.* 1990; Avery and Tobin 1993; Brady *et al.* 1994; Brady and Duncan 1994; Volesky and May-Philips 1995; Dönmez and Aksu 1999). Fungal biomass has also been widely studied as an absorbent for metals (Fourest *et al.* 1992) as have a wide variety of other biomass materials (Volesky and Holan 1995) that have been successful in detoxifying metal-rich industrial wastewaters and recovering precious metals (Greene *et al.* 1986; Kuyucak and Volesky 1989; Volesky and Holan 1995).

All algae and aquatic plants, including liverworts (Yoshimura *et al.* 2000) and cyanobacteria (Corder and Reeves 1994), accumulate metals from

their environment. Therefore it is not surprising that so much attention has been paid to their potential in bioremediation (Ornes *et al.* 1991; Sen and Bhattachayya 1994; Aksu 1998; Dönmez *et al.* 1999). Plant health and growth is not normally affected by metal uptake. For example, *Salvinia* and *Spirodella* are reported as having concentrations of Cr, Ni, Pb, and Zn, hundreds of times higher in their dry biomass than in water (Srivastav *et al.* 1993, 1994). In laboratory experiments, *Cladophora* has been shown to be able to remove 86–96% of the cadmium in water. The cadmium was added as a single spike of 5 mg l^{-1} and as several smaller does of 1 mg l^{-1} each day. Concentration factors ranged from 1,340 to 16,400 with the concentration of cadmium in the biomass as high as 1.64% dry weight (Sobhan and Sternberg 1999). The use of live plant material is primarily for the treatment of low concentrations of metals (Ray *et al.* 1993). Living biomass bioaccumulates metals to 30–50 times that found in the water, although higher levels are possible (Wang and Wood 1984; Konhauser and Fyfe 1991). Algal biomass has been successfully used to remove Cu and Cd from contaminated water (Chu *et al.* 1997; Kratochvil *et al.* 1998). Dead biomass, that has been chemically conditioned, is also used for metal removal, mainly for more concentrated waste streams (Churchill *et al.* 1995). This works by adsorption only and has the disadvantage that the biomass cannot be regenerated naturally *in situ*. Marine algae are also used for biosorption, especially the brown seaweeds of the genus *Sargassum*. This genus can adsorb most metals, including gold and uranium (Yang and Volesky 1999); some at concentrations up to 20% of the biomass dry weight (Kuyucoak 1989; Leusch *et al.* 1995). However, up to 40% of the biomass weight may be lost when it is pretreated with NaOH, 10–20% while metals are being adsorbed, and a further 5–15% during desorption of the metals by acids. So a constant supply of biomass is needed (Fourest and Volesky 1996). To increase the stability and mechanical strength of such biomass it can be immobilised onto a porous solid or embedded into a chemical matrix. Tan *et al.* (2002) have successfully immobilised *Sargassum baccularia* biomass in polyvinyl alcohol (PVA) beads that are then used in fixed columns.

Chemico-physical methods for the removal of heavy metals include chemical precipitation, electrodeposition, ion-exchange, membrane separation and adsorption. Chemical precipitation is economic but inefficient for dilute solutions, while ion-exchange and reverse osmosis are effective but expensive to operate and suffer from fouling problems. Adsorption using low cost natural materials such as dried biomass, agricultural wastes, clay materials, coal bottom ash, and seafood processing wastes are increasingly popular alternatives to conventional processes (Volesky and Holan

1995; Bailey *et al.* 1995; Lin and Yang 2002). Peat, being plentiful and inexpensive, has been extensively studied as an adsorption medium for metals although has not been widely adopted due to slow loading rates (Brown *et al.* 2000). However it is easy to use and the adsorption sites can be regenerated by acid elution. Of particular interest is chitosan, which is a hydrophilic natural cationic polymer formed by the *N*-deacetylation of chitin which is present in fungi, insects and crustaceans. Commercial chitosan is produced from the exoskeletons of crustaceans such as shrimp, crab, lobster and crawfish (No *et al.* 1989a), and is the second most abundant biopolymer after cellulose. Chitosan is a very effective ion exchanger due to the presence of a large number of amino groups. The chemical synthesis of chitin to chitosan significantly increases the presence of these amino groups increasing its adsorption capacity. Chitin is prepared by cleaning the source material (e.g. crab shells) then soaking overnight in 1% NaOH solution at room temperature. This removes the bulk of the protein. The material is then rinsed in distilled water and dried at 100°C for 48–60 hours. The material is milled so that it passes through a 4.75 mm sieve and then demineralised by soaking for 6 hours in 5% HCl solution. After washing in distilled water deacetylation is achieved by soaking in 50% NaOH solution for 6 hours at 90°C. The chitosan is then washed repeatedly in distilled water until the pH is neutral plus, air dried and sieved into size classes ready for use (Coughlin *et al.* 1990; No and Meyers 1997).

Chitosan is a particularly acetylated glucosamine biopolymer that is also found in the walls of certain fungi used as biosorbents (Felse and Panda 1999). Heavy and radioactive metals are rapidly adsorbed onto both raw and chemically modified chitosans (Knorr 1984; Coughlin *et al.* 1990; Onsoyen and Skaugnud 1990). It is an excellent metal adsorbent having been used to remove arsenic (Elson *et al.* 1980), cadmium (Evans *et al.* 2002), chromium (Udaybhaskar *et al.* 1990), cobalt (Masri *et al.* 1974), copper (McKay *et al.* 1990), lead (Masri *et al.* 1974), gold (Ngah and Liang 1999), manganese (Masri *et al.* 1974), mercury (McKay *et al.* 1990), molybdenum (Guilbal *et al.* 1999), nickel (McKay *et al.* 1990), silver (Masri *et al.* 1974), vanadium (Guibal *et al.* 1998), and zinc (McKay *et al.* 1990). The adsorbent properties of chitosan have been improved by further chemical modification. For example, Navarro and Tatsumi (2001) successfully introduced polyethyleneimine onto chitosan by its reaction with epoxide groups of grafted poly(glycidyl methacrylate) chains which enhances its metal chelating properties as well as improving its physical stability under acidic conditions. Chitosan is primarily used as a non-toxic cationic flocculant in the treatment of organically polluted water (Deans *et al.* 1995; No

et al. 1989b). It is also used in potable water treatment (Strand *et al.* 2002) where its anti-bacterial properties are also employed, and as a conditioner for sewage sludge (Lee *et al.* 2001).

10.2.4. *Phosphorus recovery*

A number of pathways are possible for the industrial recovery of phosphates in wastewaters: (i) precipitation of phosphates in a reusable form; (ii) production of high phosphate composts; (iii) extraction of phosphorus from sewage sludge; and (iv) extraction of phosphorus from sludge incinerator ash. One of the most attractive methods of recovering phosphate in a usable form is by its precipitation from liquids containing dissolved phosphates. This gives the opportunity to obtain relatively pure phosphate in a physical form that is adapted to transportation, processing and recycling as dry pellets. Biological nutrient removal produces phosphate rich sludges that produce supernatants with high concentrations of phosphates when digested. The introduction of the Urban Waste Water Treatment Directive (97/271/EEC) and the resultant imposition of nutrient limits to control eutrophication in surface waters has resulted in ever increasing quantities of phosphate rich sludges being produced. Such high levels of phosphate has restricted the use of sewage sludges to agricultural land due to lower requirements for the nutrient by farmers and increased awareness of eutrophication arising from phosphate fertilisers. Therefore, removing the phosphate allows greater utilisation of sludge permitting greater volumes to be spread per hectare of land (Sec. 8.2.2). Phosphate precipitation, either as calcium phosphate or struvite (magnesium ammonium phosphate) is currently the main recovery method employed. Calcium phosphate is essentially the same material as mined rock phosphate that is used by industry and so can be readily substituted. In contrast, struvite has limited industrial use and can only be used as a fertiliser at present. The phosphate industry in Western Europe has declared its intention to replace 25% of mined phosphate by recycled phosphates by the year 2010 (Fielding 2001) making effective recovery of phosphate from wastewaters an urgent priority.

Where calcium and phosphate ions are present in solution a number of different calcium phosphate compounds can be formed (Table 10.5). However, in practice it is the thermodynamically more stable form hydroxylapatite (HAP) that is normally formed. Precipitation of HAP can occur either spontaneously with the growth of individual crystallites in solution (i.e. homogeneous nucleation) or by aggregation of crystallites on existing surfaces of a seed material (heterogeneous nucleation). Fluidised bed

Table 10.5. The molar ratios and solubilities of calcium phosphates, where MR is the molar ratio of calcium: phosphate and SP their thermodynamic solubility product (Valsami Jones 2001).

Brushite or dicalcium phosphate dihydrate (DCPD)	$CaHPO_4 \cdot 2H_2O$	MR = 1.0	SP = 2.49×10^{-7}
Monetite (DCPA)	$CaHPO_4$ (Anhydrous DCPD)	MR = 1.0	SP = 1.26×10^{-7}
Octacalcium phosphate (OCP)	$Ca_4H(PO_4)_3 \cdot 2.5H_2O$	MR = 1.33	SP = 1.25×10^{-47}
Amorphous calcium phosphate (ACP)	$Ca_3(PO_4)_2$	MR = 1.5	SP = 1.20×10^{-29}
Hydroxylapatite (HAP)	$Ca_5(PO_4)_3O$	MR = 1.67	SP = 4.7×10^{-59}
Tricalcium Phosphate (TCP)	$Ca_3(PO_4)_2$	MR = undefined	SP = variable, more soluble than crystalline phosphates

reactors are normally used with sand granules as seed material on which the phosphate develops. Bone char, small particles of rock phosphate, calcium carbonate, activated carbon or marble have also been used for this purpose. The influent enters the bottom of the reactor fludising the medium. Supersaturation is achieved by increasing the pH by stripping the carbon dioxide from solution before entry to the reactor or by the addition of an alkaline solution along with either calcium or magnesium ions to form HAP (Valsami-Jones 2001).

Struvite has been widely reported as a problem with digested sludge supernatants, producing a crystalline deposits that blocks screens, pumps, and pipe work (Rawn *et al.* 1939; Borgerding 1972; Mohajit *et al.* 1989; Williams 1999). It is this phenomenon that has led to the exploitation of this crystalline compound as a recovery process for phosphate in wastewaters. Struvite forms distinctive white orthorhombic crystals consisting of magnesium ammonia and phosphorous in equal molar concentrations:

$$Mg^{2+} + NH_4^+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O$$

Precipitation is controlled by pH, degree of supersaturation, temperature, and the presence of impurities (e.g. calcium). Struvite crystals are formed when the concentrations of Mg^{2+}, NH_4^+ and PO_4^{3-} exceed the solubility product (K_{sp}) for the compound (Bouropulos and Koutsoukos 2000). The relationship between K_{sp} and pH shows that struvite solubility decreases with increasing pH leading to increasing precipitation (Lowenthal *et al.* 1994). Hirasawa *et al.* (1983) found that optimal struvite precipitation occurred when there was excess ammonium ions, a pH in the range of 9–10, and an Mg: PO_4 molar ratio of > 1 (Fig. 10.5) (Table 10.5).

Struvite recovery is particularly successful where BNR systems result in phosphorus-rich sludges. During digestion, the phosphorus is released into solution and as long as the K_{sp} value is exceeded, struvite precipitation can be achieved. Fluidised bed reactors are generally used with anthracite, quartz or silica sand as seed material. Calcium phosphate or struvite can also be used when a purer product is required. There are two approaches to struvite formation: (i) to raise the pH by dosing with NaOH or $Mg(OH)_2$, or alternatively aerating the liquor to strip out the carbon dioxide and by altering the carbonate chemistry raise the pH. The latter has been successfully employed at a full-scale struvite recovery plant in Treviso in Italy which achieved up to 89.6% phosphorus removal (Battistoni *et al.* 2000); (ii) an alternative method is to increase the concentration of one of the constituent ions of struvite, usually magnesium. This can be done cheaply

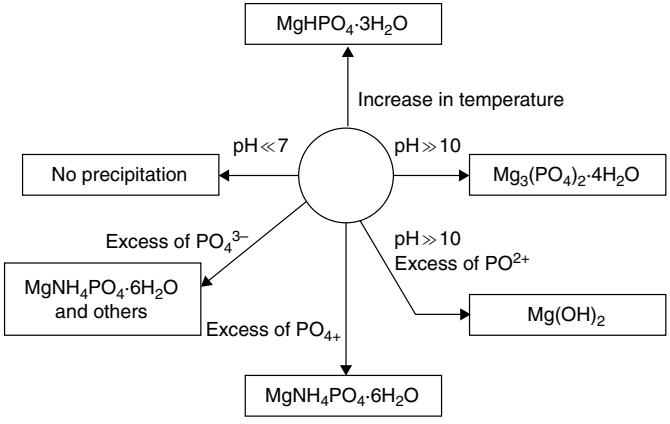


Fig. 10.5. Key factors affecting struvite solubility (Hirasawa *et al.* 1983).

using seawater as a source of magnesium or by using magnesium hydroxide that increases both the pH and ion concentration simultaneously. Munch *et al.* (2000) used 55% mg$(OH)_2$ in a 42% water slurry in a pilot scale struvite crystallisation reactor (Fig. 10.6) This supplied magnesium up to stoichiometry with the soluble phosphate and increased the pH to facilitate rapid crystallisation. The influent wastewater had a pH of 7.8, NH_4 790 mg l^{-1}, soluble Mg 11 mg l^{-1} and soluble phosphate 61 mg PO_4–P l^{-1}. The magnesium slurry was added to maintain a Mg:P ratio of 1.3, which is about 30% higher than the stoichiometry of struvite requires but ensured optimum crystallisation rates. The effluent pH was 8.5 with the overall phosphate removal at 95%. The final effluent has a concentration of 4 mg $PO_4$0–P l^{-1} at a retention time of 1–2 hours. Ratios of Mg:PO_4 lower than 1.05 result in a mixture of struvite and hydroxylapatite being formed so operationally the most effective ratio is 1.3 (Jaffer *et al.* 2002). Magnesium chloride is often used in pilot scale reactors in place of magnesium hydroxide as it disassociates faster resulting in shorter retention times.

Struvite is thought to be a potentially useful fertiliser (Munch and Barr 2001), especially because the heavy metal contamination is much lower than in natural rock phosphate (Driver *et al.* 1999). Jaffer *et al.* (2002) have estimated that 42–100 tonnes of struvite will be produced annually from the sludges at Slough Sewage Treatment Works in London worth between 14,000–34,000 euro. The economics and feasibility of struvite recovery is discussed in detail by Doyle and Parsons (2002).

Phosphorus levels in sludge incineration ash is about 50% that in mined rock phosphate, although the quality of rock phosphate is falling as reserves

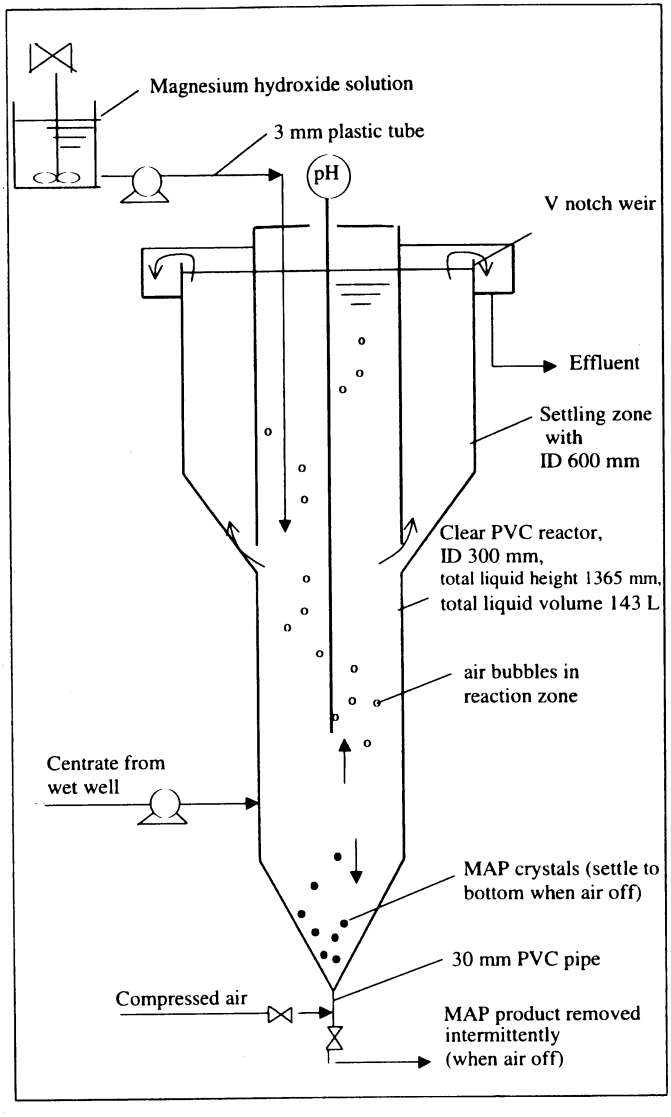


Fig. 10.6. Pilot-scale struvite reactor (Münch and Barr 2000).

are used. The phosphorus in ash is largely ferric phosphate, which is not an effective fertiliser as the phosphorus is largely unavailable to plants. Also, there is no significant industrial use for iron phosphates at present. A number of processes are being developed to recover the phosphate and metals separately from the ash (Hensen *et al.* 2000). For example, after

incineration the ashes are leached with sulphuric acid and the various anions and cations recovered separately using ion-exchange (Hultman *et al.* 2001).

10.3. Biological Conversion

10.3.1. *Bio-energy*

Energy produced from the bioconversion of wastewater sludges is mainly confined to the production of biogas from anaerobic digestion, although the production of alcohol from various other types of wastes is also important. Energy can also be produced from waste material by a variety of other processes including combustion, pyrolysis, liquefaction, and gasification.

The simplest method of obtaining energy from organic waste and biomass is to burn it, with combustion in suitable devices being one of the most efficient methods of utilising the energy potential of these substances. For example, in direct fired furnaces and steam boilers, it is possible to achieve thermal efficiencies of up to 85%, although in practice they are much less efficient. The combustion of biomass ($C_xH_yO_z$) can be expressed as:

$$C_xH_yO_z + (x + y/4 - z/2)O_2 \rightarrow xCO_2 + (y/2)H_2O$$

with the heat produced varying between 16–24 GJ t^{-1} of oven dried biomass.

The presence of water in the biomass does not reduce the thermodynamic heat yield of the combustion reaction, although in practical terms the efficiency of the reaction is severely reduced because of the need to heat the water and evaporate it off. A moisture content $> 30\%$ will prevent direct burning so that the material must be either dried, or a supplementary fuel added. Recent advances in incinerator design have increased efficiency, with fluidised bed incinerators able to accept material containing up to 55% water. Wood and straw are the most widely used biomass materials for combustion, although solid animal wastes, sewage sludges and composted sludge containing up to 75% water have all been utilised. However, preliminary drying is essential (Boyle 1984), and this is normally done by using the flue gases from the actual combustion process (Pedersen 1982) (Sec. 8.1.1).

Pyrolysis and gasification

Pyrolysis, liquefaction, and gasification are upgrading processes, converting biomass into a stable and transportable fuel that can be substituted for

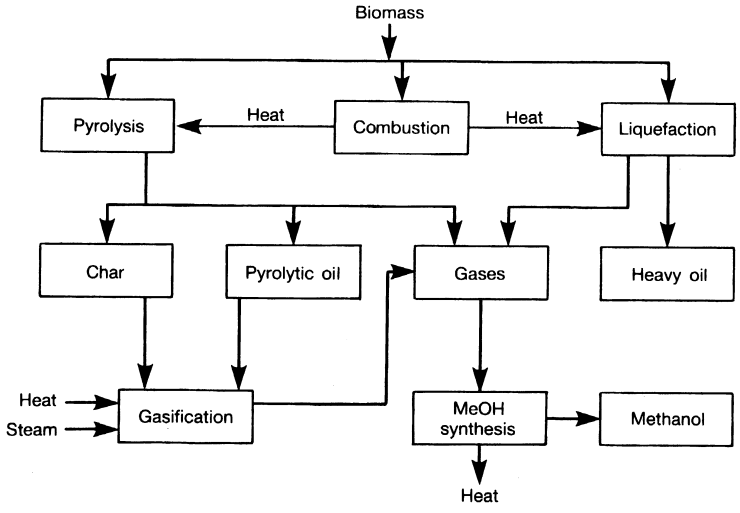


Fig. 10.7. Thermal upgrading of biomass.

conventional fuels. Solids, liquids, and gases can be produced from biomass including wastes, by a variety of processes which have similar properties to coal, oil and natural gas. When heat is applied to biomass, water is released, and above 100°C, the biomass begins to decompose. Between 250–600°C, the main products are charcoal and an oily acidic mixture of tar with variable quantities of methanol, acetic acid, acetone, and traces of other organic molecules. Before the wide availability of fossil oils, the destructive distillation of wood provided the major source for these chemicals. The reactions are very complex and are summarised in Fig. 10.7. There are various adaptations of this process that are reviewed by Boyle (1984), but it is clear that domestic and agricultural wastes have not been fully exploited by these techniques, and the potential for the production of fuel oils is particularly encouraging.

Pyrolysis is the thermal degradation of carbonaceous material at temperatures of 400–800°C in the absence of oxygen (Suzuki *et al.* 1988; Campbell and Bridle 1989; Stammbach *et al.* 1989). The products are gases, a mixture of liquid hydrocarbons (Conesa *et al.* 1998), and a char residue. Heavy metals, except mercury and cadmium, which are present as salts are safely bound to this inert carbonaceous residue (Kislter *et al.* 1987; Briddle *et al.* 1990). As a result, leaching of metals is less likely from the char than from incinerated ash (Caballero *et al.* 1997). The char can be subsequently gasified in order to significantly increase energy recovery. The kinetics of

thermal drying, pyrolysis and gasification are reviewed by Stolarek and Ledakowicz (2001).

Gasification is the thermal conversion of carbonaceous material to gaseous hydrocarbons by partial combustion with air at temperatures of 900–1100°C. The process uses sewage sludge with a high dry solids content of 90–95% and so is used in conjunction with thermal dryers as an alternative to incineration. The advantage of gasification is that it can produce sufficient fuel to power both the thermal dryer and the gasifier producing an inert residue suitable for landfill. The environmental advantages are the reduction in the use of fossil fuel and a reduction in associated transport. The process is very cost-effective with capital costs quickly recovered. Gasification is considered as a *best available technology* for sludge disposal. High temperature pyrolysis is a hybrid of both processes and produces gaseous hydrocarbons including methane, hydrogen, and higher hydrocarbons (C_2–C_6) with a calorific value of 29 MJ m^{-3} compared with 36 MJ m^{-3} for natural gas. Dried sludge is combusted in an inert atmosphere of nitrogen at 700–1000°C (Fig. 10.8). The gases are rapidly cooled as they leave the reactor and then passed through a wet scrubber. The gas mixture can be used to generate electricity using gas turbines or spark ignition engines. A number of experimental units of this design have been built in the UK.

A new method for pyrolysing sewage sludge using a microwave furnace has been proposed by Menéndez *et al.* (2002). They describe how if wet sludge is microwaved then only drying takes place, but if a suitable

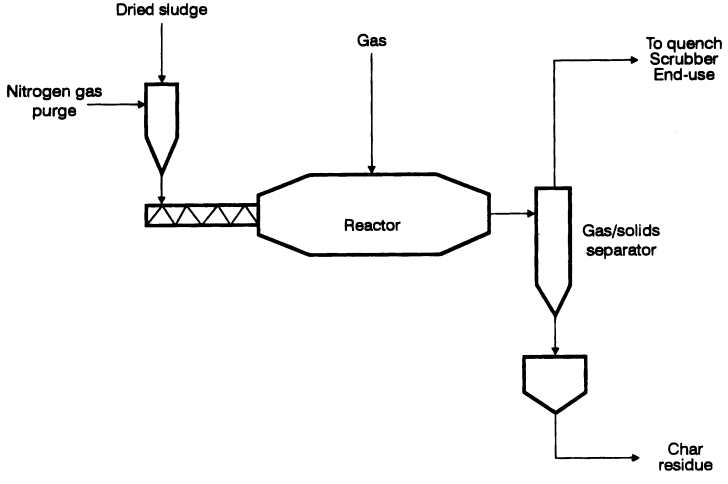


Fig. 10.8. Basic layout of a gasification plant (CIWEM 1999).

microwave absorber, such as waste pyrolysis char, is added then the temperature rises to 900°C with pyrolysis occurring instead of drying. Low temperature thermo-chemical conversion processes that turn sewage sludge into oil are also being studied (Bayer and Kutubuddin 1987; He *et al.* 2000, 2002).

Biogas

Biogas recovery is the most widespread bioconversion process used for wastewaters and for domestic sewage and animal slurries, in particular. Since its introduction in 1901, anaerobic digestion has developed from being a process for stabilising sludge with the evolved gas often vented to the atmosphere, to a highly sophisticated energy producing process. The digestion process is fully explained in Sec. 7.2.2, but suffice it to say that anaerobic digestion is based on a complex microbial community made up of three distinct groups. The hydrolytic fermentative bacteria hydrolyse the complex organic material to organic acids, alcohols, esters, and sugars, generating carbon dioxide and hydrogen. The second group is dependent upon the first, and is comprised of hydrogen-producing and acetogenic bacteria. Finally, the methanogenic bacteria convert the acetate and hydrogen into biogas which is a mixture of methane and carbon dioxide.

Biogas produced from domestic wastewater comprises 65–70% methane, 25–30% carbon dioxide, and small amounts of nitrogen, hydrogen, and other gases. Biogas has a specific gravity of about 0.86 compared with air. With the conventional digesters built at treatment plants up to the early 1970s, a 30-day retention time was typical, with only a 50% conversion of the organic matter achieved. New reactor designs today ensure efficient and rapid digestion with maximum production of methane, with digesters continuously fed and residence times as short as 10 days. Modern digesters tend to treat the wastewater rather than the sludge because in the wastewater the organic component is more likely to be in solution and so more amenable to digestion (Secs. 7.2 and 7.3). Apart from sewage treatment, the most common application of anaerobic digestion is for the treatment of vegetable and food-processing wastewaters, with reactors of between 2,000–20,000 m^3 in use. There are at present fourteen permanent major installations in the UK that are being used for the dual role of high-rate pretreatment, and the production of energy (Table 10.6). The best wastes for anaerobic digestion are the strong, warm wastewaters from the food- and drink-processing industries, where the majority of the organic material is in a soluble form. Using such ideal wastes, maximum loads of up to 25–30 kg COD m^{-3} d^{-1}

Table 10.6. Examples of some major industrial anaerobic treatment plants (Dr A.D. Wheatley, Cranfield University).

Owner	Waste	Location	Type of reactor	Contractor	Date commissioned
J. Sturge	Molasses	Selby	CSTR	Ames Crosta Esmil	1970 Rebuilt 1988
Tenstar Products	Starch	Ashford	CSTR	Biomechanics	1976
British Sugar Corporation	Sugar beet	Bury St. Edmunds	CSTR	Sorigana	1982
		Peter-borough	CSTR		1986
		Lincoln	CSTR		1988
McCains	Potato	Peter-borough	UASB/ Lagoon	ADI	1981
Swizzels Matlow	Confec-tionery	Stockport	Filter	Prototype ETA	1982
Caernarvon Creameries	Dairy	Chwilog, Caernarvon	UASB	Hamworthy	1982
Distillers	Yeast production	Stirling	Filter	Biomass	1986
Davidson	Paper mill	Aberdeen	UASB	Pacqs	1986
Wrexham Lager	Brewery	Wrexham	CSTR	Biomechanics	1987
Callard & Bowser	Confec-tionery	Bridgend	CSTR	CLEAR	1985
Hall & Woodhouse	Brewery	Blandford Forum	CSTR	CLEAR	1987
Cricket & Malherbie	Dairy	Mether-stowie, Somerset	CSTR	CLEAR	1987
General Foods	Starch/ coffee	Banbury	UASB	Biwater	1988
Tunnel Refineries	Starch	London	CSTR	Biomechanics	1988

can be achieved (McInerney *et al.* 1980). The rate of conversion of organic matter to carbon dioxide and methane is discussed in Sec. 3.4, although in practice, each kg of COD should yield about 350 l of methane. The total gas production is usually estimated from the volatile solids loading to the digester, with the volume of gas produced fluctuating over a wide range, depending on the volatile solids concentration in the sludge feed and

the level of microbial activity within the digester. Although various formulae are available for estimating the volume of biogas produced (Metcalf and Eddy 1984), it is possible to roughly estimate the volume on a per capita basis. For primary sludge, typical yields range between 15–22 m^3 per 1000 population equivalent (pe), whereas secondary sludges can produce up to 28 m^3 per 1000 pe each day. However, the methane content of the biogas is correlated to the chemical composition of the substrate, so the methane content of biogas produced from carbohydrate may be only 50% rising to 75% for alcohol substrates. Sewage sludge disintegration, the mechanical destruction of sludge cells to make them more biodegradable and so more accessible to digestion thereby increasing the methane gas yield, has been widely proposed (Baier and Schmidbeiny 1997; Bien and Wolney 1997; Kopp *et al.* 1997). Ultrasonic treatment is the most widely used (Tiehn *et al.* 1997; Chu *et al.* 2001), with the degree of disintegration of cells assessed by complex COD analysis (Kunz and Wagner 1994) or protein analysis (Schmitz *et al.* 2000).

Although methane production by micro-organisms occurs over a wide temperature range of 0–97°C, two distinct optima exist. Most digesters are mesophilic (35°C), although there is growing interest in thermophilic (60°C) digestion. The cider makers', Bulmers, have developed a thermophilic version of a fully mixed reactor to digest "still bottoms" at an operating temperature of between 60–70°C (Pickford 1984; Anon 1984b). Furthermore, the potential of digesters has been demonstrated by Caernarvon Creameries, who have perfected a high-rate digester that they use to treat 20–22 $\times 10^6$ litres of whey produced at the creamery each year, thus producing 775,000 m^3 of biogas (Anon 1983a).

In 1980, the gross energy content of sewage, wastewater from intensive livestock production, and industry-generated wastes in the then nine member states of the EEC was equivalent to 33×10^6 tonnes of oil (Anon 1982b). Most potential for biogas production appears to be from effluents from intensive animal rearing (Gibbs and Greenhalgh 1983). Although not all the 1.47×10^8 tonnes of animal waste produced in the EEC at that time was recoverable for biological conversion, as not all the animals are housed, this still represents an appreciable resource. Farm animals in the USA produce about 2 billion tonnes of waste annually, with about half of this produced by intensive animal production systems and therefore easily recoverable (Bryant *et al.* 1977). Although agricultural wastes of this kind are of variable concentration, they are generally considered to be highly polluting and a serious threat to water resources. In terms of biogas production, the stronger the effluent the greater its energy potential (Delaine

Table 10.7. Gas yields and methane content from the anaerobic digestion of various wastes (Delaine 1981).

Material	Gas yield ($m^3 kg^{-1}$ dry solids)	Methane (% volume)
Sewage sludge (municipal)	0.43	78
Dairy waste	0.98	75
Abbatoir:		
paunch manure	0.47	74
blood	0.16	51
Brewery waste sludge	0.43	76
Potato tops	0.53	75
Beet leaves	0.46	85
Cattle manure	0.24	80
Pig manure	0.26	81

1981). Therefore, all types of food industry wastes present a potentially very rich source of methane production. However, effluents with high fat and protein contents are even more productive than those containing high percentages of carbohydrates (Table 10.7).

Specific examples of anaerobic treatment plants, converting food-processing and agricultural wastewaters to biogas, are given by Dodson (1981) who also gives figures for treatment efficiency and gas production rates. Typical values are a 99% reduction in BOD and a gas production of 0.85 m^3 kg^{-1} BOD for milk-processing wastes at a retention time of 6 days. Although pea canning liquor has a shorter retention time of 3.5 days, it has a similar gas production at 0.87 m^3 kg^{-1} BOD but only a 75% BOD reduction. The effectiveness of anaerobic digestion and biogas production in treating strong effluents has been extensively reviewed. Isaac and McFiggans (1981) give details of the treatment of effluents from the malting, brewing, and distillery industries, with patented processes all claiming effective treatment and plentiful gas production (Newell 1982; Anon 1983b, 1984b). For example, the Bio-energy® system has been used in the treatment of effluent produced in the processing of wheat into starch, gluten and glucose, ham-processing effluent, cheese-processing effluent, and a range of other food-processing effluents (Anon 1983b). It is claimed that for every tonne of BOD removed, 0.45 tonnes of heavy fuel oil is recovered. The disposal of whey is the most serious problem that cheese manufacturers face, with about 10 tonnes of whey resulting from the production of each tonne of cheese, and the strength of whey varying from 32,000 to 60,000 mg BOD l^{-1}.

Hickey and Owens (1981) suggest that, on average, 35% of the operating costs of a cheese manufacturing plant could be recovered by the fermentation of the whey to methane with its subsequent use on site. The patented system, Bio-process®, is claimed to be able to recover up to 1 tonne of oil equivalent in methane for every 130 tonnes of whey treated (Anon 1984c).

It is the anaerobic treatment of domestic and municipal sewage that has received most attention and from which most of the expertise in digestion has been developed. Simplified versions of fully mixed reactors have found widespread applications in many developing countries, particularly China and India. Alaa El-Din (1980) estimated that about 5 million individual anaerobic digesters had been set up in China by 1978. About 5 m^3 of gas per day is generated by one 10 m^3 biogas plant. This is sufficient to supply a Chinese family with enough gas for cooking and lighting, with the sludge and effluent produced used as fertiliser and irrigant respectively. It is interesting that the average family does not produce enough waste to keep a digester of this size working at full capacity, so it is customary for each family to have at least one pig to help keep the digester fed with waste. Some larger collective digesters have also been built serving a number of families and even small villages. In Fu-Shang City near Shanghai, 45 m^3 capacity septic tanks each generate about 230 m^3 of gas per day which is converted to electricity. In India, extensive use of firewood for energy has led to widespread deforestation and apart from burning dung (which results in lower crop yields as there is no other source of fertiliser for the soil), there are no other locally available energy sources. Therefore, attention has been turned to locally produced biogas from animal dung and agricultural by-products. Parikh and Parikh (1977) describe in detail the potential of biogas production in India. They suggest that village plants of 170 m^3 capacity should be constructed to serve about 100 families, thus supplying all the energy for cooking and lighting. With 576,000 suitable villages in India, the widespread introduction of such a scheme could supply up to 45% of the total energy requirement of that continent.

Although it is generally agreed that small methanogenic systems are potentially viable, such as those designed for small communities, individual industries or in areas where there is no fuel resource, doubts exist on the ultimate economy of using wastes in this way (Hungate 1977; Loll 1977; Kirsop 1981). However, anaerobic digestion is now primarily used at larger treatment plants as a sludge stabilisation process, with the production of biogas often a secondary consideration. Much research has been done in the past decade to improve anaerobic digestion, especially in response to stricter guidelines for disposal of sludge to agricultural land. Thermophilic digestion

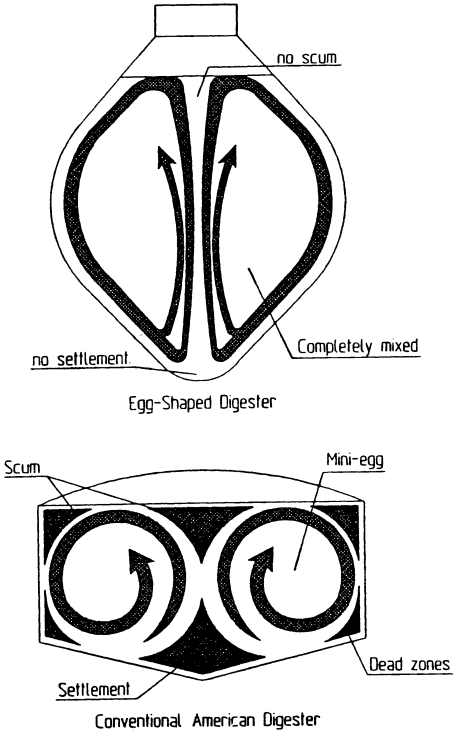


Fig. 10.9. Mixing patterns in conventional and egg-shaped digesters showing potential for scum and sediment formation (Ditchtl 1997).

is now widely used as a pretreatment step to disinfect and pre-condition the sludge (retention time 1–3 d) followed by conventional mesophilic digestion with reduced retention periods of 10–15 d compared to 20–30 d for normal single stage mesophilic digesters (Dichtl 1997). One area of rapid change is in reactor design itself. There has been a move away from the conventional tank design to egg-shaped digesters that are able to achieve near perfect mixing thereby avoiding stagnant areas of settled debris and sludge, and scum formation (Fig. 10.9). Conventional tank digesters are very susceptible to particulate settlement and scum formation, which seriously reduces mixing and operational efficiency (Fig. 10.10). This problem becomes worse as the diameter of shallow digesters is increased, with small obvoid shaped mixing zones formed within the tanks where the reaction between the liquid sludge and biomass occurs. In contrast, the shape of modern egg-shaped digesters ensures that the entire area of the reactor is utilised for treatment (Fig. 10.11) (Dichtl 1997).

