HANDBOOK OF ENVIRONMENTAL ENGINEERING™ VOLUME 11

Environmental Bioengineering

Edited by

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ISBN: 978-1-58829-493-7 e-ISBN: 978-1-60327-031-1
DOI: 10.1007/978-1-60327-031-1
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2010928632

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Dedications

The editors of the *Handbook of Environmental Engineering* series dedicate this volume to late Mr. Thomas L. Lanigan (1938–2006), who was the founder and president of Humana Press, and to late Dr. Stephen Tiong-Lee Tay, who served as a Co-editor of this volume and was an Associate Professor of Nanyang Technological University, Singapore.

Preface

The past 30 years have seen the emergence of a growing desire worldwide that positive actions be taken to restore and protect the environment from the degrading effects of all forms of pollution – air, water, soil, and noise. Since pollution is a direct or indirect consequence of waste production, the seemingly idealistic demand for "zero discharge" can be construed as an unrealistic demand for zero waste. However, as long as waste continues to exist, we can only attempt to abate the subsequent pollution by converting it to a less noxious form. Three major questions usually arise when a particular type of pollution has been identified: (1) How serious is the pollution? (2) Is the technology to abate it available? and (3) Do the costs of abatement justify the degree of abatement achieved? This book is one of the volumes of the *Handbook of Environmental Engineering* series. The principal intention of this series is to help readers formulate answers to the above three questions.

The traditional approach of applying tried-and-true solutions to specific pollution problems has been a major contributing factor to the success of environmental engineering, and has accounted in large measure for the establishment of a "methodology of pollution control." However, the realization of the ever-increasing complexity and interrelated nature of current environmental problems renders it imperative that intelligent planning of pollution abatement systems be undertaken. Prerequisite to such planning is an understanding of the performance, potential, and limitations of the various methods of pollution abatement available for environmental scientists and engineers. In this series of handbooks, we will review at a tutorial level a broad spectrum of engineering systems (processes, operations, and methods) currently being utilized, or of potential utility, for pollution abatement. We believe that the unified interdisciplinary approach presented in these handbooks is a logical step in the evolution of environmental engineering.

Treatment of the various engineering systems presented will show how an engineering formulation of the subject flows naturally from the fundamental principles and theories of chemistry, microbiology, physics, and mathematics. This emphasis on fundamental science recognizes that engineering practice has, in recent years, become more firmly based on scientific principles rather than on its earlier dependency on empirical accumulation of facts. It is not intended, though, to neglect empiricism where such data lead quickly to the most economic design; certain engineering systems are not readily amenable to fundamental scientific analysis, and in these instances we have resorted to less science in favor of more art and empiricism.

Since an environmental engineer must understand science within the context of application, we first present the development of the scientific basis of a particular subject, followed by exposition of the pertinent design concepts and operations, and detailed explanations of their applications to environmental quality control or remediation. Throughout the series, methods of practical design and calculation are illustrated by numerical examples. These examples clearly demonstrate how organized, analytical reasoning leads to the most direct and clear solutions. Wherever possible, pertinent cost data have been provided.

Our treatment of pollution-abatement engineering is offered in the belief that the trained engineer should more firmly understand fundamental principles, be more aware of the similarities and/or differences among many of the engineering systems, and exhibit greater flexibility and originality in the definition and innovative solution of environmental pollution problems. In short, the environmental engineer should by conviction and practice be more readily adaptable to change and progress.

Coverage of the unusually broad field of environmental engineering has demanded an expertise that could only be provided through multiple authorships. Each author (or group of authors) was permitted to employ, within reasonable limits, the customary personal style in organizing and presenting a particular subject area; consequently, it has been difficult to treat all subject material in a homogeneous manner. Moreover, owing to limitations of space, some of the authors' favored topics could not be treated in great detail, and many less important topics had to be merely mentioned or commented on briefly. All authors have provided an excellent list of references at the end of each chapter for the benefit of interested readers. As each chapter is meant to be self-contained, some mild repetition among the various texts was unavoidable. In each case, all omissions or repetitions are the responsibility of the editors and not the individual authors. With the current trend toward metrication, the question of using a consistent system of units has been a problem. Wherever possible, the authors have used the British system (fps) along with the metric equivalent (mks, cgs, or SIU) or vice versa. The editors sincerely hope that this duplicity of units' usage will prove to be useful rather than being disruptive to the readers.

The goals of the *Handbook of Environmental Engineering* series are: (1) to cover entire environmental fields, including air and noise pollution control, solid waste processing and resource recovery, physicochemical treatment processes, biological treatment processes, biosolids management, water resources, natural control processes, radioactive waste disposal, and thermal pollution control; and (2) to employ a multimedia approach to environmental pollution control since air, water, soil, and energy are all interrelated.

This particular book, Vol. 11, *Environmental Bioengineering,* deals mainly with engineering applications of biotechnologies, and is a sister book to Vol. 10, *Environmental Biotechnology.* Previous Vol. 10 introduces the mechanisms of environmental biotechnology processes, different microbiological classifications useful for environmental engineers, microbiology, metabolism, microbial ecology, natural and environmental engineering systems, bioengineering of isolated life support systems, classification and design of solid-state processes and reactors, value-added biotechnological products, design of anaerobic suspended bioprocesses and reactors, selection and design of membrane bioreactors, and aerobic and anoxic suspended-growth systems, aerobic and anaerobic attached growth systems, sequencing batch reactors, innovative flotation biological systems, phosphurs removal biotechnologies, and biosolids and septage management.

This Vol. 11 introduces land disposal of biosolids, heavy metal removal by crops, pretreatment of sludge for sludge digestion, bio-treatment of sludge, fermentaion of kitchen garbage, phytoremediation for sludge treatment, phyotoremediation for heavy metal contaminated soils using vetiver grass, bioremediatioon, wetland treatment, biosorption of heavy metals, rotating biological contactors (RBC) for carbon and nitrogen removal, anaerobic biofilm

reactor, biological phosphorus removal, black and grey water treatment, milk wastewater treatment, tomato wastewater treatment, gelatine and animal glue production from skin wastes, fungal biomass protein production, algae harvest energy conversion, and living machine for wastewater treatment.

These two books together (Vols. 10 and 11) have been designed to serve as comprehensive environmental biotechnology and bioengineering textbooks as well as wide-ranging reference books. We hope and expect they will prove of equal high value to advanced undergraduate and graduate students, to designers of biotechnology and bioengineering systems, and to scientists and researchers. The editors welcome comments from readers in all of these categories.

The editors are pleased to acknowledge the encouragement and support received from their colleagues and the publisher during the conceptual stages of this endeavor. We wish to thank the contributing authors for their time and effort, and for having patiently borne our reviews and numerous queries and comments. We are very grateful to our respective families for their patience and understanding during some rather trying times.

The editors are especially indebted to Ms. Kathleen Hung Li at Texas Hospital Association, Austin, TX, for her dedicated service as the Consulting Editor of Vol. 11.

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Treatment and Disposal of Biosolids

Svetlana Yu. Selivanovskaya, Saniya K. Zaripova, Venera Z. Latypova, and Yung-Tse Hung

CONTENTS

Abstract Biosolids management begins with its generation and continues through sludge treatment and sludge use and disposal. A wide variety of sludge treatment processes are used. The discussion is focused on biological methods of biosolids treatment. Most commonly, domestic wastewater sludge is biologically stabilized as a liquid in anaerobic digesters from which methane gas is a byproduct. Liquid sludge can also be treated in aerobic digesters to which oxygen (or air) must be added. Composting is a process that biologically stabilizes dewatered sludge. Several methods are widely employed to use or dispose of biosolids: land application, distribution and marketing, landfilling, and incineration.

1. WASTEWATER TREATMENT AND BIOSOLIDS FORMATION

Most countries require their municipalities to treat wastewater prior to discharging into the environment. Municipal wastewater treatment processes were designed to receive raw municipal wastewaters from both domestic and industrial sources and produce a liquid effluent of suitable quality that can be returned to natural surface waters with minimal impact on the environment and public health. A byproduct of this process, called sludge or biosolids, contains the solid fractions from the raw wastewater and the solids produced during the wastewater treatment processes. Both the effluent and sludge are treated to quality levels suitable for disposal or recycling purposes.

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_1

Municipal wastewater treatment typically comprises preliminary treatment, primary treatment, and secondary treatment. A higher degree of treatment, termed "tertiary" or "advanced" treatment, may be required at specific locations. Conventional municipal wastewater treatment is considered to include screening, grit removal, primary sedimentation, and biological treatment because it is the most common method (Fig. 1.1). Preliminary wastewater treatment generally includes screening and grit removal. The residues from preliminary wastewater treatment include coarse solids such as rags and heavy, inorganic, sandlike solids. Such residues are not usually incorporated with sludges.

Primary wastewater treatment usually involves gravity sedimentation of screened, degritted wastewater to remove settleable solids. The residue from primary treatment is a concentrated suspension of particles in water called "primary sludge." Although the goal of primary wastewater treatment is to separate readily-removable suspended solids and BOD (biochemical oxygen demand), wastewater constituents that exist as settleable solids or are sorbed to settleable wastewater solids may also be removed. Thus, primary treatment effects reduction in the effluent concentration of nutrients, pathogenic organisms, trace elements, and potentially toxic organic compounds. Constituents that are removed are contained in sludge. Primary treatment typically produces (2.5–3) $\times$ 10^3 L of sludges per 10^6 L of wastewater. The primary sludge produced contains 3–7% solids, and can be easily thickened or dewatered.

The clarified wastewater further undergoes secondary treatment, which often involves such biological processes as an activated sludge system (seeding sludge into the wastewater stream) or a trickling filter system with bacterial growth attached. Microorganisms are used to remove biodegradable organic material. A part of organic material is oxidized by the microorganisms to produce carbon dioxide and other end products. The remainder provides the energy and materials needed to support the microorganism community. The microorganisms biologically flocculate to form settleable particles, and the excess of biomass is separated in sedimentation tanks as a concentrated suspension called "secondary sludge," which is also known as "biological sludge" or "biosolids" or "waste activated sludge" or "trickling filter humus." Wastewater constituents can become associated with secondary sludge as a result of microbial assimilation, by sorption onto settleable solids, or by incorporation into agglomerate particles formed as a result of bioflocculation. Secondary treatment removes fine suspended solids and some dissolved solids and produces secondary sludge. Biological secondary treatment produces approximately (1.5–2) 10^4 L of secondary sludge for each 10^6 L of sludge treated. Secondary sludge generally has 0.5–2.0% solids, and it is more difficult to thicken and dewater than the primary sludge.

Tertiary treatment is used at municipal wastewater treatment plants when receiving water conditions, or other uses require higher quality effluent than that produced by secondary wastewater treatment. Disinfection for control of pathogenic microorganisms and viruses is a common type of tertiary treatment. Concentrations of suspended solids and associated BOD in treated effluent can be reduced by filtration or, sometimes, with the aid of a coagulant. Adsorption, usually on activated carbon, can be used to remove some persistent organic compounds and trace elements. The concentration of ammonia in secondary effluent can be reduced by nitrification. Tertiary treatment to remove nitrogen and phosphorus, so as to minimize nutrient enrichment of surface waters, is common; nitrogen is usually removed

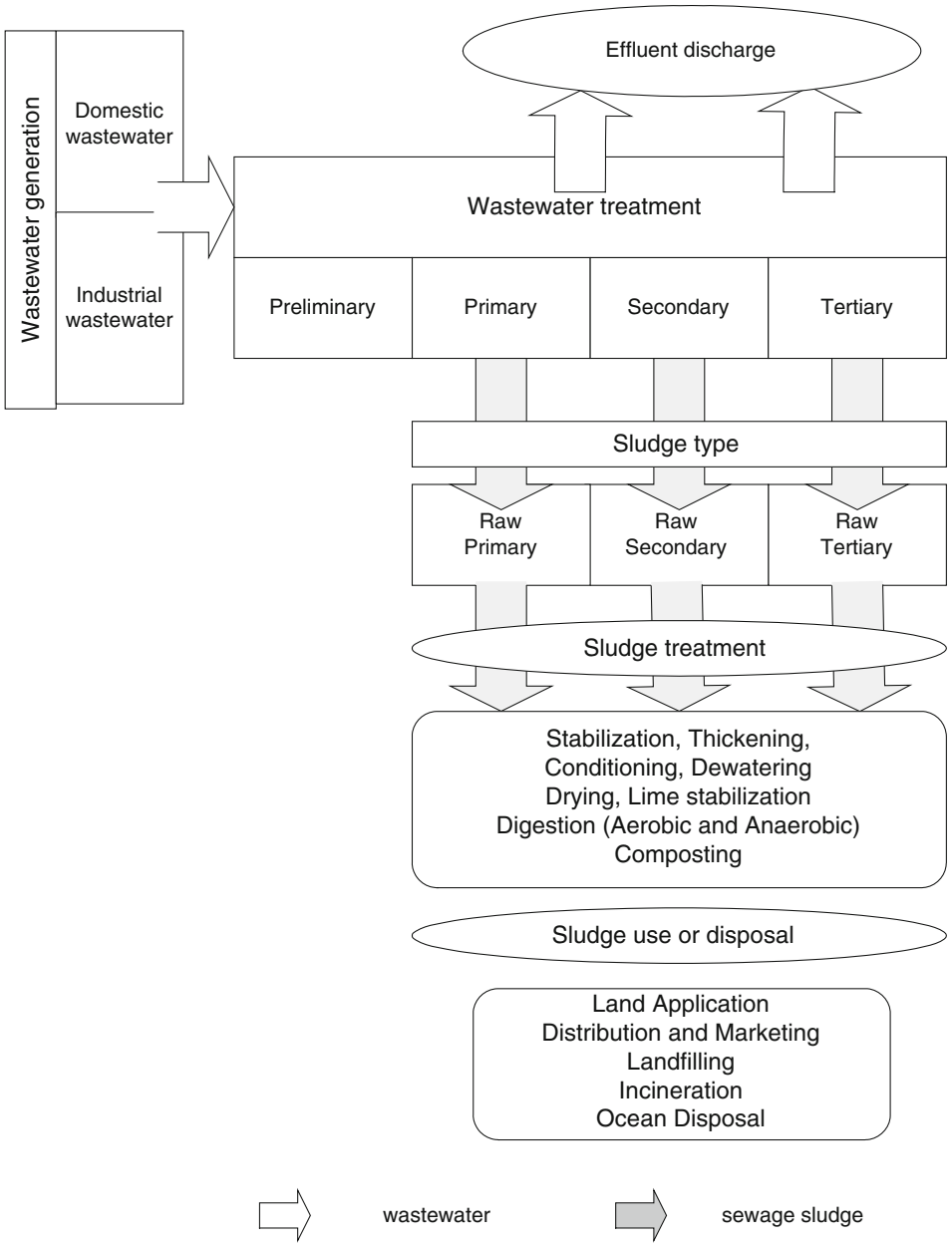

Fig. 1.1. Generation, treatment and disposal of municipal sewage sludge.

by nitrification followed by denitrification, and phosphorus is removed by microbial uptake or chemical precipitation. However, not all tertiary treatment processes follow secondary treatment. Tertiary treatment produces approximately 1×10^4 L of tertiary sludge per 10^6 L of wastewater treated. The characteristics of tertiary sludge depend on the wastewater treatment

process that produced it. Chemical sludges result from treatment processes that add chemicals, such as lime, organic polymers, and aluminum and iron salts, to wastewater. Generally, lime or polymers improve the thickening and dewatering characteristics of a sludge, whereas iron or aluminum salts usually reduce its dewatering and thickening capacity by producing very hydrous sludges which bind water.

The US EPA Part 503 rule defines sewage sludge biosolids as a solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works. Biosolids includes scum or solids removed in primary, secondary, or advanced wastewater treatment processes and any material derived from sewage sludge biosolids (e.g., a blended biosolids/fertilizer product) (1).

Sludge management begins with sludge generation and continues through sludge treatment and sludge use and disposal. It is an integral consideration in the planning and design of wastewater treatment plants and can be the most complex part of wastewater management.

2. CHARACTERISTICS OF BIOSOLIDS

The characteristics of sludge affect its suitability for the various use or disposal options. Thus, when evaluating sludge use or disposal alternatives, a municipality should first determine the amount and characteristics of its sludge. Some important properties of biosolids that needed to be characterized include the following:

Total solids content (TS)
Volatile solids content (VS)
Organic matter (OM)
Nutrients
Metals
Toxic organic chemicals
Pathogens

2.1. Total Solids Content

The total solids content of biosolids includes suspended and dissolved solids and is usually expressed as the percent of total solids present in biosolids. Typically, liquid biosolids have a solids content of 2–12% solids, while dewatered biosolids has a solids content of 12–40% solids (including chemical additives). Dried or composted biosolids typically have a solids content over 50%. TS content depends on the type of biosolids. Treatment processes such as thickening, conditioning, dewatering, composting, and drying can lower water content and thus raise the percent solids. The efficiency of these treatment processes, however, can vary substantially from time to time, producing biosolids with substantially lower solids content than anticipated.

2.2. Volatile Solids Content

Sludge volatile solids (VS) are organic compounds that are reduced when the sludge is heated to 550°C under oxidizing conditions. The VS content of sludge provides an estimate of

the organic content of the material. VS content is most often expressed as the percent of total solids that are volatile solids. Most unstabilized biosolids contain 75–85% VS on a dry weight basis. A number of treatment processes, including anaerobic digestion, aerobic digestion, alkali stabilization, and composting, can be used to reduce sludge VS content and thus the potential for odor. Anaerobic digestion is the most common method of sludge stabilization.

2.3. pH

The pH of biosolids can affect crop production at land application sites by altering the pH of the soil and influencing the uptake of metals by soil and plants. Low pH sludge (less than approximately pH 6.5) promotes leaching of heavy metals, while high pH sludge (greater than pH 11) kills many bacteria and, in conjunction with soils of neutral or high pH, can inhibit the movement of heavy metals through soils.

2.4. Organic Matter

The relatively high level of organic matter in biosolids allows sludge to be used to improve the physical properties of soil (e.g., increased water infiltration and water-holding capacity). The soil conditioning properties of biosolids are especially useful at reclamation sites such as mine spoils.

2.5. Nutrients

Nutrients present in biosolids, such as nitrogen (N), phosphorus (P), and potassium (K), among others, are essential for plant growth and endow biosolids with fertilizing properties. Nutrient levels are key determinants of biosolids application rates. Excessive nutrient levels due to high sludge application rates can result in environmental contamination of ground water and surface water and should be avoided. Table 1.1 (2–10) shows the level of nutrients typically present in biosolids (2–8). Nutrient levels, however, particularly nitrogen levels,

Table 1.1
Total concentration of selected nutrients in sewage sludge (2–10)

Source of sewage sludge	Total nutrients, % dry weight		
	N	P	K
WWPT of Michigan (USA)	3.5	2.2	0.5
WWPT of New York (USA)	2.9	1.2	0.19
WWPT of Hawaii's (USA)	3.8	0.6	0.06
WWPT Sankt-Petersburg (Russia)	4.3	2.4	0.4
WWTP of Moscow (Russia)	2.1–2.8	1.6–2.9	0.3–0.5
WWPT of Vladimir (Russia)	1.57–1.95	1.35–2.25	0.2–0.45
WWPT of Kazan (Russia)	1.7–2.6	0.12–1.2	0.14–0.36
WWPT of Sochi (Russia)	3.4	1.9	0.3
WWPT of Sipraya (Thailand)	3.43	0.11	0.08
WWPT of Triunfo (Brazil)	2.3	0.69	0.11
WWPT of Larissa (Greece)	1.8–2.8	1.2–1.65	Not determined

can vary significantly, and thus analysis should be conducted on the actual biosolids being considered for land application. Typically, nutrient levels in biosolids are considerably lower than those in commercial fertilizers, especially K, which is usually less than 0.5% in biosolids.

2.5.1. Nitrogen

Typically, treated sludges include about 1–6% nitrogen on a dry weight basis (2–9). By contrast, nitrogen in commercial fertilizers range from 11 to 82%. The nitrogen in treated sludge occurs in both organic and plant-available inorganic forms. The relative proportions of each depend upon the way sludges are processed. Thus, in anaerobically digested liquid sludges, microbial oxidation of the organic materials is incomplete, and the nitrogen occurs in both soluble ammoniacal and insoluble organic forms, primarily, in microbial cells (10). In aerobically digested sludges, microbial oxidation is greater, and there is less residual organic nitrogen than in anaerobically digested sludges. Ammoniacal nitrogen is about 10% of the total nitrogen in aerobically digested sludge and about 30% of the total nitrogen in anaerobically digested sludge (3, 4, 10). In aerobically digested sludge, the ammoniacal nitrogen is further oxidized to nitrate, of which part is lost to wastewater when sludge is dewatered. Likewise, when anaerobically digested sludges are dewatered, part of the ammoniacal nitrogen is lost with the water.

Where sludges are used as a source of nitrogen, the nitrogen application rates should not exceed the agronomic rate (a rate equivalent to the amount of fertilizer nitrogen applied to the soil for the crop grown). As with any fertilizer, nitrogen that leaches beyond the root zone could contaminate ground water. To determine the quantity of sludge needed for the crop's nitrogen requirement, it is important to know the relative proportions of inorganic and organic nitrogen. The inorganic forms of nitrogen (nitrate and ammonium) are immediately available to the crop. Organic forms of nitrogen are not available to the crop and must first be mineralized by microorganisms to inorganic forms. The rate of mineralization depends on a number of factors including sludge type, carbon-to-nitrogen ratio of the soil or sludge, climate, soil type, and water content.

2.5.2. Phosphorus

Sludges typically contain between 0.8 and 6.1% phosphorus (2–9). By contrast, commercial fertilizers typically contain between 8 and 24% phosphorus. Like nitrogen, the phosphorus in sludges is present in inorganic and organic forms. The proportions of each vary and depend on the source of municipal wastewater and on sludge treatment. Almost without exception, the amount of phosphorus applied is more than sufficient to supply the needs of the crop, where sludges are applied as a source of nitrogen.

2.5.3. Other Plant Nutrients

In addition to nitrogen and phosphorus, treated sludges contain all other nutrients essential for the growth of crops, including calcium, iron, magnesium, manganese, potassium, sodium, and zinc (9–11). Where treated sludges are applied according to agronomic rates for nitrogen, many of these essential nutrients, with the possible exception of potassium, are usually present in amounts adequate to meet the needs of the crop.

2.5.4. Metals

Biosolids may contain varying amounts of metals – cadmium (Cd), chromium (Cr), copper, lead, mercury, nickel, zinc. Arsenic, molybdenum selenium will be also viewed in this section, although strictly speaking, they are not really metals. At low concentrations in soil, some of these metals (e.g., Cu and Zn) are nutrients needed for plant growth and are often added to inorganic commercial fertilizers. But at high concentrations, some metals may be toxic to humans, animals, and plants. In fact, concentrations of metals in sludge are among the deciding factors for sludge utilization on lands because of their potential to damage crops and to enter the human food chain. Table 1.2 (3, 5, 6, 9, 12–36) lists metals content in sludges produced on WWTP of different countries (3, 5, 6, 9, 12–37). Concentrations of the metals are primarily a function of the type and amount of industrial waste that is discharged into the municipal wastewater treatment system. Industrial pretreatment and source control programs can control or reduce the metals content of sludge. Good management practices in land application, landfilling, and incineration may minimize or eliminate the potential for adverse effect. According to the data presented by Hue (2), a recent survey of U.S. wastewater treatment plants by the U.S. Environmental Protection Agency (USEPA) shows that the median concentrations of heavy metals have been decreasing over time. Based on the results of biosolids research during the past decades, a "clean sludge" category has been proposed (2). "Clean" sludge would have no limit on its application rate to land.

2.5.5. Toxic Organic Chemicals

Sludges can contain synthetic organic chemicals, from industrial wastes, household chemicals and pesticides. These chemicals are of concern because of their known and unknown hazards to public health and the environment. The following chemicals are most often contained in biosolids: chlordane, lindane, endrine, toxaphene, 2,4-D, benzene, chlorobenzene, hexachlorobenzene, 1,2-dichloroethane, toluene, naphthaline, cresols, benz(a)pyrene, polychlorinated biphenyls (PCB), etc. (9–11). Fortunately, most sludges contain low levels of these substances and do not pose a significant human health or environmental threat.

2.5.6. Pathogens

A significant proportion of the bacteria, viruses, protozoa, and eggs of parasitic worms in wastewater become concentrated in sludge during wastewater treatment. The number and types of organisms present vary, depending on such factors as population density, sanitary habits, and sludge treatments. A small percentage of these organisms may be pathogenic (disease-causing). The most common bacterial pathogens in biosolids are *Salmonella, Shigella*, and *Campylobacter* (1, 11). *Salmonella* can cause salmonellosis; *Shigella*, dysentery; and *Campylobacter*, gastroenteritis. Although *Escherichia coli* belongs to the *Shigella* spp., it is not considered pathogenic. It is often used to indicate the adequacy (or inadequacy) of a treatment process in reducing pathogens because *E. coli* is abundant in sludge. More than 110 different viruses may be present in raw wastewater and biosolids. Enteroviruses, which include Poliovirus, Echovirus, Coxsackievirus, and Hepatitis virus, can cause diseases from meningitis to infectious hepatitis. Reovirus and Adenovirus may cause respiratory infection. Of the common protozoa that may be found in wastewater and biosolids, only three species

Table 1.2
Total concentration of selected metals (mg/kg dry wt) in sewage sludge (3, 5, 6, 9, 12–36)

Country	Pb	Cd	Ni	Cr	Mn	Zn	Cu
Canada (12)	45.4	2.8	6.8	127.8	–	359	196
San-Paolo, Brazil (13)	29–719	–	289–2,815	–	–	–	130–3,510
Tai Po, Eng Lo, China (14)	52–86	1.8–2.3	53–70	53–2,239	282–537	1,108–4,692	329–458
Italy (15)	109–118	–	161–221	–	–	1,140–1,574	666–934
Spain (16)	107	2	154	112	114	454	212
China (17)	281	7				1,530	1,470
Thailand (8)	–	1.22	–	–	2,621	1,326	801
Spain (18)	140	1	46	–	–	1,043	223
USA (19)	653	81	169	111	–	4,127	1,112
Lund, Sweden (20)	59–162	1.3–3.0	13–111	28–207	–	595–1,100	651–1,333
Malmee, Sweden (20)	75–180	1.7–3.5	25–30	38–406	–	655–1,000	1,100–1,550
Germany (21)	72	13	129	–	–	1,282	515
Malabar, Australia (22)	303	11	162	235	–	2,669	1,274
Port Kembla, Australia (22)	97	32	32	66	–	1,767	648
Quakers Hill, Australia (22)	81	2	28	488	–	512	468
Snt. Marilend, Australia (22)	111	2	25	66	–	498	447
Richmond, Australia (22)	90	3	20	54	–	704	439
Domjale, Slovene (23)	126	2.78	621	856	–	2,032	433
Antonin, Zakopane, Rogozno, Dabrova, Simenovichi, Poland (24)	27–456	0.8–15	8–79	7–281	331–34,431	545–7,961	24–592
New Delhi, India (25)	150	6.9	61.4	220.0	–	237.5	139.0
Saragosa, Spain (26)	101.6–189.3	9.9–14.1	116.3–376.4	361.8–708.4	10.80–197.6	1,160–2,088	228.7–362.5
Ilaly (27)	248–295	2.4–5.0	37.5–92.5	77.5–85.5	–	850–1,827	175–290
Chicago, USA (28)	–	–	591	–	–	4,066	1,367

Dadar, Mumbai, India (29)	–	3	–	–	378	1,190	–
Tessaloniki, Greece (30)	40–155	3–4	–	56–128	175–308	403–1,878	129–147
New York, USA (30)	20–340	2.0–480	–	67–5,167	34–814	223–7,068	117–13,380
Hawaii (30)	–	3.1–10.5	–	–	44–609	421–1,308	220–462
USA (31)	13–19,700	3–3,410	2–3,520	–	18–7,100	101–27,800	84–10,400
Larissa, Metamorphosis, Greece (7)	450–500	5–12	180–200	200–600	–	–	–
Triunfo, Brazil (6)	3.5–29.7	0.51–5.1	9.8–59	1.5–127.5	1.8–606	5.5–688	1.1–26
USA (32)	136–3,065	7–444	–	169–14,000	32–527	–	90–2,890
Sankt-Petersburg, Russia (2)	57	7	33	–	97	1,671	276
Sankt-Petersburg, Russia (4)	45–137	13–90	115–530	255–3,600	187–2,625	937–3,600	580–7,321
Vladimir, Russia (5)	400–600	30–90	400–600	3,000–4,000	1,000–20,000	5,000–6,000	3,000–4,000
Shekino, Russia (33)	42–83	0.9–11	10–96	3–551	–	52–972	3–240
Ioshkar-Ola, Russia (34)	79.4	11.3	667.6	–	48.7	677.5	509.4
Velikie Luki, Russia (35)	–	–	60–630	272–646	–	478–2,095	538–1,999
Shelkovo, Russia (36)	–	51	230	1,600	–	3,300	870
Sochi, Russia (2)	70	6	100	0	760	1,669	406
Kazan, Russia (9)	120–450	15–20	280–1,400	1,400–1,850	500–1,800	150–600	150–210

are of major significance for disease transmission to humans: *Entamoeba histolytica*, *Giarda lambia*, and *Balantidium coli*. All three can cause mild to severe diarrhea. Eggs of Helminth parasites (intestinal worms), including *Ascaris lumbricoides* (round worm), *Ancyclostoma duodenale* (hookworm), *Trichuris trichiura* (whipworm), and *Taenia saginata* (tapeworm) are of particular concern because they can survive many forms of sludge treatment, and they can infect humans and animals even at small numbers.

Pathogens can present a public health hazard if they are transferred to food crops grown on land to which biosolids are applied, contained in runoff to surface waters from land application sites, or transported away from the site by vectors such as insects, rodents, and birds. Some sludge treatments, including anaerobic digestion, mesophilic aerobic digestion, and air drying, significantly reduce but do not completely eliminate pathogens. For this reason, they are called processes to significantly reduce pathogens (PSRP) in regulatory terms. To virtually destroy these disease-causing organisms, thermophilic treatments of biosolids are often required. Thus, the latter treatments are called the processes to further reduce pathogens (PFRP). These processes will be examined below.

3. REGULATIONS GOVERNING AGRICULTURAL USE OF BIOSOLIDS

Biosolids are recognized as potentially harmful because of the chemical pollutants and the disease-causing agents they may contain. In the US, the federal Part 503 rule (40 CFR Part 503) establishes requirements for land applying biosolids to ensure protection of public health and the environment when biosolids is used for its soil conditioning or fertilizing properties (37, 38). Part 503 covers biosolids sold or given away in bulk, bags, or other containers for application to agricultural land (e.g., cropland, pastures, and rangelands), forests, reclamation sites (e.g., mine spoils, construction sites, and gravel pits), public contact sites (e.g., parks, plant nurseries), and lawns and home gardens. The rule's land application requirements also pertain to material derived from biosolids. Such materials include biosolids that have undergone a change in quality through treatment (e.g., composting, drying) or mixing with other materials (e.g., wood chips) after it leaves the treatment works where it was generated.

3.1. Standards for Pathogens

Two approaches were taken in the Part 503 land application operational standards for pathogens and vector attraction reduction. In the first approach, biosolids can be treated to reduce pathogens and to reduce the characteristics of biosolids that attract vectors (39). If specified treatment-related requirements are met, nothing has to be done at the application site with respect to pathogens and vector attraction reduction. The second approach in the Part 503 rules requires a combination of biosolids treatment and management practices that must be met at the application site. For pathogens, some reduction must be achieved through treatment of the biosolids, and in addition, management practices have to be met at the application site. The management practices prevent exposure to the biosolids for a period long enough to allow the environment to further reduce the pathogens to below detectable levels, which is the goal in both approaches. The vector attraction reduction requirements that are met at the application site (i.e., injection below the land surface and incorporation

after being surface-applied) place a barrier of soil between the biosolids and the vectors. This prevents contact between the biosolids and the vectors. Part 503 contains several treatment-related alternatives for pathogen reduction and several alternatives for the combined treatment and site-related pathogen reduction. This provides the person who prepares the biosolids (i.e., the generator or a person who derives a material from biosolids) flexibility to choose the alternative that best fits a particular situation. Based upon pathogen reduction criteria, the rule divides sludge into two categories: Class A (safe for direct contact) and Class B (land and crop use restriction supply).

3.1.1. Class A Pathogen Requirements

The implicit goal of the Class A requirements is to reduce the pathogens in biosolids to below detectable levels. When this goal is achieved, Class A biosolids can be used without any pathogen-related restrictions on the site. The implicit goal of the Class B requirements is to ensure that pathogens have been reduced to levels that are unlikely to pose a threat to public health, and the environment under that must meet the Class A pathogen requirements includes biosolids that is sold or given away in a bag or other container for application to land and bulk biosolids that is applied to a lawn or home garden. Part 503 establishes six alternatives for demonstrating that biosolids meets Class A pathogen reduction requirements (Table 1.3) (1). The rule requires that the density of fecal conforms be less than 1,000. Most Probable Number (MPN) per gram total solids (dry weight) or that *Salmonella* sp. bacteria be less than 3 per 4 g total solids, as presented in Table 1.4 (1).

Table 1.3
Summary of the six alternatives for meeting Class A pathogen requirements (1)

In addition to meeting the requirements in one of the six alternatives listed below, the requirements in Table 1.5 must be met for all six Class A alternatives	
Alternative 1: thermally treated biosolids	Biosolids must be subjected to one of four time-temperature regimes
Alternative 2: biosolids treated in a high pH-high temperature process	Biosolids must meet specific pH, temperature, and air-drying requirements
Alternative 3: biosolids treated in other processes	Demonstrate that the process can reduce enteric viruses and viable helminth ova. Maintain operating conditions used in the demonstration after pathogen reduction demonstration is completed
Alternative 4: biosolids treated in unknown processes	Biosolids must be tested for pathogens – *Salmonella* sp. or fecal conform bacteria, enteric viruses, and viable helminth ova – at the time the biosolids are used or disposed, or, in certain situations, prepared for use or disposal
Alternative 5: biosolids treated in a PFRP	Biosolids must be treated in one of the Processes to Further Reduce Pathogens (PFRP)
Alternative 6: biosolids treated in a process equivalent to a PFRP	Biosolids must be treated in a process equivalent to one of the PFRPs, as determined by the permitting authority

Table 1.4
Pathogen requirements for all Class A alternatives (1)

The following requirements must be met for all six Class A pathogen alternatives
Either: The density of fecal coliform in the biosolids must be less than 1,000 most probable numbers (MPN) per gram total solids (dry-weight basis) *Or:* The density of *Salmonella* sp. bacteria in the biosolids must be less than 3 MPN per 4 g of total solids (dry-weight basis) Either of these requirements must be met at one of the following times: • When the biosolids are used or disposed • When the biosolids are prepared for sale or give-away in a bag or other container for land application; or • When the biosolids or derived materials are prepared to meet the requirements for EQ biosolids Pathogen reduction must take place before or at the same time as vector attraction reduction, except when the pH adjustment, percent solids vector attraction, injection, or incorporation options are met

Alternative 1: thermally treated biosolids. This alternative may be used when the pathogen reduction process relies on specific time-temperature regimes to reduce pathogens. The approach involves calculating the heating time necessary at a particular temperature to reduce a biosolid's pathogen content to below detectable levels. Time-consuming and expensive tests for the presence of specific pathogens can be avoided with this approach. The microbiological density portion of the requirement (i.e., the regrowth requirement) is designed to ensure that the microbiological reductions expected as a result of the time-temperature regimes have actually been attained and that regrowth has not occurred.

Alternative 2: biosolids treated in a high pH-temperature process. This alternative may be used when the pathogen reduction process relies on a particular high temperature pH process that has been demonstrated to be effective in reducing pathogens to below detectable levels. The high pH (>12 for more than 72 h) and high temperature (above 52°C for at least 12 h while pH is >12) for prolonged periods allow a less stringent time-temperature regime than the requirements under Alternative 1.

Alternative 3: biosolids treated in other processes. This alternative applies to biosolids treated by processes that do not meet the process conditions required by Alternatives 1 and 2. Alternative 3 relies comprehensive monitoring of fecal coliform or *Salmonella* sp. bacteria; enteric viruses; and viable helminth ova to demonstrate adequate reduction of pathogens.

If no enteric viruses or viable helminth ova are present before treatment (i.e., in the feed biosolids), the biosolids is Class A with respect to pathogens until the next monitoring episode. Monitoring is continued until enteric viruses or viable helminth ova are detected in the feed biosolids, at which point the treated biosolids is analyzed to see if these organisms survived treatment. If enteric virus and viable helminth ova densities are below detection limits, the biosolids meets Class A requirements and will continue to do so as long as the treatment

process is operated under the same conditions that successfully reduced the enteric virus and viable helminth ova densities. Monitoring for fecal coliform and *Salmonella* sp. bacteria, however, must continue to be performed as indicated in Table 1.4 (1).

Alternative 4: biosolids treated in unknown processes. This alternative is used primarily for stored biosolids for which the history is unknown. It also can be used when the process in which biosolids is treated does not meet any of the descriptions of a Process to Further Reduce Pathogens (PFRP). In this alternative, a representative sample of the biosolids must meet the Part 503 requirements for *Salmonella* sp. or fecal coliform bacteria (as described in Table 1.4 (1); enteric viruses; and viable helminth ova at the time the biosolids is used or disposed, prepared for sale or give-away in a bag or other container for application to land, or prepared to meet "exceptional quality" (EQ) land application requirements (to be discussed later).

Alternative 5: use of a PFRP. For Alternative 5, biosolids qualifies as Class A if it has been treated in one of the processes to further reduce pathogens (PFRPs) (Table 1.5 (1) and meets the regrowth requirement. The treatment processes must be operated according to the PFRP process descriptions summarized in Table 1.5 (1) at all times. Under this alternative,

Table 1.5
Processes to further reduce pathogens (PFRPs)(1)

1. *Composting*
Using either the within-vessel composting method or the static aerated pile composting method, the temperature of the biosolids is maintained at 55°C or higher for 3 days
Using the windrow composting method, the temperature of the biosolids is maintained at 55°C or higher for 15 days or longer. During the period when the compost is maintained at 55°C or higher, the windrow is turned a minimum of five times
2. Heat drying
Biosolids are dried by direct or indirect contact with hot gases to reduce the moisture content of the biosolids to 10% or lower. Either the temperature of the biosolids particles exceeds 80°C or the wet bulb temperature of the gas in contact with the biosolids as the biosolids leave the dryer exceeds 80°C
3. Heat treatment
Liquid biosolids are heated to a temperature of 180°C or higher for 30 min
4. Thermophilic aerobic digestion
Liquid biosolids are agitated with air or oxygen to maintain aerobic conditions, and the mean cell residence time of the biosolids is 10 days at 55–60°C
5. Beta ray irradiation
Biosolids are irradiated with beta rays from an accelerator at dosages of at least 1.0 megarad at room temperature (ca. 20°C)
6. Gamma ray irradiation
Biosolids are irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137, at room temperature (ca. 20°C)
7. Pasteurization
The temperature of the biosolids is maintained at 70°C or higher for 30 min or longer

treatment processes classified as PFRPs can continue to be operated; however, microbiological monitoring (i.e., for fecal coliform or *Salmonella* sp. bacteria) must now be performed to ensure that pathogen density levels are below detection limits and that regrowth of *Salmonella* sp. bacteria does not occur between treatment and use or disposal of the biosolids.

Alternative 6: use of a process equivalent to a PFRP. Under this alternative, biosolids is considered to be Class A biosolids if it is treated by any process equivalent to a PFRP and meets the regrowth requirement in Table 1.4 (1). To be equivalent, a treatment process must be able to consistently reduce pathogens to levels comparable to the reduction achieved by a listed PFRP. Processes must be operated at all times at the parameters described in the process description. The Part 503 rule gives the permitting authority responsibility for determining equivalency. To assist in making such determinations, the EPA's Pathogen Equivalency Committee (PEC) serves as a resource, providing recommendations on the equivalency of processes; the PEC also provides guidance to the regulated community. Equivalency determinations can be made on a site-specific or national basis.

3.1.2. Class B Pathogen Requirements

Bulk biosolids that are applied to agricultural land, forests, public contact sites, or reclamation sites must meet the Class B pathogen requirements if Class A pathogen requirements are not met. Part 503 establishes three alternatives for demonstrating that biosolids meets Class B pathogen requirements (Table 1.6) (1). The rule's implicit objective for all three approaches is to ensure that pathogenic bacteria and enteric viruses are reduced in density, as demonstrated by a fecal coliform density in the treated biosolids of 2 million Most Probable Number (MPN) or colony-forming units (CFU) per gram total solids biosolids (dry-weight basis). Viable helminth ova are not necessarily reduced in Class B biosolids. Unlike Class A biosolids, which are essentially pathogen-free, Class B biosolids contain some pathogens. Therefore, site restrictions apply for a certain period when Class B biosolids are land applied to allow environmental factors to further reduce pathogens to below detectable levels (Table 1.7) (1). The three alternatives for meeting Part 503 Class B pathogen reduction requirements are presented below.

Alternative 1: monitoring of fecal coliform. This alternative requires that seven samples of treated biosolids be collected at the time of use or disposal, and that the geometric mean fecal

Table 1.6
Summary of the three alternatives for meeting Class B pathogen requirements (1)

Alternative 1: the monitoring of indicator organisms	Test for fecal coliform density as an indicator for all pathogens. The geometric mean of seven samples shall be less than 2 million MPNs per gram per total solids or less than 2 million CFUs per gram of total solids at the time of use or disposal
Alternative 2: biosolids treated in a PSRP	Biosolids must be treated in one of the Processes to Significantly Reduce Pathogens (PSRP)
Alternative 3: biosolids treated in a process equivalent to a PSRP	Biosolids must be treated in a process equivalent to one of the PSRPs, as determined by the permitting authority

Table 1.7
Site restrictions for Class B sewage sludge applied to land (1)

Food crops with harvested parts that touch the sewage sludges/soil mixture
Food crops with harvested parts that touch the sewage sludge soil mixture and are totally above ground shall not be harvested for *14 months* after application of sewage sludge
Food crops with harvested parts below the land surface
Food crops with harvested parts below the land surface where sewage sludge remains on the land surface for 4 months or longer prior to incorporation into the soil shall not be harvested for *20 months* after sewage sludge application
Food crops with harvested parts below the land surface where sewage sludge remains on the land surface for less than 4 months prior to incorporation shall not be harvested for 38 *months* after sewage sludge application
Food crops with harvested parts that do not touch the sewage sludge/soil mixture, feed crops, and fiber crops
Food crops, feed crops, and fiber crops, whose edible parts do not touch the surface of the soil, shall not be harvested for *30 days* after sewage sludge application
Animal grazing
Animals shall not graze on land for *30 days* after application of sewage sludge to the land
Turf growing
Turf grown on land where sewage sludge is applied shall not be harvested for *1 year* after application of the sewage sludge when the harvested turf is placed on either land with a high potential for public exposure or a lawn, unless otherwise specified by the permitting authority
Public access
Public access to land with a high potential for public exposure is restricted for *1 year* after sewage sludge application
Access to land with a low potential for public exposure is restricted for *30 days* after sewage sludge application

coliform density of these sample be less that 2 million CFU or MPN per gram of biosolids (dry-weight basis). Analysis of multiple samples is required during each monitoring period because the methods used to determine fecal coliform density (i.e., membrane filter methods and the MPN dilution method) have poor precision and because biosolids quality tends to vary. Use of at least seven samples is expected to reduce the standard error to a reasonable value.

Alternative 2: use of a PSRP. Under this alternative, biosolids is considered to be Class B if it is treated in one of the processes to significantly reduce pathogens (PSRPs) (Table 1.8) (1). Unlike the comparable Class A requirement, this alternative does not require microbiological monitoring because public access to the site is restricted, allowing time for environmental conditions to reduce pathogens to below detectable levels.

Alternative 3: use of a process equivalent to a PSRP. Alternative 3 states that biosolids treated by any process determined to be equivalent to a PSRP by the permitting authority are considered to be Class B biosolids. To assist the permitting authority in making

Table 1.8
Processes to significantly reduce pathogens (PSRPs) (1)

1. Aerobic digestion Biosolids are agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C and 60 days at 15°C
2. Air drying Biosolids are dried on sand beds or on paved or unpaved basins. The biosolids dry for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C
3. Anaerobic digestion Biosolids are treated in the absence of air for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35–55°C and 60 days at 20°C
4. Composting Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the biosolids is raised to 40°C or higher and maintained for 5 days. For 4 h during the 5-day period, the temperature in the compost pile exceeds 55°C
5. Lime stabilization Sufficient lime is added to the biosolids to raise the pH of the biosolids to 12 after 2 h of contact

determinations, the EPA's Pathogen Equivalency Committee (PEC) serves as a resource, providing recommendations on the equivalency of processes; the PEC also provides guidance to the regulated community. Equivalency determinations can be made on a site-specific or national basis.

3.2. Pollutant Limits

3.2.1. U.S. Chemical Pollutant Standards for Agricultural Use of Biosolids

Philosophically, pollutant inputs to soils through land application of wastewater and biosolids may be regulated through two approaches (10). One approach is to prevent toxic chemical pollutants from accumulating above natural background levels in the soils. Another approach is to allow pollutants to accumulate so long as the soil capacity for assimilating, attenuating, and detoxifying the pollutants is adequate to minimize the risk to humans, agricultural crops, and the environment.

The underlying objective of the first approach, called "Preventing Toxic Chemical Pollutant Accumulation in Soils," is to preserve the soil's current condition and avoid an accumulation of pollutants from long-term applications of sludge and wastewater. This approach aims to prevent an increase in the concentration of pollutants based on the assumption that any increase in pollutants would compromise the soil's ability to support a productive microbial and botanical population and limit its potential use. A land application regulation based on this approach strives to prevent pollutant accumulation in the soil from exceeding levels that exist before sludge or wastewater effluent is applied. To meet this objective, pollutant input from applications of wastewater or sludge and other sources must be balanced by pollutant

output via surface runoff, leaching, atmospheric loss, and plant uptake followed by removal. In soils, pollutant output is typically very low. Consequently, the pollutant loading from all sources including land application of wastewater and biosolids must also be very low in order to maintain the balance and prevent any net accumulation. Regulations and guidelines that employ this principle must set very stringent toxic chemical pollutant loading limits for soils that can only be met by preventing all toxic chemical pollutants from entering wastewater collection and treatment systems, or by requiring the use of advanced levels of treatment to physically strip pollutants out of the effluent or sludge prior to land application. Otherwise, the sludges or effluents must be applied at very low rates to prevent no net change in the pollutant concentration in the soil. One advantage of this approach is that detailed knowledge about the fate and transport of pollutants, exposure analysis, and dose–response relationships is not necessary. The numerical limits for pollutants may be calculated by simple mass balances (pollutants in sludge transported out of soil system via surface runoff, leaching, atmospheric loss and removal by harvested plants) and this relationship can be applied to any location. However, the disadvantages are twofold: (a) meeting the numerical limits can be very costly, and (b) the allowable application rates are too low to provide any nutrient value.

The premise of the second approach, which allows pollutant accumulation in the soil, is that advantage can be taken of the beneficial qualities (moisture, organic matter and nutrients) of sludges and of the capacity of soil to attenuate toxic chemical pollutants present in the sludges. Soil is a dynamic medium consisting of mineral fragments, organic matter, biota, water, and air. Pollutants introduced into soil are subject to physical, chemical, and biological transformations. Consequently, pollutants introduced to soil in low amounts may not have an immediate deleterious effect. Over time, such pollutants will accumulate and when a specific concentration is reached, harmful effects can occur. This knowledge can be used to properly manage cropland application of treated effluents and treated sludge, so that the accumulation of chemical pollutants in the soil does not reach levels that harm exposed individuals or the environment. Under this scenario, agronomic benefits of wastewater and biosolids may be realized without harming soil quality, public health, and the environment. This approach entails developing maximum permissible pollutant loading limits and/or maximum permissible pollutant concentration for the soil.

The Part 503 Sludge Rule is based on the second approach. The rule prohibits land application of biosolids that exceeds pollutant limits termed *ceiling concentrations* in the rule for ten metals, and places restrictions on the land application of biosolids that exceeds additional pollutant limits specified in the rule (pollutant concentrations, cumulative pollutant loading rates (CPLRs), or annual pollutant loading rates (APLRs). The different types of pollutant limits included in Part 503 are discussed below and summarized in Table 1.9 (1). All biosolids applied to land must meet Part 503 ceiling concentration limits for the ten regulated pollutants. Ceiling concentration limits are the maximum allowable concentration of a pollutant in biosolids to be land applied. If the ceiling concentration limit for any one of the regulated pollutants is exceeded, the biosolids can not be land applied. The ceiling concentration limits were developed to prevent the land application of biosolids containing high concentrations of pollutants.

Table 1.9
Pollutant limits Part 503 Sludge Rule (1)

Pollutant	Ceiling concentration limits for all sewage sludge applied to land (mg/kg)	Pollutant concentration limits for EQ and PC sewage sludge (mg/kg)	Cumulative pollutant loading rate limits for CPLR sewage sludge (kg/ha)	Annual pollutant loading rate limits for APLR sewage sludge (kg/ha 365-day period)
Arsenic	75	41	41	2.0
Cadmium	85	39	39	1.9
Chromium	3,000	1,200	3,000	150
Copper	4,300	1,500	1,500	75
Lead	840	300	300	15
Mercury	57	17	17	0.85
Nickel	420	420	420	21
Selenium	100	36	100	5.0
Zink	7,500	2,800	2,800	140

Pollutant concentration limits are the most stringent pollutant limits included in Part 503 for land application. These limits help ensure that the quality of land-applied biosolids remains at least as high as the quality of biosolids at the time the Part 503 rule was developed. To derive pollutant concentration limits, the EPA assumes that the life span of a land application site is no more than 100 years and that the annual sludge application rate is less than or equal to 10 mt/ha (an agronomic rate for a typical sludge that would provide adequate available nitrogen for a number of crops). In this case, the sludge application rate at a given site will not exceed 1,000 mt/ha. The risk-based, cumulative pollutant loading rate (kg of pollutant/ha for the life span of the application site) is then uniformly distributed among 1,000 mt of sludge/ha, and a maximum permissible pollutant concentration (in kg of pollutant/ton of sludge or in mg of pollutant/kg of sludge) was calculated. This value was then compared to the 99th percentile concentration value for the pollutant from the National Biosolids Survey and the more stringent of the two was determined to be the pollutant concentration. Given these assumptions, the cumulative pollutant loading rates would not be exceeded in normal agricultural practices, and there would be little need for oversight except to assure that the sludge quality meets the criteria prior to distribution or application. In an effort to encourage the continued reduction of pollutant levels in the municipal wastewater stream, the EPA developed the concept of "exceptional quality" biosolids. Under this classification, sludges with specified low levels of pollutants, termed "pollutant concentration limits" and Class A pathogen levels, can be applied to agricultural land with a minimum of regulation and oversight. The different quality types of biosolids will be discussed below.

A cumulative pollutant loading rate (CPLR) is the maximum amount of a pollutant that can be applied to a site by all bulk biosolids applications made after July 20, 1993. CPLRs pertain only to land application of bulk biosolids, as defined in Part 503. When the maximum CPLR is reached at the application site for any one of the ten metals regulated by the Part 503 rule,

no additional biosolids subject to the CPLRs can be applied to the site. If a CPLR is reached at a site, only biosolids that meet the pollutant concentration limits could be applied to that site.

The annual pollutant loading rate (APLR) is the maximum amount of a pollutant that can be applied to a site within a 12-month period from biosolids that is sold or given away in a bag or other container for application to land. APLRs rather than CPLRs are used for biosolids sold or given away in a bag or other container for application to land because controlling cumulative applications of these types of biosolids would not be feasible.

3.2.2. Biosolids Quality and Part 503 Requirements

The Part 503 requirements that must be complied with depend on the quality of the biosolids, in terms of pollutants, pathogen levels, and vector attraction reduction control.

Exceptional quality (EQ) biosolids. Biosolids that meet the Part 503 ceiling concentration limits, pollutant concentration limits, one of the Class A pathogen reduction alternatives, and one of ft vector attraction reduction options described above can be considered "exceptional quality" (EQ) biosolids. Biosolids meeting these EQ requirements are not subject to Part 503's land application general requirements and management practices. EQ biosolids can be applied as freely as any other fertilizer or soil amendment to any type of land. While the Part 503 rule does not require EQ biosolids to be applied at the agronomic rate for nitrogen (a requirement for biosolids not meeting EQ requirements), these biosolids, like any type of fertilizer, should still be applied for good management at the agronomic rate, which supplies the nitrogen needs of the crop or vegetation grown on the site and protects ground water. To achieve EQ biosolids quality, the user or preparer of biosolids must: (a) not exceed the Part 503 ceiling concentration limits and pollutant concentration limits for regulated metals; (b) meet one of the six Part 503 Class A pathogen reduction alternatives and required bacterial monitoring; (c) meet one of the first eight Part 503 vector attraction reduction options; and (d) comply with the Part 503 frequency of monitoring and recordkeeping/reporting requirements.

"*Pollutant concentration" (PC) biosolids* meets the same low pollutant limits as EQ biosolids, but usually meets Class B rather than Class A pathogen reduction requirements. If the PC biosolids are classified as Class B pathogens, they should be land applied according to specific site restrictions discussed above to prevent exposure to the biosolids. Biosolids that meet PC criteria can be applied to all types of land, except lawns and home gardens, if these site restrictions are observed. To achieve PC biosolids quality, the biosolids must: (a) not exceed the Part 503 ceiling concentration limits and pollutant concentration limits for regulated metals; (b) meet one of three Part 503 Class B pathogen reduction alternatives and Class B site restrictions; (c) meet one of ten applicable Part 503 vector attraction reduction options; and (d) comply with the Part 503 frequency of monitoring and recordkeeping/reporting requirements.

Cumulative pollutant loading rate (CPLR) biosolids must meet more Part 503 requirements than EQ or PC biosolids. These requirements, such as tracking of cumulative metal loadings, ensure adequate protection of public health and the environment. CPLR biosolids users or preparers must: (a) not exceed the Part 503 ceiling concentration limits and cumulative pollutant loading rate (CPLR) limits for regulated metals when the biosolids is land applied in

bulk; (b) meet either Part 503 Class A or Class B pathogen reduction requirements and related requirements; (c) meet one of ten Part 503 vector attraction reduction options; and (d) comply with Part 503 frequency of monitoring and recordkeeping/reporting requirements.

Annual pollutant loading rate (APLR) biosolids, which pertain only to biosolids sold or given away in a bag or other container for application to land ("bagged" biosolids), must meet Class A pathogen reduction requirements and one of the vector attraction reduction treatment options. These provisions are required because of the high potential for human contact at sites where bagged biosolids are likely to be applied (i.e., public contact sites such as parks). APLR biosolids users or preparers must: (a) not exceed the Part 503 ceiling concentration limits and annual pollutant loading rate (APLR) limits for regulated metals when the biosolids are placed in a bag or other container, as defined in Part 503, for sale or given away for application to the land; (b) meet Part 503 Class A pathogen reduction requirements and required bacterial monitoring; (c) meet one of the first eight Part 503 vector attraction reduction options; (d) meet the Part 503 management practice that requires a label or information sheet that lists data specified in Part 503; and (e) meet the Part 503 frequency of monitoring and recordkeeping/reporting requirements.

The Part 503 labeling provision requires that the preparer of APLR biosolids provides the applier with allowable application rate information, either on a label or in a handout (usually based on the nutrient content of the biosolids). This information is based on the preparer's calculation of the annual whole sludge application rate (AWSAR) (Fig. 1.2) (1). The preparer/manufacturer should also provide the applier with information on the nutrient value of the bagged biosolids. The recommended application rate helps ensure that biosolids are applied at the appropriate agronomic rate to minimize the amount of excess nitrogen that passes below the root zone and into ground water.

While the Part 503 rule does not require it, it would also be good practice to provide information about the nitrogen content of the biosolids as well as the AWSAR on the label or information sheet that accompanies the biosolids. The Part 503 rule does, however, contain the definition of the agronomic rate for biosolids application. The agronomic rate for biosolids application is a rate that is designed to provide the amount of nitrogen needed by a crop or vegetation to attain a desired yield while minimizing the amount of nitrogen that will pass below the root zone of the crop or vegetation to the ground water. Crop-available nitrogen in biosolids that are applied in excess of the agronomic rate could result in nitrate contamination of the ground water. The Part 503 rule requires that the rate of land application for bulk biosolids be equal to or less than the agronomic rate, except in the case of a reclamation site where a different rate of application is allowed by the permitting authority. Procedures for the design of the agronomic rate differ depending on such factors as the total and available nitrogen content of the biosolids, nitrogen losses, nitrogen from sources other than biosolids (including estimates or measurements of available nitrogen already present in the soil), and the requirements for the expected yield of crop or vegetation. A sample calculation of the nitrogen supplied by biosolids based on the AWSAR is provided in Fig. 1.3 (1). Earlier in Fig. 1.2 (1), the AWSAR for the biosolids in the example calculation were determined to be 410 lb of biosolids per 1,000 sq. ft of land, assuming that biosolids need to be placed on a lawn that has a nitrogen requirement of about 200 lb of available nitrogen per acre per year. Figure 1.3 (1)

STEP 1. Analyze a sample of the biosolids to determine the concentration of each of the 10 regulated metals in the biosolids.

STEP 2. Using the pollutant concentrations from Step 1 and the APLRs from Table 10, calculate an AWSAR for each pollutant using equation below:

$$\boldsymbol{AWSAR = APLR/C \times 0.001}$$

where ***AWSAR*** = Annual whole sludge (biosolids) application rate (dry metric tons of biosolids/hectare/year) ***APLR*** = Annual pollutant loading rate (kg of pollutant/ha/yr) ***C*** = Pollutant concentration (mg of pollutant/kg of biosolids, dry weight) 0.001 = A conversion factor.

STEP 3. The AWSAR for the biosolids is the lowest AWSAR calculated for each pollutant in Step 2.

EXAMPLE:

1. Biosolids to be applied to land are analyzed for each of the 10 metals regulated in Part 503. Analysis of the biosolids indicates the pollutant concentration in the second column of the table below.
2. Using these test results and the APLR for each pollutant from Table 10, the AWSAR for all the pollutants are calculated as shown in the third column of the table below.

Metal	Biosolids Concentrations (mg per kg)	AWSAR (metric tons per hectare)
Arsenic	10	$2/(10 \times 0.001) = 200$
Cadmium	10	$1.9/(10 \times 0.001) = 190$
Chromium	1,000	$150/(1{,}000 \times 0.001) = 150$
Copper	3,750	$75/(3{,}750 \times 0.001) = 20$
Lead	150	$15/(150 \times 0.001) = 100$
Mercury	2	$0.85/(2 \times 0.001) = 425$
Nickel	100	$21/(100 \times 0.001) = 210$
Selenium	15	$5/(15 \times 0.001) = 333$
Zinc	2,000	$140/(2{,}000 \times 0.001) = 70$

3. The AWSAR for the biosolids is the lowest AWSAR calculated for all 10 metals. In our example, the lowest AWSAR is for copper at 20 metric tons of biosolids/hectare/year. Therefore, the controlling AWSAR to be used for the biosolids is 20 metric tons per hectare/year. The 20 metric tons of biosolids/hectare is the same as 410 pounds of biosolids/1,000 square feet (20 metric tons × 2,2051 *b* per metric ton/107,600 square feet per hectare). The AWSAR on the label or information sheet would have to be equal to or less than 410 pounds per 1,000 square feet.

Fig. 1.2. Procedure to determine the annual whole sludge (biosolid) application rate for biosolids sold or given in a bag or other container (1).

STEP 1. The nitrogen content of the biosolids indicated on the label is 1 percent total nitrogen and 0.4 percent available nitrogen the first year.

STEP 2. The AWSAR is 410 pounds of biosolids per 1,000 square feet, which is 17,860 pounds of biosolids per acre:

$$\frac{410\,lb}{1{,}000\,sqft} \times \frac{43{,}560\,sqft}{acre} \times 0.001 = \frac{17{,}860\,lb}{acre}$$

STEP 3. The available nitrogen from the biosolids is 71 pounds per acre:

$$\frac{17{,}860\,lb}{acre} \times 0.04 = \frac{71\,lb}{acre}$$

Conclusion.

Since the biosolids application will only provide 71 pounds of the total 200 pounds of nitrogen required, in this case the AWSAR for the biosolids will not cause the agronomic rate for nitrogen to be exceeded and an additional 129 pounds per acre of nitrogen would be needed from some other source to supply the total nitrogen requirement of the lawn.

Fig. 1.3. Procedure for applier to determine the amount of nitrogen provided by AWSAR relative to the agronomic rate (1).

shows calculations that can be useful for determining how much nitrogen is being applied to land relative to the AWSAR and the nitrogen requirements of the plants being grown.

3.2.3. Chemical Pollutant Standards for Agricultural Use of Biosolids in Russia and European Countries

Biosolids used in agricultural operations were not included in Council Directive 75/422/EEC (1975) relating to waste, but are affected by the measures provided for in Council Directive 78/319/EEC (1978) relating to toxic and hazardous waste, since biosolids may contain or be polluted by matter or substances, which represent a risk to human health or to the environment. According to this document's provisions, the management of biosolids or activities leading to its recovery must ensure that the final destination involves no danger at all while at the same time, requiring such waste and its destination to be registered in order to facilitate information collection and monitoring by the authorities.

According to Directives 75/440/EEC and 80/68/EEC, biosolids must be used under conditions that guarantee the protection of soil and surface and ground water. This regulation was necessary to ensure that the latter would be protected against the harmful effects of uncontrolled biosolids use. While biosolids exhibit certain useful agronomical properties, their application must not harm soil quality and plant production, since certain heavy metals are poisonous to plants and people. This consideration has led to establishing limits with respect to biosolids' content in soil and the type of biosolids used.

Council Directive 86/278/EEC (1986), was developed with the purpose of regulating the use of biosolids in agriculture in order to prevent noxious effects in plants, soils, animals, and human beings as well as promoting its correct use. According to this directive, biosolids must

Table 1.10
Standards for chemical pollutants in sewage sludge and soil (3,10,13,16,23 – 41)

Pollutant	EEC limit values of heavy metals concentrations in sewage sludge (mg kg^{-1} dry weight)		EEC limit values of heavy metals concentrations in soil (mg kg^{-1} dry weight)		EEC limit values for annual amounts of heavy metals (kg ha^{-1} $year^{-1}$)	Limit values of heavy metals concentrations in sewage sludge for Russia (mg kg^{-1} dry weight)	Concentration limits for different European countries (mg kg^{-1} dry weight)
	Soil with pH < 7	Soil with pH > 7	Soil with pH < 7	Soil with pH > 7			
Arsenic			–	–	–	20	10–100
Mercury	16	25	1	1.5	0.10	15	6–10
Lead	750	1,200	50	300.0	15.00	1,000	300–900
Cadmium	20	40	1	3.0	0.15	30	8–15
Nickel	300	400	30	112.0	3.00	400	26–500
Chromium	1,000	1,500	100	150.0	3.00	1,200	40–1,000
Manganese	–	–	–	–	–	2,000	500
Zinc	2,500	4,000	150	450.0	30.00	4,000	2,000–10,000
Copper	1,000	1,750	50	210.0	12.00	1,500	300–3,000
Molybdenum	–	–	–	–	–	–	Not available
Selenium	–	–	–	–	–	–	–

be treated prior to agricultural use with the exception of certain conditions; member states, for example, may authorize the burial or injection of other types of biosolids into soil, provided that no risk at all is involved. The directive's appendices outline certain limit values for heavy metals:

- Limit values for heavy metal concentration in soils: treated biosolids may not be applied to soils displaying a heavy metal concentration higher than that laid down.
- Limit values for heavy metal concentration in biosolids earmarked for agricultural use: treated biosolids for application to soil shall not exceed the limit values as laid down in its heavy metal content.
- Limit values for annual amounts of heavy metal which may be introduced into soils, based on a 10-year average: the maximum amounts of biosolids which may be applied per ha in year will be those which do not exceed the limit values set in accordance with the heavy metal content of the soil and biosolids to be used.

These limit values presented in the directive as well as the national limit values of Russia and several European countries are summarized in Table 1.10 (3, 10, 13, 16, 23, 40). In addition, biosolids and soil sampling and analysis reference methods are included, and the frequency of such analysis and of the specific parameters to be determined in them is mentioned in each case.

4. SLUDGE TREATMENT PROCESSES

Primary and secondary sludges may be expected to contain settleable materials from raw wastewater and the products of microbial synthesis. Other materials are also removed from wastewaters and incorporated into primary and secondary sludges. The large surface area of

particles incorporated into sludges provides sites for adsorption of constituents from the liquid phase. Nondegraded organic compounds in solution may partition into the organic fraction of the particles. Bioflocculation may also incorporate colloidal particles that otherwise would not be removed by sedimentation into settleable particles. These and other mechanisms result in selective enrichment of wastewater constituents in sludge. Additionally, wastewater sludges consist mostly of water and hence, wastewater constituents remaining in the liquid phase also are included in sludges.

Because primary and secondary sludges have different properties, it is sometimes advantageous to treat them separately. To illustrate, secondary sludge thickens better using the dissolved air flotation process than by gravity thickening, and it is sometimes thickened separately from primary sludge. However, the two sludges almost invariably are combined prior to the end of the treatment. A wide variety of sludge treatment processes are used to reduce sludge volume and alter sludge properties prior to disposal or use of the treated product (41–44). Hereafter, the discussion will be focused on biological methods of biosolids treatment.

4.1. Volume Reduction Processes

Biological (secondary) sludge, as produced from secondary wastewater treatment processes, often has a suspended solids content of less than 1% by weight. Primary sludges are more concentrated, but marginally; typical combined primary and secondary sludge might contain about 3% solids by weight. Because of the voluminous nature of sludges, processes categorized here as "thickening," "dewatering," "conditioning," and "drying" are common in sludge management. The removal of water from sludges improves the efficiency of subsequent treatment processes, reduces storage volume, and decreases transportation costs.

4.1.1. Thickening

Sludge thickening produces a concentrated product that essentially retains the properties of a liquid. Gravity thickening, or concentration by simple sedimentation, is the thickening process most commonly applied to municipal sludges. The product of gravity sludge thickening often contains 5–6% solid material by weight. Alternatives to gravity thickening include flotation thickening (in which a gas is incorporated with sludge solids, causing them to float), as well as the use of gravity drainage belts, perforated rotating drums, and centrifuges.

4.1.2. Dewatering

Sludge dewatering processes produce material with the properties of a solid, even though the dewatered sludge is still mostly water. Dewatered sludge can be transported in a dump truck, whereas a tank truck is required to transport thickened sludge. Dewatering may be accomplished on sand drying beds and, occasionally in lagoons, where gravity drainage and evaporation removes moisture. More often, larger municipal installations use mechanical means for dewatering sludge. Mechanical sludge dewatering equipment includes filter presses, belt filter presses, vacuum filters, and centrifuges. The solids content of mechanically dewatered sludge typically ranges from 20 to 45% solids by weight; most processes produce concentrations of solids at the lower end of that range (45).

4.1.3. Conditioning

Sludge conditioning processes do not reduce the water content of sludge. Conditioning alters the physical properties of sludge solids to facilitate the release of water in dewatering processes. Indeed, the mechanical dewatering techniques discussed in the previous paragraph would not be economical without prior sludge conditioning. Chemical and physical techniques are used to condition sludge. Chemical conditioning most commonly involves adding synthetic organic polyelectrolytes (or "polymers") to sludge prior to dewatering. Inorganic chemicals (most commonly, ferric chloride and lime) may also be used. Inorganic chemical conditioning dosages are large, and increase the mass of the solid phase of sludge. Physical conditioning techniques include heat treatment and freeze–thaw treatment.

4.1.4. Drying

If circumstances justify removal of water beyond that achievable by dewatering processes, drying is needed. Thermal drying with direct or indirect dryers is used to achieve near-complete removal of water from sludges. Solar drying is feasible in some locations. Partial drying also results from the heat produced in biochemical reactions during composting and from other chemical reactions described in the stabilization processes below.

4.2. Stabilization Processes

The purpose of sludge stabilization is to minimize subsequent complications due to biodegradation of organic compounds. Stabilization is usually accomplished by biological or chemical treatment processes.

In biological stabilization processes, the organic content of sludges is reduced by biological degradation in controlled, engineered processes. Most commonly, domestic wastewater sludge is biologically stabilized as a liquid in anaerobic digesters from which methane gas is a byproduct. Liquid sludge can also be biologically stabilized in aerobic digesters to which oxygen (or air) must be added. Composting is a process that biologically stabilizes dewatered sludge. Since it is ordinarily an aerobic process, an amendment such as wood chips or sawdust must be added to improve friability and thereby promote aeration. Composting takes place at thermophilic temperatures (often, about 55°C) because of heat released by biochemical transformations. Aerobic digesters can be made to operate thermophilically using heat from the same source. Anaerobic digesters can operate at thermophilic temperatures by burning methane produced from the process, but they typically operate at mesophilic temperatures (at about 35°C). During each of these processes, a reduction of number of indicator and pathogenic agents takes place (45).

4.2.1. Aerobic Digestion

Aerobic digestion refers to a biological transformation of organic solids in wastewater sludge to an innocuous end product. The process is conducted by agitating biosolids with air or oxygen to maintain aerobic conditions at residence times, depending on the type of sludge and temperature. During aerobic digestion, biosolids are aerated in open or covered tanks. The quantity of oxygen required to oxidize the biomass and ammonia is approximately two parts

of oxygen per one part of biomass. The destruction of biodegradable components of solids results in a reduction in the volume of waste solids that requires disposal of at least 38%.

The main objective of any type of digestion is the destruction of volatile solids that result in a reduction of volume of solids intended for disposal. Bacteria, fungi, algae, protozoa, viruses, and others represent the living organisms of biosolids. The total viable aerobic heterotrophic bacterial population in raw sewage, secondary and tertiary treated effluents exceeds 10^7 organisms/mL. Many are able to survive and proliferate during the treatment procedure. The biodegradation of organic solids, along with endogenous degradation of the biomass in the biosolids, takes place during the process. The volatile fraction of the biodegradable solids is between 44 and 67%. During aerobic digestion, soluble substrates are first completely oxidized by the microbial community in the activated sludge process. Cell material are then consumed by the bacteria for their maintain. Approximately 75–80% of biomass is oxidized. This endogenous oxidation of biomass results in the volume reduction of solids requiring disposal (44).

Temperature and sludge type influence the successful application of digestion of biosolids. Increasing the temperature subsequently increases the biochemical activity of bacterial population, along with specific oxygen utilization. Specific oxygen utilization is also dependent upon sludge age; older biosolids require less oxygen for volatile organic fraction oxidation.

Aerobic digestion is typically conducted in concrete or steel tanks. The latter is less expensive, but requires insulation. Detention times vary from 6 to 8 days, and about 60% of volatile solids are destructed. The minimum aeration time for excess activated sludge is 10–15 days. Reactors are supplied with a mixing and aeration system. The air provides oxygen required to maintain an aerobic environment; oxygen requirements depend on temperature and range 1.45 parts at temperature higher 45°C to 2 parts at mesophilic condition per 1 part of oxidizing volatile solids. A minimum value of 1.0 mg of oxygen per liter should be maintained in the digester during all time (46). Mixing suspends the biosolids and draws liquid to the aeration device. Surface foam is controlled with foam cutters. The maximum loading recommended for aerobically treated biosolids is 1.6 kg of total solids/m^3.

Aerobically digested biosolids require dewatering, usually by a vacuum filter, a cake solid concentration with $FeCl_3$, and a lime. Supernatant from aerobic digester is returned to the head of the treatment plant.

The use of aerobic digestion is limited by the high energy costs needed for aeration and mixing. Thus, the method is best used by small treatment facilities. It should be noted that aerobically digested biosolids are difficult to dewater, and that indicator microorganisms are present in the final digested sludge. Because of this, aerobic digestion is often used as pretreatment to anaerobic digestion, especially in thermophilic conditions.

4.2.1.1. Aerobic Pretreatment

The aerobic thermophilic waste sewage sludge treatment process is used as a pretreatment prior to anaerobic mesophilic digestion when hygienic quality is required. Many of the bacteria mediated in the aerobic biosolids treatment are obligate aerobes that are able to grow under limited oxygen conditions. Also, facultative anaerobic bacteria that are able to exhibit aerobic metabolism in the presence of oxygen and fermentative metabolism under

oxygen limitation are involved in that process. Two major processes occur during aerobic pretreatment: (a) death and lysis of organisms due to high temperature and heat-stable protease produced by thermophilic bacteria, and (b) "cryptic growth" of cells using lysis product (47). Both lysis and extracellular metabolic products supplement the pool of soluble nutrients for microorganisms. Aerobic conditions enable the biodegradation of compounds that are dependent on the presence of oxygen. A restricted oxygen supply results in considerable carboxylic acids production that leads to accumulation of dissolved organic compounds (DOC) in culture supernatant (48).

The combination of aerobic thermophilic biotreatment process and anaerobic mesophilic digestion could result in a higher quality of treated biosolids and accelerated waste sewage sludge stabilization.

4.2.2. Anaerobic Digestion

This process is conducted in the absence of air at residence times ranging from 15 days at 35 to 55°C to 60 days at 20°C, with a volatile solids reduction of at least 38%, and the formation of innocuous and easily dewatered substances. Anaerobic digestion is cost effective for large treatment plants.

Anaerobic digestion involves a net of biochemical reactions that leads to conversion of a portion of the organic matter in the biosolids to methane and dioxide. A consortium of acid-forming and methane-producing microorganisms conducts the process in the absence of oxygen. There are several sequential steps in this process. First, facultative heterotrophic organisms hydrolyze volatile organic solids to more simple water-soluble organic compounds. The facultative bacteria are presented by a variety of microbial genera. They can use oxygen dissolved in feed sludge introduced into the anaerobic digestion system for metabolic processes, and therefore protect the strictly anaerobic methane-forming bacteria from oxygen impact. Produced by heterotrophic bacteria, soluble organic substances are fermented by acid-producing facultative bacteria to volatile acids, carbon dioxide, and hydrogen gas. These bacteria belong to different bacterial groups, and they are tolerant of changes in pH and temperature. The primary produced acids are acetic, propionic, and butyric. Obligate anaerobic methane-forming bacteria convert these acids to methane and carbon dioxide gases. Several different species of methane-forming bacteria are necessary for the anaerobic stabilization of the organic matter of biosolids because each one can ferment a restricted group of simple compounds to methane. They are *Methanobacterium formicum*, *M. propionicum*, *M. sohgeni*, *M. omelianskii*, *M. mazei*, *M. vannielii*, *M. barkerii*, *M. methanica* (49–51).

Acid fermentation and methane formation are synchronous processes. The limiting factor in anaerobic digestion is a rate of conversion of volatile acids to methane. The methane-forming bacteria are very sensitive to changes in pH and temperature. The pH of digested mixture may decrease if volatile acids accumulate. If pH decreases below 6.0, this will inhibit methane-forming bacteria and organic acids will continue to accumulate. Therefore, the maintenance of pH balance is required.

It is essential for methane-forming bacteria to maintain a constant operating temperature as much as possible. Two temperature zones are favorable for these bacteria: the mesophilic range (between 30 and 35°C) and thermophilic range (50–60°C). The anaerobic digestion

of biosolids can be successfully operated at 20°C also but a longer duration is required. Thermophilic digestion yields a greater destruction of pathogenic organisms when compared with the mesophilic condition.

The generation time of methane-forming bacteria ranges from 2 days to more than 20 days at 35°C. This characteristic defines the detention time for anaerobic digestion of biosolids as 15–20 days. The choice of detention time is dependent on the final disposition of the digested biosolids: for land application or incineration. The hydraulic detention time of 10 days may be sufficient, but 15 days is preferable for the stabilization of biosolids.

Detention time is closely associated with volatile solids loading. The concentration of solids in the feed sludge defines the solids loading possible at the required hydraulic detention time. To achieve the needed loading, the biological sludge would have to be thickened. Concentration of solids in the feed sludge for anaerobic digestion should be between 3.2 and 7.2 kg of volatile solids per m^3 per day (49–51).

Anaerobically digested sludge must be separated and concentrated from the liquid phase. This can be done by gravity separation in a second anaerobic digester without mixing and heating. The supernatant requires treatment before disposal.

Different filters, centrifuges, and belt presses are used for digested sludge dewatering. Chemical stabilization reduces and prevents regrowth of microorganisms including pathogenic and odor-producing types. This stabilization is effectively performed with such chemicals as chlorine and lime. While chlorine is rarely used, a widely used lime is one of the lowest cost alkalis available for the wastewater industry. For stabilization purposes, sufficient lime may be added to attain and maintain a pH of 12 for 2 h.

Anaerobic digestion tanks may be either cylindrical or egg-shaped (44). External pumped recirculation, recirculation of compressed digester gas or mechanical mixing are used for mixing. Minimum power requirements for pumped circulation are 0.005–0.008 kW/m^3 or higher. Feed sludge should not be concentrated to more than 8% total solids. Daily laboratory control on volatile acids, pH, and carbon dioxide is necessary for efficient digestion performance.

The proper operation of anaerobic digestion is monitored by the ratio of carbon dioxide and methane formation. An increase of the concentration of volatile acids in digested sludge or carbon dioxide content, along with a decrease in the methane content, indicates an imbalance of the process. Reasons for this imbalance may include a sudden change in temperature, organic loading, or composition of sludge. These factors can be balanced by allowing additional time for the microbial population to adjust to the new environment and by temporarily stopping feeding of the digester. The introduction of toxic materials to the anaerobic system, as well as an extreme change in pH, can cause prolonged or even permanent imbalance of the process. In these cases, a new anaerobic digester should be started up. Substances that are toxic to the methane-forming bacteria must be removed from the feeding sludge. Among the substances able to inhibit the anaerobic process are ammonia (NH_3) in excess of 1,23 mg/L and sulfides in excess 200 mg/L (44). Gas yield from anaerobic digestion of sewage sludge could be severely inhibited by heavy metals. The degree of toxicity of metals for the methane-forming process has been observed to be in the order of Cr > Ni > Cu > Zn (50).

Anaerobic digestion has several advantages. The produced digested gas contains 60–75% methane, a usable energy source. This amount of produced energy is sufficient for maintaining the required temperature for the process, and for driving the pumps at the treatment facility. The destruction of 25–45% of influent solids results in a reduction of the mass and volume of wastes, inactivation of pathogens and parasites, nutrient presence in final product allowed to use digested solids for improving of fertility and texture of soil.

A traditional treatment method of municipal sewage solid waste and of sewage sludge is anaerobic mesophilic digestion. This process fosters the protection of the environment and energy recovery. Methane generated from this process is used to supplement electricity for sewage-treatment plants. Anaerobic digestion is successfully employed in many countries.

An excellent alternative to dumping, incinerating of household waste, simple fermentation process, and composting is the simultaneous digestion of hydrolyzed mixture of sewage sludge and organic fraction of municipal solids waste (51).

It is well known that anaerobic digester performances are very sensitive to the quality of the feed (52, 53). To enhance the solubilization of organic polymers in a mixture of primary and secondary sludge and municipal solid waste, a high temperature-alkaline pretreatment followed by bacterial hydrolysis was tested (54). Hydrolysis allowed an improved availability of organic substances, but the methane content of biogas was less (49% vs. 60%) when compared to the conventional process. On the other hand, hydrolysis of the feed leads to very low total and volatile solids concentrations in the digester, which could allow for the conversion of mesophilic reactors digestion into thermophilic ones.

Anaerobic digestion has some disadvantages. Capital costs are high for large covered tanks with pumps for raw sludge introduction and circulation, pumps for mixing, and heat exchangers. Additionally, the supernatant from anaerobic digestion, which contains suspended solids, nitrogen, phosphorous, requires additional treatment.

4.2.3. Composting

Compost is the end product of thermophilic biological biodegradation of organic wastes to stable innocuous humus-like substance. Aeration is an important parameter for composting biosolids. Oxygen is needed for the biological aerobic degradation of organic solids, for the removal of heat and excess moisture from the compost mix.

There are three types of aerobic composting: static piles, windrow piles, and in-vessel composting. These types differ in oxygen supply method. In the compost soil pile (static pile) method, the mixture is placed over a perforated pipe, through which air is pumped for oxygen and temperature maintain. In the windrow method, the mixture is stacked in long piles (1.5–2 m in high to 2–3 m in width), aeration of which is provided by periodically turning the piles by a composter. The in-vessel process enclosed systems of different structure (bioreactor) are used for the composting of biosolids and bulk mixture under controlled conditions. The three methods differ in capacity, cost efficiency, and predictability (55).

All composting processes include the following major elements: initial mixture preparation (a mixing of biosolids with bulking agents and amendments), active composting, and curing of the product (Fig. 1.4) (9).

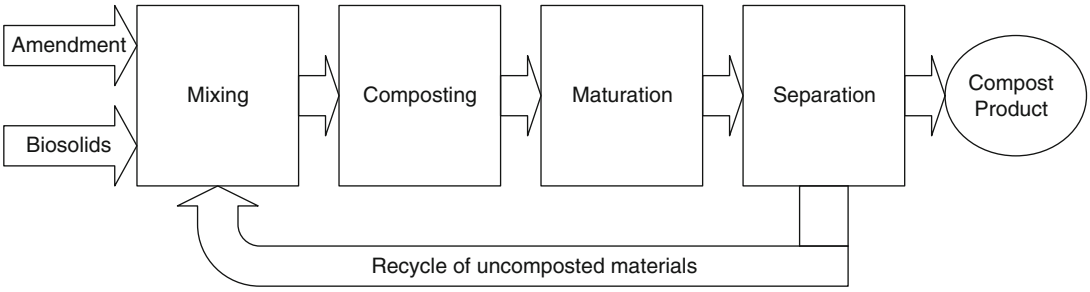

Fig. 1.4. A Scheme of composting process (9).

4.2.3.1. MIXTURE CONSIDERATION

Bulking agent and amendments. Composting is initiated by mixing of biodegradable organic matter of biosolids with bulking agents. Bulking materials increase the total solids concentration and void space porosity; improve the structure of initial mix; and increase the porosity of the composting mixture. They may provide additional biodegradable carbon for microorganisms to accelerate the starting process of microbial development. Bulking agents also can enhance product quality.

Traditional bulking agents include wood chips, sawdust, wood ash, and grains hulls. Nowadays, organic waste, processed agricultural waste, shredded yard wastes are used also as bulking amendments. Because these agents are biodegradable and easily lost in the composting process, there is a need for these to be recoverable and low in cost. Shredded tires are one such example. The smallest size rubber chip (1.27–2.54 cm), 2:1 ratio, and sawdust amendment are optimum for efficient composting (55). The type of bulking amendment chosen should depend on the solids characteristic, type of composting technology used and desired product quality.

Special processing is required to achieve a uniform desirable size of some amendments (yard wastes, tires). Hummer mills or shear shredders may be used for this processing. Reducing the particle size increases the surface area where decomposition of organic material occurs, but also reduces the pore zone, which defines the air movement in the compost piles. Fine-grained amendments absorb more moisture than coarse ones. An optimum range of particle sizes for aerated piles is 12.5–50 mm (56). Bulking materials can be recovered from the final compost by screening and reuse. Screening permits the production of a finer compost because of enhanced uniformity.

Properly sized amendments should be mixed with homogenized biosolids. The ratio of mixing components is determined by their porosity, available energy, C:N ratio, and moisture content. Dry and wet weights of biosolids and amendments must be known in order to calculate the quantity of the constituents of the mix (55). Dry solids percentages for good composting vary from 40 to 45%.

Carbon-to-nitrogen ratio. Carbon and nitrogen are the principal nutrients that affect decomposition of organic matter by microorganisms. A biodegradable carbon-to-nitrogen ratio of 20–40 should be optimum. A lesser ratio can result in ammonia being released from piles; a higher ratio results in a slowing of biochemical degradation of organic compounds and

therefore reduction of composting temperature (57). Solids in municipal wastewater treatment have a low C:N ratio and demand a high carbon amendment to reduce ammonia discharge.

Moisture. Moisture is another important factor for a successful composting process. It affects microbial activity in two ways. It was shown that microbial activity begins to decrease at a moisture content of about 40%. Moisture content exceeding 60% restrict oxygen since the pore space be blocked by water in the compost mass, and this inhibits aerobic microbial activity. So, moisture could be a limiting factor in microbial decomposition of organic matter of biosolids.

4.2.3.2. Chemical and Biological Characteristic of Composting Biosolids

The final objective of composting is a biochemical transformation of the raw organic fraction of the waste into a humus-like material. The biochemical conversion is a result of the heterogeneous microbial consortium of the composting mix – the true decomposers of organic matter of biosolids.

The organic matter of biosolids are presented by high-molecule weight compounds such as polysaccharides, fats and proteins, and small molecules such as sugars, amino acids and other simple substances, and humic substances (58). The constituents vary considerably depending on their source. Thus, biosolids of food wastes contain lower amounts of lignin or high-molecule cellulose than woody or straw, but more proteins and fats.

It is important to know the composition of the materials to be used for the design of a composting system. Combining materials high in nitrogen content (protein) with cellulose (carbon source) makes the C/N ratio more favorable for a microbial population and also reduces the potential for odors. Combining food waste with cellulose reduces the composting time of the latter through increased microbial activity due to a quick breakdown of carbohydrates in food wastes, and helps maintain the necessary microbial population in the mixture. In mature compost, most of the sugars, proteins, simple sugars, and amino acids have been metabolized by microorganisms as a source of C and N.

A wide range of microorganisms are involved in the degradation of complex organic matter of biosolids during composting. The microbial population can reach levels of 10^9 per g to 10^{10} per g of compost. The most important factors affecting the microbial population of this system include oxygen, moisture, temperature, nutrients and pH. Knowing what type of microorganisms are able to utilize specific compounds, both natural and unnatural, is very helpful for the acceleration of the composting process.

Microorganisms that participate in composting produce heat that affects the microbial population, including human pathogens (58). Destruction of pathogens is one of the main goals of composting, and it occurs at temperatures 55°C and higher. Microorganisms may be categorized as mesophiles or thermophiles, depending on the temperatures. Mesophiles are able to function at 25–45°C, thermophiles at temperature more than 45°C. A major fraction of the bacteria comprising the thermophilic hygienization process has been characterized as *Bacillus* spp., which are known to produce heat-stable extracellular proteases.

Increased temperatures accelerate the growth of organisms. Many microorganisms from different genera survive and grow at very high temperatures, up to 60–80°C (59). It has been

demonstrated that at 50°C, thermophilic fungi, bacteria, and actinomycetes were active in compost; at 65°C, bacteria and actinomycetes predominated (60). During composting, the number of aerobic mesophilic bacteria was reduced at high temperatures (around 60°C); while a decreasing of temperature afterward led to recovery of bacteria.

The microbial population fluctuates throughout the composting process. During the initial phase of composting, at the availability of such substances as sugars, alcohols, acids and proteins bacteria dominate as the principal microbes in the compost. Turning the compost mixture results in an increase in bacteria. Towards the end of the composting process, the bacterial count becomes reduced. Temperature of the compost mixture center has been observed to increase to 40–55°C within few days, and then decrease to 20–30°C. Turning of the mixture causes a rise in temperature to approximately 35–45°C, followed by a decrease. In summary, aerobic composting has three temperature phases that affect microbial population. The mesophilic phase (up to 45°C) is followed by a thermophilic phase (up to 70°C). The third phase is a return of the mesophilic (lower 45°C). Maximum growth of mesophilic bacteria occurs at the final mesophilic phase (61).

Fungi also participate in the composting decomposition process. They are able to feed on cellulose materials, which perform a large portion of composting biosolids. This group of microorganisms predominates at low-moisture environment, has less need for nitrogen.

At mesophilic conditions, different macroinvertebrates such as rotifers, nematodes, and earthworms also play a role in the composting process. Through their movement and feeding, they promote the physical breakdown of composting solids, and thereby increase the surface for microbial activities.

4.2.3.3. Aerated Static-Pile Composting

Compost soil piles are effective for above-ground application to high-strength waste streams requiring more controlled environments. It permits the management of a wide range of biosolids with regard to quantity and quality; it is economically efficient for a wide range of facility capacities, provides a high degree of pathogen destruction and gives good product stabilization. On other hand, this method has a greater land requirement than the in-vessel system, potential odor problems and is affected by climatic variability.

The bulking agents using in these technology are wood chips and shredded yard wastes. After mixing, the solids are laid over a network of pipes connected to aeration blowers. Aeration provided by forcing air through the pipes can be done on positive mode (upward through the compost pile) or negative mode (drawing air downward through the compost pile). An insulating layer of finished compost is often used to cover the pile. Aerated static-pile composting is usually practiced over a period of 14–28 days.

Aeration rate is controlled by temperature. It has been shown that the range of optimal temperature for composting process is broad, from 35 to 65°C. A high temperature in the composting process is effective for killing pathogenic microorganisms in biosolids, water evaporation from the composting mix, and for the acceleration of biosolids' organic degradation (62). Shifting the anaerobic treatment system from mesophilic (37°C) to thermophilic (55°C) conditions can also improve the efficiency of the digestion. Temperature is the most important parameter affecting the number and types of microorganisms in composting mix.

After the active composting, the compost pile is removed and screened. The total solids content suitable for screening is 55%. If drying is insufficient, an additional drying stage is necessary. A drying may be accomplished by agitating the material, or enhanced by forced ventilation. Compost is then cured before distribution. The curing provides additional time for compost stabilization. As a general rule, aerated curing piles is used to prevent odor generation formed at anaerobic condition.

4.2.3.4. Windrow Composting

A common process used in composting biosolids is the windrow system. This process, which is the least complex, involves mixing biosolids with a bulking material, placing the mixture in long raws (windrows), composting for several weeks with periodically turning the mass using mobile equipment such as a composting machine or front-end loader. An open windrow system is usually used for digested biosolids but is not suitable for raw ones because of the nuisance odors produced (63).

Biosolids and amendments must be mixed thoroughly. The height and base width of the windrow may vary from 0.9 to 2.1 m and from 3.7 to 7.0 m, respectively. The length of the raw depends on the daily biosolids quantity and quality. It should be turned at least three times per week (55). Turning reduces particle size, mixes and homogenizes composting material, maintains aerobic condition in windrow system, and promotes drying.

If the windrow is properly constructed and maintained, internal temperatures should reach 55°C within a few weeks of the composting start and stay above this level during the cycle. A windrow typically requires 30–50 days to complete the composting. After completion of the composting, the windrow is broken down using a front-end loader, and composted material is hauled away for storing or further processing. When the total solids level of the compost material reaches 60%, it is considered dry and stable enough to be used by a fertilizer company (43).

4.2.3.5. In-Vessel Composting

This method has smaller space requirements, offers a more stable and consistent product, controls odor release better, and is performed under controlled conditions (temperature, oxygen content, air flow) than the above composting piles system. On other hand, in-vessel composting system is more mechanically intensive and requires greater labor for maintenance.

The in-vessel composting process is accomplished inside enclosed containers. Two types of in-vessel composting system are known: plug-flow and dynamic. The first is a horizontal or vertical system based on a totally enclosed bin equipped with a hydraulically operated ram that pushes composted mixture through the unit without mixing. A large-diameter rotating drum works in the dynamic type by agitating the composting materials in bin reactor. Air is forced through the bed. Plug flow systems are relatively more compact and provide superior odor control. Dynamic systems offer greater control flexibility and the opportunity to use different bulking agents.

The type of amendments used in in-vessel composting can be the same as used in other system, but sawdust and recycled compost are used in practice, especially for the plug-flow system. As a rule, the amendments are not recovered in the in-vessel system.

In-vessel composting systems have a separate curing and storage step before product use. Curing can be organized in aerated static-pile or windrow pile arrangements. Detention time of this step requires 3–4 weeks before product use.

4.2.3.6. ODORS

Any composting procedure releases odor emissions (64). The anaerobic processes of decomposition produce sulfuric compounds having intense odors. During the aerobic process, odorous substances as alcohols, ketones, esters, organic acids with a low boiling point are also formed. Many of the compounds have odor thresholds in the parts per billion-concentration range. Windrows are considered the main source of odors in composting plants. There are several points of odors emission: the dumping storage and assorting of delivered material (raw rubbish odor), rotting of composting mix (most important source of emission of odor intensive gases). Odor gases easily set free at the high temperatures of the windrow, and they can be transported with evaporated water. Leaching water may contribute to a certain degree to the total emission of the odors. Odor generation results from the high moisture of the composting mix that creates anaerobic conditions and resultant odors.

Odorous emission can be prevented, reduced, collected and treated, or modified by masking with chemicals. Masking is just a modification of the odor by using chemicals with a different odor so that the resulting odor becomes less objectionable. Since this technique does not remove or reduce the odorous substances, it is the least preferred method of sludge odor control. Odors can be reduced or prevented through improved operations of treatment and by keeping the sludge treatment system clean. Odor emissions can be considerably reduced through good rotting process prevention of anaerobic digestion of organic matter. The type of bulking agent also has a substantial effect on odor emission.

Collection and treatment of odors with wet scrubbers, chemical absorbers, and soil or compost filters (biofilters) could absorb most of the exhaust air. In scrubbers, the odorous gas is passed through liquid absorbent, where the substances can dissolve or react chemically with the absorbent. The liquid phase can be water or aqueous solution of oxidizing or reducing agents. The most commonly used oxidants are chlorine, hydrogen peroxide, potassium permanganate, lime, soda, ash, and ozone. The spent liquid absorbent requires treatment.

A well-proven technique for removing odorous substances is a “dry process” – the use of activated carbon, activated alumina, silica gel, alumosilicate, or other adsorbing media. The adsorbent used should have a high surface area per unit of volume. The capacity of adsorption material dependent on pressure and temperature (positive and negative, respectively), is higher for high molecular weight substances. Adsorbents can provide chemical oxidation of some pollutants, along with physical adsorption. It is known that hydrogen sulfide is more easily oxidized to elemental sulfur in the presence of activated carbon. While the process of adsorbent odor is simple to operate, the cost of adsorbents and its regeneration is a concern.

Microorganisms can be used to remove odorous substances from contaminated air. This can be done by biological stabilization, scrubber piles, or biofilters. The first process consists of delivering the odorous air stream to an activated sludge aeration tank in which odorous matter is sorbed and then decomposed in a regular treatment procedure. Scrubber piles are used in sludge composting plants. In this case, air stream is passing through a pile of

screened compost (0.9–9 m depth), which absorbs the odors and the substances undergoing a biochemical conversion. In biofilters, the adsorption media consists of a layer of well-ventilated and biologically cultivated soil instead of compost. Both the piles and soil bed should be carefully maintained to perform efficient work. Scrubber piles and biofilters are easy to operate, but the efficiency of odor removal is not as predictable as the absorption or adsorption process.

Chemical stabilization of sludges is aimed not at reducing the quantity of biodegradable organic matter, but at creating conditions that inhibit microorganisms in order to retard the degradation of organic materials and prevent odors. The most common chemical stabilization procedure is to raise the pH of sludge using lime or other alkaline material, such as cement kiln dust. Sludge can be chemically stabilized in liquid or dewatered forms. When dewatered sludge is used, the exothermic reaction of lime with water causes heating which helps destroy pathogens and evaporates water.

4.3. Other Sludge Treatment Processes

Some processes are used to treat sludges, but are less relevant to sludge management schemes directed toward food crop production than are the processes previously discussed. These include the following:

Solidification/immobilization processes. These involve the conversion of sludge to a solid material with load-bearing capacity and the incorporation of contaminants in the solid phase so as to minimize their migration. The technology for solidifying and immobilizing waste originated in the nuclear waste industry, and although it has been widely applied in attempts to control hazardous waste, it is less commonly applied to municipal sludges.

Combustion. This process destroys organic compounds in municipal sludges and leaves an inorganic dry ash. Rarely, sludge combustion is carried out in the liquid phase under high pressure, producing an ash in liquid suspension. Because most of the organic material in sludge has beneficial attributes in agricultural systems, the combustion process is inappropriate when sludges are to be applied to cropland.

5. BIOSOLIDS USE AND DISPOSAL

Several methods are widely employed to use or dispose of biosolids: land application, distribution and marketing, landfilling, and incineration (Table 1.11) (9). Ocean dumping has been illegal since the 1990s in some countries. Their applicability to a particular municipality depends on many factors, including the source and quantity of wastewater sludge, geographic location of the community, hydrogeology of the region, land use, economics, public acceptance, and regulatory framework. Although all options have potential problems, some may be more acceptable than others for specific sludges under certain situations.

Beneficial uses of treated municipal wastewater sludges on land include agriculture and forestry uses; application to parks, golf courses, and public lands; use in reclaiming low quality or spoiled lands; and use as landfill cover or fill material. Disposal on land includes landfilling and permanent storage of dewatered sludge or sludge incinerator ash in lagoons or piles. Determining which various use/disposal options are most suitable for a particular

Table 1.11
Use and disposal of biosolids (9)

Country	Land application	Landfilling	Incineration	Ocean damping	Distribution, marketing, specific use
Sweden	60	30	Data inaccessible	–	10
Finland	40	45	–	–	15
Denmark	45	45	10	–	–
Germany	38	50	8	2	2
France	23	46	31	–	–
Belgium	10	80	10	–	–
Netherlands	53	32	3	13	2
Great Britain	45	29	3	23	–
Italy	20	60	–	–	20
USA	25	25	14	–	–
Russia	5	92	3	–	–

community is a multistage process. The first step is to define the needs, that is, to determine the quantity and quality of sludge that must be handled and estimate future sludge loads based on growth projections. Next, alternative sludge use/disposal options that meet these needs and that comply with applicable environmental regulations must be broadly defined. Unsuitable or noncompetitive alternatives must be weeded out in a preliminary evaluation based on readily available information. Resources are then focused on a more detailed definition of the remaining alternatives and on their evaluation. The final selection of an option may require a detailed feasibility study.

5.1. Land Application

Land application, defined as the spreading of sludge on or just below the surface of the land, is the most widely employed sludge use option. The sludge can serve both as a soil conditioner and as a partial replacement for commercial fertilizers. Usually, sludge is applied to land in one of four settings: on agricultural lands, forest lands, drastically disturbed lands (land reclamation), or land dedicated to sludge disposal (dedicated land disposal). Three of the four types of land application – agricultural application, forest application, and land reclamation – use sludge as a valuable resource to improve the land's characteristics. Sludge acts as a soil conditioner by facilitating nutrient uptake, increasing water retention, permitting easier root penetration, and improving soil texture (which in turn reduces runoff and erosion and makes the soil easier to work).