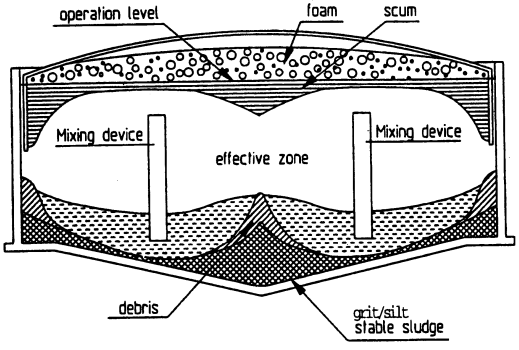


Fig. 10.10. Cross section through a conventional (American) digester (Ditchtl 1997).

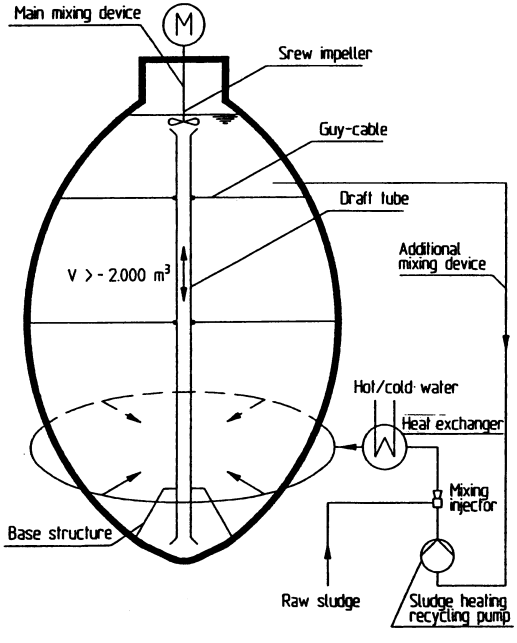


Fig. 10.11. Design of modern egg-shaped anaerobic digester (Ditchtl 1997).

There are three main options for the use of biogas: burning it to produce heat, or to generate electricity, or to fuel vehicles. The energy can be supplied to the National Grid, either in the form of gas or electricity, when sufficient biogas is produced. Each of these options requires further intermediate processing after production, ranging from simple storage until

required, to cleaning and compression in the production of fuel gas (LPG). Methane has a high calorific value of 35.8 MJ m^{-3} at standard temperature and pressure compared with 37.3 MJ m^{-3} for natural gas, which is a mixture of methane, propane, and butane. However, digester gas is only 65% methane, so without cleaning to remove the carbon dioxide, biogas has a typical calorific value of 23 MJ m^{-3}. At the sewage treatment plant, the gas is generally used in gas heated boilers to heat sludge digesters, and the excess is used to generate electricity for use on the plant. The electricity production at some intensive agricultural plants may be so high that they are able to sell the surplus energy via the National Grid.

The most exciting and cost-effective use of biogas to date has been its use to fuel vehicles. Ortiz-Canavate *et al.* (1981) have described the use of biogas in both spark ignition and compression engines. In a pilot study at the Modesto Wastewater Treatment Plant (California), compressed biogas has been used to fuel both cars and lorries. A similar study in the UK has been conducted at the Avonmouth Sewage Treatment Plant of Wessex Water where biogas has been used to generate electricity at the works for many years. Here, a full-scale trial was conducted to operate their eight vehicles on biogas rather than petrol. Before the raw gas can be used in high-efficiency internal combustion engines, the carbon dioxide content of the gas, which can be up to 45%, must be removed. The purified gas must then be compressed. A biogas processing plant was constructed (Fig. 10.12), which is able to convert up to 30 m^3 of raw gas per hour. It is a two-stage compression process, with the methane/carbon dioxide mixture scrubbed with the clarified effluent from the plant in order to remove carbon dioxide, and then further compressed to 198 bar for storage in cylinders. The cylinders are able to store 240 litres of compressed gas, which is 99% methane and produced at between 15–25 m^3 h^{-1}. The cylinders are connected to vehicle filling bays with standard LPG valves and snap-on hose connectors. Approximately 0.7 m^3 of gas is equivalent to 1 litre of petrol and the Avonmouth plant is producing up to 23 litres of petrol equivalent each hour, which is enough to satisfy 95% of their fuel requirements. In the winter, they have not been able to produce enough gas from their existing digesters, although during the summer there is a massive surplus, which at present is wasted. Modifications to the existing digesters will ensure an adequate supply of gas throughout the year in the future. Although the tax had to be paid on the gas, they estimated that the plant was saving them about £10,000 per annum in petrol costs in 1984. This figure does not include the surplus fuel that could be sold. Each converted van has two cylinders of gas located transversely behind the driver's seat. The only other

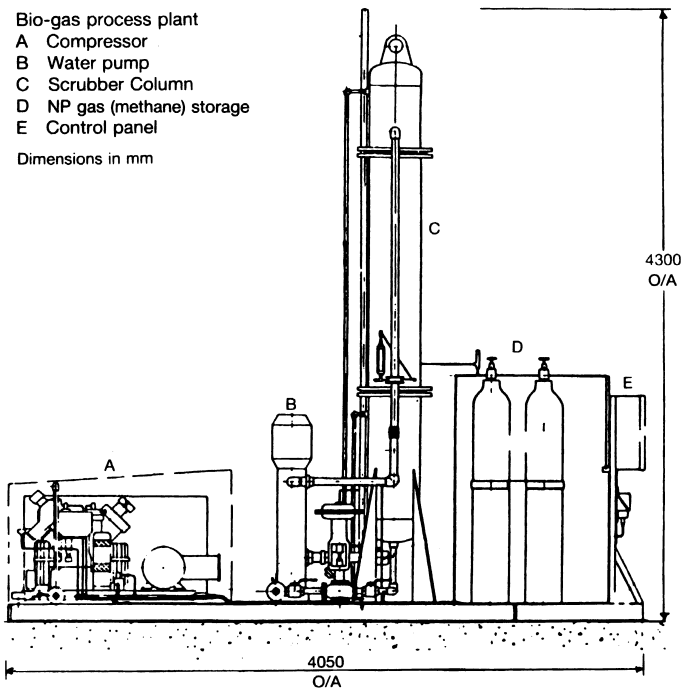


Fig. 10.12. The biogas (LPG) process plant at Avonmouth Sewage Treatment Works which is able to convert up to 30 m^3h^{-1} of 60% methane and 40% CO_2 biogas to high performance LPG with 99% methane and an output of between 15–25 m^3h^{-1} (Anon 1984e).

modifications required are the gas carburetor and the gas-petrol changeover switch. The cylinders in each van provide enough fuel for 160 km and if this is exhausted then the engine can be switched instantly over to petrol-drive without even stopping. The performance of gas is claimed to be as good as petrol, with claims of a cleaner engine which requires less maintenance, prolonged life of the engine oil, oil filter and the spark plugs, and a cleaner exhaust. The cost of the project at Avonmouth in 1982 was £30,000; approximately £25,000 for the plant and £5,000 for the vehicle conversions (Anon 1984e). A similar scheme is operated by Anglian Water at a sewage treatment plant in Essex (Anglian Water Authority 1982). They have also been working on compression engines, which are simpler to convert than spark-ignition engines. The gas can be used in mixture with the normal diesel fuel at a possible saving of 30% (Anon 1984e).

The most widespread use of biogas is for the generation of electricity (Sec. 7.2.2). For example, Athens Sewage Treatment Plant (Greece) has a

digester capacity of 40,000 m^3 offering 20 days average retention. The sludge is mixed within the reactor by biogas recirculation producing 72,150 m^3 of excess biogas each day. Combined heat and power (CHP) engines produce heat and power for the plant with excess electricity sold (Thomas *et al.* 2000). Thierbach and Hanssen (2002) provide a detailed description of an integrated system where biogas produced at the Köhlbrandhöft Wastewater Treatment Plant in Hamberg, Germany, is used in a combined gas and steam turbine process with the steam produced from sludge incineration also used to generate electricity by the process.

Fuel-alcohol

There has been increasing interest in the idea of obtaining chemical and lipid fuels from cellulose (Gallo *et al.* 1979). The production of fuel-alcohol (ethanol), in particular, has received considerable attention because of the pioneering work by the Brazilian Government who is attempting to replace all imported petrol (Gochnarg 1979; Ribeiro and Younes-Ibrahim 2001). However, it is clear that with present fermenter technology, fuel-alcohol can only be competitive with petrol when the world price of oil is high. However, it is now recognised that global warming is due in part to the carbon dioxide emissions from the use of fossil fuels in the transport sector. Thus the environmental advantage of fuel alcohol in cleaner emissions, safer production, and being renewable makes these fuels very attractive in the long term. Also, as the biomass (e.g. sugar cane) used in the production of fuel alcohol ensures almost complete reabsorption of the carbon dioxide emitted through its combustion as a fuel, it has a very positive contribution to make in controlling global warming.

Cellulose is converted to ethanol (ethyl alcohol) by a two-stage reaction (Kirsop 1981; Crueger and Crueger 1984). The crude cellulose, in the form of oligosaccharides and polysaccharides, generally requires to be hydrolysed to monosaccharides in a separate reaction before fermentation. Hydrolysis can be either chemical, using acids, or enzymatic, using cellulases obtained from bacteria and fungi, such as *Cellulomonas* spp., *Trichoderma vivide*, *T. lignovum*, *T. koningii*, *Chrysposporium lignonvum*, *C. pruninosum*, *Penicillium irieusis*, and *Fusarium solani* (Mandels 1975). Starch hydrolysis is relatively easy by using both acid and enzyme methods, whereas cellulose requires pretreatment to free the associated lignin before enzymatic hydrolysis. If the percentage of lignin in the crude cellulose is high, as is the case with a number of wastewaters, enzymatic hydrolysis is much less effective. Therefore, chemical or mechanical methods must be used instead of

enzymes, which results in high energy costs. However, a number of biological alternatives are being investigated. Using genetic engineering techniques, improved strains of the fungus *Trichoderma veesei*, may provide a suitable source of an enzyme able to cope with lignin wastes (Gallo *et al.* 1979). Kent-Kirk (1975) has reported on lignin-degrading enzymes produced by the white rot fungus that destroys wood (*Polyporus vesinosus*). He feels that whole organism rather than isolated enzymes may be more effective because the highly complex nature of 1ignin. Cooney *et al.* (1979) carried out the two processes of hydrolysis and fermentation in a single operation using the thermophilic bacterium *Clostridium thermocellum*. However, the reaction is slow and the yields poor, although they aim to eventually obtain an ethanol return of 25% of the total solids fed into the reactor. The major commercial problem with enzymes is that specific rates of cellulose hydrolysis currently achieved is low in comparison with acids (Boyle 1984). The hydrolysis reaction of cellulose or starch can be summarised as:

$$-(C_6H_{10}O_5)n- + nH_2O \rightarrow nC_6H_{12}O_6$$

The monomeric sugars are then fermented, anaerobically, by yeast such as *Saccharomyces* spp. to alcohol.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

The yeasts used for fuel alcohol production are all strains of *Saccharomyces cerevisiae* and so are genetically very similar (Gomes *et al.* 2000). Apart from sugar cane a wide range of materials can be used including oats (Thomas and Ingledrew 1995), wheat (Wang *et al.* 1999), silage (Henk and Linden 1994), and sugar beet pulp (Foster *et al.* 2001).

Crude cellulose can be obtained either as plant material or as waste. Although the latter is cheaper, the cellulose content of domestic and most agricultural wastes varies enormously. The main carbohydrates obtained from various wastes and plant materials are summarised in Table 10.8. The yield of carbohydrate varies considerably, for example, wood is composed of 60% cellulose (dry weight), where sugar beet and cane both contain between 15–20% of sucrose. Foster *et al.* (2001) have developed a pretreatment procedure for sugar beet pulp to increase hydrolysis efficiency of the cellulose component. Ammonia pressurisation-depressurisation literally explodes the pulp when the ammonia suddenly evaporates within the reactor vessel. Ultrasound, at 80 W and 38 kHz, is also used to degrade waste cellulose materials, such as paper, to produce glucose and other carbohydrates for the production of ethanol (Aliyu and Hepher 2000). There is considerable

Table 10.8. Sources of carbohydrates found in wastewaters (Boyle 1984).

Source	Carbohydrate
Monosaccharides and oligosaccharides	
Sugar cane and beet	Sucrose
Molasses	Sucrose, glucose, fructose
Dairy wastes	Lactose, galactose
Sweet sorghum	Sucrose, glucose
Polysaccharides	
Wood and crop residues	Cellulose, hemi-cellulose
Municipal and paper wastes	Cellulose
Maize and other cereals	Starch
Cassava and potatoes	Starch

interest in new enzymes. For example, alpha-L-arabinofuranosidases are able to convert a wide range of hemicellulosic substrates to fermentable sugars suitable for fuel alcohol production (Saha 2000) When used in conjunction with xylanases, they act synergistically to degrade xylan carbon sources to component sugars (Saha 2002). While frequently isolated from fungi (e.g. *Fusarium proliferatum*), xylanolytic enzymes are also isolated from some strains of bacteria (e.g. *Bacillus subtilus*) (Sa-Pereira *et al.* 2002).

Only 2% of Brazil's land will be required to supply enough cellulose to produce sufficient fuel-alcohol for their own requirements. The cellulose comes almost exclusively from sugar cane, which because of the high photosynthetic rate in the tropical climate of Brazil, grows so rapidly that three harvests a year can be taken. Alcohol is a non-polluting, anti-knock fuel that can be used instead of, or in combination with petrol (Humphrey 1975). On its own, it can also be used as boiler fuel with a thermal efficiency approaching 80%. However, compared with fuel oil its calorific value is only 57% by volume or 66% on a weight basis. Alcohol can be used directly as a substitute for petrol except that the performance is lower. Gashol is a blend of 99.9% alcohol and petrol, with some blends only containing up to 20% alcohol. Engines using gashol require only minor modifications compared to engines adapted to use alcohol alone. A major problem associated with using alcohol is that it is more corrosive than petrol so that storage tanks, pumps, and vehicle storage tanks all require to be lined with a protective material. Engine parts also wear out more quickly and engines using pure ethanol will require modification. The development of a suitable engine is continuing with current designs requiring additional lubrication and a higher compression ratio. Diesel engines do not function well on alcohol

or alcohol-diesel mixtures because up to 20% by volume of additives are required to achieve the necessary octane ratio (Rothman *et al.* 1981; Doyle 1984).

At present, the alcohol, which is a waste product of yeast metabolism, is removed constantly by removing a portion of the fermenter liquid and distilling off the alcohol, and returning the unused substrate to the reactor. The alcohol inhibits the yeast at concentrations approaching 8–10% and it must be continuously removed if the biological metabolism is not to be severely inhibited or even halted. In the production of alcohol, more energy is required to produce a unit of fuel-alcohol than the energy it contains. Many stages in the process of converting cellulose to alcohol are energy-intensive and none more so than the distillation stage. Kirsop (1981) has established that the energy costs of distillation to remove ethanol from the treated waste increases as the concentration of ethanol diminishes; Distillation is likely to be uneconomical, unless the ethanol concentration is greater than 5%, and to ensure this the carbohydrate concentration of the substrate should be in the order of 10%. An interesting proposal which will save considerable energy is to replace distillation with reverse osmosis to separate the alcohol.

New enhanced technologies are being developed to reduce the cost of ethanol production through reduced cooling costs, higher saccharification and fermentation rates, continuous ethanol removal, and reduced contamination (Wang *et al.* 1998). The use of thermophilic or thermotolerant yeasts have been an important factor in these improvements (Banat *et al.* 1998; Bayrock and Ingledew 2001). Morais *et al.* (1996) isolated a new strain of *Saccharomyces* isolated from tropical habitats that can tolerate temperatures up to 40°C, sucrose concentrations up to 50% (w/w), and ethanol concentrations up to 20 g l^{-1} during fermentation. Lactic acid bacteria (e.g. *Lactobacillus paracasei*, *Plantarum rhamnosus* and *P. fermentum*) can contaminate reactors during fermentation inhibiting ethanol production. Antibiotics such as virginiamgcin are used to control the bacterial contaminants (Hynes *et al.* 1997), while urea hydrogen peroxide has been proposed as a control when used at concentrations of 30–32 mmol l^{-1} (Narendranath *et al.* 2000).

Clearly, the production of ethanol from wastewaters is only economic when the wastewater has a high concentration of cellulose or other carbohydrate, is free from toxic materials, and when the cost of alcohol production can offset the cost of pollution control. The waste from the fermenter, which can be more than 10 times the volume of alcohol, is very polluting. The present policy in Brazil is to pump this effluent back to the cane fields

without any treatment. It may be possible, however, to devise a more integrated system where the effluent could be digested anaerobically to produce methane gas which could be used to fuel the distillation process as well as producing a useful and more handleable fertiliser. Plants are already in operation in Brazil and Australia, and orders have been placed for plants in Pakistan, France, and Germany that will produce 30,000 l of ethanol per day from cane molasses, and 90,000 l and 45,000 l of ethanol each day from beet molasses respectively (Anon 1984d). Unfortunately fuel alcohol remains unproven in the market place mainly due to a lack of investment (Bridgewater and Double 1994).

10.3.2. *Single-cell protein and biomass*

The link between biomass for food production and wastewater treatment arose from two particular developments. First, large crops of protein-rich algae grow on oxidation ponds (Sec. 6.3.2) and secondly, the conversion of wastes from various food-processing industries to yeast resulted in the purification of the wastewater as well as the production of a useful by-product. This has led to the development of numerous schemes for the utilisation of wastewater as substrates for the production of biomass or single-cell protein, and at the same time purifying the effluent (Samuelov 1983). The conversion of soluble and suspended nutrients to microbial biomass during the biological unit processes in conventional wastewater treatment has already been discussed and these gross solids in the form of activated sludge flocs or sloughed film from percolating filters have been used directly as an animal feed supplement (Grau 1980; Beszechits and Lugowski 1983; Shier and Purwono 1994). In countries where BSE is not a problem, carcass and blood meal are recovered in abattoirs and used as a feed for animals. Recovered protein sludge from the effluent stream of abattoirs can be added to carcus meal as a protein supplement (Couillard and Zhu 1993). De Villiers and Pretorius (2001) describe a modified SBR process with a sludge age of 4–7 d which produced a low SVI biomass from abattoir wastewater. Crude protein values of 27–37% were generated. Treated sewage has been used to increase fish production as well as improve final effluent quality (Henderson 1979; Hepher and Schroeder 1974), although fish for human consumption are required to be kept in unpolluted clean water for at least 2–3 weeks before use (depuration) (McGarry 1976). However, Coutinho and Gokhale (2000) studied the health of two fish species, *Oreochromis mossambius* and *Cyprinus carpio*, kept in secondary treated sewage effluents. They found that culturing *C. carpio* in effluent resulted

in starvation and extensive histopathological changes in the gills causing anoxia at cellular level. Also, by studying oxidative enzymes, they observed a shift in metabolic pathways which was reversed during depuration. The effects were not as severe in *O. mossambicus*. So great care is required when employing effluents in pisculture. The macro-invertebrates washed from fixed-film reactors have also been utilised as a source of food for non-intensive fish farming.

The use of cellulose based fibrous material and polyelectrolytes have been used to recover protein and other useful byproducts from food processing wastewaters, as well as effectively treating the wastewater. Chen *et al.* (2000) used both cellulose acetate and cellulose triacetate fibrets in conjunction with the polyelectrolyte carboxymethyl cellulose. All these materials are cellulose based and can be safely added to the feed of farm animals with the recovered protein. Carboxymethyl cellulose interacts with biomolecules by electrostatic and polymer bridging, while the fibrets facilitate floc development by adsorption and bridging of primary particles and by entrapment of small aggregates within their highly fibrillated microstructure. Carboxymethyl cellulose fibrets are oblong particles about 2 mm in length with a surface area of 20 m^2g^{-1}. When added to a liquid they become highly swollen fibrillated structures 5–60 μm wide and 100–600 μm long (Chen *et al.* 1996). Fibrets of cellulose triacetate are smaller (Smith 1988).

Single-cell protein (SCP) is microbial biomass produced by some form of fermentation process and can be used as a food or a food additive. In its simplest form, a suitable organism, such as a yeast, is cultured in a suitable substrate, normally a carbohydrate, such as a molasses solution, and under suitable conditions. The yeast or biomass is recovered from the fermentation by filtration, washed and dried to produce a free-flowing powder, rich in protein. The SCP is cheap to produce, versatile, and depending on its quality, can be used for either animal or human consumption. Its most promising applications are in the animal feed trade, although it is widely used in fortifying poor diets and in adding flavour and protein to processed foods (Rothman *et al.* 1981).

Various groups of micro-organisms have been used for the production of SCP, including bacteria, yeasts, fungi, and algae (Table 10.9). The easiest micro-organisms to be cultured and used for SCP production are the bacteria, but they are less acceptable in terms of palatability than yeasts and fungi, more difficult to separate, and contain considerably more nucleic acids. A wide range of bacterial species have been utilised, especially the photosynthetic bacteria, and these are reviewed by Kobayashi (1977) and

Table 10.9. Commonly used micro-organisms for single-cell protein (SCP) production and their substrate (Atkinson and Mavitune 1983).

Micro-organism	Substrate
Algae	
Chlorella sovokiniana	Carbon dioxide
C. regularis S-50	Carbon dioxide
Spirulina maxima (synthetic medium)	Carbon dioxide
S. maxima (sewage)	Carbon dioxide
Bacteria and actinomycetes	
Acinetobacter (*Micrococcus*) *certificans*	*n*-Hexadecane
Cellulomonas sp.	Bagasse
Methaslomonas clara	Methanol
Methylophilus (*Pseudomonas*) *methylotrophus*	Methanol
Thermomonospora fusca	Pulping fines
Yeasts	
Candida lipolytica (Toprina)	*n*-Alkanes
C. lipolytica	Gas oil
C. utilis	Ethanol
Hansenula polymorpha	Methanol
Kluyveromyces (*Saccharomyces*) *fragilis*	Cheese whey
Saccharomyces cerevisiae	Molasses
Trichosporon cutaneum	Oxanone wastes
Moulds and higher fungi	
Agaricus campestris (white var.)	Glucose
A. campestris (brown var.)	Glucose
Aspergillus niger	Molasses
Fusarium graminearium	Starch
Morchella crassipes	Glucose
M. crassipes	Sulphite waste liquor
M. esculenta	Glucose
M. hortensis	Glucose
Paecilomyces vaviota (Pekilo)	Sulphite waste liquor
Trichoderina viride	Starch

Ensign (1977). The most widely investigated groups are the yeasts and the *fungi imperfecti*. A number of higher organisms are also utilised for biomass and protein production, although they are not classed as SCP. These include worms from composting wastes and sludge (Sec. 10.3.3), fish as the top of the food chain in oxidation ponds, and the fast growing water hyacinth and other plants that are effective methods of wastewater treatment as well as being a useful by-product.

Although a wide variety of substrates can be utilised, few have been successfully exploited on a commercial scale (James and Addyman 1974;

Rose 1979a; Atkinson and Mavitune 1983) (Tables 10.8 and 10.9). The problem is that in order to produce a well-defined end product, a pure culture fermentation of a substrate with constant quality is required. Fermentations of substrates of consistent quality, such as molasses, whey or starch can successfully use pure cultures to ensure a constant product. Wastewaters, however, are rarely of constant quality. Domestic sewage, for example, is composed of multi-carbon substrates and mixed culture fermentation is required to ensure that all the carbon is utilised, thus providing adequate pollution control. Although mixed culture systems are more stable, easily maintained, and less susceptible to contamination, the product is less easily defined with an inconsistent nutrient value and possibly containing toxic metabolites and residues. Few demonstration plants have successfully produced SCP from sewage, although it is theoretically and technically feasible. The problem of contamination of the substrate by metals and toxic organic compounds that can inhibit microbial growth and become accumulated within the microbial biomass is a major drawback when municipal wastes are considered for SCP recovery. Also, inconsistency of the product and the relatively low value of the recovered protein suggests that although SCP production from sewage could be a useful by-product to partially offset wastewater treatment and sludge disposal costs, it will probably never be a major or economic source of protein.

De Villiers and Pretorius (2001) describe a modified sequencing batch reactor process used for the pretreatment of abattoir effluent that also produces SCP for animal feed. The pilot plant achieved COD reductions of $> 85\%$ and produced a sludge with a crude protein content of 27–37%. The optimum mean cell residence time to maximize protein production was between 4–5 days. Hill and Baier 1000) describe multi-level screening to pre-treat pig slurry to provide a balanced substrate for SCP production using algae. However, it is processes that produce large volumes of dilute carbohydrate wastes free of toxic materials are the most promising substrates for SCP production. These include milk and cheese processing, confectionery manufacturing, and food canning plants (Forage and Righelato 1979; Anupama and Ravindra 2000). These effluents have a high biochemical and chemical oxygen demand and are costly to treat, so the development of waste recovery using SCP production would offset the cost of treatment. The development of SCP processes using these effluents is widespread. For example, Bassetts of Sheffield are using a protein recovery process based on the dilute wastes from Liquorice Allsorts® manufacturing that was developed by Tate and Lyle Process Technology Ltd. The Swedish Symba® process is based on the production of yeast protein from

starch waste, especially from potato processing. Other investigations have used a range of wastewaters including paper mill wastes (Holderby and Moggio 1960) and cellulose materials (Callihan and Clemmer 1979), milk wastes (Wassermann *et al.* 1961; Meyrath and Bayer 1979; Wheatley *et al.* 1982), coconut and pineapple wastewater (Smith and Bull 1976; Prior, 1984) citric acid wastes (Braun *et al.* 1979), distillery-type wastes (Quinn and Marchant 1980; Tauk 1982), silage effluent (Arnold *et al.* 2000), and many more (Tomlinson 1976a,b; Rose 1979a). However, economic SCP production is limited to a small number of specific substrates (Norris 1981). A number of SCP production processes have been patented and are being operated commercially, whereas others remain at the demonstration stage (Table 10.10). The fall in the value of SCP in relation to other sources of protein, such as fish meal and soya suggests that there will be little further development in this field for the present. Research is continuing, however, with particular emphasis on dual SCP and biogas production. A commercial SCP, called BioProtein®, is widely used for animal feed and is produced by a mixed methanotrophic and heterotrophic bacterial culture using natural gas as the energy source. However, the SCP contains large amounts of nucleic acid making it unsuitable for human consumption. The manufacturers have developed a nucleic acid reduced variant to produce a product that will be acceptable for humans. Tests on the safety of this new product are currently underway (Molck *et al.* 2002). The methanogenic bacterium used is *Methylococcus capsulatus*, but to support growth over long periods in continuous cultures, a consortium of different heterotrophic bacteria are required (Bothe *et al.* 2002). Overland *et al.* (2002) describe growth trials on pigs fed with bacterial protein meal (BPM) produced by a mixed bacterial culture of *M. capsulatus*, *Alcaligenes acidovorans*, *Bacillus brevis* and *B. firmus*. They found BPM to be an excellent protein supplement and replacement. Cheaper methods of SCP production using existing systems are also being examined. Significant reductions in both capital and running costs can be made by avoiding sterilisation and controlling contamination by using high inoculum concentrations, cell recycling or a low pH. Methods of recovering the protein are generally expensive, especially those involving centrifuging, filtration, and spray or drum drying, and alternative methods of separation, such as flocculation, flotation, and screening should be used (Wheatley 1985). There has been a general movement away from dried SCP to a liquid end product. This means that the whole fermentation broth can be used after pasteurisation, which is sufficient if the protein is to be used as a supplement to ordinary animal feeds, although is probably less acceptable for use in human foodstuffs.

Table 10.10. Examples of plants producing single-cell protein (SCP) of feed grade (Atkinson and Mavitune 1983).

Plant class	Company	Plant location	Substrate	Type of organism	Plant size (tonne y^{-1})
Demonstration	British Petroleum	UK	*n*-Alkane	Yeast	4000
Demonstration	Dianippon	Japan	*n*-Alkane	Yeast	1000
Demonstration	Chinese Petroleum	Taiwan	*n*-Alkane	Yeast	unknown
Demonstration	ICI	UK	Methanol	Bacteria	1000
Demonstration	Kanegafuchi	Japan	*n*-Alkane	Yeast	5000
Demonstration	Kohjin	Japan	unknown	Yeast	2400
Demonstration	Kyowa Hakko	Japan	*n*-Alkane	Yeast	1500
Demonstration	Milbrew	US	Whey	Yeast	5000
Demonstration	Shell	Netherlands	Methane	Bacteria	1000
Demonstration	Svenska-Socker	Sweden	Potato starch	Yeast	2000
Semi-commercial	British Petroleum	France	Gas oil	Yeast	20 000
Semi-commercial	ICI	UK	Methanol	Bacteria	50 000
Semi-commercial	United Paper Mills	Finland	Sulphite waste	Yeast	10 000
Semi-commercial	USSR State	USSR	unknown	Yeast	20 000
Commercial	British Petroleum	Italy	*n*-Alkane	Yeast	100 000
Commercial	Liquichemica	Italy	*n*-Alkane	Yeast	100 000
Other systems	LSU-Bechtel		Cellulose	Bacteria	
Other systems	Tate and Lyle	Belize	Citric acid	Fungi	
Other systems	ICAITI	Guatemala	Coffee waste	Fungi	
Other systems	IFP		CO_2/Sunlight	Algae	
Other systems	General Electric		Feedlot waste	Bacteria	
Other systems	Mitsubishi		Methanol	Yeast	
Other systems	United Paper Mills	Finland	Pulp waste	Fungi	

Although SCP is of excellent nutritional value, it is very variable in quality and has a number of drawbacks that limit its widespread use. Yeasts, such as *Saccharomyces cerevisiae* and *Candida utilis*, and most fungi, are quite acceptable to animals and man, whereas algal and bacterial biomass are less so, being less palatable and containing undesirable levels of certain cellular materials. Possible health hazards include a high nucleic acid content and toxic or carcinogenic substances absorbed from the growth substrate by the microbial biomass. Also, as SCP is generally digested slowly in the digestive tract, allergic reactions or indigestion are possible (Pokvovsky 1975; Sinskey and Tannenbaum 1975; Garattini *et al.* 1979). There have been many reports of adverse reactions in humans following the consumption of microbial biomass (Scrimshaw 1975). For example, amounts of SCP comprised of *Aerobacter aerogenes* or *Hydrogenomonas eutropha* of between 12–25 g d^{-1} caused nausea, vomiting, and diarrhoea when fed to young female volunteers, although these bacteria caused no adverse effects when fed to animals (Waslien *et al.* 1968, 1969). *Candida utilis* is also known to have caused gastro-intestinal disorders when fed in quantities as little as 15 g d^{-1} (Scrimshaw 1975). If microbial biomass is to become widely used as a form of protein then more research on the physiological effects is urgently required.

Single cell protein from cultivated biomass is now widely used as a protein supplement replacing costly conventional sources of protein such as fish meal or soya meal. The current status, and developments, of SCP is reviewed by Anupama and Ravindra (2000).

Yeasts and fungi

The yeasts and fungi have been exploited more than any other microbial group for SCP production (Table 10.9). The filamentous fungi are easier to separate and dry than the yeasts, and have the advantage of having a better food texture than other micro-organisms. Full-scale trials have been conducted using *Trichoderma* and *Geotrichum* for the treatment of vegetable and fruit-processing wastes (Church *et al.* 1973; Zinno *et al.* 1999), and the latter fungus has also been used with distillery wastes (Quinn and Marchant 1980) and for the treatment of milk wastes, along with *Fusarium* (Wheatley *et al.* 1982). *Fusarium* is also used with many carbohydrate wastes (Munden and King 1973). In Iran, *Hansenula*, a fungi isolated from the effluent of ethanol fractories, has been used to treat molasses stillage from sugar beet as well as produce SCP (Shojaosadali *et al.* 1999). However, it is the yeasts that have made the most significant impact in the wastewater treatment

industry in terms of SCP production, especially strains of *Candida* and *Saccharomyces*. The production of SCP from yeasts is not new, producing yeast for food dates back to 1910, with both *Candida utilis* and *Saccharomyces cerevisia* used for this purpose. These yeasts are capable of using a range of carbohydrates, *Candida* using both pentoses and hexoses, whereas *Saccharomyces* can only utilise hexoses for growth. Sulphide liquor, for example, contains both pentoses and hexoses and in North America some 50,000 tonnes y^{-1} of yeast are produced from this source alone. World-wide yeast production is more than 5,000 tonnes per week and is used for both animal and human consumption. One particular use for this type of yeast is the production of the human foodstuff "Incaparina". The yeast is used to enrich maize flour with protein (3% yeast) and is widely available in Central and South America (Norris 1981). Brewers yeast (*Saccharomyces cerevisae*) has also been shown to be used as a partial replacement of fish meal for the farming of sea bass (*Dicentrarchus labrax*) (Oliva-Teles and Goncalves 2001).

Several strains of yeast can readily grow in silage effluent. Apart from excellent COD and phosphorus reductions, excellent yields of biomass were obtained using *Candida utilis* and a filamentous yeast *Galactomyces getrichium* (Arnold *et al.* 2000). Mixed cultures of lactic yeasts reduce whey more effectively than single cultures as they are able to utilise several sources of carbon simultaneously. This results in a greater removal efficiency of the COD and an increase in biomass yield. Cristiani-Urbina *et al.* (2000) recorded highest biomass yields using *Candida utilis* and *Torulopsis cremoris*, the former consuming some metabolic by products produced by the latter. In batch reactor tests, the average biomass yield was 0.75 g g^{-1} of lactose while COD removal was 95.8%. Yeast biomass is also rich in essential fatty acids and amino acids. For example, a detailed breakdown of the nutritional value of a yeast biomass (*Kluyveromyces fragilis*) grown on deproteinised whey and supplemented with 0.8% diammonium hydrogen phosphate and 10 ppm indole-3-acetic acid had a crude protein content of 37% and a low nucleic acid content (4.8%). The biomass was found to be rich in the essential fatty acids omega-3 and omega-6, representing 21.5% of the fatty acids present (Paul *et al.* 2002).

Carbohydrate is the most useful energy and carbon source available for SCP production. However, much of the available carbohydrate in wastewaters is in the form of large polymers, such as cellulose, and it cannot be utilised directly by many bacteria and fungi, especially on a large scale. Therefore, much attention has been focused on those wastewaters that are rich in fermentable substrates, such as sucrose-rich effluents from the

sugar industry, sulphite waste liquor from paper manufacture, and potato starch wastes. Although fermentable carbohydrates produced by agricultural industries are potentially very attractive, many are seasonal and large-scale development is unlikely to be economic.

Each year, thousands of tonnes of fruit and vegetables are wasted at packaging and canning factories. This led to the development of a novel process by Tate and Lyle to increase the protein content of such wastes, thereby converting them into a valuable product. The process involves solid substrate fermentation, using the mould *Aspergillus niger*. The company claims that the protein content of such waste with an adequate level of fermented carbohydrates can be increased to 20–30% using what is essentially a low-technology process (Davy 1981). Single cell protein and crude pectinolytic enzymes can be produced by fermentation of lemon pulp using *A. niger* or *Trichoderma vividae* (De Gregorio *et al.* 2002). *Candida utilis* can produce SCP from pineapple cannery wastes as the sole carbon and energy source (Nigam and Kukati 2002).

One of the largest SCP plants treating waste carbohydrate is that owned by the confectionery firm George Bassett and Co in Sheffield. This is a batch fermentation following heat sterilisation of the effluent (Fig. 10.13). A pure culture of *Candida utilis* is grown in the wastewater which is recovered following centrifugation and drying. The SCP is bagged and sold as a high-protein additive for animal feed. The present output for this plant is about 140 tonnes y^{-1} and it is able to reduce the COD of the wastewater by 65% and it is therefore weak enough to be discharged directly to the sewer. However, with the varibale value of protein the development of similar

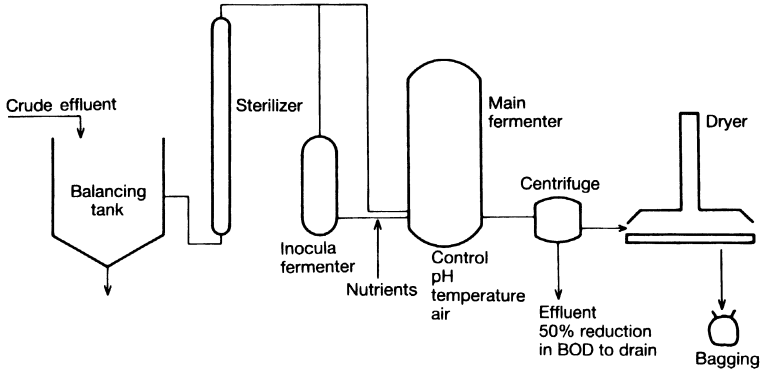


Fig. 10.13. Single cell protein (SCP) from confectionery effluent using *Candida utilis* (Wheatley 1985).

installations appears unlikely at present, although with the adoption of the "polluter-pays" principle system of effluent charges, it may be a cost-effective way of reducing the wastewater treatment bill for manufacturing and processing industries (Deering and Gray 1986).

There are many other examples. Romantschuk (1975) describes the Pekilo® process, which is a continuous process that uses large 360 m^3 fermenters to produce SCP from the polysaccharide-rich effluents from wood pulp production. The SCP yields obtained in this Finnish plant range from 2.7–2.8 kg m^{-3} h^{-1} using a mould of the genus *Paecilomyces* at a dilution rate of 0.2 h^{-1}. With a solution containing 32 g l^{-1} of reducing sugar, up to 55% of the sugar can be converted to biomass. A major process that has been developed in Ontario, Canada, is the Waterloo SCP process (Moo-Young *et al.* 1979; Moo-Young 1980) (Fig. 10.14). This is based on the mass cultivation of the cellulolytic, heat-tolerant fungus *Chaetomium cellulolyticum* or the yeast *Candida utilis* in an anaerobic fermenter using waste carbohydrate as the main carbon nutrient source. The yeast is grown in a liquid-substrate fermentation on sugars produced by acid hydrolysis of the original waste material, whereas the fungus is grown in a solid-substrate fermentation on the original waste which is pre-softened and partially delignified by thermal or thermochemical treatment. Supplementary nutrients

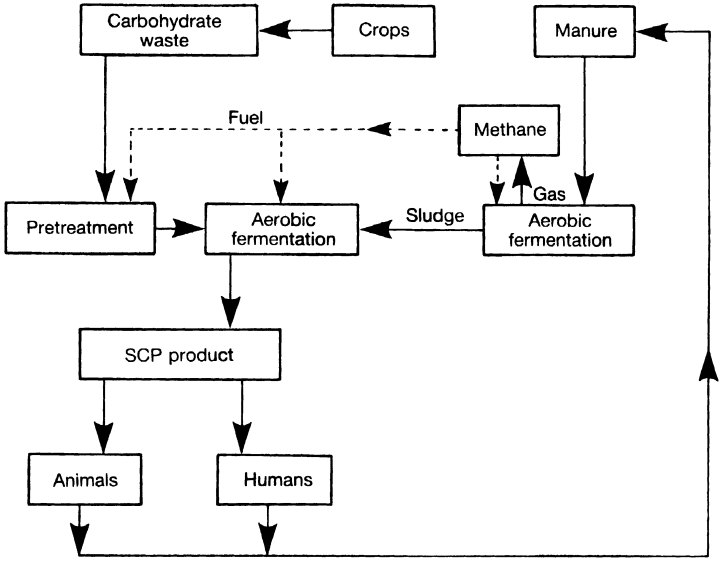


Fig. 10.14. Application of the Waterloo® SCP process to the utilisation of cattle slurry (Moo-Young 1980).

are required as is sterile air and a low pH. Anaerobically predigested manure is used for energy conservation, with the methane gas produced used to supply the energy required for the process. By recycling the processing water, the pollution potential of the final effluent is greatly reduced (Moo-Young 1980). The process appears to be particularly well suited for the treatment of wastewaters from intensive animal rearing.

Two SCP processes are of particular interest. The first is the treatment of distillery wastewaters from the manufacture of whisky. In 1978, the annual production of liquid wastes generated by whisky distilleries in Britain and Ireland was estimated to be 9.6×10^6 m^{-3} y^{-1} with a BOD equivalent to the wastewater generated by 6.1 million people (Quinn and Marchant 1980). Traditionally, distillery wastes were disposed to rivers, but now they are usually discharged directly to the sea, applied to derelict land, or evaporated and mixed with spent grain to produce distillers' dark grains that are sold as low grade animal feeds (Quinn and Marchant 1980, Quinn *et al.* 1981; Barker *et al.* 1982). The problems of safe disposal of large volumes of highly polluting wastes have increased in recent years because of high energy costs and increasing stringent anti-pollution legislation (Quinn and Marchant 1979). The spent wash produced by whisky distilleries is extremely strong, with a BOD of up to 43,000 mg l^{-1}, and are rich in carbohydrates and protein (Table 10.11). However, because of the high organic loading and the low pH, conventional sewage treatment methods are not suitable for the treatment of distillery wastes. Quinn and Marchant (1980) investigated the possibility of devising a low-technology

Table 10.11. The limit values of the main constituents of spent wash produced by whiskey distilleries in Ireland and the UK during 1976–77 (after Quinn and Marchant 1980).

Total carbohydrate	6.7–21.2 g l^{-1}
Protein	15.1–31.0 g l^{-1}
Glycerol	4.4–7.5 g l^{-1}
Total titratable acidity (as lactate)	4.9–18.4 g l^{-1}
Free amino acids	2.1–4.3 g l^{-1}
BOD	10,500–43,000 mg l^{-1}
COD	15,000–58,000 mg l^{-1}
TOC	4,920–35,000 mg l^{-1}
Total dissolved phosphorus	740–1,960 ppm
Copper	0.2–4.8 ppm
pH	2.9–3.9

microbial treatment process which would not only remove the bulk of the organic loading from the spent wash and produce an effluent that could be more easily disposed of, but which would transform the organic material into high protein biomass to serve as a dietary supplement for animals. They selected a suitable micro-organism by enrichment of inoculum from a variety of natural habitats from which they found that the predominant organism in all instances was the fungus *Geotrichum candidum*. Batch cultures of the fungus grown in 25 ml samples of spent wash, diluted 5-fold, in 25 ml Erlenmeyer flasks gave a BOD reduction of 92.2%, a COD reduction of 80.6%, and a TOC reduction of 63.7%, with a productivity of 0.106 g l^{-1} h^{-1} over the 34 h period prior to the onset of a stationary growth phase. The yield of organisms in terms of organic carbon utilised was 126.4%, with a TOC removal rate of 0.084 l^{-1} h^{-1}.

Batch cultures of undiluted samples obtained maximal TOC removal rates of 0.54 g l^{-1} h^{-1} and a biomass production of 0.68 g l^{-1} h^{-1} after 40 h incubation. The BOD removal varied from 63.5–91.4%, COD reduction from 31.7–77.8%, and TOC reduction from 22.5–75.6%. Continuous culture trials were also carried out. Single-stage continuous culture at a predetermined optimum temperature of 22°C and an optimum dilution rate for biomass productivity resulted in 50.0%, 30.0%, and 40.7% reductions for BOD, COD, and TOC respectively. With the addition of a second fermenter in line with the first giving an overall retention time of 19.75 h, the BOD removal achieved was 87.0%, with 69.8% TOC removal and a biomass yield of 34.0 g l^{-1}, and an overall productivity of 1.72 g l^{-1} h^{-1}. Biomass from the second fermenter had a protein content of 45.5%. Quinn and Marchant concluded that the process of organic matter removal by SCP production by an organism that had already been previously cultured for animal and human consumption and was readily harvested, was certainly economically viable. The process also produced an effluent of superior quality to that of the evaporation methods widely employed at that time.

Barker *et al.* (1982) found that two strains of yeast were normally isolated from spent wash samples along with *Geotrichum candidum*. Both yeasts were isolated and identified as *Hansenula anomala* and *Candida krusei* both of which had previously been used for the production of microbial protein from methanol and whey respectively. They tested the growth and substrate assimilation of all three organisms on whisky distillery spent wash in both pure and mixed, batch and continuous culture. Their results showed that although there was no difference between batch and continuous culture in terms of yield or productivity, mixed culture of all three organisms gave greater protein assimilation. The reduction in

COD from batch cultures of individual organisms ranged from 44–49%, and 55% for the combined batch cultures. Nutrient examination of the protein produced indicated that it would be suitable as a dietary protein source for non-ruminant animals. Figures of BOD removal were not included in the results so that the potential of the treatment to reduce the pollution load of the waste cannot be assessed. This work has led to a new approach to the treatment of this traditional waste. In Northern Ireland, a pilot plant for conversion of distillery wastes into microbial biomass was set up at the Old Bushmills Whisky Distillery in County Antrim. The plant operated successfully and as a result the company is in the process of changing over from the traditional evaporation treatment method to a new biomass recovery system. The produce from the plant will be used as a food supplement by a local pet food company. The new plant came on-stream early in 1986.

A number of processes producing SCP from starch-containing substrates have been commercialised, including those that treat effluent from the processing of potatoes, corn, and other starch containing foods (Forage and Righelato 1979). The potato-processing industry is another example of an industry that has to contend with the disposal of substantial volumes of highly polluting wastes. During processing, about half of the potato is lost in various forms of waste, such as peel, trim, filterable particulates, processing water and blancher water (Lemmel *et al.* 1979). The larger solids can be used as cattle feed, and the more liquid wastes are subjected to primary and secondary waste treatment. Potato-processing wastes contain a lot of starch and the purification of such wastes is difficult and expensive by conventional wastewater treatment methods. As with the distillery industry described previously, more stringent water quality standards and increasing costs of treatment have prompted research into alternative waste treatment processes, with attention focused on the bioconversion of waste into microbial biomass for feed or food.

The Symba® process was developed by the Swedish Sugar Company and the Chepmap Company to treat effluent and solid wastes from potato processing, and has been in full-scale operation since 1973 (Skogman 1976). The process is based on the symbiotic culture of the yeasts *Endomycopsis* (= *Saccharomycopsis*) *fibuliger* and *Candida utilis*. The basic substrate for SCP production in the wastewater is starch, which is not readily assimilated by most micro-organisms, including *C. utilis*, so it has to be hydrolysed to simple carbohydrates. Although starch can be hydrolysed using mineral acids, enzymatic hydrolysis is preferred as the former requires highly corrosive-resistant equipment. Also, acid hydrolysis can result in the formation of compounds that can not be used by the yeast in the second

phase of the process and gives lower yields of biomass when calculated on the basis of total starch supplied. The starch is hydrolysed by the enzyme amylase, which is produced in large quantities by *E. fibuliger*, to low molecular weight sugars, principally glucose and maltose. These are then used by *C. utilis* which has a high nutritional value and is suitable for a wide variety of uses as a food supplement (Lawford *et al.* 1979). The amylase-producing yeast *E. fibuliger*, which does not have a high nutritional value, is much slower growing than *C. utilis* and is much smaller in size, and the final fermentation product is predominantly *C. utilis* (98% by weight).

A flow diagram of the Symba® process is given in Fig. 10.15. The wastewater is strained to remove any large particles and then sterilised by heating in order to destroy any microbial contaminants that may interfere with the fermentation process. The sterile substrate passes into a small preliminary fermenter in which *E. fibuliger* is grown to supplement its population in the main fermenter, as it is much slower growing than *C. utilis* (Table 10.12). This is particularly important as the growth rate of *C. utilis* is governed by the rate at which *E. fibuliger* can hydrolyse the starch to

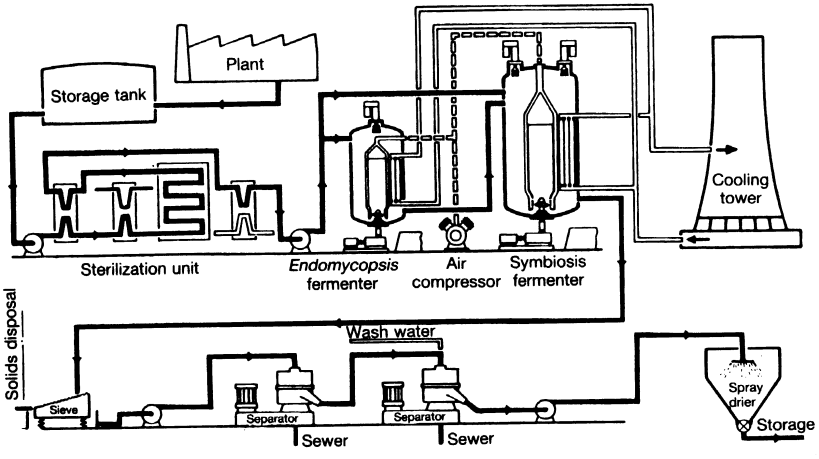


Fig. 10.15. The Symba® process (Norris 1981).

Table 10.12. Composition of mixed culture (cells ml^{-1}) as used in the Symba® process (Norris 1981).

Cultivation time (h)	*Candida utilis*	*Endomycopsis fibuliger*
0	4×10^7	5.5×10^7
20	1.2×10^9	1.4×10^8

produce the low molecular weight sugars, and if a single fermenter was used then the growth rate of *C. utilis* would be severely limited as would the rate of biomass production. Trace elements have to be added to the small fermenter to ensure that a steady supply of *E. fibuliger* cells and reduced sugars pass into the main fermenter, which has a normal capacity of about 300 m^3, where the symbiotic culture develops. Like all fermenters of this type, sterile air is required which is supplied by pumping air through a series of sterile filters. The culture within the fermenter is vigorously stirred to maintain adequate aeration. The resultant microbial activity produces large quantities of heat that is removed by cooling towers or heat exchangers. Excess biomass passes through several mechanical purification stages to bring the biomass up to the required quality, and it is then concentrated in continuous flow centrifuges in two steps, being washed in between. The concentrated biomass is then spray or drum dried before being packaged ready for use.

Skogman (1976) describes the original Symba® plant that reduces the BOD of the wastewater from 10,000–20,000 mg l^{-1} to 1000–2000 mg l^{-1} (90% removal), with 50% reduction in the concentration of both nitrogen and phosphorus. The process is able to treat 20 m^3 h^{-1} of wastewater, yielding between 250–300 kg dry yeast h^{-1} at 3% dry solids, which is equivalent to a biomass production of 45% based on the dry weight of the substrate supplied. The yeast contains about 45% protein and is low in nucleic acids but rich in vitamins, especially B vitamins. With the addition of a small amount of methionine, the protein has a nutritional value comparable to that of casein. Feeding trials have been carried out using the yeast as a skim milk replacement for young stock. It is estimated that up to 40% of milk protein used to feed calves could be replaced by Symba® SCP. All the trials carried out to date have yielded positive results without any adverse effects. The use of the Symba® process in food for human consumption is also being investigated and it seems likely that it will be used in the production of potato powders, bread, and milk products. It is a continuous process and highly automated and can be operated by one person. Although it operates at a level below the economically viable carbohydrate SCP fermenters, it is certainly cost-effective for those industries faced with the problem of disposing of a difficult and expensive waste.

Algae

The proposition that the growth of photosynthetic algae could be used for SCP synthesis, with energy derived from sunlight and carbon from carbon

dioxide in the air, has been considered for a long time. In fact, algae form part of the staple diet of the native tribes which live near Lake Chad in Africa, who collect matted algae from pools around the shore of the lake and dry it in the sun. As Norris (1981) points out, the equation of "free sunlight + free carbon dioxide + cheap inorganic nitrogen = valuable protein" is an attractive one, and it is bound to continue to attract attention.

Algae have a high nutritive value, with green algae, such as *Chlorella* and *Scenedesmus*, containing 50% protein (dry weight), and the large blue-green alga *Spirulina*, 60–70% protein. These figures are only approximate and depend on the nature of the substrate. For example, Milner (1953) found that by altering the growth conditions he could control the cellular constituents of *Chlorella* over a range of 7–88% protein (dry weight), 6–36% carbohydrate, and 1–75% true fat. The protein content in algal cells decreases and the lipid content generally increases under conditions of nitrogen limitation, and other nutritional limitations will also have strong effects on the composition of micro-algae (Waslien 1975; Benemann *et al.* 1979). Table 10.13 summarises the chemical components of the most important algae in SCP production: *Chlorella*, *Scenedesmus*, and *Spirulina*. However, SCP production using algae is frought with difficulties. Cultivation has to take place in dilute aqueous solution and as cell yields are generally low, recovery is difficult. This makes the resultant protein rather expensive in comparison with other protein sources. Light availability is the critical factor controlling algal growth and although artificial light could be used, it is not economically viable. Sunshine is the only practical light source and this restricts the location of such plants to tropical areas. Furthermore, the algae have to be grown in thin layers to ensure maximum penetration and utilisation of sunlight, unless constantly mixed. The most economic systems employ very shallow lagoons but in hot climates this results in a high evaporation rate of the water, which is normally a rare and expensive medium in those parts of the world best suited to algal lagoons. Finally, as the algal cultures are not grown under strict aseptic conditions they often become contaminated by other micro-organisms. A pilot plant has been constructed in Mexico to produce algal SCP using sunlight and carbon dioxide for energy and substrate respectively. The plant uses the alga *Spirulina platensis*, which has helically coiled cells that readily intermesh to form large aggregates which can be easily and cheaply separated, and is able to produce about a tonne of algal biomass daily. However, the limitations of high capital costs, moderate yields, poor product quality control, and a lack of markets have restricted the practical applications of algal cultures for food or feed production (Benemann *et al.* 1977a; Prentis 1984).

Table 10.13. Chemical compositions of selected micro-algae (Rose 1979a).

Component (% DW)	*Chorella* sp. 7–11–05	*Scenedesmus* sp.	*Chlorella–Scenedesmus* (10:1, sewage-grown)	*Spirulina maxima*
Protein*	55.5	53.0	41.8	55.5
Fat	7.5	13.0	7.2	12.7
Carbohydrate	17.8	13.5	27.4	17.4
Ash	8.25	6.5	19.1	7.4
Moisture	7.0	6.0	4.5	7.0
Crude fibre	3.1	8.0	—	—
Ascorbic acid	1.46	—	3.96	1.03
β-Carotene	5.02	—	6.02	2.25
Pantothenic acid	1.12	1.2	0.46	—
Pyridoxin	0.3	—	0.11	0.043
Thiamin	0.77	0.17	0.115	0.138
Riboflavin	—	0.42	0.269	0.285

*Protein contents were calculated by multiplying the value for the total nitrogen content by 6.25. True protein contents are about two-third of the values quoted.

When algal culture is combined with wastewater treatment, the production of SCP is no longer the primary objective but merely a part of the treatment process that produces a useful by-product which can make a contribution to the economics of the process. Growing algae in sewage has received much attention in recent years, and several plants have been built to take advantage of this "cash crop". In hot and arid areas, the culture of algae may be an economically competitive use of land and certainly lagoons of this type are the most cost-effective method of treating wastewaters under such climatic conditions. These systems offer a degree of flexibility so that either algal production or treatment efficiency can be optimised. What is particularly important in some arid coastal regions is that the system operates equally well with wastewater or a mixture of wastewater and sea water. Algal lagoons are very efficient in terms of BOD and nutrient removal and in arid regions the final effluent, after algal separation, may be more valuable that the algae because of the scarcity of water. In Haifa, Israel, a pilot algal lagoon system was found to be as effective as any other treatment process, more so if demand existed for irrigation water and SCP simultaneously with wastewater treatment (Table 10.14) (Shelef *et al.* 1977). Dubinsky *et al.* (1979) summarised the problems of exploiting algal mass culture that must be overcome before it becomes an economic

Table 10.14. Performance of a high-rate oxidation pond at Technion, Haifa, Israel (Shelef *et al.* 1977).

	Raw sewage (mg l^{-1})	Pond effluent (mg l^{-1})	Final effluent (mg l^{-1})*
Suspended solids	240	268	515
BOD_5			
total	330	106	10
dissolved	—	12	5
COD			
total	750	670	148
dissolved	—	64	46
Nitrogen			
total	86	71	20
dissolved	—	18	12
Phosphorus			
total	16	10	1
Coliforms per 100 ml	6×10^7	3.5×10^5	8×10^3

*Following flocculation and flotation.

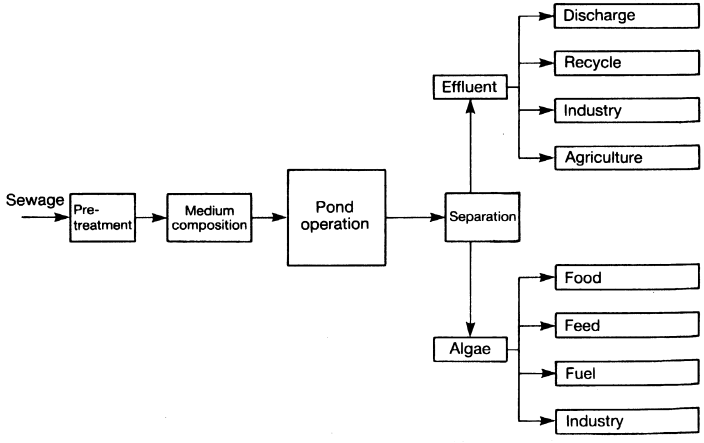


Fig. 10.16. Exploitation of algal mass culture.

reality (Fig. 10.16). Although the physical configuration of algal lagoons is not critical, with circular, square, and rectangular tanks all successfully employed; the important design criteria include water depth, mixing intensity, retention time, and organic loading. However, there are a limited number of interrelated operational variables that can be adjusted during pond operation (Table 10.15). The design and operation of such plants is examined further in Secs. 6.2 and 6.3.

The major restriction of algal lagoons for sewage treatment is the relatively large area of land required, about 1 hectare per 1,000 people, and the dependence on continuous sunlight throughout the year. Their use to date, therefore, has been restricted to smaller, isolated communities, mainly in Australia and southern USA. Between 40–100 tonnes (dry weight) of algae can be produced per hectare per year. Shelef *et al.* (1977) harvested over 110 tonnes per hectare per annum from their high-rate wastewater lagoon. Over 70% of the dry matter was algae with a protein content of between 42–48%. In Bezonbagh, Nagpur, India, a yield of 123.5 tonnes per hectare per annum was obtained from an experimental pond which was a very high yield on an area basis, compared with other agricultural crops grown in the region (Arceivala *et al.* 1970). Few species of algae are suitable for SCP production because of their indigestibility, toxicity, presence of silicone or carbonate shells, and the lack of economic cultivation technology (Benemann *et al.* 1979). The major problems of successful algal culture are harvesting the algae and maintaining the desired balance of species in the pond, which is normally achieved by separation and recirculation of

Table 10.15. Major operational variables in lagoons for the production of algal biomass (Rose 1979a).

Parameter	Control method(s)	Normal limits
Algal concentration	Harvesting, dilution, recycle, inoculation	150–700 mg l^{-1}
Depth	Dilution, harvesting	20–50 cm
Hydraulic retention time	Dilution	1.5–6.0 days
Phytoplankton retention time	Biomass recycle, dilution	1.5–6.0 days
Zooplankton retention time	Harvesting-recycling with DSM screen	0.5–6.0 days
Hydraulic loading	Dilution (applicable to oxidation ponds)	2–20 cm d^{-1}
pH value	Carbon dioxide, dilution	6.0–10.5
Nutrient additions	Add with dilution water or independently	Should not be limiting
Oxygen tension	Mixing, carbonation	0–25 mg l^{-1}
Light absorption	First four parameters, mixing	Absorption of 99–99.9% of incident light

algae. Harvesting the algae has proved the most difficult problem to resolve. Centrifugation is the most reliable and effective method but is not economically viable. Filtration and screening are usually not effective, especially for the single-celled green algae, although they may be satisfactory for the filamentous species. Algal settling and flotation using chemicals, such as lime, alum or polyelectrolytes, is effective but is expensive both in capital and operating costs. Also, the algal chemical mixtures cannot be used for SCP and have limited value for agricultural use. Benemann and Weissman (1977) have obtained 90% removal efficiencies of algae, resulting in a 10-fold increase in concentration, by using micro-strainers. The device is a rotating drum covered with a fine mesh screen (25–100 μm). The pond effluent enters the bottom of the drum and as it slowly rotates, the excess water drains through the mesh leaving the algae held to the inside of the drum. A pressurised jet at the top of the drum dislodges the algae into a collecting trough. This system costs 10% of chemical treatment and leaves the algae free from contamination so that it can be used for SCP. Ideally, strainable algae, especially the filamentous blue-green species, should be cultured on ponds, although such control is difficult.

Algae can be used to supplement the diet of intensively reared animals, including fish (Shelef *et al.* 1977). For example, Carp (*Cyprinus carpio*) and St. Peter's fish (*Tilapia galila*) in which 30% of the algae in the fish's diet replaced 85% of the fish-meal portion; which is usually 15% of the fish commercial diet (Hepher *et al.* 1975). In controlled experiments, weight gain of these fish was consistently higher when fed on the algae-supplemented diet. Even when algae have been chemically separated and contain 4% aluminium, they can be successfully used to replace 25–40% of the soya bean and fish-meal protein fed to chickens (Mukadi and Berk 1975) and fish (Hepher *et al.* 1975).

The production of algae on wastewater can be linked to biogas production (Weissman *et al.* 1979). Nitrogen and phosphorus can be removed from the effluent emanating from another secondary treatment unit by using a tertiary lagoon containing algae. Blue-green algae are especially suitable as they are filamentous and comparatively easy to harvest, and also are a suitable substrate for methane production by anaerobic digestion, with the final digested sludge being rich in nitrogen and phosphorus (Benemann *et al.* 1977a,b) (Fig. 10.17). In algal fermentations for biogas, 60–65% of the potential energy content of algae can be converted into methane, with the blue-green algae *Osciallatoria* and *Spirulina* being slightly more digestible than single-cell algae, such as *Euglena* and *Scenedesmus* (Benemann *et al.* 1977b). Anderson *et al.* (1980) have proposed a scheme that is almost totally

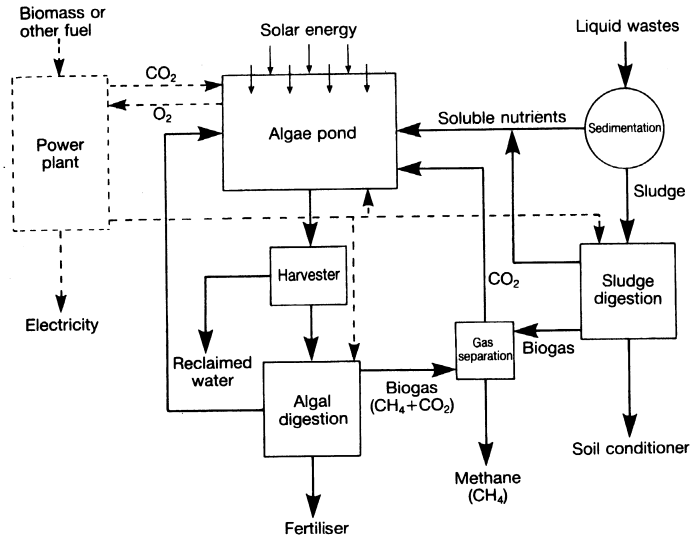


Fig. 10.17. Integrated algal biomass and methane digestion production system.

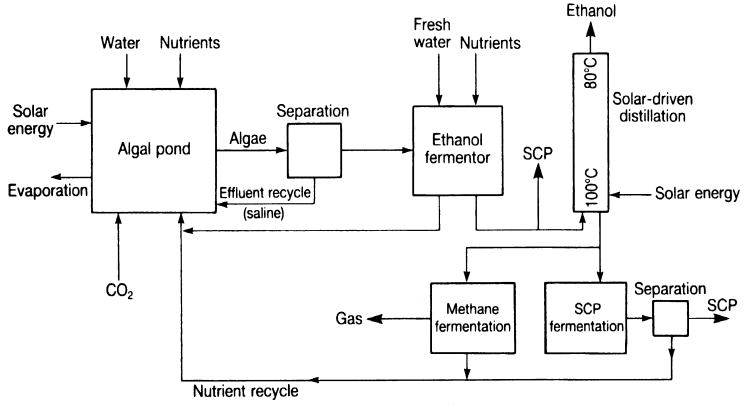


Fig. 10.18. Integrated process for the recovery of biogas, SCP, and ethanol from algal fermentation.

integrated from the recovery of biogas from fermenter effluents to almost complete recycling of nitrogen and other nutrients to the utilisation of solar energy for thermophilic digestion to produce ethanol (Fig. 10.18). Although the scheme is purely hypothetical, progress has been achieved on different elements, and this may well be the type of integrated fermentation system used for the treatment of wastewater in the future.

Although algae have only normally been exploited in facultative and high-rate lagoons, much attention has also been paid to the exploitation of algae growing in maturation ponds in the UK. Although algae in maturation ponds are beneficial in terms of phosphorus and nitrogen removal, their presence in the final effluent increases its suspended solids and turbidity, reduces the mortality rate of faecal bacteria, and because of the increase in pH caused by their photosynthetic activity, there is an increased risk of ammonia toxicity to fish (Hawkes 1983a). Suggestions have been made that cladocerans could be used as grazers to remove the algae from the effluent and that the cladocerans could then be harvested as a commercial product as food for aquarium fish. The harvesting would prevent overcrowding, encourage maximum growth rates, and minimise fluctuations in the cladoceran population (Green and Watts 1973). In practice, this has proved very difficult to operate and a rather unreliable means of removing algae, although the presence of a large cladoceran population does play a significant role in reducing the density of algal cells in ponds and final effluent (Hawkes 1983a).

Bellamy (1975) reviewed the increasing use of biological treatment systems incorporating many different trophic levels. Nutrients released during the degradation of organic matter by heterotrophs are utilised by algae which in turn are consumed by zooplankton, and so on up the food chain to the top trophic level that is usually occupied by a species of fish. The fish can then be harvested for human consumption, with coarse fish being especially popular in Central Europe. This form of treatment has been popular in China for centuries and is increasing in popularity in both developing and developed countries around the world (Edwards and Pullen 1990; Edwards 1992). However, the system requires careful management in order to ensure that the rate of organic loading does not disturb the ecological balance. European wastewater lagoons can provide ideal conditions for fish culture, especially roach, carp, and, to a lesser extent, chub and perch. Experiments on carp culture have shown that a high standing crop can be maintained in British maturation ponds, with good growth and production rates. However, problems with disease were encountered at high stocking densities, and recovery for the fish proved very difficult because of the layout of the ponds and nature of the banks. The inclusion of fish in maturation ponds has a significant effect on the ecological balance. In their original model for the control of algae by Cladocera, Green and Watts (1973) did not include predation of the grazers. Unfortunately, the fish, especially the young fingerlings which give such high productivity, feed on the zooplankton which, in turn, feed on the phytoplankton. Therefore, as

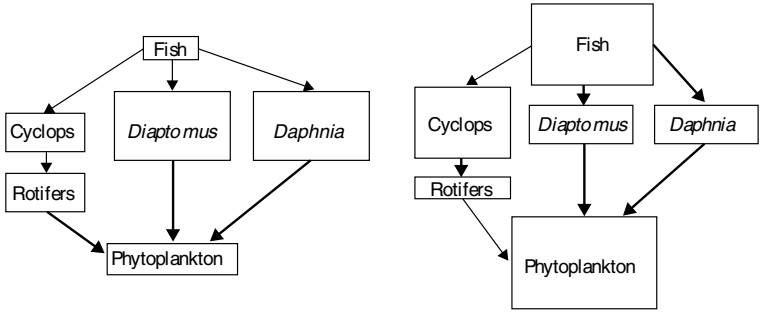


Fig. 10.19. The effect of overstocking with fish on the food web in a fish pond system (Hawkes 1983c).

the fish population increases so does the density of algae in the pond, with a corresponding increase of algae in the final effluent (Williams *et al.* 1973) (Fig. 10.19). This may not be a problem in countries where the quality of the final effluent is less important in relation to the production of fish, or if the effluent is to be used for irrigation.

The effluent from stabilisation ponds in India which is rich in both phyto- and zooplankton, the natural food of so many fish species, has been profitably exploited for rearing fish (pisciculture). As fish are extremely sensitive to oxygen depletion, even for short periods, and the dissolved oxygen concentration in stabilisation ponds fluctuates diurnally, often reaching zero at night (Sec. 6.3), fish are rarely stocked in the main stabilisation pond itself but normally in secondary ponds (Fig. 10.20). The major species of fish cultured in lagoons and ponds are all carp species, with *Catla catala*, *Labeo rohita*, *L. calbasu*, and *Cirrhinus mrigala* all grown successfully in both the stabilisation and the subsidiary lagoon. In Bhilai, 2000 fingerlings of *L. rohita* and *C. catla*, with mean weights of 18.7 g and 5.5 g respectively, were stocked in a stabilisation pond and after 12 months their weight had increased to 140 g and 990 g respectively (Chatterjee *et al.* 1967). Fish fry grown in a pond using sewage effluent in Bhopal has been shown to have a higher rate of growth than regularly grown nursery pond fry. It is clear that the main species of carp grow best in the subsidiary rather than the main stabilisation ponds, and the conditions can be more carefully controlled for pisciculture in the former and dissolved oxygen depletion is prevented. However, *Ophiocephalus* is a popular fish in some regions of India because of its high iron content and reports have indicated that it may be particularly suited to intensive rearing in stabilisation ponds as it is able to utilise both dissolved and atmospheric oxygen; hence its local name "the

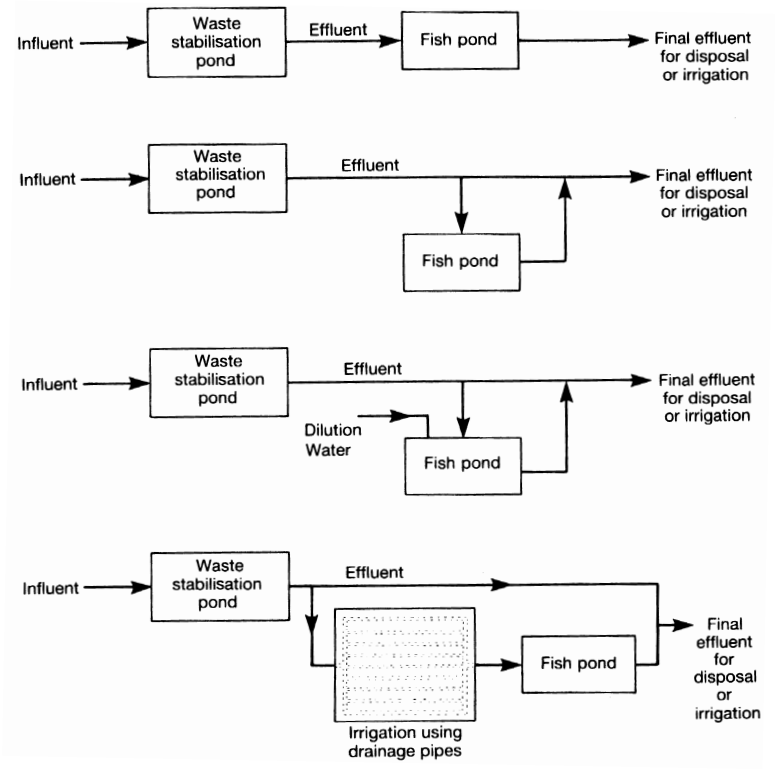


Fig. 10.20. Possible applications of fish ponds in waste stabilisation pond systems.

surface-breathing fish". Normal fish yields from freshwater ponds have also been reported as doubling (i.e. 7–8 t $ha^{-1}yr^{-1}$) when effluents from stabilisation ponds are added in the ratio of 1–2 volumes of effluent to each volume of pond water (Arceivala *et al.* 1970). Optimum yields are obtained using small ponds of up to 1 ha in area that are stocked with fingerlings, fertilised with facultative pond effluent and then harvested for months after stocking. Growth of fish within this period is rapid — from 20 g to 250 g.

There are serious microbial risks associated with the reuse of effluents (Sec. 9.4.2) and Mara *et al.* (1993) have proposed a design procedure to minimise these risks. The design ensures protection of the fish from free ammonia within the pond, minimal treatment of wastewater using anaerobic and facultative ponds in series, and the maximum production of microbially safe fish.

The fish pond receiving the effluent from the facultative pond is designed using a surface loading rate of 4 kg total N $ha^{-1}d^{-1}$. The loading rate is

critical as too little nitrogen results in a low algal biomass giving small fish yields, while too much nitrogen results in a high concentration of algae and consequent oxygen depletion at night. The area of a fish pond (A_{fp}) is estimated by a modified version of Eq. 6.13:

$$A_{\mathrm{fp}} = 10\ C_{\mathrm{i}}Q/\lambda_{\mathrm{s}}^{\mathrm{TN}}$$

Where C_{i} is the total nitrogen concentration in facultative pond effluent (mg N l^{-1}) (use Eq. 6.11), Q the flow rate ($\mathrm{m}^3\mathrm{d}^{-1}$) and $\lambda_{\mathrm{s}}^{\mathrm{TN}}$ the surface loading of total nitrogen (kg $\mathrm{ha}^{-1}\mathrm{d}^{-1}$).

The retention time (θ_{fp}) is calculated as:

$$\theta_{\mathrm{fp}} = A_{\mathrm{fp}}\ D/Q$$

where depth (D) is normally 1 m.

The concentration of faecal coliforms in the fishpond (N_{fp}) must be $\leq$ 1000 per 100 ml. This is achieved by increasing the retention time or incorporating a maturation pond after the facultative pond and before the fish pond. N_{fp} is calculated as:

$$N_{\mathrm{fp}} = N_{\mathrm{i}}/(1 + k_{\mathrm{T}}\theta_{\mathrm{a}})(1 + k_{\mathrm{T}}\theta_{\mathrm{f}})(1 + k_{\mathrm{T}}\theta_{\mathrm{fp}})$$

where N_{i} is the number of faecal coliforms per 100 ml in the influent wastewater, k_{T} the first order rate constant for faecal coliform removal (d^{-1}) (Sec. 9.3.2). θ_{a} the retention time of the anaerobic pond, θ_{f} the retention time of the facultative pond, and θ_{fp} the retention time of the fish pond.