Sludge also serves as a partial replacement for expensive chemical fertilizers. The major constituents of chemical fertilizers – nitrogen, phosphorus, and even small amounts of potassium required by plants – are found in biosolids, though usually not in optimal proportions. Biosolids contains varying amounts of micronutrients such as boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). The exact ratio of these nutrients

will not be equivalent to that of a well-balanced formulated fertilizer, but the nutrients in biosolids can be combined with nutrients from other fertilizers to provide the proper amounts of nutrients needed for crop production.

Land application also functions as a sludge treatment system. Sunlight, soil microorganisms, and desiccation help to destroy pathogens and many toxic organic substances in the sludge. Heavy metals, and to some extent, nutrients in sludge are trapped by soil as a result of the soil's various physical and chemical characteristics. Nutrients, which can cause eutrophication and other problems if released into surface waters, are instead largely converted into useful biomass such as crops or wood. However, the capacity of the land to treat sludge constituents is finite, and land application systems must be designed and managed to work within the assimilative capacity of the land and the crops grown on it.

A successful land application program must consider several factors, including (a) site characteristics, such as depth to groundwater, distance to surface water, slope of the site, soil permeability, mineralogy, pH, and public access; (b) sludge application rates which are determined mainly by concentrations of nutrients, heavy metals, or toxic organics in the sludge; and (c) method of application by which liquid or dewatered sludges are applied (11, 38, 65).

5.1.1. Application to Agricultural Lands

Biosolids applied to agricultural land must be applied at a rate that is equal to or less than the "agronomic rate." The amount of available N (or P) applied to the site is based on that required by the crop. This amount of N would otherwise be applied to the site as commercial fertilizer by the farmer. A major advantage of agricultural land application is that usually the treatment plant does not have to purchase land. The land utilized for biosolids application is kept in production, its value for future uses is not impaired, and it remains on the tax rolls. Finally, agricultural land application usually takes place in a relatively rural setting where the application of biosolids is similar to conventional farming operations, such as spreading animal manure, and is not likely to become a public nuisance if properly managed.

Biosolids application rates for agricultural land application (dry unit weight of sludge applied per unit of land area) are usually relatively low. In addition, biosolids transport, as well as application scheduling that is compatible with agricultural planting, harvesting, and possible adverse climatic conditions, requires careful management. Federal regulations also require that, prior to land application, sludges must be treated by a PSRP (11, 38). Public access to the sludge-applied land must be controlled for at least 12 months, grazing by animals whose products are consumed by humans must be prevented for at least 1 month, and growing edible crops must wait for at least 18 months. Otherwise, PFRP must be applied to the sludge (11, 38).

Methods of sewage sludge application chosen for agricultural land depend on the physical characteristics of the sludge and soil, as well as the types of crops grown. Liquid sewage sludge can be applied by surface spreading or subsurface injection. Surface application methods include spreading by farm tractors, tank wagons, special applicator vehicles equipped with flotation tires, tank trucks, portable or fixed irrigation systems, and ridge and furrow irrigation. Surface application of liquid sludge by tank trucks and applicator vehicles is the

most common method used for agricultural croplands, particularly when forage crops are grown. Surface application of liquid sludge is normally limited to soils with less than a 6% slope. After the sludge has been applied to the soil surface and allowed to partially dry, it is commonly incorporated by plowing or other tillage options prior to planting the crop (i.e., com, soybeans, small grains, cotton, other row crops), unless minimum or no-till systems are being used.

Liquid sewage sludge can also be injected below the soil surface, and injection generally is the preferred method when gaining public acceptance. Available equipment includes tractor-drawn tank wagons with injection shanks (originally developed for liquid animal manures) and tank trucks fitted with flotation tires and injection shanks (developed for sludge application). Both types of equipment minimize odor problems and reduce ammonia volatilization by immediate mixing of soil and sludge. Sludge can be injected into soils with up to 12% slopes. Injection can be used either before planting or after harvesting most crops but is likely to be unacceptable for forages and sod production.

Dewatered sewage sludge can be applied to cropland by equipment similar to that used for applying animal manures, but more sophisticated equipment has beer developed with high flotation tires and improved application design. Typically, the dewatered sludge will surface-applied and then incorporated by plowing or another form of tillage. Incorporation, however, is not used when dewatered sludge is applied to growing forages or to minimum- or no-till land.

5.1.1.1. Biosolids Application Rates for Agricultural Sites

Biosolids application rates are calculated from data on sludge composition, soil test information, N and P fertilizer needs of the crop grown, and concentrations of trace elements. In essence, this approach views biosolids as a substitute for conventional N or P fertilizers in crop production. The general approach for determining biosolids application rates on agricultural cropland can be summarized as follows:

Nutrient requirements for the crop selected are based on yield level and soil test data. If biosolids have been applied in previous years, fertilizer recommendations are corrected for carry-over of nutrients added by previous sludge additions.

Annual biosolids application rates are calculated based on N crop needs, P crop needs, and annual pollutant loading rate limits, where applicable (bagged sludge).

Supplemental fertilizer is determined from N, P, and K needed by the crop and amounts of N, P, and K provided by biosolids application.

Biosolids applications are terminated when a cumulative pollutant loading rate limit is reached it applicable.

Agricultural application rates generally range from 2 to 70 dry mt/ha/year. This is equivalent to a rate of 1–30 dry tons/acre/year. A typical rate would be 15 dry mt/ha/year (6.7 tons/acre/year). Application rates are usually limited by either the nitrogen needs of the crop grown or by the annual or cumulative metals addition to the soil. Less frequently, application rates are limited by the phosphorus needs of the crop. Phosphorus-based rates are generally

lower than metal- or nitrogen-based rates due to the relatively low phosphorus needs of most crops.

The heavy metal contact of sludge has been extensively studied as a potential source of human exposure through the food chain. Research has shown that several factors act as barriers to human exposure to heavy metals in land-applied sludge. Because metals have low solubility, uptake by plants is minimal. Metals that are taken up tend to remain in the roots, preventing buildup of toxic metal concentrations in edible plant parts. In addition, most metals visibly damage crops at concentrations far lower than those that affect human health. References should be consulted for further information on agricultural application, particularly for effects of metals on environment and calculation of the agronomic rate and annual application rate (38, 65–80).

5.1.2. Application to Forest Lands

Sludge application can greatly improve forest productivity. One major advantage of forest application over agricultural application is that forest products (e.g., wild edible berries, mushrooms, and nuts) are an insignificant part of the human food chain. Moreover, sewage sludge amends the soil by providing nutrients, especially nitrogen (N) and phosphorus (P), that are frequently limited in forest soils, and by improving soil textural characteristics. The addition of sewage sludge can improve short-term soil productivity because it provides an immediate supply of virtually every nutrient needed for plant growth in an available form. In addition, the fine particles and organics in sewage sludge can immediately and permanently enhance soil moisture and nutrient-holding characteristics.

In the long term, sewage sludge provides a continual slow release input of nutrients as the organics decompose. The primary environmental and public health concern associated with forest application is pollution of water supplies. In many areas, forest lands form crucial watersheds and ground-water recharge areas. Contamination of water supplies by nitrates can be prevented by limiting sewage sludge application rates according to the nitrogen needs of the crop in this case trees (approximately 10–100 mt dry weight per hectare in a single application every 3–5 years). Application of sewage sludge to forest land is feasible on commercial timber and fiber production lands. Sewage sludge use in nurseries, green belt management, and Christmas tree production also is possible. Three categories of forest land may be available for sewage sludge application:

- Recently cleared land prior to planting (clearcuts)
- Newly established plantations (about 3–10 years old)
- Established forests

Clearcuts offer the easiest, most economical sites for sewage sludge application. Because application takes place prior to tree planting, many agricultural sewage sludge application methods can be used. Vehicles delivering sewage sludge from the treatment plant can discharge semi-solid sewage sludge (15% or more solids) directly on the land, followed by spreading by a dozer and disking. Ease of delivery depends on the amount of site preparation (stump removal, residual debris burning, etc.), slopes, soil conditions, and weather. Site preparation and sewage sludge characteristics are also major factors in application technique

(e.g., temporary spray irrigation systems; injectors and splash plates for liquid material; manure spreaders for solid material). While sewage sludge application is easier to perform on clearcuts, these sites also may require additional management practices to control grasses and rodents such as voles. Sewage sludge injection into the soil may minimize plantation establishment problems.

Application of sewage sludge to existing stands typically is made by a tanker/sprayer system, which can apply sewage sludge with an 18% solids content over the tops of the trees (canopy) 125 ft (40 m) into a plantation. This method requires application trails at a maximum of 250 ft (80 m) intervals. A throw spreader for applying a dewatered sewage sludge up to 70 m over a plantation has developed. This method has greatly reduced application costs and allows trail spacing of greater distances (120 m with overlap for evenness of applications). A good tree age or size for this type of application is over 5 years or over 4–5 ft high because they minimize maintenance otherwise needed in clearcut areas. Liquid sewage sludge also has been successfully applied using a sprinkler irrigation system. Clogging of nozzles has been the major drawback to this method. Manure spreaders are capable of applying dewatered sewage sludge which cannot be sprayed. It is recommended that sewage sludge applications take place during the time that tree growth is reduced, but uptake of nutrients also is reduced during this time. When sewage sludge is first applied to the soil, the available N is in the NH_4 form, which does not leach.

Applications to older stands have the advantage that sewage sludge can be applied year-round. Because spraying takes place under the tree foliage, no foliage will be affected. Application methods are similar to those described for young plantations.

One clearcut application scenario is the use of sewage sludge application in nurseries, and Christmas tree stands. This type of sludge application is often used in Russia. Typically, a high level of maintenance is common and weed establishment are minimized. Thus, the field experiment carried out in nursery of Prigorodnii forestry of Tatarstan Republic (Russia) showed feasibility and positive effect of using compost from the municipal sewage sludge for the soil restoration and growth of *Pinus sylvestris* seedlings. The grey forest soil (Haplic Greyzem) was amended with compost at application rate 30, 60, and 90 $Mg\,ha^{-1}$ on a dry matter basis. Organic matter content increased with the increase in sludge amendment. The concentrations of individual heavy metal were below the current limits established for Russia and European countries. Sludge amendments enhanced the germination and the number of the seedlings and the increase were more obvious for the soil with highest sludge treatment (Fig. 1.5) (9). The application of composted sludge to soil was followed by the increase in microbial biomass and basal respiration (Fig. 1.6) (9). References should be consulted for more detail information on forest application of sewage sludge (81–88).

5.1.3. Land Reclamation

Sludge can help return barren land to productivity. Unreclaimed lands are often barren and frequently harmful to the surrounding environment. They may have such problems as acid runoff, high erosion rates, low nutrient levels, and toxic levels of trace metals. Extensive areas of disturbed land that can benefit from reclamation exist as a result of mining for clay, gravel,

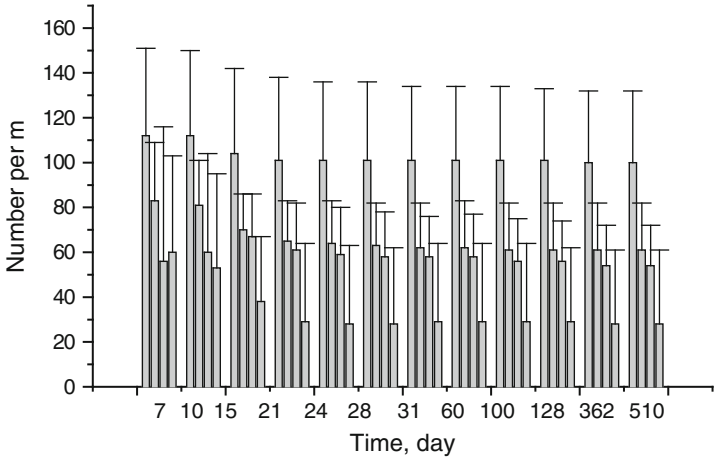

Fig. 1.5. Number of the seedlings of *Pinus sylvestris* during 2 years experiment. Column from *left* to *right* – 30, 60, 90 Mg ha^{-1} and control (without sewage sludge addition) respectively (9).

sand, stone, phosphate, coal, and other minerals. Also fairly widespread are construction areas (e.g., roadway cuts, borrow pits) and areas where dredge spoils or fly ash have been deposited. Other areas needing reclamation include clear-cut and burned forests, shifting sand dunes, landfills, and sites devastated by toxic fumes.

Application of wastewater sludge can improve all these characteristics. Sewage sludge has several traits that make it suitable for reclaiming and improving disturbed lands and marginal soils. One of the most important is the sewage sludge organic matter, which (a) improves soil physical properties by improving granulation, reducing plasticity and cohesion, and increasing water-holding capacity; (b) increases the soil cation exchange capacity; (c) supplies plant nutrients; (d) increases and buffers soil pH; and (e) enhances the rejuvenation of microorganism populations and activity. The natural buffering capacity and pH of most sewage sludge will improve the acidic or moderately alkaline conditions found in many mine soils. Immobilization of heavy metals is pH-dependent, so sewage sludge application reduces the potential for acidic, metal-laden runoff and leachates. Sewage sludge is also desirable because the nutrients contained in it may substantially reduce commercial fertilizer needs. Furthermore, sewage sludge helps to increase the number and activity of soil microorganisms.

Historically, reclamation of these lands is accomplished by grading the surface to slopes that minimize erosion and facilitate revegetation. In some cases, topsoil is added. Soil amendments such as lime and fertilizer also are added, and grass, legumes, or trees are planted. Although these methods are sometimes successful, numerous failures have occurred, primarily because of the very poor physical, chemical, or biological properties of these disturbed lands.

The amount of sludge applied at one time during land reclamation can be relatively large up to 450 dry mt/ha (200 ton/acre). This is necessary to ensure that sufficient organic matter and nutrients are introduced into the soil to support vegetation until a self-sustaining ecosystem is established. A typical one-time application would be 112 mt/ha (50 ton/acre). Usually

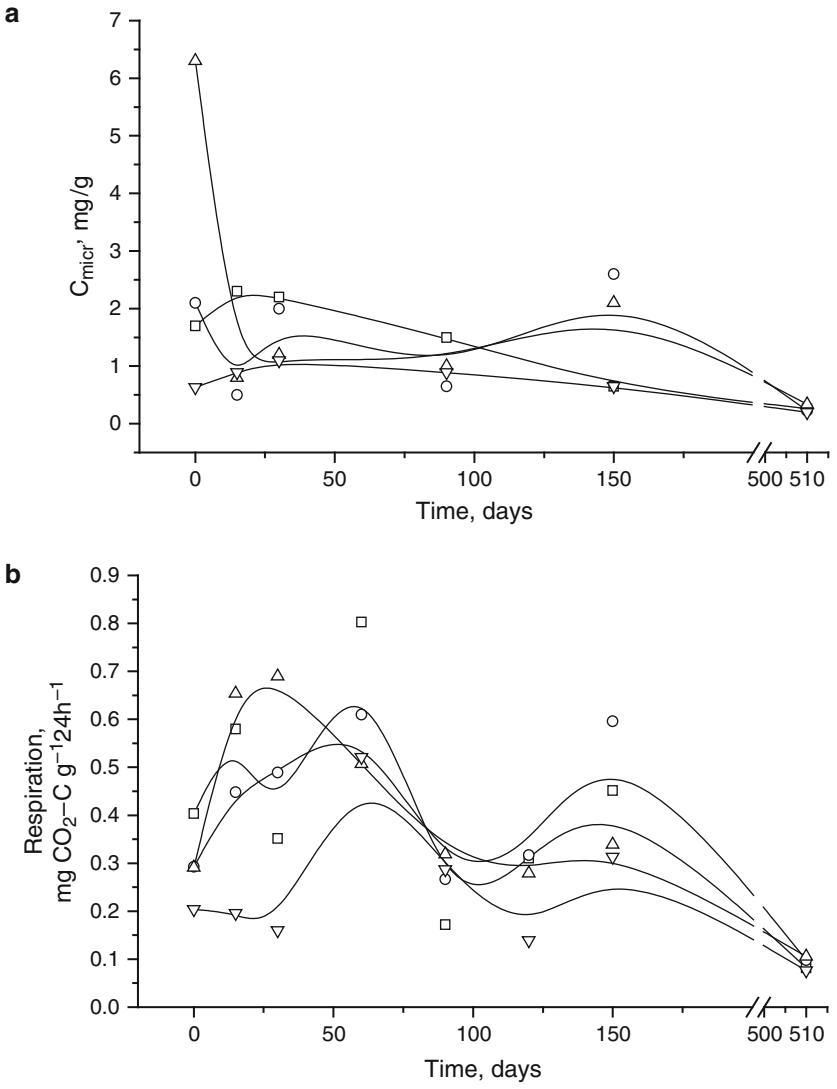

Fig. 1.6. Microbial biomass (**a**) and microbial respiration (**b**) during the 2 years experiment. Application rate: – 30 Mg ha^{-1}, *open diamond* – 60 Mg ha^{-1}, *open triangle* – 90 Mg ha^{-1}, *inverted open triangle* – without sewage sludge addition (9).

the sludge is applied and incorporated into the soil, the land is reseeded, and no further sludge is applied. Depending on site topography and sludge treatment prior to application, some contamination of ground and surface waters might occur immediately following sludge application, particularly by nitrate nitrogen. Similar problems occur during reclamation with chemical fertilizers, and such effects are usually negligible when compared to the environmental problems present prior to reclamation. Since sludge is usually applied only once, the

cumulative amount of metals and persistent organic chemicals applied during land reclamation may be less than the cumulative amount applied during agricultural application or forest land application, assuming a 20-year lifetime for forest and agricultural sites.

Sewage sludge can be used effectively to reclaim disturbed sites when the application of sewage sludge is managed properly (11, 38, 89–91). The following factors must be considered: the degree to which the sewage sludge is stabilized, sewage sludge application rates, the degree of land slope, and siting issues (e.g., quality of aquifer, depth to ground water). Good practices reduce the potential for adverse effects from sludge application during land reclamation, and also maximize the likelihood of success. For example, the type of sludge applied may be important. Research has suggested that large applications of composted sludge minimize the quantity of nitrogen leached to ground water or lost to surface waters (since the nitrogen in composted sludge has low solubility), while providing sufficient organic matter and nutrients to sustain vegetative growth for at least 10 years.

Large applications of digested sludge, on the other hand, provide sufficient organic matter, but pose a greater threat to water supplies because of the presence of large quantities of soluble nitrogen in the sludge, which can be readily oxidized to the nitrate form and enter the ground water. Smaller applications of digested sludge may provide insufficient organic matter to restore soil fertility. The benefits of applying composted sludge must be weighed against the cost of composting, and against the lower availability of nitrogen during the critical early stages of vegetation growth. Other important aspects of good practice include prompt revegetation to prevent erosion, and site preparation prior to sludge application to improve infiltration rates and reduce site slopes, thereby further reducing the potential for runoff and erosion. At the same time, there is a need for continual monitoring of ground water to evaluate the long-term effects of disposal on water quality and to provide a background to database for ascertaining environmental impacts on surface- and ground-water quality of potential future sites from sewage sludge disposal (64).

5.1.4. Other Options of Sewage Sludge Land Application

In addition to land application at agricultural, forest, and reclamation sites, sewage sludge and particularly sewage sludge products can be land applied to lawns and home gardens as well as "public contact sites" (11, 37, 38). Sometimes this option is called distribution and marketing of sludge products and it is a widely employed sludge use option. Public contact sites are defined as land with a high potential for contact by the public, such as parks, ball fields, cemeteries, plant nurseries, turf farms, and golf courses. In many cases, sewage sludge is applied to these types of sites from bags or other containers that are sold or given away, although sewage sludge also can be land applied to these types of sites in bulk form. Often the sewage sludge used at these sites is processed and marketed by municipalities or private firms as a brand-name fertilizer and/or soil conditioning product. Designing land application programs geared toward public contact sites, lawns, and home gardens may be particularly useful for municipalities with limited land available (e.g., highly populated areas with few agricultural, forest, or reclamation sites available for sewage sludge application).

Many of the strictest requirements must be met for sewage sludge that is land applied to public contact sites, lawns, and home gardens (e.g., Class A pathogen reduction; for metals,

annual pollutant loading rate limits for bagged sewage sludge or pollutant concentration limits for bulk sewage sludge). The stringent requirements are specified for sewage sludge that is land applied to public contact sites, lawns, and home gardens because of the high potential for human contact with sewage sludge at these types of sites and because it is not feasible to impose site restrictions when sewage sludge is sold or given away in bags or other containers for application to the land.

If a sewage sludge meets certain Part 503 requirements, the sewage sludge can be considered "exceptional quality" (EQ), as discussed earlier. EQ sewage sludge can be applied as freely as any other fertilizer or soil amendment to any type of land. If EQ sewage sludge requirements are met, current land application operations, including those with already successful marketing programs for sewage sludge (see Section "Natural Wetlands"), may continue with a minimum of additional regulatory requirements. For sewage sludge preparers who have difficulty meeting the Part 503 requirements for public contact sites, lawns, or home gardens, operational changes may need to be implemented to further reduce pathogen or metal levels for land application at these types of sites. Heat-dried or composted sludges usually meet these criteria and are typically used because they have a high solids content and are therefore more easily handled by the user.

5.2. Landfilling and Incineration

Landfilling and incineration are considered sludge "disposal" methods.

5.2.1. Landfilling

Landfilling is a sludge disposal method in which sludge is deposited in a dedicated area, alone or with solid waste, and buried beneath a soil cover (1, 11, 92–95). Adherence to proper sanitary landfilling procedures minimizes many potential health, environmental, and aesthetic problems associated with sludge landfilling. However, groundwater contamination by constituents in landfilled sludge remains a concern. Groundwater contamination may be difficult to detect until the damage has occurred, and even if contamination is detected, it may be extremely difficult to correct (93, 94). Proper planning and site management can help to avoid these problems. Two major types of landfilling are currently practiced: (a) sludge-only disposal, in which sludge is buried, usually in trenches; (b) codisposal, in which sludge is disposed of at a municipal refuse landfill.

5.2.1.1. Sludge-Only Disposal

Most sludge-only landfills consist of a series of trenches dug into the ground, into which dewatered sludge is deposited and then covered with soil. Sludge landfill trenches range from 1 to 15 m in width. At narrow trenches (1 to 3 m wide), dewatered sludge is usually dumped into the trench from a haul vehicle alongside the ditch. The sludge must be less than 30% solids and the trench floor must be nearly level to ensure that the sludge will spread evenly throughout the narrow trench. A wide trench (3–15 m wide) allows the haul vehicle to work within the trench itself. In this case, the sludge should be at least 30% solids (this may include bulking material, such as fine sand) to ensure that it will stay in piles and not slump.

The addition of a bulking agent is generally not cost effective if sludge solids content is less than about 20%. Instead, further dewatering of the sludge should be done at the treatment plant. The sludge should be covered with soil the same day it is deposited in order to minimize odors and to prevent insects, birds, and other vectors from contacting the sludge and spreading contaminants. As each new trench is dug, the excavated soil can be used to cover the sludge in a nearby trench. Sludges must contain at least 20% solids in order to support cover material. Narrow trenches are relatively land intensive. Sludge applications range from about 460–2,120 dry mt/ha including areas between trenches. Wide trench operations are less land-intensive than narrow trenches, with sludge applications ranging from about 1,200–5,480 dry mt/ha.

5.2.1.2. CODISPOSAL

In codisposal, wastewater sludge is deposited in a landfill together with municipal solid waste. In this way, the absorption characteristics of the solid waste and soil conditioning characteristics of the sludge can complement each other. The solid waste absorbs excess moisture from sludge and reduces leachate migration. Sludge can also aid revegetation of the completed codisposal site. Two categories of codisposal are: (a) sludge/refuse mixture, in which sludge is deposited on top of refuse and then mixed in; (b) sludge/soil mixture, in which sludge and soil are mixed and spread on top of refuse. Most sludge/refuse operations use sludges with at least 20% solids, although sludges as low as 3% solids have been codisposed by spraying the sludge on the refuse from a tank truck. However, low-solids sludge requires large refuse volumes, as much as 7 tons of refuse for every wet ton of sludge sprayed. The excess moisture in low-solids sludge increases the rate of solid waste decomposition; however, it also increases the likelihood of leachate and methane formation, and is therefore not a recommended method of operation. Spreading a sludge/soil mixture over completed refuse fill areas promotes revegetation of the site. Use of well-stabilized sludges reduces odors that could result if sludge is not completely buried. Sludge/soil covering operations have high manpower and equipment requirements.

5.2.1.3. LEACHATE

Leachate is generated from the excess moisture in the sludge, usually with some contribution from rainfall. The type and amount of constituents in leachate from a sludge landfill depend on the nature of the sludge. If landfill leachate reaches an aquifer, heavy metals and toxic organic chemicals are of particular concern because of their possible adverse health effects. If leachate enters surface waters, the resultant elevated nutrient levels can cause eutrophication and concomitant undesirable algal blooms and fish kills. Pathogen contamination of drinking water supplies could also have adverse health effects.

The potential for groundwater contamination can be reduced by properly covering landfills and installing liners to contain any leachate within the fill area and to attenuate harmful contaminants. A leachate collection system should be installed in any landfill where leachate is being contained or where water tends to pond in the fill area.

5.2.1.4. GAS CONTROL

The decomposition of organic matter in sludge and solid waste produces methane and other gases, including trace amounts of hydrogen sulfide. Methane is the gas of primary

concern. It can seep by diffusion through sludge and other materials into nearby buildings or underground structures, such as utility tunnels, where it may accumulate to explosive concentrations (5–15%). To prevent this hazard, systems to collect gases are usually installed in landfills located near buildings or underground structures. Collected gas can be vented to the atmosphere or incinerated. A third option is to recover and use the methane as an energy source. This is being done successfully at a growing number of solid waste sanitary landfills. However, the minimum landfill size required for economical gas recovery ranges from about 11 ha for a site with a 45-m fill depth to 31 ha for a site with a 15-m fill depth. Thus, gas recovery is presently not practiced at sludge-only landfills because they are normally much smaller.

5.2.2. Incineration

Incineration is the burning of volatile materials in sludge solids in the presence of oxygen. Strictly speaking, incineration is not a sludge disposal or use method, but a treatment method that converts sludge into an ash, which is then disposed of or used. Nevertheless, because incineration drastically reduces the volume and mass of residual solid materials, it has traditionally been regarded as a disposal method, and is evaluated alongside land application, distribution and marketing, landfilling, and ocean disposal as a use/disposal option (11, 96, 97).

Incineration offers significant advantages over other use/disposal options: it reduces the sludge to a compact residue consisting of about 20% of the original volume of the sludge solids, and it eliminates some potential environmental problems by completely destroying pathogens and degrading many toxic organic chemicals. Metals, however, are not degraded, but are concentrated in the ash and in participate matter are entrained in the exhaust gases generated by the process. High-pressure scrubbers or other pollution control devices are needed to prevent degradation of air quality, and appropriate means of ash disposal may occasionally be difficult to find. A major potential problem with all incineration systems is operational reliability. Because incineration is much more highly mechanized than other sludge use/disposal alternatives, it is particularly subject to varying sludge quality and quantity, equipment failure, and operator error. Although many municipalities are successfully operating sludge furnaces, many others have had to shut down operations because of repeated equipment breakdowns and operating costs much higher than originally predicted.

Two common types of incinerators for burning sludge are multiple-hearth and fluidized-bed furnaces (1, 96). Multiple-hearth incinerators have been in use for many years, and are the most common (about 76%). They are durable, simpler to operate, and more tolerant of variations in sludge quality and loading rates. The incineration takes place on the middle hearths, where temperatures can reach 760–927°C. However, multiple-hearth furnaces are not suitable for frequent stop-and-start situations, and many older units require costly upgrades to meet air quality requirements. Newer fluidized-bed furnaces, with more efficient combustion designs (even though the sludge incinerating temperatures only range between 760 and 816°C), can achieve better control on organic emissions. Currently, they constitute approximately 18% of sludge incinerators in use.

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Ultrasound Pretreatment of Sludge for Anaerobic Digestion

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CONTENTS

Abstract Ultrasound pretreatment of sludge has been examined in an effort to improve the hydrolysis rate in anaerobic digestion. The reactions that resulted from the generation and collapse of cavitation bubbles produced under the acoustic condition can significantly modify the substances present in the sludge. The principles of ultrasound that encompass acoustic cavitation and bubble dynamics, the mechanisms of biological damage and effects, the industrial applications of ultrasound, and the specific applications of ultrasound in environmental engineering are presented.

1. INTRODUCTION

Sewage sludge is an unavoidable byproduct of wastewater treatment. Raw sludge is rich not only in organic carbon and pathogens but also in heavy metals and other environmental pollutants. Therefore, the sludge must be stabilized to enable an environmentally safe disposal or utilization. Anaerobic digestion is the most commonly applied process for the stabilization of sewage sludge.

There are many positive features of anaerobic treatment, for example, mass reduction, stable products, and improved dewatering properties of the fermented sludge. Anaerobic digestion is particularly unique among several sludge stabilization methods, because it has

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_2

the ability to produce a net energy gain in the form of methane gas, leading to optimized cost effectiveness.

However, a main drawback of anaerobic digestion is its slow biological degradation rate, which results in a long fermentation period. A retention time of more than 20 days and the construction of huge digesters are usually necessary for degradation in an anaerobic process. Moreover, because of the low concentration of soluble organic matter contained in sludge, only 30–50% of the total COD or volatile solids (VS) can be degraded in very long time (1). The process of rapid industrialization and urbanization has dramatically increased the volume of sludge quantity generated. Hence, there is an urgent need to shorten the digestion period and enhance the degradation efficiency of anaerobic digestion.

The slow, rate-limiting hydrolysis process is the first step of anaerobic digestion. Extensive studies have explored ways to accelerate and enhance the performance of anaerobic digestion. The pretreatment process may include thermal pretreatment, high pressure homogenization, enzyme treatment, chemical solubilization by alkali, acid or base addition, mechanical disintegration, and ultrasound treatment. Among these processes, ultrasonication exhibits a greater potential of not being hazardous to the environment and is economically competitive.

Ultrasonic disintegration is a well-known method for breaking up microbial cells to release intracellular materials (2). Ultrasonic cavitation occurs more readily at a frequency of 20–40 kHz (3). During the sonication stage, some portion of the insoluble particulate organic matter can be transformed into a soluble state (1, 4). More than 100% increase of the maximum biological degradation rate had been achieved by ultrasonic transduction with an optimum intensity of 1.5 W/l at 25 kHz (5). The subsequent anaerobic digestion of the ultrasonically disrupted sludge may improve biogas production with a reduced sludge quantity that is vital to the economic consideration of a plant (6).

Various pretreatments of sludge have been studied in an effort to improve the hydrolysis rate. To enable a good understanding of the status of ultrasound treatment of sludge, Sect. 2 discusses other pretreatment methods including thermal, chemical, mechanical, enzyme, and irradiation.

Ultrasound generates high acoustic energy, and when this energy is applied to a liquid system, it is possible to generate physical and chemical reactions that can significantly modify the character of dissolved and particulate substances present in the liquid. These reactions result from the generation and collapse of cavitation bubbles produced under this acoustic condition. The principles of ultrasound encompass acoustic cavitation; bubble dynamics are presented in Sect. 3.

Section 4 focuses on the chemical and biological effects of ultrasound. The mechanisms of biological damage and effects are also described in this section.

Industrial applications of ultrasound are well established. With its many uses in automotive, electronic, optical, semiconductor, biomedical, and other industries, the use of ultrasound has become indispensable to modern manufacturing. Section 5 addresses the industrial applications of ultrasound, as well as its process parameters.

Section 6 discusses specific applications of ultrasound in environmental engineering with special emphasis on applications for wastewater treatment and anaerobic digestion.

2. PRETREATMENT OF SLUDGE FOR ANAEROBIC DIGESTION

2.1. Anaerobic Digestion

Anaerobic digestion is the most popular technique for wastewater sludge stabilization that results in the reduction of sludge volatile solids and the production of biogas. There are many positive features of anaerobic treatment: generated methane can be utilized as fuel; digestion has a low energy requirement; the pathogenic microorganisms in sludge are effectively killed; attention to operation is minimized; seasonal treatment is optimized; and the digested sludge is stable and may be disposed of harmlessly. However, since anaerobic stabilization is a very slow process, a long residence duration and large fermenter volumes are required.

Anaerobic fermentation converts organic materials biologically to methane and carbon dioxide in an environment devoid of oxygen. Anaerobic digestion of complex organic substances is usually considered to be a three-stage process consisting of hydrolysis, acidogenesis, and methanogenesis (Fig. 2.1).

Anaerobic digestion starts with the complex organic substances, which must initially be hydrolyzed to soluble organics of lower molecular weight. The first stage is the hydrolysis of long-chain complex organics, such as carbohydrates, proteins, and fats, to simpler molecules. Hydrolysis is a rather slow process and has been identified as the rate-limiting step. Complex organics are catalyzed by extracellular enzymes such as amylases, proteinases, lipases, and nucleases. Carbohydrates and proteins are hydrolyzed to simple sugars and amino acids, respectively. Fats are hydrolyzed to glycerol and long-chain fatty acids. These lower molecular weight organic compounds are then used by the acid formers to produce simple volatile fatty acids.

In the second stage, organic materials are converted into simple volatile fatty acids by a group of facultative and obligate anaerobes commonly termed as "acid formers". The products of this second-stage acidogenic conversion comprise predominantly organic fatty acids, and a small portion of biological cells. Although no waste stabilization is brought about during this stage of treatment, it is normally considered as an intermediate reaction to prepare the organic matter in a form amenable for the third stage of treatment. It is in the methanogenesis stage of treatment that actual waste stabilization occurs. The organic acids produced by the acid formers are converted by a unique group of microorganisms identified as "methane formers" into gaseous end products consisting of carbon dioxide, methane, and cells.

Even after some decades of optimization, a retention time of more than 20 days and the construction of huge digesters are usually necessary for efficient degradation in an anaerobic process. Nevertheless, the highest degree of degradation could be reached in an amount to about 40% for excess sludges (7).

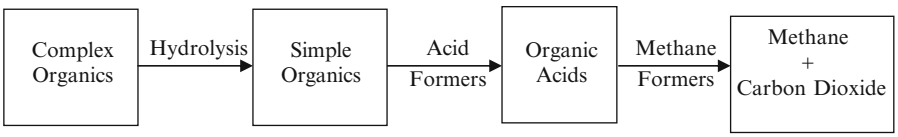

Fig. 2.1. Three-stage process of anaerobic digestion.

Hydrolysis is a slow stage that limits the speed of the entire process and leads to poor degradation results. Acceleration and better performance of the anaerobic process could be achieved by finding an alternative to the slow and rate-determining hydrolysis of the sludge. With increasing solubilization of the organic substances, more volatile solids become biodegradable. Thus, the efficiency of anaerobic digestion can be greatly enhanced by improving the rate of hydrolysis step by using physical and/or chemical pretreatment processes (8).

2.2. *Methods of Pretreatment*

Various pretreatments of sludge have been studied to improve the hydrolysis rate. These pretreatments led to rupture of the cell walls and membranes of bacteria in sludge, resulting in the release of organic substances outside of the cell. These organic substances can easily be hydrolyzed to their unit molecules by extracellular enzymes of anaerobic microbial origin, leading to an improved anaerobic digestion. The main methods of pretreatment are given below.

2.2.1. Thermal Treatment

Thermal treatment was first developed to improve the dewaterability of sewage sludge. The treatment is carried out through pasteurization by injecting steam at a temperature of 120–175°C with approximately 1×10^5 Pa pressure into a holding tank, mixing with the sludge, and raising the bulk suspension temperature to 70°C for 30 min. The thermal treatment at a temperature of 170–175°C breaks down the cells of microorganisms in the sludge into soluble organic matters, thereby improving the efficiency of anaerobic digestion and methane production (9).

Heating the sludge to above 150°C for 30 min would cause the breakdown of cell walls and the possible conversion of organics into more readily digestible forms. Experiments have indicated that a significant fraction of the volatile solids was liquefied following thermal treatment, and note an increase in gas production of approximately 34%. However, an acclimatization period was necessary for the digesters, and the quality of the supernatant liquors (in terms of the COD) was affected. Furthermore, this process requires more electrical energy than mechanical processes (10).

2.2.2. Chemical Treatment

The chemical treatment of sludge may be accomplished by using ozone, alkali, or acid treatment as discussed in the following sections.

2.2.2.1. Ozonation

The degree of biodegradability of the organic matter can be raised by partially oxidizing digested sludge with ozone (11). The objective is to apply additional treatment methods to these refractory sludge components, which cannot be disintegrated in either the one-stage or two-stage anaerobic degradation process. The refractory sludge components will then be partially oxidized either with ozone or with ozone in combination with hydrogen peroxide, which will lead to more complete degradation (12). Ozonation is not suitable for aerobic digestion due to nitrification and other problems.

2.2.2.2. ALKALI

The alkaline pretreatment may be used to hydrolyze and decompose lipids, hydrocarbon, and protein into smaller soluble substances such as aliphatic acids, polysaccharides, and amino acids (1).

The alkali treatment yields a significant reduction in microbial density and the release of COD from the sludge body, especially at pH $\geq$ 10. Lime and sodium hydroxide may be used for alkaline pretreatment of sludge to improve the solubilization efficiency of sludge. Lime treatment involves the addition of either CaO or $Ca(OH)_2$ in order to raise the pH to values of 11 or higher to kill off pathogens. However, the high pH environment may enhance the undesirable volatilization rate of ammonia, and the additional chemicals will increase the volume of the final product (1).

2.2.2.3. ACIDS

Acidification using low pH decreases the large floc size, resulting in better filterability. Nonalkaline chemicals, including either bactericides or oxidants, are seldom used because of their high cost.

Jean et al. (13) observed that adjustment of pH value for 2 h could disinfect the microorganisms in the sewage sludge by using total coliform bacteria as microbial indices. Microscopic observation revealed that in acidic conditions, the sludge floc retained its large shape and structure.

Chemical pretreatment methods have demonstrated the ability to improve the solubilization efficiency of sludge. However, the addition of inorganic chemicals has been shown to increase the volume of sludge, hence increasing the final waste volume. When acid or alkali is used, the salinity of the sludge will also be affected, thus possibly causing problems in sludge disposal (14).

2.2.3. Mechanical Treatment

Mechanical disintegration is a well-known process for obtaining intracellular products such as proteins or enzymes in biotechnological applications (15). Even for short grinding times, significant reduction of the mean particle size and an increase in surface area of the sludge was observed, because the floc structure of the sludge had been destroyed (12). The mechanical disintegration of sewage sludge destroys the floc structure of sludge and disrupts the cell walls of the microorganisms. Intracellular components are made immediately available for biological degradation, which leads to an acceleration of the process. Facultative anaerobic microorganisms are disrupted as well and become degradable, resulting in a higher degree of degradation.

However, mechanical disintegration needs high energy input. The investment for the disintegration aggregates has to be seen in relation to the reduction of digester volume and digestion time needed.

2.2.3.1. HIGH-PRESSURE HOMOGENIZATION

High-pressure treatment degrades sludge by utilizing the high shear stress produced when the sludge is released to the atmosphere. High-pressure homogenization is the most widely

known method for large-scale operations. Sludge is compressed to approximately 60 MPa and then released from the compressor through a valve at a high speed, shooting onto an impaction ring. Cell disintegration of 85% can be achieved (16).

2.2.3.2. Stirred Ball Mills

Ball-mills generate high shear stress by grinding beads to break the cell walls. The best result can be obtained when using the stirred ball mill for long grinding times, at high agitator speeds and with small particle sizes of the grinding beads (12).

2.2.4. Enzyme Treatment

The application of enzymes for the treatment of primary sludge with a high content of lignocellulosic material seems to be the most appropriate method. The use of enzymes can increase degradation; however, it is an expensive technique that produces a strong odor (17).

2.2.5. Irradiation Treatment

Irradiation can be generated directly by ionizing particles or indirectly by ionizing electromagnetic radiation obtained from radionuclide sources. Bacterial cells' structures are influenced by both the direct and indirect action of ionization products, disrupting the DNA and cell division. Viruses can be damaged by chain capture of the nucleic acid (18).

Irradiation treatment can substantially increase the concentrations of soluble organic matter. A significant improvement was seen in a 10-day-long biogas production study at a thermophilic temperature over the first 8 h (19).

However, the results of irradiation have not proven to be reproducible under the variety of conditions encountered in wastewater treatment plants, and energy costs have made it generally prohibitive.

3. FUNDAMENTAL OF ULTRASOUND

3.1. Introduction

Ultrasound is the term that is used to describe sound energy at frequencies above 20 kHz, i.e., above the range normally audible to human beings. Ultrasound is usually generated by a transducer, which converts mechanical or electrical energy into high-frequency vibrations. Ultrasound energy can be delivered into a fluid system via a horn or probe.

Sound is composed of longitudinal waves comprising rarefaction (negative pressures) and compressions (positive pressures). It is these alternating cycles of compression and rarefaction that, in high-power ultrasound applications, can produce a phenomenon known as cavitation. A broad range of frequencies and acoustic intensities can be generated by ultrasound. If high acoustic energy is applied to a liquid system, it is possible to generate physical and chemical reactions that can significantly modify the character of dissolved and particulate substances present in the liquid. These reactions result from the generation and collapse of cavitation bubbles, which are produced under this acoustic condition (20).

3.2. Acoustic Cavitation

3.2.1. Generation of Cavitation

Cavitation is the formation, growth, and collapse through implosion of microbubbles. These bubbles can be either gas or vapor filled and form in a wide variety of liquids under a wide range of conditions. Cavitation occurs in water, organic solvents, biological fluids, liquid helium, and molten metals, as well as many other fluids. Cavitation can be initiated by either setting up a tension in the liquid or by depositing energy into it (20).

The first type of cavitation observed was the formation of bubbles in liquids supersaturated with gas. The rise of cavitation as a topic for scientific research began with the development of high-powered and high-rpm steam turbines in the mid 1800s (21).

Tension appears in fluid flow, such as with ship propellers, hydrofoils, pipes, and pumps. The local deposition of energy is brought about by heat transfer in pipes or by dumping hot bodies into liquids (giving rise to eventually explosive bubble growth). It should be noted that this review is only relevant to the cavitation generated in sound fields (3).

Cavitation is accompanied by a number of effects having their origin in the dynamics of the bubbles generated. Cavitation bubbles tend to collapse extremely fast, emitting shock waves and even light (sonoluminescence). They erode solid surfaces and induce chemical reactions (21).

3.2.2. Two Types of Cavitation

Cavitation has been traditionally classified as one of two types: transient and stable. Transient cavitation involves large-scale variations in the bubble size (relative to its equilibrium size) over a time scale of a few acoustic cycles. This rapid growth usually terminates in a collapse of varying degrees of violence. Stable cavitation, on the other hand, usually involves small-amplitude (compared to the bubble radius) oscillations about an equilibrium radius. Stable cavitation in most instances results in little appreciable bubble growth over a time scale of thousands of acoustic cycles. This classification of cavitation is not strict, however. Stable cavitation can lead to transient cavitation, and the collapse of a transient cavity can produce smaller bubbles that undergo stable cavitation (3).

3.2.3. Acoustic Cavitation Conditions

When high acoustic intensities are applied, particularly in the low and mid frequency range, gas bubbles are generated that will grow by taking in gas and vapor from the liquid. These bubbles change in size in relation to the acoustic wave and can collapse in the compression cycle (implosion), with the final implosion in microseconds. This is called acoustic cavitation. At the implosion of the bubbles, extreme temperatures (5,000 K) and high pressures (500 bars) exist in the gaseous phase (22).

The bubble implosions produce short-lived (lasting micro-seconds) “hot spots” in the liquid, which can release sufficient energy to drive a variety of chemical reactions (23). The cavitation effect is influenced by a number of factors:

- Liquid temperature (it is likely to occur at higher temperatures)
- Viscosity

- Surface tension
- Ultrasonic intensity (often referred to as the acoustic energy density)
- Frequency of ultrasound vibration (usually set at 20–40 kHz)

The minimum amount of energy required to initiate cavitation is referred to as the cavitation threshold, and this varies for different fluids. Only the energy applied above the threshold will contribute to the formation of a cavitation bubble. In water, cavitation will generally occur once the ultrasonic energy rises above 1 W/cm^3 levels (23).

It is difficult to create cavitation beyond 1 MHz because the acoustic intensity that needs to be applied increases with increasing frequency. At frequencies greater than 1 MHz, the acoustic wave's impact on the liquid creates microcurrents together with stable, oscillating gas bubbles. These do not collapse, and may occasionally rise to the surface of the water body (22).

3.2.4. Effects of Acoustic Cavitation

Acoustic cavitation can affect a liquid through two possible avenues. The first is the bubble itself. The liquid is disrupted by the inhomogeneous presence of the bubbles. The second avenue through which cavitation affects a liquid interface continually changes shape and size; liquid molecules diffuse into and out of the bubble; the concentration of gas in the surrounding liquid varies; acoustic streaming occurs in the liquid in the vicinity of the bubble, often resulting in severe shear stresses; the interior pressure and temperature fluctuate rapidly; the bubble radiates acoustic energy as it oscillates; and thermal and viscous damping hinder the bubble oscillations (21).

3.3. Bubble Dynamics

3.3.1. Formation of Bubbles

Cavitation bubble collapse occurs when the expanding bubbles have reached their resonant radius. The resonant cavitation bubble radius is a function of the ultrasound frequency. In pure water and low surface tension, it can be calculated by the following equation:

$$\rho\omega_{\mathrm{r}}^2 R_{\mathrm{r}}^2 = 3\gamma P_{\mathrm{o}}, \tag{1}$$

where ρ is the density of water, ω_{r} is the resonance angular frequency, R_{r} is the resonant bubble radius, P_{o} is the pressure exerted on the liquid, and γ is the ratio of the specific heats of gases. γ correlates to the heat released upon gas compression (24) and varies from 1.66 to 1.4 and 1.33 for monoatomic, diatomic, and triatomic gases, respectively.

Taking the case of air bubbles in water at atmospheric pressure, the ultrasonic cavitation bubble radius can be approximated as

$$R_r \approx 3.28 f_{\mathrm{r}}^{-1}, \tag{2}$$

where the resonant bubble radius R_{r} is expressed in millimeters and f_{r} is the resonance frequency in kilohertz (25). The bubble radius is inversely proportional to the ultrasound frequency. The application of low frequencies creates larger cavitation bubbles. Upon bubble collapse, hard mechanical jet streams are produced that are responsible for many cavitation effects observed on solid surfaces.

3.3.2. Jet Formation

When a bubble is collapsing in a spherically asymmetric environment, the collapse changes in a remarkable way: a flat solid surface nearby causes the bubble to involute from the top (surface below the bubble) and develop a high-speed liquid jet towards the solid surface. When the jet hits the opposite bubble wall from inside, it pushes the bubble wall ahead, causing a funnel shaped protrusion (21).

3.3.3. Sonoluminescence

When a cavitation bubble field is observed in total darkness after allowing the eyes to adjust after 15–20 min, light can be seen emanating from the liquid, often in the form of filaments. Since the primary input is sound, the phenomenon is called sonoluminescence (21).

4. EFFECTS OF ULTRASOUND

4.1. Chemical Effects

As mentioned previously, acoustic cavitation generates extreme temperatures and high pressures in the gaseous phase. These dramatic conditions lead to pronounced chemical reactions with the application of ultrasound. These reactions are caused by the creation of highly reactive radicals ($H^{\bullet}$, $OH^{\bullet}$) and thermal breakdown of substances (pyrolysis), which mainly belong to the field of sonochemical reactions.

The principal products from the ultrasonic irradiation of water are H_2O_2 and H_2, and various data support the hypothesis of the intermediacy of hydroxyl radicals and hydrogen atoms, which was first reported by Neis (7).

$$2H_2O \xrightarrow{\text{Ultrasound}} 2OH^{\bullet} + 2H^{\bullet} \rightarrow H_2O_2 + H_2 \tag{3}$$

The wide range of oxidations and reductions that occurs with aqueous sonochemistry is often a consequence of secondary reactions of these high energy intermediates.

4.2. Biological Effects

4.2.1. Mechanisms of Biological Damage

Cavitation phenomena may cause damage to biological materials in several important ways. Transient cavitation generates very high pressures and temperatures, which theoretically can reach thousands of bars and degrees Kelvin, respectively, during the final stages of the collapse. The high-pressure shockwave that emanates from the location of the bubble is capable of causing mechanical damage to surrounding material. In cases where the bubble is adjacent to a solid surface, a high-velocity liquid jet may shoot through the bubble, impacting and damaging the cell walls. High temperatures can cause bond dissociations in molecules, producing free radicals that can react with biomolecular species in much the same way as those produced by ionizing radiations (26).

The inhomogeneous cyclic field established around stably oscillating bubbles can cause a steady flow of the fluid medium surrounding the bubble in a process known as microstreaming.

If streaming velocities are great enough, shear stresses resulting from the decreasing velocity with distance from the bubble can be sufficient to damage microbial cells (27).

It is quite clear that acoustic cavitation is the primary mechanism for the production of biological effects in most solutions, suspensions, plants, and insects. Some of these effects occur at levels lower than used clinically. It has also been demonstrated that cavitation nuclei exist in mammals and that 10-μm and larger bubbles are developed during sonication at low therapeutic levels (3).

4.2.2. Bioeffects of Ultrasound

If stabilized bodies of undissolved gas are present in tissues, ultrasound exposure may produce damage at relatively low values of acoustic intensity or pressure.

Carstensen et al. (28) found that exposure of plant roots to ultrasound caused reduction of growth. The reduction was most significant at a frequency of 1 and 2 MHz; subharmonic and noise signals were emitted from the tissue when the intensity was above 3 W/cm^2. The growth reduction was much less when the tissue was under 20 atm hydrostatic pressure during exposure to ultrasound.

With the development of various aspects of acoustic cavitation, acoustic radiation forces, and acoustic streaming, ultrasound is a proven application in biological and medical techniques, such as sterilization, cell disruption, dental descaling, angioplasty, extracorporeal lithotripsy, fibrinolysis, sonoporation, and treatment of Meniere's disease (29).

5. INDUSTRIAL ULTRASOUND APPLICATIONS

Ultrasound is a widely applied technique with a brilliant future. As a form of mechanical energy, its application to matter under the right circumstances can result in permanent physical changes. Because energy is a product of power and time, for a given power, the length of ultrasound exposure determines the total energy input into the material treated, which normally bears some relationship to the desired result. By definition, ultrasound pertains to frequencies above human hearing (approximately 18 kHz). Most practical applications to date have been in the lower ultrasonic spectrum, between 20 and 60 kHz.

5.1. Process Parameters

In general, power ultrasound is characterized by an ability to transmit substantial amounts of mechanical power at small mechanical movements. Ultrasonic motional amplitude is limited by the allowable stress in the ultrasonic transducer material and is dependent on frequency. To demonstrate typical values, a 20-kHz transducer operating at peak displacement amplitude of 50 μm has a peak velocity of 6.28 m/s and a peak acceleration of 8×10^4 g. The power ultrasound is characterized by high vibrational frequencies, small displacements, moderate point velocities, and extremely high acceleration (21).

Most macrosonic applications depend on compound acoustic phenomena occurring in matter, which in turn are caused by primary vibratory inputs. Thus, acoustic pressure causes cavitation and microstreaming in liquids; vibratory stress causes heating and fatiguing in

solids; and ultrasonic acceleration is responsible for surface instability occurring at liquid–liquid and liquid–gas interfaces (30).

5.2. Industrial Applications

The use of power ultrasound in industry has become well established. With its many uses in automotive, electronic, optical, semiconductor, biomedical, and other industries, power ultrasound has become indispensable to modern manufacturing.

5.2.1. Applications in Liquids

The applications of ultrasound in liquid include cleaning, soldering, deburring, erosion testing, cell disruption, extraction from plants, emulsification, dispersion of solids, sterilization, filtration, inhalation therapy, fuel atomization, drying of textiles, crystal growth, metal grain refinement, degassing, and medical surgery (21).

5.2.2. Applications in Solids

The aspects of ultrasound applications in solids include plastic welding, metal welding, metal forming, impact grinding, rotary abrasive, machining, metal cutting, fatigue testing, curing, trimming of composites, and dental descaling (21).

6. ULTRASONICATION FOR ENVIRONMENTAL ENGINEERING APPLICATIONS

While ultrasound has been used routinely for many years in fields such as medical diagnosis, cleaning, and others, the application of ultrasound technology in environmental engineering is still in its earliest phase, with only the first applications operational at a technical scale. While ultrasound shows great potential in environmental engineering, a number of scientific and technical questions exist, which include the influence of frequency, dissolved gases, and suspended solids on cavitation; optimal reactor design; economy, reliability, and life expectation of ultrasound equipment. Table 2.1 provides an overview of current ultrasound applications in water, wastewater, and sludge systems.

Table 2.1
Ultrasound applications in environmental engineering (7)

Domain	Objective
Potable water	Inactivate bacteria (disinfection)
	Improve separation of solids
	Remove encrustations in pipes and wells
Wastewater	Sonochemical pollutant degradation
	Improve biological degradation
Sludge	Disintegrate biosolids
	Decompose bulking-activated sludge flocs to allow sedimentation
	Improve dewatering

6.1. Ultrasonication on Wastewater Treatment

The biological treatment of wastewaters is usually preferred because of its low cost compared to chemical or physicochemical processes. This holds true unless bacteriotoxic or refractory pollutants inhibit biological activity, as happens in many industrial liquid wastes. If this occurs, more expensive chemical or physical methods must be used.

Ultrasound treatment shows some similarity to advanced oxidation processes (ozone, H_2O_2, UV), in which OH radicals are produced by the sonolysis of H_2O. Mechanisms involved in sonochemical transformations are still misidentified. However, acoustic cavitation appeared early as the main phenomenon responsible for chemical transformations (7).

6.1.1. Reactions of Ultrasound on Wastewater Treatment

Several modes of reactivity have been proposed: pyrolytic decomposition, hydroxyl radical oxidation, plasma chemistry, and supercritical water oxidation.

The first one, pyrolytic decomposition, takes place inside the cavities and affects the vapor from the liquid medium or dissolved organic compounds, which may penetrate into the bubbles. Indeed, energy concentrated in the bubbles is sufficient to break strong chemical bonds. In aqueous solutions, the main pyrolytic reaction is the dissociation of water. This thermal dissociation leads to the production of highly reactive radicals ($OH^{\bullet}$, $H^{\bullet}$) inside the bubbles (31).

It seems that the ratio between hydroxyl radical oxidation and pyrolysis depends on the localization of the solute (in the bulk solution, inside the bubble, or in the interfacial layer) and, therefore, on its physicochemical properties. Henglein (32) pointed out that the main property determining the entrance of a compound into the bubble was its hydrophobicity rather than its vapor pressure. Thus, hydrophilous organic compounds such as phenol and chlorophenols may undergo a hydroxyl radical attack in the bulk solution or in the interfacial film.

Other more hydrophobic compounds such as carbon tetrachloride, benzene, and chlorobenzenes may be mainly pyrolyzed inside the bubble. However, some other cases remain for which the localization of degradation is more difficult or for which there seems to be competition between mechanisms. In conclusion, hydrophobic and volatile organic compounds are destroyed very easily, whereas nonvolatile and hydrophilous compounds are more difficult to oxidize by ultrasound.

The third mode of reactivity proposed is that of plasma chemistry. Lepoint and Mullie (33, 34) observed some similarities between coronaluminescence and sonoluminescence as well as between coronachemistry and sonochemistry. This led them to assimilate the ultrasound effects to corona plasmas inside the bubbles.

6.1.2. Types of Pollutants Treated by Ultrasound

It has been shown that a variety of wastewater pollutant can be degraded using ultrasound. Different types of chemical pollutants have been investigated, for instance, chlorinated solvents and aromatics, hydrocarbons, pesticides, phenols, and polymers. Ultrasound cavitation generates pyrolytic reactions and hydromechanical forces. In many cases, these processes are the dominant factors in the ultrasound degradation of pollutants. It has been demonstrated that the reaction mechanisms vary depending on the different physicochemical properties of a particular pollutant:

- Volatile pollutants are degraded preferentially by pyrolytic reactions, which occur in the vapor phase of the cavitation bubble.
- Hydrophobic pollutants accumulate and react in the hydrophobic boundary layer of the cavitation bubble. Concentrations of OH radicals and H_2O_2 in the boundary layer are significantly higher than in the surrounding liquid. Pyrolysis and radical reactions contribute to the degradation.
- Hydrophilic pollutants in the bulk liquid are degraded by reaction with free radicals or H_2O_2.
- Macromolecules and particles are also degraded by hydromechanical forces triggered by the collapse of the cavitation bubbles.

For practical studies and experiments, the results of different chemical pollutants in wastewater after ultrasound treatment are listed in Table 2.2.

Two major mechanisms are responsible for the degradation of pollutant: radical reactions and pyrolytic decomposition. Among all the ultrasound applications, wastewater treatment

Table 2.2
Degradation of solutions of different compounds

Compounds	Sonication conditions	Intermediate products identified	Reference
Phenols	20 and 487 kHz, 30 W, air, 0.5 mM	Hydroquinone, catechol, 2,5-dioxohexen-3-dioic acid, muconic, maleic, succinic, formic, propanoic, oxalic, and acetic acids	(41)
2-chlorophenol	20 and 541 kHz, 30 W, air, 100 mg/l	Chlorohydroquinone, catechol, 3-chlorocatechol, chlorides	(53)
3-chlorophenol	20 kHz, 50 W, air, 0.05 mM	Chlorohydroquinone, 3- and 4-chlorocatechol,	(53)
4-chlorophenol	20 kHz, 50 W, air, 0.05 mM	Hydroquinone, 4-chlororesorcinol, 4-chlorocatechol, chlorides	(53)
Pentachlorophenol	500 kHz, air, 0.1 mM	Chlorides	(41)
Parathion	20 kHz, 84 W, air 0.1 mM	*p*-nitrophenol, sulfates, phosphates, formic, oxalic, and acetic acids	(54)
Benzenes	20 and 487 kHz, 30 W, air, 0.5 mM	Phenol, catechol, hydroquinone, 1,2,3-trihydroxybenzene, maleic and muconic acids, formaldehyde, acetylene	(55)
Chlorobenzene	20 and 487 kHz, 30 W, air, Air, O_2, 0.5 mM	4-chlorophenol, 4-chlorocatechol, hydroquinone, acetylene	(55)
Chloroform	20 kHz, 200 W, air	–	(55)
Carbon tetrachloride+phenol	20 and 500 kHz, 30 W, air, phenol: 0.5 mM, CCl_4: 3.8 mM	Chlorides, 2-chlorophenol, 2,4-dichlorophenol, chlorobenzoquinone	(55)

appears to be an original and expanding field of study. This process is convenient and simple in terms of temperature, pressure (ambient conditions), and reagents (no reagents). But the energy consumption for total pollutant mineralization is very high. The ultrasonication process is therefore considered a preoxidation step.

6.2. Ultrasonication on Anaerobic Digestion

As introduced in the first chapter, anaerobic digestion is the most popular technique for sewage sludge stabilization resulting in the reduction of sludge volatile solids and the production of biogas. Anaerobic digestion is a slow process, and the rate-limiting step is the hydrolysis of particulate organic matter to soluble substances (8). It has been postulated that the extreme conditions produced during sonication, if applied to sewage sludge, will cause cell disruption/lysis and release the intracellular organics, thus enhancing the digestion process. In addition, the physical action produced by the cavitation bubbles can reduce the sludge particle size distribution, which potentially increases the number of sites available for microbial action.

Neis et al. (22) investigated the effect of ultrasound pretreatment on sludge degradability by testing the increase of COD and size reduction of sludge solids (Table 2.3). Semi-continuous fermentation experiments with disintegrated and untreated sludge were conducted for 4 months on a half-technical scale. The results indicated that the fermentation of disintegrated sludge remained stable even at the shortest residence time of 8 days, with biogas production of 2.2 times that of the control fermenter. In a subsequent study (35), sonicated waste-activated sludge remained stable over a digestion time of 4 days. The effects of ultrasound frequency on the disintegration were examined by varying the frequency within a range from

Table 2.3
Technical specifications of the sonoreactors

References	(7)	(36)	(1)	(23)	(56)	(13)
Digester volume	2,000 l	1,500 l	1 l	10 l	400 ml	–
Sonicator volume, l	1.280	–	1.000	10.000	0.100	–
Frequency, kHz	31	31	20	20–35	23	–
Number of transducers	48	–	–	–	–	–
Hydraulic retention time in digester, day	4–16	8–22	–	12–15	8–12	0
Hydraulic retention time in sonicator, s	64	64	14–24/ml	<60	90	1,200–7,200
Power consumption, W	3,600	3,600	120	9,000	47	–
Acoustic intensity, W/cm^2	5–18	–	–	–	–	–
Acoustic power density, W/cm^3	2.2–7.9	–	–	–	–	0.11–0.33
Digestion temperature, °C	37	37	30–36	35	–	–
Duration, month	–	4	–	12	–	–

41 to 3,217 kHz, and the impact of different ultrasound intensities and treatment times on anaerobic digestion were also examined. Pilot-scale investigations conducted by Tiehm et al. (36) reported acceleration in anaerobic digestion of ultrasonically pretreated raw sludge.

Chiu et al. (1) investigated the use of alkaline treatment combined with ultrasound treatment for enhancing recovery of volatile fatty acid (VFA) from WAS digestion. They described the effects of ultrasound treatment on physical, chemical, and biological characteristics of waste-activated sludge. They also found a critical ultrasound power above which the floc structure was effectively disintegrated, microbial level acceptably disinfected, and particulate organic compounds sufficiently transformed into a soluble state. They also found that both ultrasonic vibration and bulk temperature rise contributed to the efficiency in treatment.

Clark and Nujjoo (23) studied the cell lysis and particle size reduction after ultrasound pretreatment. A series of laboratory-scale anaerobic digesters were operated, and following ultrasonication, significant increases in biogas yield were noted. The experiments utilized a variety of ultrasonic devices (of different geometries and construction materials) and sludge types.

Jean et al. (13) investigated the effects of ultrasound and pH values on the microbial density level in sewage sludge by using total coliform and heterotropic-plate-count (HPC) bacteria. It was observed that sonication at a higher intensity produces a smaller floc size in a shorter time. A high pH was observed to break up large flocs into smaller aggregates.

Suslick (37) reported that ultrasound of high acoustic intensities causes cavitation in water bodies when the energy forces applied exceeded the binding energy of the molecular attractive forces.

Bien and Wolny (38) observed that the effect of ultrasonic treatment on sewage sludge depends on the kind of sludge and chemical compounds used in the dewatering process. The sludge was dewatered on a vacuum filter after conditioning with polyelectrolytes and the ultrasound field.

6.2.1. Reactions of Ultrasound Pretreatment

Based on the previous studies, several factors are thought to be responsible for the disintegration of sludge with ultrasound treatment. These factors may be summarized as follows:

1. Sonochemical effects
2. High mechanical forces
3. Thermal breakdown of volatile hydrophobic substances

Through these processes, bacterial cells are disunited by pressure waves and cavitation generated from an ultrasonic generator, leading to elution of intracellular organic substances (39). The floc structure in sewage sludge is destroyed, and this increases the accessibility of hydrolytic bacteria to the released intracellular organic substances. This situation leads to greater efficiency during subsequent anaerobic digestion (40).

Inside the collapsing cavitation bubbles, the temperature and pressure can rise up to about 5,000 K and several hundred atmospheres. These extreme conditions can generate very reactive hydroxyl radicals ($H^{\bullet}$, $OH^{\bullet}$) (25, 30). In this way, sonochemical reactions can degrade volatile pollutants by pyrolytic processes inside the cavitation bubbles and nonvolatile

pollutants by hydroxyl radical reactions in the bulk liquid (41, 42). While sonochemical degradation processes can occur in a broad ultrasound frequency ranging from 20 kHz to about 1 MHz, the highest efficiency of sonochemical reactions was observed at more than 100 kHz (24, 41).

Many studies show that mechanical forces are the key contributing factor to the ultrasonic disintegration of sewage sludge. As described by Neis (7), at the first impact of the acoustic wave, sludge flocs are separated and a large number of single cells are formed. While sonication continues, single bacteria cells may act as nuclei for the formation of bubbles. This might mean they are captured and ruptured in the cavitation bubbles that, during the rarefaction cycle, can grow up to 175 μm in diameter before collapsing. The violent collapse produces very powerful hydromechanical shear forces in the bulk liquid surrounding the bubble.

Mechanical forces are most effective at frequencies below 100 kHz, which is the same frequency range of the optimal disintegration achieved. It had been shown that macromolecules with a molar mass above 40,000 are disrupted by hydromechanical shear forces produced by ultrasonic cavitation (43). On the other hand, sonochemical processes, i.e., production of hydroxyl radicals, were most significant at frequencies between 200 and 1,000 kHz (44). Therefore, hydromechanical forces produced by ultrasonic cavitation are more important for sewage sludge disintegration than sonochemical processes.

6.2.2. Influencing Parameters

6.2.2.1. Frequency

Sludge disintegration was most significant at low frequencies. Low-frequency ultrasound creates large cavitation bubbles that upon collapse initiate powerful jet streams exerting strong shear forces in the liquid. A decreased efficiency in sludge disintegration observed at higher frequencies was attributed to smaller cavitation bubbles that do not allow the initiation of such strong shear forces (35).

Theoretical considerations are useful for understanding the decrease in disintegration efficacy as ultrasound frequency increases. As depicted in Eq. (2) discussed previously, the resonant cavitation bubble radius is a function of ultrasound frequency. The bubble radius is inversely proportional to the ultrasound frequency. The application of low frequencies creates larger cavitation bubbles. Upon bubble collapse, hard mechanical jet streams are produced that are responsible for many cavitation effects observed on solid surfaces. A valid assumption might be that the energy released by a jet stream is a function of the bubble size at the moment of collapse. The number and size of cavitation bubbles in a sludge media may certainly be different to a pure water system due to the presence of a high number of solids, different density of the liquid, and the presence of dissolved gases. However, the degree of sludge disintegration could be related to the theoretical bubble size calculated by using this equation. Starting at a point where R is about 4 μm, the degree of cell disintegration increases proportionally to the logarithm of the bubble radius (35).

Comparing sonochemical efficiency at different frequencies is a complex problem because sonochemistry is associated with the bubble of cavitation. Formation and behavior of the bubble is closely linked to the sound pressure field, which depends on the reactor (design) and on the ultrasonic source (frequency, surface emitting area, intensity). In a reactor with a

well-defined configuration, a modification of the ultrasonic frequency will change the local bubble population and may affect the yield of the reaction (45).

6.2.2.2. Duration/Treatment Time

Concentrations of organic substances in the supernatant, such as protein, carbohydrate, and COD, have been observed to increase proportionally with ultrasonic pretreatment time (40). Short sonication times resulted in sludge floc deagglomeration without the destruction of bacteria cells. Longer sonication brought about the breakup of cell walls, disintegration of sludge solids, and release of dissolved organic compounds to the liquid phase (35).

An optimum pretreatment time should exist in terms of efficiency and energy cost. Chu et al. (46) concluded that the ultrasonic treatment consists of several stages. At the first stage of sonication (0–20 min) at a power input exceeding the critical level, the porous floc could be readily deteriorated into compact flocculi, while the dewaterability of sludge was markedly deteriorated. In the second stage (20–60 min), although the floc size remained almost unchanged, both heterotrophic bacteria and total coliform were effectively disinfected. The soluble COD value increased, accompanied by a reduction in microbial density levels. In the final stage (60–120 min), if the bulk temperature was controlled, ultrasonic treatment had essentially no effect on the sludge characteristics. However, the raised bulk temperature of sludge could induce continuous transformation of solid-state organic compounds into a soluble form (46). Pretreatment longer than 30 min did not lead to continued extensive increases in methane generation (40).

6.2.2.3. Energy Level (Intensity/Density)

The degree of disintegration is amplified by an increased acoustic intensity in an applied range, which can be increased by more than twofold by an increase of the sound energy from 6 to 8 W/cm^2 intensity. This is due to the higher mechanical shear forces produced at higher intensities, rupturing more microorganisms (7). Tests at 0.11 W/ml had almost no effects on the floc size. Only when the power level had exceeded 0.22 W/ml would the particle size apparently decrease (46). The cavitation threshold for water was reported to be about 0.4 W/cm^2 by Lorimer (47). But Tiehm et al. (35) observed disintegration at a rather low intensity of 0.1 W/cm^2. A lower cavitation threshold for sludge seems reasonable due to the presence of a high number of small particles and gas bubbles acting as cavitation nuclei.

Jean et al. (13) reported that the soluble COD increased by 12 times at the high-intensity level, but was almost unchanged at the low-intensity level. At a low ultrasonic intensity, the floc size and the heterotrophic-plate-count (HPC) bacteria level only mildly decreased, but the total coliform level markedly reduced after 40-min sonication. At a high intensity level, the total coliform and HPC density levels as well as the floc size were sufficiently reduced.

Neis (7) conducted a study to optimize the reduction of ultrasound energy input/degree of cell disintegration and anaerobic digestion time. Generating ultrasound waves with optimized pulsed signals reduced the power consumption considerably. While there may be other cases requiring longer treatment times, typically energy doses between 4 and 10 kWh/m^3 should be sufficient.

6.2.3. Ultrasonic Sludge Disintegration

6.2.3.1. Structural Changes

Gorczyca's floc formation model (48) consists of primary particles (≈2 μm), compact flocculi and microflocs (≈13 μm), and highly porous flocs (≈100 μm). The mechanical forces generated by ultrasonic waves at 0.44 W/ml could disintegrate the highly porous floc into microflocs or flocculi and release some extracellular polymers (4). At a low intensity, the floc size decreased gradually from 31 to 20 μm in 60 min, that was 35% reduction in size, while in the high intensity test, the floc size reduced to its plateau value (14 μm) in less than 20 min (13).

Ultrasonic treatment has no effects on the surface charge of the suspended particles. However, as floc breakage occurs, the concentrations of Ca^{2+} and Mg^{2+} ions in the supernatant markedly increase. Calcium and magnesium ions are generally accepted as the essential components to bridge the constituent particles in a sludge floc (46, 49, 50).

6.2.3.2. COD/SCOD Change

Ultrasound treatment of sewage sludge leads to a breakup of microbial cell walls. Intracellular compounds are released, resulting in an increase of COD in the aqueous phase (51). The quantity of bioavailable dissolved organic substrate measured as soluble COD is significantly increased and considerably accelerates the subsequent sludge degradation. Soluble COD was chosen as the marker analysis by a number of researchers to measure organic availability in sludge. The relationship between SCOD rise and sonication time has been reported to be linear (31).

Clark (23) observed that a significant increase in SCOD was also found in primary sludges after ultrasound pretreatment. When compared with secondary sludges, primary sludges contain relatively low levels of microbial biomass, i.e., cells. Thus, an increased SCOD is due to cavitation-induced cell lysis; otherwise, a small increase in SCOD would be expected.

6.2.3.3. Biogas

Enhanced degradation rates result in a significant increase of biogas production. Neis (7) found that the percent of methane in the biogas was always slightly higher in the fermenters operated with disintegrated sludge than in the control fermenters. Chemostat-fed sonicated sludges had 5–10% greater methane content than the untreated samples. The increase in specific methane production appears to be dependent upon the hydraulic retention time (HRT). At longer HRTs, the percentage increase in specific methane production was reduced (23). Different sludge samples have different characteristics that cause different gas production capacities. Adequate stirring of the reactor content and the maintenance of digestion temperature may ensure better digestion (6).

6.2.4. Methods to Enhance Ultrasound Efficiency

Ultrasound is a pressure wave that propagates through a medium with a vast amount of energy dissipation. Thus, reducing energy consumption and enhancing efficiency are critical for the application of ultrasound at full-scale wastewater treatment plants. It has been reported that the reduction of high concentrations of persistent organic pollutants,

viz. hexachlorobenzene and phenanthrene, can be achieved through ultrasonication on contaminated soil slurry with moisture ratio in the range of 2:1–3:1 (57).

Using simultaneous ultrasonic and alkaline treatments, the pretreatment time for municipal waste-activated sludge can be greatly shortened, resulting in a high amount of SCOD released (1). Since the two methods rely on different mechanisms to solubilize particulate organic substances, a combination of the methods will take advantage of two mechanisms and achieve better efficiency.

It has been found that the joint activity of polyelectrolytes and ultrasound are particularly favorable for the reduction of sludge volume. The mechanism of this method may be explained by a partial dehydration, i.e., the removal of the water dipoles from some part of the particle surfaces in the solid phase. This gives rise to disturbances in the stability of the hydration layer, which is replaced by an orientation of polyelectrolyte macroparticles with long chains that simultaneously "bridge" several particles. As a result, this leads to an increased number of adsorbed molecules of the reagent at the particle surface and the flocculation of minute particles present in the suspension (52).

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3
Solubilization of Sewage Sludge to Improve Anaerobic Digestion

Tsuyoshi Imai, Yuyu Liu, Masao Ukita, and Yung-Tse Hung

CONTENTS

Abstract With the sludge treatment, the solubilization process of sewage sludge invites our attention because of the shortage of a final disposal site. In this chapter, a high-speed rotary disk process was applied to solubilization of sewage sludge from a sewage plant. With anaerobic treatment, the solubilized sludge by high-speed rotary disk process could be shortened from a digestion period of 30 to 10 days. Moreover, by applying the solubilized sludge to the activated sludge process, the excess sludge produced from the final sedimentation tank could be reduced to approximately 60%.

1. INTRODUCTION

Wastewater treatment is expanding quickly both in developed countries where effluent criteria is increasingly stringent and in developing countries where wastewater plants are being built in great numbers (1). The activated sludge process method, which was first developed in England in 1914 (2), is commonly adopted. It presents a high BOD removal efficiency (between 85 and 95%) but generates 0.5 kg dry weight excess sludge/kg BOD (3). As a result, large amounts of sewage sludge are generated annually: an estimated 6.9 and 6.8 million dry tons, respectively, for the United States (1998) (4) and Western Europe (5) (Table 3.1). In Japan, 1.9 Mt/year of dry sludge was generated in sewage treatment plants, accounting for

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_3

Table 3.1
Large amounts of sewage sludge are generated annually in the United States and Western Europe (4, 5)

	Amount (million tons dry solids/year)	Disposal method (%)			
		Application to land	Land filling	Incineration	Other
Austria	320	13	56	31	0
Belgium	75	31	56	9	4
Denmark	130	37	33	28	2
France	700	50	50	0	0
Germany (West)	2,500	25	63	12	0
Greece	15	3	97	0	0
Ireland	24	28	18	0	54
Italy	800	34	55	11	0
Luxembourg	15	81	18	0	1
Holland	282	44	53	3	0
Portugal	200	80	13	0	7
Spain	280	10	50	10	30
Sweden	180	45	55	0	0
Switzerland	215	50	30	20	0
United Kingdom, 1991	1,107	55	8	7	30
United States	6,900	41	17	22	20

48% of industrial wastes (1996's value) (6). Sewage sludge is regarded as a type of Municipal Solid Wastes (MSW) because of its high moisture (nearly 90%) and organic content (59–88%, w/v) (7, 8). Additionally, excess sludge treatment and disposal usually accounts for about 60% of total wastewater treatment operation cost (9). The final disposal of sludge is rather complex and has elicited much concern.

The concept of "3R" (Waste Recycle, Reuse, Reduce) arose in the last century and continues to prevail (10). "Reduce the amount and toxicity of trash you discard;" "Reuse containers and products, repair what is broken or give it to someone who can repair it;" and "Recycle as much as possible, which includes buying products with recycled content" are required (11). Also, in Japan, until 1999, "The Basic Law for Establishing the Recycling-based Society" was promulgated along with several waste recycling regulations, which emphasized recycling the social resources, restraining the consumption of natural resources, and reducing the environmental burdens (12). Expectations for wastewater treatment include minimal environment effect, maximum energy utilization, and minimum space requirements.

Land filling, incineration, and agricultural or forest land use are often used for the controlled disposal of biological sludge (13). In Japan, nearly 45% of excess sludge is being applied for soil amendment or producing building materials, and the residue is mainly treated via dewatering, incineration and landfilling. However, decreasing landfilling space is requiring a reduction of the amount of reclamation. Although several methods may be used for the resource utilization of sludge, including energy recovery via methane and hydrogen fermentation,

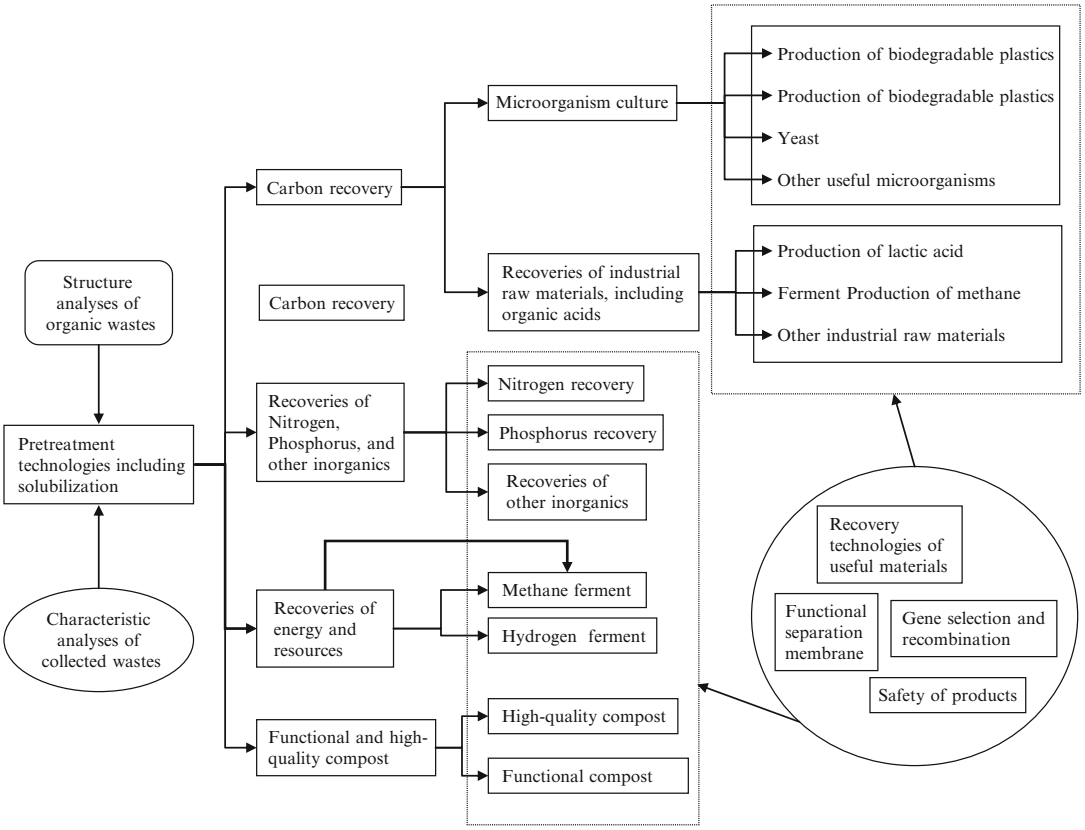

Fig. 3.1. Utilization process of organic materials (78).

developing solubilization technology as the preferred pretreatment method has become more important. Effective utilization technologies regarding organic wastes with high moisture content are largely categorized as incineration and biotechnological resources. Figure 3.1 describes the latter. Many development studies regarding sludge pretreatment technologies such as the solubilization process are being conducted. Various solubilization technologies for sludge pretreatment, such as mechanical pretreatment (14–20), ultrasound (21–41), electron beam (42), pulsed electric fields (43), thermal (heat) hydrolysis (44–56), alkaline (30, 57–61), thermochemically (62–70), chemical pretreatment (71, 72), ozone (73, 74), hydrogen peroxide (75), enzymatic (44), and thermophilic aerobic bacteria (76) have been discussed to some extent. However, these methods often result in high cost because of high energy consumption, or require the skilled personnel to manage the facilities.

In this chapter, we examined the solubilization of excess sludge using a high-speed rotary disk, an operationally convenient and economic mechanical pretreatment technology Of particular interest was the crushing of excess sludge with the aid of fluid shear stress, using a high-speed rotary disk, and the effects of temperature and preheating on the solubilization We also

examined the solubilization of excess sludge generated in sewage plants. Optimum conditions of the facility included a combination of sludge concentration, treatment period, the interval of disk, and the rotating times. Comparisons between solubilization and associated electric power were conducted to understand the optimum condition. Moreover, since sludge concentrations varied among experiments, it was difficult to directly compare the solubilization ratio via Soluble Organic Carbon (DOC). Therefore, in this study, the solubilization ratio was expressed as DOC divided by Total Organic Carbon (TOC) (77).

2. OPTIMUM OPERATING CONDITIONS OF EXPERIMENTAL APPARATUS

2.1. Experimental Apparatus and Methods

2.1.1. Experimental Apparatus

Figure 3.2 shows a sketch of the experimental apparatus used in this study. The structure consisted of a batch type rotary disk device that drew the sample (excess sludge) through two disks via the centrifugal force from the inlet of the bottom-fixed disk and crushed the sludge via the high-speed rotary of the upper motor-driving disk. The disks were made of stainless steel, and had a lubricious surface. The diameters of the upper and lower disks were 180 and 220 mm, respectively; a 30 mm hole was opened in the center of the lower disk for taking up sludge, and 75 mm of outer side could be touched. The disk intervals could be adjusted freely from several tens of micrometers to several centimeters by the arm crankcase, and could be measured using the gauge. The rotary speed of the disk was adjustable from several hundreds to 5,000 rpm.

2.1.2. Experimental Method

The excess sludge sampled in the Cleaning Center of Eastern Ube City required condensing. Since 9.0 l of condensed sludge was introduced into the experimental apparatus (Fig. 3.2), the system was set up and then samples were taken in regular intervals. The following

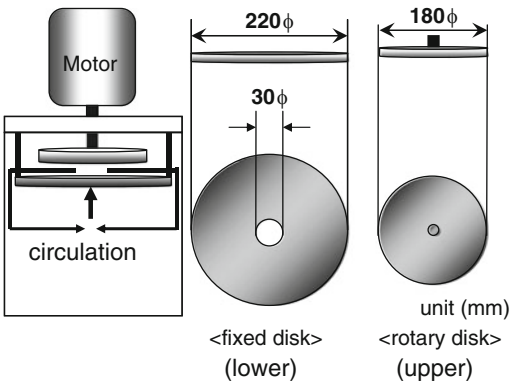

Fig. 3.2. Sketch of experimental apparatus (78).

Table 3.2
Parameters examined as experimental conditions (78)

	Concentrations (mg/L)	Treatment times (min)	Disk gaps (mm)	Rotary rate (rpm)
Examined parameters	2,000–3,000	20–120	0.1, 0.2, 0.4, 1, 2, 3, 5, 10, 20	3,500, 4,000, 4,500, 5,000

parameters were determined: Mixed Liquor Suspended Solids (MLSS), Mixed Liquor Volatile Suspended Solids (MLVSS), Biochemical Oxygen Demand (BOD), Total Organic Carbon (TOC), Dissolved Organic Carbon (DOC), and Particle size Distribution.

Table 3.1 shows the different experimental conditions for the various parameters, which were performed repeatedly to determine the optimum condition. The rotary rate was used as one parameter. Since the disk diameters were fixed in all experiments, the rotary rate can be thought as one index. Generally as for the rotary disk, the round speed, depending on the size of rotary disk and greatly affecting the fluid cut-force, cannot be regarded as an index. Therefore, Table 3.2 shows the relation between the rotary rate and the round speed of a rotary disk.

Since the disk process may cause the temperature to rise within the reaction tank, it is necessary to understand the effect of temperature on the solubilization. Experiments were thus conducted to understand the effect of preheating on solubilization. The established maximum temperature was 70°C, which was maintained for 30 min. This retention time was shorter when compared with the standard time of disk process of 45 min.

Both anaerobic and aerobic experiments were performed to understand the biodegradability of excess sludge after solubilization. In the anaerobic experiment, a vial test was adopted to observe the biodegradability under anaerobic condition. The anaerobic experiment used an effective volume 75 mL of vial bottle and sampled 40 mL (added digested sludge as seed sludge by a rate of 10 mL) of the rotary-disk-treated excess sludge (SS = 17,900 mg/L, VSS = 10,300 mg/L) from the sewage plant of Eastern Ube City; the temperature of the reactor was 35°C, and the shaking rate was 100/min. On the other hand, BOD_{20} was determined in the aerobic one. The nitration process was not controlled during the BOD_{20} measurement. In each experiment, the filtrates before (used as the control) and after treatment (rotary speed: 5,000 rpm, disk interval: 5 mm, the duration of treatment: 45 min) and treated one containing SS (as a total) were examined. Again, the filtrate paper (1.0 μm) was used. SS-contained TOC was directly determined using a TOC Analyzer after the dilution and ultrasound resolution of treated samples.

The Sewage Test Method was used to determine MLSS, MLVSS, and BOC_{20}. TOC and DOC were measured using a Total Carbon Analyzer (TOC-5000 SHIMADZU Co., Ltd.) and the particle-size distribution was achieved using the laser diffraction/scattering device (LA-920, HORIBA Co., Ltd.). Gas was measured using a Gas-chromatograph (GC-8APT, SHIMADZU Co., Ltd.).

2.2. *Optimum Operating Conditions of Experimental Apparatus*

2.2.1. Changes of Medium Radius with Disk Process

Figure 3.3 shows the change of a medium radius with treatment process. Here, the medium radius refers to the middle value. Figure 3.3 shows an obvious decrease in medium radius in the initial period (the first 1 min later) and then a slow decrease until 20 μm later. Therefore, we can infer that the most significant effect of a disk process on particle size appears in the initial stage (the first minute). Figure 3.4 shows the changes of a medium radius in the heating process (70°C, 30 min, namely preheating) and the succeeding medium treatment with heating. It was found that preheating may cause a decrease of 20 μm in the medium radius. Since the medium radius resulting from the succeeding disk process decreased to the same extent of a medium radius which had not been preheated (Fig. 3.3), we can conclude that preheating has little effect on the change of medium radius.

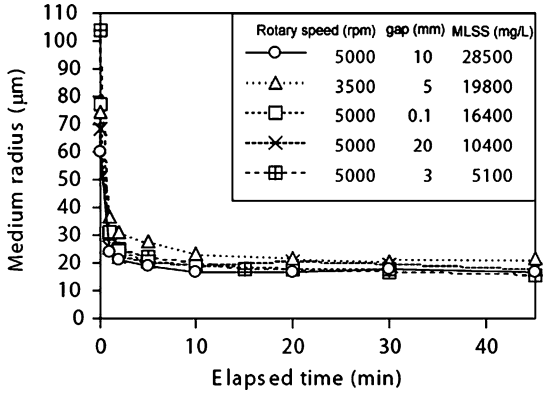

Fig. 3.3. Elapsed change of medium radius (one case) (78).

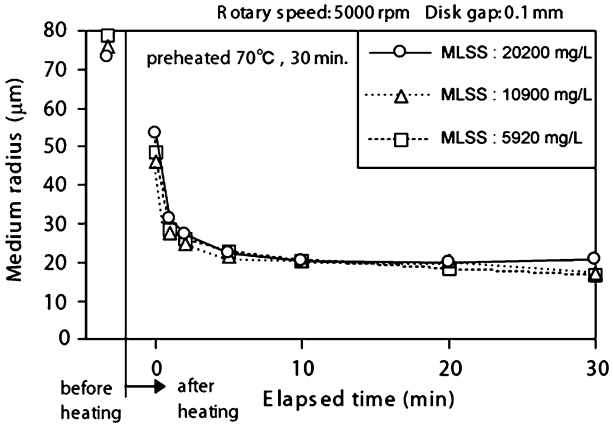

Fig. 3.4. Change of medium radius via heating (78).

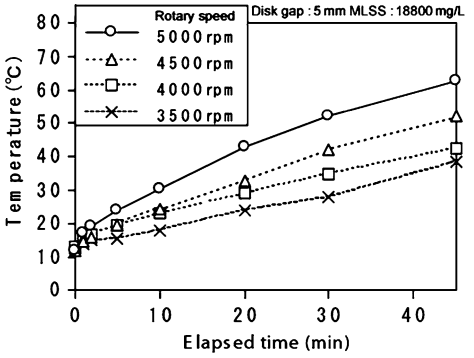

Fig. 3.5. Relationship of rotary rate and temperature (78).

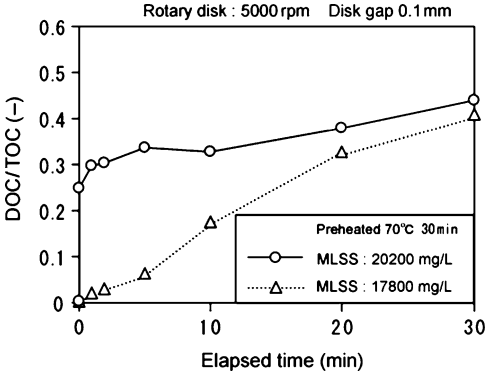

Fig. 3.6. Comparison of solubilization rates of preheated and unheated sludge (78).

2.2.2. Effects of Preheating Process on Solubilization

Figure 3.5 shows the temperature rise in the reactor tank with time in various rotary speeds, indicating that the higher the rotary speed, the more significant the temperature rise. It is assumed that since the cell membrane of bacteria is formed with the temperature rise, this facilitates solubilization. Experiments were conducted to examine the solubilization caused by preheating process. Figure 3.6 compares the solubilization rates of preheated and unheated sludge and notes a significant solubilization rate difference only in the initial 20 min of the experiment. It was concluded that preheating does little to enhance the solubilization rate of sludge by disk process. Moreover, the heating may realize 25% of sludge solubilization, implying that thorough incorporation of heating and disk process is needed for higher than 40% of solubilization rate. Therefore, the preheating seems to be unnecessary if cost is a consideration. However, because the initial solubilization can be promoted by preheating, the treatment period may be shortened.

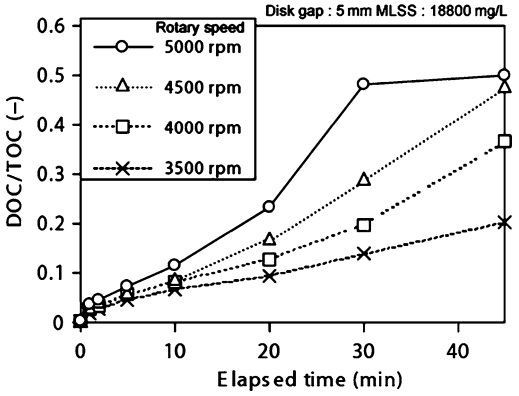

Fig. 3.7. Changes of solubilization rate with rotary speed (78).

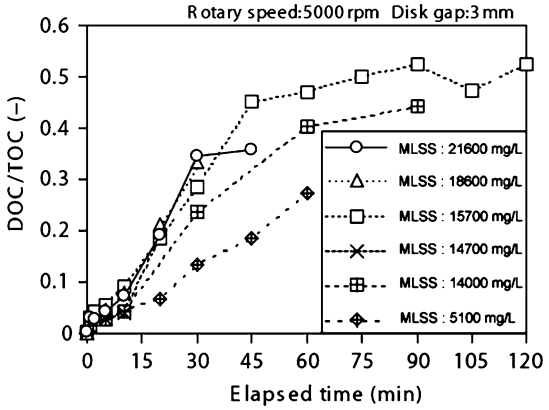

Fig. 3.8. Changes of solubilization rates with treatment (78).

2.2.3. Effects of Rotary Speed of Disk on Solubilization

Figure 3.7 indicates that since the solubilization rate of sludge depends on rotary speed, high rotary speeds may enhance the solubilization rate. At 5,000 rpm, the solubilization rate may gradually reach the steady state, and at other rotary speeds, it takes more time in treatment to reach the same solubilization rate with that at 5,000 rpm. Although the higher rotary speed may cause the higher initial solubilization rate, the lengthy treatment also can promote the solubilization rate even though the solubilization rate is low at an initial period. The decision to use this method should consider the costs or practicable conditions.

2.2.4. Changes of Solubilization Rate with Treatment Process

The changes in solubilization rate (rotary speed is 5,000 rpm) with treatment, which are given in Fig. 3.8, indicate that for sludge with an MLSS up to 20,000 mg/L, it takes more than 30 min for the solubilization rate to reach a steady state, while for that of 15,000–16,000 mg/L,

it takes 45–60 min. However, the steady state cannot be achieved for sludge with MLSS at 5,000 mg/L. Therefore, it is concluded that a lengthy treatment may promote sludge solubilization. That is to say, the higher MLSS the sludge has, the shorter time is needed for the treatment process. The reverse is also true: a sludge having a lower MLSS would require longer treatment. Which method to choose depends on both cost and treatment capacity considerations. However, experimental results show that if highly concentrated sludge were excessively treated, the burden on the motor would increase. As discussed above, if the sludge concentration exceeds 15,000 mg/L, further solubilization of sludge can hardly be observed 45–60 min later after the beginning of disk process. Thus, the duration of 50 min is thought to be appropriate for the disk process with the rotary speed of 5,000 rpm.

2.2.5. Effect of Disk Gap on Solubilization

Figure 3.9 shows that the difference of solubilization rates was negligible for different disk gaps. It means that the usual solubilization can be realized even if the gap is quite large. Since sand is often contained in sludge, a large disk gap is expected. That is to say, the solubilization also can be realized even for the highly concentrated sludge with the sand being largely mixed. If manufacture, practical operation, and maintenance are considered, it seems much better to adopt as large a disk gap as possible.

This conclusion means that the disk gap may be unlimitedly enlarged, that is, the solubilization can be realized using one rotary disk. Hence, we performed the solubilization process of excess sludge using only one disk besides the fixed one. The experiment results are shown in Fig. 3.10, in which experimental results obtained in the same conditions, with fixed disk being adopted, were also expressed for comparison. The results indicate that, compared with the experiment with different sludge concentrations and a fixed disk, the solubilization resulting from the process using one disk was 10% lower for the 30 min treatment and 15% lower for the 45 min treatment. As will be described later, since a high sludge concentration may cause high solubilization, it is estimated that a similar solubilization rate may be obtained for a sludge with the same sludge concentration. The feasibility of sludge solubilization by a single

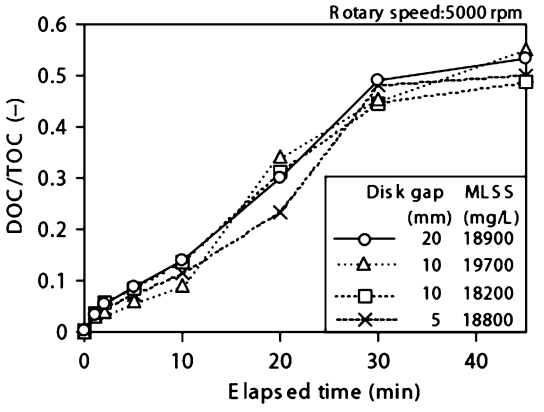

Fig. 3.9. Comparison of solubilization rates under different disk gaps (78).

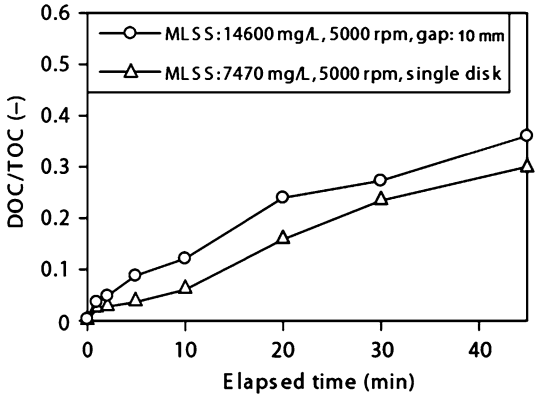

Fig. 3.10. Comparison of solubilization rates caused by two processes with single and double disks being adopted, respectively (78).

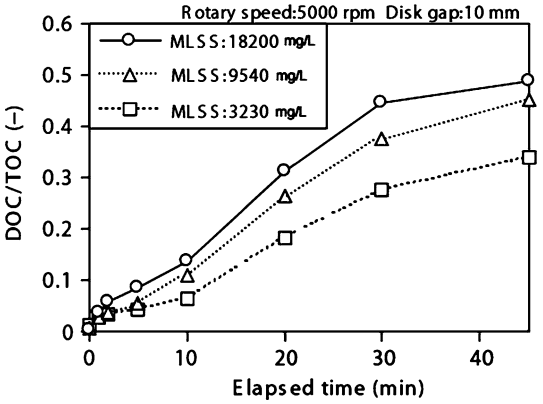

Fig. 3.11. Comparison of solubilization rates at different sludge concentrations (78).

disk implies that it is also quite advantageous when compared with the process in which a fixed disk is necessary despite considering the manufacturing fee, manufacturing precision, practical use, and maintenance of faculty.

2.2.6. Effects of Sludge Concentrations on Solubilization

Figure 3.11 shows the effect of sludge concentration, a high sludge concentration leads to significant solubilization. Although condensing sludge may enhance the solubilization efficiency, the optimum sludge concentration still depends on both the cost and time spent in sludge condensing. By comparing the solubilization rates shown in Figs. 3.3 and 3.5–3.11, we find that only the initial disk process significantly affects the medium radius and that the solubilization rate gradually increases with time. Therefore, it is considered that there is no proportional relation between them. This indicates that although the medium radius may become quite small at the initial stage of disk process, the microorganism cells still cannot be broken, and only exist apart in a frock state.

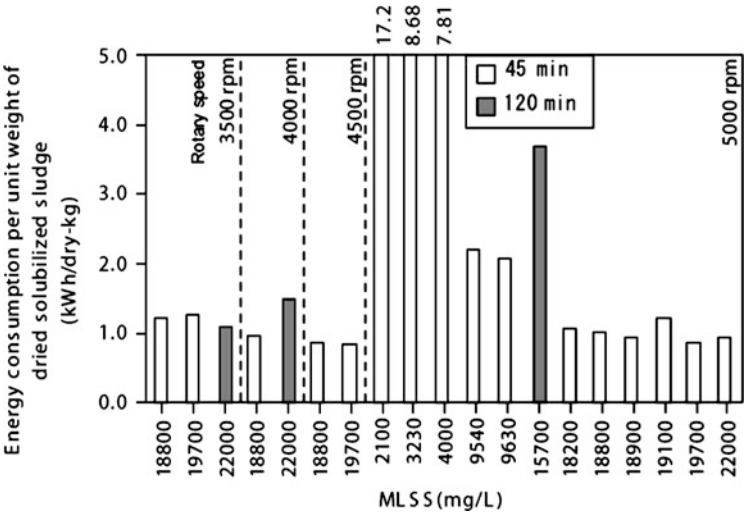

Fig. 3.12. Energy consumption for solubilized sludge (78).

2.2.7. Comparison of Treatment Cost

As described in 2.2.5, the solubilization rate has little relation with the disk gap. Hence, the energy consumption for a one-time treatment (45 min and 120 min) is shown by different rotary speeds in Fig. 3.12. Evidently, although a lower rotary speed requires less energy power, the more energy consumption per unit dry weight of solubilized sludge is also unavoidable because of its relatively low solubilization rate. However, as shown in Fig. 3.10, if a lengthy treatment is conducted on concentrated sludge at a low rotary speed, the energy consumption per unit dry weight of solubilized sludge may be reduced. Conversely, since a high rotary speed may quickly enable the solubilization rate to a steady level, a lengthy treatment will lead to the increase in the energy consumption per unit dry weight of solubilized sludge.

Figure 3.12 indicates clearly that, by the same rotary speed, a high sludge concentration may lower the energy consumption per unit dry weight of solubilized sludge. For the same rotary speed (especially for high rotary speed), a lengthy treatment will cause high energy consumption per unit dry weight of solubilized sludge even for high concentrated sludge.

Therefore, as for concentrated sludge, it is recommended to conduct a short treatment with a relatively high rotary speed (4,500–5,000 rpm), or relatively lengthy one with a low rotary speed for a higher efficiency of treatment.

2.3. Results and Discussion

The experimental results are as follows:

1. As for the sludge solubilization using a high-speed rotary disk, the medium radius diminished significantly in the initial stage of disk process, whereas the solubilization gradually increased with time. Therefore, the decrease in medium radius is not equal to the solubilization.

2. The disk process causes the temperature to rise in a reactor tank. The effect of temperature on solubilization indicates that the solubilization cannot be achieved to a large extent. Highly efficient solubilization can be achieved by the combination of disk process and heating.
3. A high rotary speed may result in high solubilization efficiency by shorter time, while a low rotary speed with a lengthy treatment time also can cause the high solubilization in the final stage. The choice of method should be decided by cost.
4. Because the solubilization rate changes little 45–60 min later after the beginning of disk process when the sludge concentration is over 15,000 mg/L, it is adequate to conduct the 45–60 min treatment if the rotary speed is 5,000 rpm.
5. Different disk intervals do not result in significantly different solubilization rates Therefore, it is considered better to adopt a disk interval as large as possible.
6. It is experimentally feasible to use only one rotary disk for sludge treatment. The decision to use this method should consider the device-manufacturing fee, manufacturing accuracy, practical use as well as maintenance.
7. Highly efficient treatment may be achieved by performing a short treatment with a high sludge concentration and considerable rotary rate (4,500–5,000 rpm), or by a lengthy treatment with a high sludge concentration and low rotary rate.
8. A half percentage of the solubilization-treated excess sludge may be digested by anaerobic digestion in a day, indicating its feasibility in practical use.

Results from the anaerobic biodegradation of solubilization-treated excess sludge significantly indicate that the application of this high-speed rotary disk process can realize the zero-sludge-charging aerobic sewage treatment.

3. BIODEGRADATION OF THE SLUDGE TREATED BY SOLUBILIZATION PROCESS

3.1. Anaerobic Biodegradation

Like the aerobic process, the anaerobic process is widely applied in sewage treatment. The anaerobic process refers to the degradation of organic matter by microorganisms living under airless conditions. In the aerobic process, organic components present in effluents are oxidized to gaseous carbon dioxide and water by oxygen; this process is similar to combustion, and the energy generated is used for the synthesis of new microorganisms. However, in an anaerobic process, organic components in effluents cannot be oxidized. They are changed to a gaseous state, i.e., methane, and then removed from wastewater. Moreover, since these organic components are transferred to such high-energy fuel as methane, there is less energy available for organism multiplication.

Solubilized sludge can be treated by the aerobic process, but has the disadvantages of high cost and deterioration of treated water quality, caused by the overcharge of influent BOD. Although heating is often required in the anaerobic process (usually 30–40°C, or 50–60°C), it is still thought to be advantageous from the energy-balance viewpoint, since highly concentrated solubilized sludge may lead to considerably large methane recovery per unit of effluent.

The following sections will discuss the anaerobic biodegradation of the sludge treated by solubilization.

3.1.1. Vial Test on Anaerobic Biodegradation

3.1.1.1. Objective

As the initial step, a vial batch test examines the anaerobic biodegradation of the sludge treated by solubilization. The vial batch test is widely used as a method to measure the bacterial activities concerning acid and methane formation in anaerobic waster treatment. Its advantage is that, under different conditions, multiple tests can be conducted simultaneously.

3.1.1.2. Methods and Experimental Conditions

Disk Process

- Excess sludge is collected and condensed if necessary
- The condensed excess sludge is introduced into the apparatus (Fig. 3.2) for treatment. Sampling is conducted at designed intervals, and then temperature and energy consumption are measured. Treatment lasts for 45 min. Sampling is done at 1, 2, 5, 10, 20, 30, and 45 min after experimental start. MLSS, MLVSS, TOC, and DOC are measured

Parameters Parameters (sludge concentration, treatment time, disk gap, rotary speed) shown in Table 3.3 were changed to examine the optimum experimental condition.

Method In this test, seed sludge and the substrate, i.e., digested sludge and excess sludge treated by disk process, respectively, were introduced into the vials (75 mL) in which the inner gases have been replaced completely by N_2 gas. The elapsed change of the volume of generated methane was observed in shaking water bath (water temperature: 36°C, shaking speed: 100/min). 30 mL of digested sludge and 10 mL of the substrate were used. Generally, the initial substrate with 1,000–3,000 mg-COD/l is considered to be appropriate in a vial test. Here, the substrate was diluted (COD from 2,920 to 1,460 mg/L). Table 3.4 describes the digested and excess sludge.

Table 3.3
Relationship of rotary rate and rotating speed (78)

Rotary rate (rpm)	Rotating speed
3,500	33.0
4,000	37.7
4,500	42.4
5,000	47.1

Table 3.4
Experimental parameters (79)

	Sludge concentration (mg/L)	Treatment time (min)	Disk gap (mn) (mn)	Rotary speed (rpm)
Experimental condition	15,800	45	10	5,000

A Gas Chromatograph (SHIMADZU, GC-8APT) was used to measure the gaseous composition. The volume of generated gases was measured using a 20-mL injector. Gaseous compositions were calculated as follows:

$$\text{Percentage of hydrogen in gases}(\%) = \text{Measured results of } H_2 \times 100/\text{Total volume of gases}$$

$$\text{Percentage of nitrogen in gases}(\%) = \text{Measured results of } N_2 \times 100/\text{Total volume of gases}$$

$$\text{Percentage of methane in gases}(\%) = \text{Measured results of } CH_4 \times 100/\text{Total volume of gases}$$

$$\text{Percentage of carbon dioxide in gases}(\%) = \text{Measured results of } CO_2 \times 100/\text{Total volume of gases}$$

Methane Transfer Rate (MTR) was calculated as follows:

(a) Total volume of gases means the sum of gases including the daily generation of gases and sample gas in vials

(b) Calculate the volume of methane contained in vials on each day using the following formula, from the percentage of methane calculated by the above-mentioned formula.

$$\text{Volume of methane}(\text{mL/day}) = \text{Total volume of gases}(\text{mL/day}) \times \text{Percentage of methane}(\%)$$

(c) Temperature correction on the volume of methane (mg/day)

$$\text{Volume of methane after temperature correction}(\text{mL/day}) = \text{Volume of methane before temperature correction}(\text{mL/day}) \times \text{Percentage of methane}(\%)$$

(d) Pressure correction on the volume of methane

$$\text{Volume of methane after pressure correction (mL/day)} = \text{Volume of methane after temperature correction}(\text{mL/day}) \times (760 - 42.2)$$

(e) Calculate the TOC by the following formula using the volume (mL) of introduced substrate and TOC of the substrate (mg/L).

$$\text{Total organic carbon of substrate (mg-TOC)} = \text{TOC of the substrate (mg/L)} \times \text{Volume of the substrate (mL)}$$

(f) Calculate the chemical oxygen demand of substrate (mg-COD) using the following formula.

$$\text{Chemical oxygen demand of substrate (mg-COD)} = \text{Total organic carbon of substrate (mg-COD)} \times 2.667$$

(g) Calculate theoretical volume (TV) of daily generated methane:

$$\text{TV (mL)} = \text{COD (mg/L)} \times 0.35$$

(h) Calculate MTR as follows:

$$\text{MTR (\%)} = [\text{Accumulative methane generation (mL)/TV (mL)}] \times 100$$

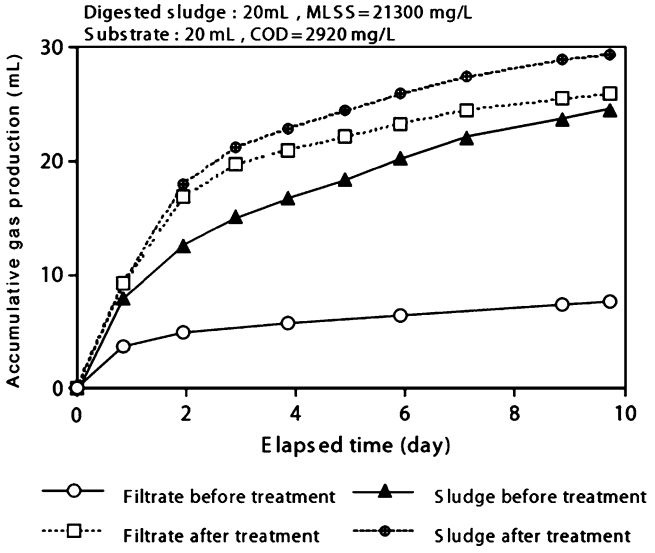

Fig. 3.13. Elapsed changes of accumulative gas generation (79).

3.1.1.3. Results and Discuss

Accumulative gas generation, accumulative methane generation, and elapsed changes of the MTR in the experiment are shown in Fig. 3.13, 3.14, and 3.15, respectively. Accumulative gas generation (Fig. 3.13) was found 30% higher for the sludge treated by disk process than for untreated sludge; also, more treated sludge could be filtered. Although the major gaseous products of anaerobic digestion are carbon dioxide and methane, the accumulative methane generation of treated sludge has been found to be 20% more than that of untreated sludge (Fig. 3.14). This indicates that the biodegradation has been improved by the disk process. Filtered treated sludge has caused higher accumulative methane generation than untreated one. However, the initial methane generation rate was also fast. Figure 3.15 describes how much substrate can be recovered in the form of methane. Methane self-generated from the digested sludge that was introduced to the bioreactor as seed sludge is not discussed here. Experimental results also show that the disk process can enhance the biodegradation of sludge because of the higher methane generation of treated sludge than that of untreated sludge. The MTR was over two times higher for filtered treated sludge than for treated and untreated sludge, indicating a high biodegradation of filtered treated sludge.

Table 3.5 gives the reduction rate of MLSS and MLVSS. It was found that for treated sludge, the reduction rate was three times lower for MLSS and three times higher for MLVSS when compared to untreated sludge. Therefore, we can conclude that the disk process is effective for the volume reduction of excess sludge.

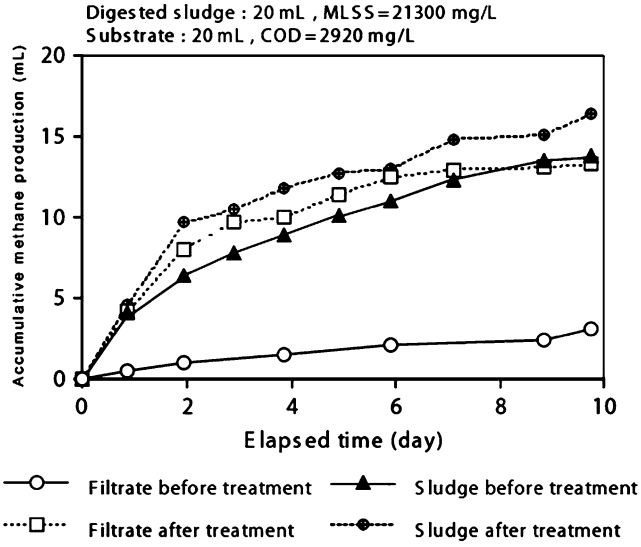

Fig. 3.14. Elapsed changes of accumulative methane generation (79).

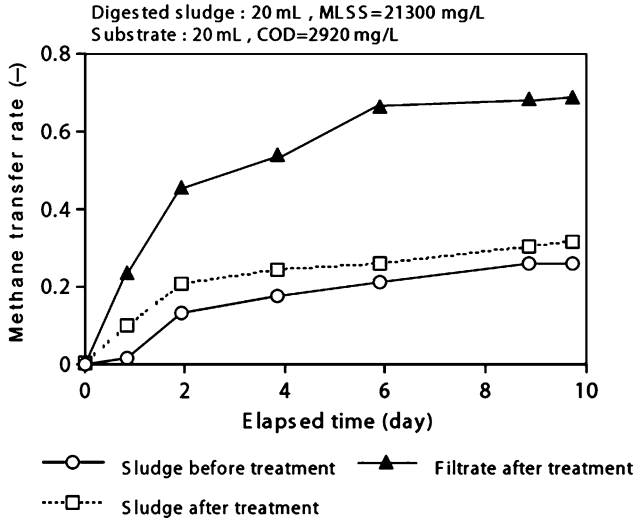

Fig. 3.15. Elapsed changes of MTR (79).

3.1.1.4. Conclusion

(a) It was proved in a vial test that the solubilization process can improve the biodegradation of treated sludge;
(b) In the case of the vial test, COD of initial substrate is suggested to be in the range of 1,000–3,000 mg/L;

Table 3.5
Digested sludge and excess sludge (79)

	MLSS (mg/L)	MLVSS (mg/L)	Solubilization rate after solubilization process (%)
Digested sludge	21,300	12,500	–
Excess sludge	15,800	10,700	36.6

Table 3.6
Reduction rates of MLSS and MLVSS (79)

	MLSS	MLVSS
Untreated sludge	0.047	0.053
Treated sludge	0.153	0.174

(c) The reduction rate is higher than as for treated sludge than untreated one, therefore the disk process is effective for volume reduction of excess sludge.

3.1.2. Continuous Experiment on Anaerobic Biodegradation

3.1.2.1. OBJECTIVE

Results of the vial test indicated the effectiveness of disk process for improving sludge biodegradation under anaerobic conditions. However, since the vial test is only a batch experiment, it is necessary to perform experiments that are much closer to a practical situation for continuous anaerobic sludge treatment in practice. Therefore, further examination on the anaerobic biodegradation of treated sludge should be made more completely in the following continuous sludge treatment.

3.1.2.2. METHOD AND EXPERIMENTAL CONDITIONS

Disk Process

- Excess sludge (10 l) is collected and condensed if necessary
- The condensed excess sludge is introduced into the apparatus (Fig. 3.2) for treatment. Sampling is conducted at designated intervals, then temperature and energy consumption are measured. Treatment lasts for 60 min. Sampling is done at 1, 2, 5, 10, 20, 30, 45, and 60 min after experimental start. MLSS, MLVSS, TOC, and DOC are measured

Parameters Parameters (sludge concentration, treatment time, disk gap, rotary speed) shown in Table 3.6 were changed to examine the optimum experimental condition.

Apparatus and Method of Continuous Experiment The apparatus is shown in Fig. 3.16. It comprises one bottle digester in which digested sludge and the substrate are mixed and then treated, and one gas capturer that was used to collect gaseous products generated in aqueous phase. Water in gas capturer is saturated with NaCl to depress the solution of gaseous products. Digested sludge (1.5 l), as seed sludge, was introduced into the digester bottle (about 2 l) in which inner gases were completely replaced by nitrogen gas. With the untreated sludge used as the substrate, HRT of treated sludge was set at operating conditions 1, 2 and 3,

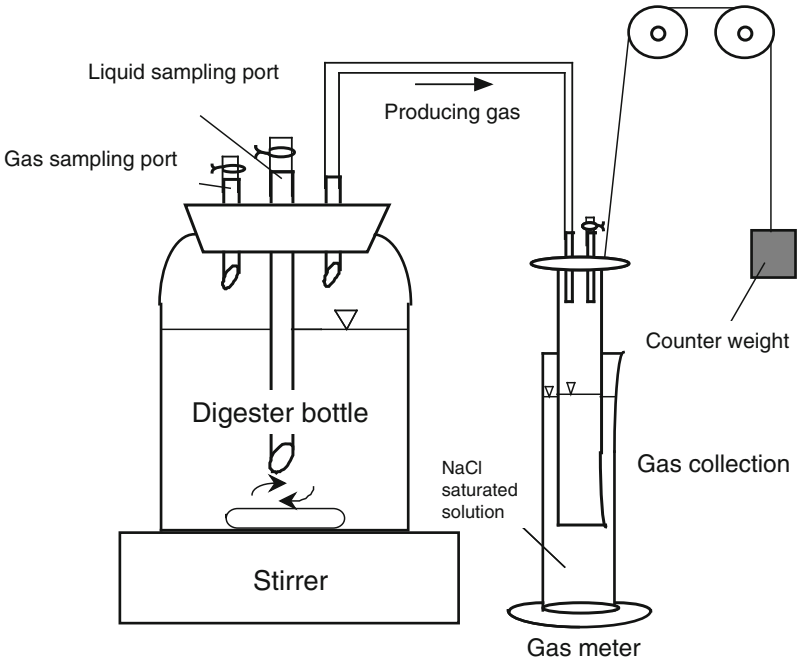

Fig. 3.16. Sketch of apparatus for continuous experiment (79).

Table 3.7
Experimental parameters (79)

	Sludge concentration (mg/L)	Treatment time (min)	Disk gap (mm)	Rotary speed (rpm)
Experimental conditions	13,000–22,500	60	10	5,000

respectively, and a daily sampling was made. After each sampling, the substrate was added. In this experiment, the bottle digesters were kept at 36°C with the inner digester stirred by a motor stirrer. MSS, MLVSS, TOC and DOC of samples were measured.

Running conditions of continuous experiments are shown in Table 3.7. The establishment of HRT was based on the fact that HRT of anaerobic process is usually 20–30 days. Digested sludge and substrates with different concentrations (see Table 3.8) were used under three different operating conditions, respectively. A maximum of three experiments could be conducted simultaneously in this study.

Sampling method

(a) Measure the temperature of the bottle digester;
(b) Measure the volume of gases generated and collected in the gas capturer;

Table 3.8
Running condition of continuous experiment (79)

Running conditions	Substrate	HRT (day)	Volume of sample (mL)	Temperature (°C)
1	Untreated sludge	30	050	
	Treated sludge	30	050	36
	Treated sludge	15	100	
2	Untreated sludge	20	075	
	Treated sludge	20	075	36
	Treated sludge	10	150	
3	Untreated sludge	10	150	
	Treated sludge	10	150	36
	Treated sludge	05	300	

(c) Analyze the compositions of gaseous products by extracting 0.5 mL of gases by the sampling outlet of the bottle digester;
(d) Liquid samples were taken from the liquid sampling outlet in each retention time. Later, a same amount of the substrate was added;
(e) Extract gases to some extent if gas accumulation is found in gas capturer;
(f) Sample analysis.

3.1.2.3. RESULTS AND DISCUSSION

Running Condition 1 (HRT 30 Days, 15 Days) Experimental results under operating condition 1 are given in Figs. 3.17 and 3.18 and Tables 3.9 and 3.10. Figure 3.17 describes the elapsed change of accumulative methane generation, Fig. 3.18 gives the MTR, and Table 3.19 gives the average methane generation rate and average MTR. Figure 3.17 shows that with the same retention time at 30 days, the volume of generated methane is 1.4 times higher for treated sludge than for untreated one, indicating that the disk process has enhanced the biodegradation of sludge. This is also demonstrated by the average methane generation rate and average MTR in Table 3.9. Even the untreated sludge for which HRT was set at 15 days (Fig. 3.18 and Table 3.9), MTR was still 1.3 times higher than that of untreated sludge with two times of RHT (30 days). It was concluded that methane fermentation may be conducted even with a shorter HRT. Therefore, it is necessary to further examine how much treatment time can be reduced by shortening HRT. Furthermore, the idea that increasing MTR at an early stage (Fig. 3.18) may be attributed to the volume of biogases generated greatly at an early experimental stage has not been deduced here. Table 3.10 gives relative results of MLSS and MLVSS with that of untreated sludge being regarded as 1, for the comparison of MLSS and MLVSS reduction rates after established HRT. In the same HRT (30 days), reduction rates of MLSS and MLVSS are 1.8 and 1.6 times higher for treated sludge than for untreated sludge. Moreover, the reduction rates of MLSS and MLVSS for treated sludge in "half" of HRT (15 days) are still 1.2 and 1.1 times higher, respectively, than that for untreated sludge in "whole" HRT (30 days). This indicates that anaerobic treatment may be shortened further.

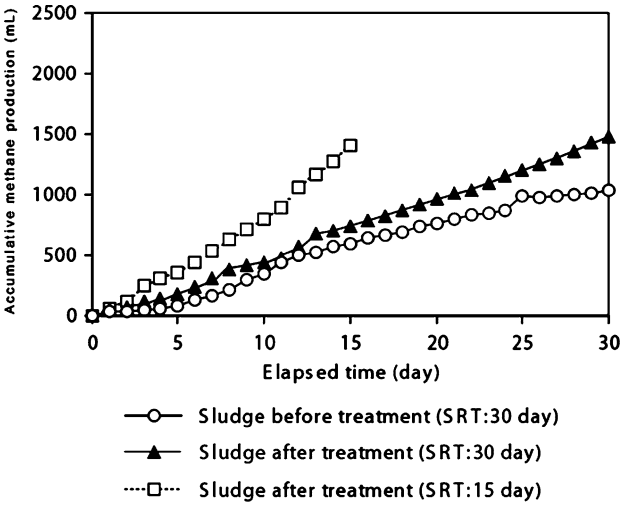

Fig. 3.17. Elapsed change of accumulative methane generation (79).

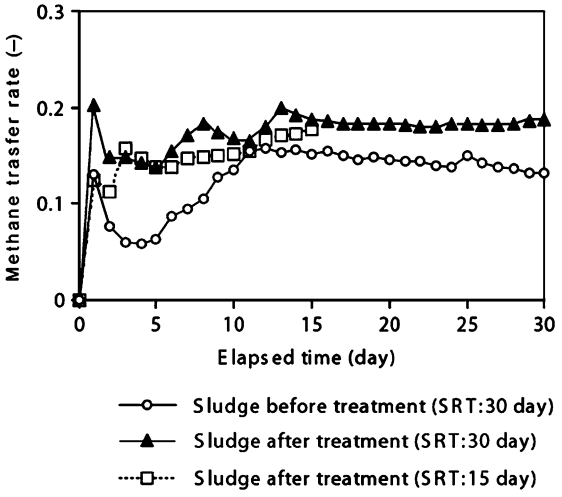

Fig. 3.18. Elapsed change of MTR (operating condition 1) (79).

Running Condition 2 (HRT 20 Days, 10 Days) Experimental results under operating condition 2 are given in Figs. 3.19 and 3.20 and Tables 3.11 and 3.12. Figure 3.19 describes the elapsed change of accumulative methane generation, Fig. 3.20 gives the MTR, and Table 3.11 gives the average methane generation rate and average MTR. Figure 3.19 shows that with the same retention time at 20 days, the volume of generated methane is 1.2 times higher for treated sludge than for untreated sludge, indicating that the disk process has enhanced the biodegradation of sludge. This is also demonstrated by the average methane generation

Table 3.9
Digested sludge and the substrates in continuous experiments (79)

Running conditions	Sludge	MLSS (mg/L)	MLVSS (mg/L)	Solubilization rate after disk process (%)
1	Digested sludge	17,500	10,300	–
	Excess sludge	13,700	10,100	31.0
2	Digested sludge	15,100	09,800	–
	Excess sludge	13,900	10,000	37.0
3	Digested sludge	18,000	11,300	–
	Excess sludge	22,500	14,600	36.0

Table 3.10
Average methane generation rate and average MTR (running condition 1) (79)

Substrate	Retention time (day)	Average gas generation rate per w. of VSS	Average transfer rate (%)
Untreated sludge	30	6.70×10^{-2}	13.5
Treated sludge	30	9.30×10^{-2}	18.5
Treated sludge	15	8.90×10^{-2}	17.7

rate and average MTR in Table 3.11. Even for the untreated sludge which had an HRT set at 10 days (Fig. 3.19 and Table 3.11), the MTR was still at the same level with that of untreated sludge with two times of RHT (20 days). It was concluded that methane fermentation may be conducted even as HRT becomes shorter. Therefore, it is necessary to further examine how much treatment the time may be reduced by shortening HRT. Furthermore, the idea that increasing MTR at early stage (Fig. 3.18) may be attributed to that the volume of biogases generated greatly at early experimental stage could not be deduced here. Table 3.12 gives relative results of MLSS and MLVSS with that of untreated sludge being regarded as 1, for the comparison of MLSS and MLVSS reduction rates after established HRT. In the same HRT (20 days), reduction rates of both MLSS and MLVSS are nearly 1.3 times higher for treated sludge than for untreated one. The reduction rates of both MLSS and MLVSS for treated sludge in "half" of HRT (15 days) are still nearly 1.2 times higher than that for untreated sludge in "whole" HRT (20 days). This indicates that anaerobic treatment may be shortened further.

Running Condition 3 (HRT 10 Days, 5 Days) Experimental results under operating condition 3 are given in Figs. 3.21 and 3.22 and Tables 3.13 and 3.14. Figure 3.21 describes the elapsed change of accumulative methane generation, Fig. 3.22 gives the MTR, and Table 3.13 gives the average methane generation rate and average MTR. Figure 3.21 shows that with the same retention time at 10 days, the volume of generated methane is 1.9 times higher for treated sludge than for untreated sludge, indicating that the disk process has enhanced the biodegradation of sludge. However, since the HRT of untreated sludge was shorter than that

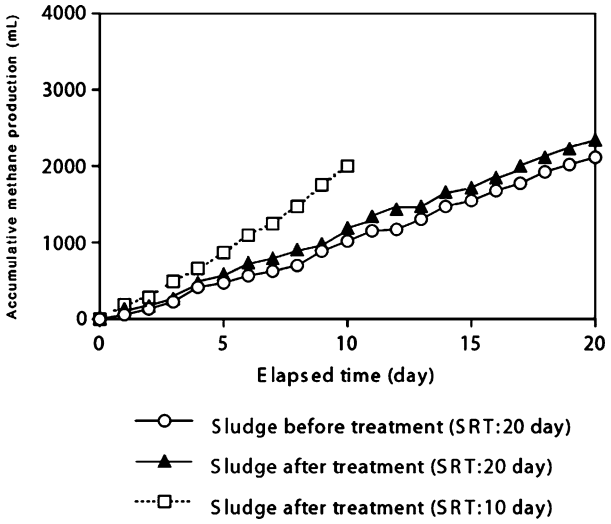

Fig. 3.19. Elapsed change of accumulative methane generation (operating condition 2) (79).

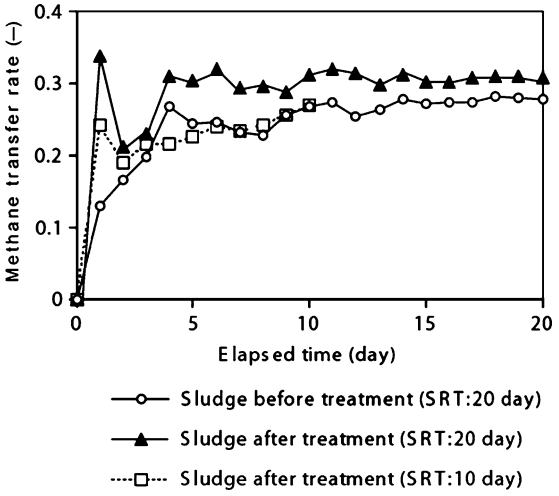

Fig. 3.20. Elapsed change of MTR (operating condition 2) (79).

of a normal anaerobic process (20–30 days), it is speculated that an incomplete anaerobic process of untreated sludge caused this difference. This reason may also be used to explain MTR (Fig. 3.22). By this MTR, we cannot determine whether methane generation may be conducted.

Table 3.14 shows MLSS and MLVSS reduction rates after HRT is established. Even in the same HRT (10 days), MLSS and MLVSS were 1.1 and 1.2 times higher for untreated sludge than for treated sludge, respectively. However, for the treated sludge with HRT established at

Table 3.11
Comparison of reduction rates of MVSS and MLVSS (running condition 1) (79)

Running condition	Substrate	Retention time (day)	Relative MLSS reduction rate	Relative MLVSS reduction rate
1	Untreated sludge	30	1.00	1.00
	Treated sludge	30	1.80	1.60
	Treated sludge	15	1.20	1.11

Table 3.12
Average methane generation rate and average MTR (running condition 2) (79)

Substrate	Retention time (day)	Average gas generation rate per w. of VSS	Average transfer rate (%)
Untreated sludge	20	12.70×10^{-2}	26.0
Treated sludge	20	15.20×10^{-2}	30.8
Treated sludge	10	12.90×10^{-2}	26.0

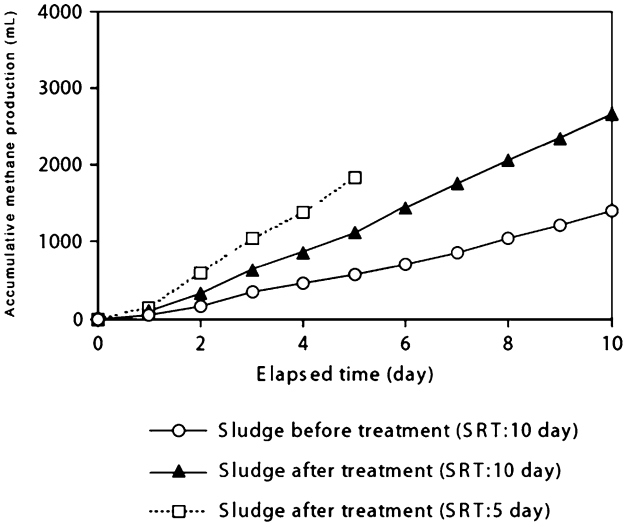

Fig. 3.21. Elapsed change of accumulative methane generation (operating condition 3) (79).

5 days, MLSS and MLVSS reduction rates were 71 and 87% of that of untreated sludge with HRT at 10 days, respectively. Accumulative methane generation seems to have not reached incomplete degradation of treated sludge under this experimental condition. Since MLSS and MLVSS reduction rates decreased for untreated sludge, it was thought that shortening the treatment time to 5 days would be difficult. Therefore, we can conclude that the anaerobic treatment time combined with the disk process is best limited to 5–10 days.

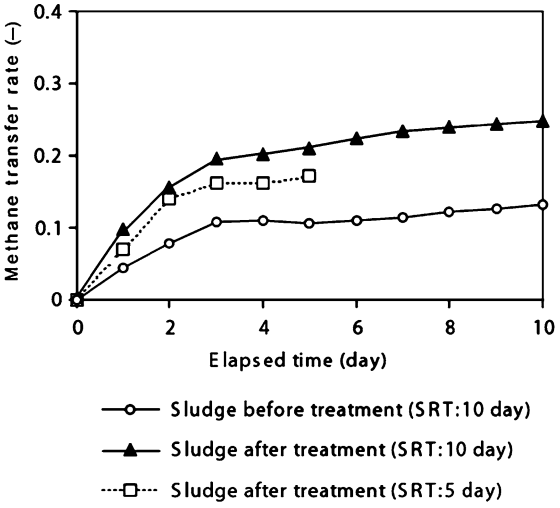

Fig. 3.22. Elapsed change of MTR (operating condition 3) (79).

Table 3.13
Comparison of reduction rates of MVSS and MLVSS (running condition 2) (79)

Running condition	Substrate	Retention time (day)	Relative MLSS reduction rate	Relative MLVSS reduction rate
	Untreated sludge	20	1.00	1.00
2	Treated sludge	20	1.33	1.26
	Treated sludge	10	1.20	1.17

Table 3.14
Average methane generation rate and average MTR (running condition 3) (79)

Substrate	Retention time (day)	Average gas generation rate per w. of VSS	Average transfer rate (%)
Untreated sludge	10	5.3×10^{-2}	13.1
Treated sludge	10	10.20×10^{-2}	24.7
Treated sludge	5	8.2×10^{-2}	17.0

3.1.2.4. Conclusion

(a) Continuous experiments showed that after the disk process, the anaerobic biodegradation of sludge would increase significantly;
(b) Higher MLSS and MLVSS reduction rates for treated sludge with "half" HRT than that of untreated sludge with whole HRT indicated that treatment time could be shortened to 10 days.

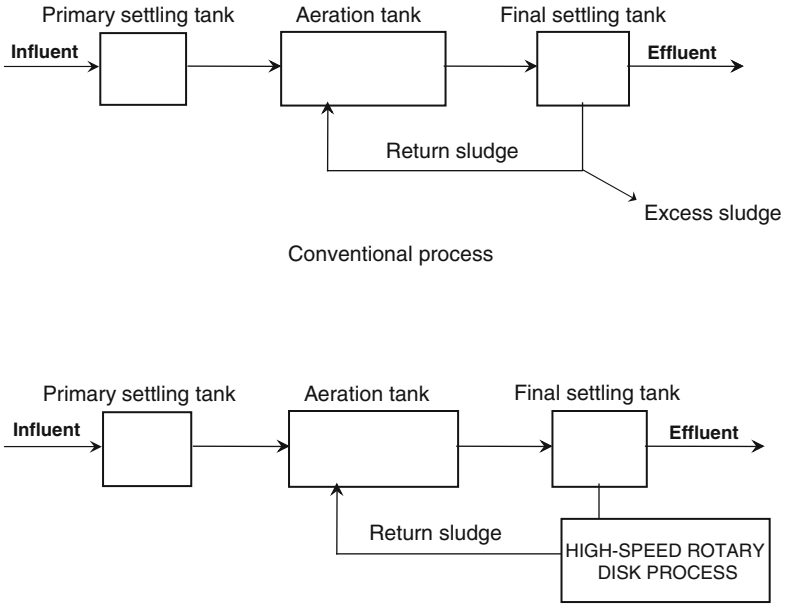

Fig. 3.23. Flow of aerobic process (79).

3.2. Aerobic Biodegradation

Both anaerobic and aerobic processes (the latter in which the solubilization-treated sludge is returned to the aeration tank) may promote volume reduction of excess sludge (Fig. 3.23). However, the aerobic process of solubilized sludge still has the disadvantages of high operating costs and the water deterioration of effluents caused by high BOD of influents.

The following section discusses the possibility of volume reduction of excess sludge, with consideration of the negative factors.

3.2.1. Aerobic Biodegradation by BOD Experiment

3.2.1.1. Objective

Biochemical Oxygen Demand (BOD) first was measured to examine the aerobic biodegradation of treated sludge. BOD may quantify the oxygen demand when microorganisms degrade organic matter.

3.2.1.2. Method and Experimental Conditions

Disk Process

(a) Condense the collected sludge (10 l);
(b) Introduce the condensed sludge into the experimental apparatus shown as Fig. 3.24 for the heating process. The apparatus should be heated with warm water as early as possible;

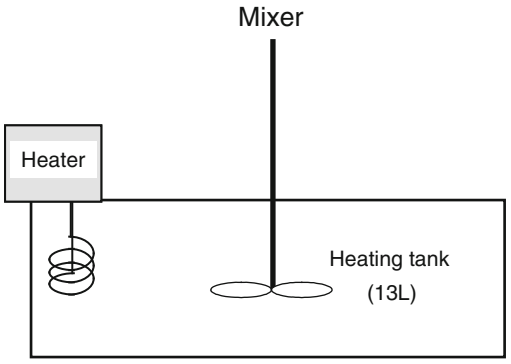

Fig. 3.24. Sketch of heating apparatus (79).

Table 3.15
Comparison of reduction rates of MVSS and MLVSS (running condition 3) (79)

Running condition	Substrate	Retention time (day)	Relative MLSS reduction rate	Relative MLVSS reduction rate
2	Untreated sludge	10	1.00	1.00
	Treated sludge	10	1.10	1.17
	Treated sludge	5	0.71	0.87

(c) Deliver the heated sludge into the experimental apparatus as shown as Fig. 3.2. Sampling is conducted at designated intervals, then temperature and energy consumption are measured. Treatment lasts for 45 min. Sampling is done at 1, 2, 5, 10, 20, 30, and 45 min after the start of the experiment. MLSS, MLVSS, TOC, and DOC are measured.

Parameters Parameters (sludge concentration, treatment time, disk gap, rotary speed) shown in Table 3.15 were changed to examine the optimum experimental condition.

Results and Discussion Filtrates of untreated sludge (control) and treated sludge (soluble) and SS-contained treated sludge (Total) have been examined in this experiment. Here, MLSS and MLVSS of excess sludge were 21,110 and 152,000 mg/L, respectively, and the solubilization rate of sludge treated by disk process was 41%. All results are shown in Table 3.16, which includes increases in BOD of the filtrate and SS-contained yield of treated sludge. A comparison of filtrates of sludge before and after the aerobic process (i.e., control and soluble) indicates that the solubilization of excess sludge by the disk process makes the sludge biodegradation easier. Therefore, it is concluded that the disk process may promote aerobic biodegradation.

Table 3.16
Experimental parameters (79)

	Sludge concentration	Preheating temperature (°C)	Treatment time (min)	Disk gap (mm)	Rotary speed (rpm)
Experimental condition	21,100	40	40	10	5,000

Table 3.17
Experimental results of BOD (mg/L) (79)

Elapsed time (day)	Before filtration	After filtration	After filtration SS being contained
00	00	0000	0000
05	07	4,880	6,435
10	20	5,850	9,275

3.2.2. Continuous Experiment on Aerobic Biodegradation

3.2.2.1. OBJECTIVE

The BOD experiment has demonstrated that the disk process can promote the aerobic biodegradation of sludge. Hence, this experiment examines the possibility of reducing the volume of excess sludge by a continuous aerobic process.

3.2.2.2. METHODS AND EXPERIMENTAL CONDITIONS

Disk Process

(a) Condense the collected sludge (10 l);
(b) Deliver the condensed sludge into the experimental apparatus shown in Fig. 3.2. Sampling is conducted at designated intervals, then temperature and energy consumption are measured. Treatment lasts for 60 min. Sampling is done at 1, 2, 5, 10, 20, 30, 45, and 60 min after the start of the experiment. MLSS, MLVSS, TOC, and DOC are measured.

Parameters Parameters (sludge concentration, treatment time, disk gap, rotary speed) shown in Table 3.17 were changed to examine the optimum experimental condition

Apparatus and Method of Continuous Experiment Figure 3.25 shows a sketch of the experimental apparatus, which consists of two parts: an aeration tank (20 l) and a sedimentation tank (7 l). The inner temperature of the aeration tank was controlled by keeping the outer temperature at 24°C using an air conditioning unit. The substrate (glucose is main carbon source) diluted to about 200 mg-BOD/l with tap water, was continuously introduced into the aeration tank (Table 3.18).

Table 3.19 shows the operating conditions for the experiment. HRT and MLSS of aeration tank were established at 24 h and 1,000–2,000 mg/L, respectively. In this experiment, excess sludge, after being treated by the disk process (MLSS: 18,800 mg/L, MLVSS: 14,600 mg/L, solubilization rate: 40%), was introduced into the aeration tank, and then the operating states of aeration tank as well as treated water were observed. In a contrasting experiment,

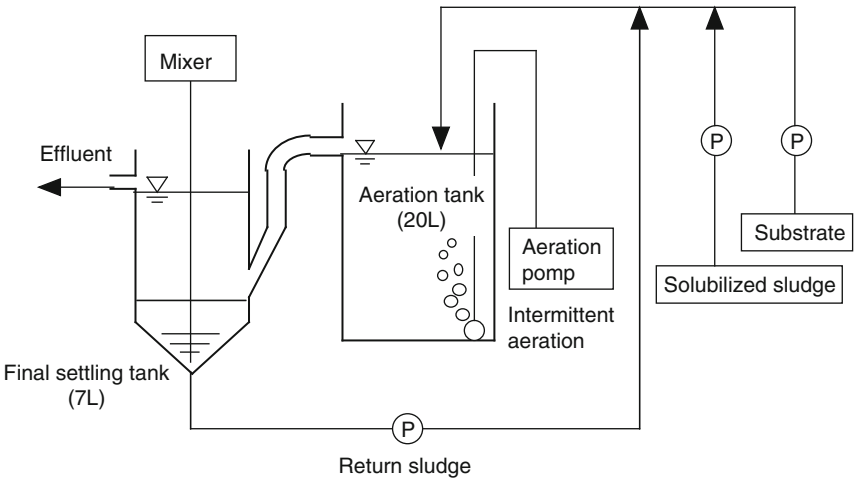

Fig. 3.25. Sketch of apparatus for continuous aerobic experiment (79).

Table 3.18
Experimental parameters (79)

	Sludge concentration (mg/L)	Treatment time (min)	Disk gap (mm)	Rotary speed (rpm)
Experimental condition	18,800	60	10	5,000

Table 3.19
Composition of the substrate (79)

Glucose substrate		Inorganic nutrient salts		
Glucose	0.28 g/L	A	$(NH_4)_2HPO_4$	350.0 g/L
Solution A	0.06 mL/L		KCl	75.0 g/L
Solution B	0.30 mL/L		NH_4Cl	85.0 g/L
Solution C	0.03 mL/L	B	$FeCl_3 \cdot 6H_2O$	42.0 g/L
$NaHCO_3$	0.12 g/L		$MgCl_2 \cdot 6H_2O$	81.0 g/L
K_2HO_4	0.12 g/L		$MgSO_4 \cdot 7H_2O$	25.0 g/L
Yeast extract	0.003 g/L		$CoCl_2 \cdot 6H_2O$	1.8 g/L
		C	$CaCl_2 \cdot 6H_2O$	150.0 g/L

solubilized sludge was not returned with only the common aerobic process being conducted. Treated waters and the solid-liquid suspending phases (SLSP) were sampled in an aeration tank once a day. MLSS, MLVSS, TOC, DOC, BOD, Total nitrogen (T-N), and Total phosphorus (T-P) were measured for treated waters, and MLSS, MLVSS, TOC, DOC, T-N, T-P, and Sludge Volume Index (SVI) were determined for SLSP.

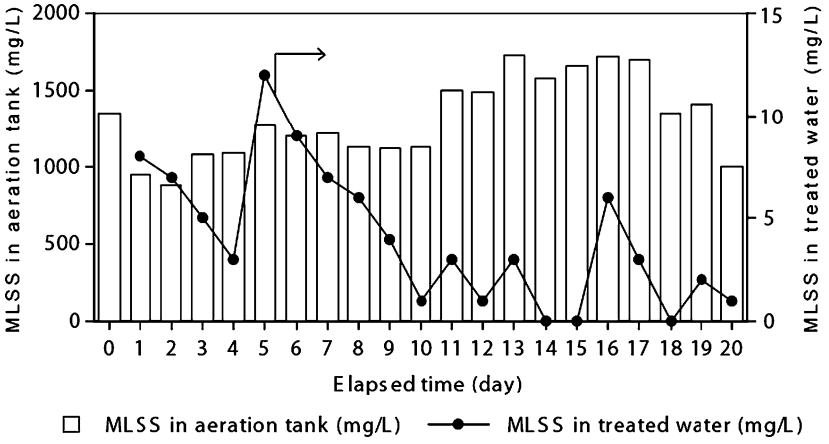

Fig. 3.26. Elapsed changes of MLSS of SLSP and treated water (contrast experiment) (79).

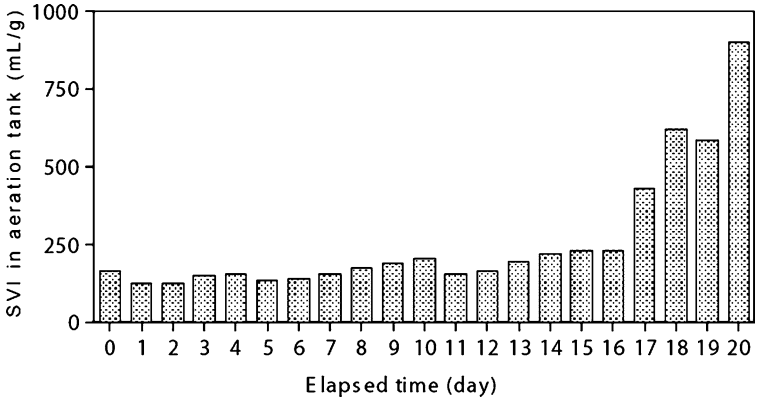

Fig. 3.27. Elapsed changes of MLSS in aeration tank (contrast experiment) (79).

3.2.2.3. Results and Discussion

Contrasting Experiment In Figs. 3.26–3.30, the results of contrasting experiments are shown, namely, MLSS of treated water and SLSP (Fig. 3.26), SVI of SLSP (Fig. 3.27), TOC and DOC (Fig. 3.28), and T-P (Fig. 3.29) and T-N (Fig. 3.30) of treated water and SLSP. Figure 3.23 shows that while MLSS of SLSP increased slightly with time, the MLSS of treated water was still lower than 10 mg/L. The decrease in MLSS of SLSP since the 18th day after the experimental start may be attributed to the increase in SVI of SLSP (see Fig. 3.27). Increasing SVI was caused by the changes of sludge in aeration tank, where the aerobic process was conducted without cleaning the excess sludge. Positive results also have been found in the TOC and DOC of treated water and T-N of SLSP and treated water (Figs. 3.28 and 3.29). T-P was found to be higher in SLSP and treated water than normal (Fig. 3.30). It is because that the phosphorus content in artificial wastewater is too high.

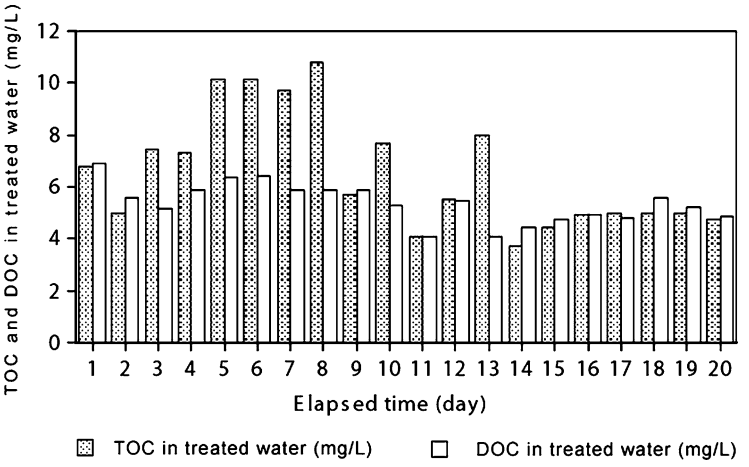

Fig. 3.28. Elapsed changes of TOC and DOC of treated water (contrast experiment) (79).

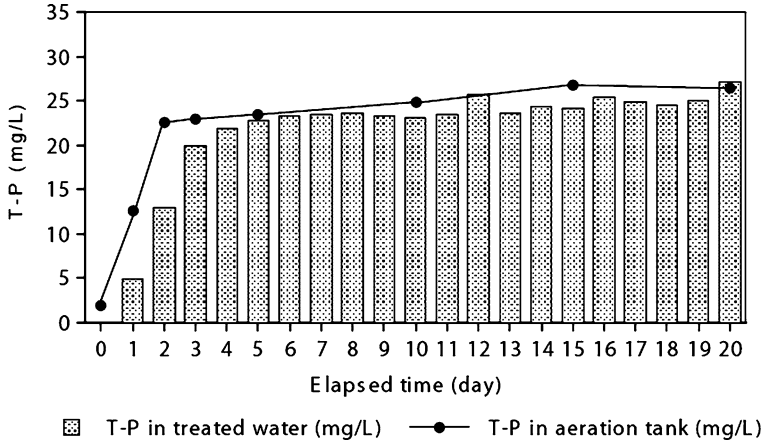

Fig. 3.29. Elapsed changes of T-P of SLSP and treated water (contrast experiment) (79).

Disk Process Experiment Figures 3.31–3.35 show the results of the disk process experiment namely, MLSS of treated water and SLSP (Fig. 3.31), SVI of SLSP (Fig. 3.32), TOC and DOC (Fig. 3.33), and T-P (Fig. 3.34) and T-N (Fig. 3.35) of treated water and SLSP. Figure 3.31 shows that MLSS were kept at a steady level in the aeration tank, although one increasing trend appeared during the first 5 days (Fig. 3.31). The MLSS of treated water was still lower than 10 mg/L. Figure 3.32 reveals the increase in MLSS of SLSP with the addition of sludge treated by the disk process. An increased SVI was brought about by the poor sedimentation of solubilized treated sludge in the aeration tank. Good results also have been found in the TOC and DOC of treated water and T-N of SLSP and treated water (Figs. 3.33 and 3.34). The T-P was higher than normal in SLSP and treated water (Fig. 3.35). It is because that the phosphorus contents in artificial wastewater is too high.

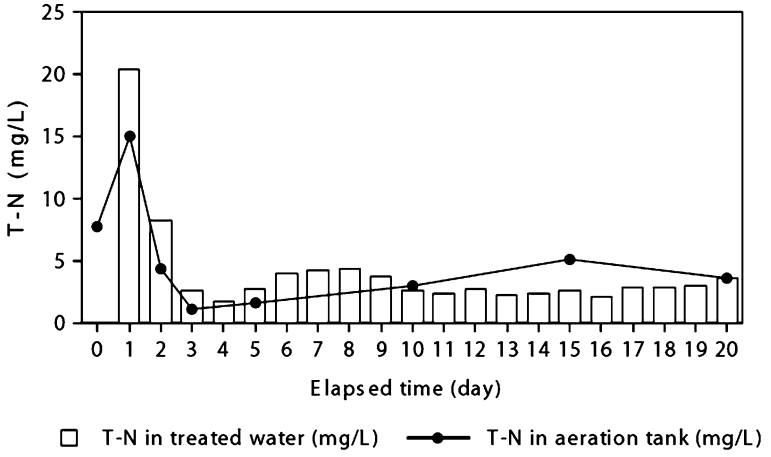

Fig. 3.30. Elapsed changes of T-N of SLSP and treated water (contrast experiment) (79).

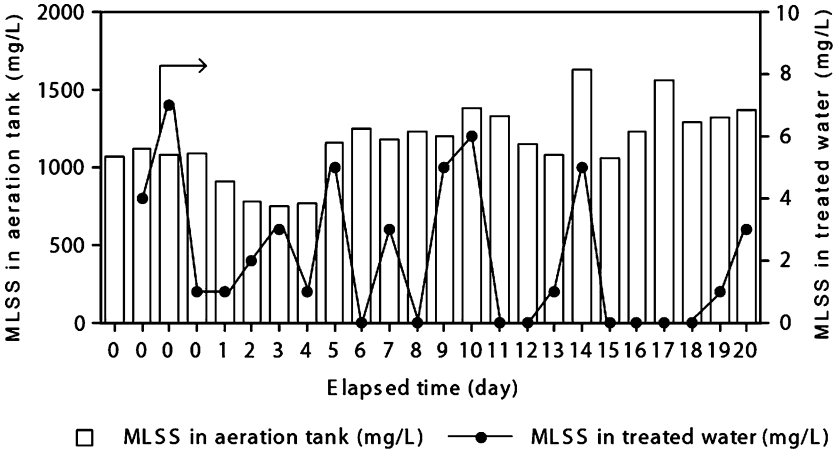

Fig. 3.31. Elapsed changes of MLSS of SLSP and treated water (disk process experiment) (79).

Comparison of Control and Disk Process Experiments Table 3.20 and Fig. 3.36 give the experimental results. The average values of water qualities of treated waters (Table 3.20) indicates that the aeration treatment associated with disk process may be carried out, with high water quality of treated water and insignificant difference with that of the contrasting system. That is, the application of a high-speed rotary disk process had little influence on the treated water. A higher than normal T-P was attributed to the fact that phosphorous-containing inorganic salts were added into the substrate, glucose (Table 3.21). Figure 3.36 compares daily sludge production between the two experimental conditions. It was found that sludge production in the disk process experiment was 60% lower than that of the contrasting system. Therefore, a high-speed rotary disk process can reduce the sludge production in an

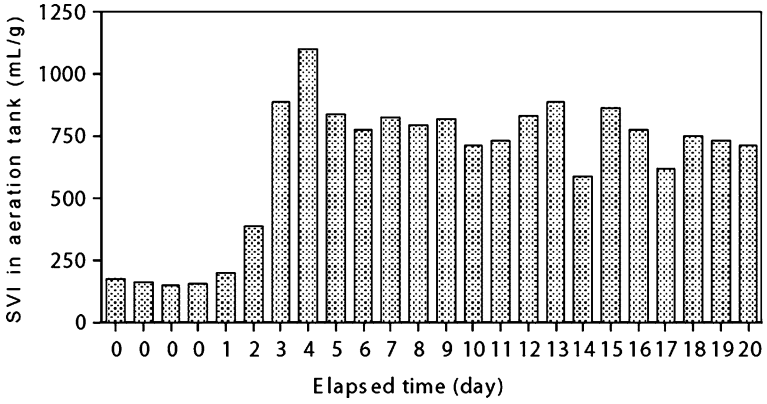

Fig. 3.32. Elapsed changes of MLSS in aeration tank (disk process experiment) (79).

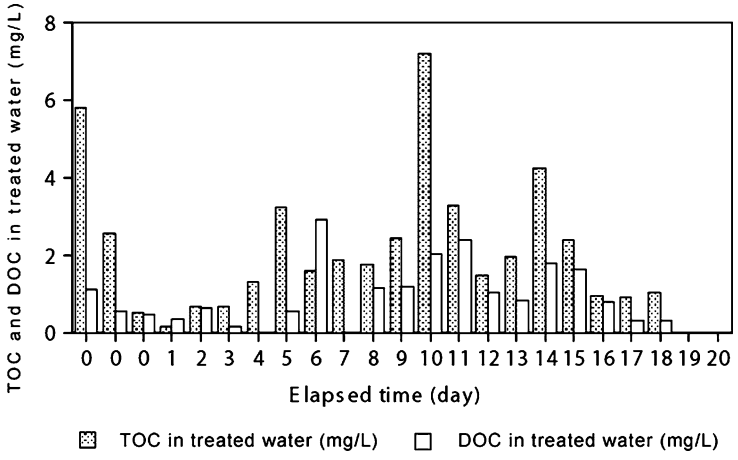

Fig. 3.33. Elapsed changes of TOC and DOC of treated water (disk process experiment) (79).

anaerobic process. This experiment lasted only for 20 days, so we recommend conducting it for a longer operating time.

Therefore, major topics for future study include a longer operating time, reproduction of experimental results, and other operating conditions.

3.2.2.4. Conclusion

Continuous experimentation indicates that the disk process can realize normal operations with the same good water quality of treated water as that of a contrasting experiment, and that the high-speed rotary disk process hardly influences the water quality of treated water. Moreover, effective sludge reduction may be realized by an aerobic process associated with a high-speed rotary disk process.

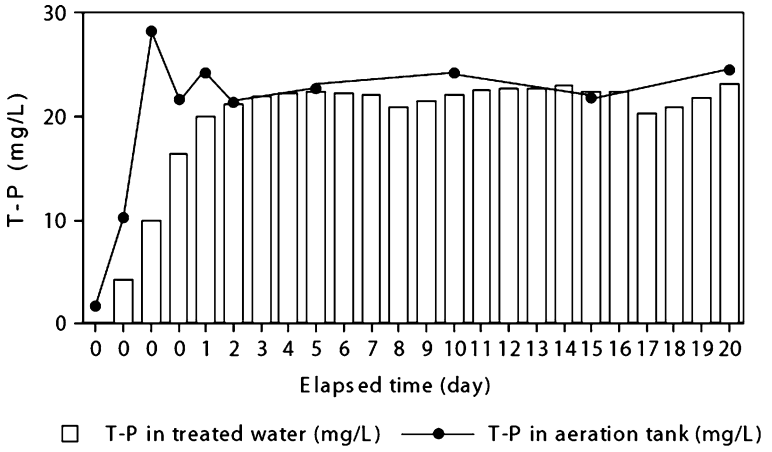

Fig. 3.34. Elapsed changes of T-P of SLSP and treated water (disk process experiment) (79).

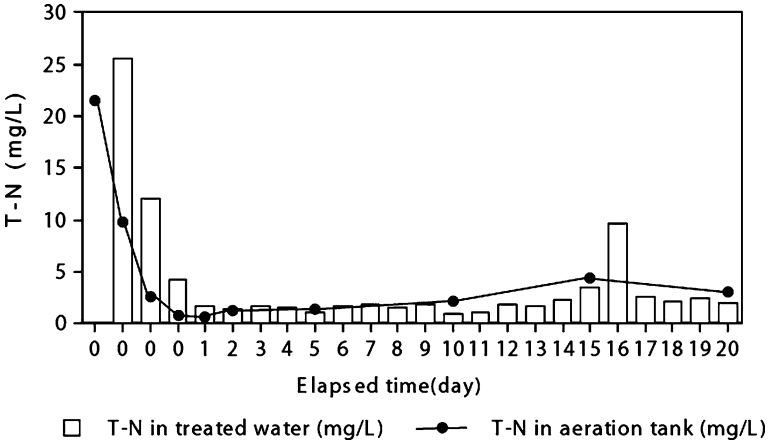

Fig. 3.35. Elapsed changes of T-N of SLSP and treated water (disk process experiment) (79).

3.3. Batch Test on Anaerobic Biodegradation of Digested Sludge Treated After Solubilization

3.3.1. Objective

Until now, this chapter has discussed the biodegradation of excess sludge via the solubilization process. This section focuses on the solubilization of digested sludge. Although carbon has been stabilized in digested sludge to a certain extent, the large amount of organic matter present makes it possible to realize the further volume reduction of sludge. Because a higher concentration of NH_4^+ in the digested sludge makes the pH higher than in excess

Table 3.20
Running condition of continuous aerobic experiment (79)

Item		Contrast	Disk process
Running time	Day	20	20
Volume loading rate of BOD	$kg/m^3/day$	0.2	0.2
Retention time (HRT)	Hour	24	24
Sludge recycling flow	L/days	6	6

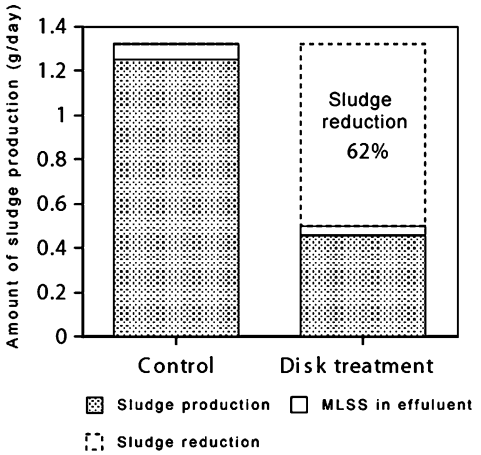

Fig. 3.36. Comparison of daily sludge production (79).

Table 3.21
Average effluent water qualities of two experiments (79)

Item	Unit	Contrast	Disk process
MLSS	mg/L	04.1	02.1
BOD	mg/L	08.9	05.6
TOC	mg/L	06.6	02.1
DOC	mg/L	05.4	01.3
T-N	mg/L	22.4	19.5
T-P	mg/L	04.1	03.6

sludge, the pH cannot be decreased even if the digested sludge is solubilized, and the digested sludge can be solubilized more easily than excess sludge. Therefore, anaerobic biodegradation of disk-process-treated digested sludge should be examined by a vial batch test. Next, the practicability of solubilizing digested sludge will be discussed as compared with that of excess sludge. ((77))

Table 3.22
Composition of the substitute (79)

Item	Unit	
Influent BOD	mg/L	200.0
Influent TOC	mg/L	090.0
Influent T-P	mg/L	026.2
Influent T-N	mg/L	009.3

Table 3.23
Experimental parameters (79)

	Sludge concentration (mg/L)	Treatment time (min)	Disk gap (mm)	Rotary speed (rpm)
Experimental condition	18,200	45	10	5,000

3.3.2. Methods and Experimental Conditions

3.3.2.1. DISK PROCESS

(a) Condense the collected sludge (10 l);
(b) Deliver the condensed sludge into the experimental apparatus shown as Fig. 3.2. Sampling is conducted at designated intervals, then temperature and energy consumption are measured. Treatment lasts for 60 min. Sampling is done at 1, 2, 5, 10, 20, 30, and 45 min after the experimental start. MLSS, MLVSS, TOC, and DOC are measured.

3.3.2.2. PARAMETERS

Parameters (sludge concentration, treatment time, disk gap, rotary speed) shown in Table 3.22 were changed to examine the optimum experimental condition

3.3.2.3. VIAL BATCH TEST

Digested sludge (as seed sludge) and digested sludge treated by the disk process (as substrate) were introduced as seed sludge and the substrate, respectively, into a vial (about 75 mL) in which inner air had been replaced earlier by nitrogen. Elapsed change of the volume of generated methane was observed in a shaking water bath (water temperature: 36°C, shaking speed: 100/min). 20 mL of digested sludge and 20 mL of the substrate were used. The digested sludge is described in Table 3.23.

3.3.3. Results and Discussion

3.3.3.1. DISK PROCESS (COMPARISON OF DIGESTED SLUDGE AND EXCESS SLUDGE)

Figure 3.37 shows the resulting solubilization rates (DOC/TOC) of the excess sludge and digested sludge. It was found that the digested sludge, after being treated by the disk process, took the same amount of time to increase the solubilization rate as the excess sludge. Viscosity was thought to be the major reason for the digested sludge to have a higher solubilization rate than excess sludge at an early stage. When compared with digested sludge, the viscosity of

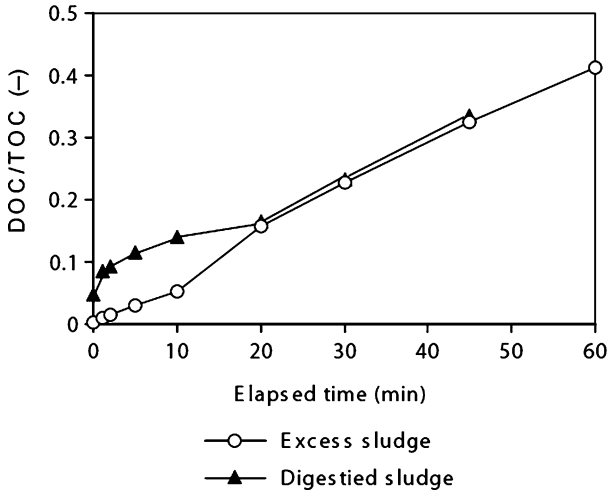

Fig. 3.37. Elapsed changes of solubilization rates of excess sludge and digested sludge (79).

excess sludge is relatively high and cannot be decreased if solubilization has not been achieved to some extent. As a result, the disk system cannot run well.

After the 45 min disk process, the solubilization rates of excess sludge and digested sludge were up to 0.324 and 0.335, respectively. This indicates that digested sludge may be solubilized to the same extent as excess sludge.

3.3.3.2. Vial Test

Figures 3.38 and 3.39 show the results of the vial test. Elapsed changes of accumulative methane production (Fig. 3.38) indicate that the accumulative methane production of treated sludge is higher than that of untreated sludge. This means that the disk process has promoted the sludge biodegradation. Moreover, since a lengthy disk process may enhance the accumulative methane production, the solubilization rate may be regarded as an index for expressing the biodegradation. Elapsed changes of MTR (Fig. 3.39) indicate that the MTR of treated sludge is also higher than that of untreated sludge. The same conclusion can be reached with Fig. 3.38.

The vial test results can be used to compare excess sludge and digested sludge. The test results for excess sludge were described previously (Sect. 3.1.1). MTR was compared instead of accumulative methane production since a direct comparison of the latter was impossible. In fact, MTR also cannot be directly compared, but it can be divided further by the MLVSS of sludge. All results are listed in Table 3.24. For 1 g of VSS seed sludge, the MTR was 0.84 and 1.13 when excess sludge and digested sludge were used as the substrate, indicating a higher activity of digested sludge than excess sludge. When digested sludge was used as the substrate, high methane activity could be attributed to a higher concentration of NH_4^+ in digested sludge than in excess sludge, keeping a high pH and making digested sludge easy to be degraded.

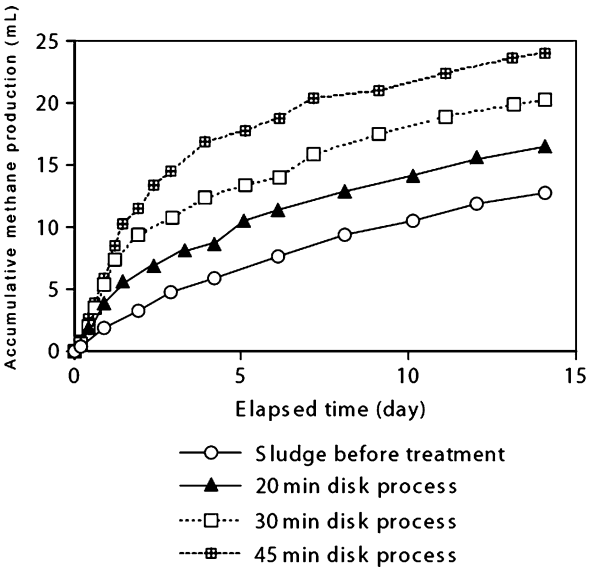

Fig. 3.38. Elapsed changes of accumulative methane production (79).

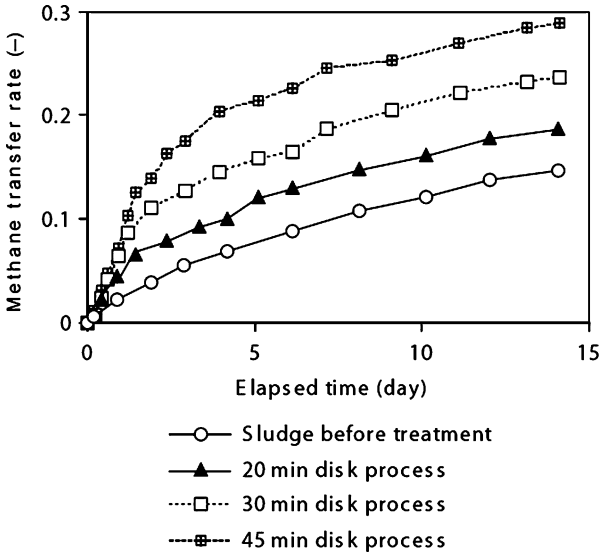

Fig. 3.39. Elapsed changes of MTR (79).

3.3.4. Conclusions

(a) Digested sludge can be solubilized to the same extent as excess sludge using a high-speed rotary disk process.
(b) Vial tests indicate that solubilization can promote the anaerobic biodegradation of digested sludge. Therefore, a larger than usual methane recovery can be realized in an anaerobic treatment system using a high-speed rotary disk.
(c) As the substrate, digested sludge is more easily degraded, when compared with excess sludge.

Table 3.24
Parameters of digested sludge (79)

	MLSS (mg/L)	MLVSS (mg/L)	Solubilization rate after disk process (%)
Digested sludge	18,200	11,400	33.5

Table 3.25
Comparison of digested sludge and excess sludge (79)

	pH	MLSS of seed sludge (mg/L)	MTR (-)	MTR per 1 g VSS (-)
Digested sludge	7.18	11,400	0.26	1.13
Excess sludge	6.52	12,500	0.32	0.84

4. COMPARISON WITH OTHER METHODS OF SLUDGE SOLUBILIZATION

4.1. Comparison of Ultrasonic Method and High-Speed Rotary Disk Process Method

4.1.1. Objective

This section discusses the practicality of the high-speed rotary disk process as compared with the ultrasonic method, one of the physical methods of solubilization technology.

4.1.2. Comparison Methods and Conditions

4.1.2.1. METHODS

(a) Calculate the energy (heat) requirement of two solubilization methods under their optimum operating conditions;
(b) Calculate the energy requirement of 1 g of solubilized sludge by dividing energy consumption using the weight of solubilized sludge obtained by two methods.

4.1.2.2. CONDITIONS

Optimum operating conditions of the two solubilization methods are listed in Table 3.25.

4.1.3. Calculation Method

4.1.3.1. ULTRASONIC METHOD

In one 100-mL container:

- When the ultrasonic intensity is 0.9 W/mL,

$$90\,\mathrm{W}, \quad \text{therefore } 90 \times 300\,(\mathrm{s}) = 27{,}000\,\mathrm{J}$$

3% of solubilization rate produces sludge, 0.03 g (dried wt)
Therefore, heat energy consumption for 1 g-dw of solubilized sludge is 900 kJ.

- When the ultrasonic intensity is 1.2 W/mL,

$$120\,\text{W}, \quad \text{therefore} 120 \times 300\,(\text{s}) = 36{,}000\,\text{J}$$

4% of solubilization rate produces sludge, 0.04 g (dried wt)

Therefore, heat energy consumption for 1 g-dw of solubilized sludge is 900 kJ.

- When the ultrasonic intensity is 1.5 W/mL,

$$150\,\text{W}, \quad \text{therefore} 150 \times 300\,(\text{s}) = 45{,}000\,\text{J}$$

6% of solubilization rate produces sludge, 0.06 g (dried wt)

Therefore, heat energy consumption for 1 g-dw of solubilized sludge is 750 kJ.

4.1.3.2. HIGH-SPEED ROTARY DISK PROCESS METHOD

In one 100-mL container:

- In the case of only disk process

From electric consumption, the heat energy consumption is calculated to be about 4,770 kJ, 35% of solubilization rate produces 70 g (dried wt) of solubilized sludge, Therefore, heat energy consumption for 1 g-dw of solubilized sludge is 68 kJ.

- 60°C of preheating (lasting time: 0 min)+disk process

From electric consumption, the heat energy consumption for preheating+disk process is calculated to be about 6,290 kJ, 39% of solubilization rate produces 78 g (dried wt) of solubilized sludge, Therefore, heat energy consumption for 1 g-dw of solubilized sludge is 81 kJ.

- 80°C of preheating (lasting time: 0 min) + disk process

From electric consumption, the heat energy consumption for preheating + disk process is calculated to be about 7,760 kJ,

39% of solubilization rate produces 82 g (dried wt) of solubilized sludge,

Therefore, heat energy consumption for 1 g-dw of solubilized sludge is 95 kJ.

4.1.4. Observations

Heat energy consumption for 1 g-dw of solubilized sludge is shown in Fig. 3.40. All results are calculated from the heat energy consumption under the corresponding optimum operating conditions, and then divided by the amount of solubilized sludge generated. It can be found that the heat energy consumption of the disk process method was one-tenth of that of the ultrasonic method. The same phenomenon also can be seen in the preheating-disk process. It is thought that the ultrasonic process is unsatisfactory for the large-scale treatment of highly concentrated sludge while the disk process is suitable. These results have been thought only in the case of the comparison of sludge solubilization rates but that of sludge volume-reduction. However, as above mentioned (Sects. 3.1 and 3.2), a high solubilization rate can be enhanced anaerobic/aerobic biodegradation. Hence, the high-speed rotary disk process is effective for sludge solubilization.

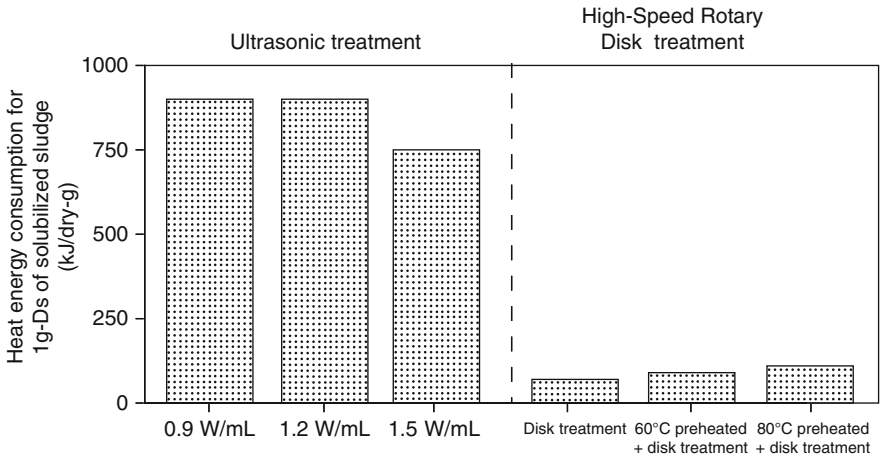

Fig. 3.40. Comparison of heat energy consumption for 1 g-dw of solubilized sludge (79).

4.2. Comparison of Pressure Exploded Process and High-Speed Rotary Disk Process

4.2.1. Objective

To examine the practicality of the high-speed rotary disk process, as compared with the pressure exploded process, a high-energy decomposition method is used.

4.2.2. Comparison Method and Condition

4.2.2.1. Comparison Method

(a) Conduct vial tests for the two methods.
(b) Calculate and compare methane rates to solubilization rate and heat energy consumption.
(c) Calculate and compare MLSS and MLVSS to solubilization rate and heat energy consumption.

4.2.2.2. Conditions

Running conditions are presented in Table 3.26.

4.2.3. Calculation Method

4.2.3.1. Methane Transfer Rate to Solubilization Rate

(Pressure exploded process)

In a 10-day vial test:
MTR (untreated sludge: 26%, treated sludge: 45%)
Solubilization rate after pressure exploded process (DOC/TOC): 53%
MTR to solubilization rate 0.84

(Disk process)

In a 10-day vial test:
MTR (untreated sludge: 26%, treated sludge: 32%)

Table 3.26
Optimum running conditions of two solubilization methods (79)

	Item	Unit	Optimum conditions
Ultrasonic method	Intensity	W/mL	0.9–1.5
	Time	min	5
	Sludge concentration	mg/L	10,000
High-speed rotary disk process	Rotary speed	rpm	5,000
	Running time	min	45
	Sludge concentration	mg/L	20,000

Solubilization rate after pressure exploded process (DOC/TOC): 37%
MTR to solubilization rate 0.87

4.2.3.2. Methane Transfer Rate to Heat Energy Consumption

(Pressure exploded process and disk process)

With the same volume, sludge, regarded as water, was calculated (primary temperature is 20°C).
Pressure exploded process (the end temperature 170°C → 150°C up)
High-speed rotary disk process (the end temperature 80°C → 60°C up)

Therefore, if the heat of pressure exploded process were regarded as 1, the heat of the high-speed rotary disk process were 0.40 (energy loss was not considered). The average electric consumption of the high-speed rotary disk process (10 l), expressed in heat energy, was calculated to be 4,770 kJ. If all energy was consumed, the sludge temperature would rise by 114°C. In practice, however, only a 60° increase is considered to be 47% of heat release. Hence, if the heat of pressure exploded process were regarded as 1, the heat of the high-speed rotary disk process would be 0.75.

The MTR is 0.45 and 0.43 for the pressure exploded process and high-speed rotary disk process, respectively.

4.2.3.3. MLSS and MLVSS Reduction Rates to Solubilization Rate (Energy Loss Is Not Considered Here)

(Pressure exploded process)

In the pressure exploded process:
MLSS reduction rate (untreated sludge: 0%, treated sludge; 43%)
MLVSS reduction rate (untreated sludge: 0%, treated sludge; 57%)
In a 10-day vial test:
MLSS reduction rate (untreated sludge: 9%, treated sludge; 14%)
MLVSS reduction rate (untreated sludge: 12%, treated sludge; 11%)
Solubilization rate after pressure exploded process (DOC/TOC): 53%

MLSS reduction rate to solubilization rate: 1.08
MLVSS reduction rate to solubilization rate: 1.28

(Disk process)

In the pressure exploded process:
MLSS reduction rate (untreated sludge: 0%, treated sludge; near 0%)
MLVSS reduction rate (untreated sludge: 0%, treated sludge; near 0%)
In a 10-day vial test:
MLSS reduction rate (untreated sludge: 5%, treated sludge; 15%)
MLVSS reduction rate (untreated sludge: 6%, treated sludge; 16%)
Solubilization rate after pressure exploded process (DOC/TOC): 37%
MLSS reduction rate to solubilization rate: 0.41
MLVSS reduction rate to solubilization rate: 0.43

4.2.3.4. MLSS AND MLVSS REDUCTION RATES TO HEAT ENERGY CONSUMPTION

(Pressure exploded process and disk process)

With the same volume, sludge, regarded as water, was calculated (primary temperature is 20°C).
Pressure exploded process (the end temperature 170°C → 150°C up) High-speed rotary disk process (the end temperature 80°C → 60°C up)

Therefore, one portion of heat energy matches 0.4 portion of high-speed rotary disk process (energy loss is not considered here). The average electric consumption of high-speed rotary disk process (10 l), expressed in heat energy, was calculated to be 4,770 kJ. If all energy was consumed, the sludge temperature would rise by 114°C. In practice, however, only a 60°C increase is considered to be 47% of heat release. Hence, if the heat of the pressure exploded process were regarded as 1, the heat of the high-speed rotary disk process would be 0.75.

MLSS reduction to heat energy:
Pressure exploded process: 0.57
High-speed rotary disk process: 0.19
MLVSS reduction to heat energy:
Pressure exploded process: 0.68
High-speed rotary disk process: 0.15

4.2.4. Observations

Both methane recovery and the volume-reduction of sludge were compared, based on the results of the vial tests.

As for methane recovery, MTRs were examined. Since a direct comparison was impossible, MTRs were firstly divided by DOC/TOC (solubilization rates) and then compared. Also, after heat energy consumption was calculated, MTRs were divided by heat energy consumption and then compared (Table 3.27). The comparison indicates that the MTR to solubilization rate for the high-speed rotary disk process was slightly higher than that for the pressure exploded process. By contrast, the MTR of heat energy consumption is relatively high.

Table 3.27
Running conditions (of pressure exploded process and high-speed rotary disk process) (80)

	Items	Unit conditions	Running
Pressure exploded process	Temperature	°C	170
	Pressure	MPa	0.7
	Treatment time	Min	60
	Sludge concentration	mg/L	18,800
	Rotary speed	rpm	5,000
High-speed rotary disk process	Treatment time	min	45
	Disk gap	cm	10
	Sludge concentration	mg/L	15,800

Table 3.28
Comparison of methane transfer rates of disk process and pressure exploded process (80)

	Running time (day)	Methane transfer rate of untreated sludge (%)	Methane transfer rate of treated sludge (%)	DOC/TOC (%)	Methane transfer rate to solubilization rate	Methane transfer rate to heat energy
Disk process	10	26.0	32.0	37.0	0.87	0.43
Pressure exploded process	10	26.0	45.0	53.0	0.84	0.45

However, heat generation in the pressure exploded process was not involved in this heat energy consumption. Even in the pressure exploded process, poor insulation results in the release of heat; therefore, the high-speed rotary disk process can be considered favorable. From the viewpoint of methane recovery, little difference was found between the pressure exploded process and high-speed rotary disk process. Therefore, system analyses indicate that the high-speed rotary disk process is favorable due to its low initial cost.

MLSS and MLVSS reduction rates were compared for the volume-reduction of sludge. Since a direct comparison was impossible, MLSS and MLVSS reduction rates were first divided by DOC/TOC (solubilization rates) and then compared. After heat energy consumption was calculated, MLSS and MLVSS reduction rates were divided by heat energy consumption and then compared (Tables 3.28 and 3.29). When MLSS and MLVSS reduction rates were compared to the solubilization rate and heat energy consumption for the high-speed rotary disk process and pressure exploded process, all values for the pressure exploded process were significantly large. Even half of the MLSS and MLVSS reduction rates of the pressure exploded process were still thought to be too large. However, since the MLSS and MLVSS reductions in the vial test fluctuate too greatly to arrive at a steady state, this evaluation

Table 3.29
Comparison of MLSS and MLVSS reduction rates of disk process and pressure exploded process (80)

	MLSS reduction rate to solubilization rate	MLVSS reduction rate to solubilization rate	MLSS reduction rate to heat energy	MLVSS reduction rate to heat energy
Disk process	1.08	1.28	0.57	0.68
Pressure exploded process	0.41	0.43	0.19	0.15

method is considered unsuitable. Moreover, although there are MLSS and MLVSS reductions in the high-speed rotary disk process, the radius of the sludge becomes increasingly smaller, making it difficult to measure. Yet, because the MLSS and MLVSS reduction rates of pressure exploded process were quite high, the high-speed rotary disk process is not favorable for the above-mentioned reasons. From the viewpoint of sludge reduction, the pressure exploded process is preferred.

4.2.5. Conclusion

With regard to methane recovery, a comparison of the MTR to the solubilization rate and heat energy consumption reveals that there is little difference between the disk process and the pressure exploded process. System analyses indicate that the high-speed rotary disk process is favorable due to the low initial cost.

With respect to sludge reduction, comparisons regarding MLSS and MLVSS rates to solubilization rate and heat energy consumption indicate that the pressure exploded process achieves greater results than the disk process; therefore the pressure exploded process is thought to be preferable.

NOMENCLATURE

BOD = Biochemical oxygen demand
MTR = Methane transfer rate
HRT = Hydraulic retention time
SLSP = Solid-liquid suspending phases
T-N = Total nitrogen
T-P = Total phosphorus
SVI = Sludge volume index
MSW = Municipal solid wastes
DOC = Soluble organic carbon
TOC = Total organic carbon
MLSS = Mixed liquor suspended solids
MLVSS = Mixed liquor volatile suspended solids
COD = Chemical oxygen demand

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4

Applications of Composted Solid Wastes for Farmland Amendment and Nutrient Balance in Soils

Tsuyoshi Imai, Yuyu Liu, Masao Ukita, and Yung-Tse Hung

CONTENTS

Abstract Presently, the actual environmental load from farmland applications of composts has been less mentioned. This chapter aims, first, at giving a detailed description on the distributions of various chemical species of elements including macronutrients, micronutrients, and heavy metals in various composted solids and composts-amended soil, and then, examining the feasibility of sustainable applications of composted biosolids, depending on the farmland nutrient-balance principle. A good understanding of the nutrient balance in environment is believed to be of great benefit to the sustainable reuses of biosolid wastes.

1. INTRODUCTION

Various composted solid wastes are largely being recycled to farmland (1–3) for two main purposes: (1) saving landfill space and disposing solid wastes at rather low operational cost (4); and (2) using composted soil wastes as soil conditioner, improving soil physic–chemical characteristics and microbial activity to prevent the soil deterioration resulted from intensive cultivation and climatic condition (5–12), and supplying plant nutrients for agricultural plant growth (13–18). Although some potential public health hazards and environmental effects related to pathogens (19) and heavy metals and organic contaminants present in composts

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_4

(20, 21) have also been well reported, composting solid wastes is still recognized as a promising option other than the traditional solid waste disposal ways, such as incineration and landfill.

The argumentation concerning agricultural applications of composts mainly focuses on the effective plant nutrient utilization as well as heavy metal accumulation in farmlands. The deficiency of plant nutrients in soil hinders plant growth and nutrient uptakes, while the excessive existence of toxic and even common nontoxic metals are seriously harmful to food chain and human health (22–31). Therefore, it is essential to understand the existence of chemical elements in various composts so as to control the environmental load on soil caused by compost application.

Many countries have drawn up their own national limits against various inorganic and organic pollutants in biosolids (32) (Table 4.1). These regulations, criteria, or thresholds are found quite different with each other. For example, some countries regulate "Contaminant acceptable thresholds" and "Permitted load" for biosolid (sludge), while others hold "Maximum allowable soil concentration" for soil.

Although total element concentrations of composts have frequently been emphasized in environmental regulations concerning disposal of biosolids in many countries, the bioavailable forms, both water-soluble and exchangeable (33), are practically more accurate in determining the bioavailability than total element content since plants can easily assimilate them (34, 35). Many researchers have ever tried to extract bioavailable metals from composts and soil with different kinds of chemical agents, such as the $CaCl_2$ (calcium chloride) solution (36), or organic extractants like diethylene triamine pentaacetic acid (DTPA) (37) (sometimes DTPA + Triethanolamine + Ammonium acetate (38)) and ethylene diamine tetraacetic acid (EDTA) (36, 39). Ciavatta et al. (40) concluded that their extraction efficiency generally decreased in the following order: EDTA > DTPA > $KNO_3 \approx H_2O$. However, both EDTA and DTPA are popularly thought to be lacking in the specificity necessary to determine the quantitative amounts of trace metals held in the given forms, only providing one semiquantitative approach to the problem of evaluating how many metals are held in soil and biosolid wastes (41). After that, the "sequential extraction" methods, instead of them, are gradually adopted to further fractionate the elements in composts and soil. Tessier et al. (42) has ever put forward a popular one. Some people have also suggested many other methods for the same purposes later (Table 4.2), but Tessier Method and revised ones are so far being popularly used.

In the past two decades, the characteristics of various elements, mainly heavy metals have largely been examined in solid composts derived from municipal solid waste (MSW) (43–47), sewage sludge (48–50), bovine excrement (51, 52), swine manure (41, 53–57), household garbage (39, 58), and mixed solid waste (47), as well as in the soil amended with sewage sludge (59–62) and MSW (63, 64). These early works have provided large amounts of information about composted solids and compost-applied soil for us. However, composted solid wastes usually vary, to different extents, with both geographic region and seasonal variation in the original input, and from one facility to another because of the differences in pretreatment and process controlling. Here, through one full-scale investigation on compost applications conducted in Japan, we will make a description of the distribution of plant nutrients and toxic elements in various composts and amended soil.

Table 4.1
Environmental criteria/thresholds in EU and USA (mg/kg) (32, 81)

		International				EPA CFR 40 part 503	US state contaminant concentration limit	
		Lowest	Countries or groups	Highest	Countries or groups	Ceiling concentration	Lowest	Highest
Compost	As	0.15	UN	75	Canada	75	5	75
	Cd	0.15	UN	20	Italy, Canada	85	10	85
	Cr	4	UN	1,750	South Africa		1,000	3,000
	Cu	12	UN	1,000	Italy	4,300	1,000	4,300
	Co	10	Austria	150	Canada			
	Pb	15	UN	750	Italy	840	300	1,000
	Hg	0.1	UN	60	Australia	57	10	57
	Mo	1	UK	25	South Africa	75	10	75
	Ni	3	UN	300	Italy	420	200	420
	Se	5	Australia	50	Australia	100	36	100
	Zn	30	UN	4,000	Demark	7,500	2,000	7,500
Soil	As	2	South Africa	50	UK			
	Cd	1	Australia	4	Norway			
	Cr	80	South Africa	400	UK			
	Cu	50	E.U.	1,000	Norway			
	Co	20	South Africa	20	South Africa			
	Pb	15	Australia	300	UK			
	Hg	0.5	South Africa	5	Norway			
	Mo	2.3	South Africa	4	UK			
	Ni	15	South Africa	80	Norway			
	Se	2	South Africa	5	Australia			
	Zn	150	Australia	1,500	Norway			

Presently the actual environmental load from farmland applications of composts has been less mentioned. Studies on heavy metal accumulation are generally aiming at several kinds of major agricultural plants (13, 65–67). Therefore, this chapter is, at first, to give a detailed description on the distributions of various chemical species of elements including macronutrients, micronutrients, and heavy metals in various composted solids and composts-amended soil, and next, to examine the feasibility of sustainable applications of composted biosolids, depending on the farmland nutrient-balance principle. A good understanding of the nutrient balance in environment is believed to be of great benefit to the sustainable reuses of biosolid wastes. This principle can be used for either the decision-making concerning the reuses of biosolid wastes in farmland or in controlling the in situ application of a given type of solid waste in the scale-limited farmland.

Table 4.2
Several selected procedures for extracting elements of chemical species in composts and soils (33, 37, 40, 41, 43, 45–49, 81, 82)

	1	2	3	4	5	6
1		20 g sample + 200 mL 0.05 M $CaCl_2$, 24 h	Sample 20 g + DI water 200 mL, 24 h	5 g + 50 mL H_2O		3 g + 20 H_2O, 2 h
2	Room temperature, 1 h, sample 1 g + 8 mL 1 M $MgCl_2$, (pH 7) or 1 M NaOAc (pH 8.3)		Residue from [1] + KCl 1 M 200 mL, 24 h	50 mL 0.05 M $CaCl_2$, pH 5; 50 mL 0.05 M $CaCl_2$ + 0.2% OH-quinone	6 g + 60 mL 0.05 M KNO_3 30°C, 24 h	20 mL 1 M $NaNO_3$, 1 h
3			Residue from [2] +$Na_2P_2O_7$ 1 M 200 mL, 24 h	50 mL aq. acetic acid	Residue from [2] 2.5% HOAc, 30°C, 24 h	20 mL 1 M NaOAc, 5 h (pH 5.0 adjusted with HOAc)
				50 mL 0.005 M DTPA and 0.1 M TEA		
4	Room temperature, residue from [2] + 8 mL 1 M NaOAc (pH 5.0 adjusted with HOAc)	Residue from [2] + 200 mL acetic acid 24 h	Residue from [6] + 200 mL 4 M HNO_3	50 mL 0.1 M HCl [5] 50 mL 0.1 M aq. KCN		30 mL 0.1 M $NH_2OH \cdot HCl$ in 25% HOAc (50°C), 60 h
5	Residue from [4] + 20 mL 0.3 M $Na_2S_2O_4$ + 0.175 M Na-citrate + 0.025 M H-citrate or 0.04 M $NH_2OH \cdot HCl$ in 25% HOAc (96 ± 3°C)	Residue from [6] (washed by 50 mL pure water) + 100 mL 0.1 M oxalate acid + ammonium oxalate 0.175 M, pH 5.25				

6	Residue from [5] + 3 mL HNO_3 + 30% H_2O_2 (adjusted to pH 2 with HNO_3), 85 ± 2°C, 2 h with occasional agitation; a second 3 mL aliquot of 30% H_2O_2 (adjusted to pH 2 with HNO_3), 85 ± 2°C, 3 h with intermittent agitation. After cooling, 5 mL 3.2 M NH_4OAc in 20% HNO_3, diluted to 20 mL, agitated for 30 min	Sample 2 g + 1.0 M $K_2P_2O_7$ (potassium pyrophosphate) (Cu-PYR)	Residue from [3] + 200 mL 0.1 M NaOH	6 g Residue from [4] + 100 mL 6%	10 mL 30% H_2O_2 + 6 mL 0.02 M HNO_3, 2 h, 50°C
				180 mL $HOOCCOONH_4$ + 6 g $C_{22}H_{38}O_7$. 1 h, 100°C	6 mL 30% H_2O_2 (pH 2.0, HOAc) Overnight Reheat in water bath at 50°C for 5 h
7	HF-$HClO_4$	Residue from [5] dried and ignited at 600°C, 0.5 g + HF	HNO_3 + $HClO_4$ + HF	HF	6 mL (65%) HNO_3 + 4 mL 30%HCl + 3 mL concentrated HNO_3, heated in 50°C water bath

[1] Water-soluble; [2] Exchangeable; [3] Complex; [4] Carbonate-bound; [5] Fe and Mn oxides-bound (reducible), [6] Organic matter-bound; [7] Residual.

2. CHEMICAL ELEMENTS IN COMPOSTED SOLIDS AND COMPOSTS-AMENDED SOIL

2.1. Sampling, Pretreatment, and Analysis of Composts and Soil

Seven kinds of composts and the soil samples that had been amended with four sorts of composts were collected from Yamaguchi and Nagano Prefecture, Japan, in the year 2002 (Fig. 4.1, Table 4.3).

Two grams of air-dried sample was mixed with 80 mL of ultrapure water, shaken for 1 h, and filtered through 0.45 μm filter membrane to extract the water-soluble phosphorous. The sample was oven-dried at 600°C for 2 h and then boiled with 1 mol/L hydrochloric acid (HCl) (Sample: HCl = 1 g: 25 mL) for extracting total phosphorous (TP). Extracted phosphorous in digests was measured with spectrophotometer at the wavelength of 800 nm according to the stannous chloride method (68) (Shimadu-UV-160U spectrophotometer, Japan).

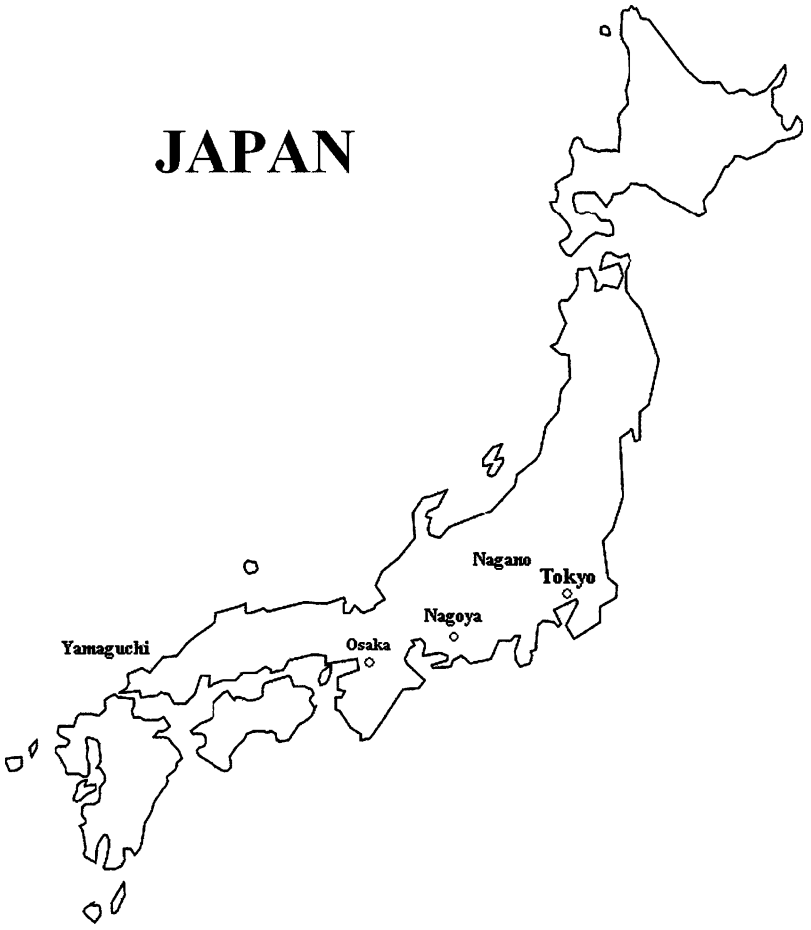


Fig. 4.1. Map of Japan.

Table 4.3
Composts and their applications of composts as well as fertilizer in farmlands (81)

(a) Composts

Compost (abbreviation)	Main raw materials
Seafood processing compost (SPC)	Fish/lobster bony parts, rice bran, EM
Garbage compost (GC)	Garbage, saw dust, etc
Sewage sludge compost (SSC)	Sewage sludge
Swine manure compost (SMC)	Swine manure, sawdust, coffee dregs
Mixed swine and cattle compost (MSCC)	Swine manure, cattle manure, sawdust
Cattle excreta compost (CEC)	Swine manure
Hen excreta compost (HEC)	Hen excreta

(b) Composts or commercial fertilizer applied soil

Compost	Soils	Application
SPC	SPS-1	SPC, rice bran, yard trimmings, 1 tons/ha/year, 19 years
	NSPS-1	Commercial fertilizers, pesticides, rice bran, straw
	SPS-2	SPC, trimmings, 1 tons/ha/year, 11 years
	NSPS-2	Commercial fertilizers, 15 years
GC	GS-1	GC, outdoor
	NGS-1	Commercial fertilizer, outdoor
	GSB	Nothing, greenhouse, 11 years
	NGS-2	Commercial fertilizer, greenhouse, 11 years
	GS_a	GC, 1 tons/ha/year, greenhouse, 11 years
	GS_b	GC, 3 tons/ha/year, greenhouse, 11 years
	GS_c	GC, 10 tons/ha/year, greenhouse, 11 years
	GS_d	GC, 1 cm deep, greenhouse, 11 years
	GS_e	GC, 3 cm deep, greenhouse, 11 years
SMC	SMS-1	SMC, 40 tons/ha, outdoor, 6–7 years
	SMS-2	SMC, 40 tons/ha, outdoor, 1 year
	NSMS	Paddy field, commercial fertilizer, outdoor, 10 years
	SMSB	Nothing Outdoor
SSC	SSS-1	SSC, 60 tons/ha/year, 7–8 years
	SSS-2	SSC and part of semi-mature SSC, covered, 60 tons/ha, 1 year
	SSSB	Nothing

Ten grams of sample oven-dried at 105°C were mixed with 100 mL of ultrapure water, shaken for 24 h, settled for 15 min, and filtered through 0.45 μm filter membrane for the extraction of water-soluble metallic elements (K, Ca, Mg, Mn, Fe, Cu, Zn, Cd, Cr, Co, Ni, and Pb). Filtrate was digested with HNO_3 and H_2O_2, filtered through 0.45-μm filter membrane, and diluted to 50 mL with acidified ultrapure water of pH 1 (adjusted with HCl). Total elemental contents were measured following USEPA standard procedure (69). K, Ca, Mg, Mn, Fe, Cu, Zn, Cd, Cr, Co, Ni, and Pb in digests were determined using atomic absorption flame spectrophotometer (Shimadzu AA-66GPC).