In order to protect the fish from ammonia toxicity, the free ammonia (NH3) should not exceed 0.5 mg N l^{-1}. The percentage of free ammonia (p) in solution is a function of the temperature and pH (Table 10.16) and can be calculated as (Emerson *et al.* 1997):

$$p = 1/[10(pk_{\mathrm{a}}^{-\mathrm{pH}}) + 1]$$

where $pk_{\mathrm{a}} = 0.09018 + (2729.92/T)$ and T is given in degrees Kelvin (°K = °C – 273.15) (Erickson 1985). The pH in fish ponds varies between 6.5 to 7.5, and so when designing such ponds a pH of 7.5 should be assumed. The total ammonia concentration is calculated using Eqs. 6.9 or 6.10 in Sec. 6.3.2.

10.3.3. *Composting*

Composting is usually applied to solid or semi-solid materials and is the biological decomposition and stabilisation of organic substrates, with

Table 10.16. Concentrations (mg l^{-1}) of total ammonia in freshwater that contain an unionised ammonia concentration of 0.02 mg NH_3 l^{-1}. The threshold concentration of 0.02 mg NH_3 l^{-1} is the long term toxic effect level of salmonid fish while the lethal level is about ten times greater.

	pH							
Temp. (°C)	6.0	6.5	7.0	7.5	8.0	8.5	9.0[a]	9.5[a]
5	160	51	16	5.1	1.6	0.53	0.18	0.071
10	110	34	11	3.4	1.1	0.36	0.13	0.054
15	73	23	7.3	2.3	0.75	0.25	0.09	0.043
20	50	16	5.1	1.6	0.52	0.18	0.07	0.036
25	35	11	3.5	1.1	0.37	0.13	0.055	0.031
30	25	7.9	2.5	0.8	0.27	0.10	0.045	0.028

[a]Criteria may be unduly low if there is low free carbon dioxide in the water.

putrescrible organic material converted to a stabilised compost by microbial action. Compared to other stabilisation processes, composting is a fast, simple, and safe approach to the bulk treatment of organic wastes. A wide variety of materials are suitable for composting including sewage sludge, animal and agricultural wastes, and household refuse (Gasser 1985; Haug 1993; de Bertoldi 1999; Epstein 1997). Almost all the widely used composting systems are aerobic, with the main products being water, carbon dioxide, and heat. Significantly less heat is produced during anaerobic composting, with methane, carbon dioxide, and low molecular weight organic acids being produced. Due to the nature of the intermittent products of anaerobic breakdown there is also a high possibility that odours will be produced. Fermentation and anaerobiosis also lead to products that will undergo further decomposition when exposed to air. In contrast, aerobic systems have a greatly reduced odour potential because the organics that remain are relatively stable so that further decomposition will continue only slowly (Edelmann *et al.* 2000). Energy, or heat production, is essential to the success of the composting process. Two distinct temperature phases occur during composting. First, a mesophilic phase in which microbial action raises the temperature up to 40–55°C, forcing the process into a thermophilic phase in which the mesophilic micro-organisms are inactivated and are replaced by thermophiles. At these higher temperatures, both animal and plant pathogenic micro-organisms are inactivated, as well as insect pests, their eggs, and weeds. Composting also reduces the water content of the composted waste thereby reducing the volume of material that has to be eventually disposed of.

Composting effectively recycles the organic matter and nutrients in wastewater and converts wastes into a useful material. Composted waste can be used as a soil conditioner to reduce the bulk density of the soil; to increase the water holding capacity and encourage proper soil structure by the addition of organic matter, thus encouraging a healthy soil microfauna/flora and healthy plant root development. Compost replaces the organic matter lost each year due to current farming practices.

Indeed, the problem of organic matter restoration in soil is becoming increasingly urgent as field size increases and desertification becomes more common (De Bertoldi *et al.* 1985). Composted waste is also a fertiliser that can improve crops by the addition of nitrogen, phosphorus, and trace elements. The nitrogen is organically bound and is therefore slowly released throughout the growing season, with minimum losses due to leaching in contrast to when soluble inorganic fertilisers are used.

Composting is used world-wide. For example, the Atsubetsu Sewage Sludge Compost Plant in Japan processes 75 tonnes of dewatered sludge daily. In 1994, 5,200 tonnes of compost were used within a 100 km radius of the plant, with half being used for agriculture and the remainder for green areas (Watanabe *et al.* 1996). There are over 280 plants currently operating in the USA (Willson and Dalmat 1983; Goldstein and Gray 1999).

Methods of composting

Wet substrates are difficult to compost because their high moisture content makes aeration difficult. As a general rule, the higher the moisture content of the substrate, the greater is the need to maintain a high voidage to ensure adequate ventilation. Dewatered sludge cake contains between 70–80% water and lacks both bulk and porosity, therefore its dense nature makes diffusion of oxygen difficult and prevents aerobic composting. When stored, unstabilised sludge cake will undergo anaerobic breakdown and as the sludge is plastic in nature, it readily compacts under its own weight, reducing the available voidage even further. Because of these factors, dewatered sludge cake is more difficult to compost on a commercial scale, requiring more bulking agent and often constant mixing so that new surfaces are continuously exposed for oxygen transfer (Haug 1980). However, sewage sludge compost is rapidly increasing in popularity with many full-scale plants in operation (Paulsrud and Eikum 1984; Bowman and Durham 2002).

To successfully compost dewatered sludge cake, the bulk density and porosity need to be reduced so that air can penetrate through the cake

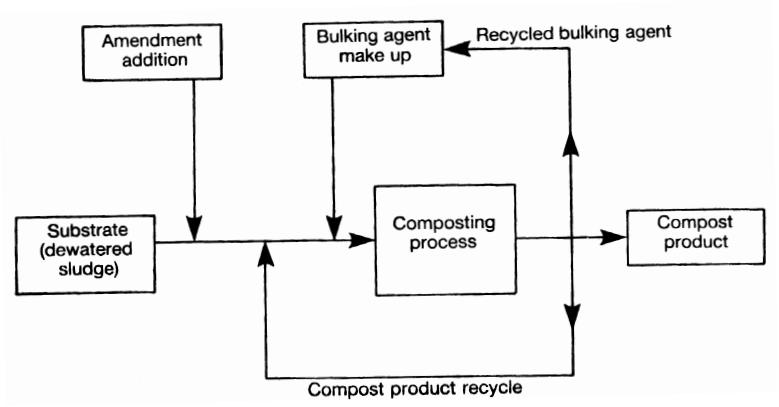


Fig. 10.21. Basic process steps in composting.

more efficiently. Three approaches can be used to overcome the problem of oxygen transfer when composting wet substrates such as dewatered sludge cake (Fig. 10.21): (1) recycling of compost and mixing with the substrate before composting; (2) addition of an organic amendment to the sludge; and (3) the addition of a bulking agent which is recovered after composting and re-used. These methods are generally used separately, although the use of amendments and recycling are occasionally used together. The most popular of the three methods is the addition of an amendment to the sludge, as it allows greatest control over the quality of the final product as well as disposing of a secondary waste material. The amendment is an organic material that is added to the substrate to reduce its bulk density and to increase the voidage, so air can penetrate providing adequate aeration. Sawdust, straw, peat, rice husks, manure, refuse and garbage, lawn, and tree trimmings have all been successfully used. The choice of amendment is generally limited to what is available locally, but ideally it should be dry, have a low bulk density and be degradable. The advantage of carefully selecting the amendment is that it can significantly increase the quality of the final compost. Bulking agents can be either organic or inorganic particles of sufficient size and shape to provide structural support to the sludge cake as well as maintaining adequate aeration (de Bertoldi *et al.* 1980). The bulking agent provides a matrix of interstices between particles in which sludge is trapped and undergoes decomposition. Enough space is left between particles to ensure sufficient ventilation. After composting, the bulking agent is recovered, normally by screening, and reused. Inert bulking agents have the longest life, although degradable materials can be used

to improve the organic quality of the compost and are less of a problem as the former is not totally removed by the screens. Wood chips are the most widely used bulking agent, although other suitable materials include pelleted refuse, shredded tyres, peanut shells, tree trimmings, and graded mineral chips.

Because composting is an exclusively biological process, those factors that affect microbial metabolism either directly or indirectly are also the factors that affect the process as a whole. The most important operational factors are: aeration, temperature, moisture, C:N ratio, and pH level (Suler and Finstein 1977; MacGregor *et al.* 1981; de Bertoldi *et al.* 1983; Finstein *et al.* 1983). In the compost pile, the critical oxygen concentration is about 15%, below which anaerobic micro-organisms will exceed aerobic ones. Oxygen is not only required for aerobic metabolism and respiration, but also for oxidising the various organic molecules that may be present. During composting, the oxygen consumption is directly proportional to the microbial activity, and a direct relationship between oxygen consumption is expected, with the high oxygen consumption occurring at temperatures between 28–55°C (Haug 1980). High temperatures in composting result from heat produced by microbial respiration. Composting material is generally a good insulator and as the heat is only slowly dispersed the temperature in the pile increases. Heat loss to the outside of the pile is a function of the temperature difference and the rate of microbial activity. As the rate of activity is limited by the rate that oxygen can enter the pile, heat production is affected by oxygen availability. In an aerated pile, 60°C can be reached within 1–2 days, whereas in unaerated windrow systems where oxygen is more limited, similar temperatures are reached after 5 days (Audsley and Knowles 1984). The compost will remain at this maximum operating temperature until all the available volatile solids have been consumed (Fig. 10.22). Excessively high temperatures inhibit the growth of most micro-organisms present in the compost severely reducing the rate of decomposition, although a number of thermophilic bacteria can withstand temperatures > 70°C (e.g. *Bacillus stearothermophilus*, *B. subtilis*, *Clostridium* sp., and *Thermus* sp.) (De Bertoldi *et al.* 1983). Therefore, for rapid and efficient composting, long periods of high temperatures must be avoided, except as an initial phase for the destruction of heat-sensitive pathogens. Moisture content is linked to aeration of the pile, and is a difficult factor to quantify. Too little moisture results in dehydration of the pile, which will reduce or even stop biological activity, resulting in a physically stable but biologically unstable compost. Too much moisture fills the interstices of the compost, excluding air, so that aerobic growth is replaced

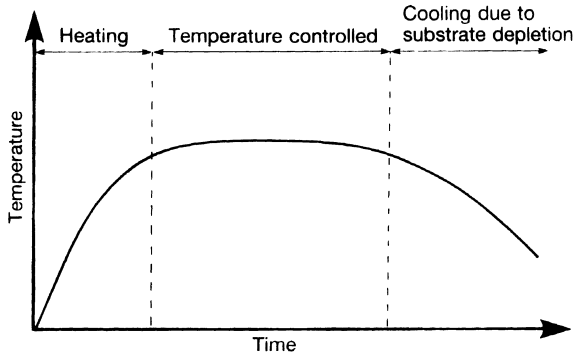


Fig. 10.22. The three stages of the composting process in a static pile system (adapted from Stentiford *et al.* 1985).

by anaerobic activity, which will result in odour production. The bulking agent can act as a reservoir for water, with straw, for example, capable of absorbing up to three times its own weight of water (Gifford 1972). The optimum moisture content for composting is discussed by Audsley and Knowles (1984) who concluded that a moisture content of at least 50% was required. The ratio of available carbon to nitrogen (C:N) should be about 12 for good microbial growth (Vogtmann and Besson 1978). All the carbon present may not be readily available, especially when bulking agents, such as wood chips are used, which are only slowly degraded and only a small proportion of the carbon is then available. In these circumstances, an optimal C:N ratio for composting may be as high as 25 (De Bertoldi *et al.* 1982, 1985). A higher C:N will slow up decomposition until excess carbon is oxidised, whereas a low C:N ratio also slows decomposition and increases the rate of nitrogen loss through ammonia volitisation, especially at the higher pH and temperatures so typical of the composting process. The final compost product must have a C:N ratio < 10–12, otherwise the soil microbial population will immediately utilise the available nitrogen, which will limit crop growth and defeat the purpose of using compost to improve soil quality. Although the optimum pH for composting is between 5.5–8.0, organic matter with a pH range of between 3–11 can be successfully composted. The pH will begin to drop as soon as composting commences because of the acid-forming bacteria breaking down complex carbohydrates to organic acids, which tends to favour fungal development. As stated before, a high pH can result in nitrogen loss if the temperature is also high.

The most critical factor in composting is the supply of oxygen and the various systems used for providing adequate aeration ranges from

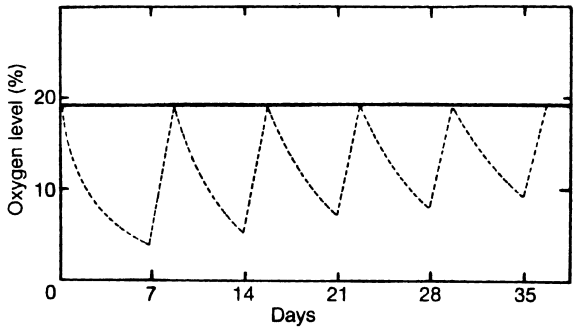


Fig. 10.23. Cyclic variation of oxygen during composting within a pile that is periodically turned. The straight line represents the oxygen demand of a composting mass, and the dotted line the oxygen availability within the pile during each period between being turned (Stentiford *et al.* 1985).

the relatively simple to the very complex. Various techniques have been developed to supply oxygen and these are used to classify the various composting systems. There are two main methods of composting. *Open systems* do not require a reactor but employ piles and windrows, whereas *closed systems* take place within a specially constructed reactor and involve a high degree of mechanisation.

Open systems are of two types: turned piles or windrows (long rows of compost triangular or trapezoidal in cross-section) and static piles. In the former, oxygen is provided by natural diffusion and periodic turning. In the latter, oxygen is supplied by forced aeration or possibly by natural ventilation.

Turned or agitated systems require that the composting mixture, which is placed in piles or windrows, to be broken up periodically during the composting cycle to allow oxygen into the pile. The compost is not fully mixed each time but rather turned or tumbled over and rebuilt back to its original shape. This can be done manually, although larger scale operations require this to be done by special machines. The height, width, and shape of the piles or rows depend on the nature of the compost mixture and the type of equipment used for turning. Generally, rows are narrower at the top than at the base to ensure stability. Oxygen is mainly supplied by gas exchange during turning, although oxygen also enters the pile by diffusion and natural ventilation caused by the warm gas escaping which induces convection currents through the pile. Although they are simple to operate and the cheapest form of composting, turned systems have severe limitations. The problem is that the pile is only fully oxygenated when turned, which results

in a cyclic variation in the oxygen concentration within the pile. In practice, this means that biological oxidation can never be maintained at maximum efficiency as the oxygen concentration is normally limiting (De Bertoldi *et al.* 1982) (Fig. 10.23). Also, pile turning requires more space than static pile systems especially if they are moved laterally. During the final stages of composting when the material is nearly dry, turning releases quantities of dust containing spores of *Aspergillus fumigatus* that are hazardous to the operator (Millner *et al.* 1977) The spores or conidia are present in soil at very low densities and rarely constitute a hazard. However, within the compost, vast densities of spores accumulate as they are heat tolerant, and are released when the pile is turned. There does not appear to be a problem with aerated static piles or closed systems of composting, with the release of spores being worst when the compost is dry. Health-associated problems are restricted to plant operators who, if they are hypersensitive or suffer from pulmonary disease, may display a severe allergic response. It is possible that the spores will germinate after inhalation and that the fungus may invade lung-parenchyma to produce typical aspergillosis (Clark *et al.* 1984a,b; Vincken and Roels 1984).

Windrowing is more popular than piles as it is more efficient in terms of space utilisation. They can be constructed either on a soil base or on concrete floors with underdrainage. Farmers composting animal wastes generally use soil-based systems, with straw as a bulking agent. They are generally constructed at the periphery or headland of fields and are left to rot slowly without turning, which allows pathogens to survive in the outer part of the pile. The final quality of the compost produced in this way is much poorer compared to piles that have been turned periodically. At sewage treatment works, concrete bases are normally employed with long windrows 1 m high and 4 m wide constructed at regular intervals so that they can be regularly turned mechanically with ease. This ensures that all parts of the compost mixture, especially the sludge cake, spends an adequate period in the centre of the pile at 55°C, so that total pathogen destruction occurs. There are a variety of turned systems in use, employing a variety of amendments and bulking agents. The Bangalore process was developed in 1925 in India and is used extensively there today. A 0.5–1.0 m deep trench is dug in the ground and filled using alternative layers of refuse, night soil, earth, and straw. The material is then turned by hand as frequently as possible, with composting completed after 120–180 days.

Static piles are not turned for aeration; instead, oxygen is supplied by natural ventilation or more commonly, by forced aeration. By using a forced air method, the amount of oxygen entering the pile can be controlled, as

can the moisture content and temperature of the compost. Static piles take up less area than turned piles and savings are made in manpower, as the compost is not touched until the process is completed. Air can be sucked or blown through the pile, or used alternately to give overall oxygen and temperature control (Haug 1980; de Bertoldi *et al.* 1985). There are a number of well-documented systems using forced aeration in operation throughout the world. A widely used system in the USA is the Beltsville process in which air is sucked through the pile at a rate of about 0.2 m^3 min t^{-1}, providing an oxygen concentration of 15% in a windrow of sludge cake and wood chips. A base of wood chips is laid down on a concrete base and the sludge/wood chip mixture is laid on top to form a 300 cm high windrow, which is covered with screened compost from earlier batches. Air is pulled through the pile by a perforated pipe laid in the wood chip base. The air that has been sucked out of the pile is dried and vented off through a pile of screened compost that eliminates any odours. Residence time in the pile is between 14–21 d, with adequate pathogens killed to allow the final product to be spread on land without causing health risks. As with all composting methods that employ a bulking agent, the wood chips are screened from the composted waste and reused (Fig. 10.21). The system devised by Rutgers University (New Jersey) essentially prevents the pile overheating. Most micro-organisms do not survive at temperatures $> 60°C$, especially fungi that degrade cellulose and lignin, thus by controlling the temperature in this way composting can continue at maximum microbial efficiency. Once the temperature rises above 60°C, microbial degradation begins to fall off rapidly and eventually stops, and composting will not continue until the temperature falls and the micro-organisms can re-invade the affected area. The system uses thermocouples placed within the pile that can activate a blower automatically if the temperature reaches critical levels. Air is blown through the pile until the temperature returns to the required optimum. Unlike suction methods, blowing air through the compost enhances evaporation and produces a highly stabilised end-product that has a low moisture content (Finstein *et al.* 1980; Willson *et al.* 1980).

It should be remembered that high temperatures destroy pathogens, and some methods combine an initial phase of suction to ensure oxygenation without any heat loss. This allows the temperature to rise for a few days so that the pathogens are killed, before blowing is introduced to control the temperature. The effect of blowing (positive pressure) and sucking (negative pressure) aeration on the temperature gradients within piles is clearly shown by Stentiford *et al.* (1985) who composted sludge using refuse as an amendment (Fig. 10.24). The shape of the pile gradually slumps as

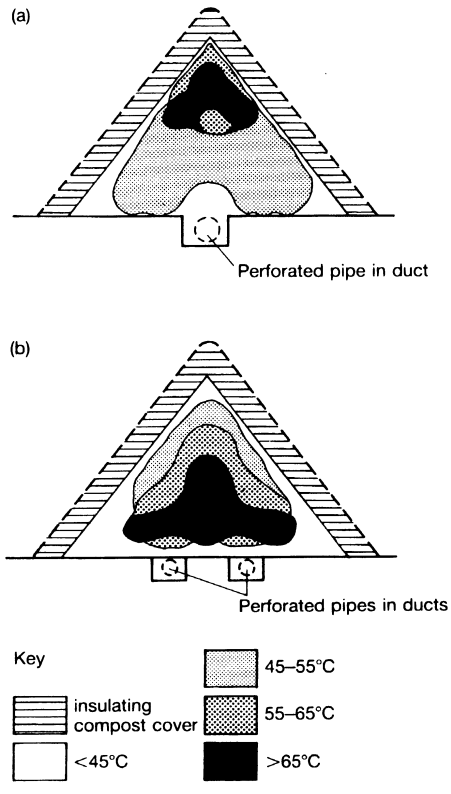


Fig. 10.24. Temperature profiles of composting sewage sludge where (a) positive pressure (blowing) aeration and (b) negative pressure (sucking) aeration is used (Stentiford *et al.* 1985).

composting progresses (Fig. 10.25), with the problem of pathogen removal in the outer layer of the pile overcome by using a thick insulating cover of mature compost (Fig. 10.26). This layer of mature compost also reduces the amount of odour produced by the piles.

Closed system composting takes place in specially constructed reactors or vessels. There are essentially two types, vertical flow reactors that are towers of up to 9 m in height, and horizontal or inclined flow reactors. Neither of these types of reactors is widely used for composting sewage sludge and is extremely difficult to manage on a continuous basis. Because of their high capital and operational costs they are not a cost-effective method of stabilising sewage sludge under normal conditions. Closed systems of composting have been extensively reviewed by Haug (1980) and de Bertoldi *et al.* (1985). An interesting closed composting system has been in operation

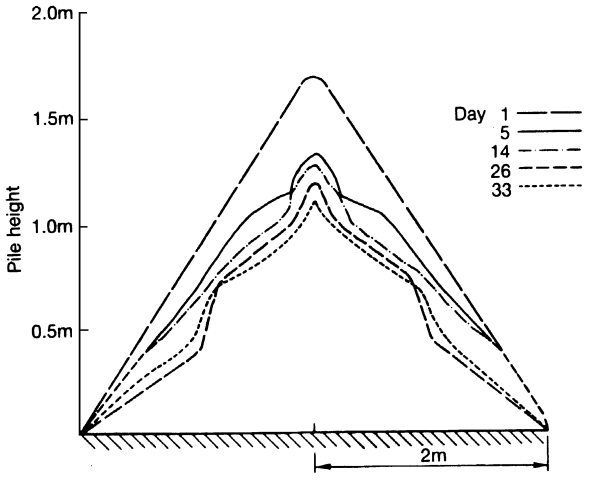


Fig. 10.25. As composting progresses the dimensions of the pile changes. In this example, sewage sludge is being co-composted with domestic refuse (Stentiford *et al.* 1985).

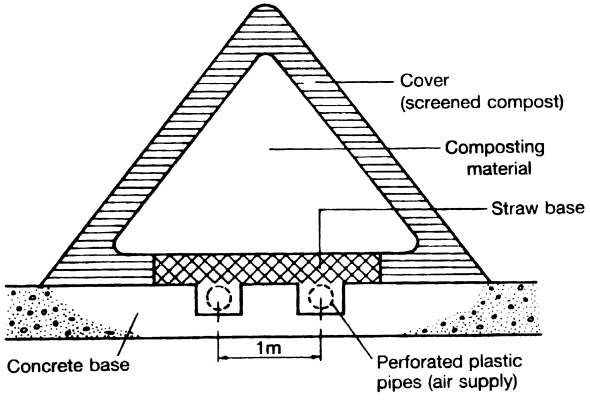
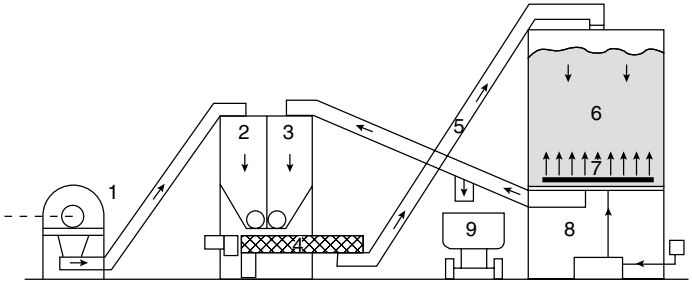


Fig. 10.26. Cross-sectional diagram of the most effective pile structure used for composting (Stentiford *et al.* 1985).

at the Bekkelaget wastewater treatment plant in Oslo since 1980. It is a BAV-type plant that is capable of composting up to 30 m^3 of dewatered sludge each day. A mixed activated sludge is used that is dewatered by centrifuge to 20% and then mixed with raw compost and sawdust before being fed into the 500 m^3 reactor, which is cylindrically shaped having an overall diameter of 9 m (Fig. 10.27). The nominal retention time in the reactor is 10–12 d. However, problems with short-circuiting have been



1) Sludge dewatering by centrifuge
2) Hopper for dewatered sludge
3) Hopper for sawdust and recirculated compost
4) Screw mixer
5) Conveyor
6) Bioreactor
7) Aeration equipment
8) Discharge of raw compost
9) Raw compost to maturing area

Fig. 10.27. The BAV composting plant at Bekkelaget sewage treatment works, Oslo (Paulsrud and Eikum 1984).

reported. The excess compost, which is not recirculated, is stockpiled for 6–9 weeks before being used by the Oslo Parks' Department (Paulsrud and Eikum 1984).

In Europe, research has centered on open systems of composting. For example, in the UK much research has been conducted into composting sewage sludge with straw. There is approximately 4.3×10^6 tonnes of wheat straw and 2.1×10^6 tonnes of barley straw surplus to requirements in the UK each year (Larkin 1982), which at present is burnt in the fields. However, with increasing pressure on farmers to curtail straw burning on environmental and conservation grounds, they are left with a serious disposal problem. There is a reluctance to plough straw into the soil because this has been shown to reduce yields by between 3–20%, depending on the weather during autumn, because of immobilisation of soil nitrogen and retardation of germination and seedling growth as it decomposes. However, in many areas of the UK, there is also a surplus of sewage sludge (Table 10.17) that could be composted with the surplus straw to provide a valuable soil conditioner and fertiliser of superior quality to either straw or sludge on their own. Research at the National Institute of Agricultural Engineering (NIAE) has shown that straw and sewage sludge can be successfully composted (Audsley and Knowles 1984). The straw can be used whole or chopped and the sludge can be either raw or digested, with the dry solids content between 4–20%, and dewatering is not essential although preferable. Dewatering removes about 30% of the nitrogen in raw sludge and up to 50% in digested sludge (Table 10.18). Therefore, in terms of nitrogen, assuming only half of the

Table 10.17. How surplus straw and sewage sludge was distributed in the UK (by country) in 1984. The sludge is expressed as 4% dry solids (Audsley and Knowles 1984).

Country	Wheat straw (tonnes)	Barley straw (tonnes)	Sludge solids (tonnes)	Surplus as tonnes sludge solids		Closest country	Final surplus sludge (tonnes)
				straw	sludge		
London	7950	3404	8740	0	7141	Herts	0
Bedfordshire	107 896	46 328	12 462	9248	0		
Cambridgeshire	396 978	115 451	10 188	64 411	0		
Essex	419 581	110 287	30 606	47 114	0		
Hertfordshire	106 436	51 847	5233	16 774	0		
Lincolnshire	533 379	235 894	13 337	94 649	0		
Northamptonshire	166 321	67 396	9366	23 715	0		
Norfolk	338 657	233 655	4730	71 886	0		
Suffolk	370 601	151 892	10 415	63 462	0		
Berkshire	42 527	26 042	21 845	0	12 540	Wilts	0
Buckinghamshire	51 247	27 188	10 727	82	0		
East Sussex	38 880	14 229	2053	5533	0		
Hampshire	136 013	79 966	13 224	16 214	0		
Kent	126 535	40 897	25 944	0	1772	E Sussex	0
Oxfordshire	133 962	90 660	12 656	17 481	0		
Surrey	15 408	13 405	22 607	0	18 854	Sussex, Hants	0
West Sussex	66 467	29 436	9036	4424	0		

Table 10.17. (*Continued*)

Country	Wheat straw (tonnes)	Barley straw (tonnes)	Sludge solids (tonnes)	Surplus as tonnes sludge solids		Closest country	Final surplus sludge (tonnes)
				straw	sludge		
Cheshire	1615	1429	13 232	0	12 836		12 836
Derby	20 575	35 026	20 041	0	13 386	Lincs	0
Greater Manchester	292	849	19 710	0	19 581		19 581
Hereford & Worcester	55 042	25 055	8808	2404	0		
Lancashire	2276	2671	39 312	0	38 691		38 691
Leicestershire	132 141	61 697	21 726	5339	0		
Merseyside	1011	1257	8573	0	8290		8290
Nottingham	113 114	67 349	29 917	0	5353		5353
Shropshire	78 073	27 245	7897	7210	0		
Staffordshire	3083	4515	41 296	0	40 396	Leics	35 029
Warwickshire	51 300	18 216	6154	3802	0		
West Midlands	2441	2493	16 515	0	15 885	Warks	0
Avon	4593	2451	3989	0	3018	H & W	614
Cornwall	164	1007	4412	0	4289		4289
Devon	5064	8615	6749	0	5111	Dorset	2042
Dorset	44 191	14 460	5389	3069	0		
Gloucester	105 926	68 358	3181	20 329	0		
Somerset	14455	5976	8372	0	5485	Wilts	2124

Table 10.17. (*Continued*)

Country	Wheat straw (tonnes)	Barley straw (tonnes)	Sludge solids (tonnes)	Surplus as tonnes sludge solids		Closest country	Final surplus sludge (tonnes)
				straw	sludge		
Wiltshire	136 376	72 777	12 905	15 901	0		
Cleveland	6661	320	620	476	0		
Cumbria	0	0	2834	0	2834		2834
Durham	13 312	798	3571	0	1364	Cleve	887
Humberside	228 128	92 593	5383	40 006	0		
Northumberland	29 390	34 683	7826	205	0		
North Yorkshire	170 249	145 539	20 447	20 764	0		
South Yorkshire	38 695	21 996	5466	2836	0		
West Yorkshire	16 836	14 581	49 090	0	44 996	Yorks	21 395
Tyne & Wear	768	2272	5262	0	4921		4921
Total	4 334 609	2 072 205	601 846	557 347	266 724		158 889

Table 10.18. Typical characteristics of raw, digested, and dewatered sewage sludge (Audsley and Knowles 1984).

	Raw sludge (%)	Digested sludge (%)	Dewatered, raw sludge (%)	Dewatered, digested sludge (%)
Solids content	4	4	20	20
Carbon content (DS)	35.5	31.7	35.5	31.7
Nitrogen content (DS)	5.2	6.7	3.6	3.4
Volatile solids (DS)	75	50	52.5	35

remaining nitrogen in sludge is available for plant growth, the value of the four types of sludge as a fertiliser in 1984 can be expressed as £ per tonne dry matter:

Raw sludge	14.9
Digested sludge	17.6
Dewatered raw sludge	8.4
Dewatered digested sludge	11.6

Clearly, dewatered sludges, with their lower nitrogen content, have a lower fertiliser value. All composting systems can be used with sludge and straw, but three systems have been particularly successful and cost-effective. The *on-field* system composts the material where it is to be used the following year. Straw is taken to the headland of each field while the sludge is stored in a lagoon until harvest. An open windrow is constructed on the headland and is not turned. The costs in 1984 were between £15–21 per tonne of compost produced, depending on farm size and transport costs (not including the cost of either digestion or straw). As the pile is not turned, pathogens will not be killed in the outer surface of the pile, and digested sludge only should be used. If dewatered sludge is used, the volatile solids content may be too low to maintain the critical temperature (Table 10.18). With *on-farm* systems, the straw is baled and stored centrally, with windrows constructed on a concrete base as the sludge is delivered. The hard base allows the farmer to use his tractor to turn the windrows regularly, and maximum pathogen removal is achieved allowing raw sludge to be used. The compost costs in 1984 were about £8–20 per tonne, depending on farm size and transport costs (the cost of straw is not included). The final system is *central-site* composting where the straw is bailed and stored at the farm and brought to the treatment

plant as required. As with the previous system, windrows are constructed on a concrete base and turned regularly, with the compost returned to the farm for spreading. Raw sludge can be used, and the cost of the compost at the central site in 1984 was as little as £5–8 per tonne. However, because of transportation back to the farm, its eventual cost was between £11–15 per tonne, depending on farm size and distance from the treatment plant, but excluding the cost of the straw (Audsley and Knowles 1984).

Maintaining an adequate C:N ratio is difficult as straw has a typical C:N ratio of 48 and sewage sludge 5–9. In order to obtain the optimum C:N ratio of 12 for good microbial growth it is necessary to mix sludge dry matter to straw dry matter in a ratio of 1.3:1, 0.7:1, 4.7:1, and 4.0:1 for raw (4% DS), digested (4% DS), dewatered raw (20% DS), and dewatered digested sludges (20% DS) respectively. More dewatered sludge is required as it contains significantly less nitrogen than non-dewatered sludges. To ensure that a high temperature is maintained for a sufficient time, large piles with a minimum cross-sectional area of 5 m^2 are required to ensure that the heat generated exceeds the heat lost. As heat is produced from microbial degradation of volatile solids, a high volatile solids content in the compost mix is desirable (Table 10.18).

In conclusion, the NIAE study found that outdoor composting required care and was liable to fail if the winter was very cold. Forced aeration improved the chance of successful composting, whereas enclosed forced aeration would, they felt, always succeed. However, methods involving forced aeration are comparatively expensive and would probably make this type of composting economically not feasible.

Experiments in Norway have been conducted on the composting of primary sludge after dewatering using a centrifuge to 20–25% dry solids. The dewatered sludge is dumped in piles on the composting site which should have good drainage during autumn to spring. During the early summer, the water content of the sludge decreases further due to evaporation, with a dry crusty layer forming with a dry solids content $> 30\%$. In midsummer, the pile is turned for the first time so that the dry crusty surface layer is mixed with the wetter sludge in the centre, and so acts as a bulking agent. At this stage, the porosity is sufficient for composting to start, and with further turning, the composting process continues for the rest of the summer and autumn (Fig. 10.28) (Paulsrud and Eikum 1984).

In a joint venture, Anglian Water and Ipswich Borough Council in the UK formed a composting company (Compost Development Venture (CDV) Ltd.) to co-compost green waste collected within a 30 km radius of Cliff Quay Sewage Treatment Plant with digested sludge cake with an average

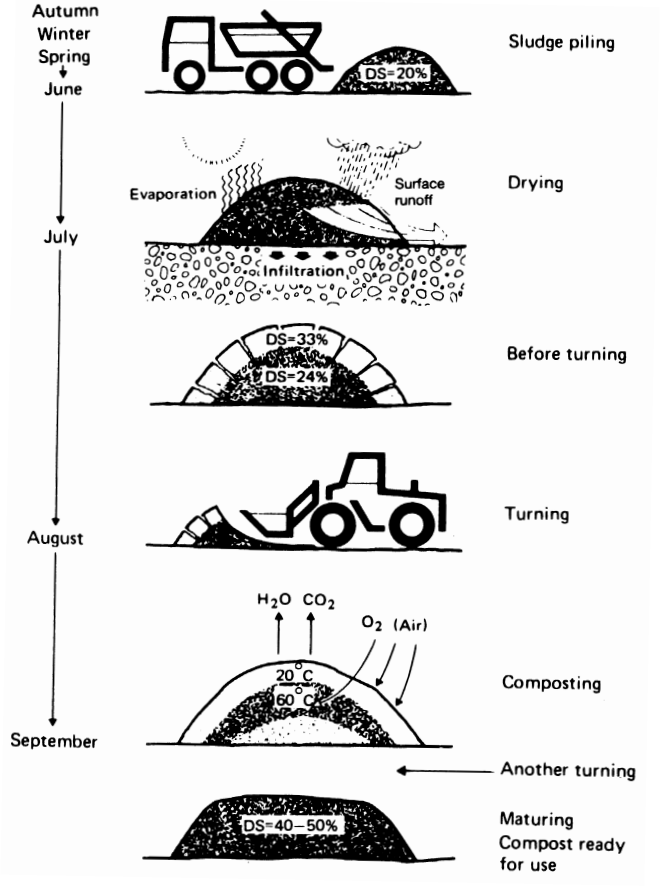


Fig. 10.28. Phases of windrow composting of sewage sludge without the aid of a separate bulking agent (Paulsrud and Eikum 1984).

dry solids content of 23.8% (Barnes 1998). A commercial tunnel system which is comprised of three concrete tunnels, 30 m × 5 m × 5 m, built over a perforated floor to allow aeration the system, uses a mixture of green waste, sludge cake, and sugar beets waste in a ratio of 1:0.42:0.32. Green waste (95 tonnes) is shredded and then mixed with sludge cake (40 tonnes) and sugar beet waste (30 tonnes) to give enough material to fully load each tunnel. The stack height within the tunnel is 2.0 m giving a floor loading of 1.1 tonnes m^2 as the mix has a density of 0.40–0.45 tonne m^3. Once loaded, the tunnel is made airtight. Monitoring the temperature of the compost, the humidity and oxygen concentration of the process air from the tunnel, and the air pressure headloss across the compost, controls the composting

process. A central processing unit controls operating conditions during the various process steps by altering the ratio of recycled to fresh air, fan speed, and water spraying. The general layout of the plant is shown in Fig. 10.29. The sequence of operation is as follows:

(I) Air is blown through the mixture to equalise the temperature. Water is also sprayed onto the compost to achieve rapid attainment of the pasteurisation temperature.
(II) Rapid warming of the mixture occurs during this stage with a temperature of 50–52°C normally achieved within 24 h. Pasteurisation is achieved by maintaining the temperature at 58°C for 12 h or 55°C for 3 d. The temperature is not allowed to exceed 60°C to prevent damaging the thermophilic bacteria. During this phase weed seeds, insect larvae, nematodes, helminths, viruses, protozoa, and pathogenic bacteria are all destroyed.
(III) Compost is initially allowed to cool rapidly to 55°C over 2–3 h and then more gradually to 50°C (4–6 h).
(IV) The mixture is allowed to condition (compost) at 48–50°C for 7–9 d. The length of time required depends on the original pasteurisation temperature achieved. The end of conditioning is indicated by a decline in the temperature of the mixture as activity slows. This is measured quantitatively by the reduction in ammonium concentration in the used process air as the C:N ratio decreases.
(V) Finally water is sprayed onto the compost and the fan speed increased to cool and aerate the material for 2 d.