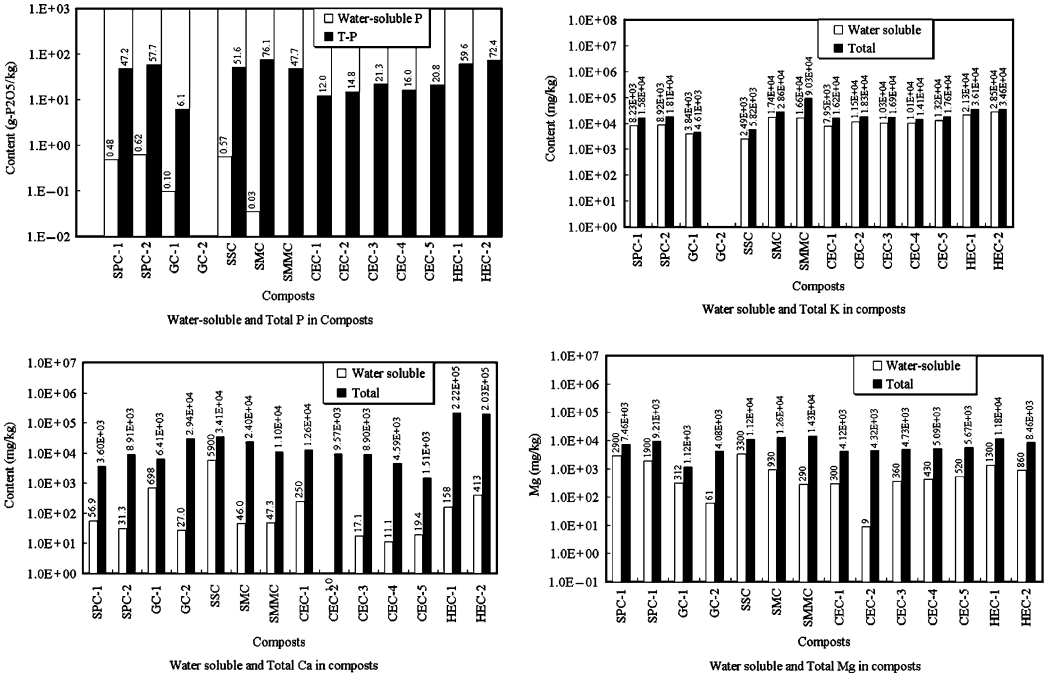


Fig. 4.2. Water-soluble and total P, K, Ca, and Mg in various composts (81).

2.2. *Macronutrient Elements (P, K, Ca, Mg) in Composted Solid Wastes and Compost-amended Soil*

2.2.1. P, K, Ca, and Mg in Composts

Figure 4.2 shows water-soluble and total P, K, Ca, and Mg in various composts. Here, the amount of total phosphorus is expressed as P_2O_5. TP has been found high in SPC (47.2–57.7 g P_2O_5/kg), SSC (51.6 g P_2O_5/kg), SMC (76.1 g P_2O_5/kg), SMMC (47.7 g P_2O_5/kg), and HEC (59.6–72.4 g P_2O_5/kg). Comparatively, CEC contained less phosphorous (12.0–21.3 g P_2O_5/kg), and GC had the least (6.1 g P_2O_5/kg). However, composts with high TP content, i.e., GC, SMC, SMMC, CEC, as well as HEC, contain only very low or even undetectable quantity of water-soluble phosphorous. Results show a relatively higher level of water-soluble phosphorus in SPC and SSC, it is supposed that SPC may also contain a small amount of heavy metals (as will be described later), which combines with the elemental phosphorus and forms insoluble heavy metal phosphates, while the existence of water-soluble/insoluble phosphates in SSC might have resulted in the high level of water-soluble phosphorus (Table 4.4).

Except GC and SSC, all composts were found at the same level of total potassium (several 10^4 mg K/kg, while GC and SSC at several 10^3 mg K/kg). Large existence of potassium in seawater may be one of the major causes of high K content of SPC. High level of potassium in SSC perhaps may be attributed to the large existence of inorganic salts containing potassium. It is in well agreement with the high content of water-soluble phosphorus in SSC, because the

Table 4.4
Procedure of sequential extraction experiment (33, 37, 40–43, 45–49, 84, 85)

Fraction	Procedure
Water-soluble	5 g + 50 mL H_2O, 125 rpm, 25–30°C, 24 h
Exchangeable	50 mL 0.05 M KNO_3 25–30°C, 125 rpm, 24 h
Carbonate-bound	Room temperature (25–30°C), 40 mL 1 M NaOAc (pH 5.0), 24 h
Fe–Mn oxides-bound	100 mL 0.04 M $NH_2OH \cdot HCl$ in 25% HOAc (~ 96°C), 2 h
Organic matter-bound	15 mL HNO_3 + 30% H_2O_2 (pH 2), 85 ± 2°C, 2 h; a second 15 mL aliquot of 30% H_2O_2 (pH 2), 85 ± 2°C, 3 h; 25 mL 3.2 M NH_4OAc in 20% HNO_3, diluted to 100 mL, 30 min

potassium phosphate is also water-soluble. High rate of water-soluble potassium in composts is also due to high solubility of element K in water.

Calcium is well found in HEC (2.03–2.22 × 10^5 mg Ca/kg), much higher than in other composts (several 10^4 mg Ca/kg SSC, SMC, SMMC, and GC, and even several 10^3 mg Ca/kg SPC and CEC). Calcium is known as one major component of chicken feed additives, since a certain level of dietary calcium is good for increasing survivability and reducing leg abnormalities (70). Water-soluble calcium has been observed evidently in various composts, and especially mostly in SSC (5.9 g Ca/kg). The second highest level (several hundreds mg/kg) of water-soluble calcium has been detected in HEC. The contents of water-soluble Ca in other composts are found mostly less than 100 mg/kg, only with two exceptions (GC-2 and CEC-2).

Total Mg is at a similar level, ranging from 1.12 × 10^3 to 1.43 × 10^4 mg/kg, and no significant difference can be found. Water-soluble Mg is found mostly in SPC (2,900 and 1,900 mg/kg) and SSC (3,300 mg/kg), but at the level of several 100 mg/kg in others, with two exceptions (GC-2 and CEC-2).

One simple comparison is made in Table 4.5, which is helpful for imagining the distributions of P, K, Ca, and Mg in composts. Generally speaking, both K and Mg are elements that easily form water-soluble inorganic salts, resulting in high level of water-soluble K and Mg in composts. On the other side, both P and Ca easily form the salts of less solubility, often leading to lower contents of water-soluble P and Ca in composts.

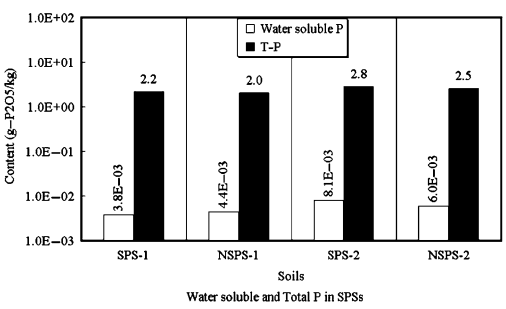
2.2.2. P, K, Ca, and Mg in Composts-Amended Soil

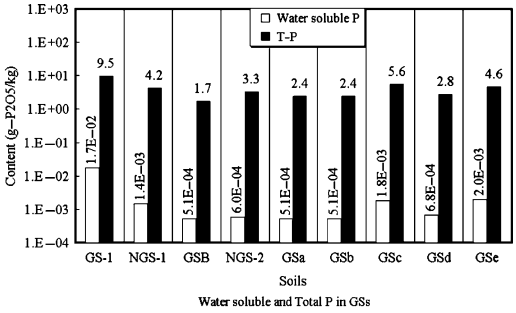
As in Fig. 4.3, there is no significant change in the contents of total and water-soluble phosphorus between the soil amended with or without SPC and GC, which can be attributed to relatively low loads of SPC and GC to the farmland, although the phosphorus content is much lower in background soil (2.19 and 2.80 g P_2O_5/kg for NSPS, and 1.73 g P_2O_5/kg for GSB) than in SPC (47.2–57.7 g P_2O_5/kg) and GC (6.1 g P_2O_5/kg). Comparatively, the applications of SMC and SSC have caused element phosphorus increases in amended soils. For SMC, the application caused the significant total phosphorus increases in the soil, while the results of SSC application indicate that the phosphorus accumulation seems have been well affected by rainfall. SSS-2 (1 year) contained more phosphorus than SSS-1 (6–7 years), indicating that the

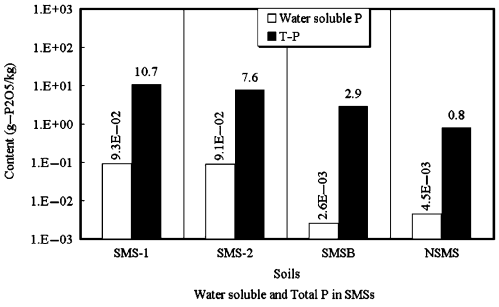
Table 4.5
Comparison of P, K Ca and Mg distributions in composts

	SPC	GC	SSC	SMC	SMMC	CEC	HEC
Total P							
Water soluble P							
Rate (sol./total)							
Total K							
Water soluble K							
Rate (sol./total)							
Total Ca							
Water soluble Ca							
Rate (sol./total)							
Total Mg							
Water soluble Mg							
Rate (sol./total)							

HIGH ⇒ LOW







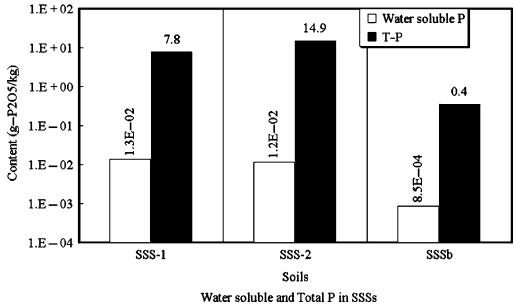


Fig. 4.3. Water-soluble and total P in amended soil (81).

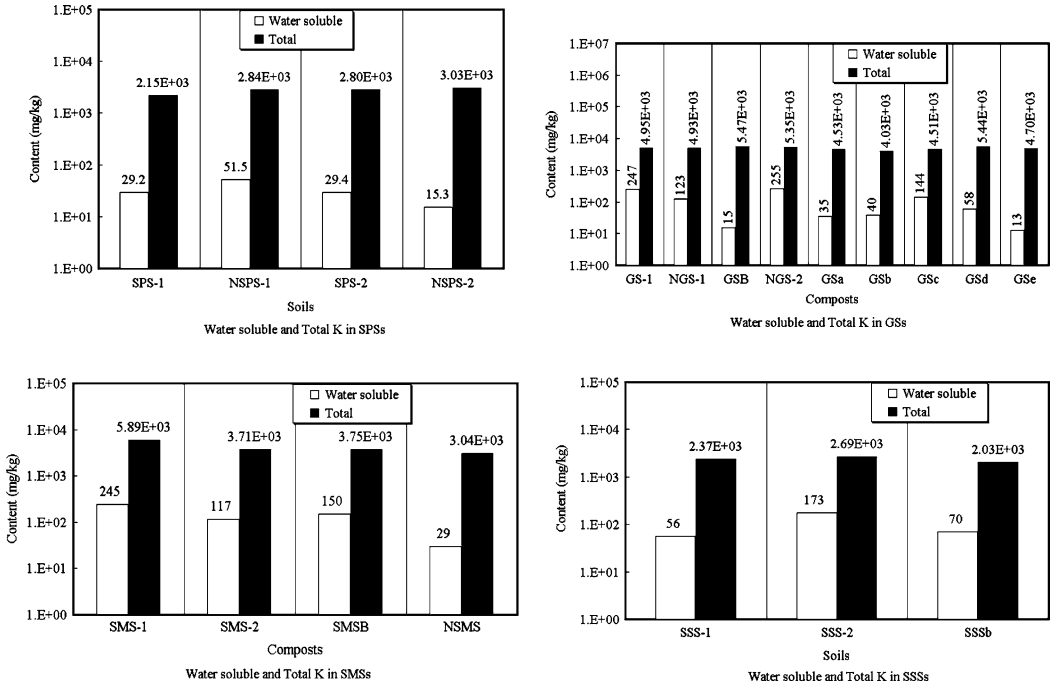


Fig. 4.4. Water-soluble and total K in amended soil (81).

content of phosphorus in soil is affected by rainfall much more strongly than by application period.

Figure 4.4 indicates that the applications of SPC, GC, SMC, and SSC have hardly resulted in total and water-soluble K accumulations in amended soils. Although composts were popularly rich in total and water-soluble K (Fig. 4.2), the outstanding solubility of K-containing compounds made it impossible for K to stay in the soil in large quantity.

Figure 4.5 exhibits slight Ca increases in the soil amended with SPC, GC, and SMC, except SSC. This is because the element Ca was a little more abundant in SPC, GC, and SMC than in the blank soil. SSC also contained more Ca, but no increase of Ca in soil has been found.

Figure 4.6 shows that the applications of SPC and GC have hardly caused the Mg increase in soil, as mainly may be attributed to the high solubility of Mg-containing salts. Element Mg in SMC-applied soils has revealed an irregular appearance. Moreover, significant Mg increases have been observed in SSC-amended farmlands. Main cause is the much higher level of Mg in SSC (1.12×10^4 mg Mg/kg soil) than that in blank soil (10 mg Mg/kg soil).

2.3. Micronutrient Elements (Fe, Mn, Cu, Zn) in Composted Solid Wastes and Composts-amended Soil

2.3.1. Fe, Mn, Cu, and Zn in Composts

Figure 4.7 shows total Fe, Mn, Cu, and Zn contents in various composts.

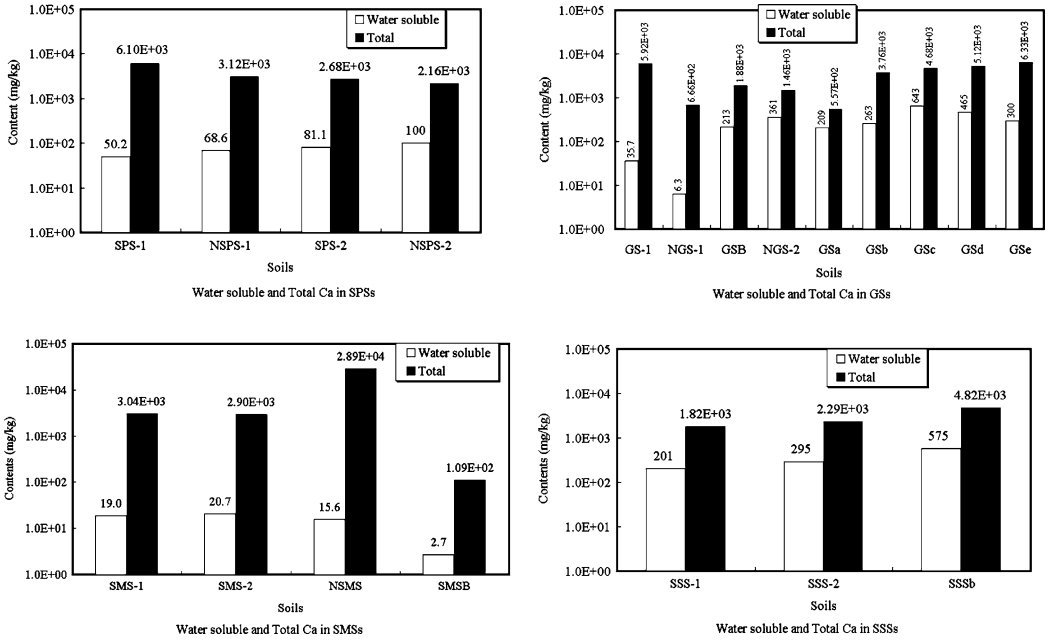


Fig. 4.5. Water-soluble and total Ca in amended soil (81).

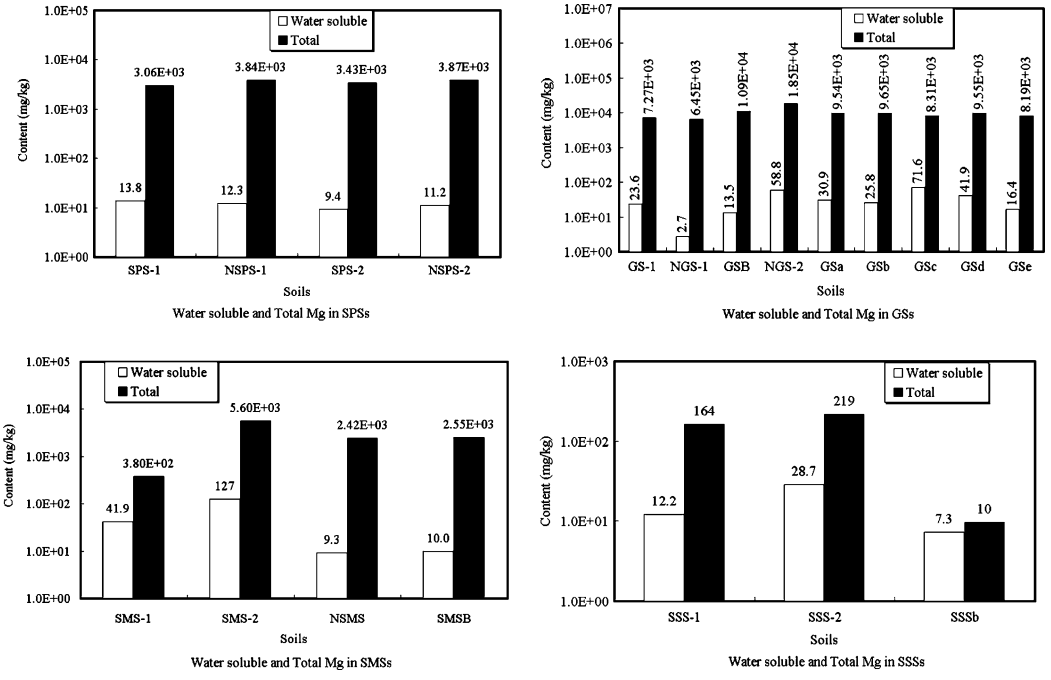


Fig. 4.6. Water-soluble and total Mg in amended soil (81).

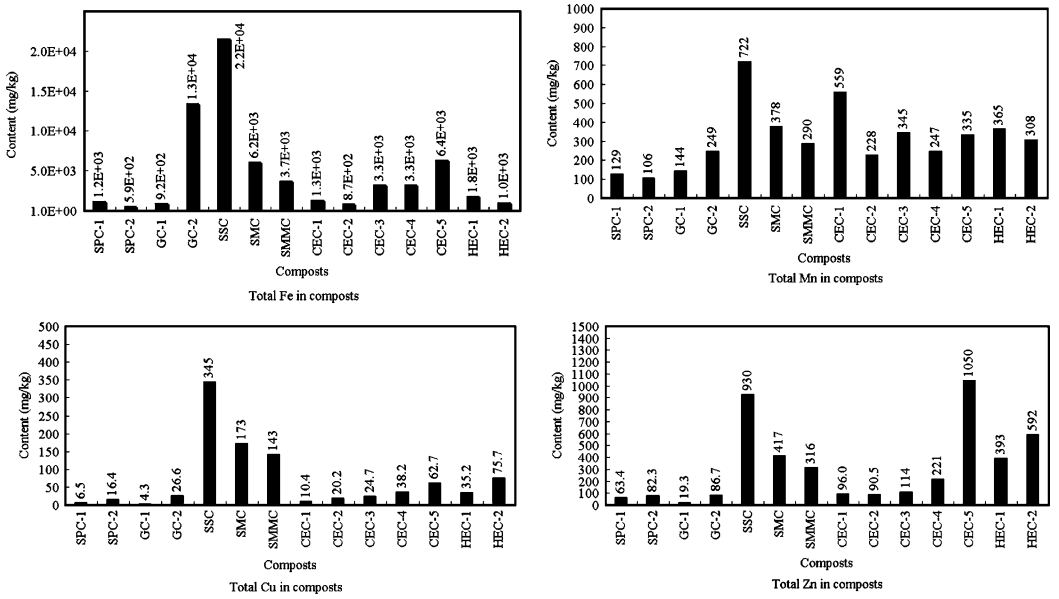


Fig. 4.7. Total contents of Fe, Mn, Cu, and Zn in various composts (81).

Total Fe, Mn, Cu, and Zn contents are mostly at much lower levels in both SPC (Fe: 593–1.17 × 10^3, Mn: 106–129, Cu: 6.5–16, Zn: 63–82, mg/kg dry wt., respectively) and GC (Fe: 923, Mn: 144, Cu: 4.2, and Zn: 19, mg/kg, respectively) than in other composts. On the other side, Fe, Mn, Cu, and Zn are mostly high in SMC (Fe: 6.2 × 10^3, Mn: 379, Cu: 173, and Zn: 417, mg/kg, respectively), SMMC (Fe: 3.7 × 10^3, Mn: 290, Cu: 142, and Zn: 316, mg/kg, respectively), and SSC (Fe: 2.2 × 10^4, Mn: 722, Cu: 345, Zn: 930, mg/kg, respectively). As reported before (24, 25, 44, 60, 71), SMC and SSC are two of the most widely concerned composts due to their high heavy metal contents. Nevertheless, Cu and Zn contents in all these composts were below the USEPA "ceiling concentration" for sewage sludge, that Cu should be no more than 4,300 mg/kg and Zn, 2,800 mg/kg (32). Both Mn and Zn were found in large quantities in HEC (310–360 mg Mn/kg and 390–590 mg Zn/kg), close to SMC and SMMC, while Fe and Cu were relatively lower. Moreover, Mn was at high levels in CEC, next to SMC, SMMC, and HEC. Therefore, generally, such an increasing order: SPC ≈ GC < HEC ≈ / < CEC ≈ / < SMMC ≈ SMC < SSC could be concluded about total contents of Fe, Mn, Cu, and Zn in composted solid wastes.

Distributions of Fe, Mn, Cu, and Zn in various targeted composts are also presented in Fig. 4.8. We can find the following: (1) The percentages of water-soluble and exchangeable Fe, Mn, Cu, and Zn are significantly higher in SPC, GC, and HEC than in SMC, SMMC, CEC, and SSC. (2) In all composts, Fe is predominant in organic matter-bound form. (3) Mn is mostly in the carbonate-bound and Fe–Mn oxide-bound forms followed by the fractions associated with organic matters-bound, water-soluble and exchangeable forms in SPC and GC; predominantly in organic matter-bound form in SSC; mainly in Fe–Mn oxides-bound form followed by

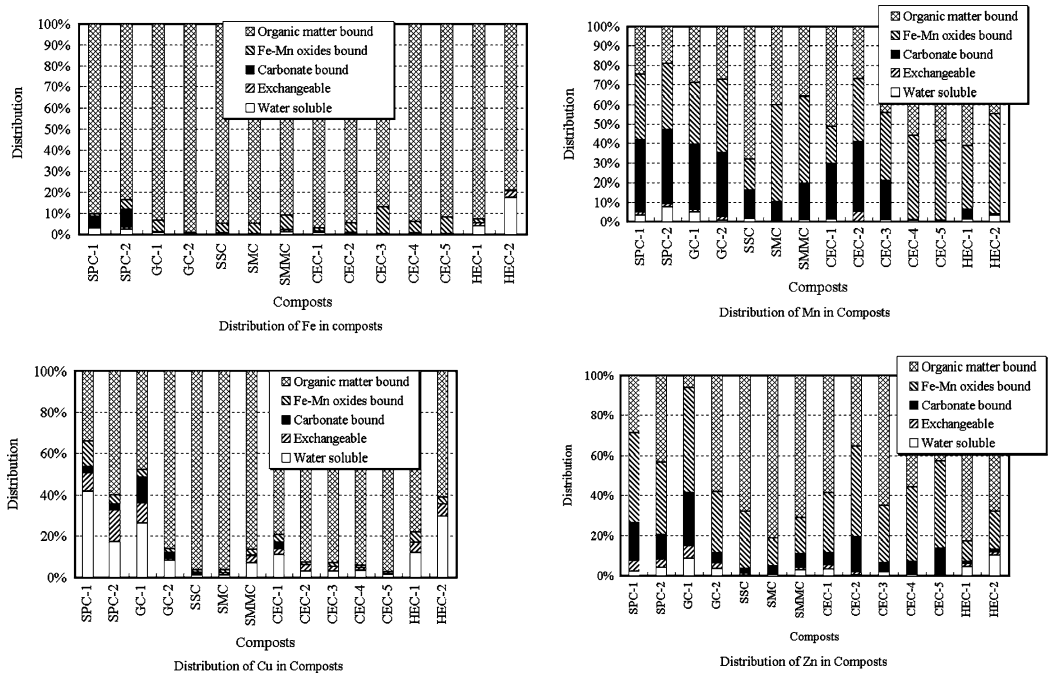


Fig. 4.8. Distribution of various chemical species of Fe, Mn, Cu, and Zn in various composts (81).

organic matter-bound, carbonate-bound, water-soluble, and exchangeable forms in SMC and SMMC; mainly in the Fe–Mn oxides-bound and organic matter-bound forms in HEC; and irregularly distributed in CEC. (4) The fractions of Cu in composts differ from that of Mn. Water-soluble and exchangeable Cu in SPC, GC, and HEC mostly exceed 10%, and sometimes as high as 40–50%, next only to the organic matter-bound form. Both water-soluble and exchangeable Cu are at considerably high levels in SMC, SMMC, CEC, and SSC, but the organic matter-bound forms of Cu are still in the majority (>80%). The degradation of organic Cu compounds will result in the slow but continuous Cu release. (5) As for Zn distribution in composts, Fe–Mn oxide-bound form is the main fraction. It is the most in SPC and GC, and the second most in SMC, SMMC, CEC, HEC, and SSC (the most was organic matter bound form). The carbonate-bound Zn counts more than exchangeable and water-soluble Zn in SPC, GC, SMC, SMMC, and SSC, except for HEC, in which water-soluble Zn is found more.

2.3.2. Fe, Mn, Cu, and Zn in Composts-Amended Soils

The total Fe, Mn, Cu, and Zn contents of various soils are shown in Figs. 4.9–4.12, respectively.

SPC applications led to the slight Fe and Mn decreases in soil, as can be contributed partly to the lower level of Fe and Mn in SPC than in NSPS (Fe: 2.96×10^4–3.41×10^4, Mn: 288–587 mg/kg). Total contents of Cu and Zn in SPS-1 and SPS-2 also fell into the

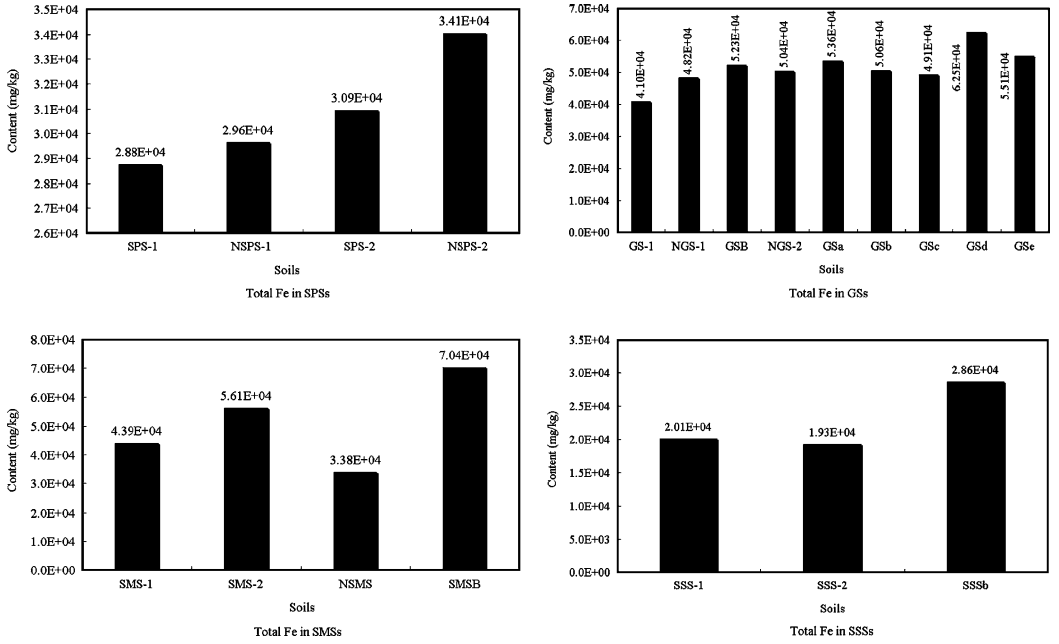


Fig. 4.9. Total contents of Fe in amended soil (81).

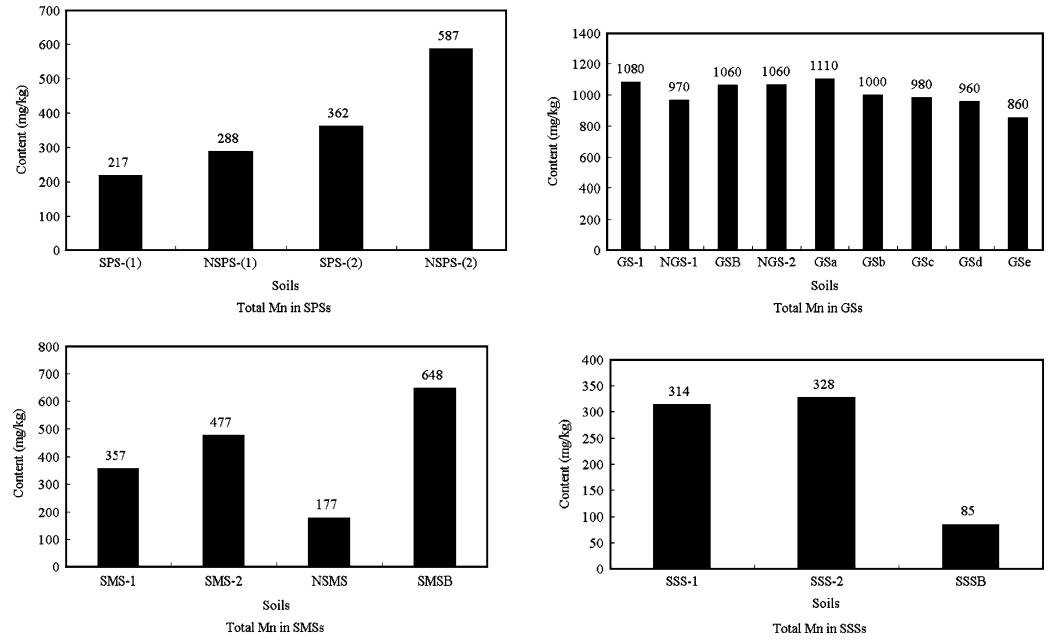


Fig. 4.10. Total contents of Mn in amended soil (81).

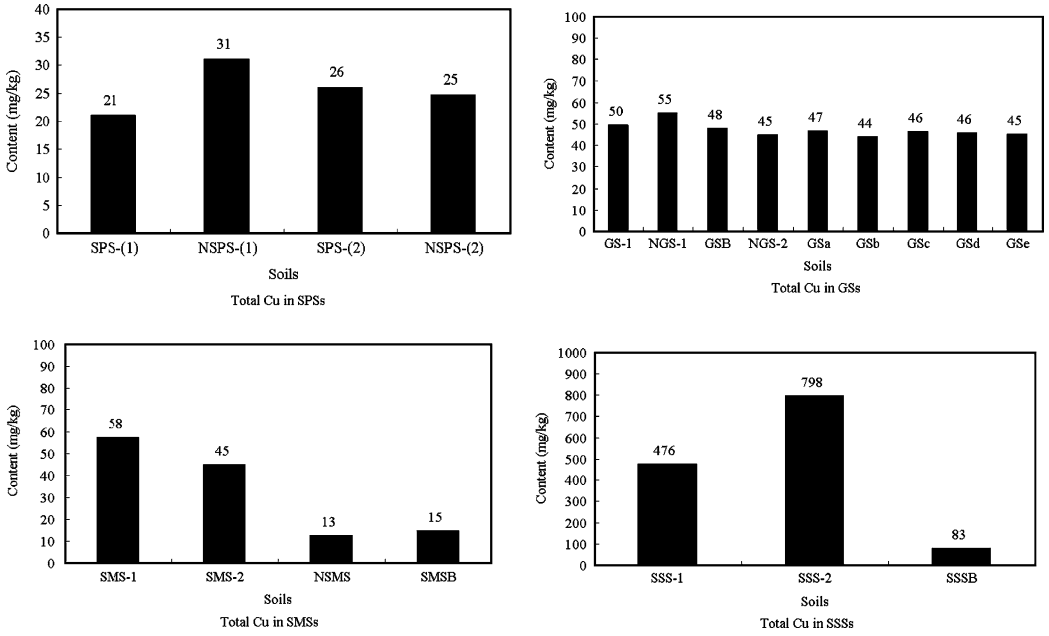


Fig. 4.11. Total contents of Cu in amended soil (81).

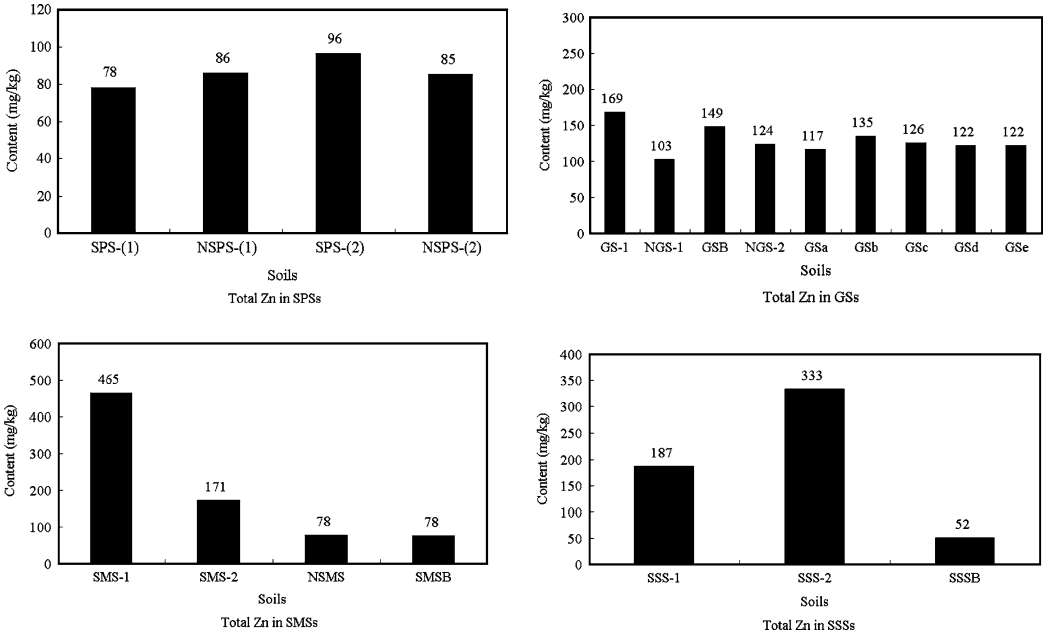


Fig. 4.12. Total contents of Zn in amended soil (81).

narrow ranges (21–26 and 78–96 mg/kg, respectively), almost close to that of NSPS (25–32 and about 85 mg/kg, respectively). Total element contents of GSs (i.e., GS, NGS, and GSB) were all in the following ranges: Fe: 4.10×10^4–6.25×10^4; Mn: 857–1.11×10^3; Cu: 44–55 and Zn: 103–169 mg/kg, respectively. They were in agreement with the literature values of uncontaminated soil: Fe: 4.0×10^4; Mn: 432; Cu: 2–250 and Zn: 10–300 mg/kg, respectively (72–74). No significant Fe, Mn, Cu, and Zn accumulation has occurred. SMSs (i.e., SMS, NSMS, and SMSB) and SSSs (i.e., SSS and SSSB) differed completely with SPSs (i.e., SPS and NSPS) and GSs in the relationships among composts-amended soil, fertilizer-used ones, and control soil. Slight decreases in total Fe and Mn contents of SMS-1 and SMS-2 were observed due to the lower contents of elements Fe and Mn in SMC than in SMSB (7.04×10^4 and 648 mg/kg, respectively). However, Fe and Mn contents of NSMS (3.38×10^4 and 177 mg/kg, respectively) were lower even than that of SMSB. This is because the land (NSMS) has been being used for rice production, and large amounts of Fe and Mn were leached into groundwater. On the other hand, total Cu and Zn contents increased greatly with compost uses. Unlikely, Fe and Mn, and Cu and Zn were at the same levels in NSMS (13 and 78 mg/kg, respectively) as in SMSB (15 and 77 mg/kg, respectively) due to both the lower solubility and the stronger organic-complex abilities of Cu and Zn than that of Fe and Mn. As for SSS, the Mn, Cu, and Zn accumulations were very significant. Mn content in SSS-1 and SSS-2 increased 2.7 and 2.9 times, Cu content did 4.8 and 9.0 times, Zn content did 2.6 and 5.5 times, respectively, relative to SSSB (Mn: 84, Cu: 82, Zn 52 mg/kg), and Cu contents even exceeded beyond the normal range of uncontaminated soil (WHO's criteria, 2–250 mg/kg). Conversely, the SSC addition caused Fe decrease in soil because Fe was at lower level in SSC than in SSSB (2.86×10^4 mg/kg). The rainfall was also an important factor that influenced the metal content in soil. It was found that Mn, Cu, and Zn contents in SSS-2, where the farmland was covered with a plastic membrane to promote the maturity of semimature composts, increased by 1.0, 1.7, and 1.8 times, respectively, relative to SSS-1, although SSS-1 has been amended for 6–7 years. These extra portions were attributed to water-soluble and exchangeable metals, as well as those contained in tiny particles.

From Figs. 4.13 to 4.16, Fe, Mn, Cu, and Zn fractionations in various soils are shown, respectively. No significant difference was observed for Fe, Mn, Cu, and Zn between SPS and GS. The distributions in soil follow the order: organic matter-bound $\geq$Fe–Mn oxides-bound $>$ carbonate-bound $>$ exchangeable $\approx$ water-soluble. For SMC and SSC, all farmland applications have lowered the percentages of the water-soluble and exchangeable Fe, Mn, Cu, and Zn, while increased those of organic matter-bound elements in SMS, carbonate-bound, and Fe–Mn oxides-bound forms in SSS. Although both water-soluble and exchangeable elements are thought to be bioavailable, it is supposed that elements in other three forms (i.e., the organic matter-bound, carbonate-bound, and Fe–Mn oxides-bound) also be the "potential" sources of available metals for plants (56), which might keep considerable concentrations of elements in soil solutions.

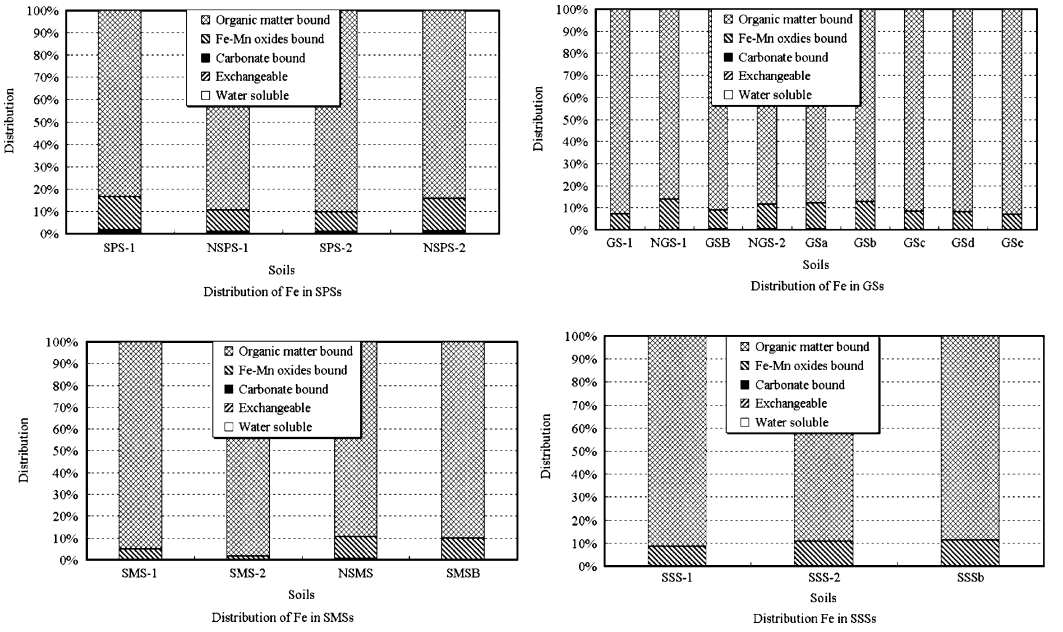


Fig. 4.13. Distribution of various chemical species of Fe in amended soil (81).

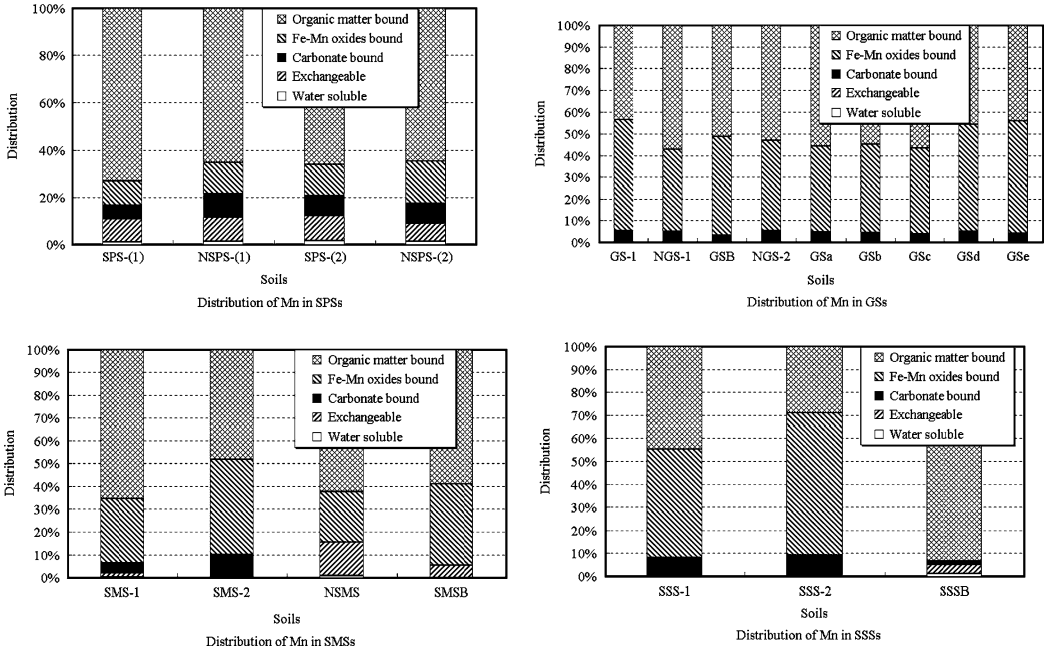


Fig. 4.14. Distribution of various chemical species of Mn in amended soil (81).

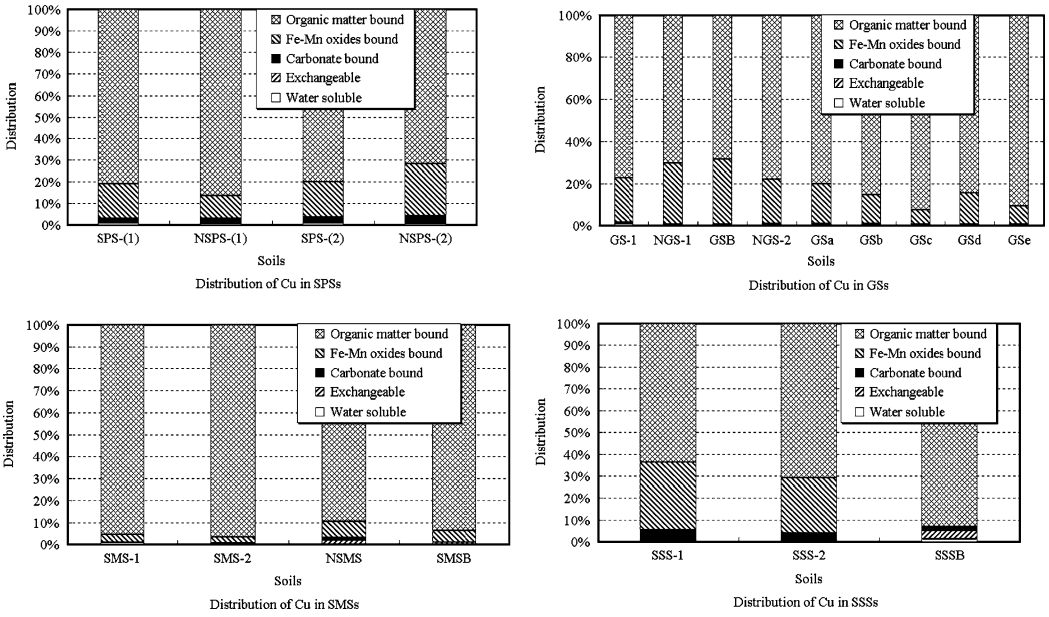


Fig. 4.15. Distribution of various chemical species of Cu in amended soil (81).

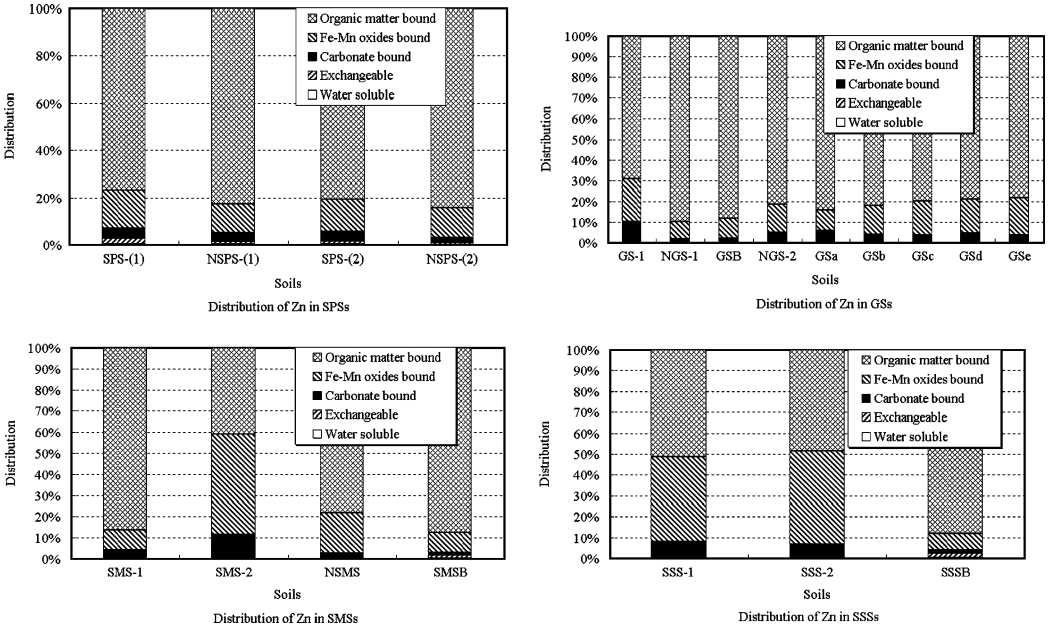


Fig. 4.16. Distribution of various chemical species of Zn in amended soil (81, 82).

2.4. *Heavy Metals (Cd, Cr, Ni, Co, Pb) in Composted Solid Wastes and Composts-Amended Soil*

2.4.1. Cd, Cr, Ni, Co, and Pb in Composted Solid Wastes

Experimental results (Fig. 4.17a) show that Cd has been found much more in SSC, 1.75 mg/kg, and in one special case of CEC, i.e., CEC-5, 1.85 mg/kg, than in SSB, 0.1–0.4 mg/kg. Moreover, both HEC and SPC have rather high Cd concentrations (0.32–0.46 mg/kg and 0.34–0.72 mg/kg, in HEC and SPC, respectively), close to that in SSB. Other composts were popularly at low level, especially GC, Cd concentrations of them are all below 0.1 mg/kg. SSC should be considered to be one major Cd source to soils among solid waste composts, and others are relatively small.

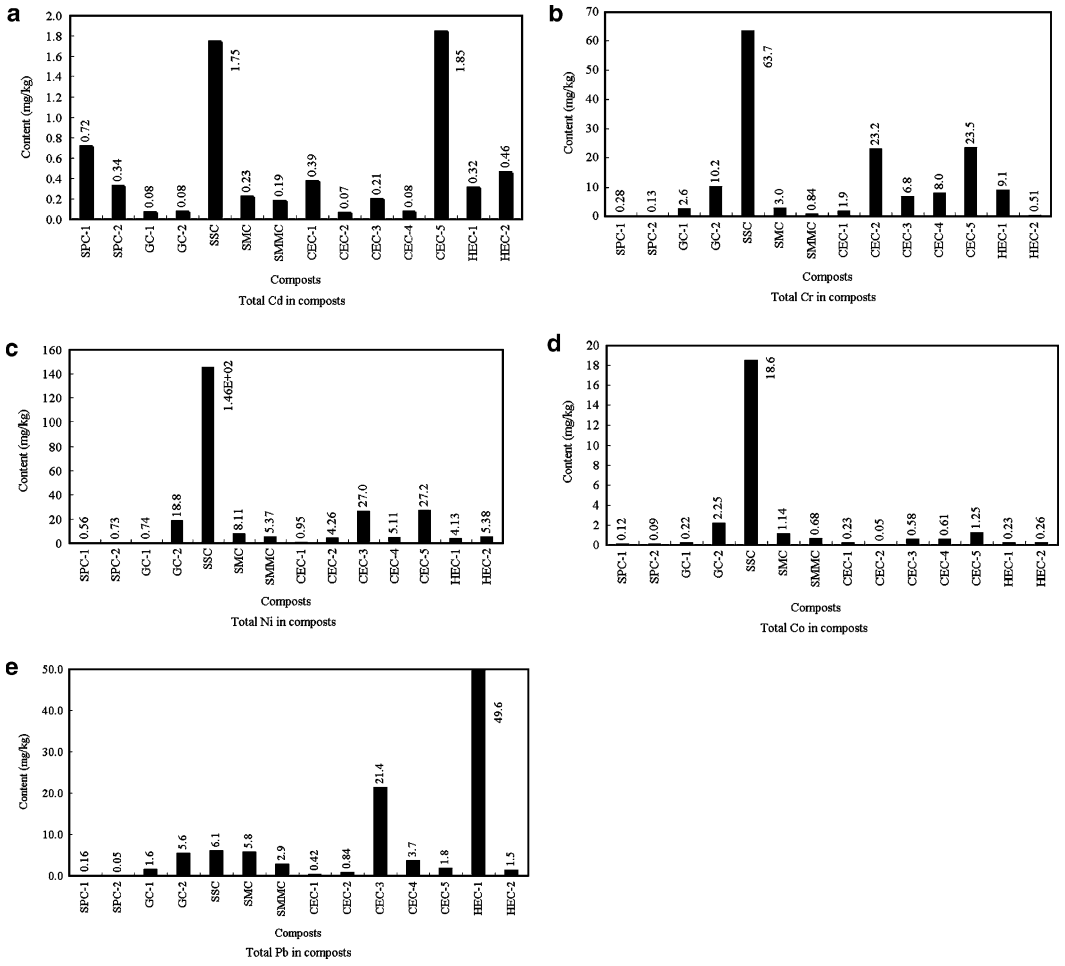


Fig. 4.17. Heavy metals in various composts (81).

Cr has also been found significantly higher in SSC (63.7 mg/kg) than in other kinds of composts (Fig. 4.17(b)). CEC also contains a considerable amount of Cr, and Cr contents of CEC except CEC-1 are over 5 mg/kg. In GC and HEC, the composts with Cr contents near 10 mg/kg also have been found. It is easily estimated that chemical impurities were introduced into composted garbage, and that the uses of inorganic salts in chicken feeding was an important cause for HEC. Apparently, large amounts of inorganic chemicals have been found in these two HEC samples collected from two different regions in Yamaguchi Prefecture of Japan. Low organic contents and surprisingly high solubility in strong inorganic acids, HCl and HNO_3, may demonstrate this conclusion; and secondly, high level of water-soluble Cr has been found and only less than organic matter-bound form. Data about Cr in both SMC and SMMC are so few that here we cannot make a clear conclusion. Two SPC samples contain much less Cr (0.28 and 0.13 mg/kg for SPC-1 and SPC-2, respectively), indicating at least that seafood (fish and/or lobster), whose bones was used to produce SPC, is not the major Cr accumulator. On the other side, although total contents are relatively low, considerable ratios of water-soluble Cr have been found.

Figure 4.17c describes the existence of Ni in various composts. SSC contains a large amount of Ni, 146 mg/kg, even exceeding the threshold level of Ni in soil in connection with phytotoxicity, 100 mg/kg. About 70% of it is in organic matter-bound form, more than 20% is in Fe–Mn oxides-bound form, and water-soluble form of nickel accounts for 3–4%. This means that 1 kg of SSC contains nearly 5 g water-soluble nickel. GC-2 and two CEC samples, CEC-2 and CEC-5, also have considerably high contents of Ni, but still lower than above-mentioned threshold level of Ni. Contents of Ni in other composts are all lower than 10 mg/kg, especially SPC.

SSC, like others (Fig. 4.17d), has a rather high content of Co, 18.55 mg/kg, higher than the mean concentration in soil, 8 mg/kg, and lower than 40 mg/kg, the limit of phytotoxicity. This level is not extremely safe for agricultural plants. All other composts have lower Co contents, and the highest content was 2.25 mg/kg in one of GC samples. The application of composts except for SSC cannot cause the Co accumulation in soil.

Different with other heavy metals in composts (Fig. 4.17(e)), the highest Lead content was found in one of HEC samples, 49.65 mg/kg. The second highest is one of CEC samples, i.e., CEC-3, 21.30 mg/kg. However, they are still in the normal Pb range of environmental soil. In other compost, Pb contents are all lower than 10 mg/kg.

2.4.2. Cd, Cr, Ni, Co, and Pb in Composts-Amended Soils

Figure 4.18 shows an extraordinarily high Cd content in the background soil, ranging from 24.7 to 34.6 mg/kg, far more than the literature value of background Cd. The Cd concentration of SPC, 0.34–0.72 mg/kg, is about 1% of it. SPC applications have caused no significant Cd increase in soil. Same with SPC, GC also contains little Cd, 0.08 mg/kg, and the accumulation has hardly been caused in soil. SMC and SSC applications have caused significant changes in Cd contents in soil. Cd contents of SMC are higher than the background Cd (about 0.037 mg/kg, which is much lower than that reported in literatures, 0.1–0.4 mg/kg) in testing sites. Therefore, it is easy to understand that experimental results, SMS-1 > SMS-2 > SMSB. At the same time, samples from fertilizer-applied soil also show an apparent increase in Cd

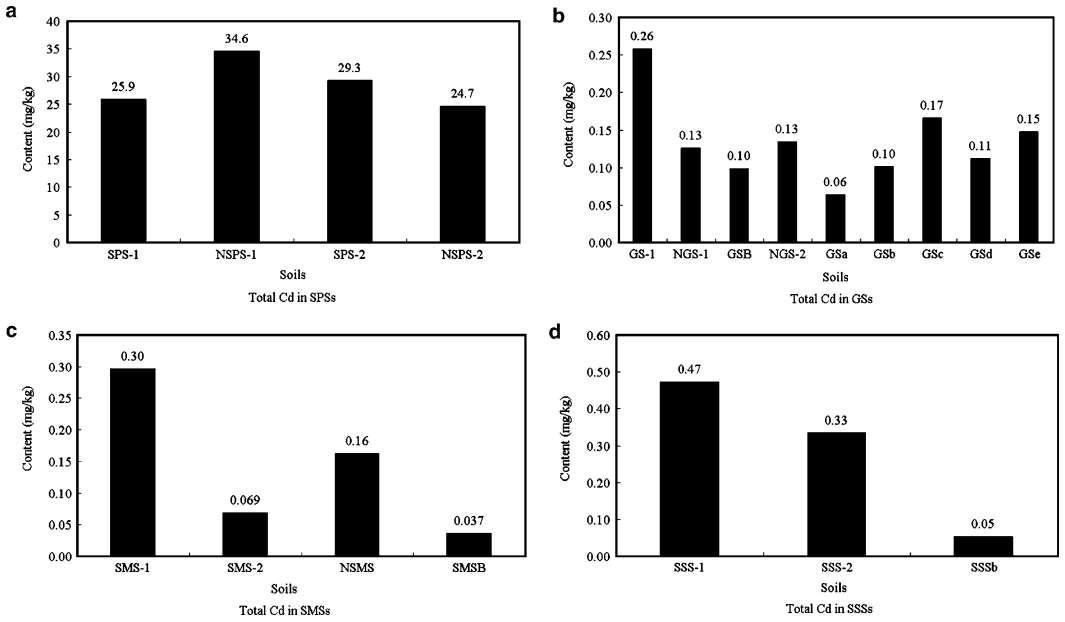


Fig. 4.18. Cd in composts-amended or unamended soil (81).

content over the background. Raw materials of chemical fertilizer usually come from natural ore, and metallic elements may be introduced into final fertilizer products. SSC applications have also caused large increases of Cd in soil. It can be attributed to the higher Cd content in SSC (1.75 mg/kg) than in SSSB, 0.05 mg/kg. It should be explained that the changes of the Cd contents of both SMC- and SSC-applied soil can be attributed to the higher Cd content of SMC and especially SSC than that of soil, and the amount of compost application is also an important factor.

No significant Cr accumulation has been found in SSS-1, which has been SSC-amended for 6–7 years and open to air (Fig. 4.19(d)). At the same time, SSS-2 show a rather increase of Cr content, mainly due to its being covered and negligible rainfall washout there. The applications of both SPC and GC (Fig. 4.19a, b, respectively) have resulted in significant Cr decreases in the compost-applied soil. This should partly be attributed to lower Cr content than soil. The SMC applications (Fig. 4.19c) also have caused Cr accumulation in soil similar to that of Cd. It is noticeable that the Cr content of SMC is found lower than that of control, but the Cr accumulation has occurred. This accumulation may mainly be attributed to the low solubility of the Cr-containing inorganic salts.

Less Ni in SPS than in NSPC may be attributed to the low Ni content of SPC (~0.6 mg/kg) or high Ni content of applied fertilizer (Fig. 4.20a). Significant difference in Ni distribution has hardly been found between SPS and NSPS. No clear change can be found after the GC application in farmland (Fig. 4.20b). Both low Ni content and the small amount of GC application seem to be unable to become the critical factor determining the existence and

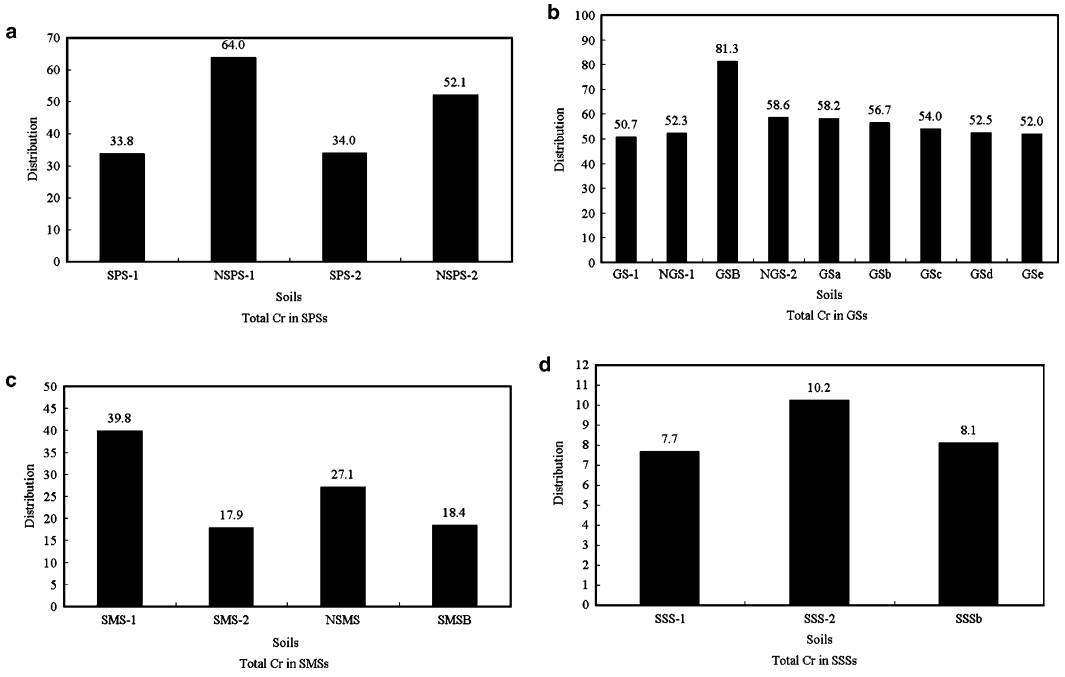


Fig. 4.19. Cr in composts-amended or unamended soil (81).

distribution of Ni in soil. Comparatively, with SMC being applied, one increasing trend of Ni in SMC-amended soils can be observed (Fig. 4.20c). Ni is much higher in SMS-1 than in SMS-2 and SMSB. This is because SMC has higher Ni content than the control, 5.9 mg/kg. Chemical fractionation analysis shows that the SMC application has increased the ratio of organic matter-bound Ni and decreased that of exchangeable and water-soluble Ni. Therefore, the direct consequence of SMC application is the increase of the ratio of the organic matter-bound Ni. The SSC application also has increased the Ni content of SSC-amended soil, as may be attributed to the higher Ni content of SSC (Fig. 4.20d). Contrary to SMC, the SSC application results in the decrease of the ratio of organic matter-bound Ni and the increase of that of Fe–Mn oxide-bound form of Ni. This is in agreement with the result that SMC has higher percent of Fe–Mn oxide-bound and less organic matter-bound Ni than that of soil, SSSB. This result implies the increase the bioavailability of Ni in soil.

In SPC- and GC-amended soils (Fig. 4.21a, b, respectively), no significant difference from unamended soil as for Co content can be found. As for the SMC application (Fig. 4.21c), slight decreases can be found in SMS-1 and SMS-2. This is because Co is less in SMC than in soil. NSMS contain less Co than SMSB, as can be attributed to the irrigation in rice field. The Co accumulation resulted from the SSC application in amended farmlands can be observed (Fig. 4.21d). On the other side, the rainfall washout also causes the Co loss of amended soil, resulting in the decrease of Co content of amended soil. Co is less in SSS-1 than in SSS-2.

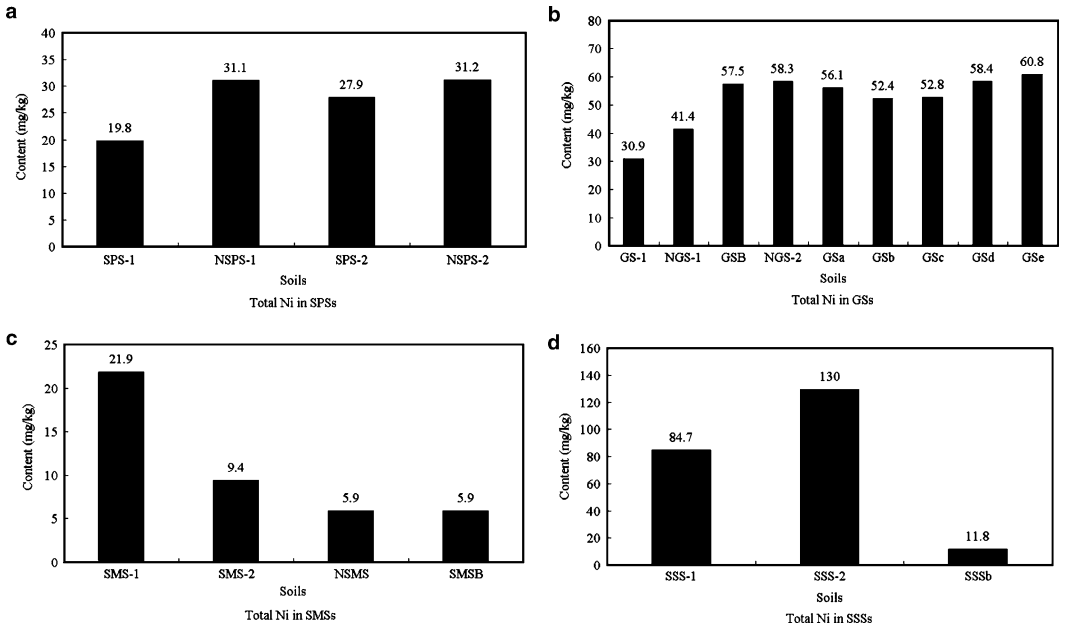


Fig. 4.20. Ni in composts-amended or unamended soil (81).

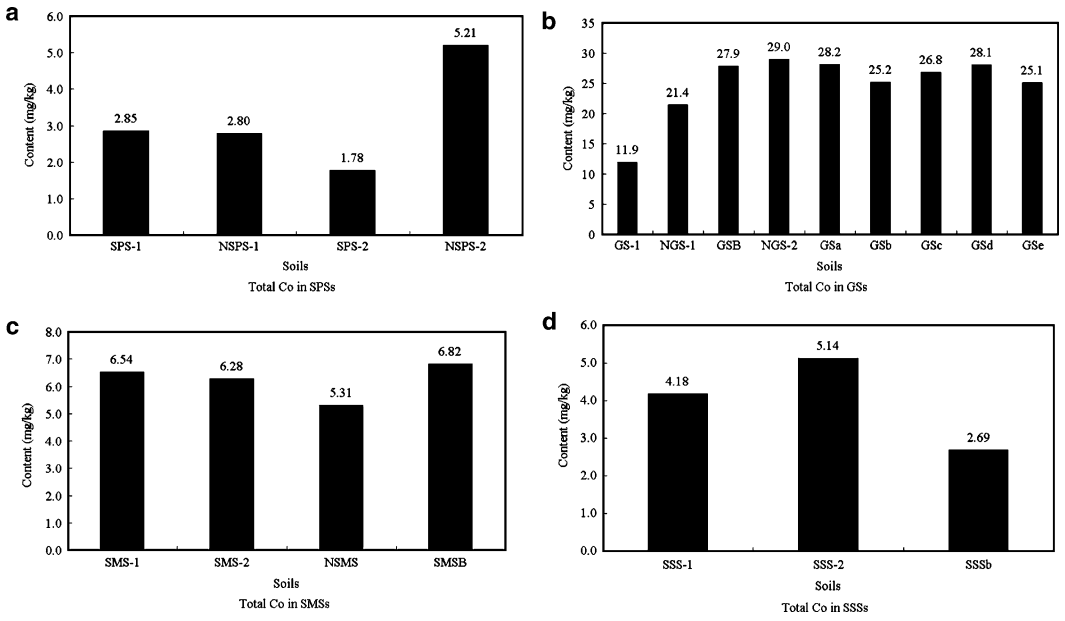


Fig. 4.21. Co in composts-amended or unamended soil (81).

There is no significant Pb change in SPC- and GC-amended soil (Fig. 4.21a, b, respectively), related to fertilizer-used soil, NSPS-1 and NSPS-2. This is because the Pb content is much lower in SPC and GC than in NSPS-1, and NSPS-2 and the amounts of SPC and GC application were so small that the Pb content of soil is difficult to be affected. Pb distribution is kept in the same order, organic matter-bound > Fe–Mn oxide-bound > other forms of Pb. However, much significant increases of Pb content have been found in SMS-1 and SMS-2 (Fig. 4.21c). The more SMC is used in farmland, the more significantly the Pb accumulates. Because SMC had ever been measured and found to be in a relatively lower Pb content than soil, its application should have not caused the Pb accumulation in soil. It should be explained here that actually the Pb content in SMC is not constant but fluctuates within quite a wide range, of which the highest value exceed that of control soil. The carbonate-bound Pb is very low, organic matter-bound and Fe–Mn oxide-bound forms of Pb are two major parts of Pb in SMC-amended soil. In SSS (Fig. 4.21(d)), the abnormal results have been obtained, which is difficult to be explained. As for the Pb distribution in SSC-amended soil, the direct consequence is that the organic matter-bound and Fe–Mn oxide-bound forms of Pb count the most parts, and Carbonate-bound form of Pb is hardly found (Fig. 4.22).

2.5. Organic Matter and Moisture Content in Composts and Unpolluted Soil

Contents of both organic matter and moisture in various composts are shown in Fig. 4.23. Organic matter contents are much lower in HEC (48%) and SSC (57%), compared with those in SMC (74%), SMCC (82%), CEC, SPC, and GC (87%). HEC, as well as SSC, contains less

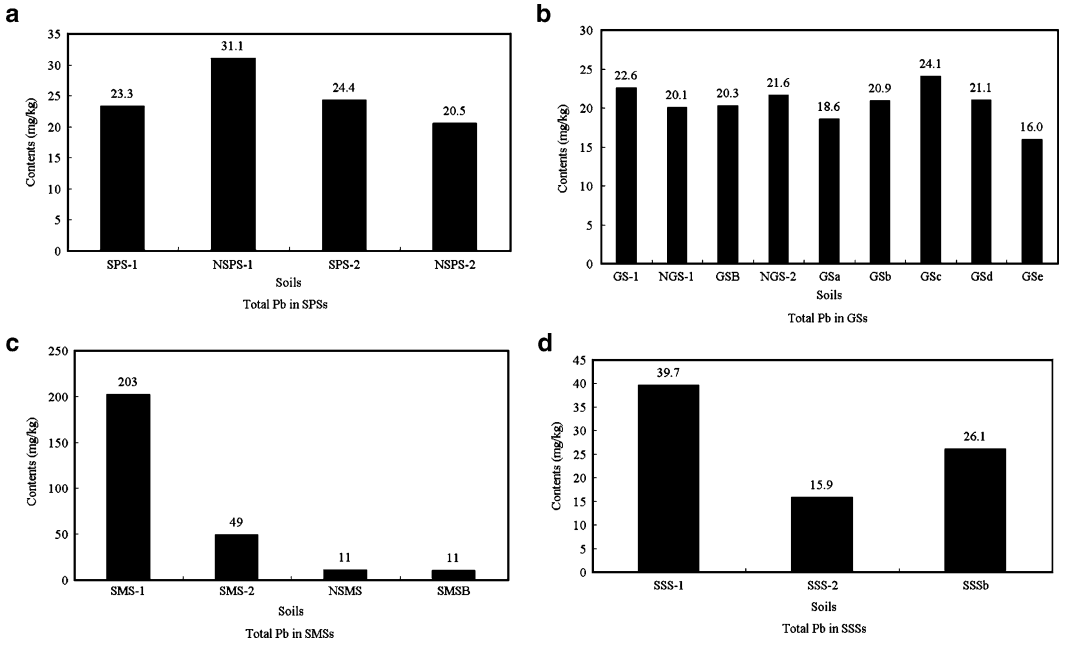


Fig. 4.22. Pb in composts-amended or unamended soil (81).

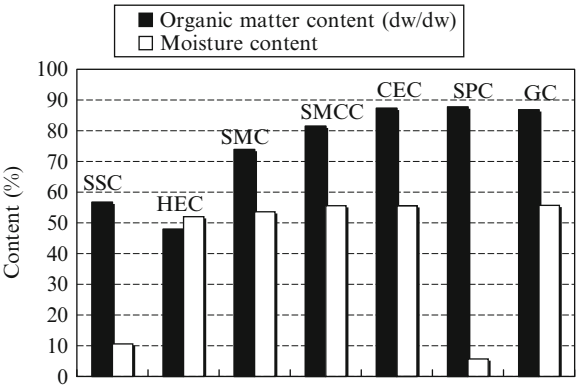


Fig. 4.23. Contents of moisture and organic matter in various composts (81, 83).

inorganic substances, this is in agreement with that reported in the literature (75), while high organic matter contents in SMC and CEC are attributed to the addition of wood chips in the composting process (of swine manure) (76) and the grass-derived feed (of cattle), respectively. The organic matter content of background soil (control) was 9.6%, much lower than that of composts. This demonstrates that the compost application could enhance the soil organic matter content.

3. FARMLAND APPLICATIONS OF COMPOSTED SOLID WASTES FOR NUTRIENT BALANCE

Plant nutrients in soil are gradually ingested by plants or redistributed to ground and underground waters, indirectly resulting in the degradation of soil fertility. Composts, although containing slightly less plant macronutrients (N P, K etc.) than chemical fertilizers, are abundant in relatively stable organic matters and plant micronutrients (Cu and Zn, etc.). This is to say, compost applications may partly make up for the deficiencies of organic matters and plant micronutrients in farmlands. To prevent farmlands from heavy metal contamination, evaluation of the current application of composted solid wastes generated in Japan was carried out, focusing on the nutrient balance in soil. It is believed of deep significance in guiding the safe reuses of composts as farmland amendment.

3.1. Principle of Nutrient Balance in Soil

For unpolluted farmlands with high soil fertility, the soil composition, including plant nutrients as well as organic matter content, should be kept in an appropriate level range, and the long-term balance between the input and output of plant nutrients should exist in soil without significant nutrient accumulation or loss (the deficiency of chemical elements).

In the farmland, as shown in Fig. 4.24, “INPUT” comes from atmospheric wet and dry depositions affected by the release of industrial spent gases and the applications of fertilizer and composted solid wastes, while “OUTPUT” is mainly caused by the washout of rainfall, farming activities, and the consumption of nutrients during the growth of agricultural plants.

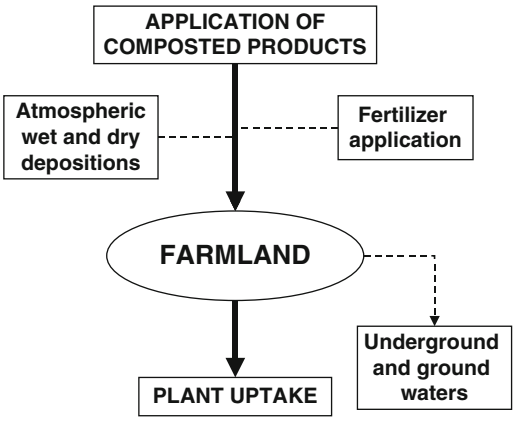


Fig. 4.24. Input and output of nutrients in farmland (81, 83).

Of these routines, only fertilizer, compost applications, and nutrient uptakes of agricultural plants are "visible, calculable and controllable." The principle suggested here that "INPUT," derived from compost applications to the object fields, must correspond to "OUTPUT," from the nutrient uptake caused by agricultural plants.

Soil environmental safety has been considered to the maximum extent here. In many countries, various national and local laws or regulations of environmental protection are currently forbidding the random and excessive releases of industrial spent gases. This makes the applications of composted solid wastes and fertilizer the major and regular "input" routines of plant nutrients to farmlands (77). Actually, the application of chemical fertilizer is not usually recommended in agricultural activities either; hence, compost application is only one way of plant nutrient input. On the other hand, the actual output is not only limited to the nutrient uptake of agricultural plants, other outputs may also lessen the heavy metal accumulation in soil although they have not been considered here. It is believed to be one of the safest ways to establish the maximum permissible application according to the nutrient uptake of agricultural plants, since in the arid regions and rainless seasons, where and when the washout of rainfall may be neglected, the heavy metal accumulation in soils never occurs.

3.2. Evaluation of the Compost Application in Farmland

Yearly yields (78) and organic matter contributions of various composts are estimated according to Eq. (1) and shown in Table 4.6.

$$\mathrm{AOM} = \sum \mathrm{AOM_C} = \sum \left\{ Y_\mathrm{C} \times (1 - \mathrm{MC_C}) \times \left(\mathrm{OMC_C} - 9.6\% \times \frac{1 - \mathrm{OMC_C}}{1 - 9.6\%} \right) \right\} \quad (1)$$

where, AOM is the total amount of organic matters available from current compost applications, 1,000 tons/year; $\mathrm{AOM_C}$ is that from compost C, 1,000 tons/year; Y_C is the yield of compost C, 1,000 tons/year (see Table 4.6); MC_C and OMC_C are the moisture content (%) and organic matter content (wt.%) of compost C, respectively.

Table 4.6
Yearly yields and organic matter contributions of various composts (Japan) (81, 83)

Compost	Yield		Organic matter	
	(1,000 tons/year)	(%)	(1,000 tons/year)	(%)
Wood composts	1,190	39.5	255	28
CEC	792	26.3	302	33
SMC	263	8.7	86.8	9.5
HEC	193	6.4	39.2	4.3
SSC	295	9.8	137	15
GC	8	0.3	1.71	0.19
Others	268	8.9	87.6	9.6
Total	3,010	100	910	100

Moisture and organic matter contents of composts in Fig. 4.23 were adopted. Moisture and organic matter contents of GC, and the averaged values of all sorts of composts were used as the corresponding parameters of "wood composts" and "others" (Table 4.6), respectively.

After the applications, composts gradually form to one part of soil. Therefore, organic matters contained in background soil should be discounted from the total contribution of composts when considering the contribution of organic matter from compost application. The organic matter content of background soil, 9.6%, was deducted. From Eq. (1), it can be seen that the contribution of organic matters from the current compost application in Japan is about 0.91 M tons/year.

Analytical results of plant nutrients in unpolluted soils collected from target fields are shown in Table 4.7. Here, literature values (73) are listed together for comparison. These areas appear to be seriously polluted due to human or agricultural activities, although K and Ca in soil are less than those reported in literatures (73). Most elements are higher in SSC than in soil, especially Cu, Zn, Cd, and Ni (Table 4.8). This can be explained as the results of industrial activities and the chemical additions during the urban and industrial wastewater treatment. SSC is one of the most important mineral element sources to soil.

3.2.1. Input–Output of Mineral Elements in Compost-Amended Farmland

Each year, a large amount of composted solid wastes are applied to farmland. Total loading rates (TR_E) of element E are estimated with Eq. (2), which is a basic expression and can be used in any scope of size, even a piece of field.

$$TR_E = \sum (C_{E,C} \times Y_C) \tag{2}$$

where, $C_{E,C}$ is the concentration of element E in compost C. Results are summarized in Table 4.9 (A).

The amount of element E in the background soil that contains the same quantity of ash with applied composts is counted from the total loading rates (TR_E) according to Eq. (3).

$$NR_E = \sum \left(C_{E,C} \times Y_C - C_{E,S} \times Y_C \times \frac{1 - OMC_C}{1 - 9.6\%} \right) \tag{3}$$

Table 4.7
Elements in unpolluted farmland (73, 81)

Elements	Contents, mg/kg		
	Experiment values		Literature values (73)
	Average	Range	
K	3.91E + 03	(2.03E + 03–5.89E + 03)	1.40E + 04
Ca	3.13E + 03	(109–6.33E +03)	1.50E + 04
Mg	5.99E + 03	(164–1.85E + 04)	5.00E + 03
T-P	1.99E + 03	(152–6.51E + 03)	800
Fe	4.29E + 04	(1.93E + 04–7.04E + 04)	4.00E + 04
Mn	676	(177–1.11E + 03)	432
Cu	41.3	(12.8–82.5)	24.8
Zn	115	(51.5–187)	54.9
Pb	23.0	(10.6–49.3)	17.1
Cd	0.16	(0.037–0.47)	0.33
Co	13.7	(1.78–29.0)	8
Ni	37.8	(5.87–84.7)	18.6
Cr	41.5	(7.68–81.3)	25.7

Table 4.8
The comparison of $C_{E,C}/C_{E,Soils}$, (DW/DW) (81, 83)

	SSC	HEC	SMC	MSCC	CEC	SPC	GC
Ca	10.9	68	7.68	3.52	2.38	2	5.73
Mg	1.87	1.69	2.1	2.38	0.8	1.39	0.43
K	1.49	9.03	7.32	23.1	4.25	4.33	1.18
Fe	0.5	0.03	0.14	0.09	0.07	0.02	0.17
Mn	1.07	0.5	0.56	0.43	0.51	0.17	0.29
Cu	8.35	1.34	4.2	3.45	0.76	0.28	0.37
Zn	8.06	4.27	3.61	2.74	2.72	0.63	0.46
Pb	0.26	1.11	0.25	0.13	0.25	0	0.15
Cd	10.6	2.39	1.42	1.16	3.16	3.21	0.48
Co	1.36	0.02	0.08	0.05	0.04	0.01	0.09
Ni	3.85	0.13	0.21	0.14	0.34	0.02	0.26
Cr	0.01	0.21	0	0	0.36	0.18	0.39
T-P	11.3	14.5	16.7	10.5	3.7	11.5	1.3

where $C_{E,S}$ is the content of element E in background soil, mg/kg, (Table 4.8). NR_E (shown in Table 4.9(B)) is thought to be a more valid parameter than TR_E, reflecting the actual net contribution of compost application to farmland.

Results of NR_E indicate that as for Japan, the compost application may provide "surplus" chemical elements such as K, Ca, Mg, P, Cu, Zn, Cd, and Ni, but Fe, Mn, Pb, Co, and Cr, for the unpolluted soil. This implies that the compost application might cause the permanent or short-term concentration increase of some elements in one area of unpolluted and uncultured land.

Table 4.9
Estimation of the input–output of elements on farmlands (83)

	Loading rates by compost application		Total nutrient uptakes of agricultural plants	
	tons/year		tons/year	kg/year/ha
	A[a]	B[b]	C[c]	D[d,e]
K	1.66E + 04	1.20E + 04	1.26E + 05	34.6
Ca	4.38E + 04	4.01E + 04	7.21E + 03	1.98
Mg	8.51E + 03	1.54E + 03	3.20E + 04	8.80
T-P	1.67E + 04	1.44E + 04	8.53E + 04	23.4
Fe	1.14E + 04	○[f]	660	0.18
Mn	493	○	541	0.15
Cu	136	88.40	89	0.02
Zn	481	346.32	591	0.16
Pb	8.56	○	–[g]	–
Cd	0.75	0.56	–	–
Co	5.90	○	–	–
Ni	49.6	5.59	–	–
Cr	14.8	○	–	–

[a]A, Total loading rate.
[b]B, Net addition = total – "background."
[c,d]C and D, total plant uptake of mineral elements.
[e]Total farmland areas in Japan is 3.638 Mha (paddy and ordinary fields are 2.199 and 1.439 Mha, respectively).
[f](○), Below zero.
[g](–), No data.

At present, the question of great concern is whether or not the amount of chemical elements introduced by compost application in Japan has exceeded the requirement for plant nutrients in farmland and agricultural plant growth. However, what is the criterion of safe compost application? A clear answer has so far not been presented yet.

In fact, the diversities of raw materials and composting processes, different element backgrounds of different soil, and different uptake capabilities of various plants for different elements, all make it meaningless to give out one single regulation for the proper compost application. Therefore, it is very important to put forward a novel and safe compost-applying model for conducting a sustainable compost application.

As described in Fig. 4.24, with agricultural plants growing, nutrients are ingested and transferred to plant tissues. Edible parts are processed to food, while residues, together with inedible parts, are collected to produce composts or feed. Plant nutrients are also redistributed. Almost all nutrients "extracted" out of soil by crops can be recycled to farmland. However some elements including copper and zinc are also introduced in other manners, e.g., the industrial activities and/or fertilizer application. Simultaneously, a considerable amount of nutrient elements may elute out of the cycle through other paths such as incineration followed

by landfill. Such exchanges between this cycle system and the "outside" may greatly affect the contents of nutrients in soil. An ideal model would maximize the recycling of resources in composted solid wastes to farmland, with zero discharge and the least negative environmental effect (heavy metal accumulation).

As mentioned before, the nutrient uptake of agricultural plants (NUAP) is an important effluent routine of mineral elements. Here, we have calculated the amount of NUAP (for Japan) by multiplying the harvest of crops (tons/ha) (Japan) with the contents of nutrients in agricultural plants. The same method also can be used to estimate the amount of NUAP for any area concerned in accordance with the sorts and yields of crops, and the contents of agricultural nutrients in plants. Here, rice, wheat, barley (wheat, two-rowed barley and six-rowed barley, rye), sweet potatoes, pulses, vegetable, and fruits, as major agricultural products of Japan (79), are considered. Industrial crops (such as tea) as well as feed and forage crops are neglected due to the higher uncertainty and much lower annual productions than those above-mentioned agricultural products.

Standard contents of nutrients in rice, wheat, sweet potato, pulses, and vegetables in Japan (80) were used to estimate the nutrient uptake of edible parts of plants. Those contained in inedible parts should also be considered. Here, several assumptions are made: (1) the weight ratio of inedible to edible tissue of rice, $\gamma = 1 : 16$, and that for wheat and barley is 1:1; (2) edible and inedible tissues of the same agricultural plants are of the same level for a given element. Thus, the equation is expressed as:

$$U_{\mathrm{E}} = \sum (C_{\mathrm{E,P}} \times Q_{\mathrm{P}} \times \gamma) \times 10^4 \tag{4}$$

where, U_{E} is total plant uptake of element E, tons/year; $C_{\mathrm{E,P}}$ is the standard content of element E in edible body tissue of plant P, mg $\cdot$ 100/g; Q_{P} is the yield of agricultural plant P in Japan, M tons/year, which are given in Table 4.10. The calculated results are given in Table 4.9.

The heavy metal accumulation in farmland should meet such a condition that the net addition caused by the long-term compost application to farmland, NR_{E} (from Eq. (3) and listed in Table 4.9, column B), should be not only higher than zero but also greater than "Total nutrient uptakes of agricultural plants," U_{E} (from Eq. (4) and listed in Table 4.9, column C). From the comparison between $\mathrm{NR_E}$ in "B" and U_{E} in Table 4.9, column C, it can be seen that the compost application can meet the demand of agricultural plant growth for Ca, while other plant nutrients including Cu and Zn seem still insufficient. This implies that Cu and Zn accumulations in compost-amended farmlands should not have happened in Japan (59), which will be explained in the following part.

Figure 4.25a, b show the nutrient uptake of various sorts of agricultural plants. Notice that the total nutrient uptake of rice is much higher than that of other plants. This may be attributed to the larger yield of rice in Japan (79), as well as much higher contents of nutrients in rice (80).

From above-mentioned analysis, it can be concluded that the farmland application of composted solid wastes can supply enough Ca for plant growth. Although toxic metals have not been estimated due to the shortage of public data about agricultural plants, the heavy metal accumulations concerned with the nutrients Cu and Zn are believed unable to occur in Japan. The feasibility of recycling composted solid wastes in the farmland amendment can be demonstrated theoretically.

Table 4.10
Major agricultural products and yields (Japan) (79)

	Agricultural plants	Yields 1,000 tons/year		Agricultural plants	Yield 1,000 tons/ year
1	Paddy	9,472	6	Japanese radishes	1,876
	Field	18		Turnips	187
				Carrots	682
2	Wheat	688		Burdocks	190
	6-row barley	38		Lotus root	76
	2-row barley	154		Taros	231
	Naked barley	22		Yams	201
				Chinese cabbages	1,036
3	Sweet potatoes	1,073		Cabbages	1,449
				Spinach	316
4	Soybeans	235		Welsh onions	537
	Red beans	88		Onions	1,247
	Kidney beans	15		Eggplants	477
	Peanuts (with shell)	27		Tomatoes	806
				Cucumbers	767
5	Mandarin oranges	1,143		Pumpkin and squash	254
	Summer oranges	85		Spanish paprika	171
	Navel oranges	19		Peas, green	38
	Tongor, pomelo mandarin	256		Soybeans, green	81
	Apples	800		Kidney beans, green	64
	Grapes	238		Maize (green)	289
	Japanese pears	393		Strawberries	205
	Occidental pears	31		Watermelons	581
	Peaches	175		Melons in the open	278
	Cherries	17		Melons under glass	39
	Japanese apricots	121		Lettuce	537
	Loquats	8.2		Celeries	40
	Persimmons	279		Cauliflowers	32
	Chestnuts	27		Broccoli	83
	Rapeseed	0.7		Lrish potatoes	2,898

1. Rice; *2*. Wheat and barley; *3*. Potatoes; *4*. Pulses (dried); *5*. Fruits; *6*. Vegetables.

3.2.2. Field Experimental Observation

Why have heavy metal accumulations been well reported? It is to be examined by the following field observations, in which GC, SMC, and SSC applications in farmland have been conducted (as Table 4.3b).

The annual addition of nutrients to farmland (Δ, kg/ha) following compost application is calculated as follows:

$$\Delta = \left(\frac{L_{\mathrm{C}} \times C_{\mathrm{E,C}} \times (1 - \mathrm{MC_C}) + 2{,}000 \times C_{\mathrm{E,S}}}{L_{\mathrm{C}} \times (1 - \mathrm{MC_C}) + 2{,}000} - C_{\mathrm{S,M}} \right) \times 2{,}000 \times 10^{-3} \qquad (5)$$

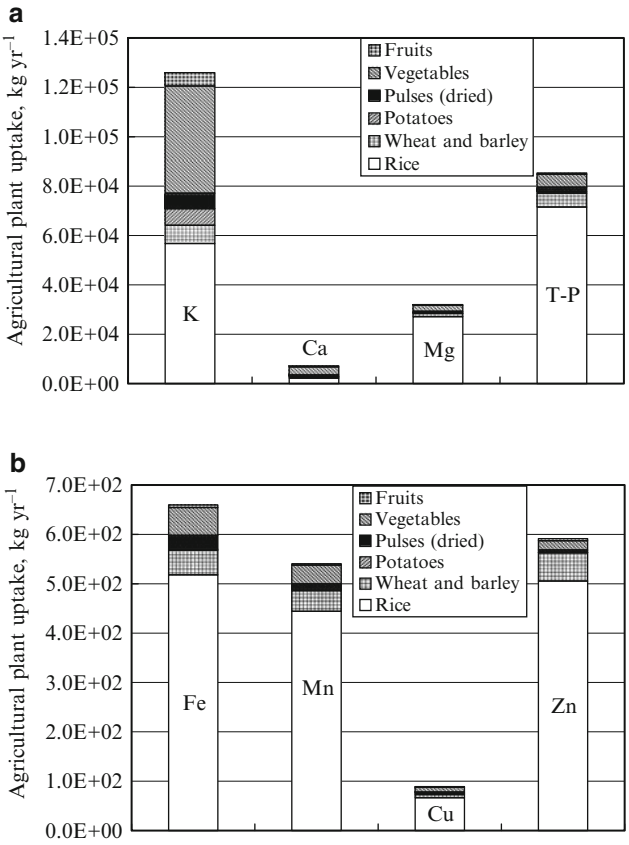


Fig. 4.25. Estimation on nutrient uptake of various sorts of agricultural plants (Japan) (81, 83).

where, L_C is the loading rate of the compost C, t ha/year; the value 2,000 t soil/ha is obtained from an assumed soil density of 1.33 g/cm^3 (see below):

$$100(\text{m}) \times 100(\text{m}) \times 0.15(\text{m}) \times 1{,}330(\text{kg/m}^3) \times 10^{-3} = 1{,}995 \approx 2{,}000(\text{t soil/ha}).$$

The soil plow depth is 0.15 m. The soil density, or bulk soil density, is the mass of oven dry soil per volume sampled, and thus includes both the soil particles and the interstitial air.

Table 4.11 gives the loads of nutrients, i.e., the net contribution of nutrients, K, Ca, Mg, P, Fe, Mn, Cu, and Zn, to the amended farmland arisen from the applications of composts GC, SMC, and SSC. The net contributions of most nutrients to the amended farmland "GS" are below zero except for K, Ca, and P (Table 4.11). This means that the (garbage) compost application may "dilute" some nutrients in soil. Hence, no heavy metal accumulation could happen. The contents of nutrients in amended soil increase in the order GS < SMS < SSS except for Fe, and the increases of nutrients in SMC- and SSC-amended soils are found to be several tens to several hundred times higher than estimated values of plant uptakes, U_E, in

Table 4.11
Loads of mineral elements in soil[a] following compost applications (81, 83)

	K	Ca	Mg	T-P	Fe	Mn	Cu	Zn
	kg/year/ha							
GSa	0.31	6.55	−1.50	0.31	−15.8	−0.21	−0.01	−0.03
GSb	0.93	19.6	−4.51	0.92	−47.5	−0.64	−0.03	−0.08
GSc	3.09	65.4	−15.0	3.05	−158	−2.12	−0.11	−0.28
SMS	435	368	117	549	−647	−5.24	2.32	5.30
SSS	100	1,617	274	1,071	−1,116	2.42	15.9	42.5

[a]The estimation is based on the assumption that the composts are equably spread and mixed homogeneously with the surface soil (below to depth 15 cm) after being applied.

Table 4.9 column D. Direct consequence might be the accumulation of heavy metals such as Cu and Zn.

Figure 4.26a–c show the real experimental comparisons of mineral elements between farmlands applied with composts and fertilizers and the blank soil (control). Nutrient elements in amended farmland have changed in different behaviors due to the existing conditions. The applications of GC have hardly caused the heavy metal accumulation in soil. The compost-amended soils are at almost the same levels of agricultural nutrients as the fertilizer-applied ones. This is in agreement with the results estimated in Table 4.11. GC applications have mainly impacted the recycling of organic matters to soil and the improvement of soil quality. On the other hand, the applications of SMC and SSC have recycled the plant nutrients in composts effectively, but they have also led to the significant heavy metal accumulation in the meantime.

The presence of both K and P in soil has been greatly affected by various processes. Practically, they are less in soil NSMS (rice field, fertilizer was used) than in SMS (grasslands) due to the washout of agricultural irrigation (Fig. 4.26(b)). However, they are found more in SSS-2 (covered field, 1 year) than in SSS-1 (open field, 6–7 years) (Fig. 4.26(c)), as may be attributed to the different washout effects of rainfall. The applications of composts (SMC and SSC) and fertilizers have caused irregular changes of Ca, Mg, Fe, and Mn in farmlands. This is attributed to the too high environmental backgrounds, which hide the effects of compost applications. Therefore, the above-mentioned principle, based on the nutrient balance in farmland, seems unsuitable for predicting the changes of plant nutrients like K, P, Ca, Mg, Fe, and even Mn in compost-amended soil.

Cu and Zn accumulations are far more evident in SMS and SSS (Fig. 4.26b, c) than in GS (Fig. 4.26a). Loading rates are ten times higher than theoretical criteria (i.e., Total nutrient uptakes of agricultural plants, kg/year/ha, U_E in Table 4.9, column D). Another possible reason is that both Cu and Zn easily form the insoluble compounds or organic complexes, resulting in the increasing Cu and Zn accumulations in soil.

In short, the occurrence of heavy metal accumulation requires two essential conditions. First, heavy metal contents are higher in composts than in amended soil; and second, the net contribution of heavy metals following compost applications is greater than nutrient uptakes

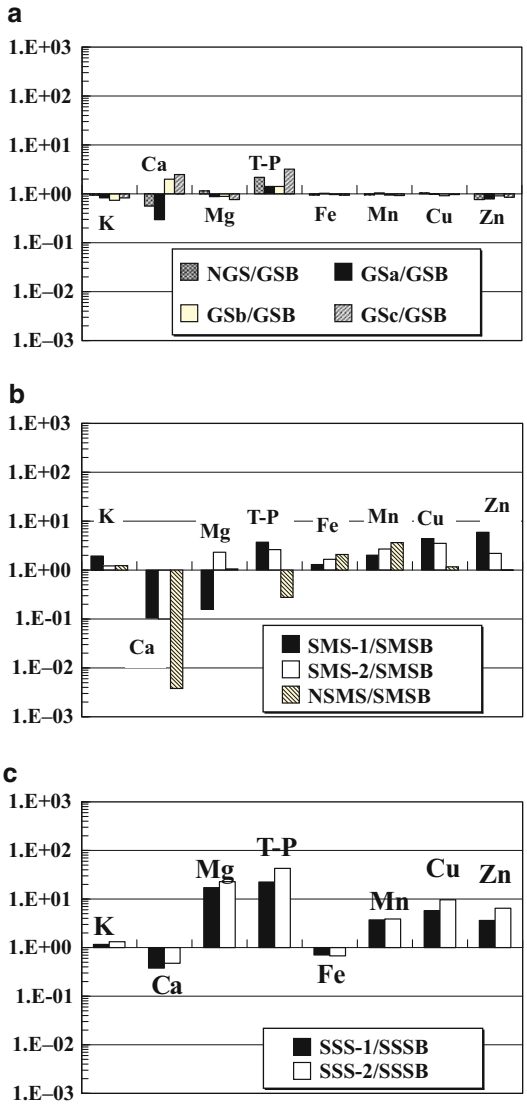


Fig. 4.26. Comparisons of mineral elements between farmlands applied with (**a**) GC, (**b**) SMC, and (**c**) SSC, as well as fertilizers, respectively, and the blank soil (control) (81, 83).

of agricultural plants. Cu and Zn accumulations occurring in the two above-mentioned cases could be attributed to the overloads of Cu and Zn caused by SMC and SSC applications. Theoretically, Cu and Zn contaminations could be avoided by controlling the loading rates of composts in limited areas. Therefore, the farmland application of composted solid wastes is believed one ideal way, beneficial not only for the safe disposal of wastes but also for resource recycling.

4. SUMMARY

Generally speaking, composts are richer in organic matters and plant essential elements, i.e., P, K, and Ca, than the background soil. Composts derived from sewage sludge and livestock excreta are at different degrees richer in plant nutrients (Mg, Cu, and Zn, except for Mn, Fe, and Co) as well as heavy metals (Cd, except for Cr, Ni, and Pb) than soil. Therefore, compost applications may provide plant nutrients to soil, making up for the nutrient deficiency of farmland, while controlling the farmland application of composts, especially sewage sludge, is also believed essential for avoiding heavy metal soil contamination in limited areas.

Results of sequential extraction can greatly sort composts into three types. The first type is the composts derived from sewage sludge and livestock excreta except hen excrete; this type contains considerable levels (i.e., total contents) of mineral nutrients and heavy metals but much lower percentages of various elements in water-soluble forms than in others. The second type is hen excrete compost, as an exception, it has not only considerably high total elemental contents but also rather high percentages of water-soluble forms of elements. The third type contains composts derived from seafood-processing wastes and garbage. Applications concerning the first and second types should be controlled; otherwise, heavy metal accumulation will happen. Comparatively, the applications of the third sort hardly cause heavy metal contamination in soil.

Model estimation indicates that the current compost farmland application in Japan could, to a considerable degree, make up for the deficiency of some plant nutrients in soil that have been resulted from the long-term and continuous nutrient uptake of agricultural plants. That is to say, the compost application to farmland could realize not only the safe disposal of solid wastes but also the effective nutrient recycling all over the Japan, without heavy metal accumulating in soil. The application method of composts greatly affects the heavy metal accumulations in farmland. The occurrence of heavy metal accumulations in farmland can often be attributed to the excessive compost application. Measuring the nutrient balance in compost-amended farmland seems suitable for estimating the changes of heavy metals, such as Cu and Zn, but unsuitable for estimating plant nutrients with high environmental background.

NOMENCLATURE

MSW = Municipal solid waste
DTPA = Diethylene triamine pentaacetic acid
EDTA = Ethylene diamine tetraacetic acid
TP = Total phosphorous
SPC = Seafood processing compost
GC = Garbage compost
SSC = Sewage sludge compost
SMC = Swine manure compost
MSCC = Mixed swine and cattle compost
CEC = Cattle excreta compost

HEC = Hen excreta compost
SPS = SPC-amended soil
NSPS = Commercial fertilizer-applied soil
GS = GC-amended soil
NGS = Commercial fertilizer-applied soil
GSB = Background soil of GS
SMS = SMC-amended soil
NSMS = Commercial fertilizer-applied soil
SMSB = Background soil of SMS
SSS = SSC-amended soil
SSSB = Background soil of SSS
US EPA = United state environmental protection agency
AOM = Total organic matters available from current application, 1,000 tons/year
AOM_C = Total organic matters available from compost C, 1,000 tons/year
Y_C = Yield of compost C, 1,000 tons/year
MC_C = Moisture content of compost C, %
OMC_C = Organic matter content of compost C, wt.%
TR_E = Total loading rates of element E, tons/year and kg/year/ha
$C_{E,C}$ = Concentration of element E in compost C, mg/kg
NR_E = Net loading rates of element E, tons/year and kg/year/ha
NUAP = Nutrient uptake of agricultural plants
γ = Weight ratio of inedible to edible tissue for agricultural plant
U_E = Total plant uptake of element E, tons/year
$C_{E,P}$ = Standard content of element E in edible body tissue of agricultural plant P, mg/100 g
Q_P = Yield of agricultural plant P in Japan, M tons/year
Δ = Annual addition of nutrients to farmlands following compost application, kg/ha
L_C = Loading rate of the compost C, tons/ha/year

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5
Biotreatment of Sludge and Reuse

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CONTENTS

Abstract Sewage sludge, a by-product of domestic wastewater treatment plant, also known as "biosolids", is generated in millions of tons each year. While sewage sludge disposal is a worldwide problem, local conditions dictate the adoption of a variety of treatment and reuse methods. Among them, composting has been practiced extensively in Malaysia. This chapter discusses the theory of the process, fundamental factors affecting the process, and the basis of solid state bioconversion technique. Numerous case studies exhibiting the large scale and continuous operation of sewage sludge composting and their utilization are also presented in this chapter.

1. INTRODUCTION

As an organic waste generated from wastewater treatment plants, sewage sludge poses problems in many parts of the world because of its requirements for special handling and disposal methods. Large land areas have been utilized to store, treat, and dispose of the sludge, but associated environmental hazards have put tremendous pressure on authorities and waste management agencies to reduce or reuse the unwanted waste sludge. Both safe and economical

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_5

methods must be utilized to dispose of, or use, the sludge materials (1). Since sewage sludge is organic in nature, biotreatment methods can be applied to sludge to convert it into reusable byproducts using a composting process. In this way, the wastes can be utilized in a sustainable way and be viewed as a renewable source of raw material to produce natural products such as biocompost.

Composting is an environmental-friendly waste management method for tackling the disposal problem of organic wastes such as sewage sludges and municipal solid waste (1). With the appropriate nutrients, carbon source, and moisture content during the composting process, microorganisms will be destroyed and the organic matter will be stabilized. The stabilized end product (compost) can contribute as a soil amendment to improve soil fertility and provide plant nutrients. These beneficial uses of compost can improve healthy plant production, reduce the use of chemical fertilizers, and conserve natural resources.

Haug (2, 3) regarded composting as the biological decomposition and stabilization of organic substrates under conditions that allow the development of thermophilic temperatures as a result of biologically, produced heat, with a final product sufficiently stable for storage and application to land without adverse environmental effects. He also added that composting is a form of waste stabilization, but one that requires special conditions of moisture and aeration to produce thermophilic temperatures. Hughes (4) stated that composting is the decomposition or incomplete degradation of organic waste materials by a mixed microbial population, usually under warm, moist, and aerobic conditions. According to Bertoldi et al. (5), composting can be defined as a biooxidative process that leads to a highly stabilized organic product, which could be used directly as soil conditioner and fertilizer. Biddlestone et al. (6) stated that composting is the decomposition of heterogeneous organic matter by a mixed microbial population in a warm, moist, and aerobic environment. Diaz et al. (7) defined composting as the biological decomposition of wastes consisting of organic substances of plant or animal under controlled conditions to a state sufficiently stable for a nuisance-free storage and utilization. According to Gaur (8), composting is a biochemical process in which diverse and mixed groups of microorganisms break down organic materials to a humus-like substance that is similar in properties to farm manure.

Mitchell and Lonsane (9) reported that composting is a process that can be carried out using low or high technology, but it is basically a socio-economic process since it removes or renders harmless a waste, which might otherwise result in an undesirable and offensive fermentation. In low-technology applications, agricultural wastes are placed in piles and occasionally turned. A succession of microbes arises from the original microflora. Readily utilizable substrates are degraded mainly to carbon dioxide and water, leaving a product containing substrates that are more difficult for microbes to degrade (especially lignocellulose); this product is then suitable for use as a soil conditioner. These biologically stable wastes represent much less of a pollutant to the environment than the original agricultural by-products.

2. SEWAGE SLUDGE

2.1. Sewage Sludge Generation

Sewage sludge, also known as biosolids, is what is left behind after wastewater is cleaned in domestic wastewater treatment works. It represents the largest in volume among the by-products of wastewater treatment plants. Sludge handling and disposal is perhaps one of the most complex environmental problems. This is because the sludge resulting from the wastewater treatment operations and processes is usually in the form of a very dilute suspension, which typically contains from 0.25 to 12% solids, depending on the operation and process used. Apart from that, sludge is composed largely of the substances responsible for the offensive, pathogenic, and toxic characteristics of the untreated wastewater. It is known to have high organic matter and plant nutrients and, in theory, makes good fertilizer. However, most developed countries regulate its use because it contains a multitude of metals, organic pollutants, and pathogens.

In the United States, the application of sewage sludge to land, especially on agricultural lands, has been contentious since the late 1980s, when national and international clean water regulations prohibiting the ocean dumping of sludge were first enacted. Research scientists and engineers in many parts of the world working on sludge management and utilization continue to advocate the natural ability of sludge, like soil, to immobilize potentially toxic metals. They point to cleaner water, as well as higher crop yields for farms that use the material.

Treatment plant operators will continue to face the challenge of disposing of millions of tons of sewage sludge generated each year (as shown in Table 5.1) (10). If not applied to land, most sludge must be burned in incinerators or land filled, which may create another form of environmental risk. Table 5.1 shows that large quantities of sludge either go into landfill or are used for agriculture purposes. The total annual US production of sludge is reported to be stable or only growing slowly, exceeding 7 million tons of dry matter; however, in Western Europe, where tougher clean water laws are beginning to take effect, sludge production is growing significantly, as small communities build and improve waste treatment plants to comply with regulations. Recent figures quoted the European Union (EU) sludge production as increasing from approximately 6 million to 8 million tons of dry matter during 1992 and 2000 (11).

In many developing Asian countries, sludge management and disposal remain relatively unattended and often receive low priority for development funding. A growing economy such as China's will be facing serious sludge production issues due to the installation of many new sewage treatment facilities; the probable estimate is 4 million tons of annual sludge generation within the next few years.

2.2. Health Impacts of Sludge Utilization

Sewage is a complex mixture of waterborne wastes of human, domestic and industrial origin. Environmental issues include a list of health risk components in sewage such as polluting organic matter, emulsified oil and grease, bacteria and virus, nitrate and phosphate, as well as heavy metals and organochlorines.

Table 5.1
Sewage sludge generation rates (10)

Country	Amount (million tons dry solids/yr)	Disposal method (%)			
		Application to land	Land filling	Incineration	Other
Austria	320	13	56	31	0
Belgium	75	31	56	9	4
Denmark	130	37	33	28	2
France	700	50	50	0	0
Germany (West)	2, 500	25	63	12	0
Greece	15	3	97	0	0
Ireland	24	28	18	0	54
Italy	800	34	55	11	0
Luxembourg	15	81	18	0	1
Holland	282	44	53	3	0
Portugal	200	80	13	0	7
Spain	280	10	50	10	30
Sweden	180	45	55	0	0
Switzerland	215	50	30	20	0
United Kingdom, 1991	1, 107	55	8	7	30
United States	6, 900	41	17	22	20

It is known that sludge contains toxic metals, although at what level and when such metals might cause harmful effects are largely unknown. In most cases, the metals are not a problem, but they could be an issue in the future. Traditionally, many European scientists favor the low estimate of toxicity, whereas many US scientists favor a higher one. If the high estimate is considered, farmers could be facing long-term risks of damaged soil, which would be almost impossible to remedy.

There is no general agreement concerning the maximum allowable concentrations of various metals in sewage sludge. Table 5.2 shows the limits of heavy metal contaminant in sludge, which is adopted by many European countries and the USA (10). Based on the US experience, the national average level of heavy metal found in sludge is about 20 times higher compared to the national average heavy metal content in the soil. Figure 5.1 illustrates the high metal content found in sludge (12).

2.3. Regulatory Issues on Sludge Disposal

Opponents of sludge have focused on the long-term buildup of heavy metals in the soil. They argue that over time, metals such as zinc, lead, copper, and cadmium, may build up to levels high enough to damage agricultural soils. Some opponents advocate a full-scale ban on the use of sludge as fertilizer. But for others, who acknowledge the benefits of sludge, questions still remain regarding the levels at which heavy metals can cause harmful effects.

Table 5.2
Heavy metal contaminant standards in sewage sludge for land application (10)

Country	Year	Cd	Cu	Cr	Ni	Pb	Zn	Hg
European Community[a]	1986	1–3	50–140	100–150[a]	30–75	50–300	150–300	1–1.5
France	1988	2	100	150	50	100	300	1
Germany[b]	1992	1.5	60	100	50	100	200	1
Italy		3	100	150	50	100	300	–
Spain	1990	1	50	100	30	50	150	1
The Netherlands[c]								
Clean soil reference values		0.8	36	100	35	85	140	0.3
Intervention values		12	190	380	210	530	720	10
United Kingdom[d]	1989	3	135	400[a]	75	300	200[e]	1
Denmark	1990	0.5	40	30	15	40	100	0.5
Finland	1995	0.5	100	200	60	60	150	0.2
Norway		1	50	100	30	50	150	1
Sweden		0.5	40	30	15	40	100	0.5
United States[f]	1993	20	750	1,500	210	150	1,400	8

[a] Values are currently being revised.
[b] Values are for soil pHs > 6. At pH 5–6, the Cd and Zn limits are 1.0 and 150 mg/kg, respectively.
[c] Soil cleanup levels which also apply to agricultural land amended with sewage sludge. Concentrations less than the clean soil reference are considered clean soil.
[d] Values shown are for soil pHs 6–7. Other values apply at pH 5–6 and > 7 (U.K. DoE, 1989).
[e] Changed following Independent Scientific Committee recommendations (see text).
[f] Calculated from maximum cumulative pollutant loading limits mixed into soil plow layer. Soil background concentrations are not taken into account.

Regulatory agencies from the EU have begun work on a new sludge directive that will place lower permissible limits for heavy metals (11). Meanwhile, another EU directive sets absolute values for contaminants in food, which could also drive down permitted levels of metals in sewage sludge in the future. Regulations on sludge disposal in the EU include the 1986 Sewage Sludge Directive 86/278/EEC, the Organic Farming Regulation (EEC) No. 2092/91, the Landfill Directive 1999/31/EC, and the Commission Decision 2001/688/EC which is related to eco-labeling of soil improvers and growth media. EU regulations on sludge disposal are currently under revision; it is foreseeable that sludge disposal will encounter much more stringent standard in the near future. Meanwhile, ocean dumping of sludge in the EU countries has been practically forbidden, but the EU Landfill Directive does not prohibit land filling of sludge.

The EU Directives are set up to safeguard public health and safety and essentially meet the following requirements:

- Pretreatment of sludge to minimize risk
- Restriction on the content of heavy metals in soil on which sludge is applied

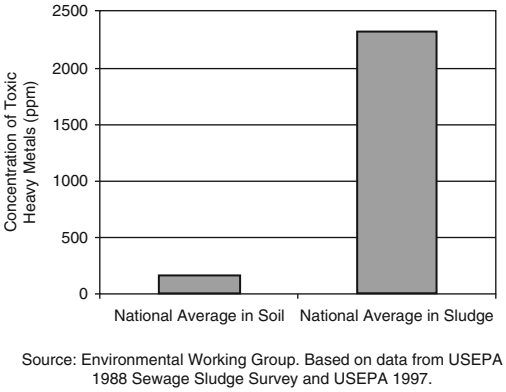

Fig. 5.1. Comparison of heavy metal content in sludge from USA (12).

- Restriction on the content of heavy metals in sludge
- Restriction on the content of micro pollutants
- Restriction on the content of nutrients added to soil (N&P)
- Restriction on the amount of dry solids/heavy metals spread per unit land area and time
- Legislative compliance control

US regulations concerning sewage sludge disposal and application on land are summarized in 40 CFR Part 503, in 1995 by USEPA (13), which is known as "Standards for the Use or Disposal or Wastewater Sludge." The US regulations contain very specific directions for sludge treatment before disposal including requirements for pathogen reduction. Under the US standard, sludge is categorized into two classes: Class A sludge – possible use without restrictions and Class B sludge – to be used under specific site restrictions. Sludge in Class B category has less stringent pre-treatment requirements.

Compared to the EU, the United States has the most relaxed standards for heavy metals content in sludge for land application. As shown in Table 5.2, proposed EU standards for heavy metals are up to 100 times higher than in any other country.

2.4. A Sustainable Approach for Sludge Disposal

While sludge disposal is a worldwide problem, local conditions dictate the adoption of a variety of disposal routes. The ultimate resting place of the sludge must be either on land or in the water. Since many countries have banned ocean dumping, the choice for sludge disposal tends to be restricted to land-based technology.

The widely practiced landfill disposal of sewage sludge is coming under increasing pressure as suitable sites become less available and controls on toxic materials become more stringent. When landfill operations and application to agricultural soil are practiced, the main issues that limit their widespread use are related to pathogens, heavy metals, toxic organics, and transport and application difficulties. A variety of technologies is available to circumvent these difficulties however, sometimes, at considerable cost. Current research findings indicate that organochlorines at low concentrations in the soil do not transfer to crops but are degraded

by soil microorganisms through a bioremediation pathway. However, heavy metals do accumulate if the amount is at high concentration and may have a significant transfer to the food chain. For this disposal method, and whenever a high level of heavy metals is detected, a cost-effective means for removing the metals from sludge is required. The most common method employed for achieving the necessary reduction of metals in sludge is the biotreatment or composting process.

Composting method used for reducing heavy metals content in sludge have shown promising results and valuable by-products can be produced without damaging the environment. These methods are very attractive for many waste management operators as the capital requirement is significantly much less compared to building incineration plants.

3. COMPOSTING OF SLUDGE

3.1. Historical Background of Composting

For many centuries, farmers in many parts of the world have practiced composting of organic wastes to some extent. The Chinese living in the river deltas were an outstanding example, whereby their recycled crop residues, human wastes, and alluvial mud went back to the soil. Using composts in agriculture can minimize organic wastes and can reduce the addition of fertilizers and fungicides in crop production (14). By practicing excellent horticulture, with high labor input, their lands have remained productive for some 4,000 years. Other noted proponents of composting are the people of the Runza Valley in the Himalayas who have practiced their agriculture in terraced fields on the mountainside.

Composting, as practiced by the Chinese, has probably changed very little over the centuries. The theory of the process has been developed over the years by the Western world namely, fundamental reaction and its application to large-scale and continuous operation. In the 1930s, the popularity of composting begun as appreciation grew of the physical, chemical, and microbial interactions involved in composting. Development in the mechanization of composting arose in response to the need for a continuous, controlled, and rapid disposal to deal with the large quantities of municipal wastes produced in towns and cities. Over the years, many composting system have been commercialized, but their basic features are very similar. The only major differences have been in the actual fermentation section, which represent the pits, heaps, cells, bins, digesters, silos, and rotating drums.

In the 1970s, composters with a capacity of over 100 tons/day were rare. During the 1990s, there were 500 tons/day units being installed, and nowadays, 1,000 tons/day ones are being considered. Many systems employ fermentation in open elongated heaps (windrows). Recent composers utilize the well-known Dano rotating drum followed by maturing in windrows. A few are starting to employ a high degree of automation in vertical, multifloored silos with continuous agitation and control of aeration and moisture (15). The problem with composting has been the handling of large volumes of waste materials; the end product is very bulky and has rather low market value. After an initial upsurge of interest in the 1950s, surprisingly few further composting units have been installed in the West. Most interest has been shown in the oil-rich Middle East states where finances are more readily available, and there is a good demand for compost for reclaiming desert soils.

Furthermore, with the sharp increase in oil prices in the 1970s and their effect on fertilizer costs, it became apparent that the agricultural systems of many less developed countries could not be based solely on mineral fertilizers. Composting of agriculture residues and sewage sludge has gained new impetus and the compost products could easily find a market in these developing countries.

3.2. Composting Process

Figure 5.2 shows an overview of the composting process. Microorganisms play a vital role in this process by using nitrogen and carbohydrate as energy sources for their activity while they multiply to produce new organisms. Like any other microbial process, the composting step requires oxygen and moisture to produce a compost product. Water, carbon dioxide, and heat are generated during the process (16).

In the degradation of organic matter into simpler substances, there are two modes of decomposition, aerobic and anaerobic. Fungi, actinomycetes, bacteria, and molds play a dominant role in both of those processes. In aerobic decomposition, living organisms utilize oxygen, feed upon the organic matter and develop cell protoplasm from the nitrogen, phosphorous, some of the carbon and other required nutrients (17). Anaerobic decomposition is characterized by low temperatures, unless heat is applied from an external source. The anaerobic process is associated with the production of odorous immediate products, and also generally proceeds at a slower rate than aerobic composting (18).

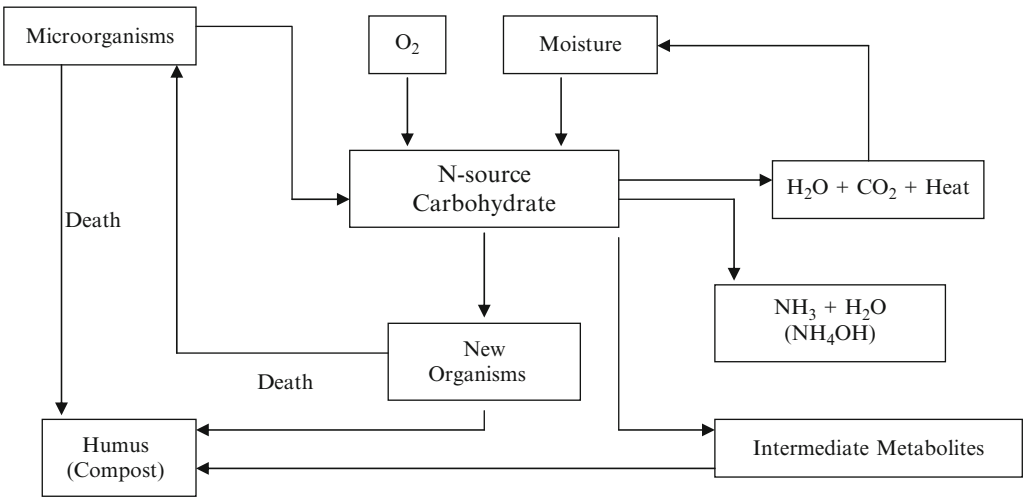

Fig. 5.2. General overview of composting.

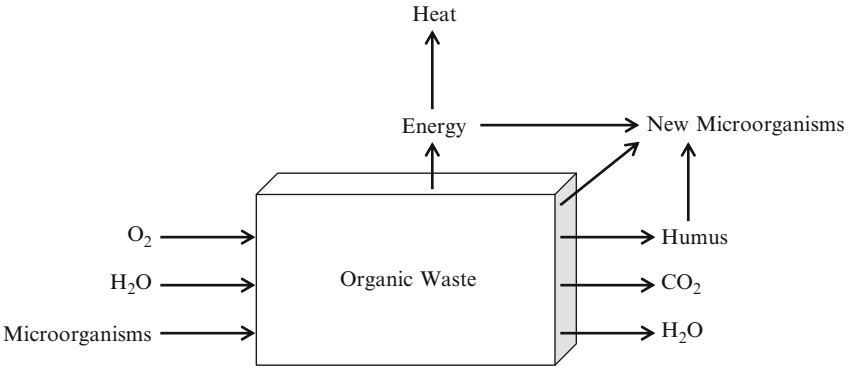

Fig. 5.3. Respiration and heat release in composting.

During the decomposition process, three distinct stages are involved:

- The rapid decomposition of some of the constituents by microorganisms
- Synthesis of new substances by these organisms
- The formation of resistant complexes by various processes of condensation and polymerization.

Bioconversion of organic matter is carried out by different groups of heterotrophic microorganisms such as bacteria, fungi, actinomycetes, and protozoa. Microorganisms involved in the process derive their energy and carbon requirements from the decomposition of carbonaceous material. The microorganisms take in moisture, O_2 from the air and food from the organic material. The organisms give off water, CO_2, and energy, and then they reproduce themselves and eventually die. Some of the energy released is used for growth and movement, while the remainder is given off as heat. The process is shown diagrammatically in Fig. 5.3.

4. TYPES OF COMPOSTING SYSTEMS

The composting system can be divided into two main categories: non reactor and reactor systems. In non reactor or non mechanical systems, the entire composting process occurs outside a reactor. Typical examples of non reactor systems are static piles and windrows. In static piles, wastes are placed into heaps and aeration can be enhanced by the periodic manual turning of the heap. Windrows are used for large quantities of waste and are considered a low-cost method of composting because of their simplicity in design.

In contrast, reactor systems allow the degradation of organic waste to occur in a more controlled environment within the wall of a vessel. Reactor systems are normally mechanical or enclosed systems in which the process can be relatively faster than that of non reactor systems. The most popular reactor composter is the Dano system developed in Europe. This composting process can be controlled rather easily as the waste can be fed through the reactor made faster or slower depending on requirement. In this method, the composting temperature can be maintained either by using insulation of the vessel or injection of air through the composter. Table 5.3 shows the comparison between reactor and non reactor composting system.

Table 5.3
Comparison of reactor and non reactor system

Type of composter	Non reactor system		Reactor system (in-vessel)	
	Turned Windrow	Aerated pile	Forced aeration with agitation	Forced aeration without agitation
Capital cost	Low	Low for small system; high for large system	Very high	High
Operating cost	Low	High	Low	Low
Control of air supply	Restricted	Complete	Complete	Complete
Requirement for subsequent drying	Not required	Not required – self-drying	Not required	Small drying required
Sensitivity to climate change	Sensitive	No	No	No
Land requirement	Very high	High	Very low	Low

Advantages of a reactor system include the following:

- The compost product can be produced in shorter time
- Parameters such as temperatures are easily controlled
- Odors are not produced
- Flies and rodents are not attracted to the end product
- A smaller area is required for operation

Disadvantages of a non-reactor system include the following:

- The system requires a large land area for creating piles or windrows
- Labor requirements are relatively high
- There is a lack of protection from rain and wind, etc. and consequently difficult to control the process
- Although it is said that this process will not cause any appreciable odors, it is believed to be almost impossible to decompose heterogeneous material of this nature with a total absence of generation of odorous gases such as sulfides, ammonia, mercaptans, and similar substances associated with anaerobic processes
- Strict pest and vector control measures would have to be employed to eliminate nuisance and to protect public health.

5. FACTORS AFFECTING COMPOSTING PROCESS

The major environmental parameters that need to be properly controlled in the operation of composting process are C/N ratio, particle size, moisture content, aeration, temperature, and pH. The optimum conditions for rapid composting are summarized in Table 5.4.

5.1. Temperature

Thermophilic temperatures (45–65°C) are favored for increasing the efficiency of the process and are lethal to pathogens. High temperatures are essential for the destruction of

Table 5.4
Optimum conditions for rapid composting

Parameters	Value	References
C/N ratio of feed	25–30:1	(6)
Particle size	10–50 mm	(22)
Moisture content	50–60%	(6)
Aeration	0.6–2.0 m^3 air/day/kg	(21)
Temperature	50–70°C	(6)
Agitation	Every 5–10 min	(21)
pH control	pH 6.0–8.0	(19)

pathogenic organisms and undesirable weed seeds. Decomposition also proceeds much more rapidly in the thermophilic temperature range. The optimum temperature range is 50–70°C, with 60°C usually being the most satisfactory (2). A prolonged high temperature of 70–75°C may inhibit some of the beneficial microbial actions and increase nitrogen loss due to the vaporization of ammonia. Temperatures should be sufficiently high for a long enough time to accomplish more a rapid decomposition rate, kill pathogenic organisms, destroy weeds and vegetable seeds, and destroy fly eggs and larvae.

5.2. Time

The quality of a compost product greatly depends on the length of time that the mixture is composted. If a high composting temperature (optimum 50–55°C) is not maintained through out the material for a sufficient length of time (> 2 days), pathogen destruction will not reach the required level. Reactor retention times and curing times may vary from system to system (2). Most composting systems are able to produce compost products within 40–60 days.

5.3. pH

Optimum pH levels are required to achieve satisfactory composting and yield neutral compost. According to Verdonck (19), optimum pH levels are 6.0–8.0 for composting and 4.0–7.0 for the end product. Both acidic and basic materials can be successfully composted to a neutral product. Aerobic bacteria thrive well at a pH range of 6.0–9.0. Actinomycetes grow at a pH of 5.5–9.5, whereas fungi develop within much wider pH ranges from 3.0 to 9.5. Control of pH is unnecessary for most municipal wastes. Normal digestion follows from an acid pH of 5.0 to a final alkaline pH of 8.0–9.0.

5.4. C/N ratio

Microorganisms use carbon as a source of energy through metabolic oxidation as well as in the synthesis of cell wall and other cellular structure and protoplasm. Microorganisms cannot live without nitrogen, as it is a major constituent of protoplasm. Gaur (20) documented 25–30 as the satisfactory C/N ratio for initial process of composting and 30–35 C/N ratios for efficient composting. The required composting time depended on the initial C/N ratio (16). The time

Table 5.5
Composting time for sewage sludge–solid waste mix based on initial C/N ratio (21)

Initial C/N ratio	Approximate composting time required (days)
27	45
32	43
52	40

required for co-composting of sludge mixed with organic solid wastes took about 40 days (Table 5.5).

5.5. Moisture Content

Microorganisms need 40–60% moisture to survive. Moisture content in waste should not exceed 80%, for the suffocation (little air availability within the waste matrix) will kill all aerobic microorganisms. In practical aerobic composting, a high moisture content must be avoided because water displaces air from the interstices between the particles, giving rise to anaerobic conditions. Previous studies indicated that the moisture content of solid wastes studied usually fell in the range of 40–60%, which is the most satisfactory range for aerobic composting.

If the amount of moisture in the waste mixture is below 40% (w/w), decomposition will be aerobic but slow. The optimum moisture level for aerobic composting is 50–60%. Moist conditions are normally applied in a composting system by the use of water sprinklers. In general, a range of 40–80% may be quite satisfactory depending upon the nature of material to be composted (20).

5.6. Aeration

Proper aeration is applied to obtain rapid nuisance-free decomposition in the composting process. Aeration also helps to reduce the high initial moisture content in the composting material. Oxygen is not only necessary for aerobic metabolism and respiration of microorganisms but also for oxidizing the various organic molecules present in the moisture. Organic composting requires aeration to provide sufficient oxygen for the aerobic microbes. In a mechanical unit with continuous aeration, the desirable amount of air was 10–30 ft^3/day per pound of volatile solids (in the initial charge) (21). This provides more than twice the amount of oxygen needed for the oxidation of the organic matter but was desirable because lower aeration rate resulted in prolonged composting while higher rates resulted in rapid cooling and drying of the refuse. The oxygen consumption in a composting mass depends on several factors:

- The state of process
- Temperature
- Degree of agitation of the mass
- Composition of the composting mass
- Particle size of the mass
- Moisture content.

5.7. Mixing

Mixing or turning affects the type and rate of composting. Turning provides sufficient oxygen for aerobic activity to take place, so that it will speed up the composting process. The mixing rate depends on the type of composting and special machines for turning/mixing and aerating are used in large-scale composting involving a windrow pile. Constant slow mixing or intermittent mixing every 5–10 min or a combination of forced air and less frequent mixing is recommended (21).

5.8. Size

Composting material that consists of small particles is more readily decomposed than material with larger particles, as the surface area of contact is greater. On the other hand, if particles are too fine, there will be less oxygen diffusion. Furthermore, very fine material tends to lose some of its usefulness as a soil amendment. Typical particle sizes of material used for composting range from 10 to 50 mm (22). It has been reported that shredding offers ample opportunity for rapid aerobic decomposition. The optimum size for raw material is about 5 cm. Fine grinding is recommended for mechanical composting with constant or intermittent stirring (20).

5.9. Microorganism

Wastes such as garbage and sewage sludge normally contain many types of bacteria, actinomycetes, and fungi. Research indicated that no pure culture of organisms could compare with a mixed culture in the aerobic composting of organic matter. Many types are necessary for composting. The required microorganisms rapidly multiply if the proper environment exists for them. Thermophilic bacteria play a major role in decomposing protein and other readily broken down organic material while actinomycetes and fungi decompose cellulose and lignin compounds (21).

5.10. Use of Inocula

Normal microbial treatment of waste requires innoculum, which is carried out by seeding using active microbes. Composting developments have been accompanied by considerable discussion of special inocula, supposedly containing several pure strains of laboratory-cultured organisms or other biological factors essential in the decomposition of organic matter and nitrogen fixation such as enzymes, hormones, preserved living organisms, activated factors, bio-catalysts, etc. In fact, several commercial composting processes have been built on the use of special inoculum, often known only to its discoverer and proponent, who claim it to be fundamental to the successful operation of the process. The need and value of such inocula have always been debatable; most composting studies have strongly indicated that they are not absolutely necessary (4).

5.11. Seeding and Reseeding

Some believe that seeding with suitable active microbial consortia is essential for rapid start-up of a composter. For mechanical digestion, seeding by recycling of actively composting

material is essential for efficiency. For the best result, reseeding should be done at the beginning of each stage of digestion (21).

6. SOLID STATE BIOCONVERSION TECHNIQUE

Similar to composting, solid state bioconversion (SSB) has been popular recently as a waste recycle method. Solid state bioconversion (SSB) can be briefly defined as a process whereby an insoluble substrate, with sufficient moisture but not free water, can be converted to compost by different microorganisms (9). The medium consists of an unrefined agricultural product such as wheat bran, wheat straw, and rice straw, which may contain all the nutrients necessary for microbial growth. In fact, SSB is a carefully engineered composting process that utilizes a bioreactor configuration to ensure that control of process can be achieved. In SSB, pretreatment of substrates is carried out simply by moistening or swelling the substrate, or cracking of the substrate surface to increase the accessibility of the internal nutrients, or milling of large substrate pieces into small particles (23, 24). The low moisture availability may favor the growth and production of fungi, but may not be favorable for the composting process.

The use of small reactor volumes, low capital and operating costs (25) are among the advantages of the SSB system. High volumetric productivity and yields in SSB reactors have been well documented. Examples include twofold higher volumetric productivity for protein production on wheat straw (26) and higher volumetric yields of celluloses from several thermophilic fungi (27). Vigorous agitation during aeration is not required, since thin films of water at the substrate surfaces have a high surface area, allowing rapid oxygen transfer (28). Downstream processing and waste disposal is often simplified or minimized. For products recovered by solvent extraction, less solvent is required. Kumar and Lonsane (29) calculated a 50–60% saving in downstream processing costs for the recovery of gibberellic acid from SSB compared to liquid state bioconversion (LSB).

Most of the processes using the SSB technique are commercialized throughout the world. SSB processes offer production of various metabolites of bacterial, fungal, and yeast origin. This trend may lead to extensive industrialization of SSB processes for diverse products. Potentially, many high value products could be produced using SSB. Various enzymes and antibiotics depend on mycelial differentiation, which may be suppressed in LSB. Improvements in socio-economic applications of SSB are desirable, as economy is becoming important for their continued operation without subsidies. The improved processes must use a cheap substrate locally available in abundance throughout the year, while the inoculum preparation method must be simplified. Hasseltine (30) also advocated the great potential for the use of mixed cultures in SSB for enhancing the productivity and the rate of bioreactions.

7. MICROBIAL BASIS OF SSB PROCESSES

7.1. Microbial Type

Many bacteria, yeasts, and fungi are capable of growth on solid substrates and therefore find application in SSB processes (43, 44). Amongst these microorganisms, filamentous fungi

are the best adapted for these processes and dominate in the research presently carried out around the world.

7.2. Bacteria

Many bacteria, yeasts, and fungi are capable of growth on solid substrates and therefore find application in SSB processes. Bacterial SSB processes are few in number. In composting, moist solid organic wastes are decomposed by a succession of microorganisms arising from the natural flora. The release of metabolic heat during early decomposition of the lignocellulosic substrate causes the pH and temperature to rise, resulting in the domination of thermophilic bacteria when the temperature exceeds 60°C (31).

7.3. Yeasts

As with bacteria, yeasts generally participate in traditional SSB processes only as minor members of the microflora (32). Yeasts are found during the early stages of ensiling, but lactobacilli are the dominant microorganisms. Yeasts have also been added to utilize excess soluble sugars formed during SSB of cellulosic substrates by cellulolytic fungi (33–35).

7.4. Filamentous Fungi

Filamentous fungi are the most important group of microorganisms for SSB processes owing to their physiological capabilities and hyphal mode of growth. Filamentous fungi are also very active in the early and late stages of composting, although they are unable to proliferate at temperatures in excess of 60°C (36). A second reason for this upsurge was the realization of the ability of many filamentous fungi to degrade macromolecular substrates, especially carbohydrates.

Solid substrates usually consist of complex arrangements involving a number of macromolecules such as starch, cellulose, hemicellulose, pectin, lignin, protein, and lipid (29). These macromolecules or specific representatives amongst them usually provide the carbon and energy for microbial growth. Of most importance are the polymers starch and cellulose. Since starch and cellulose are the most important nutritional molecules, amylases, and celluloses are necessary for their utilization. Filamentous fungi-producing amylases include species of *Mucor*, *Rhizopus,* and *Aspergillus*, while cellulase producers important in SSB include *Trichoderma reesi*, *Trichoderma lignorum*, *Chaetomium cellulolyticum,* and the white-rot basidiomycetes (31). The hyphal mode of growth gives the filamentous fungi a major advantage over unicellular microorganisms in the colonization of solid substrates and the utilization of available nutrients.

8. CASE STUDIES

8.1. Case 1: Utilization of Sewage Sludge as Fertilizer and as Potting Media

A study was carried out to investigate the possibility of using sewage sludge as fertilizer for sweet maize (36). Domestic sewage sludge was collected from oxidation ponds in a tropical Malaysian climate. The processed sewage sludge was applied on land at rates ranging from

Table 5.6
Metal concentrations in maize grain planted using sewage sludge (31)

Parameter	Value (mg kg^{-1})
Zn	4.95–19.18
Cu	0.56–2.60
Cd	0.037–0.052
Pb	0.034–0.052
Mn	1.56–8.53
Fe	8.16–24.93
Ni	0.66–1.22
Cr	0.12–0.44

186 to 746 kg N ha^{-1}. The study was conducted for three corn cycles. Sewage sludge was applied about 2 weeks prior to sowing of each crop. Maize was harvested at maturity about 75–78 days after sowing. Application of sewage sludge produced a significantly higher yield of maize than the control. The total yield ranged from 1,009 to 4,068 t ha^{-1}. However, no significant difference was observed between the inorganic fertilizer (producing 2,959 t ha^{-1}) and sewage sludge in terms of total yield produced. In addition, there were no statistical difference in economic yield (marketable yield) of maize fertilized by sewage sludge and chemical fertilizer. In summary, the sewage sludge performed as well as the inorganic fertilizer.

Results revealed that concentration for all the metals in grain corn treated with sewage sludge increased after third maize cycle (Table 5.6). However, concentrations of these metals were all below the permitted safety level (37). Metal concentrations in other parts of plants (leaves, stem, sheath and cob) were also still within the safety level in terms of consumption. In general, the distribution of metals in plants followed as: leaves and stems > sheaths and cobs > grains (36).

Furthermore, the possibility of using sewage sludge as potting media for horticulture crops such as jasmine and chrysanthemum was also investigated. Sewage sludge consisted of stabilized anaerobic sludge originating from old domestic septic tanks. Sewage sludge was mixed with coconut coir as a peat substitute in potting medium for chrysanthemum. Results showed that sewage sludge with coconut coir in the ratio of (3:1) could be used in the standard potting media as a peat substitute for chrysanthemums giving similar growth and number of flowers as peat but with only Agroblend, at the recommended rate, or with half the recommended rates of Agroblend and Agrofos. This revealed that the use of chemical fertilizers could be reduced with the use of sewage sludge in potting media for chrysanthemums (36).

Different rates of sludge application were used to investigate the possibility of sewage sludge as fertilizer for the jasmine plant. The lowest sludge rate (25%) was able to give good plant growth. Losses of nutrients were likely minimal as most nutrients in the sludge were in organic forms and released more slowly as compared to chemical fertilizers, which needed to

Table 5.7
List of microorganism utilized for composting (38)

Experiment	Inoculum
T1	Organic Gro
T2	*P. chrysosporium*
T3	*T. harzianum*
T4	*P. chrysosporium* + *T. harzianum*
T5	*Mucor hiemalis*
T6	*Mucor hiemalis* + *Mucor hiemalis*

be replenished more frequently (36). Results revealed that stabilized sewage sludge could also be used as an organic fertilizer for horticultural plants such as jasmine plant.

8.2. Case 2: Reduction of Heavy Metals in Sewage Sludge During Composting

A study was carried out to determine the effect of inoculating various microorganisms on the metals concentration during the composting progress. Table 5.7 presents the list of microorganisms used. Sewage sludge was collected from a mechanical dewatering operation. Sawdust was added as an amendment at different ratios (1:1, 1:1.5, 1:1.7, and 1:2) to the dewatered sludge to adjust the water content to 60%. The sludge was mixed together with sawdust and microorganism and then transferred into a horizontal drum bioreactor (HDB) of 300 L (38), as shown in Fig. 5.4.

Among the all-experimental runs mixing sewage sludge with 1:1.7, sawdust in the presence of *Phanerochaete chrysosporium* and *Trichoderma harzianum* was found to be the most suitable for efficient composting. In general, there was a general reduction in all-metal contents after composting (Table 5.8). The highest reduction (50%) was recorded for Cd, where combination of *P. chrysosporium* and *T. harzianum* were used (38). The highest concentration of metals in composted sewage sludge was observed for Fe and the lowest for Pb. This indicated that Fe was the most loosely bounded to the sewage sludge organic matrix and Pb was the most strongly bounded. A lower concentration of extracted metals in the composted sewage sludge revealed that composting renders part of the insoluble metals. This result confirmed that when the sludge is composted, there is lesser risk due to metals in the sludge during application on soils.

8.3. Case 3: Solid State Bioconversion of Oil Palm Empty Fruit Brunches (EFB) into Compost by Selected Microbes

The palm oil industry plays a major role in the economic development of Malaysia. In processing oil palm fruit for oil extraction, palm oil mills produce a considerable amount of solid wastes in the form of fibres, nutshells, and (EFB) empty fruit brunches (Fig. 5.5). For every 100 tons of fresh fruit bunches processed, there will be approximately 20 tons of nutshells, 7 tons of fibres, and 26 tons of empty bunches discharged from the mill (39). Disposal of the oil palm wastes requires prudent handling and consideration.

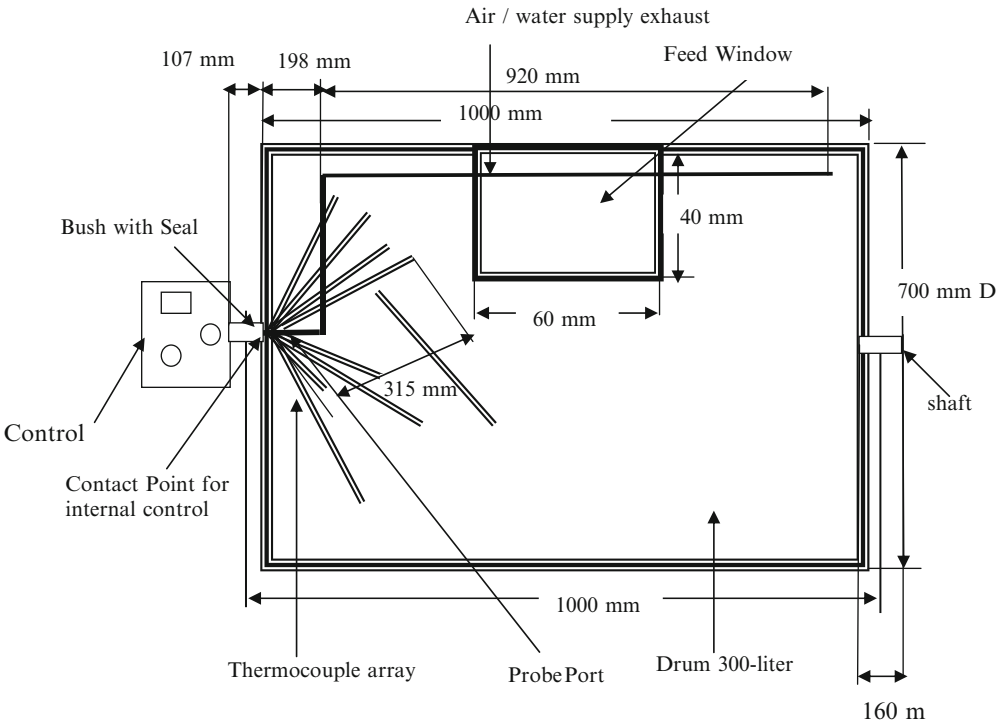

Fig. 5.4. SSB of sewage sludge in horizontal drum reactor (38).

Table 5.8
Metal content in compost produced using different microorganisms (38)

Parameter g/kg	Untreated sludge	Compost T1	Compost T2	Compost T3	Compost T4	Compost T5
Cd	0.47	0.29	0.26	0.23	0.2	0.27
Fe	19.7	12.1	14	11.2	8.98	9.89
Cu	0.60	0.49	0.33	0.54	0.22	0.50
Zn	1.84	0.27	1.11	0.01	0.98	1.5
Cr	0.31	0.25	0.19	0.1	0.08	0.21
Pb	0.30	0.28	0.19	0.18	0.17	0.20
Ca	16.5	15.2	14.3	13.2	12.3	13.6

Note: the species of microorganisms are indicated in Table 5.7.

Hassan (39) investigated the solid state bioconversion technique (SSB), by selecting microorganisms to convert EFB to compost. Shredded and partially dried EFB were allowed to compost for 4 weeks using ammonium sulfate as a source of nitrogen with the addition of single and mixed culture inoculum of *Aspergillus niger*, *Trichoderma reesie,* and *P. chrysosporium*.

Fig. 5.5. Palm oil fruit brunches.

In summary, the mixed culture of the three fungi produced better results compared to single fungi. The carbon decomposition was 54% for mixture of three followed by 53.4% for *P. chrysosporium*, 41% for *A. niger,* and 34.6% for *T. reesie*. Composting increased the total nitrogen content by 92.1% for a mixed culture followed by 77.4, 67.6, and 64.7% for *P. chrysosporium*, *A. niger* and *T. reesie*, respectively. After 4 weeks of composting, the initial C/N ratio of 47 in EFB compost dropped to 26.1 in the control and between 12.3 and 18.6 in the single cultures. The lowest C/N ratio of 11.3 was achieved by EFB compost inoculated with mixed culture. There was a 60% reduction in the C/N ratio over the control. In the EFB compost, the total phosphorus was greater in the inoculated series than in the control. The maximum content of 1.44% was recorded with mixed culture followed by *P. chrysosporium* 1.28%, *A. niger* 0.99, and *T. reesie* 0.57% (39). This indicated that the organic phosphorus present in the organic wastes was mineralized and converted to a form, which could be readily assimilated by plant.

It appears that compost produced from EFB inoculated by mixed culture contains the highest percentage of N, P, K, Ca, and Mg followed by *P. chrysosporium*, *A. niger,* and *T. reesie*, respectively. The percentage increase of N, P, K, Ca, and Mg content of the compost treated by mixed culture over the commercial product was 63.7, 37.1, 35.9, 39.8, and 20.2%, respectively. The humus content of the compost was increased significantly by inoculation with celluloytic cultures. The maximum humus content of 14.8% was noted with a mixed culture followed by 12.2% with *P. chrysosporium*, 10.5% with *A. niger,* and 8.7% with *T. reesie*, which is similar to the control (39). In addition, results revealed that the quality of the finished compost will be improved if the EFB were cut or shredded into smaller fractions.

8.4. Case 4: Composting of Selected Organic Sludges Using Rotary Drum

This study was carried out in a 75-L rotary drum modified from a cement mixer (Fig. 5.6) to compost several organic sludge including food factory sludge, palm oil mill effluent (POME) sludge, landfill leachate sludge, and sewage sludge. The temperature, moisture content, pH, and carbon–nitrogen ratio were controlled and monitored. The rotary drum was insulated with

polystyrene to maintain the temperature and was operated in a continuous rotation with a sufficient air supply (40).

The pH of composting sludge mixtures was between 5.39 and 7.75, and the moisture content for the organic sludges was more than 80%, except for leachate sludge where the moisture was 63.9%. The C/N ratio of these raw organic sludges was low, ranging from 7 to 19. The food factory sludge contained a high total microbial count at 2.7×10^{10} cfu/g and followed by POME sludge, sewage sludge, and leachate sludge at 1.0×10^{8}, 2.0×10^{7}, and 7.0×10^{6} cfu/g, respectively. The nutrient concentration of P, K, and Mg was high in POME sludge, which measured at approximately 12,602, 2,118, and 322 ppm, respectively. However, the highest concentration of Mn was found in sewage sludge, measured at 606 ppm. Overall, sewage sources contained the highest concentration of heavy metals in raw sludge (40).

The composting rate was also studied using a mixed ratio of 3:1 (sludge to bulking agent). In the study, it appeared that sludge from sewage, POME, food factory, and leachate underwent the fermentation phase of approximately 5, 5, 10, and 13 days respectively, while the curing took about 35, 30, 30, and 17 days, respectively, to achieve completion. In the final stage, decomposition rate measured was recorded to be about 60, 52, 55, and 50% for sewage, POME, food factory, and leachate sludge, respectively. The best achievement for composting of sewage sludge, POME sludge, food factory sludge, and leachate sludge were approximately 40, 35, 40, and 30 days, respectively (40).

Leachate sludge compost product measured the highest pH of 8.03. In terms of P, K, and Mg, the highest value was found in POME sludge compost, while the highest Ca and Mn were

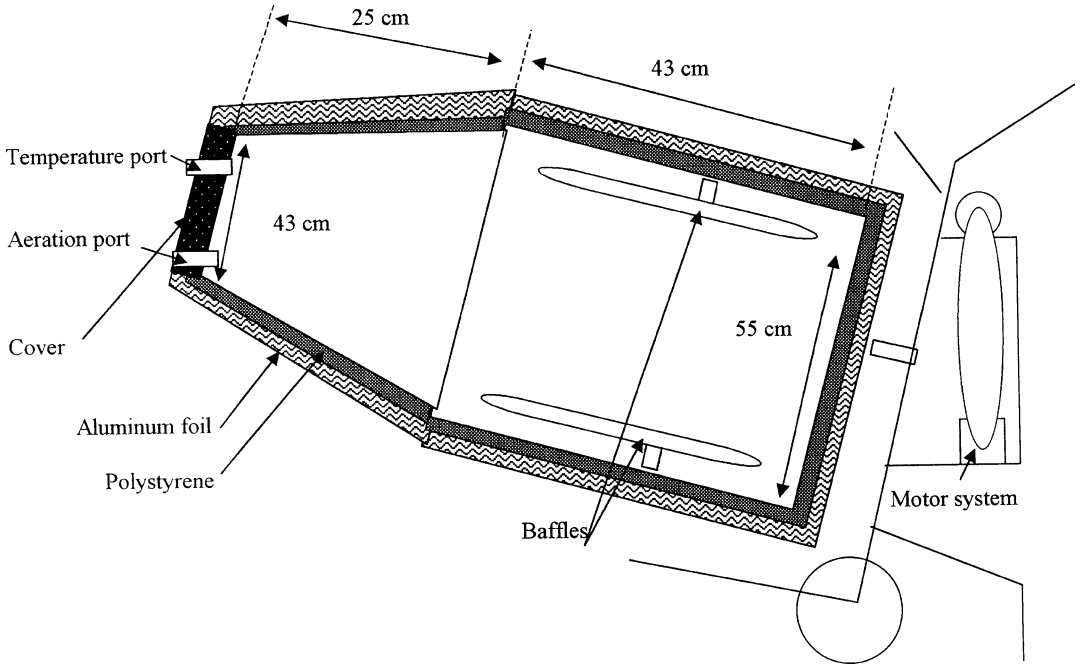

Fig. 5.6. Schematic diagram of rotary drum composter (40).

found in leachate sludge compost products. In comparison, the concentration of heavy metals in final compost products decreased in the following order: POME sludge > sewage sludge > food factory sludge and leachate sludge. In terms of physical characteristics, the research compost products were dark brown and had an earthy smell. The number of total coliform bacteria was recorded to be less than 10^2 cfu/g. Using a growth study, the germination rates using compost from POME sludge, sewage sludge, food factory sludge, and leachate sludge were 80, 90, 78, and 94%, respectively (40).

The compost product obtained in this study was applied as a biofertilizer for growing spinach. Results showed that spinach grown with sewage sludge compost produced leaves with a greener color in leaves and promoted superior growth (shown as Pot C in Fig. 5.7). Continuous growth studies after 5 weeks indicated sustained greening of the leaves and good growth especially for sewage compost (shown as Pot A in Fig. 5.8). In conclusion, it appears that sewage sludge compost, food factory sludge compost, leachate sludge compost, and POME sludge compost all showed similar characteristics as commercial composts (40).

In another study, the compost produced using a windrow system (heap method) and a rotary drum system (composter) were compared. In the windrow system, composting was performed using different percentage of inoculum with 0.1 and 1.0% Effective Microorganisms (EM) (40). For both systems, pH values were around 6.58–6.85, and moisture content was around 65–67%. An important parameter for the composting process that needed attention was the

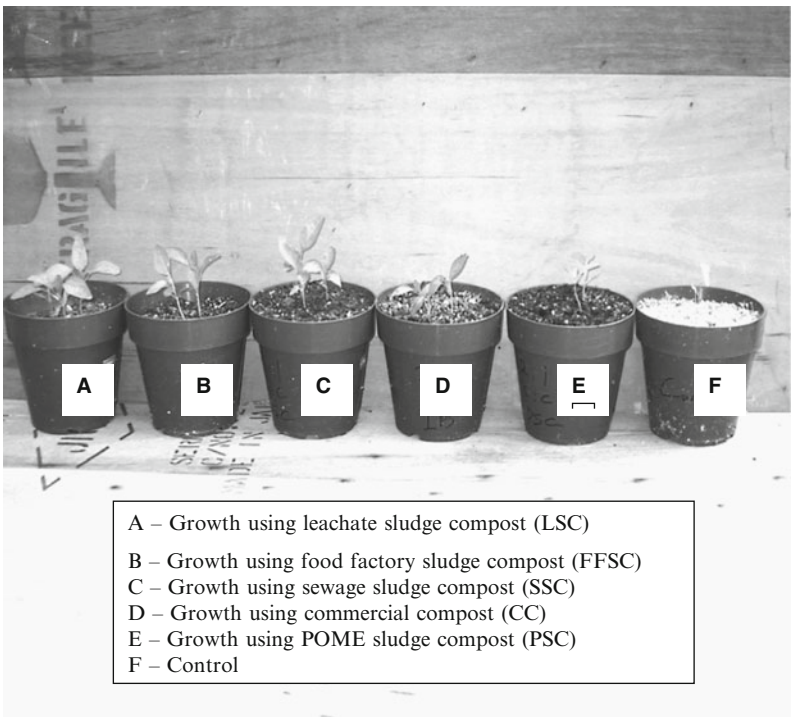

Fig. 5.7. Growth of spinach in different compost products after 3 weeks.

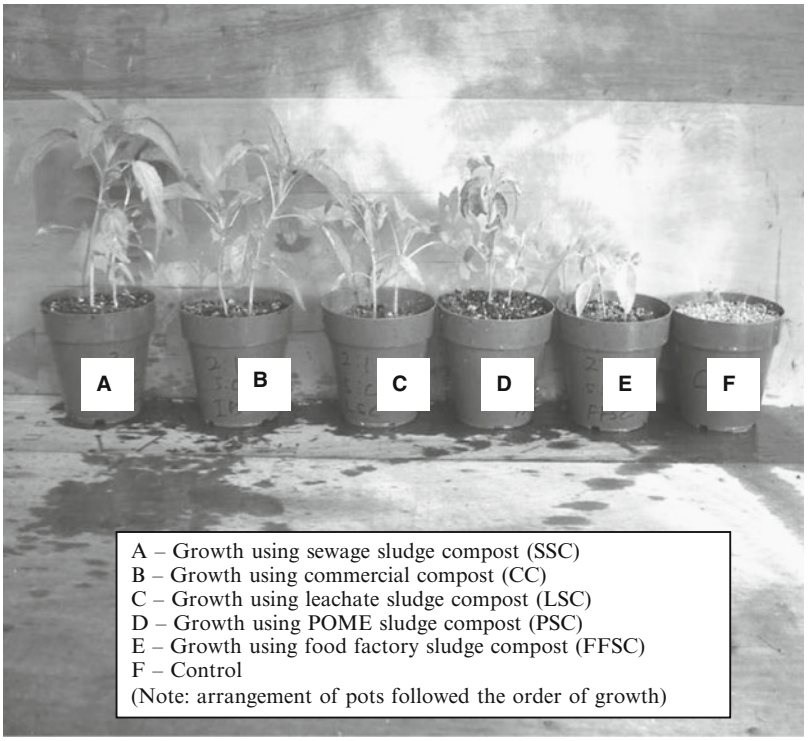

Fig. 5.8. Growth of spinach in different compost products after 5 weeks.

C/N ratio. For a windrow system, the C/N ratio started around of 22–24, while for the rotary drum system, the C/N ratio started at 28 because of the low nitrogen content in mixed substrates compared to the nitrogen value in windrow system, which is more than 2%. The total microbial count for windrow (0.1%EM), windrow (10%EM), and rotary drum were around 1.1×10^7, 1.0×10^8, and 8.6×10^8 cfu/g, respectively. The highest number of total coliform bacteria was obtained from windrow system (0.1%), measured at about 1×10^6.

The nutrient content presented a higher value in the rotary drum composter, especially for P, K, Ca, and Mn; the data recorded were 1382, 873, 1011, and 80 ppm respectively. Similarly, the concentration of heavy metals for Fe, Zn, Pb, and Ni were found to be the highest for the rotary drum system; the values were 2547, 107, 41, and 329 ppm, respectively (40). Generally, the physical, chemical, and biological characteristic showed that compost products were similar to those of the commercial composts. In terms of the number of pathogens and the concentration of heavy metals, they all complied with the standards of USEPA and were suitable for use as biofertilizer and soil conditioner.

8.5. Case 5: Bioreactor Co-composting of Sewage Sludge and Restaurant Waste

Three different types of dewatered sewage sludge, i.e., septic tank, oxidation pond, and activated sewage sludge were co-composted with municipal solid waste in a two-stage process.

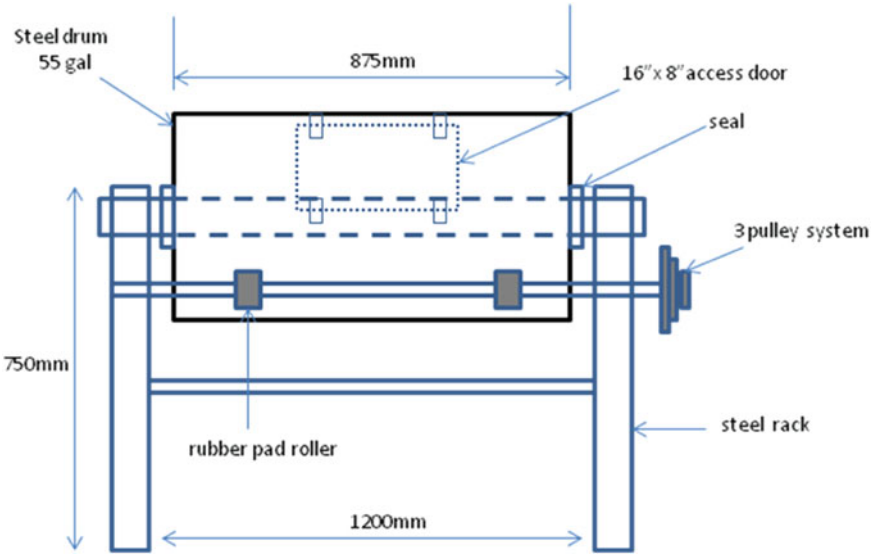

Fig. 5.9. Section view of 200 L – bioreactor composter (41).

The first phase of the co-composting process, known as the fermentation phase of sewage sludge and restaurant waste, was performed in a 200-L bioreactor (Fig. 5.9). Shredded garden waste was added as bulking agent. A 2:1 (wt/wt) ratio of municipal solid waste and sewage sludge was found to give the best initial C/N ratio for the composting process. The second phase of composting process was performed in an open space using a windrow system (heap method). The produced compost was characterized and the results were almost identical to commercial compost and also complied with US EPA standards (41).

A growth study using produced compost to grow spinach showed satisfactory results. The ratio of the compost to the soil was 2:1 based on a volume basis. It was found that the growth of spinach using compost produced from the oxidation pond and activated sewage sludge was almost identical to that of commercial compost (Fig. 5.10). The spinach that grew in the activated sewage sludge compost product produced more greenish color in the leaves (36).

NOMENCLATURE

C = carbon
Ca = calcium
CC = commercial compost
Cd = cadmium
cfu = colony forming units (coliform count)
cm = centimeter
CO_2 = carbon dioxide
Cr = chromium

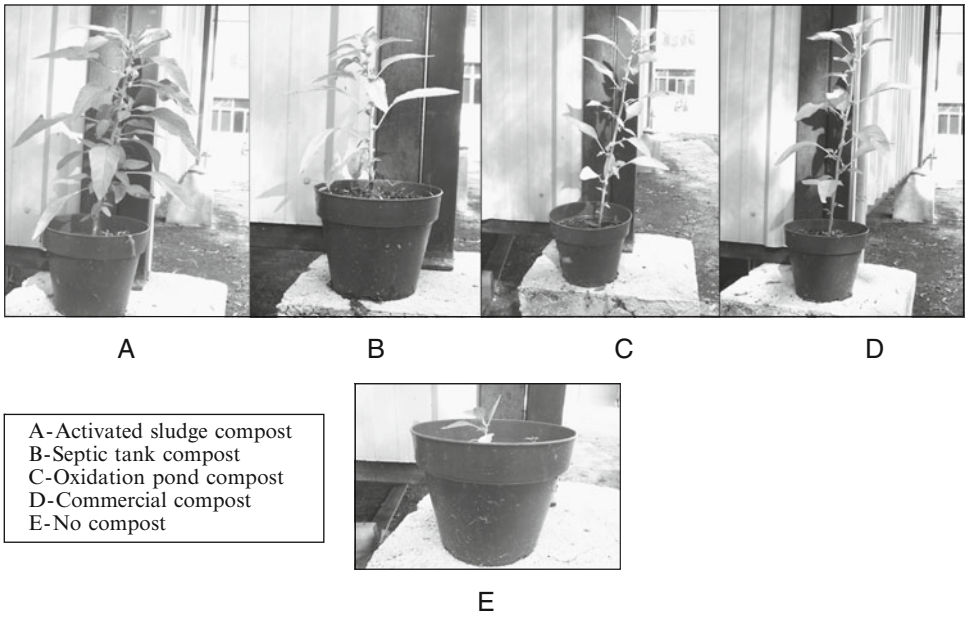

Fig. 5.10. Growth studies with spinach using sewage sludge composts (35).

Cu = copper
D = diameter
EFB = empty fruit bunch
EM = effective microorganism
EU = european union
Fe = iron
FFSC = food factory sludge compost
g = gram
H_2O = water
K = potassium
LSB = liquid state bioconversion
LSC = leachate sludge compost
mm = millimeter
Mn = manganese
N = nitrogen
NH_3 = ammonia
NH_4OH = ammonium hydroxide
Ni = nickel
O_2 = oxygen
∘C = degree celsius
P = phosphorus

Pb = lead
POME = palm oil mill effluent
ppm = parts per million
PSC = palm oil mill sludge compost
SSB = solid state bioconversion
SSC = sewage sludge compost
USA = United States of America
US EPA = Unites States environmental protection agency
Zn = zinc

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6
Kitchen Refuse Fermentation

Mohd Ali Hassan, Shahrakbah Yacob, Cheong Weng Chung, Yoshihito Shirai, and Yung-Tse Hung

CONTENTS

Abstract Controlled fermentation has been used for kitchen waste treatment. The most important factors affecting methane production from kitchen waste is organic loading rate and hydraulic detention time. Two main types of fermentation of kitchen waste are natural fermentation and controlled fermentation. The fermentation products are poly-3-hydroxyalkanoates (PHA) and poly-lactate (PLA).

1. INTRODUCTION

In the last century, the world had experienced various industrial revolutions, which were driven by fossil fuels such as petroleum and coal. These rapid changes also brought along serious environmental issues such as the dumping of nonbiodegradable polymers in landfills, uncontrolled release of greenhouse gases, and usage of nonrenewable energy. These concerns have sparked interest in finding alternative renewable materials such as industrial chemicals and biodegradable polymers that will reduce the environmental pollution. Despite intensive research and development in green technology and discussions by interested parties, there was

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_6

no major commitment in adopting green technology at a commercial level. Even though the world has acknowledged the depletion of fossil fuel reserves and increases in oil production cost, the price per unit of chemical derived from petroleum is relatively more competitive.

However, this perception is about to change as a result of current biotechnological developments in utilizing biological agents and cheap renewable resources to produce bioproducts. Biotechnology has made a strong impact by providing a sound alternative technology that contributes to the well-being of the environment. Biological agents such as enzymes and cells are more efficient than chemical reagents. Enzymes are known for their specificity and are extremely efficient in producing intermediates or chemicals and can perform as efficient as metal catalysts. Live cells can be considered as living catalysts because of their ability to assimilate or dissimilate chemical compounds while harvesting the energy released. The abundance of organic matter, particularly the biomass generated by domestic and agricultural activities, coupled with the biocatalysts mentioned, promises a great potential in producing competitive chemicals or intermediates for the chemical industries. The production of chemicals that cannot be synthesized chemically such as citric acid, monosodium L-glutamate, and L-lysine from agricultural residues have encouraged the acceptance of biotechnology as a future technology by the chemical industry.

1.1. Availability and Potential of Kitchen Refuse Biomass

The potential and definition of municipal solid wastes (MSW) as renewable materials may vary depending on the economic scale of each country. In developed nations such as Japan and the United States, MSW consists of paper and paperboard products, yard trimmings, glass, metals and to some extent electrical appliances as in Fig 6.1 (1, 2) The potential for conversion of biomass into valuable products is limited because of the low volumetric discharge of organic matter. Moreover, the organic wastes collected from the municipalities are mostly being incinerated or converted into compost rather than chemicals. On the other hand, in the

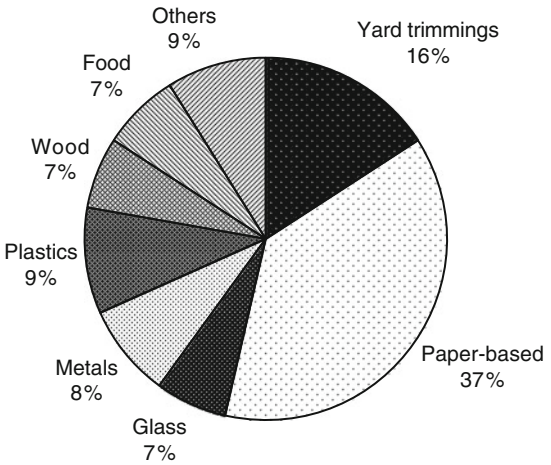

Fig. 6.1. Materials generated in MSW by weight (3).

Table 6.1
Distribution (%) of MSW wet base content in developing countries (3)

Types	Laos	Paraguay	Nicaragua	Tanzania	Philippines	Honduras	Poland	Turkey
Food waste	35	37	34–51	45	45	46	34–61	64
Grass and wood	25	19	23–26	25	16	12	2–6	2
Paper	10	10	5–7	4	16	12	14–19	15–18
Textiles	1	1	2	1	4	3	3–7	2–3
Plastic	6	4	4–6	2	7	7	4–8	6–7
Leather and rubber	1	1	1–2	1	1	2	2	1
Noncombustible	23	27	11–23	22	11	18	12–23	7–10

developing nations, the main bulk of MSW will be organic matter. Studies in eight developing countries have shown that the generation of kitchen refuse from household, restaurant and commercial venues comprised up to 60% of the total MSW content as shown in Table 6.1 (3). According to the UN estimates, 60% of the world's population will be living in urban areas by the year 2015. It is further estimated that about 90% of the population increase between now and the year 2015 will be in urban areas. Most of that increase in urban population will be in developing countries with the MSW generation rate of between 0.5 and 1.3 kg/person/day. In a recent study by the World Bank, urban waste generation is predicted to increase substantially over the next years as GNP pre capita increases. It is predicted that a total of 31.6 million tonnes per day of waste will be generated in the next few years in Asian countries (4) With an average of 50% of the total MSW being organic-based waste, it is estimated that 15.8 million tonnes of biomass, a renewable resources, are being disposed daily in Asia alone.

Leachate is one of the immediate products of MSW disposal in the open dumping sites or landfill. Pollution due to leachate contamination of groundwater system caused by organic matters, heavy metal and toxic chemicals is inevitable in poorly designed landfills. Leachate is formed when water percolates through the dumped solid waste, extracting the organic and inorganic compounds as a result of the natural hydrolytic and fermentative processes. Generally, such leachates contain high concentrations of soluble and suspended organic and inorganic matters (5), with significant levels of heavy metals. The biochemical oxygen demand (BOD) ranges from 20,000 to 50,000 mg/L and tends to vary considerably both daily and seasonally (6). As shown in Table 6.2, the chemical properties of leachate varies widely depending on factors such as temperature, water input (rain), composition, and age of MSW.

2. FERMENTATION OF KITCHEN REFUSE

2.1. Natural Fermentation Process

Based on the chemical properties and composition, the most cost-effective method in the treatment of MSW is using sanitary landfill. By exploiting the low energy requirement of anaerobic processes, the organic matter which is mainly kitchen refuse is being stabilized.

Table 6.2
Characteristics of different leachate sources (6)

Parameters	Fresh leachate	Landfill leachate
pH	4.2–5.0	7.3–8.7
BOD_5	48,000–55,000	780–22,440
COD	65,000–78,000	8,800–40,580
Total solid	35,000–52,000	4,600–25,000
Suspended solid	3,870–9,340	1,440–4,670
Ammonium	200–720	1,500–2,900
Total nitrogen	2,000–2,600	2,200–3,000
Lactic acid	13,000–19,000	50–250
Acetic acid	2,200–5,500	2,230–5,100
Propionic acid	580–3,200	120–2,500
Butyric acid	20–1,080	130–2,300
Phosphorus	100	Minimal
Manganese	7.27	1.40
Zinc	7.72	5.20
Nickel	0.52	0.70
Chromium	0.20	0.52
Copper	0.44	0.30
Iron	100	Minimal
Lead	0.45	0.14
Cadmium	0.07	0.01

All parameters are in mg/L except pH.

Alternatively, such organic matter could be easily composted aerobically. Nonetheless, the potential energy recovery from the anaerobic treatment makes it an advantage over other methods in the selection of treatment for kitchen refuse. However, the treatment in the sanitary landfill is far from the optimal conditions, resulting in prolonged existence of organic matter and environmental problems. Without any process control parameters, the anaerobic treatment of MSW is largely dependent on the presence of natural occurring microorganisms in the landfill. A few studies have shown that landfill ecosystem harbors a consortium of microorganisms with diverse biochemical properties forming a complete food chain in stabilizing the organic matter. Among the reported and identified microorganisms in landfills are *Candida* spp., *Bacillus* spp., *Cellulomonas* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Alcaligenes* spp., *Enterobacter* spp., *Pasteurella* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Yersinia* spp., *Clostridium* spp., *Syntrophomonas* spp., *Lactobacillus* spp., *Pediococcus* spp., *Leuconostoc* spp., *Weisella* spp., *Desulfuromonas* spp., *Methanobacterium* spp., *Methanosaeta* spp., and *Methanosarcina* spp. (7–11). In addition to the diversification of microbial population in the landfills, inconsistency of MSW composition, age and poorly designed landfills makes the treatment of MSW using anaerobic fermentation difficult to control or predict (51, 52).

In general, the anaerobic fermentation of organic matter can be divided into three stages. In the first stage known as hydrolysis process, all the complex substrates such as carbohydrates,

protein, and lipids are being de-polymerized into smaller compounds. The conversions are controlled by series of extra-cellular enzymes that produce long chain fatty acids and carbon dioxide. This is followed by the degradation of long chain fatty acids into carbon dioxide, hydrogen, and short fatty acids such as acetic, propionic, and butyric acids by acid forming microorganisms (acidogens). As the name implies, this stage is called acidogenesis. The final stabilization of organic matter will only occur at the final stage of the anaerobic process. At this point, the methane producing microorganisms (methanogens) which are extremophiles with narrow optimum growth conditions metabolize the short chain fatty acids mainly acetic acid to emit methane and carbon dioxide. Another pathway of methane production is via the reduction of carbon dioxide and hydrogen into methane by the hydrogen-utilizing methanogens. Methane and carbon dioxide (end-products) will be continuously emitted until all the organic matter has been depleted.

2.2. Controlled Fermentation

Unlike natural fermentation process that is being carried out in the landfill, controlled fermentation of kitchen refuse is done with two objectives, firstly to increase the treatment efficiency and secondly to produce value-added products from the conversion of organic matters. In general, the organic fraction of MSW will be subjected to properly designed bioreactor, which enables the operators to control the biochemical process toward the production of desirable products. The types of end products produced from MSW is also affected by different biochemical processes and microorganisms used. At present, there are two different biochemical processes that utilize MSW, nonsterile and sterile fermentations. In nonsterile fermentation, endogenous microorganisms are exploited to produce methane and organic acid cocktails mainly acetic, propionic, and butyric acids via anaerobic process. The sterile process is lactic acid fermentation using MSW as raw material using monoculture system.

3. PRODUCTION OF METHANE

Anaerobic degradation of a mixed composition of kitchen refuse such as lipids, carbohydrates, and proteins requires a synergistic relationship between all microbial populations which occurs only when the optimum conditions for each group of microorganisms exist. Since the activity of methanogens is the limiting factor in the final conversion of organic matter into methane and carbon dioxide, the optimum must be set within the desirable range. Methane production from organic fraction of MSW has attracted special interest as a result of the generation of renewable energy. Anaerobic fermentation of kitchen refuse is seen as an approach to mitigate the large quantity of MSW dumped in landfills. Various methane generation systems and their potential have been reviewed by Gunaseelan (12).

There are several important factors that affect the methane production from kitchen refuse. Firstly, the organic loading rate (OLR), which is equivalent to the amount of organic matter to be stabilized by microorganisms. As reported by Gallert and Winter (13), the highest OLR achieved was 9.4 kg/m^3/day at about 65% volatile solids (VS) removal. However, the performance of a full-scale plant treating kitchen refuse is wider, between 5 and 14 kg/m^3/day of OLR with 55–77% VS removal efficiency. This large variation is governed by the properties of

MSW and bioreactor configurations. As discussed by Chynoweth and coworkers (14), low VS content (<1%) can be treated with high rate bioreactors such as upflow anaerobic sludge blanket and anaerobic filter. These systems could tolerate higher OLR (>15 kg/m^3/day) because of their capability in retaining high density microorganisms. Shorter hydraulic retention time (HRT) of less than a day is required to remove high percentage of VS. For intermediate VS content of between 5 and 10%, two-stage fermentation or recycling of solid (sludge) systems is recommended. In the two-stage fermentation, the hydrolysis and acid phases is carried out in the first stage, while the methane production in the second. Insoluble matter such as lingo-cellulosic compounds is hardly digested because of their recalcitrant properties and low kinetic reaction order. Recycling of sludge assisted in stabilizing the system and compensating the biomass loss during washout. Low rate bioreactors such as plug flow digester, continuous stirred digester, or batch system are suitable for high VS content of more than 10%. Due to the high load of VS, the system can only cope up to the maximum of 5 kg/m^3/day of OLR and HRT between 20 and 30 days.

Complete removal of VS is crucial as the final composition of the treated kitchen refuse would determine the downstream treatment. The low content of VS could facilitate the physical separation between solid and liquid by settling method compared with highly viscous treated kitchen refuse. It has been showed that the nondegraded organic matter such as lignin and cellulose may represent up to 15% of the total COD (15). Therefore, this factor poses a great challenge for a commercial scale organic based MSW treatment plant as the quality of kitchen refuse is highly variable and inconsistent. Another important factor which is closely related to OLR is HRT. HRT is the function of microorganisms growth and washout. It also determines the size of the bioreactor which in turn influences the economic scale of the treatment plant. Sudden variations in COD would mean changes in volume of organic matter leading to daily fluctuation of HRT. In principle, if the same amount of substrate is fed daily, a population balance between the acidogens and methanogens will be maintained easily. Sudden addition of large amounts of readily digestible organic matter could result in the production of excess amounts of acids, thus creating an imbalance of anaerobic digestion (16). When this occurs, the methanogens activities tend to slow down and eventually acids will accumulate in the system. Nonetheless, with the introduction of sludge recycling system, formation of granules and fixed bed for microbial growth, the system will be stable and thus better able to withstand the effect of sudden increase of COD without affecting the bioreactor performance.

In a continuous anaerobic fermentation, the kitchen refuse should be introduced in a smaller quantity continuously or intermittently daily. Single loading pattern in a day would disturb the steady state condition of the microbial population or also known as loading shock (17). The loading shock is more detrimental to methanogens. A longer time is needed for methanogens to regain its optimum density because of slower growth rate and washout. Moreover, the condition after the loading is in favor of acidogens to produce more acids than what can be consumed by methanogens (18). Accumulation of acid compounds at the initial stage is one of the factors in lowering the methane content emitted as methanogens has narrow range of optimum growth conditions (13).

As mentioned before, the methanogens which caused the final conversion of kitchen refuse into stable end-products are very sensitive to conditions in the system. They can easily become

dormant or inactive when optimum conditions are not maintained. One of the most important environmental requirements is the appropriate pH. Whilst the acidogens can function satisfactorily at any pH level above 5, the methanogens are inhibited when the pH falls below 6.2. The best operating range for methanogens is between 6.8 and 7.2, while the tolerable level is between 6.0 and 8.0 (19). The pH of the system has to be maintained within the optimum range by the system's buffering capacity since the start-up operation commenced. Naturally, all biological systems are equipped with the ability to resist change of pH as a survival strategy. This is known as natural buffering capacity measured as alkalinity. During the microbial metabolic activity, some buffering materials such as bicarbonates, carbonates, and ammonia will be secreted into the solution.

The quantity of buffer produced is usually enough to counter the acid generated, so that the pH will remain at a constant level. Sudden changes in the acid production rate or the amount of buffering material can cause changes in pH. This means that the natural alkaline buffer in the system has been reduced and/or that acids are secreted faster than the neutralizing buffer and that the methanogens cannot keep up. The optimum anaerobic fermentation would require the ratio of volatile fatty acids: alkalinity of between 0.1 and 0.3. Typical causes of acidic pH are sudden changes in organic loading or temperature, lack of pH control, presence of toxic waste, and slow bacterial growth during start-up. Another important parameter in kitchen refuse fermentation is the release of ammonia from the degradation of protein. As reported by Angelidaki and Ahring (20), free ammonia may be responsible to the inhibition of methane producing microorganisms. Mesophilic methanogens are more susceptible to ammonia inhibition at the range of 80–150 mg/L compared to thermophilic methanogens at 250 mg/L (21, 22) as protein-based organic matter is being degraded at a higher rate during thermophilic process.

4. PRODUCTION OF ORGANIC ACIDS

In any anaerobic treatment of organic compounds, a consortium of different groups of microorganisms is responsible in stabilizing the organic matter. Prior to the production of methane, by manipulating the anaerobic fermentation parameters, methanogens can be suppressed for higher production of organic acids. A simple approach is by creating an acidic condition (pH $<$ 6.0) during the fermentation. It has been widely reported that accumulation of organic acids such as acetic, propionic, and butyric acids would inhibit the growth of methanogens (23, 24). This is in line with findings by Inanc and coworkers (25), explaining the role of propionic acid in promoting the growth of dominant acidogenic population in anaerobic digestion of carbohydrates. This is done by shorter HRT and higher OLR which resulted in wash out of methanogens and excess of organic acids in the fermentation broth.

Even though acid phase is part of the anaerobic fermentation of kitchen refuse, it has not been intensively studied like methane production. The production of organic acids from organic wastes using plug-flow reactor as reported by Sans et al. (26), has shown promising results. With shorter HRT of 2 days, the production of organic acids mainly acetic and butyric acids was between 11.8 and 19.5 g/L. It was reported that acidic condition (intermediate products of anaerobic fermentation) not only suppressed the growth of methanogens but

also reduce the production of organic acids by acidogens and hydrolytic microorganisms. This is because of negative feedback inhibition of intermediate products accumulation. To mitigate the situation, a cascade fermentation system was proposed by Argelier and coworkers (27). A series of three continuous stirred-bioreactors was used to achieve high production of organic acid up to 42 g/L. This system also demonstrated very high OLR at 12.5 kg/m^3/day at only 12.5 days HRT. The system created a cascade of fermentation by different groups of microorganisms in different bioreactors. Hydrolytic microorganisms are confined at the first stage, while acidogens dominated the remaining two bioreactors.

An equally potential raw material from production of organic acids which is derived from kitchen refuse is leachate. The fermentation of leachate generated from MSW was carried out in nonsterile condition for the production of organic acids (28). An optimization trial was done to determine the best condition for endogenous acid-producing microorganisms to grow. By exploiting the different optimum pH for methane and acid producers, the production of organic acids from leachate was the highest when the pH was adjusted initially to pH 7 with no further control. Approximately 45 g/L total organic acids were produced after 5 days of treatment, with 28 g/L lactic acid, 8 g/L acetic acid, and 9 g/L propionic acid. Attempts to produce organic acids using endogenous acid-producing bacteria from kitchen refuse were not as high. When the fresh leachate was autoclaved and seeded with 10% fermented kitchen garbage, the highest organic acids achieved were only between 34 and 37 g/L. The highest selectivity of lactic acid (85%) was achieved during the treatment of leachate seeded with kitchen refuse without any pH adjustment (29).

5. PRODUCTION OF L-LACTIC ACID

Homolactic bacteria, e.g., *Lactobacillus bulgaricus* and *Lactobacillus delbruckii*, have been chiefly employed for lactic acid production. The substrate may be lactose (whey), glucose (or glucose syrup), or sucrose (either pure or as beet molasses), and the fermentation is the classic example of an anaerobic process. Disaccharides are hydrolyzed to hexoses, which are catabolized via the Embden–Meyerhof pathway to pyruvate, which is finally reduced to L (−) lactic acid by lactic dehydrogenase. Under some conditions, the D,L-acid is produced, possibly by action of a racemase (Fig. 6.2). The normal medium includes carbohydrates and inorganic nutrients to supply nitrogen, phosphate and potassium. Additional vitamins may also be added.

L. delbruckii is used at temperature of up to 50°C and *L. bulgaricus* up to about 44°C. The latter must be used to ferment whey because *L. delbruckii* cannot ferment lactose. The use of a

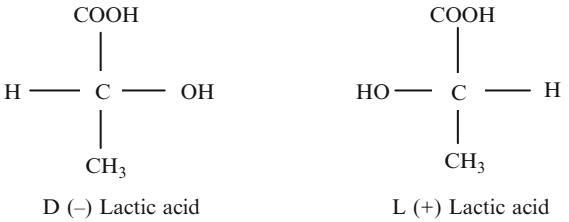

Fig. 6.2. Enantiomers of lactic acid (34).

large inoculum and the relatively high temperature make rigorous sterilization of the medium unnecessary, and pH is maintained at 5.8–6 by addition of calcium carbonate. Although lactic acid is produced by an anaerobic process, small amounts of oxygen are not detrimental. The fermentation generally takes 6–7 days and, yields are in the range of 80–90 g lactic acid per 100 g carbohydrate supplied.

Homofermentative and heterofermentative lactic acid bacteria utilize either the well known EMP pathway of glucose metabolism to produce lactic acid as the main end product, or pathways of pentose metabolism resulting in lactic acid plus other products such as acetic acid, ethanol, and CO_2 (30). Lactic acid bacteria, despite being able to produce acids as the main metabolic products, are rather sensitive to acids. Therefore, processes aiming for high consumption of carbon source to produce high concentrations of lactic acid have to be conducted at pH 5.5–6. According to Buchta (31), lactic acid fermentation is strongly inhibited at pH 5 and ceases at pH values below 4.5. The temperature range for optimal growth of mesophilic lactic acid bacteria is 28–45°C and that of thermophilic lactic acid bacteria is 45–62°C. Lactic acid bacteria are facultative anaerobic organisms. Therefore, in practice, low oxygen tension could be tolerated and exclusion of oxygen (air) is not an absolute requirement.

Unlike mixed organic acid fermentation from kitchen refuse and leachate, L-lactic acid production is carried out under sterile conditions. Intensive research was done in Japan by Sakai and coworkers (32) to develop a novel approach in utilizing MSW as a source of renewable material. The flow diagram of the biochemical process is as shown in Fig. 6.3.

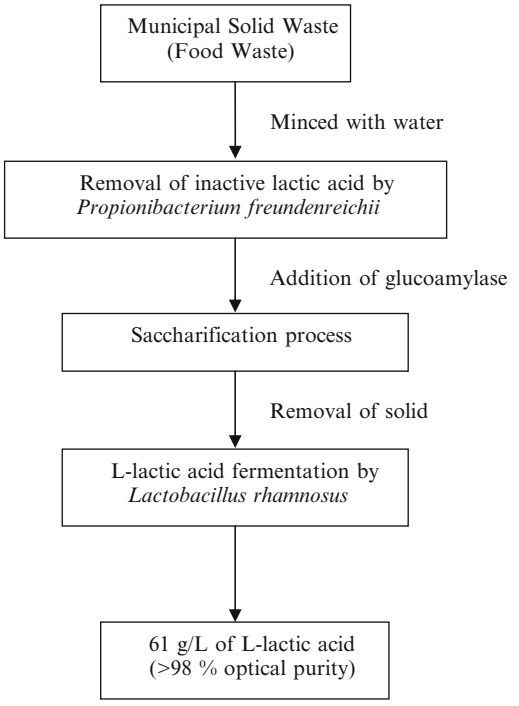

Fig. 6.3. Flow diagram of L-lactic acid fermentation using food waste from municipal solid waste (32).

There were three stages of biochemical processes involved in the production of L-lactic acid. Due to the nonsterile condition and probably partially fermented starting material (minced food waste), the presence of endogenous D,L-lactic acids were detected. Thus, the removal of D,L-lactic acids was crucial as the production of poly-lactate (PLA) requires strictly L-lactic acid. This was achieved by using *Propionibacterium freundenreichii* which had strong affinity to utilize D,L-lactic acid rather than sugar that was present in the substrate (Stage 1). This novel feature enabled maximum conversion of sugar for the production of L-lactic acid. Upon the removal of inactive lactic acid, the food waste was saccharified using glucoamylase to release sugars in the second stage. In the last stage, the enzymatically treated food waste was subjected to lactic acid fermentation using a specific L-forming homofermentative strain, *Lactobacillus rhamnosus* (33). The serial fermentation process using food waste demonstrated the true potential of biomass conversion into lactic acid. The end product of the fermentation was of high quality with more than 98% optical purity at 82% conversion of glucose released from the saccharification process into L-lactic acid (62 g/L) (32).

Another study conducted in Malaysia, a locally isolated bacteria was used to ferment various organic biomass for the production of lactic acid (34). A high lactic acid-producing bacteria was isolated from palm oil mill effluent sludge and identified as *Enterococcus gallinarium*. The ability of *E. gallinarium* to ferment glucose into L-lactic acid was never reported before. Initial fermentation trial was conducted using food waste produced a promising results with 85% optical purity and production of L-lactic acid at approximately 39 g/L. Additional trials were also carried out on sago starch and rice as raw materials for L-lactic acid production. However, food waste was far more superior in the production of L-lactic acid. This was largely due to better nutrient composition and C/N ratio for growth and acid production which is lacking in sago starch and rice.

6. POTENTIAL APPLICATIONS OF KITCHEN REFUSE FERMENTATION PRODUCTS

The introduction of biotechnology into the chemical industry should not be considered the new era of technology, but rather as the reintroduction of an old player. Prior to the industrial revolution and the readily available petroleum, biotechnology had played a major role in providing chemicals such as ethanol, methane, acetone, butanol, and acetic acid. In the past years, with growing public awareness on environmental issues, uncertainties regarding fossil fuel supply and the rising production cost of petroleum, biotechnology has been sourced out as an alternative pathway of chemicals synthesis. Since then, a few existing biotechnologies such as organic acid production have been revisited and researched to fit into the needs of the current chemical industry. One of the most promising products from bioconversion of biomass is biodegradable polymer for plastic production.

6.1. Production of Poly-3-Hydroxyalkanoates Using Organic Acids

A major obstacle in the commercial application of bioplastics, poly-3-hydroxyalkanoates (PHA), is the high production cost compared to conventional petrochemical plastics. One of the determining factors in the economics of PHA production on the industrial scale is raw

material cost. A number of nutrients, including carbon, nitrogen source, and mineral salts are required to support both cell growth and metabolite formation in any microbial reaction. Downstream processing, mainly the extraction of PHA from cells, attributes to the costly process of bioplastic synthesis (35). Much effort has been spent in optimizing the PHA production process and reducing costs such as inexpensive and scaleable PHA production and recovery schemes to produce low-cost PHAs that are competitive with traditional thermoplastics. Hassan et al. (36–38) successfully used organic acids from palm oil industry coupled with PHA production using *Rhodobacter sphaeroides* and *Alcaligenes eutrophus*. Such processes could lead to lower PHA production cost. Another potential raw material for PHA production is MSW or leachate. This organic biomass has the properties that makes it an attractive material to produce high amounts of PHA – being high in organic carbon, low in nitrogen content, and nontoxic.

Poly-3-hydroxybutyrate (PHB), the best known member of the PHA group, can be accumulated intracellularly by a number of microorganisms. The most studied organism for PHB production is *Ralstonia eutropha* (formerly known as *A. eutrophus*). The commercial interest has been focused on *R. eutropha* strain because it is capable of accumulating very high PHB levels within the cells in a short time (39). *R. eutropha* produces PHB on a variety of substrates, such as glucose, fructose, and organic acids. There have been many reports on the use of acetic and propionic acids (40–43). However, only a few have referred to lactic acid as a sole carbon source in PHB production by *R. eutropha* (44).

Based on the kitchen refuse fermentation, the mixture of organic acids produced is suitable for the synthesis of PHA using *R. eutropha*. In synthesis of PHA, acetic and propionic acid monomers will be polymerized into hydroxybutyrate (HB) and hydroxyvalerate (HV) monomers. Therefore, with the total HB monomer of 76.9 and 23.1% of HV, a P3(HB)-3-(HV) will be formed which is a good quality bioplastic (Fig. 6.4). Two-step fermentation

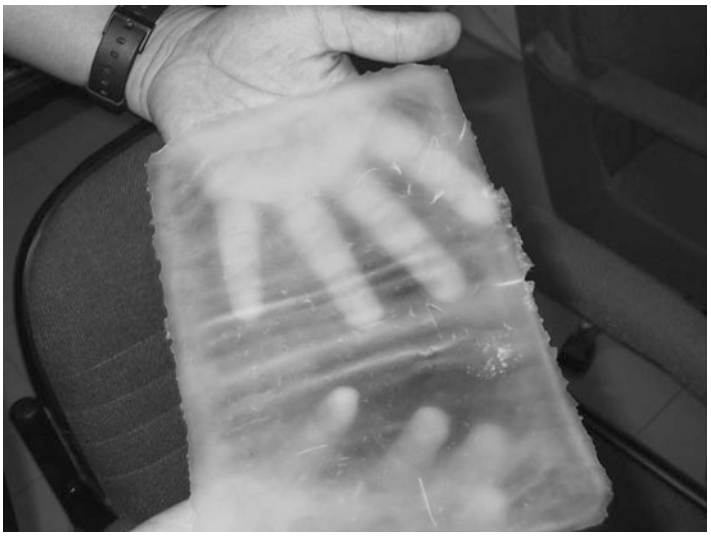

Fig. 6.4. PHA product from kitchen refuse (28).

was employed in the fed-batch fermentation of PHB using organic acids from leachate. This approach was also useful in preventing contamination with higher initial cell density seeded during initial fermentation stage. In the first step of the fed-batch fermentation, the *R. eutropha* was supplied with ammonium nitrate (nitrogen source) in addition to organic acids (carbon source) to give a final medium C/N of 20. This is crucial to build up the population density rather than the production of PHA. Once the desirable cell density is achieved, the nutrient supply is switched to nitrogen-free media with C/N higher than 30. This will minimize the cell growth and encourage the cells to conserve and store the carbon inside the cells as PHA.

Prior to the conversion of organic acids from leachate into PHA, ammonium content in the fermentation broth needs to be controlled. Direct utilization of the broth may result in low PHB yield as low C/N ratio will encourage growth rather production of PHA. Ammonium content after the fermentation was more than 500 mg/L. Mordenite zeolite (particle size < 75 μm) at concentration of 40 g per 100 ml of broth was able to remove more than 90% of ammonium. The fermentation using *R. eutropha* was carried with partially purified organic acids under sterile condition. *R. eutropha* had high affinity towards lactic acids than other organic acids. Nevertheless, most of the organic acids were finally consumed in the production of PHA but at different uptake rates. Overall, PHA fermentation process using organic acids derived from leachate yielded 6.9 g/L PHA at 85% of cell content (28).

6.2. *Production of Poly-Lactate Using Organic Acids*

One of the recent plastics emerging in the market is polylactic acid or poly-L-Lactide (PLA). PLA is a potential substitute to petroleum based plastics in a number of applications including disposables such as plates and utensils, where degradability would be the strong point. Recently, Cargill, as part of its strategy to add value to grain processing, entered the plastics derived from corn. Its new carbohydrate-based polymer was called NatureWork™ PLA, and it was strongly promoted as a renewable, biodegradable plastic (45).

PLA is the crystalline form of lactic acid polymers. PLA belongs to the family of poly(α-hydroxy) acids, one of the sub-categories of the polyesters. Notable members of this same family are polyglycolic acid (PGA), the various forms of PLA (poly-L-lactic acid, poly-D-lactic acid, stereo copolymer poly-L, D-lactic or PLA-X acid, PLA/PGA copolymers and PLA-X/PGA copolymers). However high-molecular-weight polymers of glycolic and lactic acid are possible to obtain by direct condensation reaction. Polyglycolide and polylactide are typically made by ring-opening polymerization of their respective cyclic diester dimmers, glycolide, and lactide.

Unlike PHB, the synthesis of PLA from L-lactic acid is using a series of chemical reactions (32). The L-lactic acid produced from food waste was first subjected to a purification step. Impurities such as protein, salts, acetic, and propionic acids present in the fermentation broth were separated from L-lactic acid using a combination of esterification, distillation, and hydrolysis processes. n-Butanol was used to form butyl lactate ester which has a specific boiling point of 130°C which was then distilled. The concentrated butyl lactate was then hydrolyzed at 95–110°C to produce high purity of L-lactic acid. The polymerization of L-lactic acid commences with a stepwise increment of reaction temperature from 135°C up

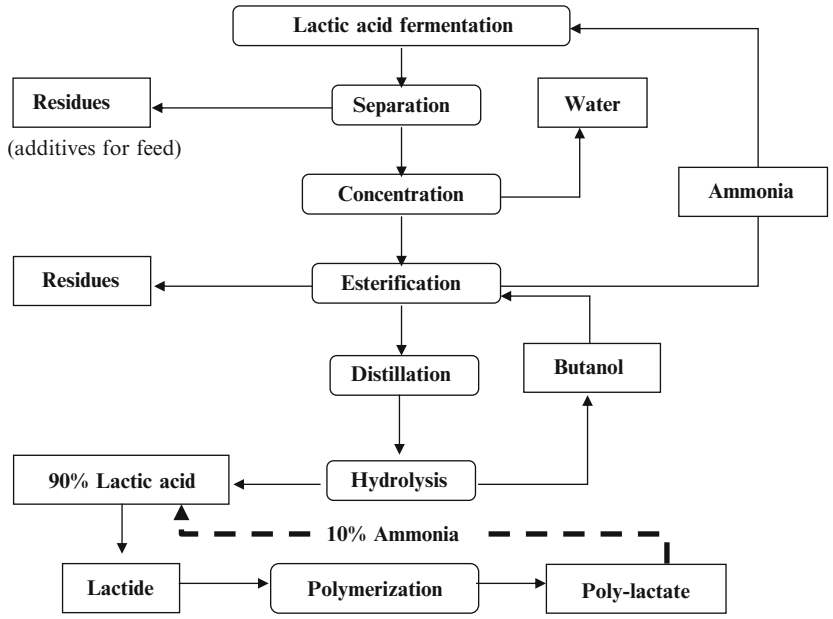

Fig. 6.5. Polymerization process of L-lactic acid produced food waste (32).

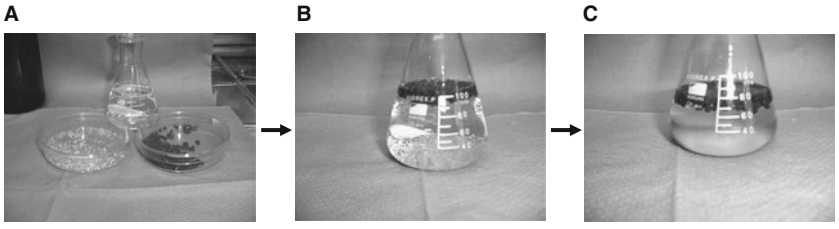

Fig. 6.6. Chemical recycle of poly-lactate by ammonia (**a**) PLA – *left*, polyethylene – *right*, 10% ammonia – *top*; (**b**) reaction at 80°C; (**c**) after 2 h) (33).

to 160°C at 10 mmHg. Subsequently, the lactide reaction mixture was distilled at 180°C at 5 mmHg to final purification step. The final product contains high optical purity of PLA at 98.8% at 95% yield (Fig. 6.5).

The main advantage of PLA compared with petroleum-based polymer is the recyclable feature of the PLA polymer. A simple solubility test was carried out between PLA and polyethylene. In the presence of 10% ammonia at 80°C, after the PLA was completely solubilized while polyethylene remains as it is (Fig. 6.6). This unique feature enables the PLA to be reused and remolded into a new product. Such technology is now ready to be commercialized with the installation of PLA demonstration plant at Kyushu Institute of Technology, Japan. The total energy requirement for the production of 1 kg PLA is 14.4 Mcal.

6.3. *Environmental Mitigation of Greenhouse Gases Effect*

The survival and sustainable growth of living organisms on the earth's surface for thousand of years is partly attributed to the balance of climatic factors such as atmospheric gases and solar energy. This equilibrium is obtained through the adsorption and reflection of solar energy from the sun and back into space. In this process, the atmospheric gases have the ability to adsorb and release the energy at a steady state. The trapped heat allows the earth's atmosphere to warm up, also known as the "natural greenhouse effect," creating a suitable environment for living organisms. These atmospheric gases or greenhouse gases (GHG) comprise of water vapor, carbon dioxide, methane, nitrous oxide, and ozone. However, the rapid industrial development which began in the eighteenth century in Europe followed by America in the nineteenth century and then in Asia shortly after that, has caused a shift in the equilibrium of the GHG. Atmospheric concentrations of carbon dioxide have increased by 30%, methane concentrations have more than doubled and nitrous oxide concentrations have risen by about 15% (46). As a result, this has enhanced the heat-trapping capability of the earth's atmosphere. The increased concentrations of GHG are likely to accelerate the rate of climate change.

Of all the GHG, methane has the highest heat trapping capacity or global warming potential (GWP) which is 23 times more than carbon dioxide (47). It has been recognized that the largest source of methane is from the anthropogenic activities mainly from landfills, municipal, and industrial wastewater treatment facilities and agricultural sectors (48–50). This is largely due to the rich organic content in the wastes generated at the end of the food and agricultural sectors. Due to the nature of the waste, anaerobic digestion/treatment is employed as it is the best and cost-effective method to reduce the polluting strength, which unfortunately emits a large quantity of methane into the atmosphere. General estimates of methane concentration from different sources are presented in Table 6.3.

Reducing methane emission from landfills seems to be a good approach in mitigating the GWP. This is done by utilizing aerobic systems for treating organic matter that will completely stop the methane release. However, the high operational cost especially high energy requirement for aeration and disposal of the large quantity of sludge produced may deter the industry from applying aerobic treatment. As such, anaerobic process is still the choice of the industry due to cost-effectiveness. Alternatively, the current landfill system could adopt a methane recovery system or installation of anaerobic bioreactor for kitchen refuse fermentation which not only reduces the release of methane, but also represents a new source of energy. Being renewable and combustible, generation of electricity from methane is a promising mitigation step to reduce the concentration of methane in the atmospheric gases. In addition, it eliminates the undesirable smell from the landfills. Lastly, the life span of the landfill system could be prolonged as the volume of wastes is reduced.

7. INTEGRATED ZERO DISCHARGE CONCEPTS OF MUNICIPAL SOLID WASTE MANAGEMENT AND HANDLING

Continuous increase in the prices of fossil fuels and rapid depletion of its reserves have renewed global interest in exploring alternative renewable energy sources. For decades, the world has largely depended on fossil fuels as its source of energy and petroleum-based

Table 6.3
Estimates of the global methane budget (Tg methane/year) from different sources (50)

Sources	Estimate (Tg methane/year)
Natural sources	
Wetland	92–237
Termites	20
Ocean	10–15
Hydrates	5–10
Anthropogenic sources	
Energy	75–110
Landfills	35–73
Ruminants	80–115
Waste treatment	14–25
Rice agricultural	25–100
Biomass burning	23–55
Other	15–20

Note: 1 Tg = 1 million tons.

chemicals. However, growing attention is now given to renewable sources such as wood fuels, agricultural wastes, animal wastes, MSW, and effluents. In addition to being renewable and sustainable, these types of energy sources are considered environmentally friendly. As such, they have great potentials for mitigating climate change. In particular, biomass as renewable resources hold great promise as a component of Kyoto Protocol strategies for the reduction of greenhouse gas emissions to acceptable levels.