The compost is then removed and any uncomposted woodchips removed by a drum screen (15 mm). It is then left to cure outside for 3 months. Details of compost quality are given in Table 10.19. All the process air that is not recycled is passed through a water scrubber, using final effluent, and then through a biofilter. The exhaust air is normally 25–40°C and has a moisture content of 60%. The scrubber water is maintained at 25–30°C while the pH varies between 7.5–8.5. Gaseous ammonia is hydrolysed to dissolved ammonium ion (NH_4^+), which is then oxidised to nitrate by nitrification. This causes the pH of the liquor to decrease and allows further ammonia dissolution to occur which would not otherwise happen if the pH were allowed to increase without control (Barnes 1998). The scrubber also removes dust. The scrubbed exhaust air is then passed through a biofilter with the moisture content maintained at 50–60%. Sulphur and remaining nitrogen in the air are metabolised by the biofilm.

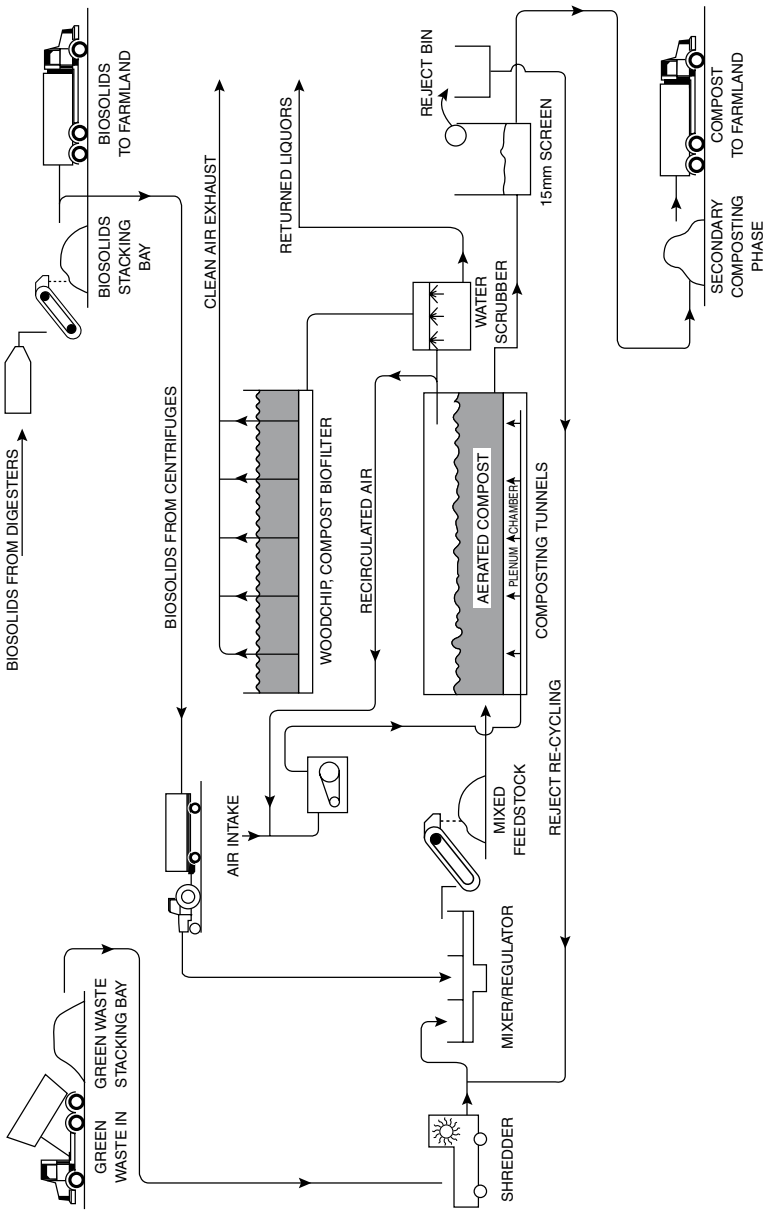


Fig. 10.29. Schematic layout of the tunnel composting plant at Ipswich, UK (Barnes 1998).

Table 10.19. Typical analysis of sewage sludge based compost from Ipswich, UK (Barnes 1998).

Parameter	Concentration
pH	7.4
Dry solids (%)	75.9
Organic matter (% on DS)	53.1
Amm.N (% on DS)	0.1
Total N (% on DS)	2.0
P (as P_2O_5) (% on DS)	1.2
K (as K_2O) (% on DS)	1.0
Mn	273
Cu	64
Zn	222
Ni	15
Pb	137
Cr	14
Cd	0.5
Hg	< 0.1

n.b. All metal concentrations are expressed as mg/kg DS

Since 1996, Thames Water has marketed a wide range of peat free horticultural-grade composts based on sewage sludge. Aerobic composting of sewage sludge can achieve an enhanced treated product with less than 1,000 *E.coli* g^{-1} DS. Originally a simple windrow system was employed at Little Marlow Sewage Treatment Works, which consisted of mixing sludge cake (12,000 wet tonnes y^{-1}) with wheat straw at a ratio of 8 parts sludge to 1 of straw by weight (Lasaridi *et al.* 2000). The straw increases the C:N ratio by providing extra C to an optimum of 20–30:1, as well as increasing porosity for air exchange. The windrows are turned three times a week for 5 weeks with the piles achieving thermophilic conditions (55°C). Full maturation takes a further 6 to 12 months.

In 2002, the company switched to a housed agitated bay composting system. The agitated bay system is comprised of long parallel bays where compostable material is contained to a depth of 2–3 m between adjacent concrete walls. Turning machines run along the top of the walls to mix the compost mixture and deposit it further along the bay to provide mixing, aeration, and continuous flow of material. This has allowed the composting process to be accelerated, permitting a greater production using a smaller area with better odour control (Bowman and Durham 2002). Negative

pressure aeration via an under-floor aeration system gives better composting performance, improved working conditions inside the building, and improved overall operational efficiency.

Several bulking agents were considered. A mixture of woodchips and shredded green material, which is a good source of carbon and initiates rapid heating, is widely employed. However, the problem with using fresh green waste as a sole or main bulking agent is that it has a high concentration of water soluble salts and a pH that is thought to inhibit the growth of some plants in the final compost. Also green material has a variable quality and seasonal availability making continuous operation difficult. Straw on its own can degrade very slowly during maturation due to its high lignin content, whereas wood chips can be screened out and recycled after a predetermined period. While other materials could be used (e.g. barley dust from the malting industry, hemp dust, and coffee grinds), only straw and wood chips are cheap, easy to obtain, can be stored, and are efficient. Woodchips were used as the sole bulking agent in the new process. With high capital costs, selling the compost for horticultural use only covers the day-to-day composting operational costs, excluding the costs of dewatering. Relative capital and operational costs are given by Bowman and Durham (2002).

Product quality

Fully composted sludge closely resembles peat, being dark brown in colour with a crumbly texture, and having a pleasant earthy smell. The fertiliser value of such compost is generally low compared with inorganic fertilisers, although it does reflect the original fertiliser value of the substrate and amendments used (Table 10.20). It is possible to fortify the compost and

Table 10.20. Fertiliser value of raw and digested sewage sludges before and after composting (Parr *et al.* 1978).

	Raw sludge	Composted sludge	Digested sludge	Composted sludge
Water (%)	78	35	76	35
pH	9.5	6.8	6.5	6.8
Organic carbon	31	23	76	35
Total nitrogen	3.8	1.6	2.3	0.9
Ammonia	1540	235	1210	190
Phosphorus	1.5	1.0	2.2	1.0
Potassium	0.2	0.2	0.2	0.1
Calcium	1.4	1.4	2.0	2.0

increase its nutrient value by adding nitrogen, phosphorus, and potassium, although the cost of this probably exceeds its value (Hileman 1982). Thames Water markets a wide range of peat-free horticultural grade compost products based on sewage sludge. Different compost products are produced by mixing composted sewage sludge with composted green waste, coir, and vermiculite (Bowman and Durham 2002). Compost has been successfully marketed as a soil additive for householders (Heaman 1975), as an ingredient for potting compost, where it is comparable to a sand-peat mixture, and for general horticulture and landscaping (Gonin 1982), where compost mixed with fertiliser produces better plant growth than the addition of fertiliser alone (Diez and Weijelt 1980). Most compost producers are spurred to improve their final product in the knowledge that 250,000 tonnes of peat are sold annually in the UK alone, at a basic price of £140 per tonne. With proper marketing, it is possible that some of that market could be replaced by compost. Straw produces a coarser compost than other types of amendments and bulking agents, and is only suitable for use as an agricultural soil conditioner (Audsley and Knowles 1984).

The quality of compost is assessed using the basic concept of stability (Foster *et al.* 1993; Lasarida and Stentiford 1998, 1999; Brinton 1999), with a wide range of chemical and physical parameters used. Respiration measurements, such as the specific oxygen uptake rate (SOUR), is normally used. The maximum rate of oxygen consumption in an aqueous compost suspension is measured at 30°C using the method of Lasaridi and Stentiford 1998). Stability of sewage sludge derived compost is acceptable for storage and handling when the SOUR is < 2.5 mg O_2 g^{-1}VS h^{-1}, while values < 1.5 mg O_2 g^{-1}VS h^{-1} indicate composts with excellent stability characteristics (Lasaridi *et al.* 2000). Ranalli *et al.* (2001) have proposed the use of a number of bioindicators and describe in detail the chemical, physical, and biochemical changes that occur during composting (Tables 10.21 and 10.22).

Heavy metals may be a problem with composted sludge as with sewage sludge, when disposed to land (Wong *et al.* 1996). Therefore, compost must be treated as sewage sludge under the existing legislation and guidelines (Sec. 8.2.2). Low concentrations of lime (< 1.0 w/w) added to sewage sludge when composted reduces the problems of heavy metal availability within the compost (Wong *et al.* 1997), although composting is increasingly inhibited with lime addition (Wong and Fang 2000). Composts are generally pathogen free although Sidhu *et al.* (2001) have reported that all composts derived from sewage sludge have the potential for *Salmonella* regrowth.

Table 10.21. Physico-chemical composition of individual and mixed raw materials prior to composting (Ranalli *et al.* 2001). Post composting details in Table 10.22.

Parameters	Grape-stalks	Grape-dregs	Rice husks	Dairy sludges	Mixed materials
PH	6.8	6.1	6.6	7.6	7.1
E.C. (mS/cm)	2.75	3.38	1.12	0.88	2.45
Bulk density (kg/L)	0.6	0.4	0.11	0.9	0.46
Total Solid (%)	45.1	21.0	89.7	17.0	29.0
Ash (%)	11.5	9.1	14.5	3.2	6.46
Organic Carbon (%)	41	54.7	30.6	36.8	43.0
Total N (%)	0.93	2.25	0.45	6.20	2.31
P-(P_2O_5) (%)	0.51	0.23	0.05	2.6	1.21
C/N	44.1	24.3	68	5.9	18.6
C/P	80.4	237.8	612	14.1	35.5
Lignin + cellulose + tannis + pectine (%)	65.4	44.3	56.6	< 2	34.8
Raw lignin (%)	41.1	29.5	16.6	< 2	19.8
Raw fiber (%)	25.6	14.9	40.1	< 2	15.3
Hemicellulose (%)	21.7	14.4	21.8	< 2	12.4

Table 10.22. Physico-chemical composition of cured compost. Average values are given on a dry weight basis (Ranalli *et al.* 2001).

Parameters	Cured compost
PH	7.4
E.C. (mS/cm)	5.48
T.S. (%)	31.4
Ashes (%)	6.67
Organic carbon (%)	26.4
Organic matter (%)	34.4
Humic acids (%)	4.83
Fulvic acids (%)	2.72
HA/FA	1.77
Total N (%)	1.75
P-(P_2O_5) (%)	0.98
C/N	15.1
C/P	26.9
Raw lignin (%)	16.4
Hemicellulose (%)	5.9

The major cause of inactivation of pathogens in composting is exposure to high temperatures (> 55°C) for a number of days. The Environmental Protection Agency in the US have produced standards to ensure pathogen destruction during composting. In aerated piles, the coolest part of the pile must be at least 55°C for 3 days, and in the windrow system, the centre must be at least 55°C for 15 out of the total of a 21–30-day composting period. During this period, the pile must be turned five times (EPA 1994, 2000). Similar regulations apply to composts in the UK (ADAS 2001). In forced aeration systems, the volatile solids can be utilised very quickly, and although a high temperature is achieved it may not last as long as the slower and slightly cooler turned system, and so give poor pathogen inactivation (Audsley and Knowles 1984). Pathogen inactivation is greater if the pile is turned, as cool spots develop within the pile, especially in the corners where pathogens can survive and even increase in numbers. Regular turning ensures all parts of the pile are subjected to the high temperatures. It is now common practice in forced aeration piles to build the new compost mixture on top of a layer of mature compost and for the pile to be sealed by a thick layer of the material, which acts as insulation. Under these conditions, high pathogen kills are possible without turning and there is no outer layer in which the pathogens can survive. However, *Salmonella* is known to survive all composting processes in low concentrations (Russ and Yanko 1981, Hussong *et al.* 1985), leading to regrowth on storage (Skanavis and Yanko 1994; Soares *et al.* 1995; Gibbs *et al.* 1997, 1998). Suppression of regrowth of pathogens is due to indigenous microflora, but this declines on storage, so composted sewage sludges should be exposed of as quickly as possible (Sidhu *et al.* 2001). Composting crop residues is an extremely efficient way of destroying all plant pathogens including fungi, bacteria, nematodes, and viruses. However, inactivation is caused not only by heat generated during the thermophilic phase but also by microbial antagonism and the toxicity of conversion products formed during decomposition (Bollen 1985; Lopez-Real and Foster 1985).

Other uses for compost have been investigated. Composting has been described as biological drying, and certainly composting is an excellent means of removing water from sewage sludge, thus reducing the volume to be transported to disposal sites. This ability could also be exploited to convert moist organic wastes into fuel (Finstein and Miller 1985). Some research has been done on direct heat recovery during composting, although so far success has been limited (Baines *et al.* 1985; Thostrup 1985).

Vermiculture

A promising approach to sludge management is the use of earthworms to convert waste sludges into a useful compost, with the worms themselves being utilised as a valuable source of protein. The process is known as vermiculture or vermicomposting depending on the emphasis placed on the final product.

It is well known that earthworms break down organic matter while feeding on the micro-organisms present (Edwards and Lofty 1977), and composting systems using windrows, piles, and boxes have been developed by worm breeders for fish bait (Tomlin 1983). Work by Hartenstein *et al.* (1979), Neuhauser *et al.* (1980), Edwards (1982, 1983) and Gajalakshmi *et al.* (2001) has demonstrated that earthworms can break down a variety of wastes and sludges including sewage sludge, pig and cattle solids and slurries, waste from chickens, broilers, turkeys and ducks, potato wastes, spent mushroom compost, water hyacinth, and paper pulp. However, not all the wastes are equally acceptable to earthworms, with the production of worm biomass varying with substrate (Fig. 10.30). Similarly, not all sewage sludges may be suitable for vermiculture. Hartenstein *et al.* (1979) report

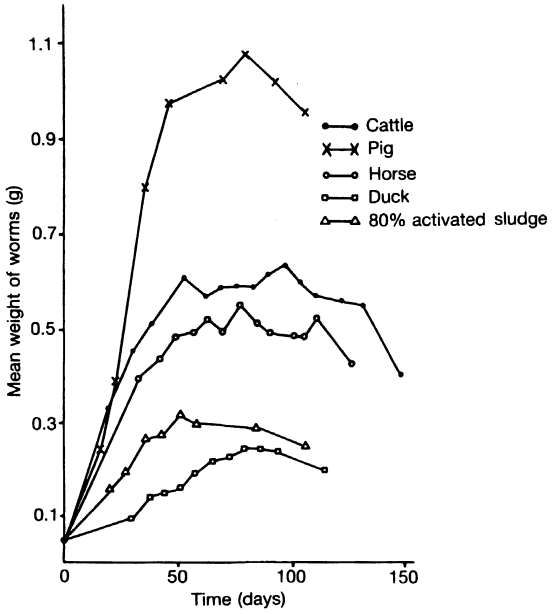


Fig. 10.30. The growth *of Eisenia foetida* on various animal wastes and surplus activated sludge (Edwards *et al.* 1985).

that *Eisenia foetida* grew faster in activated sludge than in manure, their natural habitat, although the rate of reproduction may not be as high. For this reason, they suggested that a separate culture of worms should be maintained using manure in parallel with the composting mixture and used to constantly reinoculate the composting sludge. Although a number of species of earthworm have been tested, the *tiger* or *brandling* worm (*Eisenia foetida*) is the most efficient natural species for use with vermiculture as it has rapid growth and reproductive potential. This species takes 5–7 weeks to reach sexual maturity but after that it can produce up to five cocoons per week from which up to seven hatchlings are produced per cocoon. Thus, each adult worm has the potential to produce 15–20 young per week over an average reproductive period of 22 weeks. However, the search for an even more efficient exotic species continues. *Eudrilus eugeniae*, the African night crawler, grows about twice as fast as *E. foetida* but requires a greater space per individual. It can tolerate higher temperatures than temperate worm species, having an optimum growth rate at 28°C. Below 12°C, however, the worm population is rapidly decimated (Hartenstein *et al.* 1979). In the Philippines, commercial production of compost employs the worm *Pheretima asiatica* (Anon 1984a). In general *Eudrilus eugeniae* and *E. foetida* are the preferred species although there are problems with importing them in countries where they do not exist naturally. Therefore indigenous rather than exotic species are used whenever possible (Manna *et al.* 1997; Banu *et al.* 2001; Gajalakshmi *et al.* 2001).

The sludge undergoes normal composting with the high density of worms grazing on the micro-organisms. *Eisenia foetida* requires a mixed population of bacteria, fungi, and protozoans to sustain maximum growth and reproduction, with the fungi and protozoans constituting the major proportion of its diet. Therefore, sewage sludge and agricultural wastes are ideal media for growing worms. The production of tunnels by earthworms increases aeration, allowing the material to compost more efficiently. The earthworms also accelerate the process by fragmenting organic matter and producing casts both of which increase the surface area available for microbial growth. At 25°C, an earthworm can convert up to twice its body weight of waste into compost each day, the exact amount depending on the density of the solids ingested. The casts not only decompose more rapidly than the original material, but also dry twice as rapidly. Minerals as well as organic matter are assimilated by the worms which results in an overall decrease in the ash content of the waste being composted. At the end of the composting period, the worm-worked compost is superior to normal compost. It is an odourless material resembling peat, rich in available nutrients,

with a good moisture holding capacity and excellent porosity. The action of the worms is such that most of the nitrogen is converted to nitrate by enhanced microbial activity. The amount of soluble potassium, phosphorus, and magnesium is also increased (Edwards *et al.* 1985).

Karmegan *et al.* (1999) found that vermicompost produced from decomposed pods of the green gram (*Phaseolus aureus*) using the earthworm *Eudrilus eugeniae*, produced better germination (93.3%) of *P. aureus* than normal compost (84.2%). Better growth and yields were also achieved using the vermicompost which had higher concentrations of N, P and K compared to the other composts used.

The earthworms themselves contain between 60–70% protein, 7–10% fat, 8–20% carbohydrate, and 2–3% minerals, and have a gross energy of 4,000 kcals kg^{-1}. In nutritional terms, worm tissue is excellent being equal to meat or fish. It is particularly rich in amino acids, especially lysine and the vitamins niacin, riboflavin, and vitamin B_{12}, which makes worm tissue valuable as an animal feed. Worms have already been successfully fed to a wide range of animals including fish (Guerreo 1983; Tacon *et al.* 1983), chickens and pigs (Sabine 1978; Jin-you *et al.* 1982). The tissue also contains plenty of long-chain saturated fatty acids, such as linoleic acid, dihomo-linoleic acid, and arachidonic acid that are essential to non-ruminant animals who cannot synthesise them. Their conversion efficiency of agricultural wastes into worm tissue on a dry weight basis can be as high as 10%, so that for every tonne of suitable waste composted 100 kg of worms can be produced.

Processing of worm compost falls into five stages: preparation of waste, composting/culture chamber, harvesting, worm processing, and processing of compost (Fig. 10.31). The preparation of the waste depends very much on the water content of the sludge and whether additional material, such as straw or wood chips are to be added. Worms grow most rapidly at

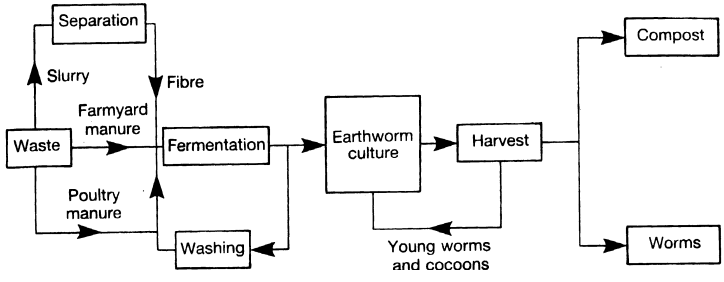


Fig. 10.31. Vermiculture system processes.

moisture contents of between 80–90%, with the optimum pH being 5, although they can tolerate a pH range of 4–9. Temperature and moisture content are the two most important environmental factors, with little growth below 10°C and temperatures above 35°C being lethal. Optimum temperature for growth, the number of cocoons produced and the number of young worms hatching per cocoon is 25°C, although there is a rapid decrease at temperatures > 25°C. The operating temperature for maximum productivity is usually between 15–20°C. The compost material is laid approximately 0.5 m deep, depending on the density of the material, on a solid floor of concrete, bricks, or railway sleepers. The larger the composting chamber, the better the economics, although the waste material has to be built up slowly in successive layers. Once the compost is active, slurries and other liquid wastes can be sprayed on top. Vermiculture cannot be carried out during October to March in most temperate climates unless there is sufficient insulation and heating. In order to maintain optimum moisture conditions, the compost may require periodic watering and some reports indicate that if worms migrate from the chamber then lighting may be required. Best yields of worms and most efficient waste conversion rates have been obtained under strictly controlled environments using polythene or fibreglass insulated tunnels (Anon 1984a). Composting is normally completed after 2 to 3 months if straw or wood chip based wastes are used, less if the separated waste only is used, with the length of time depending on the type of waste, the inoculation level of worms at the commencement of the process, and the environmental factors. This is supported by Frederickson *et al.* (1997) who found that vermicomposting, using *Eisenia andrei*, reduced the volatile solids in fresh green material significantly faster than standard composting. Combining vermicomposting with existing composting operations accelerated the stabilisation process compared to composting alone, with the duration of pre-composting determining the subsequent growth and reproductive rates of *E. andrei* which are positively correlated to the volatile solids content of the waste itself. They recommend that the pre-composting period should be kept at a minimum. Pre-composting was also found to help the vermicomposting of cattle dung (Gunadi *et al.* 2002). Harvesting presents two problems: (i) the separation of the worms from the compost and (ii) the separation of the young worms from the adults. The juvenile worms need to be recovered so they can be used to inoculate the next batch of waste to be composted. Harvesting methods are still being developed and rotary sieves have been widely used, although attempts to use the response of worms to certain stimuli such as heat, light, moisture, and chemicals (including ammonia and formalin) to

separate them from the compost have also been made with limited success. Once separated, washing and soaking in water to remove debris from the surface and the intestines respectively clean the worms. They are generally then sterilised by heat to destroy any pathogens and to help prevent deterioration of the tissue. They are subsequently preserved by freeze drying, air or heat drying, freezing or pickling and processed into either a powder or paste of uniform quality before being sold. If the compost is to be used directly on land as a soil conditioner no further treatment is necessary. However, much worm-worked waste is marketed through garden centres for horticultural and general garden use and so needs to be dried, blended, and packaged. As the compost has a moisture of 75%, it will not store without further decomposition and drying is necessary. Also, as a standardised product is required that cannot be guaranteed by the composting system itself, blending of the product is necessary. The economics of the process have been reviewed by Fieldson (1985) who estimates that from case studies in the UK, 63% of the total benefits come from the compost, 29% from the worms, and 8% from the waste disposal saving. As vermiculture can be used as a treatment process for cattle, pigs, and poultry wastewater, then the benefits in waste disposal savings alone may make this an attractive method of pollution control in some circumstances. There are considerable markets for vermiculture. Spent mushroom compost and waste paper pulp are particularly suited to vermiculture. Bann *et al.* (2001) have successfully used two exotic species of earthworm from India, *Eudrilus euginea* and *Eisenia foetida*, and the indigenous species *Lampito mauritii* to treat paper mill sludge. Another advantage of worm composting is that not only is it odour-free itself, but the compost can be used to remove odours produced from other treatment processes or from intensive rearing units. For example, the air stream extracted from a pig unit, which was in danger of closure due to odours, was vented through the worm compost which absorbed the odours completely. Vermicompost has also been used to remove heavy metals from electroplating wastewater (Jordão *et al.* (2002). The estimated market value for worms is hard to gauge but is approximately the same as other sources of animal protein, although for certain specialised uses, such as feeding young eels, their value may be 5–10 times greater, and the compost is worth the same as peat. In 1984, it was estimated that if just 10% of the available waste material in the UK was composted, assuming £400 per tonne for the worms and £80 per tonne for the compost, then its market value could be in the order of £1,800 m annually (Anon 1984a). At present, there are no plans to produce worm protein for human consumption in the UK, but in the Philippines they are producing a worm protein in the form

of a powder which is to be used as an extender in beef burgers for the Japanese market. Also, according to recent media coverage the Japanese, who consider worm protein to be an aphrodisiac, are also interested in the idea of marketing whole worms.

Research carried out by the NIAE and Rothamsted Experimental Station has resulted in the development of two types of beds for worm compostings. The first is a batch process using a simple static bed, and, more recently, a continuous bed has been developed. Fresh waste is loaded on the top surface of the worm-worked waste and the worms move slowly upwards against the flow of the waste, with the worked compost being discharged through a special floor. This system retains all the worms within the bed so that the need for worm separation is eliminated. Other advantages of the continuous system, compared with the batch process, include a faster working rate, better quality product, lower labour requirement, and easier management (Phillips 1986). Both batch and continuous flow systems are used commercially throughout the world (Riggle and Holms 1994). However, Meyer and Loots (1999) have reported problems of anisopary in worm populations, raising the question of whether such populations can be sustained for long periods without being supplemented with new worms in order to maintain conversion rates in commercial units.

Work by the US EPA (Eastman *et al.* 2001) has shown that vermicomposting can be used for class A biosolids (sewage sludge) stabilisation. Using two windrows of class B biosolids 6 m long, these were heavily inoculated with faecal coliforms, *Salmonella* spp., enteric viruses, and helminth ova. One windrow was left as a control the other was seeded with the earthworm *Eisenia foetida* at a wet weight earthworm biomass to biosolids ratio of 1:1.5. Significantly higher reductions of all pathogens occurred in the row with earthworms. After just 144 hours, the worm seeded windrow showed reductions of pathogens of 6.4, 8.6, 4.6, and 1.9 log reductions in faecal coliforms, *Salmonella* spp., enteric viruses, and helminth ova respectively compared to 1.6, 4.9, 1.8, and 0.6 log reductions in the control (worm free) windrow.

10.4. Environmental Protection

Wastewater biotechnology has an important role to play in environmental protection. Apart from removing organic matter and nutrients from wastewater before it is discharged to a watercourse, thus preventing pollution, biotechnological advances have been made in four important areas. These are: the breakdown of recalcitrant compounds, the removal of metals

from effluents, bioscrubbing, and in the use of packaged micro-organisms for seeding or uprating biological reactors. These areas are considered separately below, except for the removal of metals which has been reviewed in a previous section (Sec. 10.2.3).

10.4.1. *Breakdown of recalcitrants*

It has often been reported that certain compounds are not degraded following release to the environment, even when conditions appear adequate for microbial growth (Slater and Somerville 1979). Such compounds are termed recalcitrant and are mainly synthetic organic chemicals. Slater and Somerville advise caution in placing compounds into this category as there may be any number of relatively trivial reasons why biodegradation does not occur under a given set of conditions. However, many compounds, the so-called foreign or xenobiotic compounds in particular, are known to be recalcitrant (Leisinger *et al.* 1981). A list of recalcitrants currently under investigation is given by Leisinger (1983). Recalcitrant molecules persist for extended periods in all natural environments regardless of whether the compound is or is not inherently biodegradable (Lynch and Poole 1979). They include detergents, pesticides, and a number of common polymers, although the latter group is of little interest in aquatic systems. Vast quantities of recalcitrant organic chemicals are produced, about 150×10^6 tonnes annually, which can find their way into surface and ground waters as constituents of industrial effluents (Mitchell 1974; Hutzinger and Veerkamp 1981). However, two groups of recalcitrants are particularly significant, the aromatic compounds and pesticides.

Many micro-organisms can metabolise particular aromatic compounds (Fewson 1981) (Table 10.23). Some green, brown, and red algae contain dioxygenases allowing them to partially oxidise hydrocarbons such as naphthelene, whereas many mesophilic organisms such as protozoans, rotifers, and nematodes use the hepatic cytochrome P-450 to detoxify and eliminate aromatic xenobiotics. In the natural environment, the degradation of xenobiotics is brought about by communities rather than individual species, and this observation has been of primary importance in improving the microbial degradation of xenobiotics and recalcitrants (Fewson 1981). Two basic methods are used to obtain suitable microbial cultures to degrade xenobiotics: enrichment culture and gene manipulation. Enrichment culture is the standard method used and involves taking an inoculum of micro-organisms from a habitat that preferably has already been exposed to the compound of interest. The inoculum is then cultured and exposed

Table 10.23. Major micro-organisms involved with the degradation of aromatic compounds (Fewson 1981).

Organism	Substrate
Procaryotes	
Gram-negative bacteria	
E. coli	3- or 4-Hydroxyphenylacetate
Alcaligenes spp.	Aromatics
Azotobacter spp.	Aromatics
Flavobacterium spp.	Aromatics
Gram-positive bacteria	
Bacillus megaterium	Salicylate
B. brevis	Salicylate
B. stearothermophilus	Phenols, cresols, 4-hydroxyphenylacetate
Actinomycetes	
Nocardia spp.	Aromatic carboxylic acids, phenol, and substituted phenols
Rhodococcus spp.	Naphthalene, biphenyl
Methanotrophs	
Methosinus trichosporium	Benzene, toluene, ethylbenzene, cresols, 1-phenyl-theptane, phenylacetate, and others
Methanogens	Aromatics
Eukaryotes	
Trichosporon cutaneum	Phenol
Cuiruinghainella elegans	Biphenyl (partial metabolism)

to progressively larger concentrations of the xenobiotic compound until it eventually becomes a source of essential nutrient. Batch culture can be used to screen for suitable organisms, whereas with continuous culture, selection pressure is exerted resulting in the culture becoming dominated by the micro-organism best able to adapt to the available nutrients. It is possible to increase the rate at which adaptation takes place by increasing the mutation rate using radioactive or chemical mutagens (Harder 1981; Powledge 1983). For example, Youssef and Aziz (1999) used gamma-irradiation on *Trichoderma viride* a fungus grown on rice straw to produce SCP. The mutagenic effect increased cellulose activity of the fungus increasing biomass protein content and yield. Although this technique is both slow and random, with the selected organisms discovered by accident, genetic manipulation offers a faster and more controllable development of useful organisms. Gene manipulation using plasmid transfer, gene cloning, and transposon mutagenesis have all been used with some success. For example,

the breakdown of the alkanes, toluene and xylene is now understood quite well (Chakrabarty 1980; Williams 1981), with all the information required by the micro-organism contained in the plasmids which can be transferred by genetic manipulation. Gene cloning techniques using *Pseudomonas aeuroginosa* enables genetic manipulation procedures to be carried out on a wide range of Gram-negative bacteria that will improve degradation of aromatic compounds, such as toluene, xylene, 3-chlorobenzoate, and 2,4-dichlorophenoxyacetate (Christopher *et al.* 1981). The General Electric Company have patented a *Pseudomonas* bacterium that can degrade octane, xylene, metaxzylene, camphor, and salicylate (Davis 1979). Strains of bacteria able to decompose aromatics can be added to a normal bioreactor, usually activated sludge type systems, where they will either persist or donate their special abilities to other strains (Dagley 1981; Knackmuss 1981). The main agents for degrading crude soil, which is a mixture of many different aromatic and aliphatic hydrocarbons, are bacteria such as *Pseudomonas*, *Micrococcus*, *Coryuebacterium*, and *Mycobacterium*, with yeasts also able to degrade petroleum hydrocarbons to a lesser extent. In natural waters, this degradation is, almost without exception, an aerobic process. Davis (1979) describes an extended activated sludge process, with added nutrients, used for the biological treatment of waste machinery coolants. The reactor was originally seeded with activated sludge from a nearby municipal wastewater treatment plant and suitable micro-organisms gradually developed.

For every litre of olive oil produced, 2.5 litres of wastewater is produced, with approximately 1×10^7 m^3 of such waste produced annually in the Mediterranean region alone (Fiestas *et al.* 1996). The wastewaters are particularly polluting as they contain a range of polyphenols, sugars, volatile acids, polyalcohols, and nitrogenous compounds (Table 10.24), with the phenol concentration often as high as 10 mg/l. The antibacterial properties of phenols make conventional biological treatment ineffective for such wastewaters, with physico-chemical and oxidation processes preferred. Phenols have a similar structure to lignins making them difficult to degrade. A few micro-organisms such as the white-rot fungus *Pleurotus ostreatus* (basisiomycete) are able to degrade lignins by the production of enzymes such as manganese and lignin peroxidases, and laccases (Michel *et al.* 1991). The enzymatic system of the white-rot fungus is activated by the presence of compounds such as polyphenols, aromatic amines and dimethylo-trimethylo phenols in olive oil wastewaters resulting in the detoxification of the wastewaters and allowing them to be readily degraded anaerobically (Martirani *et al.* 1996). Fountoulakies *et al.* (2000) have used the fungus experimentally

Table 10.24. Composition of olive mill wastewaters (Fountoulakis *et al.* 2002).

Parameter	Value (mg/L)[a]
TSS	46120 ± 5250
VSS	41910 ± 6330
Total COD	105373 ± 13870
Dissolved COD	58197 ± 1750
BOD_5	47780 ± 1570
Total phenols	10.2 ± 0.14
Dissolved phenols	8.2 ± 0.09
Total phosphorus	293.9 ± 15.87
Dissolved phosphorus	212.6 ± 29.66
TKN	750 ± 24
pH	5.4 ± 0.07

[a]Except pH.

to degrade the phenols in oil mill wastewaters and found removal rates between 50–78% under varying conditions. As methanogenic bacteria are seriously affected by phenolic compounds pretreatment of such wastewaters, using *P. ostreatus* will make it more amenable to anaerobic digestion. Wood rotting fungi are used for other wastewater applications such as the treatment of pulp mill effluents using *Phanerochaete chrysosporium* and *Coriolus versicolor* (Archibald *et al.* 1990; Feijoo *et al.* 1995; Perez *et al.* 1997) and the decolourization of cotton bleaching effluents using *C. versicolor* (Summerhill and Barnes 1993; Zhang *et al.* 1999).

Pesticides are extremely persistent in soils and are usually extremely resistant to degradation (Table 10.25). There are no specific pesticide-decomposing micro-organisms, with degradation only occurring if the micro-organism has the ability to synthesise the appropriate enzyme. Degradation is often dependent on the sequential action of two or more micro-organisms (Mitchell 1974). Golovlena and Skvyabin (1981) examined the pathway of DDT degradation by *Pseudomonas aeuroginosa* (strain 64OX). They found that degradation depended on the metabolism of co-substrates and that the succession of these different substrates and the aeration conditions required were probably too complex to occur under natural conditions. However, Gasner (1979) felt that recalcitrant wastes, such as pesticides and halogenated aromatics, are often of sufficient concentration in wastewaters to support microbial growth and to be possibly biodegradable within the normal treatment process. The application of

Table 10.25. Persistence of pesticides in the soil (Alexander 1965).