Historically, an increase in the demand for cheap chemicals or intermediates to feed the rapidly growing industrial era and new discoveries of synthetic polymers in 1950s have encouraged the growth of petrochemical industries. The industries were largely based on the technology to convert petroleum into materials such as polyethylene, polypropylene, nylon, polyesters, and epoxy resins. All these polymers are synthesized from seven main precursors i.e., ethylene, propylene, butylenes, benzene, toluene, xylenes, and methane, which have become the backbone of the diverse petrochemical industry. It is estimated that 90% of the organic chemicals produced annually were synthesized from fossil fuels. Coal was once the main source of chemicals but because of the complexity of its conversion processes when compared to the more readily available and competitive petroleum and gas, the utilization of coal was limited only for energy.

There is a lot of potential for the utilization of waste for the production of organic acids and biodegradable plastics. The strategy is to have a zero waste technology, combining with the current waste management in most of the industries. Organic acids are the major key in this technology which is able to generate income and protecting the environment from pollution. A new paradigm is required that looks at waste not as a problem to be buried or burned but as an opportunity to recover valuable resources, create jobs, save money, and reduce pollution. The philosophy has arisen out of the realization that the wastefulness of our industrial society

is compromising the ability of nature to sustain our needs and the needs of future generations. "Zero Waste" is a whole system approach that aims to fundamentally change the way in which materials flow through human society. The goal is an industrial system directed toward material recovery rather than material destruction.

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7
Heavy Metal Removal by Crops from Land Application of Sludge

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CONTENTS

Abstract This chapter describes the application of phytoremediation in removing heavy metals from contaminated soils. The types of crops used as well as the characteristics and application of sludge in Malaysia are described. The standards and regulations of sludge application are also discussed. The chapter gives a detailed discussion of principles of phytoremediation and design parameters used in the design of the treatment systems. Moreover, a few case studies and design examples are covered in the chapter.

1. INTRODUCTION

The use of plants to treat wastes was investigated as early as 1967 as researchers investigated plants ability to uptake and translocate contaminants (1). Since then, this technique has evolved into a cost effective technology to remediate hazardous constituents from contaminated land. The present development of phytoremediation technology is being driven primarily by the high cost of many other soil remediation methods as well as a desire to use a "green" sustainable process. Some specific examples include the successful application of Phytoremediation technology in a small pond at Chernobyl with uranium contamination,

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_7

an engineered wetland at Milan, Tennessee for TNT removal and a riparian zone buffer strip at Amana, and Iowa for nitrate and atrazine removal from agricultural runoff. Applications at small sites were also successful in their agricultural cooperatives with pesticide and ammonia spills. It is also found that long-term monitoring and evaluation of phytoremediation technology are essential to demonstrate efficacy and further define suitable plants and applications in order to gain acceptance from regulatory agencies (2). In Malaysia, phytoremediation application in general has been limited to research only on a small-scale basis. As to date, actual remedy of heavy metals and organics has not been applied on real sites in Malaysia. Nevertheless, the Ministry of Science, Technology and Environment (MOSTE) encourages the phytoremediation research in terms of providing research funds, while the local Institution of Engineers (IEM) takes a leading role in bringing awareness of this technology to the related professional bodies and regulatory agencies.

1.1. Definition of Phytoremediation

Phytoremediation is an emerging technology that uses plants and their associated rhizospheric microorganisms to remove various pollutants from contaminated soils, sludge, sediment, groundwater, and surface water through contaminant removal, degradation, stabilization, or containment of the contaminant (3). It uses living plants for in situ and *ex situ* remediation of contaminated sites. The plants also help prevent wind, rain, and groundwater from carrying pollutants away from sites to other areas (2). It can be used to remediate various contaminants, including metals, pesticides, solvents, explosives, petroleum hydrocarbons, polycyclic aromatic hydrocarbons, and landfill leachate. It is also applicable to point and nonpoint source hazardous waste control (3).

The word "phytoremediation" is from the Greek prefix *phyto*-meaning "plant" and the Latin root word *remidium* – meaning "to correct or remove an evil." In soil, the "evil" could be anthropogenic (man-made) contaminants such as organic solvents, heavy metals, pesticides, or radionuclides (4). In general, the mechanism of phytoremediation is mainly living plants altering the chemical composition of the soil matrix in which they are growing. This is achieved by one of the five applications of phytoremediation, namely Phytotransformation, Rhizosphere, Bioremediation, Phytostabilization, Phytoextraction, or Rhizofiltration.

Phytoremediation is best applied at sites with shallow contamination of organic, nutrient, or metal pollutants. It has been utilized at a number of pilot and full-scale field demonstration tests in the United States. It is an emerging technology that should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages, and long-term applicability. Phytoremediation is well suited for use at very large field sites, where other methods of remediation are not cost effective or practicable; at sites with low concentrations of contaminants, where only "polishing treatment" is required over long periods of time; and in conjunction with other technologies, where vegetation is used as a final cap and closure of the site (5).

Initially, much interest was focused on hyperaccumulator plants that are capable of accumulating potentially phytotoxic elements to concentrations more than 100 times than those found in nonaccumulators (6–8). These plants have strongly expressed metal sequestration

mechanisms and, sometimes, greater internal requirements for specific metals (9). Some species may be capable of mobilizing metals from less soluble soil fractions in comparison with nonhyperaccumulating species (10). Metal concentrations in the shoots of hyperaccumulators normally exceed those in the roots, and it has been suggested that metal hyperaccumulation has the ecological role of providing protection against fungal and insect attack (7). Such plants are endemic to areas of natural mineralization and mine spoils (11). Examples include species of Thlaspi (*Brassicaceae*), which can accumulate more than 3% Zn, 0.5% Pb, and 0.1% Cd in their shoots (12, 13), and Alyssum (Brassicaceae), some species of which have been shown to accumulate over 1% Ni (14).

Exploitation of metal uptake into plant biomass as a method of soil decontamination is limited by plant productivity and the concentration of metals achieved (12). For instance, *Thlaspi caerulescens* is a known Zn hyperaccumulator, but its use in the field is limited because individual's plants are very small and slow growing. The ideal plant species to remediate a heavy metal-contaminated soil would be a high biomass-producing crop that can both tolerate and accumulate the contaminants of interest (15). Such a combination may not be possible; there may have to be a trade-off between hyperaccumulation and lower biomass, and vice-versa. Furthermore, the cropping of contaminated land with hyperaccumulating plants may result in a potentially hazardous biomass (16).

1.2. Heavy Metals in Soil

A heavy metal is a term usually applied to a large group of trace elements with an atomic density greater than $6\,g/cm^3$. The heavy metals, which tend to give rise to the greatest amount of concern with regard to the human health, agriculture, and ecotoxicology are As, Cd, Cr, Hg, Pb, Tl, and U (17). Heavy metals occur naturally in soils, usually at relatively low concentrations, as a result of the weathering and other pedogenic processes acting on the rock fragments on which the soils develop (soil parent material). Although heavy metals are constantly encountered in soil parent materials, such as igneous or sedimentary rocks, the major anthropogenic source of metals to soils and the environment are as follows:

(a) Metalliferrous mining and smelting
(b) Agricultural and horticultural materials
(c) Sewage sludge
(d) Fossil fuel combustion
(e) Metallurgical industries – manufacture, use, and disposal of metal commodities
(f) Electronics – manufacture, use, and disposal of electronic commodities
(g) Chemical and other manufacturing industries
(h) Waste disposal
(i) Sports shooting and fishing
(j) Warfare and military training.

Plants have the potential to enhance remediation of the following types of contaminants:

(a) Petroleum hydrocarbons
(b) Benzene, toluene, ethyl benzene, and xylene (BTEX)
(c) Polycyclic aromatic hydrocarbons (PAH)

(d) Polychloroethene biphenyls (PCB)
(e) Trichloroethene (TCE) and other chlorinated solvents
(f) Ammunition wastes and explosives
(g) Heavy metals
(h) Pesticide waste
(i) Radio nuclides
(j) Nutrient waste (such as phosphates and nitrates) (18).

1.2.1. Natural Content of Heavy Metals in Soil

Trace elements are those inorganic chemical elements that in very small quantities can be essential or detrimental to plants and animals. Trace elements occur as trace constituents of primary minerals in igneous and sedimentary rocks. Since soil is considered the products of in situ weathering of all rock types, the trace element concentrations in soil may then be linked to the types of parent material and the interactions with climate, organisms, and time. Table 7.1 shows the mean concentrations of selected trace metals in a range of representative types of igneous and sedimentary rocks, while Table 7.2 shows the mean concentrations of various types of surface soils.

Although Table 7.1 illustrates that trace metals are commonly present in soil parent materials, anthropogenic sources may also increase the background concentration of the soil sometimes to dangerously high levels. Table 7.2 shows that the creation of lead free housing is not nearly sufficient, as lead in soil has been shown to be a major contributor to the high lead levels present in children. There is a need for a plan, which will eliminate the soil lead exposure pathway because there is major health associated with lead exposure, especially in children (4).

Table 7.1
Mean selected trace metal contents of major rock types (mg/kg) (17)

Elements	Earth's crust	Igneous rocks			Sedimentary rocks		
		Ultrabasic	Basic	Granitic	Limestone	Sandstone	Shales and clays
Arsenic	1.5	1	1.5	1.5	1	1	13
Cadmium	0.1	0.12	0.13	0.09	0.028	0.05	0.22
Chromium	100	2,980	200	4	11	35	90
Cobalt	20	110	35	1	0.1	0.3	19
Copper	50	42	90	13	5.5	30	39
Lead	14	14	3	24	5.7	10	23
Manganese	950	1,040	1,500	400	620	460	850
Mercury	0.05	0.004	0.01	0.08	0.16	0.29	0.18
Molybdenum	1.5	0.3	1	2	0.16	0.2	2.6
Nickel	80	2,000	150	0.5	7	9	68
Selenium	0.05	0.13	0.05	0.05	0.03	0.01	0.5
Tin	2.2	0.5	1.5	3.5	0.5	0.5	6
Zinc	75	58	100	52	20	30	120

Table 7.2
The mean concentrations of various types of surface soils (17)

Element	Sandy soils	Silty and loamy soils	Organic soils
Arsenic	4.4	8.4	9.3
Cadmium	0.37	0.45	0.78
Chromium	47	51	12
Cobalt	5.5	10	4.5
Copper	13	23	16
Lead	22	28	44
Manganese	270	525	465
Mercury	0.05	0.1	0.26
Molybdenum	1.3	2.8	1.5
Nickel	13	26	12
Selenium	0.25	0.34	0.37
Zinc	45	60	50

1.3. Heavy Metals from Sludge

All sludge contains a wide range of metal and other contaminants in varying concentrations. Industrial sludges usually contain higher metal contents than suburban domestic sludges. However, domestic inputs of metals to the sewerage system are still not insignificant, being derived from the corrosion of metal plumbing fittings, excretion of metals in the human diet, cosmetics, healthcare products, and other domestic products. It has been estimated that in the UK, 62% of the Cu and 64% of the Zn were from domestic sources. The heavy metals most likely to cause problems for crop production on sludge-amended soils are Cd, Cu, Ni, and Zn (17). The ranges of values found in the literature for the concentrations of heavy metals in sewage sludges are given in Table 7.3.

1.4. Land Application of Sludge

1.4.1. Sewage Sludge Generation

Increasing industrialization and urbanization have resulted in a dramatic increase in the volume of wastewater produced around the world. Treatment of this wastewater resulted in various pollutants being concentrated or thickened into a sludge containing between 1 and 2% by weight dry solids. The dramatic increase in the volume of wastewater treated also resulted in large volumes of sludge, which required proper disposal in a safe manner. In the early 1990s, the UK produced 1.1 million tones dry sludge solids per year, while the USA produced 5.4 million tones. In the whole of European Community, 6.3 million tones of sewage sludge were being produced, including West Germany producing 2.5 million tones, France 0.7, the Netherlands 0.28, and Switzerland 0.215 million tones. Japan produced 1.1 million tones of dry sludge solids per year, while Australia produced 0.3 million tones.

In 1984, 45% of the sludge produced in the UK was used in agricultural land, compared to West Germany 32% and in the USA 25%. Japan on the other hand incinerates 55% of the sludge produced (20). Land disposal of sludge is a simple physical operation with the main

Table 7.3
Trace elements in sludge

Metal	United States[a]		European Union[a]		Malaysia[b]	
	Range	Mean	Range	Mean	Range	Mean
Arsenic	1.1–230	10				
Cadmium	1–3,410	10	158–1,770		1.2–6.6	2.1
Chromium	10–99,000	500			7.2–1,326	15
Cobalt	11.3–2,490	30				
Copper	84–17,000	800	500–17,000		123–769	153
Iron	1,000–154,000	17,000			10,000–31,300	22,000
Lead	13–26,000	500	800–8,030		15.3–338	32
Manganese	32–9,870	260			297–460	367
Mercury	0.6–56	6			2.2–7.1	3.5
Molybdenum	0.1–214	4				
Nickel	2–5,300	80	100–1,000		14.1–162	19
Selenium	1.7–17.2	5				
Tin	2.6–329	14				
Zinc	101–49,000	1,700	1,000–15,000		669–7,110	1,090

Units: mg/kg dry sludge.
[a] Ref. (17).
[b] Ref. (19).

variations centering on the rates and techniques of application. Land spreading, soil injection, and landfill are the three main options, with environmental and safety considerations dictating application rates and the degree of pretreatment.

In general, land disposal is considered the ideal option for sewage sludge disposal for a number of reasons. If suitable land, which is located less than 20 km from the treatment plant, is available, then excessive processing of the sludge can be avoided and benefit gained from the nutrient content of the sludge as well as its soil conditioning properties. However, one of the major limiting factors on the application of sewage sludge to agricultural land is the presence of heavy metals. Even domestic sludge may contain high amounts of zinc, copper, lead, and cadmium. Table 7.3 gives comparative data from selected countries on the maximum allowable concentrations of heavy metals in sludge.

1.4.2. Land Application of Sludge in Malaysia

There are three types of sludge produced in Malaysia, namely septic tank sludge, drying bed sludge, and lagoon sludge. Different types of sludge will exhibit different physical, chemical, and biological properties and thus need to be classified prior to utilization.

Sewage sludge has many characteristics that are good for soils and plants, if applied properly. Research has shown that the organic matter in sludge can improve the physical properties of soil. Treated sludge is also known as biosolids, a slightly more attractive name used as a soil additive. Sludge improves the bulking density, aggregation, and porosity of the soil. In other words, if added properly, sludge enhances soil quality and makes it better for vegetation. Plants also benefit from the nitrogen, phosphorus, and potassium in sludge. When

applied to soils at recommended volumes and rates, sludge can supply most of the nitrogen and phosphorus needed for good plant growth, as well as magnesium and many other essential trace elements like zinc, copper, and nickel within existing approval levels.

The application of treated sewage sludge to agricultural land is generally the most economical means of waste disposal and also provides an opportunity to recycle beneficial plant nutrients and organic matter to soil for crop production. However, sewage sludge also contains varying amounts of heavy metals that may pose hazard to metal toxicity in crops and to consumers of the crops. Thus, the uptake of heavy metals by crops and the fate of these heavy metals in soils need to be monitored.

1.4.3. Characteristic of Sludge

Sludge consists of organic solids, grit, and inorganic fines. Sewage sludge comprises lumpy, flaky, and colloidal solids interspersed with water. The volatile organic substance of the sewage sludge is either solid or liquid. If water is totally removed, the remaining organic volatile matter and inorganic matter (ash) are known as dry solids (DS). Tables 7.4 and 7.5 present the sludge characteristics for selected cities in Malaysia. Table 7.6 provides the sludge characteristics of three residential estates compared with an industrial estate.

1.4.3.1. Physical Properties

The application of treated sludge on soil has shown to alter the physical properties of the soil texture. Hydraulic properties like porosity, permeability, and flow velocity can influence

Table 7.4
Sludge characteristics in Malaysia for selected cities (19)

Selected city in Malaysia	Dry matter (% DS)	Organic matter (% DS)	pH 25°C	Total (N %)	Total (P %)	Zn (mg/kg DS)
Alor Setar	3.25	55.32	7.1	2.82	0.43	963.3
Gombak	4.27	62.36	7.1	2.87	0.28	912.2
Ipoh	12.12	67.35	7.4	3.04	0.57	1,178.9
Klang	2.20	62.57	7.1	2.47	0.46	1,123.4
Kluang	5.76	70.12	7.1	2.37	0.34	1,068.3
Kuala Terengganu	1.02	69.16	7.3	3.25	0.37	1,240.3
Kuala Lumpur	3.8	66.83	7.3	2.92	0.27	1,101.5
Kuantan	3.25	64.41	7.2	2.7	1.02	1,215.7
Labuan	3.43	63.31	7.2	2.64	0.45	2,156.8
Langkawi	0.71	66.51	7.3	2.92	0.9	1,250.4
Melaka	2.68	75.08	7.2	3.09	0.19	960.2
Penang	1.08	78.35	7.5	3.8	0.17	669.4
Seremban	3.04	67.14	7.3	2.75	0.32	1,096.5
Prai	2.29	73.17	7.2	3.08	0.36	1,167.7
Taiping	2.59	72.05	7.2	3.08	0.31	1,162.5
Ulu Tiram	4.77	57.68	7.2	2.3	0.25	928.4
Range	0.71–12.12	55.32–78.35	7.1–7.5	2.3–3.8	0.1–1.02	669.4–2,156.8

Table 7.5
Physical characteristics of sludge (21)

Parameter	Primary sludge	Secondary sludge	Dewatered sludge
Dry solids	2–6%	0.5–2%	15–35%
Volatile solids	60–80%	50–70%	30–60%
Sludge specific gravity	∼1.02	∼1.05	∼1.1
Sludge solids specific gravity	∼1.4	∼1.25	∼1.2−1.4
Shear strength (kN/m^2)	<5	<2	<20
Energy content (MJ/kg VS)	10–22	12–20	25–30
Particle size (90%)	<200 μm	<100 μm	<100 μm

Table 7.6
Sludge characteristics in Malaysia for selected cities (19)

Selected district in Malaysia	Cu (mg/kg DS)	Ni (mg/kg DS)	Cd (mg/kg DS)	Pb (mg/kg DS)	Hg (mg/kg DS)	Cr (mg/kg DS)
Alor Setar	135.7	21.1	1.8	35.0	4.7	23.3
Gombak	135.4	20.2	1.2	35.7	3.5	13.5
Ipoh	171.1	19.0	3.1	42.8	4.3	13.5
Klang	143.2	24.8	2.5	44.8	7.1	19.1
Kluang	147.1	15.6	2.0	33.0	2.2	14.4
Kuala Terengganu	153.1	17.2	2.5	23.2	2.2	9.3
Kuala Lumpur	153.9	18.6	2.4	29.5	4.2	16.5
Kuantan	151.9	30.5	2.4	42.0	7.1	9.9
Labuan	131.7	20.2	1.7	29.0	2.9	15.6
Langkawi	140.9	26.1	1.7	33.9	2.4	16.6
Melaka	155.3	27.2	2.2	20.2	4.6	17.0
Penang	131.6	15.8	2.1	19.8	3.3	7.2
Seremban	130.0	14.1	1.9	26.5	2.9	12.1
Prai	165.1	22.6	2.2	36.4	3.1	15.5
Taiping	154.4	17.5	2.2	25.5	3.9	12.5
Ulu Tiram	129.2	14.6	1.8	27.2	3.3	16.2
Range	129.2–165.1	14.1–30.5	1.2–3.1	19.8–44.8	2.2–7.1	7.2–23.3

soil moisture content and aeration respiration. The color varies from the source of sludge i.e., individual septic tanks (IST), Imhoff tanks, and mechanical process. Shear strength of sludge is a relevant parameter for consideration as sludge is being more and more disposed of on land. In the case of landfills (specifically mono-landfill), sludge should have a DS > 35% and a shear strength of more than $30\,kN/m^2$. However, there is some doubt as to the ability of sludge to retain shear strength as some research indicates that sludge loses its strength over time (i.e., more than 2 years) as shown in the Tables 7.4 and 7.5.

1.4.3.2. Chemical Properties

Chemical properties of sludge include metals, polymers, pH, alkalinity, and nutrients. The organic volatile matter may be characterized by its net calorific value. Chemical properties such as pH, alkalinity, and organic content of sewage sludge vary with industrial discharge into the system. The inorganic content of sewage sludge also varies widely, but for waste activated sludge, it is typically 20–35% DS, and for primary sludge, 30–45% DS.

1.4.4. Some Statistics on Sludge

Malaysia produces about 5 million cubic meters of sewage sludge per year, and this is expected to increase continuously. By the year 2022, the amount has been estimated to reach 7 million cubic meters per year (1). This is a tremendous amount of waste that has to be disposed off. World wide, the traditional means of sludge disposal is on land and into the sea. Due to increasing environmental awareness, disposal of sewage sludge will be costly, and an alternative disposal method that will enhance soil property and plant life without land contaminating them needs to be studied. Thus, pressures exist for useful or beneficial utilization of treating this waste. Research on utilization of treated domestic sewage sludge on crop lands has been in progress, and it is one aspect of sludge utilization that needs to be studied in detail in order to reduce rising costs. An efficient and economical disposal or safer application of this waste as a fertilizer is eminent.

Since 1994, individual septic tanks in Malaysia are desludged on a 2-year routine basis. Treated sludge from sewage treatment plants has been periodically taken to drying beds in regional plants. Table 7.7 shows the volume of sludge managed by Indah Water per month, while Table 7.8 gives the different sludge facilities adopted to treat the current sludge produced in Malaysia. The characteristic of sludge taken from several existing sewerage treatment plants in Negeri Sembilan and Klang Valley, Malaysia are provided in Table 7.9.

Table 7.7
Liquid sludge handled in Malaysia per month (19)

Sludge type	Quantity (m^3)
IST sludge	7,500
STP sludge	18,000
Pour flush sludge	4,500
Total	30,000

Table 7.8
Sludge treatment facilities used in Malaysia in treating sludge (19)

Treament/disposal Method	Quantity (%)	Volume (m^3)
Trenching	10	3,000
Drying beds	20	6,000
Sludge lagoons	10	3,000
STPs (with spare capacity)	60	18,000

Table 7.9
Characteristic of sludge taken from several existing sewerage treatment plants in Negeri Sembilan and Klang Valley (19)

Sludge Characteristics	Residential estates			Industrial estates
	Tmn Tasik Jaya, Seremban	Tmn Sri Gombak, Selangor	Tmn Sg. Besi Indah, KL	Tmn Perindustrian Puchong Utama
Total dry solids (TS, %)	46.2	36.7	54.5	94.3
Volatile solids (VS, %)	50.0	59.5	57.5	51.0
pH	6.87	5.16	6.00	6.06
Ash content (%)	50.0	40.5	42.5	49.0
Moisture content (%) (wet, wt)	53.8	63.3	45.5	5.7
Organic material (%)				
Carbon (C, %)	29.0	34.51	33.35	29.58
Nitrogen (N, %)	1.4	3.23	4.39	3.19
C/N Ratio	20.71	10.68	7.59	9.27
Phosphorus (P, %)	1.52	0.71	1.09	1.98
Inorganic material (% or mg/kg)				
Potassium (K, mg/kg)	696.73	539.50	521.10	861.61
Sodium (Na, mg/kg)	246.21	433.70	235.22	401.38
Calcium (Ca, %)	1.27	0.89	2.06	1.01
Iron (Fe, %)	2.8	2.01	1.02	3.13
Copper (Cu, mg/kg)	122.83	171.21	258.72	768.56
Zinc (Zn, mg/kg)	1,280.30	1,316.99	7,110.10	5,752.92
Lead (Pb, mg/kg)	73.60	93.55	223.40	338.28
Magnesium (Mg, mg/kg)	1,769.50	667.57	1,766.05	2,982.50
Silika (Si, mg/kg)	406.92	423.70	430.73	224.02
Chromium (Cr, mg/kg)	15.15	112.17	90.83	1,325.56
Cadmium (Cd, mg/kg)	3.35	3.77	6.28	6.57
Nickel (Ni, mg/kg)	28.14	25.88	43.12	162.25
Aluminum (Al, %)	0.91	1.8	1.18	1.67
Manganese (Mn, mg/kg)	389.07	296.55	322.48	460.23

2. PRINCIPLES OF PHYTOREMEDIATION

2.1. Types of Crops and the Uptake Relationship of Heavy Metal

The US EPA's Phytoremediation Resource Guide definition of the six types of phytoremediation and their application in listed below (22):

2.1.1. Phytoaccumulation

Also called phytoextraction, refers to the uptake and translocation of metal contaminants in the soil by plant roots into the aboveground portion of the plants. Certain plants, called hyperaccumulators, absorb unusually large amount of metals in comparison with other plants

and the ambient metal concentration. These plants are selected and planted at a site based on the type of metal present and other site conditions. After the plants have been allowed to grow for several weeks to months, they are harvested. Landfilling, incineration and composting are options to dispose of or recycle the metals, although this depends upon the results of the Toxicity Characteristic Leaching Procedure (TCLP) and cost. The planting and harvesting of plants may be repeated as it is necessary to bring soil contaminant levels down to allowable limits. A plan may be required to deal with the plant waste. Testing of the plant tissue, leaves, roots etc., will determine if the plant tissue is a hazardous waste. Regulators will play a role in determining the testing method and requirement for the ultimate disposal of plant waste.

2.1.2. Phytodegradation

Also called phytotransformation, is the breakdown of contaminants taken up by plant through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of compound (such as enzymes) produced by the plants. Pollutants are degraded, used as nutrients, and incorporated into the plant tissues. In some cases, metabolic intermediate or end products are re-released to the environment depending on the contaminant or plant species (see Sect. 2.1.4).

2.1.3. Phytostabilization

Phytostabilization is the use of certain plant species to immobilize contaminants in the soil and groundwater through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone, and physical stabilization of soils. This process reduces the mobility of the contaminant and prevents migration to the groundwater or air. This technique can be used to re-establish a vegetative cover at sites where natural vegetation is lacking due to high metal concentrations. Metal tolerant species may be used to restore vegetation to such sites, thereby decreasing the potential migration of contamination through wind erosion, transport to exposed surface soils, and leaching of soil contamination to groundwater.

2.1.4. Phytovolatilization

Phytovolatilization is the uptake and transpiration of the contaminant by a plant, with release from the plant. Phytovolatilization occurs as growing trees and other plants take up water and the organic and inorganic contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations. Many organic compounds transpired by a plant are subject to photodegradation.

2.1.5. Rhizodegradation

Rhizodegradation, also called phytostimulation, rhizosphere biodegradation, enhanced rhizosphere biodegradation, or plant-assisted bioremediation/degradation, is the breakdown of contaminants in the soil through microbial activity that is enhanced by the presence of the rhizosphere. Microorganisms (yeast, fungi, and/or bacteria) consume pollutants to degrade or transform organic substances such as nutrient substances. Certain microorganisms can degrade organic substances for use as nutrient substances such as fuels or solvents that are hazardous to human and eco-receptors and convert them into harmless products through

biodegradation. Natural substances released by plant roots – such as sugars, alcohols, and acids – contain organic carbon that act as nutrient sources for soil microorganisms, and the additional nutrients stimulate their activity. Rhizodegradation is aided by the way plants loosen the soil and transport oxygen and water to the area. The plants also enhance biodegradation by other mechanisms such as breaking apart clods and transporting atmospheric oxygen to the root zone.

2.1.6. Rhizofiltration

Rhizofiltration is the absorption or precipitation of contaminants onto plant roots or the absorption of contaminants into the roots when contaminants are in solution surrounding the root zone. The plants are raised in greenhouses (with their roots in water rather than in soil). Once a large root system has been developed, contaminated water is diverted and brought in contact with the plants or the plants are moved and floated in the contaminated water. The plants are harvested and disposed as the roots become saturated with contaminants (22).

Plant species are selected for use according to their ability to treat the contaminants of concern and achieve the remedial objectives to redevelopment, and for their adaptability to other site-specific factors such as adaptation to local climates, depth of the plant's root structure, and the ability to the species to flourish in the type of the soil present. Often the preferred vegetation characteristics include the following:

(i) The ability to extract or degrade the contaminants of concern to nontoxic or less toxic products
(ii) Fast growth rate
(iii) Adaptability to local condition
(iv) Ease of planting and maintenance
(v) The uptake of large quantities of water by evapotranspiration.

Several types of plants and sample species frequently used for phytoremediation are listed below:

(i) Hybrid poplars, willow, cottonwood, and aspen trees
(ii) Grasses (rye, Bermuda grass, sorghum, and fescue)
(iii) Herbaceous plants such as legumes, clover, alfalfa, and cowpeas
(iv) Aquatic and wetland plants (water hyacinth, reed, bulrush, and parrot feather)
(v) Hyperaccumulators for metals (such as alpine pennycress for zinc or alyssum for nickel). Other plants that are being investigated for their potential to remediate heavy metals contaminated soil include Indian mustard (*Brassica juncea*), oats (*Avena sativa*), barley (*Hordeum vulgare*), and alfalfa (*Medicago sativa*).

2.1.7. Impact of Heavy Metals on Plants

Zinc (Zn) and Cadmium (Cd) concentration exceeded normal values reported for these two elements in the leaves of all crops studied except maize. Cd tends to accumulate in leafy vegetables (17) like lettuce and spinach as well as in potato leaves. Sugar beet has been reported to accumulate Zn (23). Result shows how much metal uptake from the same soil can vary between different crops and within different parts of the same plant. Because of the low availability of the metals in relation to the high total loads, no phytotoxicity was observed, but metal accumulation was still high enough to make crop products on the highly polluted plots

unacceptable for consumption by humans or animals according to the current legal standards in Switzerland.

The fate and effects of sewage sludge constituents in a soil–plant system are influenced by factors such as climate (rainfall and temperature), management (irrigation, drainage, liming, fertilization, addition of amendments), and composition of the sewage sludge. In addition, soil properties affect the chemical reaction and process, which occur after the application of sewage sludge to a soil. Soil properties that affect the reaction and resultant plant uptake of sewage sludge constituent include pH, organic matter, cation exchange capacity (CEC), iron and aluminum oxides, texture, aeration, specific sorption sites, and water availability. Many of these factors are interrelated and thus create a rather complex medium involving chemical and microbial reactions. The factors, such as pH, water content, and aeration (relates to water content), vary frequently or are easier to adjust. For example, soil pH can be increased by lime additions.

Soils cation exchange capacity (CEC) is dependent on soil properties such as organic matter, pH type, and percentage of clay. Thus, it serves as an easily measured; integrating parameasured soil property, which provides background information on soil, pH measured in the laboratory is the representation of that site in the soil may be significantly different from the pH of other sites. For example, the pH at the root–soil interface may be lower because of exuded organic acids. Due to differential uptake of cations and anions, the pH in the root cylinder of active root hairs may be lower than that in order parts of the root system. Also, pH reductions with time in sludge-treated soils are due to the protons generated during the oxidation of reduced forms of N and S mineralized from sludge organic matter. Similar pH reductions occur after the addition of fertilizers, particularly those containing ammonium.

Plant uptake of elements from soil solution initially requires positional availability to the plant root. Either the element must be moved to the root through diffusion or mass flow processes, or the root must grow to the element. The element must then occur in a form, which can move into the plant via the uptake mechanism. This transfer requires that the element move through a solution phase, thus water solubility and a variety of complexation, chelation, and other chemical reaction become important.

In general, researchers agree that effects of organic compounds, certain pesticides, and metals are not dangerous when managed properly at regulated levels. However, they caution that additional study of organic compounds and longterm fate of materials is needed before unlimited application of sludge can occur safely on all lands.

2.2. Design Parameters

The design consideration includes

(a) Contaminant levels
(b) Plant selection
(c) Treatability
(d) Irrigation, agronomic inputs (P, N, P, salinity, Zinc, etc.), and maintenance
(e) Groundwater capture zone and transpiration rate
(f) Contaminant uptake rate and clean-up time required.

The design of a phytoremediation system varies according to the contaminants, the conditions at the site, the level of clean up required, and the plants used. Contaminant and site conditions are perhaps the most important factors in the design and success of a phytoremediation system. Other factors that influence the selection and design of a phytoremediation system are as below:

(a) Technical factors
(b) Strategies for contaminant control
(c) Innovative technology treatment trains
(d) Design team (soil science or agronomy, hydrology, plant biology, environmental engineering, regulatory analysis, cost engineering and evaluation, risk assessment and toxicology, and landscape architecture) (4).

Design of a phytoremediation system includes

(a) Plant selection
(b) Treatability
(c) Planting density and pattern
(d) Irrigation, agronomic inputs, and maintenance
(e) Groundwater capture zone and transpiration rate
(f) Contaminant uptake and clean up time required
(g) Analysis of failure modes.

2.2.1. Monitoring Plan

Usually, a monitoring plan is also submitted to the authorities before the approval of a project is given. The success of a phytoremediation project would depend on the monitoring of the reduction of contaminant levels in the soil or the accumulation of contaminant concentrations in the plants. Pilot studies should be performed before field-scale phytoremediation project are implemented. Information collected during monitoring may indicate the need for design modification. The monitoring plan should include evapotranspiration, erosion control, contaminant reduction in soil or contaminant accumulation in plants, and the process of succession. Evapotranspiration is measured by assessing the hydrologic balance of the site, while erosion control is usually measured by the quality of water runoff. The effectiveness of the contaminant reduction may be estimated through soil nutrients data, soil oxygen content, root development, and the measured levels of contaminants. The change of site vegetation over time must also be considered in the monitoring plan. The ideal species for remediating a site may be replaced by naturally occurring plants or pioneer species. The ideal species should be introduced to the site prior to pioneer species invasion.

2.2.2. Limitations

Phytoremediation has its own limitations. The presence of the contaminants in plants may be bioavailable to the food chain at an unacceptable concentration. The potential absorbed dosages must be estimated and compared with maximum safe food intake limits. Authorities such as the Department of Environment or Local Government may impose certain restrictions, for example, by erecting fencing around contaminated sites and/or by having buffer zones around such sites.

Table 7.10
Pollutant limit for land application (24)

Pollutant	Ceiling concentration limits for all biosolids applied to land (mg/kg) (Dry wt.)	Cumulative pollutant loading rate limits for CPLR biosolids (kg/ha)	Annual pollutant loading rate limits for APLR biosolids (kg/ha) APLR 365-day period
Arsenic (As)	75	41	2.0
Cadmium (Cd)	85	39	1.9
Chromium (Cr)	3,000	3,000	150
Copper (Cu)	4,300	1,500	75
Lead (Pb)	840	300	15
Mercury (Hg)	57	17	0.85
Nickel (Ni)	420	420	21
Selenium (Se)	100	100	5.0
Zinc (Zn)	7,500	2,800	140
From Part 503	Table 1, Section 503.13	Table 2, Section 503.13	Table 4, Section 503.13

2.3. Empirical Equations

Determination of the annual whole sludge application rate is given by the following formulae (22):

$$\text{AWSAR} = \frac{\text{APLR}}{0.001\,C}, \tag{1}$$

where, AWSAR is the annual whole sludge application rate (dry metric tons of biosolids/ha/year), APLR is the annual pollutant loading rate (kg of pollutant/ha/year) from Table 7.10, C is the Pollutant concentration (mg of pollutant/kg of biosolids, dry weight), and 0.001 is the conversion factor.

2.4. Health Effects

Heavy metals, including lead, are present in soils either as natural components or as the result of human activity. Metal-rich mine tailings, metal smelting, electroplating, gas exhausts, energy and fuel production, downwash from power lines, intensive agriculture, and sludge dumping are the human activities that introduce the largest quantities of lead into soils.

Today, more is known about the effects of lead and the pathways of exposure. Currently, lead is listed as a known or suspected carcinogen in the EPA's Toxic Release Inventory (TRI). If ingested, lead can accumulate in body organs, including the brain, and result in various degrees of lead poisoning. At high levels of exposure, lead can not only damage the brain and kidneys of adults and children severely, but also cause death.

Major pathways of exposure to lead:

(a) The inhalation of lead-containing car exhausts or industrial emission
(b) The ingestion of lead-based pain

(c) The ingestion of contaminated soil or dust from hand-to-mouth activities of those living in lead polluted environment
(d) The inhalation of leaded dust carried on clothing or by the wind.

Children face the most devastating effects of lead poisoning. The effects of lead are listed as below:
Effects for fetuses,

(a) Premature births
(b) Smaller birth weight
(c) Decreased mental ability in the infant
(d) Abortion.

Effects for children,

(a) Impair development
(b) Result in a lower IQ
(c) Shortened attention span
(d) Cause hyperactivity
(e) Cause progressive mental deterioration (includes a loss of motor skill, severe aggressive behavior disorders, and poorly controlled convulsive disorder).

Effects for adults,

(a) Decrease reaction time
(b) Possibly affect the memory
(c) Cause weakness in fingers, wrists, and/or ankles
(d) Cause anemia, weakness, lassitude, insomnia, facial pallor
(e) Weight loss, anorexia, malnutrition, constipation, nausea, abdominal pain, and vomiting
(f) May increase blood pressure in middle-aged man
(g) High levels of exposure may damage the male reproductive systems.

Future research direction,

(a) Determination of uptake rate of contaminant among different species of plants
(b) Determination of heavy metals uptake by harvestable crops such as palm oil.

3. STANDARDS AND REGULATIONS

3.1. Sludge Application on Land

Sewage sludge has many characteristics that are good for soils and plants, if applied properly. Research has shown that the organic matter in sludge can improve the physical properties of soil. Treated sludge, also known as biosolids, is a slightly more attractive name used as a soil additive. Sludge improves the bulking density, aggregation, and porosity of the soil. In other words, if added properly, sludge enhances soil quality and makes it better for vegetation. Plants also benefit from the nitrogen, phosphorus, and potassium in sludge. When applied to soils at recommended volumes and rates, sludge can supply most of the nitrogen and phosphorus needed for good plant growth, as well as magnesium and many other essential trace elements such as zinc, copper, and nickel within existing approval levels.

Fifteen chemical elements are considered essential for plant growth, that is, plants will not complete their lifecycle if they are not supplied. Each of these essential elements has defined physiological functions (25). Macronutrients are needed in concentrations greater than 0.15% of dry matter or are present at greater than 5 kg/ha in the mature plant tops. Micronutrients are those in concentration less than 0.01% of dry matter or are present at less than about 1 kg/ha in the mature plant tops.

Some plant species may require other elements for growth. Sodium may be necessary for plants with the C_4 photosynthetic pathway, and some halophytes, such as saltbush (*Atriplex* spp.). Silicon is important for the strength of stem tissue of some crops, such as rice and sugar cane. Legumes fixing N symbiotically require cobalt. This is a requirement of the *Rhizobium* spp., but has been observed also in growth responses by subterranean clover (Trifolium subterranean) to cobalt sulfate applications.

The application of treated sewage sludge to agricultural land is generally the most economical means of waste disposal and also provides an opportunity to recycle beneficial plant nutrients and organic matter to soil for crop production. However, sewage sludge also contains varying amounts of heavy metals that may pose hazard to metal toxicity in crops and to consumers of the crops. Thus, the uptake of heavy metals by crops and the fate of these heavy metals in soils need to be monitored. The following sections highlight the probable contamination levels of untreated domestic sludge on land and the advantages of nourishing the soil by safe and controlled application of treated domestic sludge or biosolids. Besides, there is a need for regulatory body to formulate the rate and frequency of application of biosolids to different types of soil and recommended characteristics of treated biosolids.

3.2. Standards and Regulations of Sludge Applications in Malaysia, the USA, and Europe

For advanced countries such as Europe and the United State, there are regulations on the criteria of waste disposal to be protected of an environment quality, including heavy metals content in those wastes. The USEPA and European Community Limit have regulated a guideline on heavy metals content for biosolids disposal as shown in the Tables 7.10 and 7.11. There are some differences on concentration of heavy metal allowable limit between them as an example; allowable concentration limit of cadmium is 85 mg/kg (dry wt.) for USEPA instead allowable concentration limit of cadmium range of 20–40 mg/kg for European Community Limit.

In Malaysia, the Government has regulated the *Environmental Quality* (*Sewage and Industrial Effluents*) (*Amendment*) *Regulations 2000* [*P.U (A) 398/00*], but has not mentioned about the allowable concentration limit of heavy metals in the sludge before it can be disposed off into the landfarming. Malaysian's domestic sludge is processed separately from industrial and commercial sludge; thus the heavy metals content are very low, as shown in the result of this study. Anyway, sludge generation would increase tremendously, so the government should revise and update an existing regulation. Hopefully, this study would help the government agencies to revise a regulation, especially on heavy metal allowable limit.

Table 7.11
European community limit (after CEC 1986) [a] (26)

Pollutant	Concentration in soil (mg/kg)	Concentration in dry sewage sludge (mg/kg)	Annual application rate (kg/ha/year)[b]
Cadmium	1–3	20–40	0.15
Copper	50–140	1,000–1,750	12
Nickel	30–75	300–400	3
Lead	50–300	750–1,200	15
Zinc	150–300	2,500–4,000	30
Mercury	1–1.5	16–25	0.1

[a] Assume soil pH range of 6–7.
[b] Based on average 10 years.

4. CASE STUDIES AND RESEARCH FINDINGS

Researches showed that majority of crops were able to adsorb almost heavy metals and concentrated in the tissues with or without effect to the crop's yield depending on the types and concentration of heavy metals applied. One of the factors that influences an uptake of heavy metal by the crops is soil pH. Normally, maximum yield of crops are achieved in the soil pH range of 5.5–6.5 and decrease below or above the range. Based on the study, the soil pH falls slightly below this range (pH = 5.2). However, there are exceptions in the case of lupines and treacle performing well in more acidic soils, whereas medics such as Lucerne prefer alkaline soils. The problem of low soil pH occurs in regions of excess rainfall of 500 mm per annum and irrigated areas. The problems of high pH are common in lower rainfall environments with calcareous sands and cracking clays as well as with many nonsaline sodic soils. Soil composition varies widely, and it reflects the nature of the parent material. The principle factors determining these variations are the selective incorporation of particular elements in specific minerals during igneous rock crystallization, the relative rates of weathering, and the modes of formation of sedimentary rocks.

Studied showed that most of pH values of all treatment ponds was in the normal range (pH $\approx$ 7.0), while COD, TS, and TVS parameters vary from each other. Domestic sludge sample from Community septic tank treatment plant was the highest concentration to COD, TS, and TVS, which were 79,900, 16.0, and 12.54 mg/L, respectively, while Activated sludge was the lowest concentration to TS and TVS, which were 1.34 and 0.28 mg/L as shown in the Table 7.12.

Studies on heavy metals content in the domestic sludge showed that cadmium range from 0.001–0.100 mg/kg (dry weight), chromium from 0.091–0.285 mg/kg (dry weight), copper from 0.131–0.569 mg/kg (dry weight), lead from 0.212–0.555 mg/kg (dry weight), nickel from 0.300–2.324 mg/kg (dry weight), and zinc from 0.180–3.129 mg/kg (dry weight). The concentration of those heavy metals after application to soil was 1.1211, 54.450, 57.113, 397.62, 844.42, and 183.38 mg/kg (dry weight) for cadmium, chromium, copper, lead, nickel, and zinc as shown in Table 7.13. Metal concentrations of sludge are presented in Table 7.14. Based on the U.S. Environmental Protection Agency (22) Part 503 and European Community

Table 7.12
Characterization of domestic sludge in Malaysia

Parameter	Community septic tank (CST)	Activated sludge (AcS)	Oxidation pond (OP)	Aerated lagoon (AL)
pH	7.22	7.16	6.92	7.03
COD, mg/L	79,900	29,600	31,500	26,400
Total solids, mg/L	16	1.34	3.99	13.61
Total volatile solids, mg/L	12.54	0.28	1.25	10.05

Table 7.13
Heavy metals content in the domestic sludge sample

Subject	Concentration of elements, mg/kg (dry wt.)					
	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
Heavy metal in studied domestic sludge (range)	0.001–0.100	0.091–0.285	0.131–0.569	0.212–0.555	0.300–2.324	0.180–3.129
Heavy metal in soil after applied sludge	1.1211	54.450	57.113	397.62	844.42	183.38

Table 7.14
Comparison of heavy metals content to USEPA and European community limit

Element	Concentration of elements, mg/kg (dry wt.)					
	Cd	Cr	Cu	Pb	Ni	Zn
This study (average)	0.003	0.203	1.202	0.37	1.077	1.46
USEPA, Part 503	85	3,000	4,300	840	420	7,500
European Community Limit	20–40	N.S	1,000–1,750	750–1,200	300–400	2,500–4,000

Limit, the content of heavy metals substance in domestic sludge studies remains well below the limit values.

The heavy metal concentration range was different in the plants after being applied by domestic sludge as shown in the Table 7.15. Three types of plants were chosen to be studied of heavy metal uptake by crops; *Ipomoea aquatica*, *Spinacea oleracea* and *Brassica juncea*. *Spinacea oleracea* has shown a good uptake of metal cadmium and zinc, while *ipomoea aquatica* and *Brassica juncea* have shown a good uptake of metal chromium, copper, lead, and nickel. It also showed a good sign of heavy metal mobility in plant–soil system.

Table 7.16 shows a distribution of heavy metals content in plants cross-section (%). The distribution of heavy metal in different parts of the crops is variable depending on the type of heavy metal. Most metals are more concentrated in root tissues of plants than in stem and leaves tissues, especially for lead, nickel, and copper.

Table 7.15
Heavy metals content in the plants sample

Type of plants	Average concentration of heavy metals content, mg/kg (dry wt.)					
	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
Ipomoea aquatica	0.251	10.83	37.68	32.96	213.2	63.47
Spinacea oleracea	1.26	8.4	17.45	23.05	24.06	118.25
Brassica juncea	0.15	9.27	22.42	26.25	32.24	88.83

Table 7.16
Distribution of heavy metals content in plants cross-section (%)

Cross-section	Type of plant	Cd	Cr	Cu	Pb	Ni	Zn
Leaves	*Ipomoea aquatica*	29.055	38.085	27.644	12.862	31.646	30.586
	Spinacia oleracea	26.928	12.545	24.563	12.508	11.681	18.908
	Brassica juncea	39.092	16.15	13.315	12.803	19.367	26.075
Stems	*Ipomoea aquatica*	40.685	23.363	21.527	13.342	26.806	36.411
	Spinacia oleracea	13.459	32.173	18.76	9.1241	15.702	28.595
	Brassica juncea	22.504	29.608	44.155	23.595	30.74	35.008
Roots	*Ipomoea aquatica*	30.818	38.573	50.959	73.666	41.562	32.927
	Spinacia oleracea	59.652	55.275	56.706	78.378	72.625	52.531
	Brassica juncea	38.403	54.242	42.53	63.602	49.893	38.917

Table 7.17
Design example for sample from Indah Water Konsortium (IWK), Malaysia

Heavy metal	Biosolids concentrations (mg/kg)	APLR (kg/ha/year)	$\text{AWSAR} = \dfrac{\text{APLR}}{(0.001)\,\text{Conc. In biosolids}}\,(\text{tons/ha})$
Cadmium, Cd	2.0	1.9	$1.9/(0.001 \times 2.0) = 950.0$
Chromium, Cr	14.4	150	$150/(0.001 \times 14.4) = 10{,}416.7$
Copper, Cu	147.1	75	$75/(0.001 \times 147.1) = 509.9$
Lead, Pb	33.0	15	$15/(0.001 \times 33.0) = 454.5$
Nickel, Ni	15.6	21	$21/(0.001 \times 15.6) = 1{,}346.2$

5. DESIGN EXAMPLE

By using data from Indah Water Konsortium (IWK), Malaysia for Kluang location as shown in Table 7.6, the determination of the annual whole sludge application rate could be calculated as shown in Table 7.17.

6. FUTURE DIRECTION RESEARCH

Studies show that landfarming method is capable of reducing the concentration of heavy metals in the samples. From the result of this study, landfarming technique is suitably applied to the palm oil farm because the concentration of heavy metals could be reached into an eatable

tissue lesser. Anyway, further study should be done to make sure the concentration of heavy metals in an eatable tissue. For future research, the determination of heavy metals uptake rate for several of plants could be done. Further study would be able to gain the range of heavy metal constant uptake rate by the crops (27, 28).

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8

Phytoremediation of Heavy Metal Contaminated Soils and Water Using Vetiver Grass

Paul N. V. Truong, Yin Kwan Foong, Michael Guthrie, and Yung-Tse Hung

CONTENTS

Abstract Phytoremediation includes utilization of plants to remediate polluted soils. In this chapter, application of Vetiver grass in phytoremediation of heavy metal contaminated soils is discussed. Case studies in Australia, China, and South Africa are presented. The future application may be in the areas of mine site stabilization, landfill rehabilitation, leachate treatment, wastewater treatment, and other land rehabilitation. It is a low-cost remediation method.

1. GLOBAL SOIL CONTAMINATION

Due to ever increasing industrial, agricultural, and mining activities worldwide, heavy metal pollution of land and water is becoming a globally important environmental, health, economic, and planning issue. There is an increase in world population, and unpleasant disposal of industrial effluents, especially in the developing countries, causing soil pollution. Utilization of these lands for agricultural purposes and urban developments requires a safe

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_8

and efficient decontamination process. With the increasing use of agrochemicals to maintain and improve soil fertility, unwanted elements such as cadmium into soils due to contaminated sources of fertilizers, especially in developing countries, are being introduced into agricultural soils, which poses a potential threat to the food chain (1, 2). Mining and industrial operations also lead to significant challenges for the management of the natural environments during and after these activities.The increased public awareness of the environmental impact of such activities demands an interdisciplinary, inter-organizational, and international effort (3). Soil and water contaminated with heavy metals pose a major environmental and human health problem that needs an effective and affordable technological solution (4).

2. REMEDIATION TECHNIQUES

2.1. Physical and Chemical Techniques

Various physical and chemical techniques to decontaminated soils have been undertaken during the last 25 years (5–8) and millions of dollars being spent by governments all over the world on preventive measures. However, all of them are labour intensive and costly, and cannot be applied to thousands of hectares of land contaminated with inorganic heavy metals (8, 9). These technologies results in rendering the soil biologically dead and useless for plant growth as they remove all flora, fauna, and microbes including useful nitrogen fixing bacteria and P-enhancing mycorrhizal fungi (10).

Many sites around the world remain contaminated with no remediation in sight simply because it is too expensive to clean them up with the available technologies (11). If these wastes cannot be economically treated or removed, steps must be taken to prevent off-site contamination of the food chain processes through wind and water erosion, leachate generation (9).

2.2. Bioremediation Techniques

Microbial bioremediation technology, well known for decontamination of organics (12), is not available for large-scale biodegradation of inorganic heavy metals. The health hazards caused by the accumulation of toxic metals in the environment together with the high cost of removal and replacement of metal-polluted soil have prompted efforts to develop alternative and cheaper techniques to recover the degraded land (10).

2.3. Phytoremediation

The restoration of derelict land by establishing a plant cover is important before it poses serious health hazard by transferring the trace metals into the surroundings. Current research in this area includes utilization of plants to remediate polluted soils and to facilitate improvement of soils structure in cases of severe erosion, the innovative technique being known as phytoremediation (1, 8, 10, 13).

Phytoremediation is widely considered to be not only an innovative but also an economical and environmentally compatible solution to many engineering and environmental issues across the world. Although essentially simple, this new technology branches further and into a variety of different fields and techniques. A review of tropical hyperaccumulator of

heavy metal plants and concluded that there is a lack of investigation for the occurrence of hyperaccumulator plant species. No botanical or biogeochemical exploration of trace metal tolerant and/or accumulating plant species has yet taken place in many parts of the world. Many plant species, which can accumulate high concentrations of trace elements, have been known for over a century (17). Renewed interest in the role of these hyper-accumulating plants in phytoremediation has stimulated research in this area (8, 17). Several plant species or ecotypes, associated with heavy metal enriched soils, accumulate metals in the shoots. These plants can be used to clean up heavy metal contaminated sites by extracting metals from soils and accumulating them in aboveground biomass (10, 13, 14).

2.3.1. Phytoextraction

This is a technique that utilizes plants known as heavy metal hyper accumulators and metal accumulating plants with large biomass to extract heavy metals such as Pb, Zn, Cu, and Cd. The plants are then harvested to allow the removal of contaminants from site (15).

2.3.2. Phytofiltration

This technique uses plant roots, grown in aerated water to concentrate and precipitate heavy metals from polluted effluents. Plants that can adapt to wetland conditions are the most suitable (15).

2.3.3. Phytostabilization

This technique relies on plants to stabilize contaminants in soils, rendering them harmless. Plants with low metal accumulating properties but that are tolerable to high heavy metal concentrations are most suited to this technique (15).

2.3.4. Phytovolatilization

This technique is useful for the removal of volatile metals such as Hg and Se. Plants extract these metals and volatilize them from the foliage.

2.3.5. Phytomining

There are several plant species or ecotypes, associated with heavy metal enriched soils, accumulate metals in the shoots. These plants can be used to clean up heavy metal contaminated sites by extracting metals from soils and accumulating them in aboveground biomass (13, 14). The metal enriched biomass can be harvested and smelted to recover the metal.

2.3.6. Limitations of Phytoremediation

Although phytoremediation is the least destructive method among the different types of remediation because it utilizes natural organisms and the natural state of the environment can be preserved, it has its limitations like all other biological methods: it has not yet been found to remove or reduce contaminants completely (16). Furthermore, any vegetative method of remediation may be more suited to a long-term application due to the time it takes for the plants to grow.

The use of a vegetative and effective erosion and sediment management program has proven to be viable. Vegetative methods are the most practical and economical; however, revegetation

of these sites is often difficult and slow due to the hostile growing conditions present, which include toxic levels of heavy metals (9).

2.3.7. Plants for Phytoremediation

Plants that are used to extract heavy metals from contaminated soils have to be the most suitable for the purpose, i.e. tolerant to specific heavy metal, adapted to soil and climate, capable of high uptake of heavy metal(s), etc. Plants either take up one or two specific metals in high concentrations into their tissues (hyperaccumulator) with low biomass (1), or extract low to average heavy metal (not metal specific) concentrations in their shoots with high biomass. Low biomass hyperaccumulators, generally, have a restricted root system (17). In contrast, nonaccumulators, high biomass producing and tolerant plants have physiological adaptation mechanisms, which allow them to grow in contaminated soils better than others (18). The tolerance and specific behaviour at the root level must be taken into consideration while selecting plants for phytoremediation (19). Root system morphology allows some plants to be more efficient than others in nutrient uptake in infertile soil or stressed soil conditions (20).

Phytoremediation is considered an innovative, economical, and environmentally compatible solution for remediating some heavy metal contaminated sites (4) among others. The next step is to find suitable species of vegetation with the ability to develop this technology on a large scale. This chapter deals with some experiments conducted in Australia using Vetiver.

3. VETIVER GRASS AS AN IDEAL PLANT FOR PHYTOREMEDIATION

The success of phytoremedial efforts is dependent largely upon the choice of plant species. Among the plants involved in phytoremedial measures, Vetiver grass (*Chrysopogon zizanioides* L (Roberty), formerly *Vetiveria zizanioides* L. (Nash)), should receive special attention (Fig. 8.1).

Fig. 8.1. Vetiver – Shoot and Root. *Left* Vetiver grass has stiff and erect stems with sterile flower heads, reaching 3 m high under good growing conditions. *Right* Deep, extensive and penetrating root system, capable of extending to 3.3 m in the first year of growth, and to 4.5 m in 3 years.

Vetiver is one of those few plants which possess both economical and ecological capabilities, i.e. essential oil distilled from its roots in over 70 countries (21) and its conservation properties, such as up to 2 m high plant with a strong dense and mainly vertical root system often measuring more than 3 m, useful in soil erosion control (15, 22–25). It is propagated vegetatively and is noninvasive (26). It is extremely resistant to insect pests and diseases (27) and is widely used worldwide for soil and moisture conservation and soil restoration. It is immune to flooding, grazing, fires, and other hazards (28). Vetiver grass is regarded as a tool for environmental engineering (32) and as one of the most versatile crops of the third millennium (33).

3.1. Unique Morphology and Physiology

Vetiver is a fast growing, perennial grass native to the South and South-East Asian regions. It will grow to approximately 1–2 m in height and has long been used in Asia for slope stabilization in agricultural lands because of a deep (up to 3 m), strong root system. Traditionally, these roots were woven into mats, fans, and fragrant screens (34).

Vetiver is used throughout the world in various cultivars; however, it has been shown that although Vetiver does adapt to its environment over time, most nonfertile genotypes such as Monto, Sunshine, Vallonia, and Guiyang are genetically identical (35). It can then be said that most application with specific results obtained by research can be applied with confidence throughout the rest of the world.

Vetiver grass is both a xerophyte and a hydrophyte and, once established, is not affected by droughts or floods (17)

The unique characteristics of Vetiver can be summarized as follows:

- Adaptability to a wide range of soil and climatic conditions
- Can be established in sodic, acidic, alkaline, and saline soils
- Tolerant to drought due to deep and extensive root system
- Mature plants are tolerant to extreme heat (50°C) and frost (−10°C)
- Vetiver can withstand burning, slashing, and moderate tractor traffic
- Resistant to infestations from most pests, diseases, and nematodes
- Absence runners or rhizomes, and only spreads by tillering

3.2. Tolerance to Adverse Soil Conditions

Extensive researches over a decade by the senior author has uncovered the ability of Vetiver grass to grow on both acidic and alkaline soils and tolerate a wide range of heavy metals at various concentrations. It has been demonstrated that Vetiver has a very high tolerance to heavy metals such as Arsenic, Cadmium, Copper, Chromium, Lead, Mercury, Nickel, Selenium, and Zinc when compared to most other plants

3.3. Tolerance to High Acidity and Manganese Toxicity

Experimental results from glasshouse studies show that when adequately supplied with nitrogen and phosphorus fertilizers, Vetiver can grow in soils with extremely high acidity and manganese. Vetiver growth was not affected, and no obvious symptoms were observed when the extractable manganese in the soil reached 578 mg/kg, soil pH was as low as 3.3, and plant

manganese was as high as 890 mg/kg. Bermuda grass (*Cynodon dactylon*) which has been recommended as a suitable species for acid mine rehabilitation, has 314 mg/kg of manganese in plant tops when growing in mine spoils containing 106 mg/kg of manganese (36). Therefore, Vetiver, which tolerates much higher manganese concentrations both in the soil and in the plant, can be used for the rehabilitation of lands highly contaminated with manganese.

3.4. Tolerance to High Acidity and Aluminum Toxicity

Results of experiments where high soil acidity was induced by sulfuric acid show that when adequately supplied with nitrogen and phosphorus fertilizers, Vetiver produced excellent growth even under extremely acidic conditions (pH = 3.8) and at a very high level of soil aluminum saturation percentage (68%). Vetiver did not survive an aluminum saturation level of 90% with soil pH = 2.0; although a critical level of aluminum could not be established in this trial, observation during the trial indicated that the toxic level for Vetiver would be between 68 and 90% (37, 38). This level was later confirmed by field observation, where Vetiver survived on a sandy soil with an aluminum saturation level of 86% (Fig. 8.2)

3.5. Tolerance to High Soil Salinity

Results of saline threshold trials showed that soil salinity levels higher than $EC_{se} = 8\,dS/m$ would adversely affect Vetiver growth, while soil EC_{se} values of 10 and 20 dS/m would reduce yield by 10 and 50%, respectively (Fig. 8.3).

These results indicate that Vetiver grass compares favourably with some of the most salt tolerant crop and pasture species grown in Australia (Table 8.1) (Fig. 8.3).

In an attempt to revegetate a highly saline area (caused by shallow saline groundwater), a number of salt tolerant grasses, Vetiver, Rhodes (*Chloris guyana)*, and saltwater couch (*Paspalum vaginatum*) were planted. Negligible rain fell after planting. So plant establishment

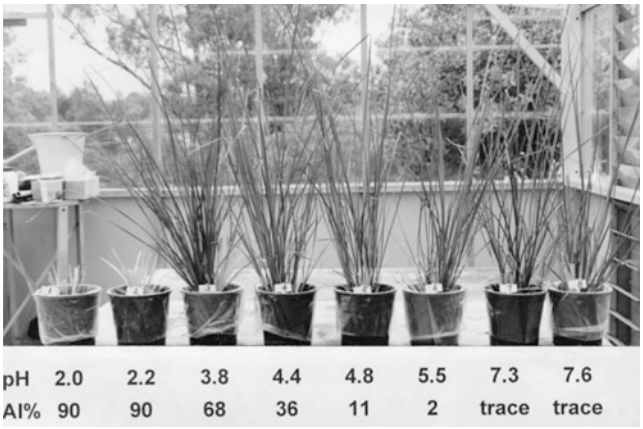

Fig. 8.2. Vetiver growth on aluminum saturated soil. When adequately supplied with N and P fertilizers, Vetiver growth was not affected when soil aluminum saturation extract (ASE) reached 68%, and soil pH at 3.8. ASE higher than 45% is highly toxic to both crop and pasture plants. Field sampling indicated that Vetiver grew on site with ASE at 86%.

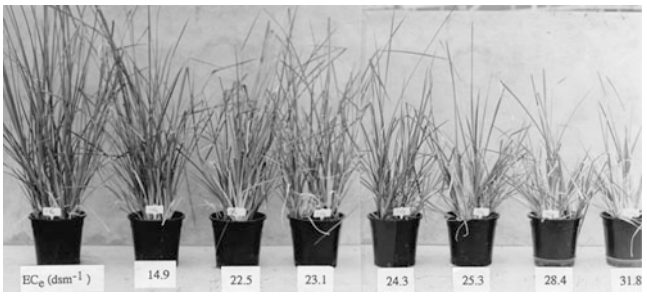

Fig. 8.3. Vetiver growth in highly saline soil. Soil salinity level higher than $EC_{se} = 16\,dS/m$ is considered to be highly saline. Vetiver growth was not greatly affected until soil salinity reached 23 dS/m.

Table 8.1
Salt tolerance level of Vetiver grass as compared with some crop and pasture species grown in Australia

Species	Soil EC_{se} (dS/m)	
	Saline threshold	50% yield reduction
Bermuda grass *(Cynodon dactylon)*	6.9	14.7
Rhodes grass (C.V. Pioneer) *(Chloris guyana)*	7.0	22.5
Tall wheat grass *(Thynopyron elongatum)*	7.5	19.4
Cotton *(Gossypium hirsutum)*	7.7	17.3
Barley *(Hordeum vulgare)*	8.0	18.0
Vetiver (*Chrysopogon zizanioides*)	8.0	20.0

Table 8.2
Soil salinity levels corresponding to different species establishment

Species	Profile soil EC_{se} (dS/m)	
	0–5 cm	10–20 cm
Chloris guyana	4.83	9.59
Paspalum vaginatum	9.73	11.51
Vetiveria zizanioides	18.27	18.06
Bare ground	49.98	23.94

and growth were extremely poor, but following heavy rain during summer (9 months later), vigorous growth of all species was observed in the less saline areas. Among the three species tested, Vetiver was able to survive and resume growth under the higher saline conditions (Table 8.2), reaching a height of 60 cm in 8 weeks (39). These results are supported by observation in Fiji and Queensland, where Vetiver was found growing in highly saline tidal flats next to mangrove.

3.6. Tolerance to Strongly Alkaline and Strongly Sodic Soil Conditions

Vetiver was satisfactorily established on a coal mine overburden and bentonite tailings with ESP (Exchangeable Sodium Percentage) of 33 and 48%, respectively. Soil with ESP higher than 15 is considered to be strongly sodic (40). Moreover, the sodicity of this coal overburden is further exacerbated by the very high level of magnesium (2,400 mg/kg) compared to calcium (1,200 mg/kg).

3.7. Tolerance to Heavy Metals

3.7.1. Tolerance Levels and Shoot Contents of Heavy Metals

Literature search indicated that most vascular plants are highly sensitive to heavy metal toxicity, and most plants were also reported to have very low threshold levels for arsenic, cadmium, chromium, copper, and nickel in the soil. Results shown in Table 8.3 demonstrate that Vetiver is highly tolerant to these heavy metals. For arsenic, the toxic content for most plants is between 1 and 10 mg/kg, for Vetiver, the threshold level is between 21 and 72 mg/kg. Similarly for cadmium, the toxic threshold for Vetiver is 45 mg/kg and for other plants between 5 and 20 mg/kg. An impressive finding was that while the toxic thresholds of Vetiver for chromium is between 5 and 18 mg/kg and that for nickel is 347 mg/kg, growth of most plants is affected at the content between 0.02 and 0.20 mg/kg for chromium and between 10 and 30 mg/kg for nickel. Vetiver had similar tolerance to copper as other plants at 15 mg/kg (28–31).

3.7.2. Distribution of Heavy Metals in the Vetiver Plant

Table 8.4 shows that the distribution of heavy metals in Vetiver plant can be divided into three groups:

Table 8.3
Threshold levels of heavy metals to Vetiver growth (30, 31)

Heavy metals	Thresholds to plant growth (mg/kg)		Thresholds to Vetiver growth (mg/kg)	
	Hydroponic levels (4)	Soil levels (5)	Soil levels	Shoot levels
Arsenic	0.02–7.5	2.0	100–250	21–72
Cadmium	0.2–9.0	1.5	20–60	45–48
Copper	0.5–8.0	NA	50–100	13–15
Chromium	0.5–10.0	NA	200–600	5–18
Lead	NA	NA	>1,500	>78
Mercury	NA	NA	>6	>0.12
Nickel	0.5–2.0	7–10	100	347
Selenium	NA	2–14	>74	>11
Zinc	NA	NA	>750	880

NA not available.

Table 8.4
Distribution of heavy metals in Vetiver shoots and roots

Metals	Soil (mg/kg)	Shoot (mg/kg)	Root (mg/kg)	Shoot/root (%)	Shoot/total (%)
Arsenic (As)	959	9.6	185	5.2	4.9
	844	10.4	228	4.6	4.4
	620	11.2	268	4.2	4.0
	414	4.5	96	4.7	4.5
	605	6.5	124	5.2	5.0
Average				4.8	4.6
Cadmium (Cd)	0.67	0.16	7.77	2.0	2.0
	0.58	0.13	13.60	1.0	0.9
	1.19	0.58	8.32	7.0	6.5
	1.66	0.31	14.20	2.2	2.1
Average				3.1	2.9
Copper (Cu)	50	13	68	19	16
Chromium (Cr)	50	4	404	1	1
	200	5	1170	<1	<1
	600	18	1750	1	1
Average				<1	<1
Lead (Pb)	13	0.5	5.1	10	9
	91	6.0	23.2	26	20
	150	13.2	29.3	45	31
	330	41.7	55.4	75	43
	730	78.2	87.8	87	47
	1,500	72.3	74.5	97	49
Average				57	33
Mercury (Hg)	0.02	BQ	0.01	–	–
	0.36	0.02	0.39	5	5
	0.64	0.02	0.53	4	4
	1.22	0.02	0.29	7	6
	3.47	0.05	1.57	3	3
	6.17	0.12	10.80	11	6
Average				6	5
Nickel (Ni)	300	448	1040	43	30
Selenium (Se)	0.23	0.18	1.00	53	15
	1.8	0.58	1.60	36	27
	6.0	1.67	3.60	46	32
	13.2	4.53	6.50	70	41
	23.6	8.40	12.70	66	40
	74.3	11.30	24.80	46	44
Average				53	33
Zinc (Zn)	Control	123	325	38	27
	100	405	570	71	42
	250	520	490	106	51
	350	300	610	49	33
	500	540	830	65	39
	750	880	1,030	85	46
Average				69	40

BQ below quantification.

Very little of the arsenic, cadmium, chromium, and mercury absorbed were translocated to the shoots (1–5%), a moderate proportion of copper, lead, nickel, and selenium were translocated (16–33%), and Zinc was almost evenly distributed between shoot and root (40%).

The important implication of these findings is that when Vetiver is used for the rehabilitation of sites contaminated with high levels of arsenic, cadmium, chromium, and mercury, its shoots can be safely grazed by animals or harvested for mulch as very little of these heavy metals are translocated to the shoots. As for copper, lead, nickel, selenium, and zinc, their uses for the above purposes are limited to the thresholds set by the environmental agencies and the tolerance of the animal concerned (Table 8.4).

3.8. Tolerance to Extreme Nutrient Levels

Vetiver also tolerates extremely high N and P in the growing medium. Research results indicate that Vetiver grass has a very high capacity of absorbing N at elevated levels of N supply. Vetiver growth will respond positively to N supplied at rates of up to 6,000 kg/ha/year, with no adverse growth effects apparent up to 10,000 kg/ha/year. As a result, vetiver has a very high N uptake as compared with other pasture grasses. (Fig. 8.4).

Vetiver requirement for P was lower than that for N, and no growth response was observed at rates exceeding 250 kg/ha/year. Its growth was not adversely affected at P application rates up to 1,000 kg/ha/year. However, in combination with a high growth rate and high yield, the total amount of P uptake by Vetiver was found to exceed those of other tropical and subtropical grasses (41).

The combination of these features makes Vetiver highly suitable for treating both domestic and industrial wastewater and landfill rehabilitation.

3.9. Tolerance to Agrochemicals

Herbicides applied to farmlands are important for controlling weeds in crops but this practice, if not properly managed, can lead to serious off-site contamination of the surrounding

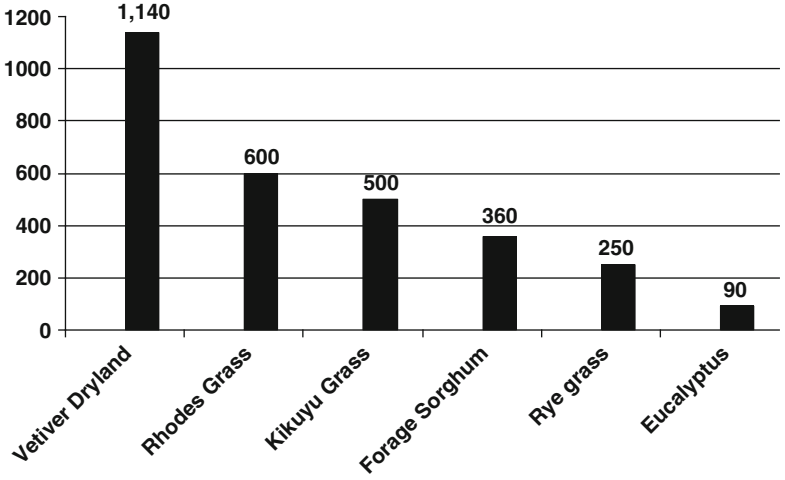

Fig. 8.4. Comparisons between Vetiver and other plants of nitrogen uptake capacity.

environment. In particular, residues of these chemicals can adversely affect flora and fauna in downstream aquatic ecosystems.

A glasshouse trial was conducted to determine the effects of varying concentrations of Atrazine and Diuron on the growth of Vetiver in a simulated wetland environment. Sixty-four to 65 days after planting, the Vetiver plants were exposed to either Atrazine or Diuron at concentrations of 0, 20, 200, or 2,000 μg/L in the free water. Effects on growth were measured for 28–30 days after herbicide application. Growth parameters measured included water use, cumulative leaf area, chlorophyll fluorescence, and whole plant dry weight at harvest.

Results showed that growth of Vetiver was not adversely affected by application of Atrazine or Diuron at rates up to 2,000 μg/L. By contrast, growth in *Phragmites australis* was significantly reduced at the highest rate of application of both herbicides. Not only does Vetiver establish and grow well under wetland conditions, it is also able to tolerate relatively high levels of Atrazine and Diuron (42).

3.10. Breaking Up of Agrochemicals

Wetlands have been recognized for their unique role in the natural landscape. The physical and chemical properties in the wetland environment allow the wetland trap and eliminate or enhance degradation of many agricultural and industrial pollutants. Wetland plants have adaptations, which allow them to tolerate and thrive in this low oxygen environment. These plant species have been shown to play an essential role in enhancing the degradation of Atrazine in the wetland environment.

Experimental results have shown that plots vegetated with iris and Vetiver species significantly reduced total Atrazine levels in the pot environment. The mechanisms responsible for the enhanced degradation have not been clearly identified. However, soils microorganisms play an important role in reducing soil Atrazine levels. This may explain the enhanced degradation in iris and Vetiver, particularly Vetiver, as it was not seen to sequester a significant amount of Atrazine into its tissues. These results have identified Iris and Vetiver as two species that promote the degradation of Atrazine (43).

Recently, research conducted at the Laboratory for Environmental Biotechnology, Swiss Federal Institute of Technology Lausanne, Switzerland, has confirmed the Australian results that Vetiver is highly tolerant to elevated Atrazine under the hydroponic system. The Swiss research also found that roots were able to hyper accumulate Atrazine and Vetiver resistance toward Atrazine was best explained by conjugation in the leaves and sequestration in the roots. Vetiver oil was also found to concentrate Atrazine, with a comparable value of Atrazine partition into octanol (5).

3.11. Growth

Given adequate nitrogen and phosphorous, Vetiver is a very fast growing grass in most climatic conditions producing large biomass yields when compared to other grasses. Under favourable growing conditions such as high N, P, soil moisture levels in the soil and warmer weather, Vetiver can produce up to 33 T/ha of dry biomass every 3 months (37). This is particularly important in bioremedial applications such as phytoextraction and phytofiltration. Although Vetiver is not classed as a hyper-accumulator, the name given to plants that

accumulate contaminants in their tissue at accelerated rates, it compensates by producing large biomass yields.

3.11.1. Root System

Vetiver has a very deep and massive root system that enables it to stabilize soils at varying degrees of slope. Typically, the shoot to root ratio of Vetiver is approximately between 1:1.2 and 1:1.8, which is illustrated by some specimens achieving 2 m-root depth within the first year of growth. Even in soil conditions high in salinity and acid sulphates, the root system can grow to over 1 m deep in the first year (44).

This growth will not occur in every instance of planting as it largely depends on the availability of moisture to the roots. In examples of hydroponics growth, or saturated soil, roots will not grow deeper than they require in search of moisture.

3.11.2. Shoots

Since Vetiver does not possess any runners or rhizomes, coupled with the presence of some nonfertile genotypes such as Monto, Vetiver spreads by what is known as tillering. This is the growth of new shoots, or tillers from the base of the plant giving the grass only limited lateral movement. Because of this tillering quality, Vetiver can be propagated by splitting up young plants into slips with each one containing around three to four tillers. When being buried, Vetiver will start rooting and shooting from the nodes, with this method of growth allows Vetiver to rise with rising soil levels in location where sediment build up can occur. The tillers simply grow begin to shoot at ground level.

3.12. Weed Potential

Vetiver is noninvasive, has no runners nor rhizomes, and only spreads by tillering (34). Although flowering under certain conditions, Monto Vetiver has been rigorously tested and proven to be completely sterile and has been approved for release by the Queensland Environmental Protection Agency. There are certain genotypes of Vetiver available throughout Australia, which do set fertile seeds, therefore should be avoided.

4. PHYTOREMEDIATION USING VETIVER

In Australia, Vetiver has been used successfully for the stabilization and rehabilitation and reclaiming of acid sulphate and trace metals contaminated soils and to stabilize mining overburden and highly saline, sodic, magnesic, and alkaline or acidic tailings of coal and gold mines (9, 45). Chen et al made a comparative study of the effects of chemical methods on the growth and uptake of trace elements by many plants including Vetiver grass and found this perennial grass having a greater ability to remove Cd, Pb, and Zn from soil, the values of Cd accumulation close to those of hyperaccumulator *Thlaspi caerulescens*. The authors discussed the effectiveness of phytoremediation with this grass with great biomass and concluded that 'Vetiver Grass Technology, VGT, is an effective, low-cost, and environmentally friendly technology to clean Cd contaminated soils'. The authors suggested developing a genetically modified Vetiver grass incorporating genes of hyperaccumulator. In southern China, it was reported that enhanced trace metal extraction in field experiments using Vetiver grasses

for re-vegetation of Pb/Zn mine tailing. VGT is emerging as an alternative technology for rehabilitation of degraded, saline, or trace metal contaminated soils, and for purification of water polluted with trace elements, agrochemicals, and industrial-effluent disposals (15).

Plants chosen for mine rehabilitation should also be poor translocators of metal contaminants to aboveground plant tissues that could be consumed by humans or animals. Additionally, the plants must grow quickly to establish ground cover, have dense rooting systems and canopies, and have relatively high transpiration rates to effectively dewater the soil (46). Another important phytoremedial property, particularly in plants that are not hyperaccumulators of contaminants such as heavy metals, is the ability to grow quickly producing large biomass. This enables them to accumulate large amounts of contaminants purely by volume as opposed to faster rate per plant mass. The most conspicuous characters of Vetiver grass include its fast growth, large biomass, strong root system, and medium to high level of metal tolerance; therefore, Vetiver grass is an important choice for stabilization of metal-contaminated soils (4).

On the issue of large biomass, an experimental trial showed that Vetiver could be particularly useful in phytoextraction applications. Although the metal contents in the shoots of *V. zizanioides* were significantly lower than three other grasses (hyper accumulators), the total amount of metals (Lead and Copper) accumulated in the shoots was the highest among the four plants tested, due to its highest biomass (15).

5. CASE STUDIES

5.1. Australia

5.1.1. Gold Mine

A series of glasshouse and field trials were carried out to determine the nutritional requirement of Vetiver grass during establishment phase on three types of gold mine wastes: oxide and barren waste materials, alkaline new tailings, and acidic old tailings on a goldmine in northern Australia. Results indicate that all waste and tailings materials are extremely low in N and P. Old tailings materials are extremely acidic and required high liming rate for satisfactory establishment, while fresh tailings only need N and P fertilisers.

When organic sources of N and P supply were compared, it was shown that there was little difference between organic N and P and chemical fertilizers on Vetiver growth. It was also established that As and Cd contents in Vetiver tops were very low; therefore, animals can safely graze Vetiver grown on theses tailings.

Barren and oxide waste materials: Chemical analyses of the materials show that both N and P levels are rather low particularly in the oxide material (Table 8.5).

Results showed that Vetiver can be readily established on both barren and oxide waste materials provided that DAP (Di-Ammonium Phosphate) at the level of at least 500 kg/ha was applied and adequate soil moisture is available (Table 8.6).

New gold tailings: Fresh gold tailings are typically alkaline (pH = 8–9), low in plant nutrients and very high in free sulphate (830 mg/kg), sodium and total sulphur (1–4%) (Table 8.7). Vetiver established and grew very well on these tailings without fertilizers, but growth was improved by the application of 500 kg/ha of DAP (Table 8.8).

Table 8.5
Chemical analysis of overburden

Analyses	Barren	Oxide
pH	7.7	9.1
EC (mS/cm)	0.80	0.17
Cl (mg/kg)	77	37
NO_3-N (mg/kg)	13	3
P (mg/kg)	36	8
SO_4-S (mg/kg)	610	9
Ca (meq 100 g^{t1})	16	10
Mg (meq/100 g)	1.4	7.1
Na (meq/100 g)	0.33	1.50
K (meq/100 g)	0.47	0.14
Cu (mg/kg)	4.10	0.87
Zn (mg/kg)	20.00	0.53
Mn (mg/kg)	9.6	5.0

Table 8.6
Vetiver grass dry matter yield after 10 weeks grown on overburden

Fertiliser (DAP kg/ha)	Barren (g/pot)	Oxide (g/pot)
0	14.17	13.89 a
100	13.45	12.82 a
200	12.44	14.59 a
300	16.64	13.82 a
500	14.00	20.16 b
L.S.D 5%	Not significant	Significant difference between a and b

The above results indicate that Vetiver can be established readily on fresh tailings when adequately supplied with N and P fertilizers and water. Therefore when established at appropriate intervals, Vetiver hedges could provide effective wind barrier for dust control on fresh tailing dams. Vetiver was used on a large-scale application to control dust storm and wind erosion on a 300 ha tailings dam. When dry the finely ground tailings material can be easily blown away by wind storms if not protected by a surface cover (Fig. 8.5). As gold tailings are often contaminated with heavy metals, wind erosion control is a very important factor in stopping the contamination of the surrounding environment. The usual method of wind erosion control in Australia is by establishing a vegetative cover, but due to the highly hostile nature of the tailings, revegetation is very difficult and often failed when native species are used. The short-term solution to the problem is to plant a cover crop such as millet or sorghum, but these species do not last very long. Vetiver can offer a long term solution by planting the rows

Table 8.7
Chemical analysis of new gold tailings

Analyses	New tailings
pH	7.8
EC (mS/cm)	0.88
Cl (mg/kg)	131
$NO_3 - N$ (mg/kg)	1
P (mg/kg)	7
$SO_4 - S$ (mg/kg)	830
Ca (meq/100 g)	12.5
Mg (meq/100 g)	0.84
Na (meq/100 g)	1.42
K (meq/100 g)	0.27
P (%)	0.042
K (%)	2.7
S (%)	1.59

Table 8.8
Vetiver grass dry matter yield after 10 weeks growth on new gold tailings

Fertiliser (DAP kg/ha)	New Tailings (g/pot)
0	16.79 a
100	13.70 a
200	15.20 a
300	12.43 a
500	17.60 b
L.S.D 5%	3.55 (significant difference between a and b)

at spacing of 10–20 m to reduce wind velocity and at the same time provide a less hostile environment (e.g. shading and moisture conservation) for local native species to established voluntarily later (Fig. 8.6).

Although excellent establishment was achieved, Vetiver growth varied greatly along the rows, ranging from very poor growth of between 0.2 and 0.3 m in height, to excellent growth of up to 1.5 m. As planting materials and fertilizer rate were similar, this difference in growth can be attributed to the variance in the amount of water supplied by the drip irrigation system. This was a result of the difficulty experienced in ensuring an even distribution over the entire 250 m length. However, the results clearly show that with adequate water and fertilizer supply, optimal Vetiver growth can be achieved with one wet season. It is expected that this poor growth would improve greatly during the coming wet season, if additional fertilizer applications were carried out before the wet season.

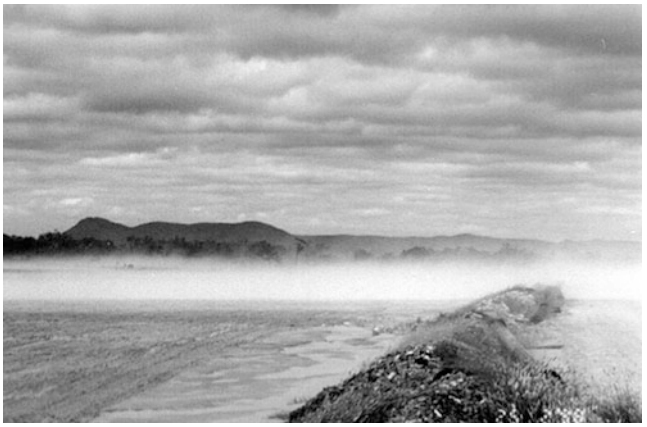

Fig. 8.5. Highly erodable gold mine tailings. A typical windstorm on a new gold mine tailings dam spreading fine particles loaded with heavy metals to the environment.

Fig. 8.6. Vetiver hedges minimizing wind erosion on gold tailings. A thick cover crop is commonly used to control this strong wind problem. But establishment of the cover crop is very difficult due to wind erosion. Vetiver planted in rows is the most effective and economical measure to protect the cover crop from wind erosion.

At its optimal growth, 1-year-old Vetiver hedge can form a very effective barrier to slow down wind velocity and control dust storms. The 1-year-old hedge is about 1.3 m high, and a very thick hedge was formed up to 0.8 m level. It is undoubted the hedge height and thickness will increase as the plants mature, providing a more effective barrier later. Although irrigation will not be needed in the future, further fertilizer applications, especially P at the rate of 500 kg/ha/year of DAP or equivalent, are recommended for the next 2–3 years to ensure the best growth in the future.

Fig. 8.7. Vetiver hedges providing protection from wind erosion. Vetiver hedges form a thick barrier protecting the cover crop from wind erosion.

Under the local conditions, 10 m spacing is probably too wide to be effective after 1-year growth. A closer spacing is needed but it is difficult to determine, as the hedges are not mature yet. However, for economical reasons, the application of Vetiver hedges system for dust control purpose should be combined with a ground cover crop, a pasture species such as Rhodes grass and green couch as demonstrated at this site. Therefore, the growth, height, and survival rate of the ground cover species will have to be taken into account to determine the most effective spacing of Vetiver hedges (Fig. 8.7).

When comparing with the wind barrier built on the same site, the main advantages of the Vetiver hedges are:

Low cost: Vetiver hedges would be much cheaper to establish than the fence barrier built with shade-cloth, geo-fabric, wire mesh, and star pickets.
Resistant to wind damage: once established, Vetiver hedges cannot be damaged by strong windstorms, and its tall growth will bend over and with its deep root system, Vetiver cannot be blown off. This is in sharp contrast to the steel reinforced barriers, which were damaged by strong wind.
Low maintenance: once established, Vetiver hedges do not require further maintenance except for the application of maintenance fertilizer once every few years.

Table 8.9
Acidity levels of old gold tailings

Surface conditions	Depth (cm)	pH	EC (mS/cm)	TAA (mole H + /T)	TPA (mole H + /T)
1. White powdery crust	0–5	3.1	14.5	1,063	1,590
2. Same as 1	5–10	3.0	5.2	262	726
3. Yellow hard crust	0–5	2.6	7.0	490	499
4. Coarse sandy	0–5	2.9	0.4	22	222

TAA total actual acidity, *TPA* total potential acidity.

Table 8.10
Heavy metal contents of representative gold mine tailings in Australia

Heavy metals	Total contents (mg/kg)	Threshold levels (mg/kg)
Arsenic	1,120	20
Chromium	55	50
Copper	156	60
Manganese	2,000	500
Lead	353	300
Strontium	335	NA
Zinc	283	200

NA not available.

The main disadvantages of the Vetiver hedges are:

Its slow growth in the first year,
The need for an effective irrigation system during the first 3–4 months

Old tailings: due to high sulphur content, old gold mine tailings are often extremely acidic (pH 2.5–3.5), high in heavy metals and low in plant nutrients. Revegetation of these tailings is very difficult and often very expensive and the bare soil surface is highly erodible (Table 8.9).

These tailings are often the source of contaminants, both above ground and underground to the local environment. Table 8.10 shows the heavy metal profile of gold mine tailings in Australia.

At these levels, some of these metals are toxic to plant growth and also exceed the environmental investigation thresholds (47) (Table 8.11).

Field trials conducted on two old (8 years) gold tailings sites; one is typified by a soft surface and the other with a hard crusty layer. The soft-top site had a pH of 3.6, sulphate at 0.37% and total sulphur at 1.31%. The hard top site had a pH of 2.7, sulphate at 0.85%, and total sulphur at 3.75% and both sites were low in plant nutrients (Table 8.12) (Fig. 8.8).

Results from both sites indicated that when adequately supplied with nitrogen and phosphorus fertilizers (300 kg/ha of DAP) excellent growth of Vetiver was obtained on the soft top

Table 8.11
Investigation thresholds for contaminants in soils (47)

Heavy metals	Thresholds (mg/kg)	
	Environmental[a]	Health[a]
Antimony (Sb)	20	–
Arsenic (As)	20	100
Cadmium (Cd)	3	20
Chromium (Cr)	50	–
Copper (Cu)	60	–
Lead (Pb)	300	300
Manganese (Mn)	500	–
Mercury (Hg)	1	–
Nickel (Ni)	60	–
Tin (Sn)	50	–
Zinc (Zn)	200	–

[a] Maximum levels permitted, above which investigations are required.

Table 8.12
Chemical analyses of an 8-year-old gold tailings

Analyses	Soft top	Hard top
pH	2.7	3.5
EC (mS/cm)	5.0	3.3
Cl (mg/kg)	5	19
$NO_3 - N$ (mg/kg)	Below quantifiable	Below quantifiable
P (mg/kg)	207	37
$SO_4 - S$ (mg/kg)	3,740	8,500
Ca (meq/100 g)	24	31
Mg (meq/100 g)	8.2	11.0
Na (meq/100 g)	0.02	0.01
K (meq/100 g)	0.01	0.02
Cu (mg/kg)	28	68
Zn (mg/kg)	237	198
Mn (mg/kg)	449	142
P (%)	0.059	0.078
K (%)	2.78	2.91
S (%)	1.31	3.75

site (pH = 3.6) without any liming. But the addition of 5 t/ha of agricultural lime significantly improved Vetiver growth. On the hard top site (pH = 2.7) although Vetiver survived without liming, the addition of lime (30 t/ha) and fertiliser (500 kg/ha of DAP) improved Vetiver growth greatly (Table 8.13) (Fig. 8.9).

Exchangeable arsenic in tailings and plant arsenic and cadmium: As the total Arsenic levels of old tailings were rather high (Table 8.14), and Vetiver growth did not achieve its full

Fig. 8.8. Vetiver trials on old gold mine tailings. This old gold mine tailings site had a pH of 3.8, high in As (590 mg/kg), Zn, Pb, and Mn. With adequate supply of fertilizers, good growth of Vetiver was noted 11 months after planting.

Table 8.13
Dry matter yield and nutrient contents (mean values of two nitrogen rates)

Liming rate (T/ha)	pH[a]	DM yield (g/pot)	Plant N (%)	Plant P (%)	Plant Mn (mg/kg)	Plant Zn (mg/kg)	Plant Cu (mg/kg)
Soft top							
0	3.60	0.20a	1.59	0.29	IS	IS	IS
5	5.00	5.15b	1.00	0.09	1150	91	5.2
10	6.40	6.72bc	0.99	0.09	1135	52	5.5
15	6.70	8.92c	0.91	0.10	930	52	4.8
LSD 5%		2.55					
Hard top							
0	2.70	0	–	–	–	–	–
5	2.90	0	–	–	–	–	–
15	3.90	0	–	–	–	–	–
30	5.50	3.31	0.95	0.11	430	32	4.9
40	6.40	3.05	0.68	0.07	445	96	4.0
50	7.00	3.40	0.73	0.07	455	95	3.8
60	7.30	4.60	0.78	0.08	410	54	3.0
LSD 5%		n.s.					

IS insufficient samples.
[a]Final pH at 11 weeks.

potential even at very high lime and phosphate rates, the effect of As on Vetiver growth was further investigated.

Table 8.15 shows that soluble Arsenic leached out from the hard top tailings is higher than from the soft-top tailings over the period of 5 weeks. These results support the total As shown in Table 8.14.

Fig. 8.9. Vetiver trials and old gold mine tailings. This old gold mine tailings site had a pH of 2.7, high in As (970 mg/kg), zinc, lead, and manganese. Very good growth was recorded 11 months after planting with adequate supply of lime (20 T/ha) and fertilizers.

Table 8.14
Total As and pH of soft top and hard top gold tailings

Tailings type	pH	As (mg/kg)	EC (mS/cm)
Soft top	3.59	590	2.78
Hard top	2.80	1,100	2.84

Table 8.15
Soluble As and pH levels in tailings under different lime treatments

Liming rate	pH	Soluble As (ppb of leachate)						Total AS leached (μg/kg)
		Week 1	Week 2	Week 3	Week 4	Week 5	Average	
Soft top (control)[a]	3.80	28.6	85.6	45.6	32.2	27.0	43.8	16.1
Soft top (20 T/ha)[a]	4.64	30.5	68.8	59.1	22.9	31.2	42.5	15.6
Hard top (control)[a]	3.09	91.6	287.6	186.2	99.4	34.3	139.82	39.1
Hard top (20 T/ha)[a]	2.73	406.8	587.0	424.9	184.2	55.70	331.72	214.1
Hard top (40 T/ha)[a]	4.62	231.7	116.7	146.3	NA	NA	164.9	49.5
Hard top (Control)[b]	2.96	120.1	81.2	111.7	248.8	153.5	143.06	62.0
Hard top (5 T/ha)[b]	4.89	34.1	19.2	88.8	100.5	53.6	59.24	32.6
Hard top (10 T/ha)[b]	8.10	25.1	35.8	58.7	59.6	36.9	43.22	15.4
Hard top (20 T/ha)[b]	7.98	45.15	115.7	154.3	282.4	223.9	164.29	61.2
Hard top (30 T/ha)[b]	8.10	36.28	155.1	155.2	220.1	93.5	132.03	75.9
Hard top (40 T/ha)[b]	8.14	27.2	178.5	206.4	220.1	88.9	144.22	77.8
Hard top (60 T/ha)[b]	8.08	35.9	155.2	184.1	166.0	88.2	125.88	46.8

NA not available.
[a]Field trial.
[b]Glasshouse experiment.

Table 8.16
Total exchangeable as affected by liming rates

Liming rate (T/ha)	Hard top from field trial		Hard top from glasshouse trial	
	pH	Total exch. As (mg)	pH	Total exch. As (μg)
Control	3.09	39.1	2.96	61.9
20	2.73	214.1	7.98	61.1
40	4.62	49.5	8.14	77.8

Table 8.17
As contents in Vetiver tops and roots as affected by liming rates

Tailings	Liming rate (T/ha)	Tailings As (mg/kg)	Total exch. As (μg)	Shoot As (mg/kg)	Root As (mg/kg)	Shoot As/total As (%)
Soft top	Control	590	16.1	4.5	96	4.5
	20	605	15.6	6.5	124	5.0
Hard top	Control	1100	39.1	9.6	185	4.9
	20	844	214.1	10.4	228	4.4
	40	620	49.5	11.2	268	4.5
Average						4.6

Table 8.18
Cadmium levels in tops and roots of Vetiver as affected by different liming rates

Tailings	Liming rate (T/ha)	Tailing pH	Tops Cd (mg/kg)	Roots Cd (mg/kg)	Tops Cd/total Cd (%)
Soft top	Control	3.80	0.31	14.20	0.9
	20	4.64	0.58	8.32	2.0
Hard top	20	2.73	0.13	13.60	2.1
	40	4.62	0.16	7.77	6.5
Average					2.9

These results also indicate that although liming had a strong effect on soil pH, it had little effect on the level of exchangeable As (Table 8.16)

The As contents in shoot and root of Vetiver plants collected from the field trial sites are presented in Table 8.17. These results indicate that very little As was absorbed by Vetiver plants and liming again had little effect on the amount absorbed. These results confirmed earlier finding that on the average only 4.6% of the amount of As absorbed was translocated to the tops, the majority was retained in the roots (95.4%).

Similar to As content, Cd contents in shoot and root of Vetiver are not greatly affected by liming rates and pH level. Again, most of the Cd absorbed was retained in the roots (97.1%), only 2.9% was translocated to the tops (Table 8.18).

Table 8.19
Chemical analyses of the coal mine overburden in Central Queensland

Soil pH (1:5)	9.6	Calcium (mg/kg)	1,200
EC dS/m	0.36	Magnesium (mg/kg)	2,400
Chloride mg/kg	256	Sodium (mg/kg)	2,760
Nitrate mg/kg	1.3	Potassium (mg/kg)	168
Phosphate mg/kg	13	ESP (%)	33
Sulphate mg/kg	6.1		

ESP exchangeable Na percentage (Na % of total cations).

From the above results, it is quite evident that liming did not greatly affect exchange As in the tailings and also both As and Cd in the Vetiver plants. The distribution of As and Cd in Vetiver tops and roots are quite similar. The majority of the absorbed heavy metals were retained in the roots. In the case of As, only 4.6% was translocated to the tops and only 2.6% for Cd. At these levels, animals can safely graze Vetiver.

In addition, the As contents in Vetiver tops (between 4.5 and 11.2 mg/kg) are well below the As toxic threshold level shown in Table 8.3 (between 21 and 72 mg/kg). Similarly, the Cd contents of Vetiver top (between 0.13 and 0.58 mg/kg) are also well below the toxic threshold level of between 45 and 48 mg/kg shown in Table 8.3. These results clearly indicate that Vetiver, grown on both hard top and soft top tailings, was not affected by either As or Cd toxicities.

5.1.2. Coal Mine

Coal mine overburden: The overburden of open cut coalmine in Central Queensland is generally highly erodible. These soils are usually sodic and alkaline (Table 8.19). Vetiver has established and stabilized successfully the spoil dump with 20% slopes and promoted the establishment of other sown and native pasture species (Figs. 8.10 and 8.11).

Coal mine tailings: In an attempt to rehabilitate an old coalmine tailings dam, (surface area of 23 ha and capacity of 3.5 million cubic metres) a trial was set up to select the most suitable species for the rehabilitation of this site. The substrate was saline, highly sodic, and extremely low in nitrogen and phosphorus. The substrate contained high levels of soluble sulphur, magnesium, and calcium. Plant available copper, zinc, magnesium, and iron were also high. Five salt tolerant species were used: Vetiver, marine couch (*Sporobolus virginicus*), common reed grass (*Phragmites australis*), cumbungi (*Typha domingensis*), and Sarcocornia spp. Complete mortality was recorded after 210 days for all species except Vetiver and marine couch. Mulching significantly increased Vetiver survival, but fertilizer application by itself had no effect. Mulching and fertilizers together increased growth of Vetiver by 2 t/ha, which was almost ten times higher than that of marine couch (48) (Fig. 8.12).

5.1.3. Bentonite Mine

One of the major ecological concerns for Bentonite Mine is the effect of run-off water from disturbed areas to surrounding catchments, particularly with sediment being the principal transport mechanism for a range of pollutants entering watercourses. The site is one of the

Fig. 8.10. Highly erodable coal mine overburden. This stockpile of coalmine overburden with 40° slope is highly erodible. It is saline and sodic and remained mostly bare of vegetation in the last 30 years.

Fig. 8.11. Vetiver applications on coal mine overburden. Vetiver was planted in the gullies to stop further erosion and to encourage the re-establishment of native species. Excellent growth was obtained 6 months later.

Fig. 8.12. Vetiver and marine couch trials on coal tailings dam. Vetiver grass was one of the only two survivors on this coal tailings dam, which is highly saline, sodic, and high in heavy metals. Vetiver biomass was about ten times greater than marine couch.

major disturbed areas on this mine. This consisted of two hectares that has been modified and levelled to provide a support base for stockpiling and solar drying of sodium Bentonite. The entire area required vegetation coverage to protect the soil from erosion. Due to the high sodium content, limited water holding capacity, and low nutritional value of the bentonite waste material, vegetation required for rehabilitation of this site has to be a specifically resilient species.