Common name	Chemical structure	Persistence
Aldrin	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-endo-1,4-exo-5,8-dimethanonaphthalene	> 15 yr
Chlordane	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene	> 15 yr
DDT	1,1,1-Trichloro-2,2-*bis*(*p*-chlorophenyl)-ethane	> 15 yr
Dicamba	3,6-Dichloro-*o*-anisic acid	4 yr
Diuron	3-(3,4-Dichlorophenyl)1,1-dimethylurea	> 15 mth
2-(2,4-DP)	2-(2,4-Dichlorophenoxy)propionic acid	> 103 days
Endrin	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-endo-5,8-dimethanonaphthalene	> 14 yr
Fenac	2,3,6-Trichlorophenylacetic acid	> 18 mth
Fluometuron	N'-(3-Trifluoromethylphenyl)-N,N-dimethylurea	195 d
Heptachlor	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-endomethanoindene	> 14 yr
Lindane	1,2,3,4,5,6-Hexachlorocyclohexane	> 15 yr
Monuron	3-(*p*-chlorophenyl)-1,1-dimethylurea	3 yr
Parathion	*O*,*O*-Diethyl *O*-*p*-nitrophenyl phosphorothioate	> 16 yr
PCP	Pentachlorophenol	> 5 yr
Picloram	4-Amino-3,5,6-trichloropicolinic acid	> 5 yr
Propazine	2-Chloro-4,6-*bis*-(isopropylamino)-*s*-triazine	2–3 yr
Simazine	2-Chloro-4,6-*bis*-(ethylamino)-*s*-triazine	2 yr
2,4,5-T	2,4,5-Trichlorophenoxyacetic acid	> 190 d
2,3,6-TBA	2,3,6-Trichlorobenzoic acid	2 yr
Toxaphene	Chlorinated camphene	> 14 yr
Trifluralin	α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine	> 40 wk

biofilms has been successfully employed in drinking water treatment to remove and degrade a range of hazardous water xenobiotics such as chlorinated benzenes, ethylbenzene, styrene, and naphthalene to mineralised end products (Manem and Rittman 1992; Carraro *et al.* 2000).

Bacteria can also detoxify metals. For example, selenium, an essential trace element for organisms, is present in the environment in a number of different forms. There are two soluble oxidised forms, selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), which are toxic at higher concentrations; elemental

selenium (Se^o) which is insoluble and non-toxic under normal environmental conditions; and the highly toxic gas selenide (Se^{2-}) which is spontaneously oxidised to non-toxic elemental selenium in the presence of air. Selenium is widely used by industry in the production of glass, pigments, pesticides, stainless steel, and photoelectric cells, with wastewaters from these industries containing large quantities of soluble selenium. It is also common in oil refinery wastewaters and in the water used to flush fly ash from coal-fired power stations (Ohlendor and Santolo 1994). Several bacteria strains are capable of reducing selenate into elemental selenium via selenite under anaerobic conditions, thereby detoxifying soluble selenate in aquatic environments (Maiers *et al.* 1988; Lortie *et al.* 1992; Fujita *et al.* 1997). The bacteria have been isolated mainly from Se rich or Se polluted sediments. However, Ike *et al.* (2000) collected material from unpolluted sediments and found them all to exhibit selenate and selenite reduction capabilities, showing the ability to be widespread. From these sediments three different selenate-reducing bacteria were isolated, two strains of *Pseudomonas stutzeri* and one strain of *P. fluorescens*, although the rates of reduction were lower than other isolated strains. The use of bacteria in bioaugmentation is considered in Sec. 10.4.3.

10.4.2. *Bioscrubbing*

Waste gases are a major pollution control problem for many industrial processes, with unpleasant odours particularly difficult to remove because of their low concentrations. For example, some mercaptans have an odour threshold of below 1 ppb. The control of odours and waste gases requires complex and very expensive technology, with incineration, dispersion, catalytic oxidation, scrubbing, and adsorption the major control options available. However, many of these physical and chemical processes are not very flexible if the volume, concentration or composition of the gas alters, resulting in a general reduction in performance. This is largely overcome by the biological control of gases, first proposed in 1930, which provides combined liquid and gas treatment with no chemical costs and, compared with other methods, negligible energy costs. Packed beds (biofilters), similar to percolating filters, are used as the medium supports a large area of biofilm that provides a large gas-liquid surface area for adsorption (Le Roux and Mehta 1980; Le Roux 1982). Removal occurs by adsorption on to the biofilm followed by solubilisation of the compound, which is subsequently degraded by microbial action. Biofilters have been used to treat all volatile organic

compounds including chlorinated and non-chlorinated compounds (Cox and Deshusses 1999; Bibeau *et al.* 2000; Deshusses and Webster 2000). Walshe (1988) describes a biofiltration unit, which is used to remove the offensive odours produced at an animal rendering plant in South Tipperary, using a mixture of 25% peat and 75% heather. The odorous air is sprayed with water to lower its temperature and increase its humidity before it is pumped under pressure up through the filter. Efficiencies of such plants in West Germany are in the order of 95% reduction in odour. Other examples include the treatment of odours from animal by-product rendering (Kock *et al.* 1982), compost wastes (Gust *et al.* 1979), and intensive livestock rearing (Dragt *et al.* 1987).

Odour nuisances at sewage treatment works vary from plant to plant but typically arise from the inlet works, high-rate percolating filters, and sludge treatment facilities, especially sludge thickening and dewatering, primarily due to volatile sulphur compounds such as mercaptans and hydrogen sulphide (North 1979; Frechem 1988; WRc 1995; Gostelow *et al.* 2001) (Table 10.26, Fig. 10.32). Odour production in sewers, leading to inlet problems, is reviewed by Spooner (2001). It is now common practice

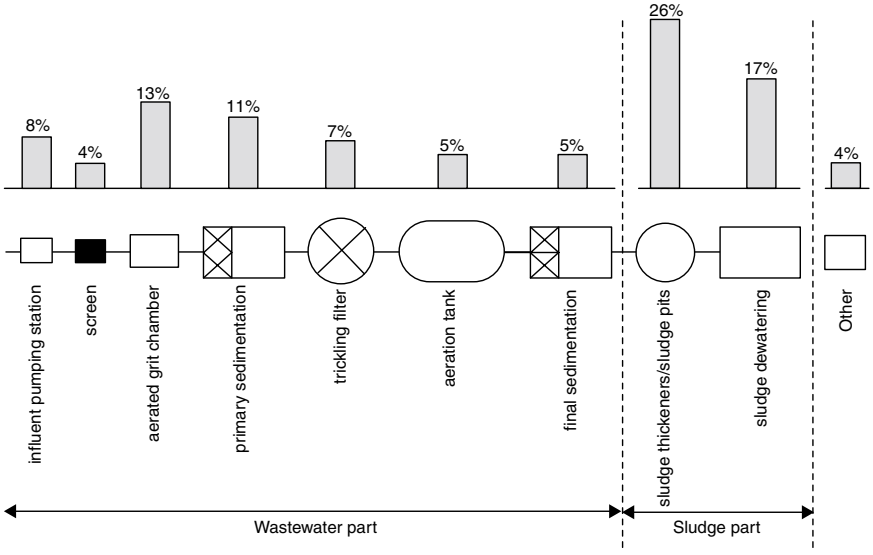


Fig. 10.32. Major sources of odour from a survey of 100 German sewage treatment works. Figures are the percentage of respondents who identified the process as an odour source (Frechen 1988).

Table 10.26. Expected concentrations ranges of odour and sulphur compounds in gas emissions from sewage treatment works (Brennan *et al.* 1996).

Installation	Typical concentration ranges		
	Odour (odour units/m^3)	Hydrogen sulphide (ppm)	Methyl mercaptan (ppm)
Inlet works (gravity feed)	50–5000	< 1	< 1
Inlet works (rising mains)	1000–10 000	1–10	0.5–5
Screening plant	100–5000	1–10	0.5–5
Primary sedimentation tank	50–1000	≤ 1	< 1
Biological filter	50–5000	≤ 1	< 1
Aeration basin	50–5000	1–5	< 1
Final clarifier	< 500	< 1	< 1
Desludging chamber	1000–10 000	10–100	1–10
Primary sludge tank	1000–500 000	10–500	1–250
Activated-sludge tank	500–5000	1–5	≤ 1
Picket-fence thickener	500–10 000	5–100	1–10
Sludge dewatering plant	1000–50 000	0.5–50	0.5–15

to cover these critical areas of the plant and venting the buildings through a scrubber to remove odours. Systems are also treating odours from other treatment units, such as digesters or composting systems. However, it is possible to seed reactors with specially cultured micro-organisms that are capable of removing specific gases.

The media used in biofilters varies widely, although media which also has high adsorptive capabilities such as peat (Brennan *et al.* 1996), compost (Rands *et al.* 1981), and bark (van Langenhore *et al.* 1986), can also play a major role in the removal of odourous compounds while at the same time providing a surface on which the biofilm can develop. The most frequently cited reason for failure is insufficient moisture content of the air entering the biofilter so that a healthy biofilm cannot be maintained. The mechanical stability of the media is also important. Inhibition due to acidification and accumulation of inorganic salts can also significantly affect the effectiveness of such systems (Vab Lith *et al.* 1997). Although there are now many biofilters in operation to treat odorous gaseous emissions from treatment plants, the injection of such gases into activated sludge aeration tanks is also widely practised.

In bioscrubbers, biological degradation of odourous compounds takes place in a liquid phase with the micro-organisms in suspension within a reactor. Similar to activated sludge these are compact units especially constructed solely for odour removal. Bioscrubbers are much more compact systems than biofilters and can be operated at much higher air loadings, 3,000–4,000 $m^3m^2h^{-1}$ compared to just 100–115 $m^3m^2h^{-1}$ respectively, making the former ideal for sewage treatment plants where air ventilation rates are very high. For example, Hansen and Rindal (2001) describe the actions taken at the Damhusaaen Sewage Treatment Plant in Copenhagen, Denmark, to minimise odour nuisance. An odour survey identified the inlet works, particularly the screens and grit-grease separation chambers as the major sources of odour. These were covered and some 6,000 m^3h^{-1} of ventilated air was cleaned by a bioscrubber. Cleaning efficiency for hydrogen sulphide was $> 90\%$ with less than 0.1 mg m^3 of organic sulphur left in the air. Odour has been significantly reduced around the plant to the extent that it is no longer detectable. Bioscrubbers can also be used for simultaneous nitrification and denitrification when treating odourous N compounds (Rasmussen *et al.* 1994).

Little is known about the ecology of biofilters or bioscrubbers. Protozoa have been shown to reduce the biomass in biofilters used to treat toluene (1g m^3) contaminated air by 15% (Cox and Deshusses 1999).

10.4.3. *Bioaugmentation*

The use of specially cultured micro-organisms, mainly bacteria, is known by a variety of terms such as seeding, biomass enhancement, inoculum addition, and more widely as bioaugmentation. Bioaugmentation, or the use of commercially grown micro-organisms to supplement or replace naturally occurring microbes, is now widely practised in wastewater treatment and pollution control (Huban and Plowman 1997). The micro-organisms used are usually naturally occurring species collected from special sites where natural selection has already favoured microbes adapted to unusual conditions. For example, up to half the micro-organisms in coastal waters can degrade hydrocarbons, while meat-processing plants and even septic-tanks can yield microbes capable of degrading lipids and proteins. These organisms are grown in the laboratory and are cultured on a medium which is rich in the pollutant they are required to degrade. From this, the best strains can be isolated and grown for commercial exploitation (Fig. 10.33). This enrichment technique can be developed by using mutagenesis to find more efficient degraders or by producing new custom-built microbes by gene manipulation (Powledge 1983) (Sec. 10.4.1). In practice, only organisms selected from the environment are used, with mutation or

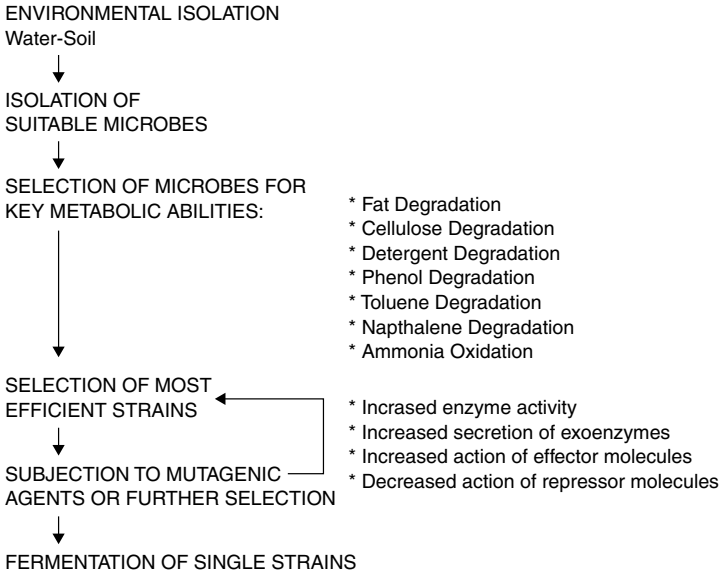


Fig. 10.33. Process development and potential applications of packaged micro-organisms (Thibault and Tracy 1979).

gene manipulation only used for enhanced industrial processes (for example, the production of enzyme), so that there are no problems about releasing new genetically engineered species into the environment.

The microbial culture which is eventually produced is grown in special reactors in order to produce the large quantities needed, then freeze-dried and packaged for sale. Most companies market their microbes as a powder that also contains important additives, such as wetting agents, emulsifers to aid dispersion, and nutrients. To activate the microbes all that is required is to add warm water and stir. Bioaugmentation is used for the following key application: (i) improved removal efficiencies in terms of BOD or COD removal; (ii) to improve the degradation of target substances that are often recalcitrant or cause operational problems; (iii) to reduce process instability often caused by fluctuation in the organic loading; to restart or commission plants; (iv) to reduce the inhibitory effects of toxic compounds in wastewater; (v) to improve mixed liquor flocculation and separation; (vi) to induce and enhance nitrification; and (vii) to reduce the accumulation of sludge and scum from aerobic and anaerobic digesters and lagoons by ensuring complete stabilisation.

For most of the above applications, the commercial product, either as micro-organisms or enzymes, is added directly to the biological reactor. The amount of product used is based on the activity level of the product, the concentration of the target substrate, and the desired effluent quality. In some circumstances (e.g. applications (ii) and (iv)), the most efficient micro-organisms must be isolated and selected from the existing treatment plant on site, then grown on site in a separate reactor and added to the main influent waste stream either continuously or when required (Glancer and Soljan 1998). The off-line reactor used to grow the micro-organisms for use in the main reactor is called an enricher-reactor and is now commonly used (Cardinal and Stenistrom 1991; Babcock *et al.* 1992; Saravanane *et al.* 2001).

Although most of their products are supplements for wastewater treatment they also produce a range of other pollution control products including dried microbial seeds for the BOD test.

There is a wide range of products for improving the BOD removal of all types of wastewater treatment plants, eliminating filamentous bulking in the activated sludge process, and improving methane generation in anaerobic digesters. Numerous studies have shown that bioaugmentation with nitrifiers can produce nitrification at sludge ages that would otherwise preclude nitrification. Also, that the necessary reactor volume needed for nitrification can be reduced by bioaugmentation (Sinjaer *et al.* 1996; Li and

Hultman 1997; Plaza *et al.* 2001). Although bioaugmentation with *Acinetobacter* spp. has not shown a positive correlation between bacterial density and phosphorus removal (Oerther *et al.* 1998). The purple non-sulphur bacteria *Rhodobacter* spp. have been successfully used to bioaugment the treatment of anerobically pretreated piggery wastewater by the activated sludge process. The bacteria are chemo-heterotrophs that grow aerobically in the light or dark. They are able to utilise fatty acids, other organic acids, primary and secondary alcohols, and some aromatic compounds (Stanier *et al.* 1976; Huang *et al.* 2001).

There has been considerable success in using specialised cultures to clean up chemical spillages and also decontaminating soil (Cooke *et al.* 1983). Commercial micro-organisms for cleaning up spillages of crude oil or hydrocarbons are produced by most companies. Also, microbial cultures that are able to degrade most of the chemicals that are transported in bulk are now available world-wide, including pesticides and even Arochlor 1260 which is one of the most highly chlorinated of the polychlorinated biphenyls (PCBs). An interesting example occurred in northern California where a spillage of 21,000 gallons of 50% formaldehyde solution was successfully treated by containing the liquid in a sealed drainage ditch and pumping it through a temporary bioreactor, where it was aerated and mixed with a hydrocarbon degrader made up of several mutant bacteria produced by the Polybac Corporation. The formaldehyde concentration was reduced from 1,400 mg l^{-1} to < 1 mg l^{-1} after just 14 days, with the whole operation costing about 10% of physically removing the spillage. As well as dealing with spillages to surface water, opportunities also exist for cleaning up contaminated wells and aquifers. Fat and grease-degrading bacteria can be added to drains and sewers to prevent blockages that would normally have to be removed by hand. They are also better than degreasing chemicals which are often problematic to treat at the treatment plant and who's action is only temporary, just transferring the problem further downstream. A good example was in New York City in 1982, when $25,000 was spent on bacteria to degrade the grease that clogged the sewers beneath the city's restaurants. The biological result was the same as the previous method, which was using augers to physically drill through the grease, but at a third of the price (Powledge 1983).

A major use of bioaugmentation is a microbial seed to inoculate the BOD test (Sec. 3.1.3). The seed normally comes in a capsule that contains 100 mg of specialised microbial culture made up of 15 to 20 different strains including *Pseudomonas* sp., *Nocardia* sp., *Streptomyces* sp., *Bacillus* sp., and *Micromonospora* sp. Each capsule provides enough seed for up to 500

BOD tests, with the culture being activated by adding the contents of a capsule to 1 litre of nutrient water and leaving overnight, but kept continuously stirred. Then, 5 ml of the activated culture is then added to each litre of dilution water used. Fitzmaurice (1986) compared the performance of one BOD seed with raw sewage. He found that the commercial seed produced BOD results with a reaction rate similar to that obtained using raw sewage as seed, but with a higher repeatability estimate. He concluded that apart from the obvious convenience factor, its widespread use in water pollution laboratories could significantly reduce inter-laboratory variation (Fitzmaurice and Gray 1987a). Various combinations of bacterial species have been tested to achieve the optimal breakdown of organic waste compounds during the BOD test. Kumar *et al.* (1999) compared a number of different combinations of heterotophs and found a consortium made up of *Yersinia*, *Enterobacter*, *Pseudomonas*, *Serratia*, *Klebsiella* and *Enterobacter* to be most effective.

There can be no doubt that commercially produced micro-organisms can achieve success in treating many difficult and recalcitrant wastes as well as uprating and seeding wastewater treatment plants, providing the conditions are suitable and the correct culture of microbes is utilised. Packaged micro-organisms and their enzymes have to survive and function in the sub-optimal conditions of a complex ecosystem. The strains that are marketed are generally selected for their ability to metabolise certain pollutants, rather than on their ability to survive environmental stress. Although they are ill-adapted to cope with predation by protozoans, myxobacteria, and slime moulds, like all natural populations they will adjust to the predation level. However, other factors, such as abiotic stresses, including sunlight and temperature, may have a significant effect on numbers and hence efficiency. Clearly, in these situations, enhanced cultures may be more successful than either mutated or gene manipulated cultures. The use of these products is very expensive and their use should be limited to those situations where normal treatment is at risk. They should not be used as a substitute for good operational management of existing biological reactors. However, they are now considered as a normal remediation option. For example, wastewater treatment plants, and in particular activated sludge systems, can be significantly inhibited by the presence of toxic compounds such as phenolics in their influent. Three strategies could be considered to deal with this problem: (i) pretreatment (Lin and Chuang 1994); (ii) removal by the addition of activated carbon or chitosan into the reactor (Speccia and Gianeto 1984; Galil and Rebhun 1992); or (iii) bioaugmentation of the biomass (Cardinal and Stenstrom 1991; Abllah and Lee 1991).

There is often an over expectation by the purchaser of what packaged micro-organisms can achieve in the field. This is often due to suppliers overstating what their products can actually achieve (Gasner 1979). Stephenson and Stephenson (1992) reviewed the use of bioaugmentation of biological wastewater treatment systems and examined in depth the reasons why actual results in the field were often disappointing. Among the more common reasons cited were (i) substrate concentration was too low to support growth; (ii) presence of inhibitory substances; (iii) suboptimal or inhibitory growth conditions (e.g. temperature); (iv) insufficient acclimatization period; (v) too few organisms to effect significant change; or (vi) introduced organisms are not put into an environment where it can effectively consume the desired substrate.

Nutritive supplements that don't contain bacteria are also widely available. For example, Nutriflok 50S® contains nutrients and flocculating agents sold to improve activated sludge performance. Independent studies have shown that such suppliments can improve both performance and settleability (Vansever *et al.* 1997).

The Environmental Protection Agency (EPA) in the USA regulates the release of mutagenic and genetically engineered organisms into the environment, and there is also considerable public concern about these organisms. This means that until suitable tests have been developed by the EPA to evaluate new organisms, there are going to be long delays before permission is given to allow these organisms to be used, and future research is going to be at a standstill. Without the prospect of newly developed products, the investment in pollution control will decline, even though biotechnological processes are much cheaper and safer than traditional disposal alternatives (Powledge 1983).

Chemical pesticides, although effective, have numerous unwanted side effects such as pollution, destruction of non-target organisms, resistance, and poor degradability leading to accumulation in the environment and food chain. Biological insecticides are now seen as a safer and more sustainable method of pest control. Bacteria, protozoa, viruses, and fungi are all used as biological control agents (Aronson *et al.* 1986; Burges 1986). Perhaps one of the most successful examples is the use of *Bacillus thuringiensis* for the control of insect vectors primarily in the agricultural and forestry sectors, with different strains effective against lepidopteran, dipteran, and coleopteran pests (Rowe and Margaritis 1987). The bacteria produces a crystal inclusion during the sporulation phase called δ-endotoxin which is comprised of protein sequences that are toxic to the target insect (Höfte and Whiteley 1989). The bacterium is not toxic to non-target

organisms and the toxin produced is readily degradable thereby minimising environmental risk.

Bacillus thuringiensis based biopesticides are very expensive due to high production costs, a susbstantial part of which is due to the expensive synthetic media required to grow the bacteria (30–40%) (Sachdeva *et al.* 1999). This is based on soya bean meal, fish meal, glucose, yeast extract, peptone, and trace elements (Ejiofor 1991; Lisansky *et al.* 1993) Therefore, in order to reduce the cost of these biopesticides alternatives, cheaper, growth media are required (Tirado Montiel *et al.* 1998). Tirado Montiel *et al.* (2001) and Vidyarthi *et al.* (2002) have demonstrated that sewage sludge contains all the necessary nutrients to sustain growth and sporulation of the bacteria and have proposed it as a cheap alternative growth medium.

10.4.4. *Immobilised cells and biosensors*

Immobilised cells for wastewater treatment provide compact specialist treatment units ensuring high biomass concentrations without the risk of biomass loss. Immobilisation is mainly by entrapment in polymeric materials such as agar, agarose, alginate, carrageenan, cellulose, chitosan, collagen, gelatine, polyacrylamide, polyurethane, and polyvinyl alcohol (PVA) (Leenen *et al.* 1996).

Polyacrylamide has been identified as having a detrimental effect on cell viability and has been replaced by PVA. Polyvinyl alcohol is extremely popular due to its mechanical strength, durability, cell viability and cost (Hashimoto and Furukawa 1987). The PVA is crosslinked with boric acid for a short time (10–120 minutes) to form spherical beads that are solidified by esterification with phosphates (Lin and Chen 1993). A small amount of alginate can also be used during crosslinking to improve surface properties and prevent agglomeration of beads (Wu and Wisecover 1992). An and Lo (2001) used this technique to immobilise activated sludge for use in a fluidised bed reactor.

Ozaki *et al.* (1991) have co-immobilised ferromagnetic particles so that they could recover beads using a magnet. The beads are normally 2–3 mm in diameter and may form distinct oxygen gradients within the beads. However, microspheres of a diameter of 2–50 μm have been developed for use in groundwater restoration (Stormo and Crawford 1992).

Natural algal polysaccharides have been widely studied for use as an immobilisation entrapment medium. Carrageenan is extracted from *Rhodophyceae* while alginate is extracted primarily from *Phaeophyceae*. The organisms to be immobilised are mixed in a solution of sodium alginate. The

alginate reacts with most divalent cations, especially calcium, to form a gel. So by slowly dripping the mixture over a solution of $CaCl_2$ beads are instantaneously formed. Gel formation takes about 60 minutes to complete in the chloride solution (Cheetham and Bucke 1984; Bucke 1987). The beads can be damaged by gas production by the immobilised organisms, by microbial growth on the surface of the beads, or by leaching of calcium ions from the calcium alginate bead by phosphates or any other calcium-chelating agent. For this reason PVA has become the most widely used entrapment medium.

Immobilised cells have been used in a wide variety of wastewater treatment and pollution control applications (Crawford and O'Reilly 1989; Cassidy *et al.* 1996). For example, immobilised fungi have successfully broken down recalcitrant compounds. Nazaly and Knowles (1981) demonstrated that immobilised fungi could produce the enzyme cyanide hydratase that can break down cyanide to formamide. The white rot fungus (*Coriolus versicolor*) has been immobilised to treat brown lignin compounds in paper mill effluents (Livernoche *et al.* 1983). Immobilised bacteria can successfully treat phenolic compounds (Bisping and Rehm 1988; Heitkamp *et al.* 1990), and chlorinated phenols (O'Reilly *et al.* 1988; Sofer *et al.* 1990; Wada *et al.* 1992, 1993). Another successful application has been in enhanced nitrification and denitrification. Nitrifying bacteria immobilised in polyethylene glycol resin beads was shown to rapidly increase the rate of nitrification when added to activated sludge reactors (Tanaka *et al.* 1991). Vogelsang *et al.* (1997) found that their nitrifying bacteria, immobilised in crosslinked PVA-alginate beads maintained their activity for 10 months, showing that immobilised nitrifyers are more stable than free cells (Asano *et al.* 1992). Denitrifying bacteria immobilised in PVA or cellulose triactetate have been reported as being 99% efficient (Lin and Chen 1993). Residual methanol added to denitrifying units can be effectively removed by methanogens, if they are co-immobilised with the denitrifying bacteria, turning it into methane gas (Chen *et al.* 1997). The advantages and disadvantages of immobilised cells are summarised in Table 10.27.

Biosensors comprise a biological sensing element, normally immobilised micro-organisms, that produce a signal, when they interact with a specific analyte, that is transmitted to a transducer that is able to convert it into an electrical signal (Fig. 10.34). Other biological sensing elements include enzymes, nucleic acid, or antibodies. Turner *et al.* (1987) has listed the key biological sensing elements and the transducers commonly employed (Table 10.28).

The most successful wastewater application of biosensors has been in rapid determination of BOD. Biochemical oxygen demand sensors comprise

Table 10.27. Some advantages and disadvantages of immobilised cells used in wastewater treatment.

Advantages	Disadvantages
Continuous reactor operation	Diffusion problems due to high cell density
No wash out of biomass	Cell physiology changes may effect productivity
Simple separation of biomass and liquid	Beads can be overgrown by other microbes
High cell densities possible	Cost of immobilisation can be high when using artificially captured systems
Cells reused many times	Possible loss of bead stability with time
Comparatively long operational life	
Control of settlement problems (e.g. bulking)	
Microbes performing different reactions can be easily spatially separated	
Smaller reactor volumes possible due to high cell concentration	
Immobilized cells are very stable	
Increased plasmid stability	
Higher productivity per unit volume	
Immobilised microbes are protected from environmental stress and toxicity	
Easy and low-cost operation	

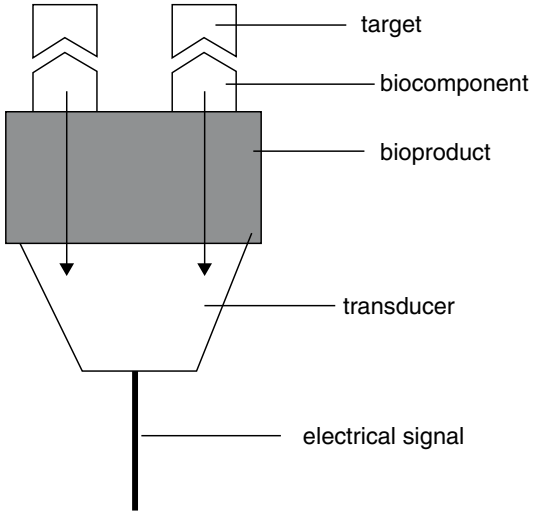


Fig. 10.34. Main components of a biosensor (Bitton 1999).

Table 10.28. Transducers used for different biological elements in biosensors.

Biological element	Transducer
Organisms	Potentiometric
Tissues	Amperometric
Cells	Conductimetric
Organelles	Impedimetric
Membranes	Optical
Enzymes	Calorimetric

an oxygen electrode consisting of a platinum cathode and silver anode bathed in saturated KCl solution, and a gas permeable Teflon membrane. The micro-organisms are immobilised on a porous membrane and trapped between the porous and Teflon membranes (Fig. 10.35). Oxygen is consumed by the immobilised micro-organisms, causing a decrease in current until a steady state is reached. The drop in current is correlated with BOD. Riedel *et al.* (1990) immobilised *Trichosporum cutaneum* in PVC and found a good linear correlation with the standard five day BOD test with a response of < 30 seconds (Fig. 10.36). While the actual biosensor BOD:BOD_5 ratio varies from 0.4–2.81, most reported biosensors lie within

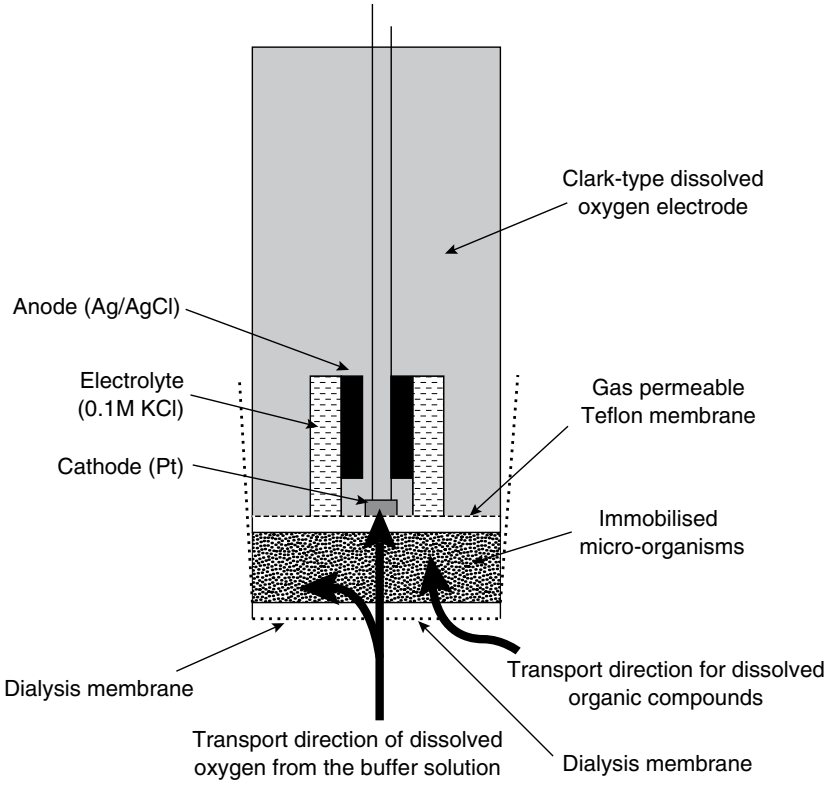


Fig. 10.35. Schematic diagram of a BOD sensor (Liu and Mattiasson 2002).

the range of 0.8–1.2. Samples with more readily assimilable compounds give less variable and more accurate biosensor BOD values compared with more complex industrial and municipal wastewaters. However, biosensor response is generally linear with standard BOD, so for specific wastewaters the linear regression can be used to calculate ratios (Riedel *et al.* 1997, 1998). A wide range of micro-organisms have been successfully employed in BOD biosensors including pure cultures of *Arxula adeninivorans*, *Bacillus polymyxa*, *Bacillus subtilis*, *Hansenula anomala*, *Klebsiella oxytoca*, *Pseudomonas putida*, *Serratia marcescens*, *Torulopsis candida*, *Trichosporon cutaneum*; mixtures of two strains (e.g. *Bacillus subtilis* and *Bacillus licheniformis*, *Rhodococcus erythropolis* and *Issatchenkia orientalis*); and microbial consortia (e.g. activated sludge). Commercial BOD sensors currently available are summarised in Table 10.29. The use of biosensors for BOD analysis is extensively reviewed by Liu and Mattiasson (2002).

Table 10.29. Some commercially available BOD biosensor instruments (Liu and Mattiasson 2002).

Model	BOD–2000 BOD–3000	DKK^{TM} BOD sensor 7842	BODypoint	BSBmodul	ARAS	BIOX–1010[c]	RODTOX 2000	BOD-BioMonitor	QBOD metre & EZ-BOD metre[e]	RACOD^{TM} metre
Manufacturer	Nisshin Denki & Central Kagaku Co. Ltd., Tokyo, Japan	DKK Corporation, Japan	Aucoteam FmbH, Berlin, Germany	Prüfgerätewerk Medingen GmbH, Dresden, Germany	Dr. Lange GmbH, Berlin Germany	STIP Isco GmbH, Groß-Umstadt, Germany	Kelma, Belgium	LAR Analytik & Umweltmesstechnik GmbH, Berlin, Germany	Bioscience, Inc., Bethlehem, USA	USFilter Vineland, NJ, USA
System configurations	Biofilm type, flow-through system	Biofilm type, flow injection with 3 ml measuring chamber	Biofilm type, flow-through system	Biofilm type, flow-through system	Biofilm type, with 2 ml stirred measuring chamber	A bioreactor combined with a dilution system	Respirometer type (BOD & toxicity analyser)	Respirometer type	Respirometer type (bioreactor)	Respirometer type
Microbial species	*T. cutaneum*	*T. cutaneum*	*T. cutaneum*, *C. parapsilosis*	*T. cutaneum*, *C. parapsilosis*	*R. erythropolis* *I. orientalis*	Bacteria isolated from wastewater	Activated sludge	Activated sludge	Activated sludge	Activated sludge
Measuring time (min)	20–40	5	< 1	3 or < 1	1–3	3–15	20–40	3–5	20[d], 15–60[c]	10 (high range) or 30 (low range)
Measuring ranges (mg/l BOD)	0–100, 0–200, 0–500[a] 3–1000[b]	0–60	5–500	0–22 or 2–33	2–300	5–1500, 20–1500, 20–100000	0–500000	0–50, 0–200000	0.5–300 or 0.5–5000[d]	100–4000 (high range) or 0–100 (low range)
Working Stability (days)	30[a,b]		30	30	30		14		60–90[d]	
Precision (±%)	3[a,b]	5	< 10	< 10	< 5	3	< 5		10 (low range)[d] 5 (high range)[d]	
Calibration Standard	GGA	GGA	Glucose	Glucose	Glycerol		Stabilised wastewater of the plant being monitored			

[a]BOD–2000.
[b]BOD–3000.
[c]Measuring principle based on dynamic dilution of two gear pumps depending on the O_2 respiration of microbes.
[d]QBOD metre.
[e]EZ-BOD metre.

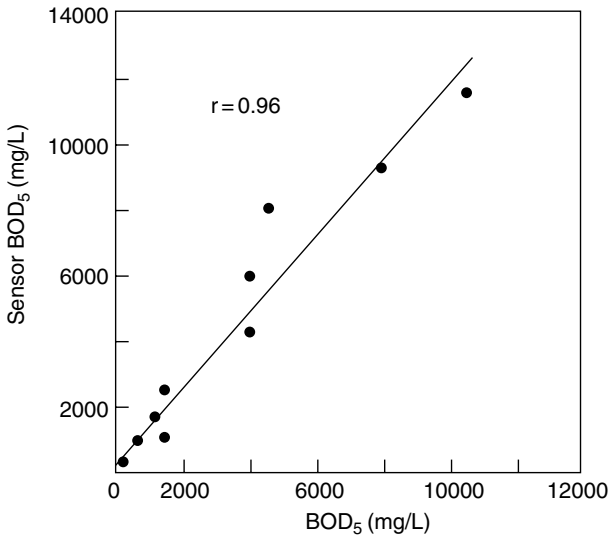


Fig. 10.36. Correlation between the 5-day BOD of wastewater and that measured by a BOD biosensor (Riedel *et al.* 1990).

Ammonia biosensors consist of a dissolved oxygen electrode and an immobilised microbial membrane in which nitrifying bacteria, usually *Nitrosomonas europacea*, are contained (Fig. 10.37). Research is underway to couple nitrification to a more sensitive detection system than reduction in dissolved oxygen concentration (Nielsen *et al.* 2002). Nitrate can be monitored by biosensors based on its conversion to N_2O by immobilising the denitrifying bacterium *Agrobacterium radiobacter* (Larsen *et al.* 1996, 1997). A nitrate sensor has been developed by substituting *A. radiobacter* with a bacterial strain that can only reduce NO_2^- and not NO_3 to N_2O (Nielsen *et al.* 2002). This provides a full range of biosensors for wastewater treatment plant nitrification. It is also possible that a reliable phosphate biosensor could also be developed in the near future. Inui *et al.* (2002) have successfully used an ammonia biosensor as a toxicity monitor to screen wastewaters. They found that when constantly fed with a sample containing a standard ammonia solution the output current of the dissolved oxygen electrode became stable at a level corresponding to the normal respiration rate of the bacterium. Chemicals added to the sample solution that can inhibit ammonia oxidation resulting in respiratory inhibition of the nitrifying bacteria. The differences in output correspond to inhibition and toxicity. Amtox® is a commercially available on-line toxicity screening system used for assessing both the toxicity and treatability of wastewaters

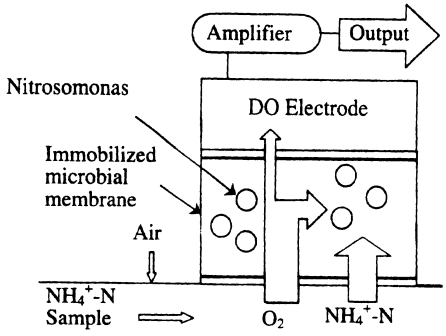


Fig. 10.37. Principal of an ammonia biosensor (Inui *et al.* 2002).

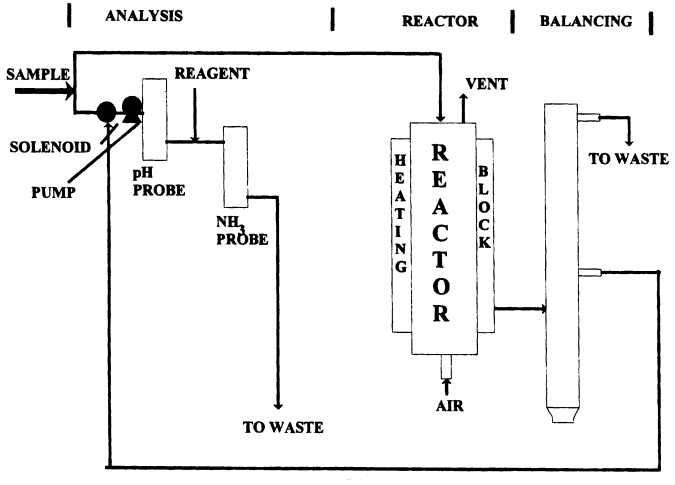


Fig. 10.38. Schematic diagram of the Amtox toxicity biosensor (Hayes and Upton 1999).

using immobilised cultures of nitrifying bacteria that are maintained in a miniature heated (30°C) aerobic reactor (Hayes and Upton 1999). This is an automated version of the nitrification inhibition test (DOE 1980). Probes determine the pH and the ammonia concentration of the sample at the start of the test so that adjustments can be made to optimise conditions in the miniature reactor for nitrification. The sample is then introduced into the reactor at a fixed rate during which the ammonical N in the reactor effluent is measured (Fig. 10.38). The level of inhibition is calculated by comparing the inlet and outlet ammonical N concentrations with the results continuously displayed on a PC. The system is completely automatic.

An algal biosensor using the cyanobacterium, *Spirulina subsalsa*, attached to a dissolved oxygen electrode has been developed by Campanella *et al.* (2000). The evolution of photosynthetic oxygen is affected by pollutants and is used for toxicity assessment in estuarine waters.

Further reading

Biotechnology: Bull *et al.* 1982; Cooke 1983; Sikyta 1983; Wheatley *et al.* 1983; Wheatley 1985; Sidwick and Holdom 1987.

Fertiliser value: Davis 1980; Smith 1996

Reuse of effluents: Shuva1 1977; Dean and Lund 1981; Prescod and Arar 1988; WHO 1989; EPA 1992; Asano 1998; Bonomo *et al.* 1999; Okun 2000.

Metal recovery: Murr *et al.* 1978; Ferraiolo and Del Borghi 1987; Volesky and Holan 1995.

Biosorbents: Bailey *et al.* 1999.

Phosphorus recovery: Galarneau *et al.* 1997; Battistoni *et al.* 2002; Doyle and Parsons 2002.

Bio-energy: Boyles 1984; Lewis 1983, 1988; Bridgewater and Double 1994; Batstone *et al.* 2002b; Thierbach and Hanssen 2002.

Single-cell protein and biomass: Rose 1979a,b; Benneman *et al.* 1979; Norris 1981; Samvelov 1983; Goldberg 1985; Wheatley 1987; Edwards 1992; Anupama and Ravindra 2000; Villas-Boas 2002.

Immobilised cells: Burlage 1997; Revsbech *et al.* 2000.

Composting: Haug 1980; de Bertoldi *et al.* 1983, 1988; Gasser 1985; Anderson and Smith 1987; Martin 1992; Haug 1993; de Bertoldi *et al.* 1996; Epstein 1997; Goldstein and Block 1997; Edwards 1998; de Bertoldi 1999.

Vermiculture: Riggle and Holmes 1994.

Breakdown of recalcitrants: Leisinger *et al.* 1981, Leisinger 1983.

Bioscrubbing: Le Roux and Mehta 1980; Hansen and Rindel 1992; Cox and Deshusses 1998.

Bioaugmentation: Powledge 1983; Kobayashi 1983; Hakulinen 1988; Rittman and Whiteman 1994; Huban and Plowman 1997.

11

Sustainable Sanitation

11.1. Introduction

Current biological wastewater treatment processes have changed remarkably little since their introduction in the late nineteenth century. The reliance on a few key processes, combined with the conservative nature of engineers, has meant that the wastewater industry has not been well-placed to embrace new concepts, especially that of sustainability. Their primary response has been to increase the efficiency of existing unit processes largely in terms of energy use and odour control. However, sustainability and efficiency are not the same thing as the motivating factors behind each are quite different. Also, the conflicting factors of capital and operational costs have increasingly resulted in less reliable, less robust, compact, high-energy systems being selected.

The industry is constantly looking over its shoulder, trying to conform to increasingly stringent legislation by retrofitting existing systems. However, sustainability requires long-term planning and a change to the basic concept of wastewater treatment away from current end of pipe solutions towards better resource utilisation. By anticipating the needs and potential problems of the next twenty to fifty years, innovation will speed up. Solely reacting to problems as they arise has the opposite effect of stifling innovation and slowing it down. The problems of water use, wastewater generation, and treatment must be considered holistically and without the constraining necessity of incorporating existing technology. As our lifestyles have become more complex, so has our waste. Hence, there is an urgent need to move away from the end of pipe treatment mentality, and to place some of the responsibility of treatment back to where it was generated.

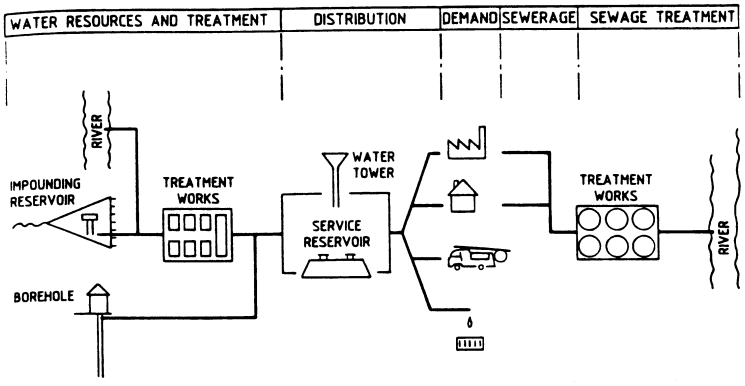


Fig. 11.1. Urban water cycle showing how water is used by humans during its movements within the hydrological cycle (Latham 1990).

Rather than looking at wastewater treatment in isolation, it should be seen as an integral part of the urban water cycle, which itself forms part of the natural hydrological cycle. In a sense water is just intercepted from the hydrological cycle, treated, used and finally returned after wastewater treatment back into the cycle, often at almost the same point it was originally removed (Fig. 11.1). The water invariably becomes contaminated during use and so will change catchment water quality once it has been returned. Van der Graaf *et al.* (1997) examined the urban water cycle in detail and found that at every stage within the cycle, significant improvements could be made to reduce overall contamination. So in terms of sustainability, the best scenario would be to ensure that the urban water cycle becomes a closed system isolated from the natural water cycle, to protect resources and their ecology, with only treated effluents of the highest quality returned to the catchment (Van der Graaf 2001). In this short chapter, an attempt will be made to identify the problems and some of the possible solutions in order to achieve an urban water cycle that is truly sustainable.

11.2. The Problems

Two factors currently make sustainability implementation difficult. First is the requirement to currently treat wastewaters and to continue to do so effectively in the future. The second is the problem of expecting private companies not only to deliver current service requirements but also to develop the technology and achieve the resource use changes needed to achieve a closed urban water cycle in the future.

There are so many different definitions of sustainable development that it is difficult to get a clear picture of what is required in practice to achieve sustainable sanitation. These definitions are largely abstract and frequently used inappropriately. Many argue that it is important to have broad definitions as different cultures and differing local circumstances will often require different solutions (Mitcham 1995). While most people agree in principle to the idea of sustainable development few agree on how it can be achieved.

The most widely used definition of sustainable development is that proposed by the World Commission on Environment and Development (WCED 1987). This definition was given the status of a global mission by the United Nations Conference on Environment and Development held in Rio de Janeiro in 1992 and so is pivotal to the development of any sustainability strategy:

> *Development that meets the needs of the present generation without compromising the ability of future generations to meet their own needs.*

The Commission identified three dimensions to sustainability: environment, development, and security. It is often forgotten that the World Commission on Environment and Development definition of sustainability was also about equity between all inhabitants of the planet and equity among generations to come. The term '*needs*' in the definition refers in particular to the essential needs of the world's poor. So the remediation of current social and economic problems is paramount.

There does appear to be some confusion as to what can be achieved in the short-term and what should be the long-term objectives. Sustainable sanitation, like transportation, lacks clear criteria and subsequent objectives on which to develop clear guidelines for the future. Increasing development, increasing water usage, reasonably priced technology, compromise environmental quality standards are all factors that limit the development of sustainable sanitation. The industry is currently trying to adapt the existing infrastructure and technology to meet these increasing demands without taking time to investigate the outcome of these decisions for future generations. Our existing technologies have evolved over the past 150 years in a stopgap fashion. Sustainability requires fundamentally different solutions and technologies. The future direction for water cycle sustainability is going to require significant changes for both industry and consumers alike resulting in a new partnership incorporating novel and radical changes in our use and management of water.

To explore the possibilities for the future a number of critical issues have been identified which are briefly discussed below. Together they present a basic agenda for sustainable sanitation and highlight possible areas for further examination. What is paramount is the transformation of the concept of sustainability into practical criteria and objectives.

The global perspective

The world population in 1996 was 5.8 billion, which will double by the year 2100. With an average world production of 300 g of faeces $ca^{-1}d^{-1}$ and 1.2 kg of urine $ca^{-1}d^{-1}$, this is equivalent to a world production of 1.75 million tonnes of faeces and 7 million tonnes of urine each day. Worldwide only 15% of the wastewater produced is currently treated (Chocat *et al.* 2001). In developing countries waste disposal was traditionally achieved locally, while in developed countries waste disposal has become increasingly centralised, resulting in large and damaging point sources of pollution. The trend of centralisation is no longer a developed world phenomenon, with increasingly fewer urbanised people with satisfactory drinking water or adequate sanitation. Currently Mexico City and Calcutta have populations in excess of 32 and 20 millions respectively. The provision of water services cannot cope with the rate of population growth, especially where such services are prohibitively expensive. Over one billion people lack access to clean water and 1.7 billion have no sanitation. The rapidly increasing demand for water and sanitation, especially in developing countries, is an obvious obstacle to sustainability. Conversely, the urgent necessity for its provision is similarly an obstacle. The industry is struggling to cope with current demands with little or no opportunity to look to the future. Mitcham (1995) suggests that many developing countries see sustainable development as a Western idea meant to maintain the western way of life. In developed countries population growth is often seen as the major threat to sustainability while in developing countries over-consumption is more frequently cited as the major problem. Communities in which people are denied opportunity, face poverty and exclusion, and whose economies are in long-term recession, are not sustainable (Petts 2001).

Climate change

There is now overwhelming evidence that the 21st century will witness significant changes in climate, although it is not yet possible to identify exactly what this will mean at a local level in terms of weather patterns. Globally mean temperature is expected to rise by 2°C by 2100 with rising

surface temperatures leading to a more vigorous water cycle with more extreme events (e.g. droughts and floods) in some areas.

In the UK average temperatures have risen by approximately 1°C over the past 100 years with further temperature increases of between 1.2 to 3.4°C predicted by 2080. This will result in drier summers, especially in the southeast and wetter winters, especially in the northwest, with severe storms becoming more frequent (Petts 2001). While the predicted changes in temperature will occur too rapidly for many species to adapt, some species may benefit. However, it is clear that such changes must be considered in terms of overall planning and management of the water cycle in the future. Increased incidence of flooding, higher water demands in dry areas with fewer resources to meet such demands, reduced flows in some rivers, changes in the impacts on lakes and rivers, and increased demand for water for irrigation, are major issues that will need to be addressed if a sustainable water cycle is to be achieved.

Definition of sustainability in relation to sanitation

Water is a sustainable resource that is recycled naturally. So it is not a question of exhausting a limited resource, but rather the optimisation of available supplies, preservation and enhancement of quality, and the protection of water environments. The Water Framework Directive (2000/60/EEC) makes this bold statement at the outset:

> *Water is not a commercial product like any other but, rather, a heritage, which must be protected, defended and treated as such.*

Traditional sanitation has several disadvantages: (i) it requires too much water on a per capita basis, (ii) it results in dilute wastewater, (iii) it raises levels of nutrients, metals, and organic contaminants via effluent and sludge disposal, (v) it facilitates the spread of disease organisms and encourages the development of antibiotic resistance during treatment, and (v) is often prohibitively expensive both in energy, capital, and operational costs. Current trends in design and practice are to look for marginal reductions or improvements in these areas. This fine-tuning of existing technology, while improving the current situation, cannot lead to a sustainable industry.

A widely used definition of wastewater treatment is:

> *The treatment of domestic and industrial wastewaters, and the disposal of effluent and sludge, in order to minimise impacts on human health and the environment.*

An alternative definition is:

> *The treatment and disposal of effluent in a manner that minimises environmental impacts while providing an economically viable long-term solution to disposal.*

In both definitions the term *minimise* appears rather vague and ill-defined, certainly open to a wide interpretation and probable abuse. Perhaps the term *minimise* in the above definition should be replaced with the unambiguous term *eliminate* which certainly more accurately reflects the ultimate sustainability goal. However, both of these definitions are inadequate when compared to the basic sustainability principle, i.e. preserving the world for today's as well as for future generations. This, taken in conjunction with the fact that global water quality has never been poorer, implies the need for more radical action to preserve what we currently have and what we will require in the future. So the challenge is to translate sustainability into real environmental objectives from which operational criteria can be developed. Our starting point is to define what is sustainable in terms of (i) source contamination, (ii) treatment and (iii) final disposal. So a better term for sustainable sanitation is water cycle sustainability.

Sustainability criteria

Before unambiguous objectives can be set, sustainability criteria must be selected and relevant information gathered. Balkema (1998) has divided sustainability criteria for the comparison of wastewater treatment systems into economic, environmental, social-cultural, and functional. These have been further broken down in Table 11.1 and provide a useful set of criteria from which long-term objectives could be derived. Balkema *et al.* (2002) have reviewed the indicators that are currently used for sustainability assessment of wastewater treatment systems.

The driving force in the introduction of sanitation was disease control following the cholera and typhoid outbreaks of the mid nineteenth century. The discovery of antibiotics and the development of better health care in the mid to late twentieth century allowed the key objective of wastewater treatment, i.e. the control and prevention of water borne diseases, to be replaced with a primary concern for the environment. These concerns have shifted over the years from organic pollution to eutrophication, acidification and now organic-micropollutants. With more than 10 million people dying annually from water-related diseases in the developing world (IRC 1997), and increasing problems with new micro-organisms and resistant strains of

Table 11.1. Sustainability criteria for wastewater treatment (Balkema 1998).

Functional:	
– Performance	– Expressed in removal of: BOD/COD, heavy metals, organic micro-pollutants, pathogens, and nutrients.
– Adaptability	– Indication of possibilities for implementation on different scales, increasing/ decreasing capacity, and anticipate on changes in legislation etc.
– Durability	– Lifetime of installation.
– Flexibility	– Indication of sensitivity of the process concerning toxic substances, shock loads, seasonal effects etc.
– Maintenance required	– Indication of maintenance required: frequency/costs and time needed for maintenance.
– Reliability	– Indication of sensitivity of the process concerning malfunctioning equipment and instrumentation.
Economic:	
– Affordability	– Costs relative to national/regional budget.
	– Foreign exchange required relative to national/regional foreign exchange requirements.
– Costs	– Net Present Value of the Investment costs (specified for: land, materials, equipment and labour), Maintenance costs and Cost for destruction.
– Cost effectiveness	– Performance relative to costs.
– Labour	– Number of employees needed for operation and maintenance.
– Willingness to pay	– The amount of money spent by users on sanitation relative to their total budget.
	– Indication of the amount of money the user is willing to pay for (improved) sanitation.
Environmental:	
• *Emissions:*	
– Acidification	– Acidification potential
– Depletion[2]: Abiotic	– Mineral material depletion potential (ADP, yr^{-1})
Biotic	– Biodiversity

Table 11.1. (*Continued*)

– Depletion of fossil fuels	– Fossil energy carrier depletion potential (EDP, GJ)
– Global warming	– Global warming potential (GWP, kg aeq. CO_2)
– Nutrification	– Nutrification potential (NP, kg aeq. PO_4)
– Ozone Depletion	– Ozone depletion potential
– Photochemical air pollution	– Photochemical ozone creation potential (POCP, kg aeq. C_2H_4)
– Toxicity: Aquatic	– (ECA, m^3 aeta)
Human	– (HT, kg hta)
Terrestrial	– (ECT, kg teta)
– Waste production	– Final waste (kg)
	– Toxic final waste (kg)
	– Nuclear final waste (kg)
Additional detailed information on emissions during operational phase:	
– Heavy metals	– Balances of Cu, Cr, Zn, Pb, Cd, Ni, Hg, Ar
– Nutrients	– Balances of N P, K
– Organic matter	– Balance of C, S
– Organic pollutants	– Indication of emissions of pesticides and other toxics
– Pathogens	– Bacteria, viruses, helmiths
• *Resource Utilisation:*	
– Energy	– Energy used, produced and 'lost' during installation, operation and destruction of the wastewater treatment system. Energy 'lost' indicates the amount of energy no longer available due to emissions of waste disposal.
	– Indications of possibilities to apply sustainable energy sources.
– Land area	– The total land area required.
	– Indication of the possibilities to integrate the wastewater treatments system (partly) in green area's.

Table 11.1. (*Continued*)

– Nutrients	– Amount of nutrients suitable for reuse. – Indication of nutrient quality.
– Organic matter	– Amount of organic matter recycled through sludge reuse. – Indication of sludge quality. – Amount of organic matter recycled through biogas production.
– Resource effectiveness	– Performance relative to resource utilisation.
– Water	– Amount of water suitable for reuse. – Indication of water quality.
Social:	
– Institutional requirements	– Indication of the efforts needed to control and enforce the existing regulations. – Indication of embedding of technology in policymaking.
Cultural:	
– Acceptance	– Indication of the cultural changes and impacts: convenience and correspondence with local ethics.
– Expertise	– Number of engineers needed specified to installation and operation. – Indication whether a system can be designed and built or can be repaired, replicated and improved locally/in the country/or only by specialised manufacturers.
– Stimulating sustainable behaviour	– Indication of the possibilities for technical stimulation of sustainable behaviour. – Indication of the possibilities for economic stimulation of sustainable behaviour. – Indication of the possibilities for participation of the end user.

[2]Depletion is listed under emissions, although this is not completely correct. Depletion is used as an indication of resource utilisation in the installation and destruction phase. Note that the resource utilisation of the operational phase is studied in detail.

existing bacteria in developed countries (Gleeson and Gray 1997), protection of public health must always be the primary treatment objective of sanitation.

Formulation of sustainability objectives

The most widely cited objectives for sustainable wastewater treatment are: (i) finding the most sustainable disposal or reuse route for sludge and effluent; (ii) reduction in the volume of wastewater entering treatment plants; (iii) development of wastewater treatment systems for individual houses to reduce waste and protect local surface and ground waters. This does not only involve the environmentally sound disposal of end products but also includes the aspiration of decreasing the volume of wastewater requiring treatment and subsequent disposal. It has already been proposed that we should only discharge completely clean, nutrient, contaminant and microbe free final effluents which in theory could already be achieved by simply spending lots of money on advanced (tertiary) treatment. In this case all we need is better technology within a free market and unlimited exploitation of water resources.

This raises the question again as to whether or not efficiency and sustainability are analogous. Efficiency is the selection of the most appropriate technology and implementing it in such a way that consumes as few resources as possible. So efficiency is good design and operational practice. Sustainability allows us to set new objectives possibly requiring the development of new technologies and practices. There are four areas to be considered: *hardware*, such as machinery and plant (i.e. the treatment works); *software* to optimally operate the system; *human resources*, the education and training personnel to operate and maintain the system; and finally *organisation* to manage the systems. The selection of sustainable systems may not in practice require new or innovative technology so long as it is appropriate for solving the given problem. It should however be flexible enough to allow for further development if needed. As the quantity and quality of resources vary spatially and temporally so the most sustainable technological solutions will also vary. They must be effective, efficient, acceptable and convenient, not only for users, but for operation and maintenance. All sustainable technology should encourage sustainable behaviour.

Before objectives can be formalised then it must be accepted that the water issue is not only a problem of water supply and wastewater treatment, it engulfs the whole socio-economic basis of all societies. Therefore

mechanisms for the achievement of sustainability will vary between rural, suburban and urban environments, as well as between climatic zones, and economic zones. Also, current standards are not capable of coping with emerging problems (e.g. oestrogen mimicking compounds, trace organics, pharmaceuticals, new pathogens). Using current technology and legislative procedures will lead to ever increasing complexity and costs.

It would appear, therefore, that there are only two possible objectives for wastewater treatment:

(i) The elimination of all material in used water that was not present before its use, regardless of concentration (i.e. there can be no acceptable level for an introduced substance).
(ii) The protection and if necessary the reclamation of water resources to a condition that can be deemed to be wholly natural.

Assessment of sustainability

There already exist a number of established methods for assessing sustainability but few have been applied to wastewater treatment. *Life cycle assessment* (LCA) is the most widely used which encompasses the cradle to grave concept where the environmental impact of construction through operation and decommissioning are all considered before projects are selected with the object of minimizing such impacts. The energy and materials used in construction and operation, transportation and disposal of sludge, use and reuse of materials, and the wastes released are all critical considerations. Impacts include human health, ecological health, resource depletion, conventional costs, and economic-based costs of environmental or social externalities (Emmerson *et al.* 1995; Bengtsson *et al.* 1997). Other approaches include *life cycle management* (LCM), which is used to structure and conceptualise environmental activities; *product stewardship*, which is a set of tools to manage wastewater treatment systems over their full life cycle; and *design for the environment* (DFE), which are methods and tools to assist engineers to incorporate environmental criteria into their designs. Risk assessment is always an important factor.

It is not always easy to compare results from LCA when different systems are being assessed. In the Netherlands, Balkema (1998) has proposed using a functional unit based on a population equivalent of 10,000 to enable plants to be compared effectively. The author explains that as the scale of a plant is not a sustainability criterion, sustainability assessment should incorporate scale-analysis to indicate the optimal size of any technologies affected by scale.

New designs and technologies need to be evaluated by some form of sustainability index, and once built some form of benchmark performance assessment (e.g. effluent quality index) will also be required. Table 11.1 lists the basic sustainability criteria for wastewater treatment plants that could be quantified to produce an index of sustainability for the selection and design of future plants.

11.3. Sustainable Options

Many new process developments have already been described in the text, especially in Chapter 10. However, examples of recent advances in wastewater treatment that have potential sustainable applications are given below under the general headings of (i) source contamination, (ii) treatment and (iii) final disposal.

11.3.1. *Source contamination*

Storm water separation and treatment

The major objectives of urban drainage are public hygiene, flood protection and pollution control. In most developed countries these first two objectives have been achieved, while in developing countries public hygiene and flood protection remain major problems. Combined sewer systems are the most widely used for wastewater collection with combined sewer overflows (CSOs) a major source of surface water pollution. There is little control of discharges to sewers with only industrial wastewaters requiring pre-treatment and consents. Also, not all the wastewater discharged to sewer will be transported to the treatment plant as all sewer networks leak to a certain extent varying from 5 to 40% with 20% taken as an average value. This of course depends on the water table with groundwater infiltration into sewers also possible which results in massive increases in wastewater volume and a significant reduction in strength. In the past the design of these systems were standardised and took no consideration of CSOs on receiving waters resulting in often serious and widespread pollution (Ellis and Marsalek 1996). In contrast, modern designs look at urban drainage not in isolation but as an integrated component of a larger system that includes both the catchment and wastewater treatment (Ellis 1995; Larsen and Gujer 1997; Welker *et al.* 1999).

It is now understood that sewerage systems can also play a significant role in the biological oxidation of wastewater. There is an active biomass

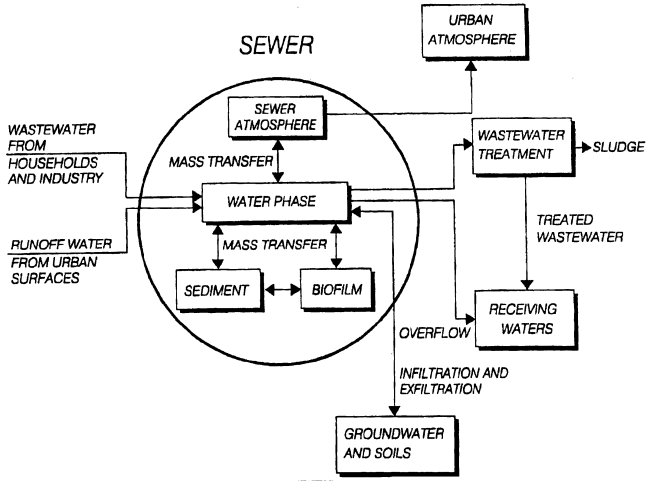


Fig. 11.2. Wastewater flow and subsystems for the processes within and related to a sewer network (Hvitved-Jacobsen *et al.* 2002).

present both as attached growths on the walls of the sewer as well as dispersed growths in suspension, and as the hydraulic retention time of the sewerage network is similar to that of an activated sludge tank, treatment will occur as long as aerobic conditions are maintained in the wastewater (Hemming *et al.* 1983; Hvitved-Jacobsen *et al.* 2002). Sewers also have a plug flow configuration making them ideal biological reactors. The sediment in the sewer also plays a role by providing aerobic, anoxic and anaerobic zones where a wide range of reactions can occur (Figs. 11.2 and 11.3). The dissolved oxygen concentration of the wastewater in the sewer must be maintained at a minimum of 1 mg l^{-1} to ensure oxidation, so where necessary, oxygen must be supplemented (Newcombe *et al.* 1979). Malik (1998) recorded significant removals of soluble BOD and COD (i.e. the oxygen demand was measured on samples filtered through 1 μm filter) of 78% and 60% respectively.

Storm water must be seen as a water resource and whenever possible intercepted and reused. To achieve this, both centralised and decentralised systems have been proposed (Larsen and Gujer 1996 and Otterphol *et al.* 1997). Where reuse is not feasible then storm water must be disposed of locally via swales or infiltration ponds, or treated locally using reed beds or another sustainable system in order to prevent pollution. Chocat *et al.* (2001) have explored the future needs of urban drainage, while Brelot *et al.* (1999) and Matos (2002) have reviewed the latest innovations in this area.

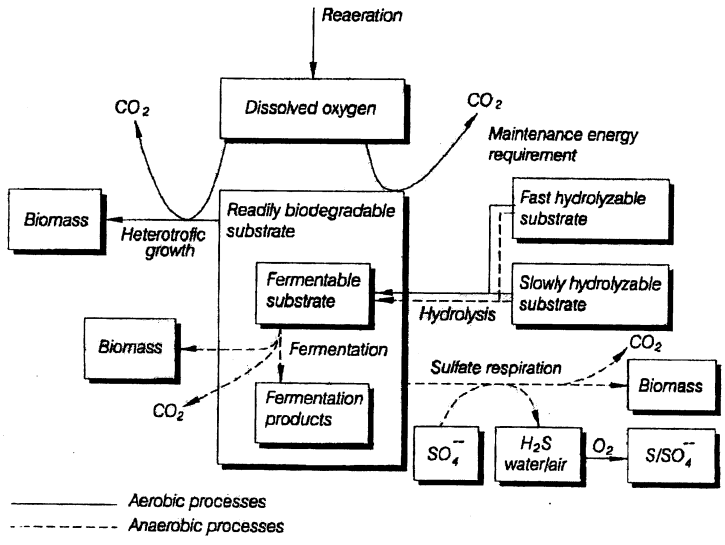


Fig. 11.3. An integrated aerobic and anaerobic concept for in-sewer transformations of wastewater organic carbon and sulphur (Hvitved-Jacobsen *et al.* 2002).

The control of diffuse pollution arising from damaged sewers, agricultural nutrients, run-off, and pesticides are reviewed by Burkart and Heath (2001).

Reduction in contamination of water at point of use

The nature of sewage has significantly altered over the past half century with many new micro-pollutants (e.g. residual pharmaceuticals, body care products, fuel additives, laundry and washing compounds) and pathogens (e.g. anti-biotic resistant bacteria, exotic pathogen species and prions) being recorded. These pose a significant risk to the quality of water resources and to the environment as a whole. Unfortunately our current wastewater treatment designs are not able to deal with many of these new problems without retrofitting with expensive advanced treatment processes. While the tracing of industrial contaminants back through the sewerage system has been effective in eliminating many contaminants, especially heavy metals, these new problem compounds are largely domestic in origin and so are ubiquitous in wastewaters.

Thus the reduction of the polluting nature of domestic wastewater is a priority for a sustainable future. Reduction in nutrients, metals, removal of pharmaceuticals and drugs, especially antibiotics, from wastewaters and the re-evaluation in the use of hormones (e.g. HRT, contraception) are all urgently required to protect water quality.

Endocrine disrupting substances have been associated with a number of effects on wildlife including hermaphrodite fish, reproductive malformation, and sex changes in other species. A study on UK sewage identified that the key endocrine disrupting compounds were the natural female steroid hormones oestrogen and 17β-oestradiol and the synthetic hormone ethinyl oestradiol from the contraceptive pill, which arise from the urine of the female population (Environment Agency 1998b). Oestrogen mimicking compounds such as alkylphenols (APs) and alkylphenol ethoxylates (APEs) arise from industrial wastewaters. For example, hermaphroditism in the River Aire (Scotland) was caused by these compounds, which arose from pollution by wool-scouring detergents (DOE 1995). A full list of these compounds and their effects has been produced by the Environment Agency (1998a). Walker (2000) reports that a number of physico-chemical processes can be effective in reducing the oestrigenicity of wastewaters. Treated sewage effluent entering the River Chelmer in Essex (England) is mildly oestrogenic but dilution by river water at a 3:1 ratio eliminates measurable oestrogenic activity in fish. Activated carbon removes 94.8% of the oestrogens, which is equivalent to a dilution of almost 20:1. So the treatment of the final effluent with powered activated carbon during low river flows eliminates any effect. Ultra-violet radiation is also known to be a factor in reducing oestrogenicity in rivers downstream from sewage outfalls, although Walker reported low removal rates during his experiments using treated wastewaters. Ozone is also partially effective, so a combination of these technologies offer significant control of these compounds.

After being administered, pharmaceuticals and their metabolites are excreted, and along with unwanted pharmaceuticals, end up in the wastewater stream. Mass balance studies of these drugs show that removal at the wastewater treatment plant is generally poor resulting in their discharge into surface waters (Ternes 1998) (Table 11.2). The effects on the biota remain unknown but as these chemical compounds are designed to have a direct biological effect, then they must be of great concern (Fig. 11.4). Where water is reused for drinking water supply then these drugs pose a real threat to public health. Pharmaceuticals have also been recorded in groundwaters that have been recharged using river and canal water that received treated sewage effluents (Heberer *et al.* 1997, 1998). As some 70–80% of pharmaceutical compounds in wastewater originate from urine, the only practical method of controlling them in the environment may be by dealing with urine separately. Oxidation using a combination of hydrogen peroxide and ozone has been shown to be reasonably effective at reducing the concentration of the commonest pharmaceutical compounds in final effluents (Zwiener and Frimmel 2000).

Table 11.2. Amounts of some common pharmaceutical used in Germany in 1993 and concentrations found in secondary effluents and surface waters (Zwiener and Frimmel 2000).

Agent	Applied mass in t a^{-1}	Concentration in sewage in μg l^{-1}	Concentration in surface water in μg l^{-1}
Acetylsalic acid	23–116	0.05–1.51	< 0.05
Ibuprofen	48–96	0.05–3.35	0.05–0.28
Diclofenac	48–72	0.005–1.59	0.005–0.49
Bezafibrate	38–57	0.25–4.56	0.005–0.38
Clofibric acid	15–21	0.46–1.56	0.005–0.30
Fenofibric acid	11–15	0.05–1.19	0.005–0.17

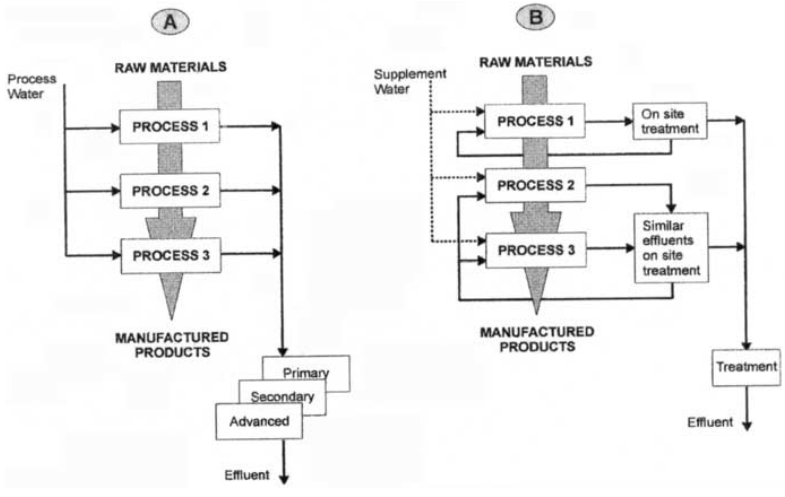


Fig. 11.4. (A) Conventional industrial wastewater treatment; (B) and a more sustainable approach (Lema and Omil 2001).

The consumer will be vital in controlling what ends up in the wastewater stream not only by being pro-active in its management but in demanding new sustainable products and appliances. In terms of domestic wastewater generation this will have to include revision of current household design criteria and the development of new building regulations to encourage water conservation (e.g. rainwater harvesting), reuse of laundry water, and separation of urine from faeces. Separation of the different types of wastewater generated at source will provide more freedom and flexibility for the selection of the most sustainable disposal option. These include avoidance, reduction, reuse and retention (Chocat *et al.* 2001). The main waste streams identified are summarised in Table 11.3.

Table 11.3. Domestic wastewater can be separated into a number of different waste streams offering the potential for a more sustainable disposal option.

Code	Content
Black water	Urine and faeces
Brown water	Faeces only
Yellow water	Urine only
Grey water	Washing water
White water	Runoff

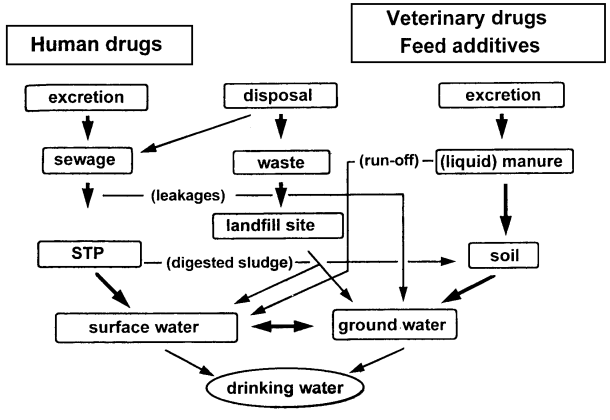


Fig. 11.5. Main fate of drugs in the environment after application. STP is the abbreviation for sewage treatment plant (Ternes 1998).

Larsen and Gujer (2001) describe the concept of source control practice. This will contain one or more of the following elements: (i) substitution of chemicals not wanted in the waste stream; (ii) enhancement of the treatability of user chemicals without losing the characteristics desired by the consumer; (iii) on-site treatment of concentrated industrial and trade waste with problem specific technology; (iv) development of new appliances that will save water or segregate waste streams in the desired way; and (v) development of waste handling processes for individual households.

Removal of the industrial waste component of municipal sewage is critical if sustainable strategies are to be achieved. Van Lier *et al.* (2001) conclude that a zero-discharge option is already feasible for paper mill wastewaters provided in-line treatment is applied (Fig. 11.5).