The natural topsoil of the region is predominantly a shallow, texture contrast soil (Podzolic) with a hard setting sandy loam surface. However, the trial zone has been modified and leveled to suit drying and stockpiling of Bentonite through the use of strongly sodic and semi-impermeable overburden. It is strongly sodic with Exchangeable Sodium Percentage (ESP) as high as 48%, highly dispersive (Montmorillonite clay) and susceptible to erosion if proper conservation practices are not applied. The occurrence of tunnel erosion had initiated in the north-east corner of the trial zone prior to Vetiver planting. The soil contains very low levels of major nutrients, this combines with its extreme reflective nature provides an environment hostile to germinating seedlings, but it is capable of hosting established specimens (Table 8.20).

On the site several rows of Vetiver were planted on contour line. The rows were carefully surveyed to ensure that the rows are levelled with zero fall at either ends to provide a water spreading mechanism. It was envisaged that this method would slow the flow of water, control against surface erosion, and aid in the building of a seed bank along the excess drying area (Fig. 8.13).

The following results were observed 10 months after planting:

Mulching of the areas had encouraged extensive shoot growth, with an average of 3 cm/week over the first 3 weeks. The mulched areas appear to be tolerable to high temperature and other weather changes.

Table 8.20
Chemical analyses of the soil at the trial site

Analyses	Overburden	Bentonite waste
pH	5.4	5.4
EC (dS/m)	0.18	0.14
Cl (mg/kg)	135.0	47.4
NO_3-N (mg/kg)	1.9	0.7
P (mg/kg)	2.0	5.0
SO_4-S (mg/kg)	66.0	101.0
Ca (meq/100 g)	0.19	0.93
Mg (meq/100 g)	4.75	6.44
Na (meq/100 g)	2.7	7.19
K (meq/100 g)	0.16	0.43
Organic matter (%)	0.45	0.35
ECEC (meq/100 g)	8	15
ESP (%)	35	48

Fig. 8.13. Vetiver hedge applications at bentonite mine. Vetiver grass planted on this highly sodic bentonite waste dump to control wind and water erosion, and to promote the establishment of other endemic plants.

Heavy rain had inundated the Vetiver rows, with some plants being submerged for 2.5 weeks. After the water had evaporated, the plants still appeared to be in healthy condition with general height retained; they did not appear to have any growth whilst the soil was water logged.

Runoff water samples were collected and their sediment content was measured by the rate of flow through a 2 mm sieve. Water samples were taken at positions upstream and downstream of the Vetiver hedges during peak flow and compared to those of distilled water. Results in Table 8.21 indicate that the Vetiver hedges trapped almost 100% of solids from clay contaminated storm water.

Table 8.21
Time taken for 300 mL of water to pass through a 2 mm sieve

Water samples	Time
Upstream from row	20.54 s
Downstream from row	11.76 s
Distilled water	11.20 s

Fig. 8.14. Established Vetiver hedge at bentonite mine. Fourteen months after planting, note the establishment of native grasses along the Vetiver hedges.

The amount of sediment trapped by the hedges varied with the conditions of the hedges. When the hedges were complete (with no gaps), up to 200 mm deep of sediment was trapped, with the sediment texture being greatly made up of sand and clay and less than 5% silt.

Random test holes show that the root systems have progressed quite substantially, with positive identification down to 500 mm. The hedges have encouraged 100% soil saturation within a 3.4 m arc along the rows; this has encouraged cracking of the clay to 220 mm (depth) and 30 mm (width). Surface cracking had appeared prior to row planting only to a depth of 30 mm.

Areas with extended growth from the use of fertigation techniques were found to be extremely palatable to cattle and were constantly chewed down to more than 150 mm.

Vetiver has flourished under the harsh conditions of the trial zone including an air temperature range of -3 to 42°C, wet extremes of 1 in 10 year rainfall event and prolonged dry periods. Growth height has averaged 600 mm, and plant base diameter is an average of 100 mm after 10 months (Fig. 8.14).

The grass has formed a semi-impermeable hedge which is slowing the flow velocity of the water, allowing minor rills to fill with sediment and altering the volume of water meeting the storm drain at any one time (time of concentration). Areas where a perfect level was not

achieved, some erosion occurred because of the concentrated flow of water; this has now been rectified through placing a concave row at the end of the hedge. The sediment trapped by the Vetiver rows has played host to several annual and perennial species. These species are currently only found on the southern side of the hedges within 1 m from the actual rows.

With the aims of determining the ability of Vetiver grass hedges to establish on extremely sodic soils, the effect of the hedges in spreading concentrated flows, in trapping sediment over major flow areas to provide a support mechanism for other plant growth and in reducing signs of visible erosion.

Current results have indicated that the Vetiver will establish satisfactory on sodic soils when adequately supplied with fertilizers and water. The use of mulches to 100 mm deep will provide a constant growing temperature for the plant roots allowing for a continual growth.

Vetiver Grass Technology (VGT) has achieved all the aims by effectively spreading concentrated flows of water and trapping sediment, providing favourable conditions for the establishment of other species. This process has also reduced the visible signs of erosion.

5.1.4. Bauxite Residue or Alumina Redmud

Bauxite residue is commonly extremely high in alkalinity, sodicity, and salinity as shown in Table 8.22.

These high levels of alkalinity and sodicity were resulted mainly from the addition of sodium hydroxide to the ore processing treatment. The hydroxide ion per se is not toxic to plant growth, but it can interfere with the availability of other plant nutrients such as phosphorus. Na per se is not toxic to plant growth, but it can interfere with the availability of other nutrients. Therefore, the addition of Ca and Mg (dolomite) may be needed to reduce ESP level, and a relatively high level of P application such as super phosphate will be needed. Literature shows that Vetiver can tolerate this level of alkalinity provided P and N are adequately supplied.

The salinity levels of the two samples are extremely high. However, this high salinity could not be attributed to sodium chloride (NaCl) as the chloride levels in both samples are not

Table 8.22
Analytical results of residues from redmud ponds

Analyses	Units	Residue textures	
		Cloddy	Sandy
pH		10.90	10.20
EC	dS/m	9.26	13.32
Cl	mg/kg	258.00	591.00
NO_3-N	mg/kg	BQ	BQ
P	mg/kg	38.00	28.00
Ca	meq/100 g	4.50	1.80
Mg	meq/100 g	0.14	0.19
Na	meq/100 g	1,900.00	2,600.00
K	meq/100 g	0.21	0.20

BQ below quantification.

extremely high (high level of Cl is toxic to plant growth); the high salinity recorded was most likely due to NaOH rather than NaCl. Therefore, plant establishment can be achieved on these tailings when adequately supplied with essential nutrients.

A simple glasshouse trial was conducted with the following treatments:

Control: a mixture of cloddy and sandy residues
Fertilizer treatment: complete NPK and S fertilizer
Fertilizer, dolomite, and low level of sulphuric acid: dolomite was used to supply Ca and Mg to counterbalance the high Na level and sulphuric acid to reduce alkalinity.
Fertilizer and high level sulphuric acid: to further reduce the acidity level

First symptoms of dieback appeared 3 days after planting, leaves became pale green, then yellowish and eventually completely bleached and dry up. Within 10 days after planting most plants were dead in treatment 1 and 2. Soil pH taken after this test showed that pH level of the red mud remained very high at 10.5 in the first two treatments; the acid addition reduced the pH to 8.0 for treatment 3 and 7.6 for treatment 4.

Results at 5 weeks after planting are very encouraging in that although plants in both treatments 3 and 4 suffered initial dieback of leaf tips and young shoots, and growth resumed after 2 weeks in both treatments. It was noted that more young shoots emerged in the dolomite treatment and more growth on older leaves in the acid treatment, with one leaf growing 310 mm in 3 weeks. It observed that the dolomite treatment, although with higher pH (8.0) producing similar growth to the high acid treatment with pH 7.6.

5.1.5. Landfill Rehabilitation and Leachate Treatment

As Vetiver grass has a very high water use and nutrient uptake rates, and it is tolerant to elevated levels of heavy metals and other adverse conditions such as salinity, sodicity, high nutrient load, it is best suited for landfill rehabilitation and leachate disposal. The following case study will illustrate its effectiveness.

Stotts Creek Landfill is a major waste depot of the Tweed Shire receiving wastes from both Tweed Heads and Murwillumbah townships and neighbouring local government areas in northern New South Wales. Disposal of leachate is a major concern of the Shire as the landfill site is close to agricultural areas. An effective and low cost leachate disposal system is needed, particularly during summer high rainfall season.

Leachate quality at Stotts Creek Landfill is low in heavy metals but relatively high in salts and nutrients (Table 8.23).

Currently, leachate and runoff from the landfill site are stored in ponds at the foot of the mound. During dry periods, the leachate is irrigated onto the top of the completed waste mound, where it evaporates or transpires into the atmosphere. During heavy rainfall, the leachate overflows into a system of wetlands and then to a local creek. Following capping and topsoiling, Vetiver has been planted on the surface of the completed waste mound and irrigated with leachate from collecting ponds. So far an area of 6 ha has been planted with Vetiver (Fig. 8.15).

As soon as an area was planted, it was irrigated with leachate by overhead spray irrigation, and almost 100% establishment was achieved. Results to date has been excellent, within

Table 8.23
Long term average levels of pollutants in Stotts Creek leachate

Tests	Units	Levels (ranges)
pH	–	7.2–9.3
Conductivity	μS/cm	199–11,150
Alkalinity (as $CaCO_3$)	mg/L	256–1,262
Redox potential	mv	−86 to +144
Dissolved oxygen	mg/L	0.2–30
Nitrate	mg/L	<0.01−10.5
Nitrite	mg/L	1.4–5.9
Ammonia	mg/L	0.01–410
Total N	mg/L	31.8–48.1
Total phosphorus	mg/L	0.04–3.5
Chloride	mg/L	215–1,700
Fluoride	mg/L	0.2–1.1
Sodium	mg/L	153–2,680
Calcium	mg/L	<1−658
Potassium	mg/L	78–1,650
Magnesium	mg/L	20–96
Sulphate	mg/L	3.8–134
BOD_5	mg/L	<2−640
Total suspended solids	mg/L	6–3,243
Total organic carbon	mg/L	43–1,440
Aluminium	mg/L	<0.1−1.0
Arsenic	mg/L	<0.01−0.12
Boron	mg/L	0.5–2.1
Cadmium	mg/L	<0.01−0.03
Copper	mg/L	<0.01−0.06
Chromium	mg/L	0.01–0.34
Iron	mg/L	0.09–7.0
Lead	mg/L	<0.01−0.03
Manganese	mg/L	0.01–1.74
Mercury	mg/L	<0.0001−0.001
Zinc	mg/L	<0.1−0.4

18 months, Vetiver growth had reached almost 3 m in height and have successfully disposed off all the leachate produced at this landfill (49).

5.1.6. Domestic Wastewater Treatment

The Esk Shire Council has recently installed a Vetiver Grass Wetlands System to treat sewerage effluent at Toogoolawah in South East Queensland. The sewerage treatment plant is situated on a 22-ha site on the northern edge of town. The aim of this scheme was to improve water quality before the effluent discharges to the natural wetlands. The biggest problem with

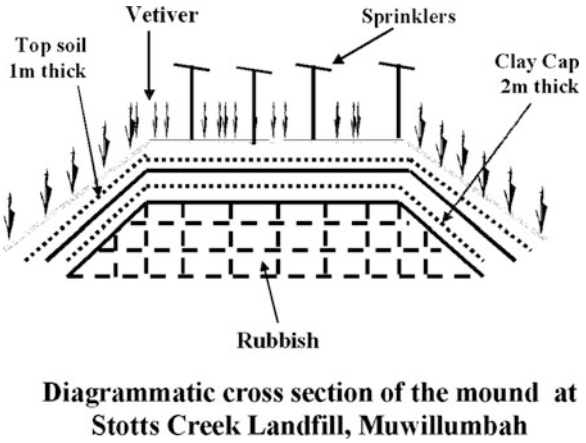

Fig. 8.15. Cross section of the Stotts Creek Landfill Cell.

the quality of the effluent is its high nutrient loading. With the recent changes to license conditions imposed by the Environmental Protection Agency, the existing treatment plant no longer complies with the license and an upgrade of the plant was required.

Instead of traditional upgrades, a new and innovative phyto-remedial technology recently developed in Queensland by the Department of Natural Resources and Mines, is being implemented at Toogoolawah. Under the Vetiver Wetlands System, the effluent is being treated in two stages:

- Preliminary treatment of the pond effluent *in situ* by floating pontoons placed in the ponds, and by Vetiver planting around the edges of the three sewerage ponds.
- Main treatment by Vetiver wetlands, once the effluent exits the sewerage ponds it passes through a Vetiver Grass contoured wetlands constructed over 3 ha of the land. The Vetiver Grass wetlands have been constructed in rows following the contours to allow good contact between the grass and the effluent. The Vetiver Grass takes up the water and in particular, the grass will remove the nutrients from the water that passes through it.

Vetiver grass pontoons: Results of a preliminary trial conducted on site with the first three pontoons show that Vetiver established and flourished (up to 1.5 m in 3 months) under hydroponics conditions. These pontoons have been removed and the grass harvested to produce about five new tillers of grass from each original tiller placed on the pontoons. The pontoons have now become the source of Vetiver grass for the project. Vigorous growth has been seen in the Vetiver grass plants that were placed onto the 21 new pontoons.

Growth on the pond edges: Planting Vetiver just above the pond supply level is the second part of the plan to pretreat the effluent in the ponds. At this position, the extensive Vetiver root has full access to the high nutrient load of the pond effluent.

Growth in the ephemeral wetlands: For the wetlands, the growth of the Vetiver grass has been varied for the first 3 months. Where the grass was able to dry out between watering, the growth was good. The growth was poor in places where the water laid around the grass. Growth was much reduced during winter and frost only burnt some of leaf tips of young

Table 8.24
Effluent quality before and after Vetiver treatment

Tests	Plant influent	Previous results 2002/03	New results (effluent) 2004
pH (6.5–8.5)[a]	7.3–8.0	9.0–10.0	7.6–9.2
Dissolved oxygen (2.0 minimum)[a]	0–2 mg/L	12.5–20 mg/L	8.1–9.2 mg/L
5 day BOD (20–40 mg/L max)[a]	130–300 mg/L	29–70 mg/L	7–11 mg/L
Suspended solids (30–60 mg/L max)[a]	200–500 mg/L	45–140 mg/L	11–16 mg/L
Total nitrogen (6.0 mg/L max)[a]	30–80 mg/L	13–20 mg/L	4.1–5.7 mg/L
Total phosphorous (3.0 mg/L max)[a]	10–20 mg/L	4.6–8.8 mg/L	1.4–3.3 mg/L

[a]Licence requirements (N and P levels are possible future requirements).

plants. Good growth resumed in spring and continued to grow vigorously in early summer. Ten months after planting, most plants were at least 1.5 m tall.

Irrigation schedule: In the early stage, best Vetiver growth is obtained when the wetland is irrigated on a 4-day cycle, one wet day, and three dry days. When the plants are fully mature and more Vetiver grass is planted in the bay, it is expected that a 2-day cycle will be possible.

Water quality: Even at this early stage, there is already evidence that the quality of the effluent is improving in respect to nutrient loads. The total Phosphorous level for the plant influent varies between 10 and 20 mg/L and the effluent results have dropped to between 1 and 3 mg/L. Similarly, the total N influent results are 30–80 mg/L, and the effluent results are now 4–6 mg/L. Table 8.24 show that the levels of nutrient in the effluent after passing through the Vetiver treatment were well within the EPA guidelines.

It is expected that it will take a further 12 months of growth before the wetland grass is properly established. However, the results so far already show that the Vetiver Grass wetlands can improve the effluent quality to the same quality as a high tech BNR sewerage treatment plant.

Conclusion: As Vetiver Grass system is very effective in removing nutrient loads, results to date has been excellent, within 18 months, Vetiver growth had reached over 2 m in height and have successfully disposed off all the sewerage effluent from the treatment plant except in times of heavy rainfall.

The Vetiver Grass wetland has already shown itself to be a suitable alternative to more expensive solutions to upgrade existing sewerage treatment plants. A high technology solution is not necessarily the best available option.

This scheme will provide a large-scale prototype of possible sewerage treatment schemes that can be used throughout western Queensland and other locations, where there is plenty of land and where the local government does not want to pay for installing and operating high cost solutions (50).

5.1.7. Industrial Wastewater Treatment

The disposal of industrial wastewater in Queensland, Australia is subjected to the strict environmental guidelines enforced by the Environmental Protection Authority. The most common method of treating industrial wastewater in Queensland is by land irrigation, which is presently based on tropical and subtropical pasture plants. However, with limited land area available for irrigation, these plants are not efficient enough to sustainably dispose of all the effluent produced by the industries. Therefore, to comply with the new standards, most industries are now under strong pressure to upgrade their treatment processes.

The conventional solutions such as chemical treatment plant and transportation to sewage treatment plant were considered, but both of which are impractical and, most importantly, very costly to build and to operate. Therefore, a more innovative and natural solution was needed.

The GELITA factory extracts gelatine from cattle hide using chemical processes involving strong acids, lime, and hydroxides. Tree planting was one of the earlier options considered, it has been trialled for several years but has not provided an effective solution to the problems faced by the company. Preliminary findings have established that an estimated 16.5kg/ha/year dry matter yield of pasture will result in an N export of 458 kg/ha/year from between tree rows if an assumed N level of 2.9% occurs.

Due to the limit of the land area, TEYS Bros abattoir in Beenleigh, Queensland, which processes in the order of 210,000 cattle per year for both domestic consumption and export. TEYS Bros abattoir will pipe excess effluent output to the Logan City Council for treatment. The cost of treating this effluent is based on both quantity and quality of the effluent.

Over the past 2 years, a series of research projects conducted at GELITA and Teys Bros. abattoir in Beenleigh to determine a viable means to achieve these goals. The Vetiver System has been identified as having the potential to meet all the criteria:

- Vetiver has the potential of producing up to 132 kg/ha/year of dry matter yield as compared to 23 and 20 kg/ha/year for Kikuyu and Rhodes grass respectively (Fig. 8.16).
- With this production, Vetiver planting has the potential of exporting up to 1,920 kg/ha/year of N and 198 kg/ha/year of P as compared to 687 of N and 77 kg/ha/year of P for Kikuyu and 399 of N and 26 of P for Rhodes grass respectively (Fig. 8.17).

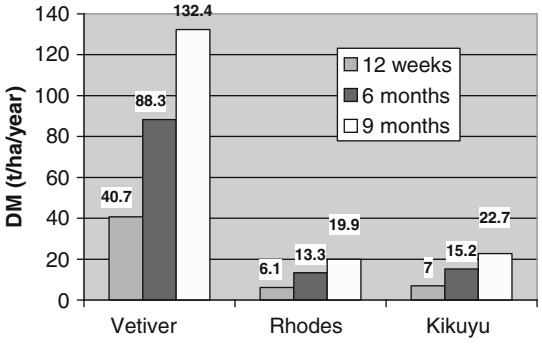

Fig. 8.16. Potential dry matter yield of the three grasses over time.

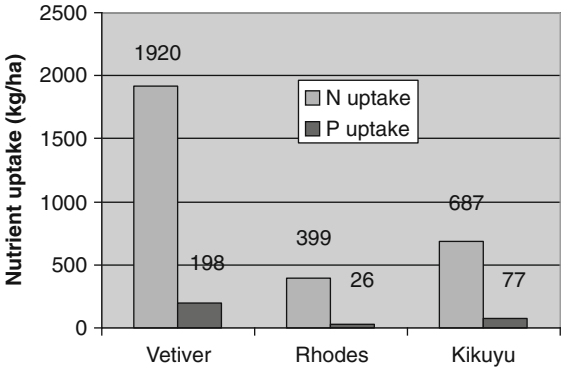

Fig. 8.17. Potential N and P uptakes by the three grasses over 9-month period.

Table 8.25
Effectiveness of Vetiver planting on quality of effluent seepage

Analytes	Nutrient levels		
	Inlet	Mean levels in monitoring bores	
		20 m down slope from inlet	50 m down slope from inlet
pH	8.0	6.5	6.3
EC (μS/cm)	2,200	1,500	1,600
Total Kjel. N (mg/L)	170	11.0	10.0
Total N (mg/L)	170	17.5	10.6
Total P (mg/L)	32	3.4	1.5

- Vetiver growth can respond positively to N supply up to 6,000 kg/ha/year, and to ensure this extraordinary growth and N uptake, P supply level should be at 250 kg/ha/year (Table 8.25).
- Based on the above results, the two companies have developed long term implementation plans for effluent and other solid waste product disposal (51).

5.2. China

It is well known that metalliferous mining activities produce a large quantity of waste materials, such as tailings and wastewater such as acid mine drainage (AMD) which is of major environmental concern due to potential hazards of surface or groundwater pollution. They contain excessively high concentrations of heavy metals and therefore result in severe pollution problems and lots of land degradation.

The first mine using Vetiver in Guangdong was the Lechang Pb/Zn mine located in the north of the province, where the first experiment comparing growth and performance of Vetiver and three other grasses, Bahia (*Paspalum notatum*), Bermuda (*Cynodon dactylon*), *Imperata cylindrica*, in the mine tailings was carried out. The result indicates that the height and biomass

of Vetiver are significantly greater than those of the other three grasses; moreover, the growth performance of Vetiver is the best among the four species. Thereafter, a pot experiment showed that Vetiver has strong uptake ability to two heavy metals, Pb and Zn, stronger than Bahia; but it is inferior to Bahia with regard to uptake of Cu. In addition, Vetiver roots had a larger retention capacity to heavy metals than Bahia roots, inferring that Vetiver keeps relatively more amounts of heavy metals in its roots than Bahia (4).

To rehabilitate the degraded ecosystem of a shale oil waste dump of Maoming Petro-Chemical Company located in Southwest of Guangdong Province, Vetiver, Bahia grass (*Paspalum notatum*), St. Augustine grass (*Stenotaphrum secundatum*), and Bana grass (*Pennisetum glaucum* × *P. purpureum*) were used. Among them, Vetiver had the highest survival rate, up to 98.6%, followed by Bahia and St. Augustine, 96.5 and 90.9% respectively, whereas Bana has the lowest survival rate of 61.7%. The coverage and biomass of Vetiver were also the highest 6-month after planting. Fertilizer application significantly increased biomass and tiller number of the four grasses, of which St. Augustine was most pronounced, up to 70.1%, while Vetiver was least pronounced, only 27.4%. Two heavy metals, lead and cadmium tested in this trial had different concentrations in the oil shale residue, and also had different contents and distributions in the four grass species. Concentrations of Pb and Cd in the four grasses presented a disparity of only 1.6–3.8 times, but their uptake amounts to the two metals were apart up to 16–35 times, which was chiefly due to the significantly different biomasses among them. In summary, Vetiver may be the best species used for vegetation rehabilitation in oil shale disposal piles (4).

5.3. South Africa

Rehabilitation trials conducted by De Beers on both tailings dumps and slimes dams at several sites, have found that Vetiver possessing the necessary attributes for self-sustainable growth on kimberlite spoils. Vetiver grew vigorously on the alkaline kimberlite; containing run off, arresting erosion, and creating an ideal microhabitat for the establishment of indigenous grass species. Rehabilitation using Vetiver was particularly successful on kimberlite fines at Cullinan mine where slopes of 35° are being upheld. It is clear that Vetiver is likely to play an increasingly important role in rehabilitation and, as a result of this; nurseries are being established at several mines (52).

At Premier (800 mm annual rainfall) and Koffiefontein (300 mm rainfall) diamond mines where surface temperature of the black kimberlite often exceeds 55°C, at this temperature most seeds are unable to germinate. Vetiver planted at 2 m VI (Vertical Interval) provided shades that cool the surface and allowing germination of other grass seeds.

Vetiver has also been used successfully in the rehabilitation of slimes dams at the Anglo American platinum mine at Rastenburg and the Velkom, President Brand gold mine.

6. RECENT RESEARCH IN HEAVY METAL PHYTOREMEDIATION USING VETIVER

A small scale trial was undertaken in 2004 at the Environmental Engineering Department, Queensland University of Technology to reconfirm earlier findings and to ascertain the capability of Vetiver to provide a practical solution to remove heavy metals in contaminated soils

Table 8.26
Trial concentrations As compared with previously determined toxicity thresholds

Contaminant	Trial concentration (mg/kg)	Toxicity thresholds (mg/kg)	Qld EPA exposure setting (Table 9.1 of contaminated land guidelines)
Copper	50 and 100	Up to 100	A
Chromium	25 and 50	Up to 600	Environmental investigation
Lead	150 and 300	Up to 800	A
Zinc	100 and 200	Up to 180	Environmental investigation

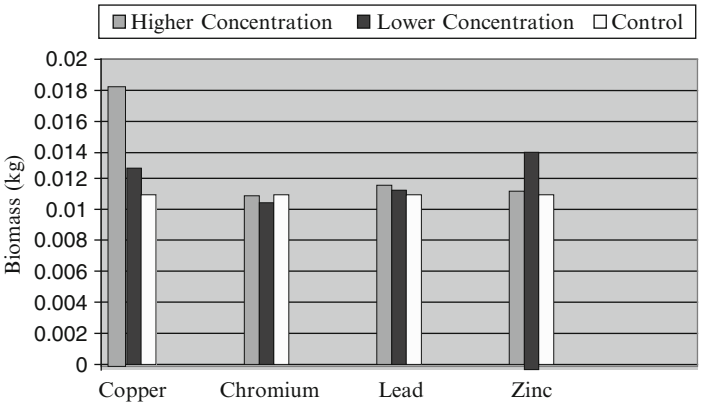

Fig. 8.18. Comparisons of dry biomass yields between Vetiver grown in contaminated soils.

in agricultural land, landfills and industrial sites and to comply with the standards, shown in Table 9.1 of the of the Department of Environment Draft Guidelines for the Assessment and Management of Contaminated Land in Queensland (53).

Remediation capability of Vetiver on Cu, Cr, Pb, and Zn was tested under the concentrations outlined in Table 8.26. These concentrations were chosen to relate back to the environmental and health-based thresholds described in Table 9.1, referred in Table 8.26.

The soil was supplied with 3,000 kg/ha/year of Nitrogen (Ammonium Nitrate) and 500 kg/ha/year of Phosphorous (Potassium di-Phosphate). Previous research indicated that under average growing conditions, Vetiver developed best at these levels of N and P.

6.1. Growth

During this trial, Vetiver achieved growth at the same level as control plants in terms of dry biomass for all heavy metal treatments. The average growth yields in dry biomass are summarized graphically in Fig. 8.18.

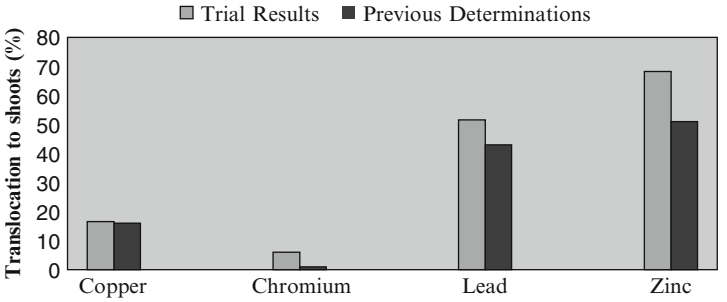

Fig. 8.19. Comparisons between shoot translocation results and previously recorded levels. Translocation: shoot content/total uptake (shoot + root content).

6.2. Results

On the whole, results obtained from this trial confirmed earlier findings that

Vetiver growth was not affected when exposed to Copper, Chromium, Lead, and Zinc at concentrations below previously determined toxic thresholds of these heavy metals.

Although the results indicated that there may be a case for Vetiver being able to translocate higher percentages than previously determined, but it must be noted that this trial had a smaller number of samples. Therefore, in the broader translocation relationship, these results supported previous research findings (see Fig. 8.19). That is

- Minimal translocation of Chromium
- Moderate translocation of Copper and
- Fairly even distribution of Lead and Zinc throughout the root and shoot

This information reconfirms the quality of Vetiver in terms of animal grazing suitability. It can be said that when using Vetiver for the rehabilitation of sites contaminated with Chromium only, the land could be used for grazing animals or use as feedstock or mulch; however, for contaminated land containing Copper, Lead, and Zinc, this application will be limited to animals' thresholds to the individual contaminants.

In addition to the above findings, this trial also showed that:

Plant tissue concentration: Vetiver growth did not appear to be affected by uptake of all contaminants at higher levels within the plant tissue than previously determined, in particular Lead concentrations for up to 360 mg/kg and Zinc at concentrations of up to 3,500 mg/kg within the plant tissue as compared with 78 and 880 mg/kg respectively as outlined in Table 8.27.

Leaching potential of harvested shoots: Shoot tissue samples of Vetiver that were exposed to Zinc at both 100 and 200 mg/kg were found to contain significantly high heavy metal concentrations (2,280 and 3,530 mg/kg respectively) and were subsequently returned to the laboratory for TCLP testing. This was to determine the suitability of disposing the shoots for further use as mulch at landfills. The results of this testing indicates the following:

It can be seen in Fig. 8.20 that the Vetiver shoots containing up to 3,530 mg/kg of Zn only leach approximately 20 and 2% of the allowable limit for unlined and lined landfills

Table 8.27
Threshold levels of Vetiver for heavy metals trialled (30, 31)

Heavy metals	Threshold to Vetiver growth (mg/kg)	
	Soil	Plant tissue
Copper	50–100	13–15
Chromium	200–600	5–18
Lead	>1,500	>78
Zinc	>750	880

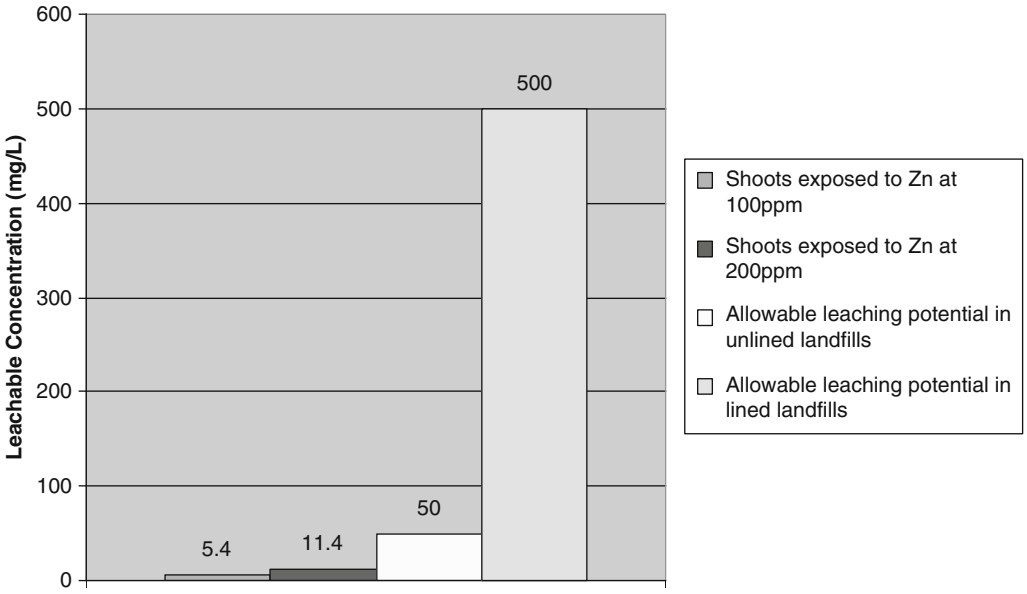

Fig. 8.20. Leaching potential compared with ANZECC (1994) national guidelines for the management of wastes – National manifest and classification system.

respectively. This allows the possibility of further investigation into using Vetiver as mulch or a cover material additive in modern waste management practice.

7. FUTURE LARGE SCALE APPLICATIONS

The future of phytoremediation will become increasingly applied, explored, and refined. The general consensus throughout all of the literature is that Vetiver, due to its diverse, unique physiological and morphological properties, is an ideal candidate for a range of effective phytoremedial applications, particularly in heavy metal contaminated mediums (54).

7.1. Phyto-extraction

Although Vetiver grass is not classified as a hyper-accumulator, as is the case for other plants used for this application, the ability of Vetiver to grow quickly with large biomass, coupled with tolerance to a wide range of adverse soil conditions suggests Vetiver grass is ideal in this application (15) also raise the potential for the use of chemical or chelating agents, a new development whereby a chemical is added to the plant encouraging increased uptake of contaminants such as heavy metals.

7.2. Phyto-stabilization and Mine Site Rehabilitation (55–57)

Vetiver can be employed to reduce the spreading of contaminants because of wind or water erosion. This application is particularly useful for barren mining land, where Vetiver can tolerate its harsh soil conditions. It is well known that metalliferous mining activities produce a large quantity of waste materials, such as tailings and wastewater. They contain excessively high concentrations of heavy metals and therefore result in severe pollution problems and lots of land degradation (4).

7.3. Landfill Rehabilitation and Leachate Treatment (58, 60)

Landfill rehabilitation has become an increasingly popular application of Vetiver. In Australia and China, landfill and industrial waste sites are usually contaminated with heavy metals such as Arsenic, Cadmium, Chromium, Nickel, Copper, Lead, and Mercury, which are highly toxic to both plants and humans. The movement of heavy metals and other toxic leachate from landfills can be controlled by a Vetiver system uniquely tailored to individual sites.

7.4. Wastewater Treatment (59)

The main advantages of using Vetiver grass in wastewater treatment are that it is low cost, simple, effective, and an environmentally friendly solution to an increasingly serious problem in both industrialized and developing countries. In fact using Vetiver grass in wastewater treatments a recycled process, where wasted nutrients are turned into useful fodder or organic mulch. This is in sharp contrast with other processes, such as chemical treatment, which often introduces another waste problem. Therefore, phytoremediation using Vetiver is expected to be very popular in both industrialized and developing countries.

7.5. Other Land Rehabilitation

In Australia, Vetiver is highly successful in the rehabilitation of both old and working quarries, where very few species can be established because of the hostile environment. Vetiver is able to stabilize the lose surface first, so other species can colonize the areas between the hedges later. Most recently, quarry rehabilitations also being carried out successfully in China (59, 60).

8. BENEFITS OF PHYTOREMEDIATION WITH VETIVER GRASS

As the world gears toward increasingly sustainable technology, the use of environmentally friendly, or better yet, naturally occurring technology is indeed the direction that environmental scientists and engineers should explore. This remediation technology not only

has the ability, with today's scientific advances, to be manipulated but is only a fraction of the cost of other physical and chemical remediation methods, and does not require manufacture and large scale machinery and equipment.

The financial benefit of implementing Vetiver for phytoremediation can be quantified readily as in the case of the Toogoolawah sewage treatment plant.

9. CONCLUSION

The use of Vetiver for phytoremediation is not only highly effective but also a step in the right ecologically sustainable direction. As beneficial research and development advances and worldwide exposure is increased, further research opportunities must be identified and implemented.

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9
Bioremediation

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CONTENTS

Abstract Environmental pollutants such as polycyclic aromatic hydrocarbons (PHAs), polychlorinated biphenyl's (PCBs), pesticides, petroleum hydrocarbons, and heavy metals are released into the environment, where they cause deleterious effects to wildlife and humans, owing to their inertness and being recalcitrant. However, the existence of microorganisms and plants capable of utilizing or accumulating such compounds has made the applications of such organisms in cleaning up of the environment a workable strategy. Therefore, Bioremediation (the application of bacteria and fungi) and Phytoremediation (the application of plants) to clean-up the environment are the two feasible and safe approaches that offer promise regarding environmental reclamation and sustainable use.

1. INTRODUCTION

1.1. Environmental Pollution: An Overview

The natural global environment (land, air and groundwater) is heavily polluted by human activities such as mining, discharge of industrial wastes, agrochemical usage and long-term application of urban sewage sludge in agriculture soils, waste incineration and vehicle exhausts, as well as anthropogenic organic pollutants. The above activities introduce into the environment a diverse array of pollutants including heavy metals, volatile organic compounds,

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_9,

nitroaromatic compounds, phenolic compounds, xenobiotic aromatic hydrocarbons: polycyclic aromatic hydrocarbons (PAHs), and pesticides, and polychlorinated biphenyls (PCBs) (1–6). Once the pollutants are in the environment, they pose great health risks to both humans and wildlife, which is due to their toxicity and recalcitrance. For example, PCBs, which were phased out in many countries in the mid-1980s because of their toxicity and adverse effects on humans and wildlife, are still ubiquitous all over the global environment and its biota because of their resistance to biodegradation. Similarly, pesticides and organophosphates have been or are being phased out for similar reasons. Owing to their toxicity and recalcitrance, PCBs and similar pollutants are generally referred to as persistent organic pollutants (POPS) (7). Heavy metals, on the other hand, pose the greatest health risk because of the difficulty associated with their removal from the environment, which arise from the fact that they cannot be chemically or biologically degraded and are thus ultimately indestructible (2).

1.2. Environmental Remediation Strategies

The health risks associated with environmental pollution have made it necessary to develop strategies to reclaim the environment from the various pollutants. Over time, a number of approaches or strategies have been devolved. To date, the most commonly used conventional approaches to remediate contaminated sites include, among others, landfilling, recycling, pyrolysis and incineration (8). Landfilling involves digging up contaminated soil and moving it to a landfill. Alternatively, the contaminated site is demarcated and contained (9). This method simply moves the contamination elsewhere and may create significant risks in the excavation, handling and transport of hazardous materials. Coupled with this drawback, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. Therefore, this method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long time into the future, with all the associated costs and potential liability (9).

Incineration at high temperature and various types of chemical decomposition (e.g., base-catalyzed dechlorination, and UV oxidation) may be effective at reducing levels of a range of contaminants but are limited in a number of ways. For instance, several technologies for in situ remediation such as chemically enhanced soil flushing using extracting solution (organic and inorganic acids) and complexation agents have been proposed for remediation. In a number of cases, these approaches are not only technologically complex, labour intensive and expensive to run, but also result in extensive changes in the physical, chemical and biological characteristics of the soil. Besides, they are often associated with an increase of exposure to contaminants for both workers at the site and nearby residents. Consequently, not only do they lack public acceptance but their applications are also limited to a small scale. Typically, they are unsuitable for very large areas such as mining sites or industrially/agrochemically contaminated soils (6, 9).

1.3. Bioremediation: A Concept

Microorganisms are ubiquitous, being widely distributed in a diverse array of habitats ranging from marine to terrestrial environments. Some of these habitats include those that have been heavily contaminated by heavy metals, as well as chemical and organic pollutants

emanating from human activities (Sect. 1.1). The inhabitation of polluted environments by microorganisms means that they are equipped with the necessary metabolic machinery to enable them survive in such environmental conditions. It is assumed that microorganisms may utilize such contaminants as carbon source and/or as terminal electron acceptors. These, in turn, enable microorganisms utilize such compounds for energy conservation and their eventual mineralization (9, 10). Besides microorganisms, some plant species are endowed with the capacity to concentrate, degrade and volatilize contaminants (6). These activities by some microorganisms and plant species are useful in reclaiming the environment of pollutants and are the basis of the bioremediation concept. Bioremediation, as a concept, relies or seeks to utilize the metabolic capacities of microorganisms and plants to decontaminate the environment of pollutants. The term bioremediation is primarily applied to the use of microorganisms (bacteria and fungi), while phytoremediation is applied with reference to the use of plants and their associated microbes in the decontamination of polluted environments. Considering the two processes, i.e., using microbes and plants, bioremediation may be defined as the process by which living organisms (bacteria, fungi, earthworms and plants) degrade or transform and detoxify hazardous organic and inorganic contaminants or waste under natural conditions into innocuous compounds such as carbon dioxide and water or to less toxic forms (8, 9, 11). Transformations of environmental pollutants are achieved through reactions that take place as part of their metabolic processes. Therefore, living organisms of potential for bioremediation possess enzymes and novel pathways that enable them to detoxify and/or mineralize those pollutants.

1.4. Advantages of Bioremediation

Bioremediation offers a number of advantages over physico-chemical approaches. Typically, bioremediation techniques are more economical than traditional methods such as incineration and other chemical methods, and can achieve complete degradation of organic pollutants without collateral destructions of the site material or its flora and fauna. Besides being economical, bioremediation can be used in situ for pollutants that are present at low but environmentally significant concentrations. This, in turn, prevents their gradual build-up in the environment. Furthermore, pollutants can be treated on site, thus reducing exposure risks for clean-up personnel or potentially wider exposure as a result of transportation accident. This approach also renders it unnecessary to transfer the contaminants from one environmental medium to another, for example, from land to water or air, as complete destruction of target pollutants is possible. Owing to the disadvantages associated with the applications of physico-chemical remediation approaches, bioremediation approaches remain the only versatile and ecologically acceptable clean-up technology (6, 7, 9, 12, 13).

Inasmuch as some instances of pollution can be readily bioremediated using existing technologies (Sect. 1.3), this is not normally the case with pollution involving toxic, inert and chemically stable compounds such as PCBs, PAHs, pesticides, heavy metals and synthetic polymers. These pollutants are not known to be degraded efficiently by many microorganisms and therefore require development of new innovative technologies (3, 6, 8, 12). These pollutants degrade slowly under natural conditions and depending on their respective half-lives, tend to enter the food web, where they are subsequently biomagnified (8, 9). The recognition

of bioremediation as clean technology and the apparent limitations of its operationalization, has directed research into innovative ways of enhancing the capability of natural bioflora to effectively mineralize the environmental pollutants at acceptable rates (expounded in Sect. 2.3).

2. ENVIRONMENTAL CONTAMINANTS

2.1. Environmental Contaminants

Environmental contaminants targeted for bioremediation may, for convenience, be grouped into six major groups comprising: (a) Chlorinated contaminants (these include chlorinated solvents, PCBs and chlorinated phenols), (b) PAHs, (c) Petroleum hydrocarbons (d) BTEX (Benzene, toluene, ethylbenzene and xylene), (e) Pesticides, and (f) Heavy metals (Table 9.1). These environmental contaminants pose serious health problems to both humans and wildlife owing to their high toxicity and persistence within the environment (6, 7, 9, 14, 15). Each group is explored in detail in the following sections:

2.2. Chlorinated Contaminants

Chlorinated contaminants comprise chlorinated solvents, polychlorinated biphenyls (PCBs) and chlorinated phenols (Table 9.1). Chlorinated organic compounds are among the most significant pollutants in the world. They comprise, among others, trichlorethene (TCE), tetrachloroethene (PCE), 1,1,1-trichloroethane (TCA) and chlorobenzene. Polychlorinated biphenyls (PCBs), on the other hand, are a class of chemicals consisting of theoretically about 209 compounds, collectively known as congeners. In PCBs, the aromatic biphenyl carbon skeleton carries between one and ten chlorine atoms. Even though there are 209 possible congeners, typical industrial preparations obtained by random chlorination of biphenyls contain 20–60 PCB congeners (7, 9, 14, 15).

Chlorinated compounds and PCBs in particular exhibit peculiar properties. For example, polychlorinated biphenyls are thermally and chemically very stable, flame- and oxidation-resistant, have low vapour pressure, are super hydrophobic and have excellent dielectric properties. These properties explain the surge in their application in a number of industrial processes such as the manufacture of flame retardants, oil condensers, dielectrics, plasticizers, heat exchangers, extender of insecticides, insulation of transformer and hydraulic fluids. It is therefore, not surprising that the annual tonnage of PCBs produced rose from 100-ton quantities in the early 1930s to a peak of 200,000 tons in 1975. By mid-1980s, about 1.5 million tons of PCBs had been produced worldwide and a substantial fraction entered the environment, while the remaining fraction will ultimately enter the environment (3, 7).

Inasmuch as PCBs possess properties desirable in a number of industrial applications, their continued use is limited by their toxicity and persistence in the environment. Environmental persistence results in their bioaccumulation in the food chain, with the accompanying disastrous effects on humans and wildlife. In humans and most mammals, incomplete degradation of most of these pollutants by the different mammalian enzymes of non-specific activity, tend to transform them into more toxic and harmful intermediates. Currently, oxygenated metabolic intermediates of some congeners are known to be teratogenic, immunogenic and/or

Table 9.1
Environmental contaminants targeted for bioremediation

Class of contaminant	Specific examples	Organism(s) involved			Microbial process	Reference(s)
		Bacterial species	Fungal species	Plants		
Chlorinated solvents	Trichloroethylene	*Methanotrophs*			Co-metabolism	(9)
	Perchloroethylene Trichloroetheure (TCE)	Dehalococcoides ethenogenes strain 195			Halorespiration	(14)
	Tetrachloroethene (PCE)	*D. ethenogenes* strain TCAI			Reductive dechlorination	(15)
	1,1,1-trichloroethane (TCA)	*D. restrictus*				
	Chlorobenzene					
Polychlorinated biphenyls (PCBs) or congeners (about 209 different compounds)	4-chlorobiphenyl	*Clostridium* sp.	*Phanerochaeta*		Meta or-ortho-cleavage oxidation route	(3)
	4,4-dichlorobiphenyl	*Burkholderia cepacia* strain-LB, 400	*Chrysosporium*			(9)
	2,6-dichlorobiphenyl	*Pseudomonas* sp.	*Tranaetes versicolor*		Co-metabolismsss	(7)
	2,5,2,S-tetrachlorobiphenyl					(15)
	2,2-, 2,3,6-and 2,4,6-chlorobiphenyls	*Rhodococcus globerulus* p6			CYP 450 monooxygenase system-dependent degradation	(5)
		Sphingomonas sp.	*Pleurotus ostreatus*			

(Continued)

Table 9.1
(Continued)

Class of contaminant	Specific examples	Organism(s) involved			Microbial process	Reference(s)
		Bacterial species	Fungal species	Plants		
Chlorinated phenol						(9)
Polyaromatic hydrocarbons (PAHs)	Naphthalene	*Alcaligenes* sp.	*Myceliopthora thermophia*		CYP 450-mixed function oxidase mediated oxidation	(9)
	Anthracene	*Pseudomonas* sp.				
	Flourene	*Mycobacterium* sp.				
	Pyrene	*Beijerinckia* sp.				(4)
	Benzo(a)pyrene	*Rhodococcus* sp.	*Phanerochaete chrysospo-rium*			
	Phenanthrene	*Sphingomonas* sp.				(5)
	Benzo(a) anthracene	*Streptomyces* sp.	*Tremetes versicolor*			(14)
	Benzo (b) flouranthene	*Burkholderia* sp.				
		Vibro sp.				
	Benzo (k) fluranthene	*Gardona* sp.				
	Dibenz (a,h) anthracene	*Cyclotrophicus* sp.				
		Stenotrophomonas sp.				
	Indeno (1,2,3-cd) pyrene	*Moraxella* sp.				
		Aeromonas sp.				
		Flavobacterium sp.				
		Bacillus sp.				
		Nocardia sp.				

Petroleum hydrocarbons	Paraffins (alkanes)	α-Proteobacteria		Oxidation	(14)
	Cycloalkanes	γ-Proteobacteria			
	Resin	*Syntrophus* sp.			
	Asphaltene	*Methanosaeta* sp.		Co-metabolism	(13)
		Methanospirillum sp.			(11)
		Desulfotomaculum sp.			
		ε-Proteobacteria sp.			
		Sphingomonas sp.			
		Alcahivorax sp.			(18)
		Marinobacter sp.			
BTEX	Benzene	*Dehalococcoides* sp.	Liginolytic fungi	Attenuation	(9)
	Toluene	*Methylocystis* sp.			
	Ethylbenzene	*Pseudomonas* sp. ADP	*Phanaerochaete chrysosporium*		(19)
	Xylene				

(Continued)

Table 9.1
(Continued)

Class of contaminant	Specific examples	Organism(s) involved			Microbial process	Reference(s)
		Bacterial species	Fungal species	Plants		
Pesticides	Atrazine	*Pseudomonas* sp.	White rose fungi		CYP450 monooxyagenase system dependent degradation	(5)
	Carbaryl	ADP	*Phanerochaete cyrosospo-rium*			
	Carbofuran Coumphos					(9)
	Diazinon	*Pseudomonas diminuta*	*Trametes versicolor*			(7)
	Glycophosphate Parathion					
	Propham		*Pleurotus ostreatus*			
	Organophosphate					
Heavy metals	Copper	*Rhizobacterium* sp.	Mycorrhizal fungi	Elsholzia	Metallothioneins (MTs) & phytochelatins (PCs)	(2)
	Cobalt			Splendens		
	Zinc	Cyanobacterial strains of genus *Synechococcus*				
	Cadmium			*Biscutella laevigata*	Bioaccumulation and sequestrations	(6)
	Lead Nickel					
	Mercury Arsenic			*Thlaspi* sp.	Compartmentalization	

carcinogenic. Moreover, the oxygenated metabolites may act as environmental oestrogens (the so called endocrine disruptors), thereby affecting the normal functioning of endocrine system. Accordingly, many investigators in this field think that PCBs and their oxygenated metabolic intermediates may be one of the causes of decreasing fertility in industrialized nations (3, 7, 8).

2.2.1. Microbial Degradation of Chlorinated Pollutants

In order to use microorganisms for bioremediation of chlorinated pollutants, such organisms need to be isolated and studied to evaluate their suitability. Since microbes capable of degrading chlorinated pollutants are likely to be found in environments where such pollutants are dumped, such environments have often been explored for potential chlorinated pollutant-degrading microbes. The predominant microorganisms fall into two groups: bacteria and fungi. Microbial degradation of chlorinated pollutants has widely been studied in regard to their degradability, molecular characteristics of enzymes involved, as well as the associated genes from a variety of soil microbes (15). Studies in a number of laboratories worldwide have identified microbes and enrichment cultures that metabolize and utilize PCBs as carbon and/or energy source. Through these studies, it has been established that the ability of microorganisms to degrade PCB depends heavily on their possession of the necessary enzymes and specialized pathways (7).

Microbial degradation of polychlorinated biphenyls (PCBs) occurs both aerobically and anaerobically. As a general rule, highly chlorinated congeners (which are highly stable and highly hydrophobic) are good substrates for anaerobic degradations, but are poor substrates for aerobic degradation. Anaerobic utilization of PCBs proceeds possibly via chlororespiration whereby the PCBs are initially used as electron acceptors. This process, also known as dechlorination, progressively converts higher-chlorinated congeners to lower chlorinated forms or more hydrophobic congers to less hydrophobic forms. The lower-chlorinated congers are, in turn, poor substances for anaerobic dechlorination, but are good substrates for aerobic degradation, in which they act primarily as electron donors (3). From the abovementioned, it is evident that microorganisms that are useful for bioremediation of sites polluted by chlorinated compounds are those that can couple reductive dehalogenation of chlorinated solvents and PCBs with energy conservation by electron-couple phosphorylation. In essence, these bacteria should be able to carry out what is known as halorespiration (15).

Bacterial species such as *Dehalococcoides ethenogenes*, strain 195, *D. ethenogenes* strain TCA1, *Dehalobacter restrictus* strain TEA and *Dehalococcoides* sp. strain CBDB degrade chlorinated solvents through halorespiration or reductive dechlorination processes, with an accompanying energy conservation. To date, *Dehalococcoides ethenogenes* strain 195 is the only strain known that is able to completely dechlorinate tetrachloroethene (PCE) to ethane; while strain TCA1 is capable of conserving energy for growth through the reductive dechlorination of 1,1,1-trichloroethane (TCA), converting it sequentially to 1,1-dichloroethane and chloroethane. *Dehalobacter restrictus* strain TEA, which is a strict anaerobe, couples PCE and trichloroethene (TCE) dechlorination to hydrogen oxidation for growth in a respiratory process. Such metabolic capabilities of these strains have found application in bioremediation of TCA contaminated aquifer sediment (15). Other bacterial species, especially the methanotrophs, can co-metabolize pollutants such as trichloroethylene (TCE) and aromatics using

their methane monoxygenase enzyme systems. The oxygenases have broad substrate specificity and have been shown to co-oxidize pollutants such as aromatics and trichloroethylene (TCE) (15, 16).

On the other hand, PCBs can be degraded either by microorganisms via a meta-cleavage pathway to yield tricarboxylic acid cycle intermediate and (chloro) benzoate (CBA) or are transformed by a co-metabolic process using biphenyl dioxygenase enzymes, and fungal ligninolytic enzymes (3, 7, 15). The degradation or transformation of PCBs to form chlorobenzoates involves four enzymes. They include biphenyl dioxygenase (Bph Dox), which introduces molecular oxygen to one of the biphenyl rings, usually at the 2 and 3 positions, a dehydrogenase, a dihydroxy biphenyl dioxygenase (DHBD), which cleaves the biphenyl ring, and a hydrolase (7, 15). White-rot fungi such as *Phanerochaete chrysosporium* and *Trametes versicolor* utilize three principle ligninolytic enzymes: Lignin peroxidase (Lip, E.C.1.11.1.14), Mn-dependent peroxidase (MnP, 1.11.1.13) and phenol oxidase or Laccase (LAC, E.C.1.10.3.2). Besides, Cytochrome P450 monooxygenase system may enhance the rate of biodegradation of PCBs (5).

Biphenyl dioxygenases are distributed in a number of bacteria genera and several genes have been studied. The notable species include *Pseudomonas pseudoalcaligenes* strain KF707, *Burkholderia cepacia* strain LB400, *Rhodococcus globerulus* P6, *Pseudomonas aeruginosa*, *Arthrobacter globiformis* and *Sphingomonas* sp. (3, 7). Bacterial degradation of PCBs requires the participation of a consortium of different species. This is due to the fact that each bacteria species exhibits a particular activity spectrum with regard to the type and extent of PCB congeners metabolized, with some strains having a narrow spectrum and others, notably *Burkholderia cepacia* strain LB400 and *Rhodococcus globerulus* P6, being able to transform a broad range of congeners. These differences reflect parallel differences among the respective biphenyl dioxygenases from these bacterial species. As a matter of fact, knowledge gained from comparative studies of genes encoding substrates recognition subunit of multi-component biphenyl dioxygenase enzymes, indicate that they differ greatly in substrate specificity (3, 7). It is probable that these differences in substrate specificity of biphenyl dioxygenases may explain the different capabilities these enzymes have to catabolize PCB congeners.

In order to understand the degradation pathways of PCBs, a large number of bacteria have been isolated and their capabilities to mineralize the substrate (degrade both the biphenyl rings) evaluated. From these studies, it has been established that a great majority of culturable bacterial species degrade only the least chlorinated rings, and release the second ring as chlorobenzoate. If this also applies to other unculturable microorganisms, it could then be inferred that bacteria capable of mineralizing both of the aromatic rings of chlorobiphenyls are, for some unknown reasons, rare in nature. For this reason, mineralization of chlorobiphenyls appears to require the presence of communities of chlorobiphenyl transforming and chlorobenzoate-degrading organisms at the contaminated site(s) (3, 17). For bioremediation application, an elegant system involving complementary interaction of a consortium comprising microorganisms capable of metabolizing chlorobiphenyls (such as *Burkholderia* sp. LB400 and some fungal species) to release chlorobenzoate, and a consortium comprising chlorobenzoate-mineralizing microorganisms (such as *Pseudomonas* sp. B13FR1 and some

fungal species), may be assembled. Furthermore, chlorinated pollutants are normally intermixed with other organic pollutants such as monocyclic aromatics, polycyclic aromatics, among others. This substrate overlap means that other pollutants on a site may act as co-substrates that can influence the composition and activity of biphenyl-metabolizing communities. At times, PCBs may be co-metabolized by pathways not dedicated to biphenyls. For example, it has been shown that biphenyls can be metabolized by *Pseudomonas putida* CE2010, tod (toluene) and cmt (cumate) pathways, which complement one another, thereby providing the ability to mineralize PCBs (3). Therefore, this co-dependence of different microbial communities constituting a global microbial biota could be used in remediation of heavily contaminated sites, thus reclaiming them for beneficial human activity.

2.3. Polycyclic Hydrocarbons and Petroleum Contaminants

Polycyclic aromatic hydrocarbons (PAHs) are aromatic compounds made up of two or more fused benzene rings. Polycyclic aromatic hydrocarbons found in the environment originate from a number of activities comprising, among others, (a) incomplete combustions of organic fuels e.g. emission sources such as automobiles exhausts, (b) stationary matter e.g., coal-field, electricity-generating power plants, (c) domestic matter e.g., tobacco smoke and residential wood or coal combustion, and (d) area source matter e.g., forest fires and agricultural burning (4, 7). Like PCBs, polycyclic aromatic hydrocarbons (PAHs) are recalcitrant and can persist in the environment for long periods. Likewise, PAHs are also grouped among pollutants generally referred to as persistent organic pollutants (POPs). Their wide distribution in the environment is directly linked to their utilization in a number of industrial and domestic products whereby they also form major waste products. Some products in which PAHs like naphthalene and phenanthrene are constituents include pesticides, fungicides, detergents, dyes and mothballs (4, 7). Major groups of PAHs are summarized in Table 9.1. Examples include naphthalene, phenanthrene, acenaphthene, fluranthen, benzo(a)pyrene, benzo(a)anthracene, benzo(b) flouranthene, benzo (k) flurantheru dibenz (a, h) anthacene, 1-nitropyrene, and indeno (1,2,3-c,d pyrene) (4, 9).

Petroleum contaminants, on the other hand, are categorized into four divisions: saturates, which are hydrocarbons containing no double bonds, aromatics, which are hydrocarbons having one or more aromatic rings with or without alkyl substitution(s), and the resins as well as the asphaltenes. In contrast to the saturate and aromatic divisions, both resins and asphaltenes contain non-hydrocarbon polar compounds. The elements present in resins and asphaltenes, in addition to carbon and hydrogen, are trace amounts of nitrogen, sulphur and/or oxygen. Resins and asphaltenes are largely solids, and not only are their chemical structures complex but they also have remained, to a greater extent, unknown. Furthermore, according to chemical structures, saturates are classified into alkanes (paraffin) and cycloalkanes (naphthalenes) (18).

Environmental contamination by petroleum hydrocarbons can be attributed to oil-tanker accidents, rupture of storage tanks, pipeline leakages and transport accidents. Oil contaminants, which are a complex mixture of hydrocarbons, often enter into the ecosystem where they are exposed to a number of abiotic and biotic factors. These factors may either alter or lead to loss of some components. For example, abiotic factors such as evaporation, dissolution, and photochemical oxidation significantly alter the composition of petroleum hydrocarbons

whereby low molecular weight volatile fractions and water-soluble components are removed. Such volatile petroleum components as n-alkanes with chain lengths shorter than C14 and monocyclic aromatic hydrocarbons (e.g., benzene and xylene) are subjected to both evaporation and dissolution. Under sunlight, petroleum undergoes photochemical modification resulting in an increase in the polar fraction and decrease in aromatic fraction (13, 18). After these physical processes, long chain and complex hydrocarbons are left in the environment. These are recalcitrant and are slowly degraded by microorganisms: bacteria and fungi. In the process, microorganisms remediate the environment of these pollutants.

Polycyclic aromatic hydrocarbons as well as petroleum hydrocarbon contaminants pose pubic health concern owing to their persistence in the environment and they have potentially deleterious effects on both wildlife and humans. Many PAHs, for example, have toxic, mutagenic and/or carcinogenic properties. Naphthalene, a common micro pollutant in potable water, exhibits cataractogenic activity. Studies conducted on laboratory animals have revealed that naphthalene binds covalently to molecules in the liver, kidney and lung tissues, thereby enhancing its toxicity through its inhibitory effects on mitochondrial respiration. In humans, acute naphthalene poisoning can lead to haemolytic anaemia, nephrotoxicity as well as dermal and ophthalmologic changes among occupationally exposed workers. Besides naphthalene, phenanthrene is known to be a photo sensitizer of human skin, a mild allergen, a potent inhibitor of gap-junction intracellular communication, and mutagenic to bacterial systems under specific conditions. Little information is available on PAHs such as acenaphthene, fluranthene and flourene with respect to their toxicity in animals. However, the toxicity of benzo (a) anthracene, benzo (b) flouranthene, benzo (k) fluranthene, dibenz (a, h) anthracene and indenol (1,2,3-d,c) pyrene has been studied and there is sufficient experimental evidence to show that they are carcinogenic (4).

One important property of PAHs is their high solubility in lipids. This makes them readily absorbed from the gastro intestinal tract of mammals. As a result they are distributed in a wide variety of tissues with marked tendency for localization in body fat (4). Owing to their toxicity, PAHs and petroleum-based hydrocarbon have been listed by the US Environmental Protection Agency as priority pollutants for bioremediation.

2.3.1. Microbial Degradation of Polycyclic Aromatic and Petroleum Hydrocarbons

In order to enhance the bioremediation processes, a number of microorganisms capable of growth on various PAHs and petroleum hydrocarbons from contaminated sites have been studied for their suitability for application in bioremediation of contaminated environments. For example, a large number of naphthalene-degrading microorganisms including *Alcaligenes denitrificans*, *Mycobacterium* sp., *Pseudomonas putida*, *P. flourescens*, *P. paucimobilis*, *P. vesicularis*, *P. cepacia*, *P. testosteroni*, *Rhodococcus* sp., *Corynebacterium venale*, *Bacillus cereus*, *Moraxella*, sp., *Streptomyces* sp., *Vibrio* sp., *Sphingomonas*, *Burkhodelria*, *Methanosaeta* sp., *Methanospirillum*, *Desulfotomaculum*, *Geobacter* sp., and *Cyclotrophicus* sp. have been isolated and examined for mineralization of PAHs and petroleum hydrocarbons (4, 14). Among fungi, a few genera have been isolated and studied. They comprise species such as *Phanerochaete chrysosporium*, *Tremetes versicolor*, *Pleurotus ostreatus* and *Myceliophthora thermophia* (5, 7).

Bacterial and fungal degradation of PAHs and petroleum hydrocarbons is dependent on their ability to grow on such compounds as carbon and energy sources. Alternatively, these pollutants may be co-metabolized in the presence of other substrates or transformed into less toxic degradation products. Therefore, several enzyme systems in the past several years have been identified, and their genes are characterized. Enzymes such as oxidoreductase (laccases and cytochrome P450 mono-oxygenase (CYPs)) are being exploited for the enzymatic degradation of PAHs and have been isolated in a diverse species of bacteria and fungi (7).

The first step in the microbial degradation of PAHs involves the incorporation of oxygen atoms on two carbon atoms of the benzene ring of a PAH by dioxygenase enzymes. The *cis*-dihydrodiol formed, undergoes re-aromatization by a dehydrogenase to form dihydroxylated intermediates, which subsequently undergo ring cleavage to form tricarboxylic acid (TCA) cycle intermediates. Specifically, PAHs can be oxidized by CYP enzymes to form catechols, which are then oxidized by dioxygenases (catechol dioxygenase) to harmless products and incorporated into the TCA cycle of microorganisms. Besides the CYP enzymes, PAHs are also oxidized by ligninolytic enzymes and particularly the Laccases. These enzymes, belonging to a group of multicopper enzymes, also catalyze the oxidation of a variety of phenolic compounds. A laccase from a thermophilic fungus, *Myceliophthora thermophia* (MtL) for example, has been extensively studied. The gene for laccase was subjected to several rounds of gene shuffling in order to improve its catalytic activity and stability. The improved enzyme exhibited a 22-fold increase in the K_{cat} for 2,2-azinobis (3-ethylbenzthiazoline-6 sulphonic acid) (ABTS) and a 170-fold higher total activity than the wild type. These findings indicate that the MtL enzyme holds a great potential for bioremediation of PAHs. This is due to its high thermal stability that enables it to work at elevated temperatures needed to increase the solubility of highly recalcitrant PAHs as well as the highly improved catalytic activity (4, 7).

The effectiveness of these enzyme systems in degrading PAHs pollutants is limited to PAHs with at most five rings. For example, although benzo (a) pyrene (BaP), a five-ring molecule abundantly present as an active component of coal tar has been detected in a variety of environmental samples, so far, no microorganisms has been reported that can use BaP as a sole source of carbon and energy. However, a partial degradation of BaP in a six component PAHs mixture by *Mycobacterium* sp. may allude to the possibility that complex PAHs are degraded via co-metabolism strategy with other substances. This strategy is also employed by several microorganisms to metabolize recalcitrant and less bio-available environmental pollutants (4).

2.4. BTEX and Pesticides Contaminants

BTEX contaminants comprise benzene, toluene, ethyl benzene and xylene, while pesticides contaminants comprise atrazine, carbaryl, carbofuran, coumphos, diazinon, glycophosphate, parathion, propham and organophosphate (Table 9.1) (9, 19). BTEX and pesticide pollutants mostly originate from anthropogenic sources, which include, among others, oil production and storage facilities, gas work sites, paint manufacturing plants, chemical manufacturing industries, timber treatment plants and pesticide manufacturing industries. BTEX and pesticide pollutants from these sources are released into the environment as waste from the various industries or as a result of accidents occurring at a manufacturing or storage facility.

Pesticides such as atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-5-triazine) belong to a class of s-triazine herbicides first introduced in the 1950s. It has since been widely used for weed control in agricultural production of crops such as maize, sorghum and sugarcane. Despite containing only one chlorine constituent, atrazine is recalcitrant to biodegradation, with a reported half-life of greater than 170 days in soils containing atrazine degrading microorganisms. Due to its recalcitrance, atrazine is frequently detected in surface and ground water samples, posing a direct risk to humans via potable water consumption (7). Organophosphates (OP) are highly toxic neurotoxins used in insecticides and chemical warfare agents. Included in the organophosphate group are paraxon, parathion, chloryrifos disulfoton, ruelene, carbophenothion and dimeton. The neurotoxic properties of this class of compounds are mainly due to its ability to suppress acetyl cholinesterase. As a result, the breakdown of acetylcholine at the synaptic junction by acetyl cholinesterase is inhibited. Further, these compounds have also been associated with pathology and chromosomal damage connected with bladder cancer (7).

The main problem associated with these pollutants is their long half-lives, which means that they persist for long periods in the environment. Like other pollutants already described, the danger associated with recalcitrance is the eventual accumulation of the pollutants in the food chain. This, therefore, calls for their removal or reduction to acceptable levels by remediation processes. As pointed out earlier (Sect. 1.3) bioremediation offers promise to completely detoxify the pollutants. For BTEX and pesticide pollutants, several species of bacteria have been isolated from contaminated environments and several genes of interest have been studied.

2.4.1. Microbial Degradation of BTEX and Pesticides

BTEX can be biodegraded under both aerobic and anaerobic conditions. This means aerobic and anaerobic bacteria with capabilities of degrading BTEX for carbon and energy exist. This is of great importance in the development of bioremediation strategies for soil pollutants and groundwater pollutants, which may require aerobic and anaerobic degraders, respectively (20, 21). Aerobic BTEX degraders have been isolated from surface soils at contaminated sites as well as from non-contaminated soils. Two mains groups of BTEX degraders exist. They comprise *Actinobacteria*, encompassing strains such as *Rhodococcus* sp., *Microbacterium*, *Mycobacterium* sp., *Arthrobacter* strains and *Proteobacteria*, encompassing strains such as *Pseudomonas* sp., *Azoarcus* sp. and *Bradyrhizobium*. These species constitute the culturable BTEX degrading bacteria, most of which utilize benzene as the only carbon source. However, there are strains that utilize toluene as the only carbon source, which indicate that the ability to utilize TEX compounds as carbon source is not always accompanied by the ability to utilize benzene in the bacterial community (21).

Initial degradation of BTEX requires the concerted action of monooxygenases and dioxygenases enzymes to form catechol. Catechol 2,3 dioxygenase, thereafter cleaves the aromatic ring, converting it into intermediates that are further degraded via the Krebs cycle. BTEX catabolic genes have been isolated from various bacterial strains and also from metagenomes-contaminated soil. The genes relevant to BTEX degradation have been identified and included are *xyl* (*xylA*, $xylE_1$ and $xylE_2$), *tbu* (*tbuA*, *tbuE*), *tmo* (*tmoA*), *tmb* (*tmbD*), and *tod* (*todC*1, *todE*). These genes encode for either BTEX monooxygenases or dioxygenases. Proteins

involved in BTEX degradation can be found in subfamilies 1.2.A, 1.2.B and 1.2.C within family 1.2 and in subfamily 1.3.B within family 1.3 of the catechol 2,3 dioxygenase (C23O) amino acid sequences. Subfamily 1.2.A contains the C23O sequences of mainly fluorescent Pseudomonas bacteria, whereas subfamily 1.2.B contains C23O sequences of mainly Sphingomonas bacteria (21). Subfamily 1.2.C comprises two C23O sequences involved in the BTEX degradation i.e., the *cdo* gene encoding for the C23O II Cdo in *Pseudomonas putida* MT15 and the *tbuE* gene encoding for the C23O TbuE in *Rastonia pickettii* PKO1. The subfamily 1.3.B contains the 3-methylcatechol 2,3 dioxygenase TodE similar to those found in *Pseudomonas putida* F1 and *Pseudomonas putida* DOT-T1, as well as TodE of *Pseudomonas putida* PB4071, which are involved in toluene degradation (21). Complete remediation of BTEX contaminated environments would therefore require the interplay of metabolic activities of different bacterial genera, whereby the different metabolic pathways operate synergistically to completely mineralize these pollutants from contaminated environments.

As with BTEX pollutants, pesticides degradation by microbes has attracted attention, and several microorganisms have been recommended and their metabolic capacity to mineralize pesticides evaluated. Several enzyme systems have been studied for their suitability in bioremediation application. A typical example is a study conducted using *Pseudomonas* sp. ADP. In this study, the genes and encoded enzymes responsible for atrazine metabolism were isolated and characterized. From these studies, it is now known that degradation of atrazine to cyanuric acid requires the action of three different enzymes; AtzA, B and C enzymes. In the biodegradation of atrazine, *Pseudomonas* sp. ADP enzyme (AtzA) transforms atrazine to hydroxyatrazine while AtzB catalyses the hydrolytic deamination of hydroxyatrazine to yield *N*-isopropylammelide. Finally, the enzyme AtzC converts *N*-isopropylammelide to cyanuric acid, which is subsequently mineralized to carbon dioxide and ammonia by other soil microorganisms (7). For organophosphates, bacterial phosphotransferases (PTE), also known as organophosphorus hydrolase (OPH), are highly efficient enzymes that hydrolyze the cleavage of P–O, P–F or P–S bonds in a number of organophosphates (7).

2.5. Heavy Metal Contaminants

Pollution of the environment with heavy metal is a global environmental problem. Heavy metal contaminants result from human activities such as mining and smelting, agricultural activities such as agrochemical usage and long term application of urban sewage sludge in agricultural soils, industrial activities such as sewage disposal, waste incineration, as well as from anthropogenic sources (2, 6, 22). Heavy metals ions of health concern include lead, arsenic, cadmium, copper, zinc, nickel, selenium, cobalt and mercury (Table 9.1). Heavy metal speciation in the environment is determined by their mobilities and solubilities, which in turn, determine their relative effects on soil ecosystems, and the associated ill-health effects. Once in the environment, metals and metalloids often accumulate in the agricultural soils and water, ending up in food due to transfer from soil to plant. The co-existence and persistence of heavy metals in soils as multiple contaminants and human exposure to them through ingestion of heavy metal contaminated food or drinking water, can lead to their accumulation in humans, plants and animals (6).

Heavy metals such as cadmium (Cd), mercury (Hg), and lead (Pb) induce deregulation of a number of physiological activities resulting in ill-health. Lead intoxification, for example, interferes with the synthesis of haem in humans. This is through its inhibitory effects on enzymes of haem synthesis pathway. Apart from interference with haem synthesis, lead toxicity is also associated with renal function impairment including interstitial fibrosis, tubular atrophy and decreased glomerular filtration at concentrations $\geq .40\ \mu g/dL$. In addition, exposure to high doses of lead during foetal development is currently associated with adverse effect among children. Elevated blood Pb levels in children ($\geq 70\ \mu g/dL$) can lead to mental retardation due to brain injury (23, 24). Cadmium toxicity, on the other hand, is also associated with renal tubular dysfunction, cardiovascular disease and malignant neoplasm, such as prostate cancer and lung cancer (25).

Besides the effect of individual metal intoxication, mixed metal contaminations seem to exert a synergistic effect on the overall toxic effects. For example, exposure to multimetals such as Lead and Arsenic may cause inhibition of myeloperoxidase release, thus further decreasing the immune competence of the splenic macrophages. Further, high degree of DNA fragmentations of splenic macrophages on exposure to multimetals indicates that a greater number of cells undergo apoptosis on heavy metal exposure and thus disturb their functional integrity (26).

2.5.1. Remediation of Metal Contaminants

Owing to their inertness to both chemical and biological degradation, heavy metals are extremely persistent in the environment, where they readily accumulate to toxic levels. The accumulation of metal ions, therefore, becomes not only an environmental hazard, but also a public health concern. It is because of these concerns that it is necessary to devise strategies of removing metal contaminants from the environment to acceptable levels.

Various physico-chemical and biological remedial technologies have been developed over the last three decades in order to address metal contamination problems. The selection of each technology is dependent on the specific site of contaminants and the type of metal contaminant(s). Physico-chemical technologies involve chemically enhanced soil flushing using extraction solutions such as organic and inorganic acids, and use of complexation agent. However, these technologies are associated with many problems. Typically, they are expensive, labour intensive, and result in extensive changes to the physical, chemical and biological characteristics of treated soil (6). Further, the health hazards associated with soil contamination with heavy metals, together with the high cost of removal and replacement of polluted soil require that alternative and cheaper technologies be developed to reclaim or recover the degraded land. Current research has been focused on the use of both microorganisms (bacteria and fungi) and plants to remediate metal ion-polluted soils. This would later on facilitate improvement of soil structure, and hence its usability for productive human activities (2, 6, 27).

2.5.2. Microbial Removal of Heavy Metal Contaminants

Microbial removal of heavy metal contaminants from contaminated water, wastewater streams and soil involves sequestering of metals from soils and sediments, and/or solubilizing

metals to facilitate their extractions (2). Bacteria and other organisms inhabiting metal contaminated niches possess resistance mechanisms to toxic metals, which make metal toxicity harmless to them, and in other instances, microorganisms may use various defence systems. Resistance mechanisms, such as the active efflux pumping of the toxic metal out of the cell as well as the enzymatic detoxification (generally redox chemistry), convert toxic ions into less toxic or less bioavailable metal ions. Defence systems, on the other hand, involve exclusion, compartmentalization, metal complexion using metallothioneins (MTs), and enzymatic transformation of metals. Therefore, it is possible to find naturally occurring organisms with unique abilities of metal absorption, accumulation and precipitation. Further, these systems can be utilized in engineering microorganisms for bioremediation of polluted water and soil (2, 27).

2.5.2.1. Microbial Defence Systems

Many microorganisms inhabiting heavy metal contaminated environments have developed a number of defence systems, which they use to detoxify or remove the toxic metal ion(s) from the environments. Detoxification of toxic metals is achieved either enzymatically through transformation of metals to metalloids or through synthesis and production of metal binding proteins such as metallothioneins (MTs).

Enzymatic activities of various microorganisms transform certain metal species through oxidation, reduction, methylation and alkylation reactions. These biological processes have important implication for bioremediation applications because they generate less poisonous metal species. Valls and Lorenzo (27) described the enzymatic process of detoxification of mercury and arsenic. The mechanisms for bacterial resistance to mercury (Hg^{+}) depend on the reduction of mercury by the enzyme mercury reductase to a less toxic and volatile mercury (Hg^{o}) species which is released into the atmosphere. Sometimes mercury derivatives or compounds such as organo-mercurials (methyl mercury for example), which are highly poisonous, exist among contaminants. These organo-mercurials are transformed to mercury (Hg^{+}), which is subsequently transformed to volatile Hg^{o}. The reductase activity thus provides a means of mercury removal by mobilization of the metal to the atmosphere (27).

The proficiency of natural mercury tolerant bacterial isolates in mercury volatilization is being investigated under different conditions and experimental systems. For instance, a *Pseudomonas putida* strain was shown to remove over 90% of the metal from a 40 mg/L solution in 24 h. The gene encoding for mercury reductase (*merA*) activity has been cloned and introduced into *E. coli* as well as *Deinococcus radiodurans* strains. The later strain, being resistant to radiation, is instrumental in the decontaminations of a mixture of mercury and radioactive waste, since it can grow in the presence of both radiation and ionic mercury (27), effectively volatilizing the metal.

Anaerobic microbial transformation of metalloids through reactions such as the methylation has also been reported for arsenic, selenium and tellurium. The process of methylation may be coupled to methane biosynthesis among arsenic-transforming methanogenic bacteria, which converts arsenic to volatile compounds, dimethyl-or trimethyl arsine. Arsenic volatilization may thus be used as a mechanism for its detoxification. Alternatively, arsenic (As (III)) may be oxidized to the more readily absorbed species As (V), which subsequently forms insoluble sulphides upon exposure to hydrogen sulphide (H_2S). For bioremediation

purposes, microbial oxidation would be useful in precipitating As from solution if combined with a separate step of exposure to hydrogen sulphide produced by sulphate-reducing bacteria (SRB). As for arsenite, its oxidation to arsenate in nature is predominantly a microbially driven process. This is due to the fact that chemical oxidation is slow under most environmental conditions. For instance, As (III) was oxidized by *Thermus* sp. at a rate approximately 100-fold greater than abiotic rate (27). However, making these mechanisms operational to bioremediation applications awaits further knowledge of their molecular basis (27).

Heavy metals toxicity may also be removed by use of metal chelating proteins such as metallothioneins (MTs) and phytochelatins (PCs). MTs are low molecular weight (6–7 kDa), cystein (Cys) rich proteins found in animals, higher plants, eukaryotic microorganisms and some prokaryotes. Further, MTs are divided into three different classes on the basis of their cysteine content and structure. The Cys-Cys, Cys-X-Cys and Cys-X-X-Cys motifs (in which X denotes any amino acid) are characteristic and invariant features of MTs. Like MTs, phytochelatins are also cystein (Cys) rich peptides that are enzymatically synthesized from glutathione (GSH) by phytochelatin synthase (PC synthase). They also chelate heavy metals and have a general structure (γ-Glu-Cys) n-Gly where $n = 2$–11 (28). PCs, however, are so far found in some plant species and none have been identified in animals and prokaryotic microorganisms (2, 27).

The biosynthesis of MTs is regulated at transcription level and is induced by several factors which comprise, among others, hormones, cytotoxic agents, and metals such as cadmium (Cd), zinc (Zn), mercury (Hg), Copper (Cu), gold (An), silver (Ag), Cobalt (Co), Nickel (N) and Bismuth (Bi). Like MTs, biosynthesis of PCS is also induced by metals including Cd, Hg, Ag, Cu, Ni, Pb, and Zn. Once synthesized, MTs and PCs sequester heavy metals by forming complexes with them. Consequently, the environment is mitigated of the heavy metal toxicity (2, 27). While MTs are essentially metal-chelating protein from higher animals and eukaryotic microorganisms, they have been only found in a few cyanobacterial strains of the genus *Synechococcus*. The MT from this strain is encoded by *smtA* gene and contains fever cysteine residues than mammalian MTs.

In view of the fact that other bacterial metal resistance mechanisms such as active metal efflux mechanisms protect only the bacteria without necessarily remediating the contaminated environment, it is desirable for bioremediation purposes, to enhance the defence mechanisms that may accompany active removal of metal contaminants from the environment and thus its mitigation. Enhancement of such capabilities may be achieved by genetic engineering of bacteria to produce MTs or enhancement of their capacity to transform toxic heavy metals or metalloids into less toxic or completely harmless products. Toward this goal, several bacterial genes responsible for conferring a metal resistance phenotype have been cloned and expressed in *E. coli* as fusion protein to other proteins (Table 9.2). This is advantageous because it makes it possible to target MTs to cell surface of the bacteria, thus greatly enhancing their capabilities to complex metal contaminants from the environments (2, 27).

The first studies in genetic engineering of metal chelating proteins involved the cloning of human MTs and their intracellular expression in bacteria. This involved fusing the human MT to an arabinose (*araB*) gene of *E. coli*. The resultant cytoplasmic production human MTs fused to *araB* in *E. coli* brought about a three- to fivefold increase in Cd and Cu

Table 9.2
Recombinant metal binding proteins and their effect on the cell upon exposure to metal contaminants

Protein	Expressed site	Host	Effect	References
Monkey MT	Intracellular	*Escherichia coli*	Though metal accumulation was effected expressed protein has short half-lines and were less stable	(2)
Yeast MT	Intracellular	*Escherichia coli*	Though metal accumulation was effected expressed protein has short half-lines and were less stable	(2)
Human MT-11	Intracellular	*Escherichia coli*	Increase in metal resistance reported	(2)
Mouse MT-I	Intracellular	*Escherichia coli*	Increase in metal resistance reported	(2)
Rainbow Trout MT	Intracellular	*Escherichia coli*	Increase in metal resistance reported	(2)
Plant MT	Intracellular	*Escherichia coli*	Increase in metal resistance reported	(2)
Yeast (CUP1) MT	Fusion to LamB	*Escherichia coli*	Increased metals binding capacity	(2)
Mammalian (HMTIA) MT	Fusion to LamB	*Escherichia coli*	Increase metal binding capacity 15–20-fold	(2)
Neurospora crassa MT	Maltose-binding protein in the periplasm	*Escherichia coli*	Cd-binding capacity increased 65-fold	(2)
Recombinant MT	LamB	*Escherichia coli*	Increase 15–20-fold in $Cd2^{+}$ binding	(27)
Recombinant MT	β-domain of IgA protease auto transporter	*Escherichia coli*	Increase in $Cd2^{+}$ binding	(27)
Recombinant MT	β-domain of IgA protease auto transporter	*Escherichia coli*	Increase in $Cd2^{+}$ binding	(27)
Recombinant MT	β-domain of IgA protease auto transporter	*Pseudomonas putida*	Increase in $Cd2^{+}$ binding	(27)
N. Nacrassa MT	Maltose-binding protein	*Escherichia coli*	6.5-fold enhancement in metal uptake	(27)

bioaccumulation. In addition, the chelating efficiency of MT was proven to be higher when targeted to the periplasmic space. However, targeting to the cell membranes or periplasmic space was shown to circumvent the problems associated with cytoplasmic expression such as: metal uptake limitation, toxicity associated with intracellular metal accumulation, and interference with redox state of the cytosol. For that matter, systems that target MTs to either the periplasmic space or to the other membrane compartments have been developed in *E. coli*, *R. metallidurans*, and *Pseudomonas putida* (2, 27) (Table 9.2).

An alternative to the surface display coordinating moieties is cytoplasmic expression combined with the introduction of specific heavy metal transporter. This approach further overcomes metal uptake limitations across the cell membrane. Unfortunately, it too, is restricted to those metals for which there are active transport systems such as mercury, copper, lead, and nickel. This approach has been used with reasonable success when yeast and pea MTs fused to glutathione S-transferase gene, were cloned into *E. coli* together with a nickel transporter from *Helicobacter pylori*. A threefold increase in Ni accumulation was produced in cell expressing MTs. Similarly, genetically engineered bacteria co-expressing the merT–merP mercury transporter with MTs or metal-binding peptides in the cytoplasm showed an Hg bioaccumulation comparable to that of cells directly expressing the binding peptides on the cell surface (27).

2.5.2.2. Plant Removal of Heavy Metals

Apart from microorganisms, plants too, are endowed with the ability to accumulate metal ions and concentrate them into harvestable parts (phyto-extraction), absorb metals from contaminated water (Rhizo-filtration), immobilize and reduce the mobility and bioavailability of contaminants (phyto-stabilization), and volatilize the contaminants from soil to the atmosphere (phyto-volatilization). These bioremediation strategies are chiefly achieved by plants and may further be enhanced by plants-associated microbes (rhizo-microorganisms). Besides, plant-microbe associations have also been used to degrade chloronito aromatic pollutant such as 4-chloronitrobenzene (4CNB): thus the application of plants in bioremediation is not limited to heavy metals (104). Collectively, plant based remediation process are referred to as phytoremediation (2, 6).

For a plant to be useful for phytoremediation purpose, it should possess the following attributes: (a) the plant should be able to accumulate high levels of metal and translocate it to the harvestable segments of the plant; (b) it should grow rapidly and reach a high biomass; (c) the plant should be metal tolerant, thus allowing it to grow in high metal concentrations. Another category includes metal-tolerating plants which may not be metal accumulators. Such plants also offer possibilities for bioengineering by introduction of metal-binding protein/peptides genes (2, 6). In nature, it is not common to find plants that combine all these attributes. It is, therefore, not surprising that many metal hyper accumulating plants not only grow very slowly, but also have a low biomass owing to their small sizes. Moreover, many fast growing and high biomass producing plants such as Vetiver grass and hemp, though metal tolerant, they are not metal accumulators. Besides these factors that are intrinsic to plants, phytoremediation may be restricted by limitation of Contaminants bioavailability. In order to enhance metal uptake, soil amendments with metal-chelator such as EDTA, citrate, and

hydroxylamine may be applied to make metals bioavailable and thus absorbed by plant roots. Even then, the type of chelator and its time of application are important considerations (6).

To make phytoremediation practicable, both plant biomass and metal accumulation capabilities should be enhanced. Efforts to have plant biomass increased have centred on the use of plant growth regulators (PGR) such as auxins, cytokinins and plant hormone indo-acetic acid (IAA). Auxins and cytokinins enhance phytoremediation abilities of non-hyper-accumulating plants by increasing their growth and biomass. Indo-acetic acid, on the other hand, encourages hyper-accumulation of metals through enhancement of the bioavailability of metal contaminants to plants. Typically, IAA enhances bioavailability of iron (6). While some plant growth regulators and hormones are produced by some plants, some PGRs are produced by rhizobacterial (PGPR) strains and mycorrhizal fungi that live symbiotically with plant-root system. Plant growth promoting rhizobacterial strains such as Pseudomonands and Acinetobacter produce IAA, which results in enhanced uptake of iron, zinc, magnesium, calcium, potassium, and phosphorus by crop plants. Furthermore, PGPR fix nitrogen, produce phytohormones and specific enzyme activities, lower ethylene levels, protect plants from diseases by producing antibiotics as well as other pathogen-depressing substances such as siderophores (6).

Like in bacteria, metal accumulation may also be enhanced by genetically modifying plants capable of growing in metal contaminated environments to express MTs and PCs. Transgenic plants that express MTs have been scored to enhance Cd tolerance, Cd accumulation, or modified Cd distribution. For example, a human MT-11 gene introduced into tobacco and oilseed rape, enabled growth of these transgenic seedlings in Cd contaminated environments at concentrations of 100 μM. In some instances, an increased Cd tolerance of up to 200 μM Cd^{2+} or an altered distribution of Cd have been observed in transgenic plants expressing MTs, while in other instances, expression of MTs achieved a modified distribution of the accumulated metal (2). For instance, the human MT-11 gene fused to the β-glucuronidase gene was expressed in tobacco. In vitro grown seedlings expressing the fusion protein accumulated 60–70% less Cd in their shoots than the control plants. In the control plants, 70–80% of the Cd was translocated to the leaves whereas in the MT-expressing plants only 40–50% was translocated (2). Reduced translocation to leaves was accompanied with increased Cd levels in both roots and stem. A modified distribution is of a particular interest for crops in the objective of translocating of metal contaminants to non-consumed segments of the plant or to harvestable parts for phytoremediation. Apart from introducing mammalian MTs into plants, modifications on plant detoxifying proteins, the phytochelatins (PCs), or over-expression of enzymes that are involved in the synthesis of glutathione and PCs have been used to further enhance heavy metal tolerance and accumulation in plants (2).

It is comprehensible from the above discussion that the successful application of plants to reclaim environments heavily contaminated with heavy metals would require careful integration of plant-types of divergent capabilities to accumulate or tolerate metals. While it may be necessary to develop transgenic plants, it would be more beneficial to exploit natural means of enhancing growth and increasing biomass especially through the integral use of plant growth regulators and hormones, as well as free-living or symbiotic plant growth-promoting rhizobacteria and mycorrhizal fungi.

3. BIOREMEDIATION STRATEGIES

As already pointed out (Sect. 1.3), bioremediation is a natural process by which microorganisms either immobilize or transform environmental contaminants to innocuous end products. Bioremediation includes all processes and actions that take place in order to biotransform an environment, already altered by contaminants, such as pesticides, herbicides, insecticides, cleaning chemicals and chemicals used in the food chain, to its original status. There is variation in the processes employed; however, similar principles apply as in the use of microorganisms or their enzymes. The enzymes may be indigenous which may be stimulated by the addition of nutrients or optimization of conditions, or may be seeded into the soil. The objective is to transform the contaminants into substances that can be absorbed and used by the autotrophic organisms with no toxic effect on them (29, 30). Bioremediation has been used in the treatment of contaminated soil and ground water through: (a) stimulation of the activity of indigenous microorganims by the addition of nutrients, regulation of redox conditions, optimizing pH conditions, (b) inoculation of the site by microorganisms with specific biotransforming abilities, (c) application of immobilized enzymes, and (d) use of plants (phytoremediation) to remove and/or transform pollutants (31). Specific methods used for bioremediating contaminated soil and water include: landfarming, compositing, intrinsic bioremediation, and slurry bioreactor (Table 9.3).

3.1. Landfarming

Landfarming is a managed treatment and disposal process that involves the controlled application of waste to soil or soil-vegetation system (32). It relies on agricultural principles and aims to control the biocycling of natural compounds. Conditions of soil microbial populations are optimized by the dilution of contaminated soil with clean soil, tilling of the soil to reduce initial toxicity, as well as by controlling physical parameters, such as aeration, pH, soil moisture content, and temperature. Aeration is obtained by tilling the soil, or by forced aeration after covering the soil and exiting air cleaned through filters. Temperature control is achieved through the introduction of hot air, or the 'greenhouse effect' in a closed system.

3.2. Composting

Composting is a biological aerobic decomposition of organic matter under strictly controlled conditions. This helps thermophilic microorganisms transform organic materials into a stable, soil-like product (33, 34). The process is natural in soil where microorganisms decompose materials. However, the natural processes may be so slow that some materials hardly get decomposed. In order to increase the rates and use composting for industrial purposes, microbial growth may be optimized through optimizing oxygen concentration, pH, moisture content, carbon to nitrogen (C:N) ratio, and particle size (33, 34). Composting could also be enhanced through the use of bulking agents such as wood chips and vermiculite, which through increasing the void space in the compost (35), would allow for the maintenance of adequate oxygen to enable the obligatory process to proceed.

Table 9.3
Bioremediation technologies and their application

Technology	Principals	Advantages	Disadvantages	Applications	References
Land farming	Solid-phase treatment system for contaminated soils; may be done in situ or in a constructed soil treatment cell	Simple procedure. Inexpensive. Currently accepted method	Slow degradation rates. Residue contamination often removed. High exposure risks. May require long incubation periods	Surface contamination. Aerobic process. Low to medium contamination levels	(32)
Composting	An anaerobic microbial driven process that converts solid organic wastes into stable, sanitary, humus-like material	More rapid reaction rates. Inexpensive. Self-heating	Need bulking agents. Requires aeration. Nitrogen addition often necessary. High exposure risks. Residual contamination. Incubation periods are months to years	Surface contamination. Aerobic process. Agricultural and human wastes. Sewage sludges, industrial wastes, yard wastes, municipal solid wastes	(33) (34) (35) (36)
Intrinsic bioremediation	Relies on the natural assimilative capacity of the ground to provide site remediation and control contaminant migration	Relatively inexpensive. Low exposure risks. Excavation not required	Low degradation rates. Less control over environmental parameters. Needs good hydrogeological site characterization. Incubation periods are months to years	Deep contamination. Aerobic or nitrate reducing conditions. Low to medium contamination levels. Oil and gasoline. Chlorinated hydrocarbons	(39) (40) (44) (45) (46)
Slurry bioreactor	Soil and water agitated together in reactor	Good control over parameters. Good microbe/compound contact. Enhance desorption of compound from soil. Fast degradation rates. Incubation periods are days to weeks	High capital outlay. Limited by reactor size. High exposure risks	Surface contamination. Recalcitrant compounds. Soil that binds compound tightly. Aerobic and anaerobic processes	(35) (48) (49) (50)

Table 9.4
Composting methods

Method	Composting time	Cost	Usage	Disadvantages
Windrow	2–6 months for municipal solid waste	Low	Used mainly in combination with in-vessel technology for curing the compost	Difficult control of conditions, temperature, water concentration odour
Aerated piles	6–12 weeks	Medium	Used for sewage sludges, municipal solid waste, yard wastes and industrial organic wastes	Continued electrical costs
In-vessel	Less than a week to 2 weeks	High due to installation costs	All types of waste	High costs, intense and skilful management

Composts constitute a valuable soil amendment and may be used, as a fertilizer substitute to supplement plant nutrient needs because of the high organic matter content. Therefore, composting can be used as a method to stabilize and decrease sewage sludges, industrial wastes, yard wastes, and municipal wastes. It has also been used in the treatment of hazardous waste such as explosives (36). There are three types of composting including: windrow, aerated static pile, and in-vessel (37, 38) (Table 9.4). The three types of composting share similar stages, but differ in the time to complete the tasks, capital and operating costs, and the ways in which to achieve the necessary conditions for bacterial growth.

3.3. In Situ Intrinsic Bioremediation

In situ or intrinsic bioremediation is a natural process, which exploits natural ways of recycling nutrients through the cycles of nitrogen and carbon (39). The decomposition of the contaminant is carried out by indigenous microorganisms, which grow on the contaminated soil and can only survive in that environment by using the contaminants as a source of energy (40, 41). The process could be exploited to enhance the degradation and recycling of wastes and to clean contaminated soils (42). To enhance the process of decomposition, the microorganisms could be genetically modified (43) or strictly selected nutrients could be added to the soil (39). The requirement for no excavation and special equipment means low cost of operation and no disturbance of the natural environment. The method is therefore suitable for treating rocky or underground water areas (39, 44). A major disadvantage of in situ bioremediation is that it is slow and may not be suitable for use where immediate site clean up is required. The method also produces toxic by-products in some cases. Addition of nutrients may not reach the target, hence prolonging the process of remediation (39, 45). The process is also more difficult to keep under control than *ex situ*, or engineered bioremediation due to the lack of experimental conditions in the contaminated soils (46).

3.4. Ex Situ or Slurry Bioremediation

In *ex situ* or slurry bioremediation, the contaminated soils are excavated and mixed with water to form a slurry that is mechanically aerated in a reactor vessel. Agitation of the reactor ensures the breakdown of soil aggregates, desorption of contaminants from soil, increased contact between wastes and microorganims, and improved oxygenation of the slurry (35). In order to improve the treatment of the contaminated soils and to increase the biodegradation capability, use of surfactants, dispersants and materials supporting microbial growth, control of temperature, and concentration of biomass is key (35, 47). To ensure efficiency, the contaminated soils are pre-treated before introduction into the reactor, the soils are graded physically to reduce the cost of mixing and agitation, soils may be fractionated to reduce the total volume to be treated and increase the rate of biodegradation of the contaminants (48). Alternatively, sodium hydroxide and sodium chloride may also be added to neutralize soil acidity and dispersion of clay particles, and to trap the contaminants. *Ex situ*/slurry bioremediation is faster than the *in situ* method, although higher costs than for the *in situ* systems are involved because of the high degree of engineering (49).

3.5. Bioaugmentation

Bioaugmentation involves the use of specialized competent strains or consortia of microorganisms, which may be indigenous or genetically modified organisms, to improve the capacity of a contaminated environment. The process relies on the immense metabolic capacities of the microbes to transform organic man-made pollutants into harmless or, less dangerous compounds. Biodegrading microorganisms do occur in nature, however, their potential to degrade and mineralize target pollutants may be limited by low numbers, unfavourable local conditions, and the presence of complex molecules or a mixture of compounds that require specific microorganisms and/or pathways (50).

Bioaugmentation may be attained through: the addition of pre-adapted pure bacteria strains (51, 52); pre-adapted consortia, i.e. degrading enrichment cultures (53); genetically engineered bacteria, to avoid the accumulation of potentially toxic pollutants and biodegradation-relevant genes transferred by conjugation into microorganisms in the biotope under remediation (54).

Bioaugmentation has been used to (a) improve the flocculation of activated sludge, and (b) to enhance the removal efficacy of recalcitrant compounds. Bioaugmentation enhances the removal of 3-chorobenzoate, 4-methyl benzoate, toluene, phenol, and chlorinated solvents (55, 56). However, the technique has not yet received wide application due to the fact that the bioaugmentation of activated sludge is less predictable and controllable than direct physical or chemical destruction of pollutants. The removal of refractory and inhibitory compounds in coke plant wastewater, that was unachievable by conventional methods, such as solvent extraction, steam stripping, and/or biological treatment, was achieved recently using bioaugementation, with a quinoline-biodegrading aerobic bacterium, *Burkholderia pickettii*, obtained from activated sludge (57).

4. APPLICATION OF BIOREMEDIATION

4.1. Case Studies of Bioremediation

Bioremediation is key in the food industry, having been used in the treatment of wastes from processing of fruits and vegetables, olive oil, fermentation, dairy, meat, and poultry products.

4.1.1. Fruit and Vegetable Processing Industry

The fruit and vegetable processing industry includes among others: fruit and vegetable canning, frozen vegetables, vegetable dehydration, fruit and vegetable drying, fruit pulping, tomato juice and fruit concentrates, etc. Since fruit and vegetable production are seasonal, environmental pollution from waste generated from the industry is equally seasonal. A big proportion of the waste from the fruit and vegetable industry is solid suspensions and high biochemical oxygen demand (BOD). Other parameters also affected by such waste include pH, chemical oxygen demand (COD), dissolved oxygen, and total solids. The pH is mainly acidic. The chemical composition varies depending on the type of fruits and vegetables processed, and the pesticides, herbicides and cleaning chemicals used during production. Separate treatment is therefore used for the different wastes. For solid waste treatment, composting, slurry bioreactors and landfarming may be used. The waste is pretreated to remove the water and neutralize the pH to allow for efficient microbial growth and development. Bulking agents such as sawdust, paper, mature compost, straw, and coffee residuals may be added to improve the porosity of the sludge and decrease the bulk density (38). Increased porosity helps in the drainage of water. The bulking agents have the double effect of also increasing the C: N ratios of the waste due to their high carbon content and the pH (58).

4.1.2. Olive Oil Industry

The olive oil industry generates wastewater, a liquid waste that contains dark-coloured juice, organic substances such as sugars, organic acids, polyalcohols, pectins, colloids, tannins and lipids. These products have very high BOD, COD, and concentration of organic substances, such as phenols, which are difficult and expensive to degrade (59, 60). Biotreatment of the olive oil mill wastewater (OMW) may be conducted aerobically or anaerobically.