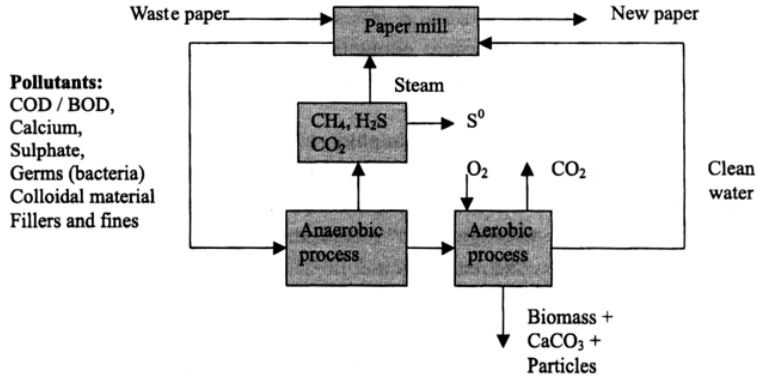


Fig. 11.6. Example of a zero-discharge system. This paper mill has in-line anaerobic-aerobic treatment (van Lier *et al.* 2001).

Manufacturing processes have been based on the consumption of considerable amounts of water resulting in the production of waste process waters in equally large quantities each with different levels of contamination making treatment complex and expensive. Cleaner production technology aims to reduce water usage with processes employing specific on-site treatment plants for the wastewater generated by each process (Fig. 11.6). This uses smaller and specific treatment units producing effluents that can be reused. Only the waste generated from these units would be treated by conventional treatment or discharged to sewer.

11.3.2. *Treatment*

Within Europe nearly all wastewater is now treated in wastewater treatment plants. In the near future the majority will also include nutrient removal. There is, however, much more interest in selecting appropriate technologies and where possible employing more sustainable and more energy efficient systems (Qian 2000). Natural treatment systems, high efficiency anaerobic processes (Lema and Omil 2001), advanced bioreactors and membrane bioreactors (Wilderer 2002) are all currently being considered as replacement technologies for the more conventional activated sludge systems. Photo-catalytic and chemical oxidation processes are also being used for the treatment of complex and often highly toxic waste streams (Witzig *et al.* 2001). Our understanding of conventional biological processes is improving all the time and this is leading to greater efficiency. For example, it has been shown that micronutrient supplements can have

Table 11.4. Nutrient requirements of activated sludge and recommended amendment concentrations (Burgess *et al.* 1999).

Nutrient	Range of theoretical micronutrient requirements (mg l^{-1})[a]	Concentration of micronutrients detected in the wastewater (mg l^{-1})	Dose added (mg l^{-1})
Macronutrients			
N	15.0[b] minimum	32.00	32.00
P	3.0[b] minimum	1.69	1.69
S	1.0 minimum	100.0	100.0
Trace elements			
Ca	0.4–1.4	0.44	1.0
K	0.8–>3.0	95.0	none
Fe	0.1–0.4	1.20	none
Mg	0.5–5.0	10.0	none
Mn	0.01–0.05	< 1.0	1.0
Cu	0.01–0.05	< 1.0	none
Al	0.01–0.05	0.02	1.0
Zn	0.1–1.00	< 1.0	1.0
Mo	0.1–0.7	< 1.0	0.5
Co	0.1–0.5[c]	< 1.0	1.0
Vitamins			
Biotin	0–1.0 μg/l[d]	–	1.0 μg/l
Niacin	0–1.0[d]	–	1.0
Thiamine (B_1)	0–1.0[d]	–	1.0
Lactoflavin (B_2)	0–1.0[d]	–	1.0
Pyridoxine (B_6)	0–0.01 μg/l[d]	–	0.01 μg/l
Pantothenic acid	0–1.0[d]	–	1.0

[a]Wood and Tchobanoglous, (1975); Metcalf and Eddy (1991).
[b]From suggested COD:N:P ratio, Beardsley and Coffey, (1985).
[c]Sathyanarayana Rao and Srinath, (1961).
[d]Lind *et al.* (1994).

either an inhibitory or stimulatory effect on the treatment of industrial wastewaters by the activated sludge process. Burgess *et al.* (1999) have shown that treatment efficiency can be optimised by the use of such supplements (Table 11.4). Improved operational management is also important in achieving more sustainable systems. For example, many new microscopic and genetic methods have been developed to analyse and control biological systems such as fluorescence *in situ* hybridisation (FISH) and confocal laser scanning microscopy (CLSM) for structure-function analysis (Table 11.5). These have allowed precise identification of microbial populations as well as advanced our knowledge of biofilm and floc development.

Table 11.5. Comparison of high resolution methods for investigation of microbial aggregates (Wilderer *et al.* 2002).

Method	Suitable for[a]	Advantages	Limitations
Environmental 16S rRNA and functional gene libraries	D, PS	High phylogenetic resolution; required to design new gene probes and to identify novel bacteria	Time consuming; DNA extraction, PCR and cloning biases; not quantitative in regard to community composition
16S rRNA and functional gene fingerprinting techniques	D, PS	Inexpensive; high sample throughput	DNA extraction and PCR biases; not quantitative in regard to community composition; multiple rRNA operons per organism can complicate analysis
FISH	D, PS, A	Relatively straightforward to perform; allows direct visualisation of non-cultured microbes; amenable to direct combination with microautoradiography and microsensors	Adaptation of protocols required for some gram-positive *Bacteria* and for *Archaea*; inactive cells with low ribosome content not detectable; sample embedding required to preserve aggregate architecture
FISH/fluorescent staining and CLSM and image analysis	A, D, PS	Quantitative analysis of population structure and/or floc architecture; non-invasive technique; amenable to automation	Expensive instrumentation; time consuming

Table 11.5. (*Continued*)

Method	Suitable for[a]	Advantages	Limitations
FISH — Microautoradiography	D, PS, MA	Simultaneous in situ identification and determination of substrate uptake pattern on a single cell level; quantification of important physiological microbial groups (e.g. denitrifiers) possible	Specialised knowledge required; isotope lab needed; time consuming and expensive
FISH — Microsensors	D, PS MA	Chemical concentration gradients can be directly measured and correlated with spatial arrangement of microbial consortia	Invasive technique; usually one-dimensional measurements: problems might arise due to architectural heterogeneity of aggregates; spatial resolution not on single microbial cell level; not all compounds measurable; possible interference by other ions

[a]Estimation of microbial diversity (D); determination of microbial population structure and dynamics (PS), aggregate architecture (A), specific microbial activities (MA), chemical concentration gradients (C).

Nutrient losses using the best affordable treatment systems are still over 20% for N, 5% for P and 90% for K. Pathogen levels are also high in final effluents. Thus, where the quality of the receiving water is determined primarily by the quality of the effluent discharged to it, further treatment is required. There are many new advanced treatment processes available and these are compared in Table 11.6. Currently most promise lies in the area of filtration including membrane filtration, deep bed filtration and flocculating filtration (STOWA 2000).

The use of large sewerage networks to take wastewater to centralised treatment plants are considered by many authors to be highly undesirable and like much in wastewater treatment a system that has been historically inherited and not one that would be envisaged if starting from scratch today (Van Lier and Lettinga 1999) (Table 11.7). It arose from the use of sewers to remove polluted and infectious water away from residential areas. However, concentrated wastes are relatively easy and cheap to treat compared to more complex and energy demanding systems for weak wastewaters. Therefore, by reducing water usage by reuse and recycling, the volume of wastewater is reduced, although its strength will be increased. Any method that helps to reduce water consumption and increase the percentage of recycled organic matter and water is to be encouraged. Water saving reduces the amount of energy and chemicals used in its treatment as well as reducing the cost of water and wastewater treatment. It also reduces the impact on receiving waters. The separation and treatment of individual wastewater process streams will become increasingly important in making end of pipe treatment less complicated and more reliable (Sekoulov 2002).

Anaerobic wastewater treatment is becoming increasingly important in providing more flexible treatment solutions, especially of stronger wastewaters. Compared to aerobic systems, anaerobic treatment offers: (i) lower treatment costs, (ii) high flexibility being able to deal with a wide variety of wastewaters, (ii) higher loading rates at higher concentrations, (iii) smaller footprint per unit of waste treated, (iv) smaller volumes of excess sludge, (v) organisms that can survive long periods without food making them ideal for intermittent and seasonal loadings, (vi) greater biotransformation and biodegradation of xenobiotics, (vii) retention and degradation of volatile hazardous compounds, and (viii) greater stability and fewer operational problems, especially when immobilised biomass is used (Lema and Omil 2001). Of course anaerobic treatment also produces energy in the form of biogas at a rate of 1.27×10^7 J per kg COD converted while aerobic systems have a high-energy requirement (Speece 1996).

Table 11.6. Advanced treatment methods for effluents (van der Graaf 2001).

Process	**Main principle**	**Removal**	**Applications**	**Status**	**Costs**	**Prospects**
coagulation/flocculation sedimentation	chemical precipitation	broad spectrum of components 50–90%	P-removal	available	medium	medium
deep bed filtration	filtration biol. oxidation	SS (90–95%) ammonia	polishing	available optimization	low	high
flocculating filtration	chem. prec. filtration biol. oxidation	SS (90–95%) P (–90%) ammonia	P-removal	available optimization	low	high
microsieves	sieving cake filtration	SS (60–70%)	reduction of SS	available	low	low
(primary) membrane filtration	ultrafiltration microfiltration	SS (100%) bacteria (100%) viruses (100%)	high quality purposes	development	high	high
(advanced) membrane filtration	hyperfiltration reverse-osmosis osmosis nanofiltration	soluble components (–100%)	high purity water	development	high	medium/high

Table 11.6. (*Continued*)

Process	**Main principle**	**Removal**	**Applications**	**Status**	**Costs**	**Prospects**
activated carbon	adsorption	soluble comp. micropollutants	removal of micropoll.	available	high	medium
ion exchange	ion exchange	ammonium heavy metals	demi water	available development	high	low
ozone	chemical oxidation	organic subst. micro-org.	disinfection	available	high	medium
chlorination	chemical oxidation	organic subst. micro-org.	disinfection	available	low	medium
UV	radiation	micro-org.	disinfection	available	medium	high
polishing ponds	biological sedimentation	broad spectrum	polishing	available	high	low
reed bed filters	biological filtration	broad spectrum	polishing	available	high	medium
biofilms	biological filtration	broad spectrum	P-N removal	available	high	low

Table 11.7. Disadvantages of implementing large scale sewer networks (van Lier and Lettinga 1999).

• Requirement of relatively high tap water consumption in order to prevent sewer clogging resulting in the contamination of large amounts of water.
• Relatively high risks of spreading contaminants over the environment (e.g. strom water, leaking of sewers); even where some off-site treatment systems have been installed.
• Relatively high risks of hazardous compounds dischange into the sewer by residents, industries, etc. ('out of sight, out of mind'). This frequently leads to a situation where excess sludges become unsuitable for re-use in agriculture.
• Need to employ (more expensive) treatment methods, suitable for (very) dilute types of waste waters (higher volumetric rates).
• Possible exportation of rainwater from the residential areas, leading to an undesirable drop of the groundwater level, or even to a severe 'city/regional dryness'.
• Very high costs for the construction and maintenance of sewers and pumping station. Once every 50–60 years a complete renovation is needed. Sub-optimal maintenance leads to high losses of reclaimable water to the soil.
• Centralised urban sanitation systems depend highly on central services like electricity supply, consequently they are insufficiently robust in e.g. periods of economic and/or political instability.

A number of different authors have attempted to compare and evaluate treatment plants and offer advice on the optimum design (Ellis and Tang 1990; Tang and Ellis 1994; Chen and Beck 1997; Mels *et al.* 1999; Foxton 2000). However, none of these include options such as use of rainwater, separating wastewater streams, reusing greywater for toilet flushing or garden irrigation etc. (Table 11.3) (Balkema *et al.* 2001). Another factor that is rarely considered is the advantage of standardising treatment plant design and unit processes. This leads to the development of better operational management and a greater reliability of systems by ensuring availability of replacement machinery parts.

Van Lier and Lettinga (1999) define sustainable wastewater treatment in terms of the minimum consumptive use of energy, chemicals, and water and the maximum reuse of treated wastewater and of the residues produced from the pollutants present in the wastewater. Their criteria for sustainable sanitation are summarised in Table 11.8.

11.3.3. *Final disposal*

Recovery and reuse of domestic wastewaters

There has been much interest in the household recyclying of wastewaters (Sec. 11.3.1). Utilisation of non-potable domestic reuse systems is restricted by the need for a complete dual distribution system. It proved to be uneconomic to install a dual water supply system in the UK, although it has

Table 11.8. Criteria for sustainable environmental protection concepts and networks (van Lier and Lettinga 1999).

1. No dilution of high strength residues (wastes) with (clean) water, e.g. for conveying them from the site where they are produced (e.g. installation of expensive sewerage).
2. Maximum of recovery and reuse of treated water and byproducts obtained from the polluting substances, e.g. for irrigation, fertilisation etc.
3. Application of efficient, robust and reliable treatment/conversion technologies, which are low in cost (in construction, operation and maintenance), which have a long lifetime and are simple in operation and maintenance.
4. Applicable at any scale, very small and very big as well.
5. Leading to a high self-sufficiency in all respects.
6. Acceptable for the local population.

been done in a limited way in a number of other countries (Gray 1994). It is generally prohibitively expensive in large cities although limited use in small towns is feasible. For example, at the Grand Canyon in Arizona, wastewater has been reused for toilet flushing since the 1970's (Middleton 1977). There is great potential for reuse in isolated communities, especially islands where water is restricted. Reuse of treated effluent on land is a safer disposal route than directly into surface or ground waters. Partially treated supplies can be used for all purposes, with point of use systems utilised for water used for consumption and hygiene.

Climate models predict that rainfall in the British Isles will fall by 14% over the next 30–35 years. Increased evaporation from reservoirs could result in an overall 15% reduction in stored water. So, in the UK where 33% of total household water consumption is used for flushing the toilet, grey water recycling systems could reduce household water demand by up to 30% (Minting 1998). The use of low flush toilets, showers instead of baths and general water conservation all reduce grey water production. Effluent and sludges can be used for crop irrigation and soil fertility/conditioning respectively. Composting toilets can be zero or low flush in design. As up to a maximum of 85% of the total nitrogen and 80% of the total phosphorus in domestic wastewater originates from the toilet, separation of the toilet waste from the waterborne route results in significant reduction in waste loads to the sewer. Human urine is also the largest contributor of nitrogen (80%) and phosphorus (55%) and much attention has been paid to its separate collection and reuse as a fertiliser. Separation toilets use a double section bowl with urine collected in the front and the faecal matter in the rear section. The urine is stored separately and then collected by local farmers for use as a fertiliser (Hanæus *et al.* 1997: Jönsson *et al.* 1997; Hellström *et al.* 1999).

Non-potable use for treated wastewater effluents include recreational and wildlife purposes. In Hong Kong treated wastewater is used extensively for toilet flushing, irrigation of parks, public gardens and racecourses (Biswas and Arar 1988). Such uses ensure that nutrients and other contaminants are removed from the irrigation water in the active soil layer. The concept of the zero-water garden is also gaining popularity with the use of roof runoff collection, conservation of soil water by the use of mulches, gravel and pebbles to reduce evaporation, and the planting of species that can cope with semi-arid conditions. There are many new treatment systems for individual and small groups of houses. For example, domestic wastewater has been effectively treated when used for the horticultural production of roses using the nutrient film technique. Final effluent quality (and percentage removal) was COD 39 ± 13 mg l^{-1} (89%), BOD 7 ± 4 mg l^{-1} (95%), and suspended solids 8 ± 6 mg l^{-1} (94%). The roses removed up to 20–23% more P than systems without roses (Monnet *et al.* 2002).

Larsen and Gujer (2001) have identified a number of key steps towards a sustainable household wastewater system (Table 11.9). The result is to eliminate combined sewer overflows and the eventual elimination of combined sewer systems.

Reuse

An example of sustainable reuse would be the discharge of treated effluent to a river where after dilution it is abstracted for supply or some other beneficial use downstream. Industry is increasingly taking responsibility for their environmental impacts through the development of environmental management systems and reuse within the company both of which are now commonplace.

Groundwater recharge is common in the US and Israel using direct injection, surface flooding, riverbank or stream infiltration. The use of treated effluents for groundwater recharge is not widely practiced in Europe. However, with the availability of groundwater decreasing throughout the world and use exceeding natural replenishment, the use of effluent as infiltration water must be seriously considered. It has been shown to be a viable option in areas where groundwater usage is high and recharge is low, where aquifers can be used as storage areas for reclaimed water, and to prevent saltwater intrusion (Rowe and Abdel-Magid 1995). Only the highest quality effluents should be used, with ultrafiltration the minimum acceptable level of treatment. Like irrigation, groundwater recharge requires careful monitoring and strict controls to ensure long-term sustainability.

Table 11.9. Possible milestones for wastewater treatment design (Larsen and Gujer 2001).

Advantages as compared to today's conventional system (CSS)	
1. No source control	Does not exist in Western Europe.
2. As today (regulation)	Regulation prevents clogging of sewers, contamination of sludge, poisoning of the biocenosis in wastewater treatment plants, etc.
3. As today minus rainwater	Infiltration results in fewer storm water retention basins and/or fewer CSO events. With time, the diameter of sewers will decrease. Groundwater is replenished to a certain degree, depending on local infiltration possibilities.
4. As today minus faeces	If the faeces are kept out of the system, the organic loading of the treatment plants is reduced by at least a third. The hygienic problems caused by wastewater will be drastically reduced.
5. As today minus urine	With urine separation, nutrient control at the treatment plant will be obsolete. Nutrient recycling to agriculture will be facilitated. Emissions of pharmaceuticals and hormones from human metabolism to the combined sewers will be drastically reduced.
6. Only gray water collection	This solution combines the advantages of 4 and 5.
7. Production of "un-polluted" wastewater only	Unpolluted wastewater may be disposed of together with storm water. Storm sewers or infiltration possibilities have to be provided anyway in non-arid areas. There will be no use for the rest of the CSS.
8. No export of wastewater	In non-arid parts of the world, this is comparable to 7.

An interesting example of wastewater reuse is the recirculation of aquaculture water to eel ponds after treatment by a simple biofilter treatment system. Without water reuse, eel culture would be environmentally unsustainable due to the large raw water usage (Yang *et al.* 2001).

Sewage sludge treatment and disposal

The disposal of sewage solids, often referred to as biosolids, has emerged as one of the major sustainability issues in wastewater treatment. As wastewater treatment becomes more widespread, the amount of sludge produced will rise accordingly. Anaerobic digestion of sludges, when considered in the context of Life Cycle Analysis, offers a number of advantages. For example the recovery of energy (i.e. biogas for heat or LPG), reduced emissions of volatiles (e.g. ketones, aldehydes and ammonia), and the reduction of xenobiotic compounds. Strauss and Wiedemann (2000) have demonstrated that in terms of the contribution to global warming anaerobic processes are

less detrimental to the environment than other treatment processes. Hwang and Hanaki (2000) compared individual life cycle carbon dioxide emission units (CEUs), i.e. the amount of emitted carbon dioxide for treating a unit weight of sludge, for a range of sludge treatment processes allowing carbon dioxide production during treatment to be simulated and found anaerobic digestion the most efficient (Table 11.10).

The most sustainable options for the disposal of sludge in Europe are currently considered to be incineration and agriculture. However, there are significant problems in public opposition to both these options. In Denmark sewage sludge is mainly incinerated with the ash used in cement manufacture and the construction of roads and pavements thus ensuring any contaminants are either thermally destroyed or permanently immobilised. Much work has been done on turning sludge into value-added products such as compost, cement, bricks and other useful products (Ho and Skrypsi-Mantele 2000; Eenglande and Reimers 2001). Bridle and Skrypski-Mantele (2000) have used a life cycle assessment approach for evaluating sludge management strategies and highlighted the following issues that must be included: compliance with world-best practice with respect to environment and protection of health, energy usage, greenhouse gas emissions, odour control, volume reduction and public acceptability. They conclude that to be sustainable only beneficial reuse sludge options can be considered, with the value of the recovered resource outweighed by the total resources invested. Stypka *et al.* (2002) have defined four basic rules to enable efficient sludge recycling: (i) limitation at source of the amount of harmful and toxic substances entering the treatment plant and effecting sludge quality; (ii) efficient sludge processing using technically, ecologically and economically feasible methods to reduce its quantity and improve its utilisation properties; (iii) energy and product recovery through advanced sludge processing methods; (iv) safe and environmentally sound sludge utilisation, preferably as an agricultural supplement.

Strong public opinion in Germany and Sweden following the salmonella and BSE health scares put pressure on food retailers not to supply food in their outlets that had been grown with the use of sewage sludge. This has resulted in a virtual ban on the use of sewage sludge in agriculture in those countries for fear of direct or indirect pathogen contamination of food. In the UK a similar reaction has been seen with retailers informing farmers that they would not accept food grown on land that had received sewage sludge. This has led to a revision of the spreading of all waste materials to land (Table 11.11) and the production of a new set of regulations controlling the use of sludge in agriculture, 'The Safe Sludge Matrix' (Tyson 2002).

Table 11.10. Life cycle carbon dioxide emission units of main treatment processes (Hwang and Hanaki 2002).

Process	**Unit process**	**Prerequisite**	**CEU (kg-C/t-DS)**
Thickening	Gravity settling		6.4
	Air floatation		12.8
	Centrifugal		25.9
Anaerobic digestion	2-stage A.D. equipped with electric power generation system	Thickening	–46.9
Dewatering	Filter press	Thickening only	199.5
	Belt press		22.9
	Solid bowl centrifuge		36.9
	Filter press	Thickening+A.D.	224.9
	Belt press		32.2
	Solid bowl centrifuge		53.1
Incineration/melting	Fluidised bed	Thickening+Dewatering	82.8
	Fluidised bed boiler		154.9
	Cyclonic melting		47.5
	Fluidised bed	Thickening+A.D.+Dewatering	113.9
	Fluidised bed boiler		185.5
	Cyclonic melting		79.7
Reject water treatment	Conventional A.S. process	Containing A.D. supernatant	29.5
		not containing	21.9

Table 11.11. Estimates of the quantities of waste spread annually on land in the UK (Tyson 2002).

Origin of waste	**Quantity (tonnes $\times 10^3$ dry weight)**
Farm animal	21,000
Sewage sludge	430
Paper industry	520
Food industry	600
Sugar industry	200
Vegetable and food processing	70
Textile industry	22
Water treatment	17
Meat processing (blood etc. from abattoirs)	15
Beverage production (breweries, soft drinks)	11
Dairy industry	7
Leather tannery	1
Septic tank sludge	*
Biological treatment plant sludge	*
Waste wood, bark or plant	*
Waste soil	*
Dredgings from inland waters	*> 500 000**
Lime, cement industries	*> 500 000**
Compost	*∼ 600 000**

* — reliable data not available, figures in italics are crude estimates and expressed as wet weight (WRc 1998).

The Safe Sludge Matrix (ADAS 2002) offers clear guidance to the level of treatment required for sewage sludge before it can be used for specific crops and rotations. Treatment is defined as either conventional (i.e. treatment leading to at least 99% reduction of pathogens) or enhanced (i.e. sludge is free from *Salmonella* as well as a 99.9999% pathogen reduction). It is no longer permitted to use either raw or untreated sewage sludge on agricultural land for food production, neither is the surface spreading of conventionally treated sludge on grazed grassland. Conventionally treated sludges can only be applied to grazed grassland if directly injected into the soil and left ungrazed or harvested for a minimum of 3 weeks. Conventionally treated sludge can be applied to land used to grow vegetables in rotation provided at least 12 months has elapsed between sludge application and the harvest of the crop. If the crop may be eaten raw this interval must be at least 30 months or if enhanced treated sludges are used this can be

Table 11.12. The current and future predicted average final effluent quality from wastewater treatment plants in the Netherlands (van der Graaf 2001).

Parameter		**1981**	**1997**	**future**
COD	mg O_2/l	112	57	< 40
BOD	mg O_2/l	29	9	< 5
N-Kjeldahl	mg N/l	18	9	< 4
P-total	mg P/l	10	2.4	< 0.5
Suspended solids	mg DS/l	–	9	5

reduced to 10 months. Where grassland is reseeded then conventionally treated sewage sludge can be applied but must be either deeply ploughed or deep injection used. Conventionally treated sludge can also be applied to the surface of grassland or forage crops (e.g. maize) as long as the crops are subsequently harvested and there is no grazing of that land within the season of application. Current sludge management practices are reviewed by Leschber (2002).

Effluent quality

There has been a steady overall improvement in average effluent quality in most European countries over the past two decades. Van der Graaf (1999) estimates that further improvements will eventually bring current final effluent values in the Netherlands down to COD < 40 mg O_2 l^{-1}, BOD < 5 mg O_2 l^{-1}, Kjeldahl-N < 4 mg N l^{-1}, total-P < 0.5 mg P l^{-1} and suspended solids 5 mg l^{-1} (Table 11.12). He points out that at these concentrations the oxygen-consuming substances COD, BOD and Kjeldahl-N will hardly disturb the oxygen balance in receiving waters, while eutrophication will be largely controlled at these discharge nutrient concentrations. While the residual suspended solids fraction is unlikely to be a significant input to the receiving waters, it does prevent its potential reuse for aquifer recharge. This fraction also significantly contributes to the remaining organic and nutrient burden of the final effluent. The application of membrane filtration to remove the residual suspended solids would produce further significant reductions in COD to < 30 mg O_2 l^{-1}, BOD to $<$ 2–3 mg O_2 l^{-1}, and total-P to < 0.2 mg P l^{-1}. Also, removing this fraction will reduce the concentrations of heavy metals in the final effluent. (Table 11.13).

Until now little attention has been paid to the microbial quality of final effluents, and the increasing use of activated sludge, often at higher rates of

Table 11.13. Current and predicted future concentrations of heavy metals in final effluents in the Netherlands (van der Graaf 1999).

heavy metals in μg/l	**As**	**Cd**	**Cr**	**Cu**	**Hg**	**Pb**	**Ni**	**Zn**
present, average	1.5	0.2	4	12	0.1	6	10	55
future	1.0	0.1	2	5	0.05	3	5	50
, after filtration	1.0	0.05	1	2	0.05	2	2	50
, after ultrafiltration	1.0	0.05	0.5	1	0.05	1	2	50

loading has lead to a general decline in microbial quality of effluents. Also, there has been a discernible reduction in the number of activated sludge plants that have a diverse and healthy population of grazers (e.g. ciliate protozoa and rotifers) that significantly contribute to the reduction in the number of pathogens in effluents (Sec. 5.5). With the rapid development of gene probes it is only a matter of time before microbial quality standards for wastewater effluents are introduced. These exist already where sewage treatment plants are discharged to designated bathing areas or shellfish waters (Sec. 9.4.1). Currently few European wastewater treatment plants disinfect their final effluent, however with increasing worries about antibiotic resistance in surface waters, new pathogens, and pathogen transfer then disinfection will increase. While the use of sodium hypochlorite is still popular in the US, concerns over haloginated toxic by-products has resulted in a general transfer to UV sterilisation (van der Graaf 2001). Treatment technologies are considered in detail in Sec. 9.5.3.

To achieve true sustainability, many feel that alternative approaches should be considered. For example, for many industrial processes a zero discharge regime should be the ultimate goal; and for domestic and municipal wastewaters final effluent quality should be equal to or better than receiving water quality for all parameters. The concept of restorative discharges that improve receiving water quality is not unique, and there are many instances of wastewater discharges being the cleanest tributary within an urban catchment. Modern technologies could already deliver this option for many more river catchments.

Marine dumping

A quality status report on the North Sea concluded that less than 3% of P, and about 1% of Hg, Cd, and N came from the disposal of sewage sludge (including marine incineration). Much greater inputs come from rivers. The UK and Irish Governments' decision to phase out dumping

of sewage sludge at sea in spite of scientific evidence indicating that there would be little environmental benefits was justified on the basis of the precautionary principle. Dumping at sea was finally phased out on 31st December 1998. However, it is possible that marine disposal was the most sustainable method of disposal, especially linked with better control of wastewater contamination by metals and nutrients. The challenge is now to find an equally safe land-based disposal routes for the resulting sludge.

11.4. Implementation

Treatment hierarchy

The solid waste hierarchy encourages waste producers to choose waste management options from the top of the hierarchy, thereby making consumers and waste disposers think and act sustainably. The most favoured option is waste reduction followed by reuse and then recovery (Burton 1998). This approach is equally useful when dealing with wastewaters and should also be applied to all producers of wastewater including individual households (Fig. 11.7). At the top of the wastewater treatment hierarchy is the reduction in water use to reduce the overall generation and dilution of wastewater. Next is the reduction of wastewater contamination by careful selection of products used or if necessary their removal before discharge to the main wastewater stream. The separation of waste streams offers extensive scope for water recycling. This can be expanded by employing simple intermediate treatment steps such as sand filtration to permit a greater reuse of waste streams. Whenever possible wastewater should be treated at source, especially if this waste contains materials that could contaminate other

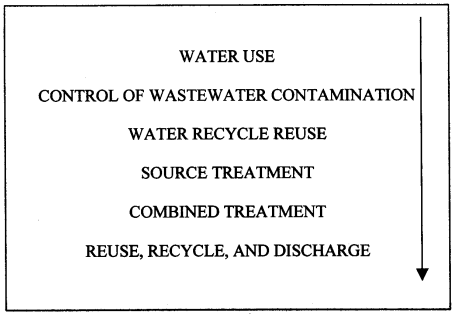


Fig. 11.7. Wastewater treatment hierarchy. The most favoured options are reduction of water use followed by control of contaminants entering the wastewater stream.

wastewaters or sludges. Otherwise combined treatment must be provided, especially for high housing densities and for those wastes requiring advanced treatment. Final effluents and sludges from treatment plants must be considered as valuable resources and opportunities for reuse or recycling exploited before discharge.

Public awareness and acceptance

While most people accept the principles of sustainable development and the need to preserve resources for future generations, few are prepared to alter their current lifestyle in order to achieve it. This is reflected in car ownership and usage rates as well as the per capita energy usage that are all continuing to rise throughout the developed world. In fact sustainability is often a difficult and expensive alternative for consumers compared with less sustainable options. Successful sustainable sanitation will involve a varying degree of consumer participation and acceptance, which will raise both social and cultural issues. Currently people's perception of sanitation is that it is an institutional problem not a personal one. Sanitation is seen as an unobtrusive service that does not impinge or limit the consumers' lifestyle.

Cultural problems should not be underestimated. These range from perceptions about waste, personal habits, to even discussing the problems. Separation toilets have been removed from ecological centres and villages due to the need to explain to visitors how to use them and the reluctance of visitors to comply with the instructions given (Fittschen 1997). So a vital step in achieving sustainable solutions is education of the need and cost of sanitation and its important role. It has been shown at all levels that sustainable behaviour can be stimulated through awareness. A greater responsibility for their own wastewater disposal will also raise people's awareness and participation in sustainable options (e.g. the requirement for new housing to install separation toilets). Financial incentives, for example a levy on water charges, will offset the effort and inconvenience that may arise, but unless they are significant, few will avail of them. Therefore, stricter regulation will inevitably have to follow to ensure full compliance. The willingness to pay depends on the priority the user places on sustainability. Where the technologies include user participation (e.g. urine separation, controlled use of pharmaceuticals etc.), they must be convenient and acceptable. For example, few people in developed countries would be prepared to empty a compost toilet by hand. Making sustainable options cheapest will result in slower compliance than by ensuring sustainable behaviour is

the most convenient option through better design. The concept that paying more is an acceptable alternative to behaving sustainably will make a sustainable option non-viable and ineffective.

Catchment management

The closest we have come to water cycle management is through integrated catchment management where water quality is protected by controlling land use activities. This also includes the urban water cycle. In 1992 a blueprint for future survival on plant earth was established at the United Nations Conference on Environment and Development held in Rio de Janeiro. This blueprint has become known as Agenda 21, and in this a number of key principles for integrated water management are listed. These are: (i) water is a scarce resource; (ii) all those interested in water allocation and use should be involved in decision making; and finally (iii) water should be managed within a comprehensive framework including water supply and waste management. Petts (2001) has recognised three additional principles specifically for sustainable river management: (i) rivers are influenced by all activities within their drainage basins (i.e. catchments); (ii) river ecosystems involve longitudinal, lateral and vertical fluxes including important interactions with floodplains and riparian zones during the flood season; (iii) river ecosystems are in large part driven by abiotic factors (e.g. hydrological, hydraulic, water quality, morphological dynamics). The main concerns about river catchments in the UK are summarised in Table 11.14.

Integrated catchment management leading to sustainable agricultural is now widely accepted as the key to environmental protection of terrestrial and aquatic resources and has been widely adopted worldwide (Greenfield and Oliver 2001; Shield and Good 2002). In Europe this has been achieved through the introduction of the Water Framework Directive (2000/60/EEC). Four key preconditions have been identified as necessary to achieve sustainability in catchments. These conditions are often expressed as the BAPP formulae of sustainability:

$$\frac{\mathrm{BAP}}{\mathrm{P_1}}$$

Where B is the definition of boundaries (e.g. jurisdiction, biological, discipline boundaries); A is administrative alignment and integration between and within organizations and levels of government; P the protection of non-urban lands (e.g. agricultural, natural, potable water supplies, fauna); and $\mathrm{P_1}$ the full participation of all stakeholders at all levels (e.g. community,

Table 11.14. Current concerns about river catchments in the UK (Petts 2001).

Flows	Local flooding due to blocked culverts
	Low flows, saline intrusion and wetland degradation
	Rising groundwater
Pollution	Misconnections
	Storm overflows and CSOs
	Pollution incidents/accidents
	Eutrophication
	Runoff from contaminated land
	Persistent chemicals
Ecology	Lack of instream habitat
	Loss of wetlands
	Loss of floodplain habitats
	Conservation of rare species
	Impact of invasive, non-native species
Amenity	Lack of access
	Derelict sites along river corridor
	Litter and illegal tipping
	Algae
	Drain-like channels

local, regional, national). The first three are all tasks, which is why they appear on the top of the equation above. The participation of all stakeholders underpins the success of the tasks and so appears below in the equation. The equation is of course non-quantitative but illustrates the need for full participation at all levels if sustainability is to be successful. The term stakeholder is widely used in catchment management and implies an investment either in terms of financial commitment or effort (Nemmo 2001). However, for sustainability to be fully achieved, everyone living within a catchment must be seen as a stakeholder with a responsibility to preserve the water quality within it through whatever means are necessary (Carroll *et al.* 2002; Scolz *et al.* 2002).

Economic sustainability of sanitation

Table 11.1 outlines the key economic criteria that are decisive when selecting a wastewater treatment system. Affordability will be a critical issue especially for developing countries requiring some compromise in design selection. Costs should not only include construction, operation, and maintenance, it should also include waste disposal costs and the cost of dismantling

parts of the treatment plant as individual unit processes reach the end of their useful life. Careful consideration should be given to labour, as different systems require different amounts of labour and expertise. Balkema (1998) suggests that a country with high unemployment should consider a labour intensive system. Finally, the success of sustainable sanitation as with other sustainable systems is the willingness of the user to pay and it is this criterion that in the past has been difficult to achieve.

To ensure financially and economically sustainable water and sewage operations and maintenance, financial autonomy of operating authorities is essential. The performance of services depends not only on the level of financing for them but also the actual source of such financing. In practice it appears that the performance of operating authorities in the provision of services is often linked to the degree that users are involved in financing them. Critical factors in financing services are (i) that the charges are applied to all customers; (ii) the tariffs are realistic and fair, and (iii) that there is a high level of success in revenue collection (Serageldin 1994). Apart from making people more careful and aware of water usage, charging also provides an opportunity to reward those for incorporating waste minimisation technology. For example, where charges are based on potable water metering, then the charge per cubic metre of waters supplied should increase dramatically with usage to encourage water harvesting and reuse of water. Minimum user charges encourage water usage and should not be used.

Social sustainability of sanitation

No sanitation system can be effective without user collaboration. This is particularly true of waste minimisation and exclusion of certain wastes from the water cycle. Therefore it would appear that sustainable sanitation is unlikely to succeed in isolation and must be part of an overall community acceptance of the sustainability principles into every aspect of our lives. Individual rejection of the principles must be penalised severely as the provision of a dual system will be both undesirable and counter-productive. The old ways of waste generation and disposal are now just too expensive both economically and environmentally. The risks to our health are also rapidly increasing which will threaten our current way of life, a problem exacerbated by the effects of global warming.

New attitudes amongst governments and the public, new technological developments and new management frameworks are all enabling the concepts of sustainability to become gradually accepted. However, at worse, the

concept of sustainability forces people to question where development is going. Also, that using economic costs and benefits as the sole criteria for development is no longer acceptable. At best, sustainability offers us the opportunity to create a cleaner and safer environment that could provide an acceptable standard of living for all future generations. Sustainable sanitation is a vital part in achieving these goals.

Further reading

General: Chen and Beck 1997; Bultler and Parkinson 1997; Lundin 1999; Mels *et al.* 1999; Hellström *et al.* 2000.

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