In the aerobic process, the oxygen is provided by an external source. However, the biodegradation proceeds very slowly due to operational problems and requires a high concentration of the feed to operate more efficiently (61). The aerobic process cannot efficiently remove certain persisting pollutants, such as polyphenols and colouring substances. Suggestions have been made to mix sewage wastewaters with OMW to improve biodegradation and reduce the cost as well (62). In order to improve biodegradation of OMW, the polyphenols and lipids have to be removed prior to the aerobic treatment. In addition the colouring substances could be removed using the fungus Pleurotus.

The anaerobic process has been shown to produce better results than the aerobic process on organic pollutants, sugars, polyphenols, and pectins. The growth rates of the microorganisms are lower than the corresponding rates for the aerobes. Examples of anaerobic processes include: anaerobic lagooning, anaerobic contact and the upflow anaerobic sludge blanket.

4.1.3. Fermentation Industry

Waste from the fermentation industry may be generated from brewing, distilling and wine manufacture. Fermentation waste is characterized by high BODs and CODs, although differences have been observed in the concentration of the organic compounds. The high concentrations of tannins, phenols and organic acid in fermentation wastewater enhance the anaerobic bioremediation processes (63). These processes may be enhanced further by optimizing the acidity (5–6 pH) and temperature (40°C) of highly concentrated brewery wastewater using the upflow anaerobic sludge blanket (64). Treatment of winery waste is limited by the presence of vinasse, which must be biologically treated for 4–8 days to reduce the COD by 90% (65).

4.1.4. Dairy Industry

Dairy industrial waste is one of the most important pollutants of soil and surface water. It may contain proteins, salts, fatty substances, lactose, and different cleaning chemicals, which may be alkaline or acid (66, 67). It is mainly characterized by: high organic load (e.g. fatty acids and lactose), large variations in waste supply, considerable variations in pH (4.2–9.4), and relatively large load of suspended solids (SS) (400–2,000 mg/L) (67). The cleaning chemicals comprise the biggest pollutants, since in addition to either being alkaline or acid; they also may contain phosphates, sequestering agents, surfactants, dispersing agents, anti-foaming agents, and inhibitors (68). Although the presence of detergents in dairy wastewater hardly influences the total COD in contrast to milk, cream, or whey, it presents some difficulties in their treatment. According to Wildbrertt (69), sodium carbonate passed through a treatment process almost unchanged. Both aerobic and anaerobic treatment systems have been employed in the bioremediation of dairy wastes (70–74). A new promising technology in diary wastewater treatment is thermophilic aerobic treatment, which could be used for treating high-strength organic waste streams. The technology combines the advantages of low biomass yields and rapid kinetics associated with high temperature operation and stable process control of aerobic systems. Additionally, the technology has potential for producing pathogen-free products and for the exchange of energy generated by the process (75).

4.1.5. Meat, Poultry and Fish Industries

The meat, poultry, and fish industries produce the highest loads of waste within the food industry. In the meat industry, wastes are generated in the slaughterhouses and processing units. The slaughterhouse wastes, which is separated into wastewater and solid waste, contains various quantities of blood, fats, residues from intestines, paunch grass, and manure (76). The slaughterhouse wastewater is rich in moisture (90–95%), nitrogen, BOD, and is odorous. The management of nitrogen in the meat processing industry is key in waste treatment. The waste must be pretreated to reduce the moisture to 60–75%, and bulking agents must be used to increase the porosity of the waste for efficient aeration. The pre-treatment also aids in the control of pathogens that may interfere with the process (76). According to Starkey (77), a treatment system for poultry waste should consider land availability, previous site history, publicly owned treatment work discharge, conventional waste treatment systems, and land application systems. Similarly, pre-treatment of poultry waste to reduce moisture and kill

pathogens and the use of bulking agents to increase the porosity, which also increase aeration and carbon levels in the wastewater, are considered *a sine qua non*.

4.1.6. Oil Refinery Sludge

The petrochemical industry generates a series of liquid effluents during the petroleum-refining process. These effluents are treatable through depuration processes. The oil refinery sludges that result from this depuration process have a high content of petroleum-derived hydrocarbons, which may be alkanes and paraffin of 1–40 carbon atoms, cycloalkanes and aromatic compounds (78). This makes it a potentially very dangerous waste product, which may have serious environmental consequences (79). Petroleum hydrocarbon wastes may be treated using natural biological, chemical, and physical processes (80).

4.1.7. Coke Plant Wastewater

Coke plant wastewater is generated in the coal coking, coal gas purification, and by-product recovery processes of coke plants. The wastewater contains ammonia, thiocyanate, phenolics, and other organic compounds, such as mono- and poly-cyclic nitrogen-containing aromatics, oxygen- and sulphur-containing heterocyclics, and polynuclear aromatic hydrocarbons (PAHs) (81, 82). These wastes are very harmful and carcinogenic. Conventional treatment of coke plant wastewater includes solvent extraction, steam stripping and biological treatment. However, due to the presence of refractory and inhibitory compounds, the conventional biological treatment is not efficient in removing COD. Use has been made of anoxic–oxic (A–O) and anaerobic–anoxic–oxic (A1–A2–O) processes to treat coke plant wastewater with good results (82). However, this could not reduce the effluent COD to less than 200 mg/L.

Bioaugmentation of activated sludge systems with specialized microorgamisms could be used to improve the flocculation of activated sludge and to enhance the removal efficiency of recalcitrant compounds. Bioaugmentation has been reported to enhance the removal of 3-chlorobenzoate, 4-methly benzoate, toluene, phenol, and chlorinated solvents. However, bioaugmentation of activated sludge is less predictable and controllable than direct physical or chemical destruction of pollutants.

Quinoline, a heterocyclic compound, which is poorly removed in the A1–A2–O system, was isolated from activated sludge of a coke oven wastewater treatment plant by enrichment shaking culture (57). This was achieved through bioaugmentation with a quinoline-degrading bacterium, *Burkholderia pickettii*. *B. pickettii* has a degradative role and is tolerant to refractory and inhibitory organic compounds in coke plant wastewater.

4.1.8. Marine Bioremediation

Sources of pollution in the marine environment could be due to: nutrients; sediments; pesticides; sewage outfalls; stormwater; exotic species; coastal development; hydrocarbons; heavy metals; litter and aquatic organisms (83). Three approaches to reduce marine associated environmental health risks have been suggested as: cleanup, isolation, and prevention. Marine bioremediation efforts often target hydrocarbon contaminants, but do have applications also to nutrient loading, heavy metals, haloorganic compounds and other pollutants.

Nutrient loading is a widespread phenomenon in many coastal areas. Although generally not directly toxic to indigenous organisms, it could promote excessive algal growth resulting

in hypoxia or anoxia (84). The removal of nitrate from wastewater helps prevent downstream eutrophication and can be accomplished using wastewater treatment systems, modified to remove organic compounds under anaerobic conditions. By switching to anaerobic conditions with methane as a carbon and energy source, methylotrophic bacteria convert the nitrate to nitrite and then to molecular nitrogen. Denitrifying bacteria have now been shown to also contribute significantly to biological phosphate removal through processes in which the organisms are cycled between anaerobic conditions that favour nitrate removal and the aerobic conditions that favour phosphate removal (85). This results in reduced chemical oxygen demand and expands the operational range of the biological process (86).

Metals are not degradable by microorganisms. However, microorganisms could detoxify heavy metals and radionuclides from contaminated waters by precipitating, volatilizing, solubilizing or adsorbing them (87, 88). Bacterial strains are known, which have the capacity to concentrate or remediate the metal contaminants into forms that are precipitated or volatilized from solution and hence less toxic and easily disposable. For example, sulphate-reducing bacteria were used to immobilize metals at what was once a zinc-refining site at Budelco in the Netherlands. Contaminated groundwater was pumped through a bioreactor in which ethanol, ammonia and phosphate support the growth of sulphate-reducing bacteria. The bacteria converted the sulphate in the water to hydrogen sulphide, which reacted with the heavy metal contaminants to form insoluble metal sulphides. Biosurfactants such as glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants enhance the desorption of heavy metals in two ways:

(i) They may complex free forms of the metal residing in solution, which decreases the solution phase activity of the metal and, promotes desorption according to Le Chatelier's principle.
(ii) Alternatively, through direct contact to sorbed metal at solid solution interface under conditions of reduced interfacial tension, allows biosurfactants to accumulate at solid solution interface.

The effectiveness of the use of biosurfactants for metal remediation increases in terms of cost involved at sites co-contaminated with organic compounds. However, the addition of biosurfactants may also inhibit some microorganisms. Therefore, the best strategy would be to stimulate biosurfactants produced by indigenous population present at the contaminated site. This is not only environmentally compatible but also more economical than using metal chelators such as EDTA.

Haloorganics such as polychlorinated biophenyls (PCBs), solvents and pesticides are recalcitrant to degradation. However, others may be mineralized or only partially biodegraded under anaerobic conditions. For example, consortia of indigenous microorganisms were able to degrade the PCBs in Hudson River (89), in which both anaerobic and aerobic biodegradation played roles in the otherwise slow process. Increased degradation rates were obtained on addition of inorganic nutrients, the organic co-metabolite biphenyl and oxygen. Dehalogenation is a key initial step in degradation, which may occur by oxygenolytic, hydrolytic or reductive mechanisms (90).

Crude oil or refined petroleum includes hundreds of different alkanes and aromatic hydrocarbons, among which are polycyclic aromatic hydrocarbons (PAHs), which are carcinogenic. Marine ecosystems may be affected by disastrous oil spills, spills that occur during refuelling

in ports, terrestrial spills and run-off, which are major sources of oil pollution (91). The biodegradation of petroleum compounds occurs through diverse enzymatic capabilities within bacterial populations that are ubiquitous in the marine environment and rapidly increase, in relative proportions, in the presence of petroleum contamination (92). PAHs with fused aromatic rings are refractory to biodegradation because they are hydrophobic and hence they tend to adsorb to the soil and sediment. In nature, bioemulsifiers and biosurfactants may play a role in desorption and bioavailability of the hydrophobic contaminants (93). The biodegradation of floating oil is limited by surface area (94). In order to stimulate biodegradation in such circumstances, a dispersant may be added to the oil slick. This dramatically increases the surface area available for microbial colonization at the oil–water interface. Surfactants used in some dispersants have been shown to further enhance biodegradation of dispersed floating oil by serving as a biodegradable substrate and stimulating growth of biodegradative bacteria (94).

4.2. Factors for Designing a Bioremediation Process

The design of improved biocatalysts involves different aspects of optimization, including: creating new metabolic routes; expanding the substrate ranges of existing pathways; avoiding substrate misrouting into unproductive routes or to toxic or highly reactive intermediates; improving the substrate flux through pathways to avoid the accumulation of inhibitory intermediates; increasing the genetic stability of catabolic activities; increasing the bioavailability of hydrophobic pollutants; and improving the process-relevant properties of microorganisms. A variety of strategies for designing new or improved catalysts for bioremediation are available including in vivo and in vitro strategies.

4.2.1. Biodegradative Performance

Consortia that exhibit novel catabolic activities can be obtained by sustained selective pressure in a chemostat. The consortia could be developed for the mineralization of chlorinated biphenyls, chlorinated dibenzofurans (95), and aminonaphthalenesulfonates (101). One member of the consortium transforms the substrate into the corresponding chlorinated benzoate or salicylate and grows at the expense of the initially attacked aromatic ring. Thereafter, a second member mineralizes the formed benzoate or salicylate.

4.2.2. Anaerobic–Aerobic Processes

Another approach to the mineralization of highly chlorinated congeners is the development of anaerobic–aerobic processes. Since microbial degradation of PCBs occurs in sediments, and anaerobic dehalogenation is enhanced by an increase in halogen substitution, in contrast to aerobic degradation, for which the persistence increases with increasing halogen substitution, the process could be used to transform highly chlorinated biphenyls into less-chlorinated congeners, which are more amendable to aerobic degradation. There are, however, only a few cultures that are able to dechlorinate PCBs reductively to date.

Additionally, the metabolic division of labour in co-cultures of aerobic microorganisms may not constitute the most effective situation and prolonged selection may lead to the transfer of genetic determinants of catabolic functions between members of the consortium and the

emergence of a single organism with the complete catabolic sequence. These natural gene-transfer events are the basis of numerous in vivo design experiments and are facilitated by the fact that naturally occurring pathways for the metabolism of organic compounds are often encoded by broad-host-range plasmids (98). Plasmid cloning vectors may, however, suffer from the same instability as natural plasmids and moreover, have antibiotic-resistance selection markers, which are undesirable for environmental applications. In order to circumvent these problems, mini-transposon cloning vectors have been developed to insert heterologous genes stably into the chromosomes of host bacteria without the use of antibiotic-resistance markers or, with markers that can be selectively eliminated after gene transfer.

4.2.3. Catalyst Performance

An increase in the rate of pollutant removal may be obtained through identification of enzymatic or regulatory step of the pathway that is rate limiting, followed by experimental elevation of the activity of the rate-limiting protein. The activity of the rate-limiting protein could be elevated through an increase in the transcription or translation of its genes, or in its stability or kinetic properties. This involves the use of mutants of regulatory proteins that either mediate higher levels of transcription than the wild-type regulator or respond to new effectors (96). The use of artificial regulatory systems allows the expression of catabolic genes to be uncoupled from the signals that ordinarily control their expression and offers considerable flexibility for process control (100).

Protein engineering could be exploited to improve an enzyme's stability, substrate specificity and kinetic properties. The rational design of proteins performed by site-directed mutagenesis requires an understanding of structure–function relationships in the molecule and a detailed knowledge of the three-dimensional structure of the enzyme itself. However, the number of degradative enzymes whose structure has been elucidated is still small and this constitutes a major limitation for rational protein design. Additionally, proteins with new activities could be developed through combining the best attributes of related enzymes by exchanging subunits or subunit sequences, or through shuffling their genes sequences (97).

4.2.4. In-Complete and Complete Metabolic Pathways

In bioremediation, co-metabolic processes need an input of energy, which may present a metabolic burden for the microorganism involved. Further, the end metabolites produced by incomplete pathways may be toxic or subject to further transformation by other microorganisms, forming reactive or toxic molecules. For example in PCB metabolism, microorganisms usually metabolize only one aromatic ring and accumulate the others as the corresponding chlorobenzoates, which have been shown to be inhibitory to further PCB metabolism (46, 47). The use of complete pathways could help overcome the problem associated to incomplete pathways. Although a complete pathway for a particular substrate may not exist in a single organism, partial and complementary pathway segments may exist in different organisms (Sect. 2.2.1). In order to form a complete pathway sequence for a target substrate for an organism exhibiting a desired catabolic phenotype, determinants for complementary pathway segments may be combined.

4.2.5. Pollutant Bio-availability

Bioremediation is limited not only by the recalcitrance of the target pollutants but also by the toxicity of such compounds and, in particular, the limited bio-availability of hydrophobic, poorly water soluble pollutants such as PCBs. Biological reactions occur in or at the interface of the aqueous phase and the surfactants have the ability to desorb and disperse poorly soluble compounds in small, high-surface-area micelles within the water phase. Surfactants can thus improve the accessibility of these substrates to microbial attack (102). The high surface activity, heat and pH stability, low toxicity and biodegradability of bio-surfactants constitute important advantages over synthetic surfactants, particularly for environmental applications. However, a major limitation of the application of bio-surfactants is the high cost involved. Efforts are currently geared towards the design of recombinant biocatalysts that exhibit a desired catabolic trait and that produce a suitable bio-surfactant (99).

4.2.6. Catalyst Survival in the Environment

Improving inoculant survival is an important goal in the further development of bacterial inocula for biotechnological applications in the environment, where the microorganisms are exposed to a variety of stresses such as toxic metals, solvents and extremes of temperature and pH. A combination of resistance to environmental stresses and catabolic phenotypes in appropriate bacterial strains, such as strains of *Deinococcus radiodurans*, solvent-resistant bacteria able to mineralize hydrophobic pollutants would yield microbial catalysts with significantly improved survival characteristics in hostile habitats.

4.3. Bioremediation Process Design and Implementation

Bioremediation process design depends on a clear understanding of the nature of the polluted environments. These environments, which include soil, surface and ground water, need to be assessed for constituent pollutants as well as natural flora. Pollutants may be classified as either organics or heavy metal, while the natural flora include microbial consortia, which comprises microbial flora (bacteria and fungi) and plants. Assessment of the polluted environment is essential in determining the nature of the pollutant and associated natural flora (Fig. 9.1). Other factors of importance include pH, temperature, and nutrient availability. Subsequent to careful assessment of these factors it is possible to determine the bioremediation strategy to undertake. For example, an environment polluted by organics would require the action of microbial consortia, while that polluted by heavy metals would require the action of both microbial consortia and plants for remediation. Issues concerning cost-effectiveness of any bioremediation process design should be addressed, before the implemetation of the process.

5. LIMITATION OF BIOREMEDIATION STRATEGY

1. It is often difficult to evaluate the success of an in situ bioremediation programme. This is true whether using genetically engineered or intrinsic microorganisms. For instance, it is not easy to deduce to what extent a certain microbe is actually contributed to the degradation process. Where genetically engineered microorganisms (GEMs) are used, it is difficult to distinguish between GEM-specific degradation and biodegradation due to indigenous microbial consortia.

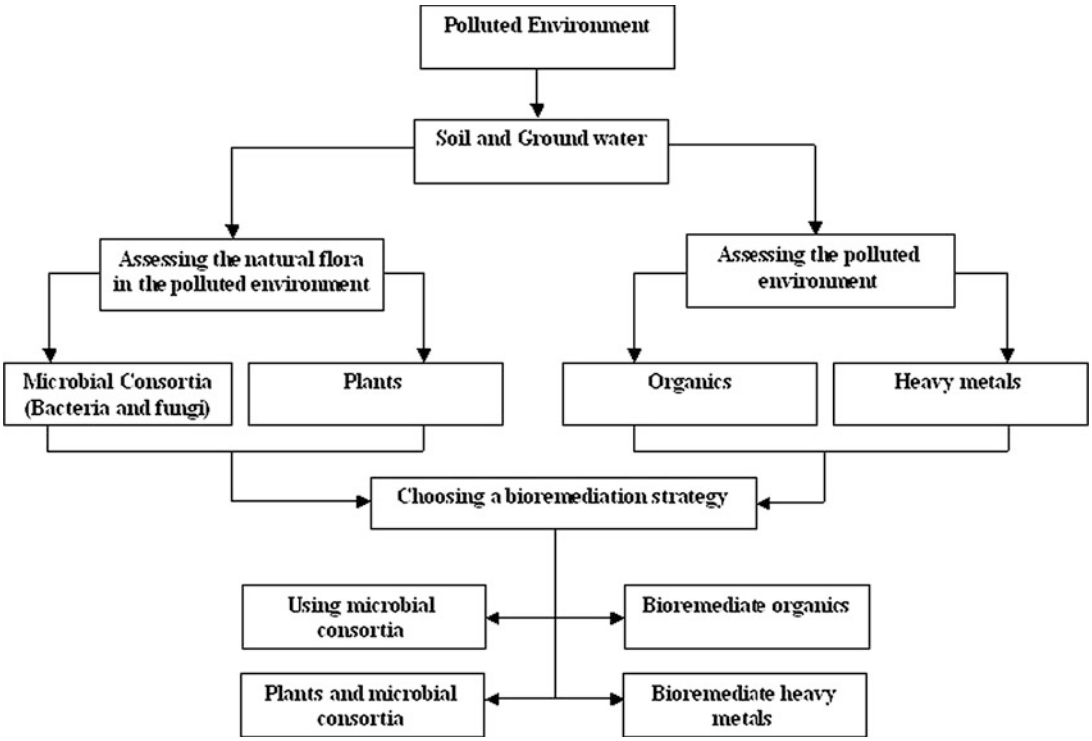

Fig. 9.1. Schematic representation of factors to consider in designing a bioremediation process.

2. Due to the highly heterogeneous distribution of the contaminants in the environment, it is difficult to statistically gauge bioremediation efficacy.
3. There are no rules to predict if a contaminant can be degraded.
4. Some contaminants such as chlorinated organic or high aromatic hydrocarbons are resistant to microbial attack. They are degraded slowly or not at all; which makes it not easy to predict the rates of clean up for a bioremediation exercise.
5. The mineralization of pollutants by cultivable bacteria has not been reported because the fraction of microbial diversity that is culturable does not contain the metabolic potential for mineralizing all the different xenobiotic pollutants present in the environment.
6. Recalcitrant and toxic xenobiotic compounds such as highly nitrated and halogenated aromatic compounds as well as some pesticides and explosives are highly stable and chemically inert under natural conditions.
7. Environmental concern on the use GEMs. It has generally not been agreed on the use of GEMs over concerns of their uncontrolled survival/dispersal into the environment.

6. FUTURE PROSPECTS

The future of bioremediation lies in the use of genetically engineered microorganisms (GEMs) (103). GEMs have shown potential for application in bioremediation of contaminated soil, groundwater, and activated sludge environments. Rate limiting steps in known metabolic

pathways could be genetically manipulated to yield increased degradation rates. More recent developments on bioremediation can be found from the literature (104–108).

NOMENCLATURE

ABTS = 3-ethylbenzthiazoline-6 sulphonic acid
Ag = Silver
Au = Gold
araB = Arabinose
BaP = Pyrene (BaP)
Bi = Bismuth
BOD = Biochemical oxygen demand
BTEX = Benzene, toluene, ethylbenzene and xylene
C23O = Catechol 2,3 dioxygenase
CBA = Benzoate
Cd = Cadmium (Cd)
Co = Cobalt
COD = Chemical oxygen demand
Cu = Copper
CYPs = Cytochrome P450 mono-oxygenase
Cys = Cystein
DHBD = Dihydroxy biphenyl dioxygenase
DNA = Deoxyribonucleic acid
EDTA = Ethylenediamine tetraacetic acid
GEMs = Engineered microorganisms
GSH = Glutathione
H_2S = Hydrogen sulphide
Hg = Mercury (Hg)
IAA = Indo-acetic acid
LAC, E.C.1.10.3.2 = Laccase
Lip, E.C.1.11.1.14 = Lignin peroxidase
merA = Mercury reductase
MnP, 1.11.1.13 = Mn-dependent peroxidase
MtL = *Myceliophthora thermophia*
MTs = Metallothioneins
N = Nickel
OMW = Olive oil mill wastewater
OP = Organophosphates
OPH = Organophosphorus hydrolase
PAHs = Polycyclic aromatic hydrocarbons
Pb = Lead
PCBs = Polychlorinated biphenyls
PCE = Tetrachloroethene = Tetrachloroethylene = PERC

PCs = Phytochelatins
PGPR = Rhizobacterial
PGR = Plant growth regulators
POPs = Persistent organic pollutants
PTE = Phosphotransferases
SRB = Sulphate-reducing bacteria
SS = Suspended solids
TCA = 1,1,1-trichloroethane
TCA = Tricarboxylic acid
TCE = Trichloroethylene
UV = Ultraviolet

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10
Wetlands for Wastewater Treatment

Azni Idris, Abdul Ghani Liew Abdullah, Yung-Tse Hung, and Lawrence K. Wang

CONTENTS

Abstract This chapter discusses the use of natural and constructed wetlands for treatment of wastewaters. Mechanisms of treatment processes for wetlands were described. Function, roles, types, and selection of wetland plants were discussed. This chapter also covers design, monitoring, and maintenance of wetland treatment systems for wastewater. Case studies in Malaysia and UK were discussed.

1. INTRODUCTION

In recent years, the selection of treatment methods for wastewater discharge from both municipalities and industrial sources has open for wider options to include natural and constructed wetlands. The increasing capital and operation costs associated with modern mechanical treatment processes are a major driving force that calls for rethinking of using natural system to solve river pollution problems. Constructed wetlands are "designed and man-made complex of saturated substrates, emergent and submergent vegetation, animal life, and water that simulates natural wetlands for human use and benefits" (1). Constructed wetlands are considered to be a low-cost system for treating wastewater discharged from municipal,

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_10,

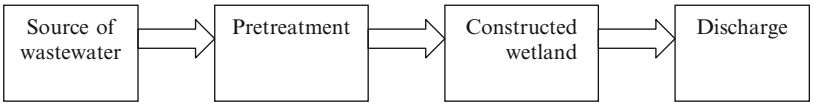

Fig. 10.1. A schematic process flow of a constructed wetland system.

agricultural, and industrial sources. A schematic process flow for a constructed wetland system is shown in Fig. 10.1.

Constructed wetlands represent an emerging eco-technological treatment system in which they are designed to overcome the disadvantages of natural wetlands. They have the qualities of reliability, cost effectiveness, and versatility on top of the conventional engineering measures. Constructed wetlands have a great potential in treating wastewater as they can tolerate higher organic loading rate and shorter hydraulic retention time (HRT). In addition, they also have the capability of treating more than one type of pollutants simultaneously to some satisfactory levels as compared to other conventional treatment systems. Constructed wetlands can be created from existing marshlands or built at any land with limited alternative uses.

2. WHAT ARE WETLANDS?

Wetlands are defined by the Convention of wetland of International Importance (the Ramsar Convention 1971) as: "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water, the depth of which at low tide does not exceed 6 m. Wetlands include marshes, swamps, vleis, pans, bogs, ponds, reed beds, and estuaries"(2). Broadly defined, wetlands are land areas that have a prolonged high water or at least that are covered with shallow water. As a transition habitat between dry land and a deep water environment, in which they support plants that are adapted to grow in wet conditions.

Wetlands are transitional areas between land and water. The boundaries between wetlands and uplands or deep water are, therefore, not always distinct. The term "wetlands" encompasses a broad range of wet environments, including marshes, bogs, swamps, wet meadows, tidal wetlands, floodplains, and ribbon (riparian) wetlands along stream channels. All wetlands (natural or constructed) have one characteristic in common, i.e., the presence of surface or near-surface water, at least periodically. In most wetlands, hydrologic conditions are such that the substrate is saturated long enough during the growing season to create oxygen-poor conditions in the substrate. The lack of oxygen creates reducing. (oxygen-poor) conditions within the substrate and limits the vegetation to those species that are adapted to low-oxygen environments.

The hydrology of wetlands is generally one of slow flows and either shallow waters or saturated substrates. The slow flows and shallow water depths allow sediments to settle as the water passes through the wetland. The slow flows also provide prolonged contact times between the water, substrates, and the surfaces within the wetland. The complex mass of organic and inorganic materials and the diverse opportunities for gas/water interchanges foster a diverse community of microbes that break down or transform a wide variety of substances. Most wetlands support a dense growth of vascular plants adapted to saturated conditions. This

vegetation slows the water, creates microenvironments within the water column, and provides attachment sites for the microbial community. The litter that accumulates as a result of dead plants in the wetland creates additional material and exchange sites, and provides a source of carbon, nitrogen, and phosphorous to fuel microbial processes.

2.1. Wetland Functions and Values

Wetland functions are the inherent processes occurring in wetlands; wetland values are the attributes of wetlands that society perceives as beneficial. Many values that wetlands provide result from their functions (hydrological, biogeochemical, and ecological). Hydrological functions may include floodwater retention, groundwater recharge and discharge, and sediment retention. Biogeochemical functions may include nutrient retention/removal and in-situ carbon retention. Ecological functions may also include ecosystem maintenance and food web support.

Here a function can be defined as an activity that results from the interactions that occurs between natural processes (physical, chemical, and biological) and the structural components such as geomorphology, hydrology, soil, flora, fauna, and microbes of the ecosystems.

Under appropriate circumstances, constructed wetlands can provide extremely effective water quality improvement, flood storage and the desynchronization of storm, rainfall and surface runoff, cycling of nutrients and other materials, habitat for fish and wildlife, passive recreation, such as bird watching, and photography, active recreation, such as hunting, education and research, aesthetics, and landscape enhancement (3). Figure 10.2 shows the inter relationship between wetland functions and values.

3. NATURAL WETLANDS

Natural wetlands are sometimes called swamps, marshes, bogs, fens, wet meadows, or sloughs. Natural wetland definitions are not necessarily the same. Plant types and species, water, and geographic conditions vary, creating different kinds of wetlands in many different countries.

The 1977 Clean Water Act Amendments provide a broad definition of wetlands: "The term 'wetlands' means those areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support, and that under normal circumstances do support, a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs, and similar areas."

Wetlands are natural receptacles. Occurring in low-lying areas, wetlands receive runoff water and overflow from rivers and streams. In response, various wetland biological mechanisms or processes evolved over geologic time to treat inflows. These mechanisms trap sediments and break down a wide range of pollutants into elemental compounds. Wetlands have a natural, innate ability to treat wastewater. Water moves slowly through wetlands, as shallow flows, saturated substrates or both. Slow flows and shallow waters cause sediments to settle. The slow flows also act to prolong contact times between the water and surfaces within the wetland.

The organic and inorganic materials within a wetland form a complex mass. This mass along with the occurrence of gas/water interchanges promotes a varied community of

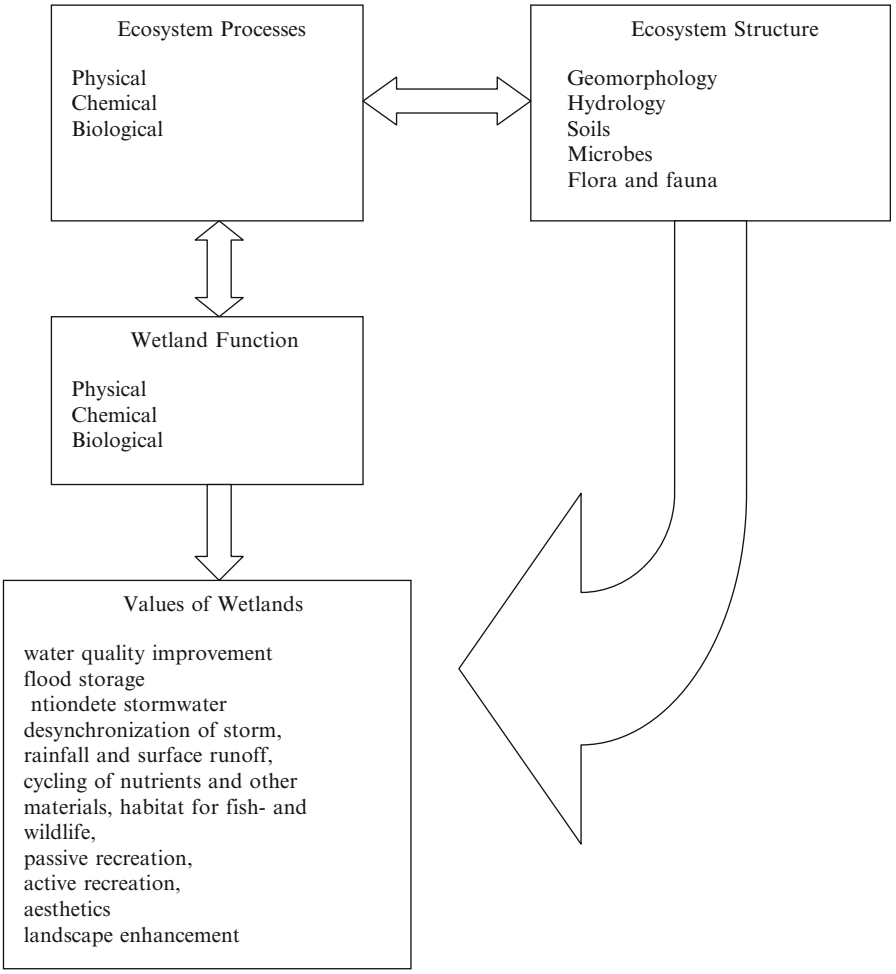

Fig. 10.2. Interrelationship between wetland processes, functions, and values.

microorganisms to break down or transform a wide variety of substances. Dense growths of vascular plants adapted to saturated conditions often thrive in wetlands and contribute to its treatment capacity. Along with slowing the flow of water, the vegetation creates microenvironments and provides the microbial community enormous attachment sites. Furthermore, plants die in some seasons and tend to accumulate as litter. This phenomena creates additional material and exchange sites as well as providing a source of carbon, nitrogen, and phosphorous to fuel microbial processes.

4. CONSTRUCTED WETLANDS

The role of wetland in water resource management is fast gaining ground resulting in the construction wetland in most developed countries. This trend has evolved because wetlands have been added to wastewater facilities that provide only basic levels of primary or secondary

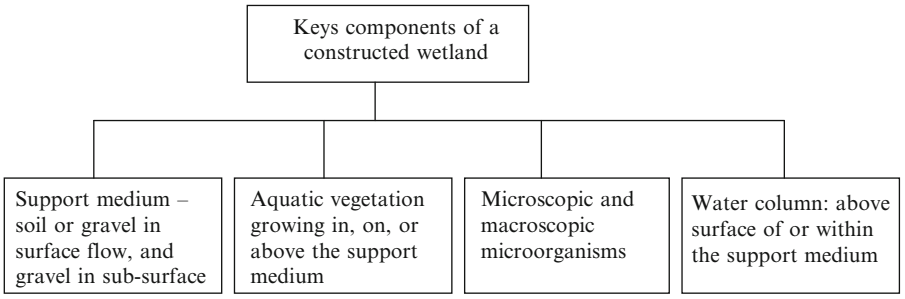

Fig. 10.3. Key components in constructed wetlands.

treatment. Because of the potential for creating nuisance conditions in wetlands that receive poor quality wastewater, the European design preference has been to use subsurface flow through soil or sand planted with common reed.

Constructed wetlands are man-made system that involves altering the existing terrain to simulate wetland conditions. They primarily attempt to replicate the treatment that has been observed to occur when polluted water enters the natural wetland. These wetlands have been seen to purify water by removing organic compounds and oxidizing ammonia, reducing nitrates, and removing phosphorus. The mechanisms are complex and involve bacterial oxidation, filtration, sedimentation, and chemical precipitation.

Most constructed wetland attempts to imitate the ecosystem's biochemical function as filtration and cleansing agents, followed closely by the hydrological function that is centered on flood mitigation. These constructed wastewater treatments may include swamps and marshes. Most of the constructed wetland systems are marshes. Marshes are shallow water regions dominated by emergent herbaceous vegetation including cattails, bulrush, reeds, rushes, and sedges.

4.1. Components of Constructed Wetlands

A constructed wetland consists of a properly designed basin that contains water, a substrate, and, most commonly, macrophyte vegetation. These components can be manipulated in constructing a wetland. Other important components of wetlands, such as the communities of microbes and aquatic invertebrates, develop naturally. The essential components of both a natural wetland and a constructed wetland are shown in Fig. 10.3.

4.2. Advantages of Constructed Wetlands for Wastewater Treatment

Constructed wetland is a cheaper alternative for wastewater treatment using locally available resources. Aesthetically, it is a presentable, scenic, and more landscaped looking wetland site compared to the conventional wastewater treatment plants. This promotes sustainable use of local aquatic plants, which is a more environment friendly biological wastewater treatment system.

Constructed wetlands can be created at lower costs than other treatment options, with low-technology methods where no new or complex technological tools are needed (essentially grading, dike construction, and vegetation planting). Properly designed and construction systems do not require chemical additions and other procedures used in the conventional treatment systems (4). The system relies on renewable energy sources such as solar and kinetic energy, and wetland plants and microorganisms, which are the active agents in the treatment processes.

The system can tolerate both large and small volumes of water with varying contaminant levels. These include municipal and domestic wastewater, urban storm runoff, agricultural wastewater, industrial effluents, and polluted surface waters in rivers and lakes. The system could be promoted to various potential users for water quality improvement and pollutant removal. These potential users include the tourism industry, governmental departments, private entrepreneurs, private residences, aquaculture industries, and agro-industries.

Utilization of local products and labor helps to reduce the operation and maintenance costs of a treatment system. Less energy and raw materials are needed, with periodic onsite labor, rather than continuous full-time attention. This system indirectly will contribute greatly in the reduction of use of natural resources in conventional treatment plants, and wastewater discharges to natural waterways are also reduced. The constructed wetland system also could be used to clean polluted rivers and other water bodies. This derived technology can eventually be used to rehabilitate grossly polluted rivers in the country. The constructed wetland treatment system is widely applied for various functions. These functions include primary settled and secondary treated sewage treatment, tertiary effluent polishing and disinfecting, urban and rural runoff management, toxicant management, landfill and mining leachate treatment, sludge management, industrial effluent treatment, enhancement of in-stream nutrient assimilation, nutrient removal via biomass production and export, and groundwater recharge.

The primary purpose of constructed wetland treatment systems is to treat various kinds of wastewater (municipal, industrial, agricultural, and stormwater). However, the system usually serves other purposes as well. A wetland can serve as a wildlife sanctuary and provide a habitat for wetland animals. The wetland system can also be aesthetically pleasing and serve as an attractive destination for tourists and local urban dwellers. It can also serve as a public attraction sanctuary for visitors to explore its environmental and educational possibilities. It appeals to different groups varying from engineers to those involved in wastewater facilities as well as environmentalists and people concerned with recreation. This constructed wetland treatment system also provides a research and training ground for young scientists in this new research and education arena.

4.3. Types of Constructed Wetlands

Constructed wetland systems are classified into two general types: the horizontal flow system (HFS) and the vertical flow system (VFS). HFS has two general types: surface flow (SF) and subsurface flow (SSF) systems. It is called HFS because wastewater is fed at the inlet and flows horizontally through the bed to the outlet. VFS are fed intermittently and drains vertically through the bed via a network of drainage pipes.

4.3.1. Surface Flow System

The use of SF systems is extensive in North America, whereby more than 200 constructed wetlands are in operation. These systems are used mainly for municipal wastewater treatment with large wastewater flows for nutrient polishing. The SF system tends to be rather large in size with only a few smaller systems in use. The majority of constructed wetland treatment systems are surface flow (SF) or free water surface (FWS) systems. These types utilize influent waters that flow across a basin or a channel that supports a variety of vegetation, and water is visible at a relatively shallow depth above the surface of the substrate materials. Substrates are generally native soils or other suitable medium to support emergent vegetation, and clay or impervious geotechnical materials that prevent seepage. Typical emergent plants that are found in surface flow wetlands are cattails (*Typha spp.*), bulrushes (*Scirpus spp.*), and various sedges (*Carex spp.*). The shallow water depth with low flow velocity of water and presence of plants help to regulate flow, especially in a long narrow channel to ensure that plug-flow conditions are met. Typically, bed depth of wetland is about 0.3–0.4 m. Figure 10.4 below shows a profile of a three-zone SF constructed wetland cell.

4.3.2. Subsurface Flow System

The SSF system includes soil-based technology, which is predominantly used in Europe with more than 500 wetlands are operational and the vegetated gravel beds are found in Europe, Australia, South Africa, and almost all over the world. In a vegetated SSF system, water flows from one end to the other end through permeable substrates that are made of mixture of soil and gravel or crusher rock. The substrate will support the growth of rooted emergent vegetation. It is also called "root-zone method" (RZM) or "rock–reed filter" (RRF) or "emergent vegetation bed system" or "vegetated submerged bed" (VSB). The media depth is about 0.6-m deep and the bottom is a clay layer to prevent seepage. Media size for most gravel substrate ranged from 5 to 230 mm with 13–76 mm being typical. The bottom of the bed is sloped to minimize water that flows overland. Wastewater flows by gravity horizontally through the root zone of the vegetation about 100–150 mm below the gravel surface. Many macro and microorganisms inhabit the substrates. Free water is not visible. The inlet zone

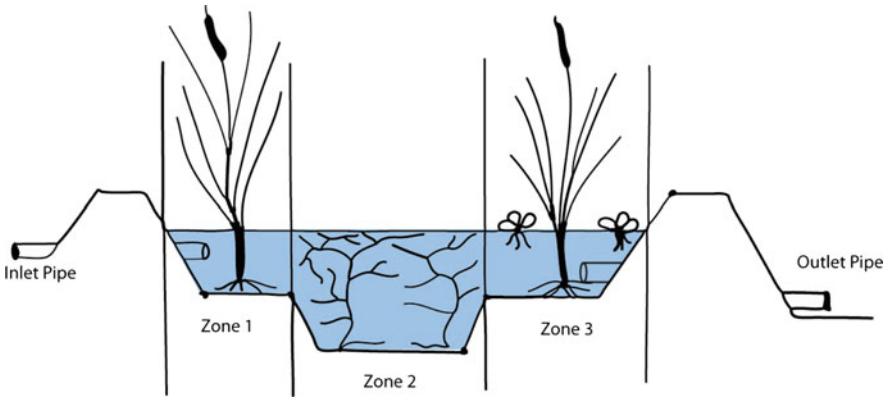

Fig. 10.4. Profile of a three-zone SF/FWS constructed wetland cell.

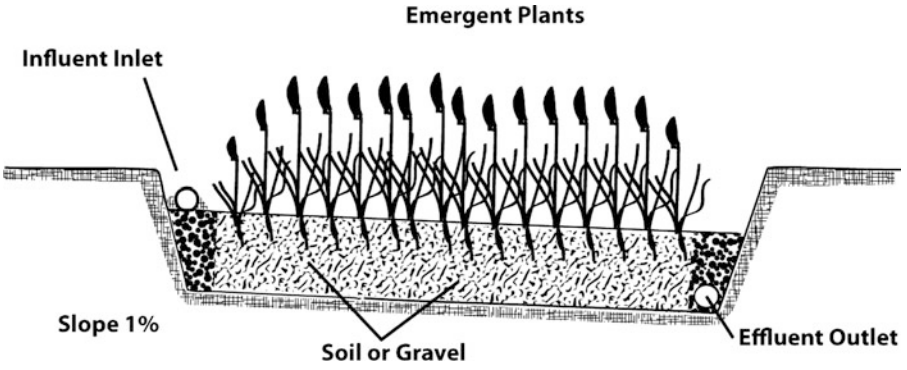

Fig. 10.5. A typical cross section of subsurface flow wetland system.

has a buried perforated pipe to distribute maximum flow horizontally through the treatment zone. Treated water is collected at outlets at the base of the media, typically 0.3–0.6 m below bed surface. In both systems, the flow of wastewater is maintained approximately 0.15–0.3 m below the bed surface (2). Figure 10.5 shows a typical SSF wetland system.

5. MECHANISMS OF TREATMENT PROCESSES FOR CONSTRUCTED WETLANDS

An understanding of the treatment mechanisms is necessary to ensure that constructed wetlands are designed effectively with improved treatment performances. Wetlands have been found to be effective in treating BOD, SS, N, and P as well as for reducing metals, organic pollutants, and pathogens. The principal pollutant removal mechanisms in constructed wetlands include biological processes such as microbial metabolic activity and plant uptake as well as physico-chemical processes such as sedimentation, adsorption, and precipitation at the water–sediment, root–sediment, and plant–water interfaces (5). Table 10.1 shows the summary of removal mechanisms in a constructed wetland.

5.1. Biodegradable Organic Matter Removal Mechanism

Microbial degradation plays a dominant role in the removal of soluble/colloidal biodegradable organic matter in wastewater. Biodegradation occurs when dissolved organic matter is carried into the biofilms that attached on submerged plant stems, root systems, and surrounding soil or media by diffusion process. Wetland plants provide support medium for microbial degradation to take place and convey oxygen to the rhizosphere for aerobic degradation to occur.

Organic matter contains approximately 45–50% carbon (C), which is utilized by a wide array of microorganisms as a source of energy. A large number of these microorganisms consume oxygen (O_2) to break down organic C to carbon dioxide (CO_2), a process that provides energy for growth. Therefore, the release of excessive amounts of organic C to surface waters can result in a significant depletion of O_2, and subsequent mortality of fish and other O_2-dependent aquatic organisms.

Table 10.1
Summary of removal mechanisms in a constructed wetland

Pollutant	Removal mechanism
Biochemical oxygen demand (BOD)	Oxidation Absorption Filtration Sedimentation Microbial decomposition
Suspended solids (SS)	Filtration Sedimentation
Nitrogen (N)	Adsorption Assimilation Absorption Ammonification–nitrification–denitrification
Heavy metals	Adsorption Cation exchange Bioaccumulation
Pathogenic bacteria and viruses	Adsorption Predation Sedimentation Sterilization by UV
Other pollutants	Precipitation Evaporation Evapotranspiration

Wetlands contain vast numbers of organic C-utilizing microorganisms adapted to the aerobic (O_2-rich) surface waters and anaerobic (O_2-depleted) soils at the bottom. Thus, wetlands are capable of highly effective removal of organic compounds from a variety of wastewaters. Organic C in wetlands is broken down to CO_2 and methane (CH_4), both of which are lost to the atmosphere. Wetlands also store and recycle copious amounts of organic C, contained in plants and animals, dead plant material (litter), microorganisms and peat. Therefore, wetlands tend to be natural exporters of organic C as a result of decomposition of organic matter into fine particulate matter and dissolved compounds.

The more readily degradable organic C compounds typically found in municipal wastewater can be rapidly removed in wetlands. Biological removal of a variety of recalcitrant (not readily decomposed) organic C compounds, including lignin-based compounds and petroleum products, can also be achieved in wetlands, although removal rates may be substantially lower.

5.2. Suspended Solids Removal Mechanism

Settleable solids are removed easily by gravitational settlement, since wetlands system generally have long HRTs. On the other hand, nonsettling or colloidal solids are removed via processes such as filtration, adsorption on plants and wetlands media, and biodegradation.

The types of removal mechanism at work are very dependent on the size and nature of solids present in the wastewater and the type of filter media being used. In most cases, wetland plants have insignificant impact on the removal of suspended solids.

5.3. Nitrogen Removal Mechanism

Nitrogen (N) can exist in various forms, namely ammoniacal nitrogen (NH_3 and NH_4^+), organic nitrogen, and oxidized nitrogen (NO_2^- and NO_3^-). The removal of nitrogen is achieved through three main mechanisms: nitrification/denitrification, volatilization of ammonia (NH_3), and uptake by wetland plants. A majority of nitrogen removal occurs through either plant uptake or denitrification. Nitrogen uptake is significant if plants are harvested and biomass is removed from the system. At the root–soil interface, atmospheric oxygen diffuses into the rhizosphere through the leaves, stems, rhizomes, and roots of the wetland plants, thus, creating an aerobic layer similar to those that exists in the media–water or media–air interface. Nitrogen transformation takes place in the oxidized and reduced layers of media, the root–media interface, and below ground portion of the emergent plants. Ammonification takes place where organic nitrogen is mineralized to NH_4^+–N in both oxidized and reduced layers. The oxidized layer and the submerged portions of plants are important sites for nitrification in which Ammoniacal Nitrogen (AN) is converted to nitrites N(NO_2^-) by the *Nitrosomonas* bacteria and eventually to nitrates N (NO_3^-) by the *Nitrobacter* bacteria. At higher pH of 10, some AN, which exists in form of NH_3 will be lost to the atmosphere by volatilization process.

Nitrate in the reduced zone is removed through denitrification, leaching and some plant uptake. However, it is replenished by NO_3^- from the oxidized zone by diffusion. At the root–soil interface, atmospheric oxygen diffuses into rhizosphere through the leaves, stems, rhizomes, and roots of the wetland plants thus creating an aerobic layer that is similar to that existed at the media–water or media–air interface. Nitrification process occurs in the aerobic rhizosphere where AN is oxidized to NO_3^-, which is either taken up by the plants or diffuses into the reduced zone where it is converted to N_2 and N_2O by the denitrification process.

Nitrate removal efficiency typically is extremely high in wetlands. The biological process of denitrification, i.e., conversion of nitrate to nitrogen gas, provides a means for complete removal of inorganic N from wetlands, as opposed to storage within the vegetation or soil. Denitrification usually accounts for the bulk of the inorganic N removal in wetlands.

5.4. Heavy Metals Removal Mechanism

Wetlands soils are potentially effective traps, or sinks for metals, due to the relative immobility of most metals in wetland soils. Aquatic macrophytes remove heavy metals by absorption into living tissue. Decomposing plant litter also contributes to removal of heavy metals by adsorption and precipitation as metal hydroxides in the aerobic zones and as metal sulfides in the anaerobic zones. Cation exchange may involve the binding of positively charged metal ions in solution to negatively charged sites on the surface of the particulates. A significant clay content may also enhance the potential for metal removal. Heavy metals are removed as insoluble sulfides formed during the anaerobic decomposition of dead vegetation. Complexation or chelation with organic materials and media material is also a possible

pathway. Heavy metals are also reduced through direct uptake by wetland plants. However, overaccumulation may kill the plants.

Data on wetland performance for removal of metals are relatively sparse. Based on a limited data set for treatment wetlands, metal removal efficiency is potentially very high, but also highly variable among sites.

5.5. Pathogenic Bacteria and Viruses Removal Mechanism

Pathogenic bacteria and viruses are removed mainly by sedimentation, filtration, and absorption by biomass and by natural die-off due to prolonged exposure to unfavorable environmental conditions such as temperature, pH, solar radiation, nutrient starvation, and predation.

5.6. Other Pollutants Removal Mechanism

Evapotranspiration is one of the mechanisms for pollutant removal. Atmospheric water losses from a wetland that occurs from the water and soil is termed as evaporation and from emergent portions of plants is termed as transpiration. The combination of both processes is termed as evapotranspiration. Daily transpiration is positively related to mineral adsorption, and it could be used as an index of the water purification capability of plants. Precipitation and evapotranspiration influence the water flow through a wetland system. Evapotranspiration slows water flow and increases contact times, whereas rainfall, which has the opposite effect, will cause dilution and increased flow. Precipitation and evaporation are likely to have minimal effects on constructed wetlands in most areas. If the wetland type is primarily shallow open water, precipitation/evaporation ratios fairly approximate water balances. However, in large, dense stands of tall plants, transpiration losses from photosynthetically active plants become significant (18–20).

6. SELECTION OF WETLAND PLANT

6.1. Function of Wetland Plants

In general, the most significant functions of wetland plants (emergents) in relation to water purification are the physical effects brought by the presence of the plants. The plants provide a huge surface area for attachment and growth of microbes. The physical components of the plants stabilize the surface of the beds, slow down the water flow, thus assisting in sediment settling and trapping process, and finally increasing water transparency. Wetland plants play a vital role in the removal and retention of nutrients and help in preventing the eutrophication of wetlands. A range of wetland plants has shown their ability to assist in the breakdown of wastewater. The common reed (*Phragmites spp.*) and cattail (*Typha spp.*) are good examples of marsh species that can effectively uptake nutrients. These plants have a large biomass both above (leaves) and below (underground stem and roots) the surface of the substrate. The subsurface plant tissues grow horizontally and vertically and create an extensive matrix, which binds the soil.

This accumulation of particles enable the creation of a large surface area for the uptake of nutrients and ions. Hollow vessels in the plant tissues enable oxygen to be transported from

Table 10.2
Functions of wetland plants (8)

Plant parts	Functions
Roots and/or stems in the water column	Surface on which the bacteria attach and grow Media for filtration and adsorption of solids
Stems and/or leaves at or above the water surface	Attenuate sunlight and thus can prevent the growth of algae Reduce the effects of wind on the water, i.e., the transfer of gases between the atmosphere and water Important in the transfer of gases to and from the submerged parts of plants

the leaves to the root zone and to the surrounding soil (6, 7). This enables the active microbial aerobic decomposition process and the uptake of pollutants from the water system to take place. Some specific functions of wetland plants are summarized in Table 10.2.

6.2. Roles of Wetland Plants

The roles of wetland plants in constructed wetland systems can be classified into six categories as follows:

Physical. Macrophytes stabilize the surface of plant beds, provide good conditions for physical filtration, and provide a large surface area for attached microbial growth. Growth of macrophytes reduces current velocity, allowing for sedimentation and increase in contact time between effluent and plant surface area, Thus, to an increase in the removal of nitrogen.

Soil hydraulic conductivity. Soil hydraulic conductivity is improved in an emergent plant bed system. Turnover of root mass creates macropores in a constructed wetland soil system allowing for greater percolation of water, thus increasing effluent/plant interactions.

Organic compound release. Plants have been shown to release a wide variety of organic compounds through their root systems, at rates up to 25% of the total photosynthetically fixed carbon. This carbon release may act as a source of food for denitrifying microbes (9). Decomposing plant biomass also provides a durable, readily available carbon source for the microbial populations.

Microbial growth. Macrophytes have above and below ground biomass to provide a large surface area for growth of microbial biofilms. These biofilms are responsible for a majority of the microbial processes in a constructed wetland system, including nitrogen reduction (9).

Creation of aerobic soils. Macrophytes mediate transfer of oxygen through the hollow plant tissue and leakage from root systems to the rhizosphere, where aerobic degradation of organic matter and nitrification will take place. Wetland plants have adaptations with suberized and lignified layers in the hypodermis and outer cortex to minimize the rate of oxygen leakage.

Aesthetic values. The macrophytes have additional site-specific values by providing habitat for wildlife and making wastewater treatment systems aesthetically pleasing.

6.3. Types of Wetland Plants

Wetland plants can be classified into three broad types. These broad types are:

- *Floating*. These are plants that are free floating and not attached to any substrate.
- *Submerged*. These are plants that are attached to the substrate or free floating, but whose leaves and stems are permanently submerged. It includes plants whose flowers may be emergent.
- *Emergent*. These are plants that are attached to the substrate and whose leaves and stems either float on the surface or protrude above the surface. It includes plants that are periodically or seasonally as well as permanently inundated.

Each of these types of plants has a different role to play in constructed wetlands and will produce different microhabitats. Use of the different types of plants leads to diversity within the wetland, which results in more biodiversity, better functioning, and a more stable wetland. Figure 10.6 illustrates the different types of wetland plants.

6.4. Selection of Wetland Plants

In selecting plants for use in a constructed wetland it is necessary to consider the factors that affect their natural distribution both within the state and locally, as these will have a major impact on the success of the plants that are used for wetland planting. Table 10.3 shows the characteristics of plants for constructed wetlands.

When selecting plants for constructed wetlands, it is necessary to consider the following factors:

- The species available or suitable for the proposed wetland site
- The substrate on which the plants will prefer to grow (e.g., sand, mud, clay, peat)
- Aerobic vs. anaerobic conditions and when and where this is likely to occur within the wetland
- The depth of water in which the plants normally grow, e.g., shallow or deep water
- The frequency and depth of inundation
- Periods of drying and the ability of the plants to withstand drying

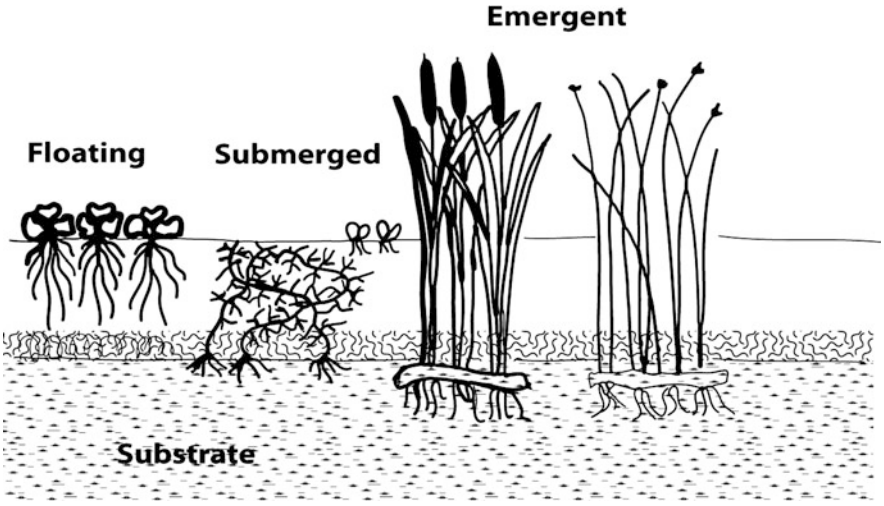

Fig. 10.6. Types of wetland plants.

Table 10.3
Characteristics of plants for constructed wetlands (10)

Types of plants	Characteristics and common examples	Function or importance treatment process	Function or importance for habitat	Design & operational considerations
Free-floating	Aquatic roots or root-like structures suspended from floating leaves. Will move about with water currents. Will not stand erect out of the water. Common duckweed (*Lemna*), Big duckweed (*Spirodela*)	Primary purposes are nutrient uptake and shading to retard algal growth. Dense floating mats limit oxygen diffusion from atmosphere duckweed will be present as an invasive species	Dense floating mats limit oxygen diffusion from the atmosphere and block the sunlight from submerged plants. Plants provide shelter and food for animals	Duckweed is a natural invasive species in North America. No specific design is required
Rooted floating aquatic	Usually with floating leaves, but may have submerged leaves. Rooted to bottom. Will not stand erect water. Water lily (*Nymphea*), Pennywort (*Hydrocotyle*)	Primary purposes are providing structure for microbial attachment and releasing oxygen to the water out of the column during daylight hours. Dense floating mats limit oxygen diffusion from the atmosphere	Dense floating mats limit oxygen diffusion from the atmosphere and block. Sunlight from submerged plants. Plants provide shelter and food for animals	Water depth must be designed to promote the type of plant (i.e., floating, submerged, emergent) desired while hindering other types of plants
Submerged Aquatic	Usually totally submerged; may have floating leaves. Rooted to bottom. Will not stand erect in air. Pondweed (*Potamogeton*), Water weed (*Elodea*)	Primary purposes are providing structure for microbial attachment and providing oxygen to the water. column during daylight hours	Plants provide shelter and food for animals (especially fish)	Retention time in open water zone should be less than necessary to promote algal growth which can destroy these plants through sunlight blockage

Emergent aquatic	Herbaceous (i.e., nonwoody). Rooted to the bottom. Stand erect out of the water. Tolerate flooded or saturated conditions. Cattail (*Typha*), Bulrush (*Scirpus*), common reed (*Phragmites*)	Primary purpose is providing structure to induce enhanced flocculation and sedimentation. Secondary purposes are shading to retard algal growth, windbreak to promote quiescent conditions for settling, and insulation during winter months	Plants provide shelter and food for animals. Plants provide aesthetic beauty for humans	Water depths must be in the range that is optimum for the specific species chosen (planted)
Shrubs	Woody, less than 6-m tall. Tolerate flooded or saturated soil conditions. Dogwood (*Cornus*), Holly (*Ilex*)	Treatment function is not defined: it is not known if treatment data from unsaturated or occasionally saturated phytoremediation sites in upland areas is applicable to continuously saturated wetland sites	Plants provide shelter and food for animals (especially birds). Plants provide aesthetic beauty for humans	Possible perforation of liners by roots
Trees	Woody, greater than 6-m tall. Tolerate flooded or saturated soil conditions. Maple (*Acer*).Willow (*Salix*)	(Same as for shrubs)	(Same as for shrubs)	(Same as for shrubs)

- The pH of the water and its likely variance over time
- The local climate including the length and nature of the growing season.

Other important factors that need to be taken into consideration may include:

- Containment, especially for free-floating species
- Potential interaction with animals and their likely destruction by animals, e.g., as nest sites
- Potential weediness of the species selected both within and also outside the wetland.

Another factor to consider is the nature of the plants and their growth habits, e.g., free floating, bottom anchored, upright, spreading, or creeping. These different plant types can have an impact on the amount of shading of the wetland and this can be important in algal control. In addition, the different sorts of plants provide different habitats for the various microflora and microfauna that will live in the wetlands. Not all wetland species are suitable for wastewater treatment since plants for treatment wetlands must be able to tolerate the combination of continuous flooding and exposure to wastewater or stormwater containing relatively high and often variable concentrations of pollutants.

Floating and submerged plants are used in an aquatic plant treatment system. A range of aquatic plants have shown their ability to assist in the breakdown of wastewater. The water hyacinth (*Eichhornia crassipes*), and duckweed (*Lemna*) are common floating aquatic plants, which have shown their ability to reduce concentrations of BOD, TSS, and total phosphorus and total nitrogen. Figure 10.7 shows some examples of common floating plants.

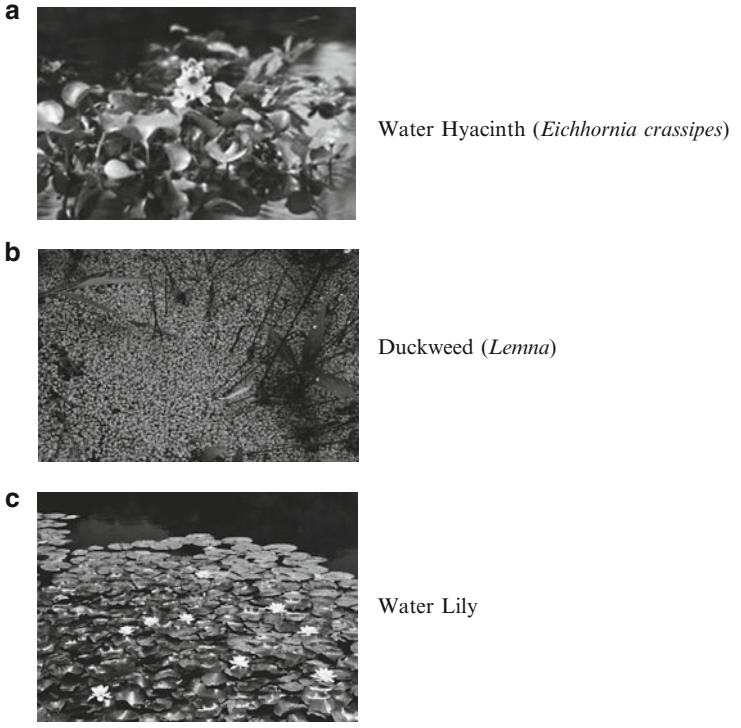

Fig. 10.7. Examples of common-floating plants.

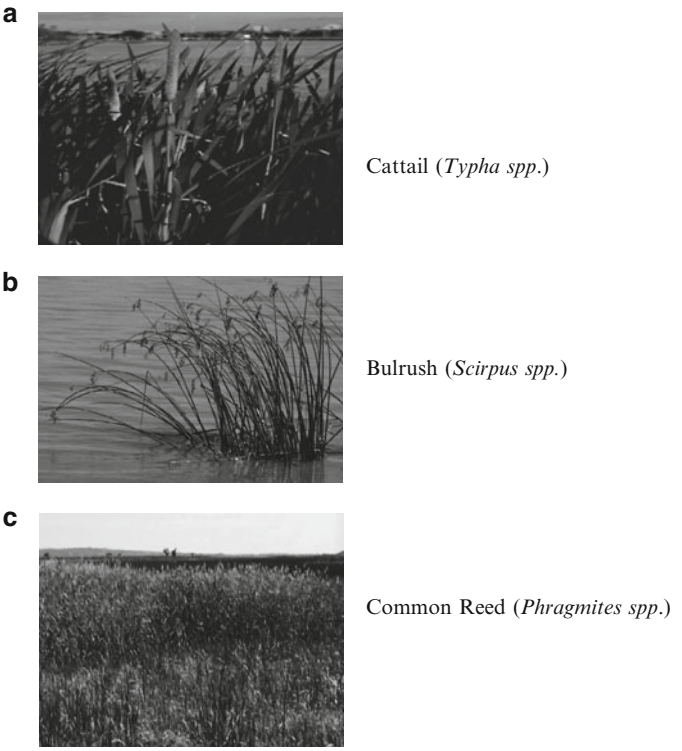

Fig. 10.8. Examples of common-emergent plants.

The common reed (*Phragmites* spp.) and cattail (*Typha* spp.) are good examples of emergent species used in constructed wetland treatment systems. Plant selection is quite similar for SF and SSF constructed wetlands. Emergent wetland plants grow best in both systems. These emergent plants play a vital role in the removal and retention of nutrients in a constructed wetland. Although emergent macrophytes are less efficient at lowering nitrogen and phosphorus contents by direct uptake due to their lower growth rates (compared to floating and submerged plants), their ability to uptake nitrogen and phosphorus from sediment sources through rhizomes is higher than from the water. Figure 10.8 shows some examples of common emergent plants.

Only selected species of wetland plants are chosen by wetland designers, the species must have a rapid and relatively constant growth rate. In a tropical system, wetland plants have a higher growth rate. These wetland plants are easily propagated by means of runners and by bits of mats breaking off and drifting to new areas. This will help in increasing the capacity of pollutant absorption by the plants. The plants should also be able to tolerate waterlogged-anoxic and hypereutrophic conditions. The plant species should be a local species and widely available in the country. Use of exotic plants in constructed wetland systems should be avoided, as they are highly invasive and difficult to control. The plant should be a perennial with a life cycle of more than one year or two growing seasons to ensure the sustainability of

the constructed wetland system. Wetland plants with aesthetic appeal will provide a landscape-pleasing environment.

To assist in plant selection, a number of species that have been used successfully in the northeastern United States are listed in Table 10.4.

7. DESIGN OF CONSTRUCTED WETLAND SYSTEMS

7.1. Design Principles

Characteristics of wastewater to be treated, as well as desired and/or required discharge limits, need to be taken into consideration in designing a constructed wetland treatment system. Main characteristics of the wastewater include both soluble and solid organic compounds, i.e., biochemical oxygen demand (BOD), suspended solids (SS), nitrogen compounds, phosphorus compounds, heavy metals, pathogenic bacteria, and/or viruses. Constructed wetlands could be designed to remove these characteristics. Design considerations in constructed wetland system may include hydraulic capacity, loading rate, retention time, plant type, and species. These in turn, are constrained by regulations and effluent discharge limits. Constructed wetlands are dynamic systems influenced by a wide suite of factors ranging from the regional climatic conditions and geological characteristics to the local vegetation to land-use patterns.

7.2. Hydraulics

The hydraulic capacity of a wetland can be defined as the ability of the wetland to process a given volume of wastewater in a given time. This period of time is known as HRT, which is the expected average time in which a molecule of water will flow from one end to the other of the wetland. Requirement vary depending on the pollutant and the desired level of treatment. Typical detention times are 2–5 days for BOD removal and 7–14 days for nitrogen removal (12).

The HRT in the wetland can be calculated using Eq. (1) below:

$$t = \frac{V}{Q} = \frac{LW(d_{\mathrm{m}}n + d_{\mathrm{w}})}{Q} = A\frac{(d_{\mathrm{m}}n + d_{\mathrm{w}})}{Q}, \tag{1}$$

where t is the hydraulic retention time, d; L the length of the wetland cell, m (ft); W the width of the wetland cell, m (ft); d_{m} the depth of media, m (ft); d_{w} the depth of water from media surface, m (ft); n the porosity, or the space available for water to flow through the wetland, porosity is percent, expressed in decimal. Typically in mature wetlands, they are in the range of 0.65–0.75; Q the average flow through the wetland, $\mathrm{m}^3/\mathrm{d}(\mathrm{ft}^3/\mathrm{d})$; V the volume of water in the system, m^3 (ft^3); and A_s is the surface area of wetland, m^2, (ft^2).

The hydraulic loading rate (HLR) is a term that provides a measure of the volumetric application of wastewater into the wetland. It is often used to make comparisons between wetland systems and indicates their potential to be overloaded by wastewater.

HLR is calculated using the following expression:

$$\mathrm{HLR} = \frac{Q}{A_{\mathrm{s}}}, \tag{2}$$

Table 10.4
Emergent plants for constructed wetlands (11)

Recommended species	Maximum water depth[a]	Notes
Arrow arum (*Peltandra virginica*)	12 in.	Full sun to partial shade. High wildlife value. Foliage and rootstocks are not eaten by geese or muskrats. Slow grower. pH: 5.0–6.5
Arrowhead/duck potato (*Saggitaria latifolia*)	12 in.	Aggressive colonizer. Mallards and muskrats can rapidly consume tubers. Loses much water through transpiration
Common three-square bulrush (*Scirpus pungens*)	6 in.	Fast colonizer. Can tolerate periods of dryness. High metal removal. High waterfowl and songbird value
Softstem bulrush (*Scirpus validus*)	12 in.	Aggressive colonizer. Full sun. High pollutant removal. Provides food and cover for many species. of birds. pH: 6.5–8.5
Blue flag iris (*Iris versicolor*)	3–6 in.	Attractive flowers. Can tolerate partial shade but requires full sun to flower. Prefers acidic soil. Tolerant of high nutrient levels
Broad-leaved cattail[b] (*Typha latifolia*)	12–18 in.	Aggressive. Tubers eaten by muskrat and beaver. High pollutant treatment, pH: 3.0–8.5
Narrow-leaved cattail[b] (*Typha angustifolio*)	12 in.	Aggressive. Tubers eaten by muskrat and beaver. Tolerates brackish water. pH: 3.7–8.5
Reed canary grass (*Phalaris arundinocea*)	6 in.	Grows on exposed areas and in shallow water. Good ground cover for berms
Lizard's tail (*Saururus cernuus*)	6 in.	Rapid grower. Shade tolerant. Low wildlife value except for wood ducks
Pickerelweed (*Pontedaria cordata*)	12 in.	Full sun to partial shade. Moderate wildlife value. Nectar for butterflies. pH: 6.0–8.0
Common reed[b] (*Phragmites australis*)	3 in.	Highly invasive; considered a pest species in many states. Poor wildlife value. pH: 3.7–8.0
Soft rush (*Juncus effuses*)	3 in.	Tolerates wet or dry conditions. Food for birds. Often grows in tussocks or hummocks
Spikerush (*Eleocharis palustris*)	3 in.	Tolerates partial shade
Sedges (*Carex* spp.)	3 in.	Many wetland and several upland species. High wildlife value for waterfowl and songbirds
Spatterdock (*Nuphar luteum*)	5 ft (2 ft minimum)	Tolerant of fluctuating water levels. Moderate food value for wildlife, high cover value. Tolerates acidic water (to pH 5.0).
Sweet flag (*Acorus calamus*)	3 in.	Produces distinctive flowers. Not a rapid colonizer. Tolerates acidic conditions. Tolerant of dry periods and partial shade. Low wildlife value
Wild rice (*Zizania aquatica*)	12 in.	Requires full sun. High wildlife value (seeds, plant parts, and rootstocks are food for birds). Eaten by muskrats. Annual, nonpersistent. Does not reproduce vegetatively

[a]These depths can be tolerated, but plant growth and survival may decline under permanent inundation at these depths.
[b]Not recommended for stormwater wetlands because they are highly invasive, but can be used in treatment wetlands if approved by regulatory agencies.

where HLR is the hydraulic loading rate, m/d (ft/d); Q is average flow through the wetland, $m^3/d(ft^3/d)$; and A_s is the surface area of wetland, m^2, (ft^2).

7.3. General Design Procedures (13)

A constructed wetland system can be considered to be attached growth biological reactors system, and their performance can also be estimated using first-order plug-flow kinetics for BOD and nitrogen removal.

The relationship for plug-flow models is given below by Eq. (3):

$$\frac{C_e}{C_o} = \exp(-k_T t), \tag{3}$$

where C_e is the effluent pollutant concentration, mg/L; C_o the influent pollutant concentration, mg/L; k_T the temperature dependent first-order reaction rate constant, d^{-1}; and t is the hydraulic retention time, d.

The rate constant k_T at temperature T(°C) can be determined using the following Eq. (4).

$$k_T = k_{20}(\theta)^{T-20}, \tag{4}$$

where k_{20} is the rate constant at 20°C and θ is the temperature coefficient.

Table 10.5 gives apparent rate constant values for SF and SSF wetland systems.

7.3.1. Surface Flow Wetland

Hence, it is possible to determine the surface area of the wetland by combining Eqs. (1) and (3). Therefore, general design equation is as follows:

$$\frac{C_e}{C_o} = \exp\left[-K_T A_s(d_m n + d_w/Q)\right], \tag{5}$$

$$A_s = LW = Q(\ln C_o - \ln C_e)/K_T(d_m n + d_w). \tag{6}$$

Nitrogen removal is a temperature-dependent process and is highly sensitive to cold temperature. In winter time, once the temperature falls below 5°C, nitrogen removal will be difficult. It is much easier for wetlands to remove nitrates than ammonia, hence if nitrogen removal is

Table 10.5
Apparent rate constant values for SF and SSF wetland systems (13)

Wetland type	Pollutant removal	Temperature (°C)	Apparent rate constant (d^{-1})	Temperature coefficient (θ)
SF	BOD	20	0.678	1.06
	NH_4	20	0.2187	1.048
	NO_3	20	1.000	1.1
SSF	BOD	20	1.104	1.06
	NH_4	20	K_{NH}	1.048
	NO_3	20	1.000	1.15

Note: $K_{NH} = 0.01854 + 0.3922(rz)^{2.6077}$

a goal, then the treatment process should provide for nitrification, with subsequent discharge into wetlands for denitrification.

For nitrification process, the following assumptions are being made:

1. All the organic nitrogen entering the system will be converted to ammonia nitrogen (AN)
2. AN removal is due to entirely to nitrification

Nitrification process is described by a plug-flow first-order model the same as that in Eqs. (3) or (5) with C_e = effluent ammonia (NH_4) concentration and C_o = influent TKN concentration as follows:

$$\ln(\text{TKN}/\text{NH}_{4\text{eff}}) = A_s k_T (d_m n + d_w)/Q, \tag{7}$$

where TKN is the influent Kjeldahl nitrogen, mg/L and NH_{4eff} is the effluent ammonia, mg/L.

The following are temperature-dependent functions to compute the rate constant for nitrogen removal:

$$k_T = 0\text{d}^{-1} \quad \text{where} \quad T = 0°\text{C}$$
$$k_T = 0.1367(1.15)^{(T-10)}\,\text{d}^{-1} \quad \text{where} \quad T = (1 - 0°\text{C})$$
$$k_T = 0.2187(1.048)^{(T-20)}\,\text{d}^{-1} \quad \text{where} \quad (T > 0°\text{C}).$$

Nitrate removal via denitrification process can be estimated using Eq. (6) with C_e = effluent nitrate (NO_3) concentration, and C_o = influent nitrate (NO_3) concentration as follows:

$$\ln(\text{NO}_{3\,\text{inf}}/\text{NO}_{3\text{eff}}) = A_s k_T (d_m n + d_w)/Q, \tag{8}$$

where $NO_{3\,inf}$ is the influent nitrate, mg/L and NO_{4eff} is the effluent nitrate, mg/L.

However, the temperature-dependent rate constant, k_T was suggested to be as follows:

$$k_T = 0d^{-1} \quad \text{where} \quad T = 0°\text{C}$$
$$k_T = 1.0(1.15)^{(T-20)}d^{-1} \quad \text{where} \quad T \geq 1°\text{C}.$$

Suspended solids (SS) is essentially involves filtration and retention times. SS removal is affected by velocity, thus Eq. (9) below can be used for SS removal calculation in SF wetland system.

Water depth should not exceed 0.45 m (18 in.).

$$\text{SS}_e = \text{SS}_o \times [(0.1139 + 0.00213) \times \text{HLR}], \tag{9}$$

where HLR is the hydraulic loading rate, m/d; SS_e the effluent SS, mg/L; and SS_o is the influent SS, mg/L.

7.3.2. Subsurface Flow Wetland

The basic mechanisms for BOD removal in SSF wetlands are similar to SF/FWS wetlands as described above. However, for SSF wetlands, the $d_w = 0$. Therefore, Eqs. (4) and (5) will

be as follows:

$$\frac{C_e}{C_o} = \exp[-K_T A_s(d_m n)/Q], \tag{10}$$

$$A_s = LW = Q(\ln C_o - \ln C_e)/k_T(d_m n). \tag{11}$$

Nitrogen removal formulas are the same as for surface flow (SF) system, except that the reaction rate constants are different. For the nitrification process, this type of system is very dependent on the emergent plants to supply oxygen to the root zone for nitrification process to occur. Therefore, the nitrification rate constant should be a function of the root zone as follows:

$$k_{20} = 0.01854 + 0.3922(rz)^{2.6077}\,\mathrm{d}^{-1}, \tag{12}$$

where k_{20} is the nitrification rate constant at $20°C$ and rz is the percent of wetland bed depth occupied by root zone (decimal 0–1).

The temperature dependence of k_T is given by the following Eq. (13).

$$k_T = k_{20}(1.048)^{(T-20)}\,\mathrm{d}^{-1} \quad \text{for} \quad T \geq 10°\mathrm{C}. \tag{13}$$

Therefore, the design model or nitrification will be as follows:

$$\ln(\mathrm{TKN}/\mathrm{NH_{4eff}}) = A_s(0.01854 + 0.3922(rz)^{2.6077})(1.048)^{(T-20)} \times (d_m n)/Q, \tag{14}$$

where TKN = influent Kjeldahl nitrogen, mg/L and $\mathrm{NH_{4eff}}$ is the effluent ammonia, mg/L.

For denitrification process, the design model is described by Eq. (10), with C_e and C_o defined as effluent nitrate (NO_3) and influent nitrate (NO_3) concentrations, respectively. The temperature-dependent, k_T is the same as that for SF wetland.

$$\ln(\mathrm{NO_{3\,inf}}/\mathrm{NO_{3eff}}) = A_s k_T \times (d_m n)/Q, \tag{15}$$

where $\mathrm{NO_{3\,inf}}$ is the influent nitrate, mg/L and $\mathrm{NO_{3eff}}$ is the effluent nitrate, mg/L

Suspended solids (SS) essentially involves filtration and retention times. SS removal is affected by velocity, thus Eq. (16) can be used for SS removal calculation in SSF wetland system (13).

$$\mathrm{SS_e} = \mathrm{SS_o}[(0.1058 + 0.0011 \times \mathrm{HLR})], \tag{16}$$

where HLR is the hydraulic loading rate, m/d; $\mathrm{SS_e}$ the effluent SS, mg/L; and $\mathrm{SS_o}$ is the influent SS, mg/L.

Example 1

Determine the area of a SSF constructed wetland for a residential area of 100 houses, each with a septic tank. Assume that all the wastewaters are collected using the existing septic tanks as pretreatment tanks. Average number per dwelling is 3.2 and average per capita flow is 50 gallons per day. Given the following data:

Influent BOD = 140 mg/L
Effluent BOD = 10 mg/L

Depth = 0.6 m (2 ft)
Porosity = 0.44
Assume the water temperature is the same as the ground temperature, i.e., 10°C.

Solution

(a) Calculate the flow: 100 houses × 3.2 people × 50 gpdpc = 16,000 gpd = 60.5 m^3/d
(b) Calculate rate constant: $K_T = 1.104 \times 1.06^{(10-20)} = 0.62\,d^{-1}$
(c) Calculate the area: $A_s = \frac{60.5(\ln 140 - \ln 10)}{0.62 \times 0.6 \times 0.4} = \frac{160}{0.145} = 1{,}103\,m^2$

Example 2

Determine the area required for a FWS wetland system with the following data:

Influent BOD = 250 mg/L
Effluent BOD (desired) = 10 mg/L
Wastewater flow = 500 m^3/d
Mean temperature (winter) = 10°C
(summer) = 25°C
Assume $n = 0.75$, and bed depth of 0.6 m and water depth of 0.1 m throughout the year round.

Solution

(a) Calculate the value of K_T at 10°C : $K_T = 0.678 \times 1.06^{(10-20)} = 0.379\,d^{-1}$

$$K_T \text{ at } 25°C : K_T = 0.678 \times 1.06^{(25-20)} = 0.907\,d^{-1}$$

(b) Calculate hydraulic retention time (HRT) from Eq. (3): $t = \dfrac{\ln C_o - \ln C_e}{K_T}$

$$\text{winter} : t = \frac{\ln 250 - \ln 10}{0.379} = 3.5\,d,$$

$$\text{summer} : t = \frac{\ln 250 - \ln 10}{0.907} = 8.5\,d.$$

(c) Calculate the area:

$$\text{Winter} : A_s = \frac{500 \times 3.5}{(0.6 \times 0.75) + 0.1} = \frac{1{,}750}{0.55} = 3{,}182\,m^2 = 0.32\,ha,$$

$$\text{Summer} : A_s = \frac{500 \times 8.5}{(0.6 \times 0.75) + 0.1} = \frac{4{,}250}{0.55} = 7{,}728\,m^2 = 0.77\,ha.$$

Example 3

Compare the sizes of the SF/FWS and SSF wetlands for the same nitrogen removal design conditions:

Influent TKN = 25 mg/L
Effluent AN (desired) = 3 mg/L
Effluent TN (desired) = 3 mg/L
Mean water temperature = 25°C

Solution
For SF/FWS Wetland:

(a) Determine the value of the rate constant for AN removal, k_{25} from:

$$k_T = 0.2187(1.048)^{(T-20)}.$$

Thus, $k_{25} = 0.2187(1.048)^{(25-20)} = 0.2187(1.048)^5 = 0.2765\,\mathrm{d}^{-1}$

(b) Determine the HRT, t, which is given by:

$$t = \ln(25/3)/0.2765 = 7.7\,\mathrm{d}.$$

Thus, the area of SF/FWS required for AN removal will be as follows:

$$A_\mathrm{s} = Qt/(d_\mathrm{m}m + d_\mathrm{w}) = 500 \times 7.7/(0.6 \times 0.75) + 0.1 = 7{,}000\,\mathrm{m}^2.$$

(c) Determine the rate constant for nitrate, N, removal as follows:

$$k_T = 1.000(1.15)^{(25-20)} = 1.000(1.15)^{(5)} = 2.011\,\mathrm{d}^{-1}.$$

(d) Determine the effluent nitrate N and TN:
Wetland nitrate, N $= 25 - 3 = 22$ mg/L
Effluent nitrate, N $= 22\exp[-(2.011)(7.7)] \approx 0$ mg/L
Effluent TN $= 3$ mg/L

For SSF wetland:

(a) Determine rate constant for AN removal assuming 50% root zone:

$$k_{25} = [0.01854 + 0.3922(0.5)^{2.6077}](1.048)^{(25-20)} = 0.3157\,\mathrm{d}^{-1}.$$

(b) Determine the HRT, t:

$$t = \ln(25/3)/0.3157 = 6.7\,\mathrm{d}.$$

Then determine the required area for SSF wetland for AN removal:

$$A_\mathrm{s} = Qt/d_\mathrm{m}n = 500 \times 6.7/0.76 \times 0.35 = 12{,}594\,\mathrm{m}^2.$$

(c) Determine the effluent nitrate N and TN:

$$\text{Effluent nitrate, N} = 22\exp[-(2.011)(6.7)] \approx 0\,\text{mg/L},$$

$$\text{Effluent TN} = 3\,\text{mg/L}.$$

Notice that the both area requirement for SF/FWS and SSF wetlands for N removal would be about 2–3 times larger than that required for BOD removal from 250 to 10 mg/L.

8. WETLAND MONITORING AND MAINTENANCE

Monitoring the water quality and maintenance of the wetland areas are essential components of a wetland operation. Wetland monitoring is required to obtain sufficient data to assess the wetland performance in fulfilling the objectives. Wetland maintenance is required to manage macrophytes and desirable species, to remove invading weeds, to remove sediment from the wetlands, and to remove litter from the wetlands (14).

Effective wetland performance depends on adequate pretreatment, conservative constituent and HLRs, collection of monitoring information to assess system performance, and knowledge of successful operation strategies. Sustaining a dense stand of desirable vegetation within the wetland is crucial to ensure treatment efficiency. Aggressive species will out-compete less competitive ones and cause gradual changes in wetland vegetation. Certain undesirable plant species or weeds may be introduced to the wetland from the catchment. Natural succession of wetland plants will take place. However, some aquatic weeds may require maintenance by periodic removal. Weed invasion can dramatically reduce the ability of wetlands to meet its design objectives. For example, pondweed (*Azolla*), duckweed (*Lemna*), water fern (*Salvinia molesta*), and water hyacinth (*Eichhornia crassipes*) can form dense mats, exclude light and reduce dissolved oxygen in the water. Manual removal of noxious and undesirable weeds column is necessary, and eventually lead to an increase in the movement of nutrients through the system.

Water level management is crucial to control weed growth. Floods will cause plants to be scoured from the wetland and/or drowned. If a large area of plants is lost, re-establishment will need to be carried out. Small areas will generally recover naturally while larger areas above $5\,m^2$ may require replanting. Plant viability is vital to water quality improvement in wetlands. Visible signs of plant distress or pest attack should be investigated promptly. Severe infestation could lead to severe stunting and death of plants. Biopesticides or narrow-spectrum pest-specific insecticides could be used if pest population exceeds a certain threshold value.

Water levels are important in wetlands, which may have significant effects on hydrology and hydraulics and impact on wetland biota. Water level should be monitored using water level control structures to ensure successful plant growth. A recirculation system should be in place to allow water from outlet points to be fed back to the wetlands to supplement catchment flows during dry periods. Suspended solids from effluents and litter fall from plants will accumulate in time and gradually reduce the pore space, which has to be flushed to prevent short-circuiting. In terms of health consideration, monitoring of mosquito populations should be undertaken to avoid diseases, which can result in a local health related problem. Selected fish population can be introduced into wetland as a means to kill mosquito larvae.

8.1. Water Quality Monitoring

When constructed wetlands are used to treat wastewater, the main objective of measuring performance is to assess if the regulatory discharge limits are being met. Therefore, water quality data are a good indication of wetland performance. Water quality should be monitored through assessment of inflow and outflow water quality parameters.

Some important water quality parameters which could be monitored may include dissolved oxygen, redox potential, water temperature, pH value, and turbidity, which are the in-situ parameters while laboratory analysis parameters include total suspended solids (TSS), chemical conductivity, ammoniacal nitrogen (AN), nitrate–nitrogen, phosphorus, potassium, magnesium, soluble Fe, mercury, lead, zinc, iron, cyanide, arsenic, phenols, chemical oxygen demand (COD), biochemical oxygen demand (BOD), faecal coliforms, and oil and grease.

9. CASE STUDY

9.1. Putrajaya Wetlands, Malaysia

Constructed wetland is a new area of research in Malaysia. The use of constructed wetlands started in Malaysia in 1999 with the introduction of 200 ha of Putrajaya Wetlands, which is one of the largest constructed freshwater wetlands in the tropics. Putrajaya Wetlands is a pioneer venture in constructed wetland system. It functions as a flood control system and as a natural treatment system that filters most of the pollutants in river water and inflows to the wetlands before finally discharging to the lake. Apart from providing an expansive area for recreation and education, it forms an essential part of the eco-system.

Table 10.6 shows the components that form the Putrajaya Wetlands (15).

The salient features of Putrajaya Wetland are as follows (16):

- Putrajaya Wetlands are the first man-made wetlands in Malaysia
- It is also one of the largest fully constructed freshwater wetlands in the tropics
- It is one of the largest man-made lakes in an urban setting
- At a level of 21 m, the resulting surface area is some 400 ha
- Average depth is 6.6 m
- Deepest depth of some parts are in the range of 12–13 m

The wetlands were constructed in March 1997 and was completed in August 1998. The water levels in the cells varies from level 32 m to level 23.5 m with water from each cell cascading down over each cell weir. The 400 ha Lake was created by construction of a dam on the lower reaches of the Chua River. Construction was undertaken in two phases. The first phase of, approximately, 110 ha involved the construction of a temporary dam across Chua River. This allowed inundation of the upper half of the Lake.

The dam was completed in May 1998 and the impoundment of the first phase of the Lake commenced in September 1998 and was fully inundated in January 1999. The second phase of the Lake begun, thereafter, with the construction of the permanent dam in 2000. Two years later after the Dam was completed, the Lake was completely inundated by March 2003 reaching to level 21 m.

It is the intention of Perbadanan Putrajaya (local authority) that the lake will be utilized for various purposes, not only as an aesthetic one. The many uses envisaged included both primary and secondary contact recreation. To that end, guidelines were developed to manage the Lake by Perbadanan Putrajaya and to regulate and manage lake activities.

The Putrajaya Wetlands are the first man-made wetlands in Malaysia. The Lake is recognized as the most important feature of the city – providing the focal point for the development.

Table 10.6
Features of Putrajaya wetlands (15, 16)

Total area (ha)	Planted area (ha)	Open area (ha)	Weirs & Islands (ha)	Zone of intermittent inundation (ha)	Maintenance tracks (ha)
197.20	77.70	76.80	9.60	23.70	9.40

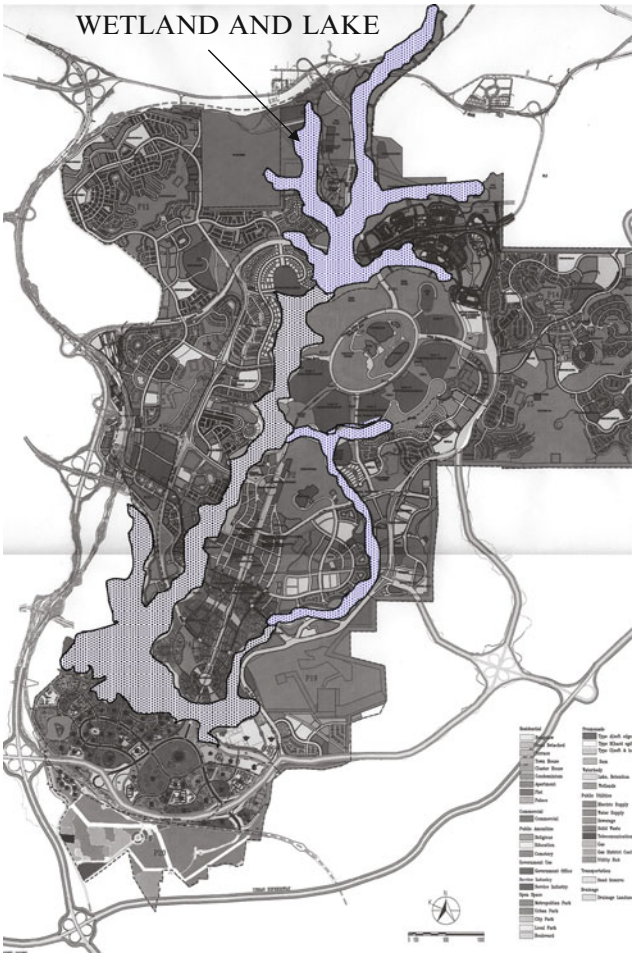

Fig. 10.9. Layout diagram of Putrajaya wetland (16).

At a water level of 21 m the resulting surface area of the lake measures some 400 ha. The average depth of the wetland is 6.6 m and storage volume stands at 265 million cubic meters. It is one of the largest man-made lakes in an urban setting. It is expected to provide focus for many watersport activities as well being used for relaxation. Figure 10.9 shows the overall layout of Putrajaya Wetland system.

9.2. Acle, Norfolk, United Kingdom (17)

A constructed wetland typed SSF was built in Acle, Norfolk, England, United Kingdom (UK) in 1985, which was owned by Anglian Water, to treat tertiary treatment of domestic sewage. It has the area of 3,500 m^2 with layout consisting of two beds of 50 m(length) $\times$ 35 m(width). The wetland was designed for population equivalent (p.e.) of 1,300 (1 p.e. $\equiv$ 150 L/d $\equiv$ 0.15 m^3/d).

Table 10.7
Average performance of wetland in Acle, Norfolk in 1988 (17)

Parameter	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)
BOD	38	4.8	87
AN	6.1	5.3	13
SS	76	28	63

The support medium used was 0.60-m soil from sugar beet washing, and the vegetation was *Phragmites australis*. The floor slope was in the ratio of 1:50. The wastewater flow through a slotted pipe buried in gravel and the treated effluent was discharged using a height adjustable bellmouth.

The wetland has an average flow of 240 m^3/d, average hydraulic load of 0.07 $m^3/m^2/d$, and plan surface area of 2.92 m^2/pe. The performance of the wetland can be seen in Table 10.7.

9.3. Arcata, California (10)

Arcata is located on the northern coast of California about 240 miles north of San Francisco. The population of Arcata is about 15,000. The major local industries are logging, wood products, fishing, and Humbolt State University. The surface flow (SF) constructed wetland located in Arcata is one of the most famous in the United States.

The community was originally served, starting in 1949, with a primary treatment plant that discharged undisinfected effluent to Arcata Bay. In 1957, oxidation ponds were constructed, and chlorine disinfection was added in 1966. In 1974, the State of California prohibited discharge to bays and estuaries unless "enhancement" could be proven, and the construction of a regional treatment plant was recommended. In response, the City of Arcata formed a task force of interested participants, and this group began research on lower-cost alternative treatment processes using natural systems. From 1979 to 1982, research conducted at pilot-scale wetland units confirmed their capability to meet the proposed discharge limits. In 1983, the city was authorized by the state to proceed with development, design, and construction of a full-scale wetland system.

Construction was completed in 1986, and the system has been in continuous service since that time. The wetland system proposed by the city was unique in that it included densely vegetated cells dedicated for treatment followed by "enhancement" marsh cells with a large percentage of open water for final polishing and habitat and recreational benefits. This combined system has been successful since start-up and has become the model for many wetland systems elsewhere.

Two NPDES permits are required for system operation: one for discharge to the enhancement wetlands for protection of public access and one for discharge to the bay. The NPDES limits for both discharges are BOD 30 mg/L and TSS 30 mg/L, pH 6.5–9.5, and fecal coliforms of 200 CFU/100 mL. Since public access is allowed to the enhancement marshes, the state required disinfection prior to transfer of the pond/treatment marsh effluent. The state then required final disinfection/dechlorination prior to final discharge to Arcata Bay. The effluent

from the final enhancement marsh is pumped back to the treatment plant for this final disinfection step.

The basic system design for the treatment and enhancement marshes was prepared by researchers at Humbolt State University. The design was based on experience with a pilot wetland system that was studied from 1979 through 1982. The pilot wetland system included 12 parallel wetland cells, each 20-ft wide and 200-ft long (L:W 10:1), with a maximum possible depth of 4 ft. These were operated at variable hydraulic loadings, variable water depths, and variable initial plant types during the initial phase of the study. Hardstem bulrush (*Scirpus validus*) was used as the sole type of vegetation on all cells. The inlet structure for each cell was a 60° V-notch weir, and the outlet used an adjustable 90° V-notch weir, permitting control of the water depth. Heavy clay soils were used for construction of these cells, so a liner was not necessary and seepage was minimal. The second phase of the pilot study focused on the influence of open water zones, plant harvesting, and kinetics optimization for BOD, TSS, and nutrient removal. Some of the cells, for example, were subdivided into smaller compartments with baffles and weirs along the flow path. The results from these pilot studies not only provided the basis for full-scale system design but have contributed significantly to the state-of-the-art for design of all wetland systems.

The full-scale treatment wetlands, with a design flow of 2.9 mgd, utilize three cells operated in parallel. Cells 1 and 2 have surface areas of about 2.75 acres each ($L \approx 600$ ft, $W \approx 200$ ft), and cell 3 is about 2.0 acres ($L \approx 510$ ft, $W \approx 170$ ft). The original design water depth was 2 ft, but at the time of the 1997 site visit for this report they were being operated with a 4-ft depth. Hardstem bulrush was again used as the only plant species on these treatment marshes. Clumps of plant shoots and rhizomes were hand planted on about 1-m centers. Since nutrient removal is not a requirement for the full-scale system, the treatment marshes could be designed for a relatively short detention time primarily for removal of BOD and TSS. The HRT in these three cells is 1.9 d at design flow and a 2-ft water depth. These treatment marshes were designed to produce an effluent meeting the NPDES limits for BOD and TSS (30/30 mg/L) on an average basis. These wetland cells utilized the bottom area of former lagoon cells. A schematic diagram of the operating system is shown in Fig. 10.10.

The final "enhancement" marshes were intended to provide for further effluent polishing and to provide significant habitat and recreational benefits for the community. These three cells are operated in series at an average depth of 2.0 ft and have a total area of about 31 acres. Retention time is about 9 d at average flow rates. The first cell (Allen Marsh), completed in 1981, was constructed on former log storage area and contains about 50% open water. The second cell (Gearheart Marsh), completed in 1981, was constructed on former pasture land and contains about 80% open water. The third cell (Hauser Marsh) was constructed in a former borrow pit and contains about 60% open water. These 31 acres of constructed freshwater (effluent) marshes have been supplemented with an additional 70 acres of salt water marshes, freshwater wetlands, brackish ponds, and estuaries to form the Arcata Marsh and Wildlife Sanctuary, all of which has been developed with trails, an interpretive center, and other recreational features. The shallow water zones in these marshes contain a variety of emergent vegetation. The deeper zones contain submerged plants (Sago pondweed) that

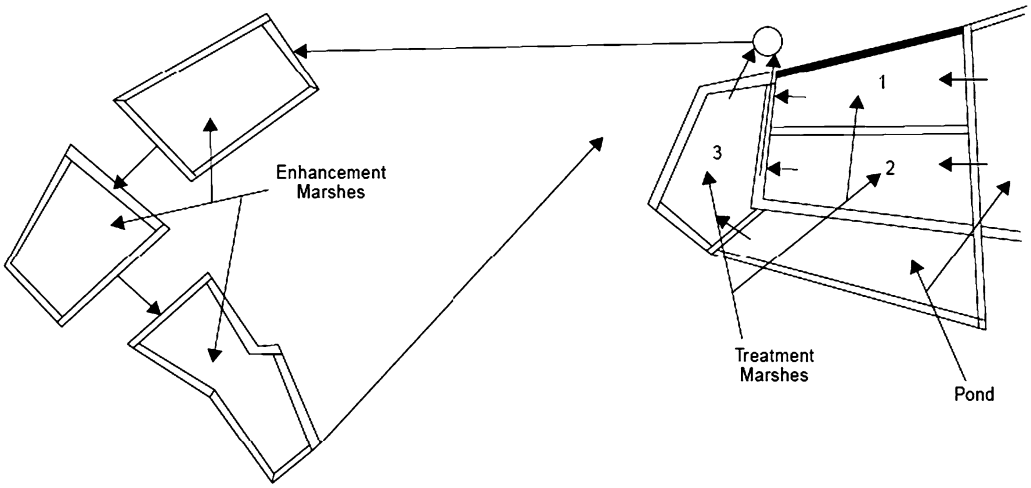

Fig. 10.10. Schematic diagram of wetland system at Arcata, CA (11).

provide food sources for ducks and other birds and release oxygen to the water to further enhance treatment.

The construction costs for the entire system, including modifications to the primary treatment plant, disinfection/dechlorination, pumping stations, and so forth were USD 5,300,000 (1985). Construction costs for the treatment wetlands are only estimated to be about USD 225,000, or USD 30,000 per acre, or USD 78 per 1,000 gpd of design capacity (including removal of sludge from this site, which was previously a sedimentation pond for an aerated lagoon). This does not include pumping costs to transfer final effluent back to the chlorination contact basin, disinfection facilities, or the pumping and piping costs to reach the enhancement marshes. Land costs also are not included since the treatment wetlands were located on city-owned property.

Performance data were collected for a two-year period during the Phase 1 pilot testing program. This program varied the flow rate and water depth in each of the two cells to compare BOD removal performance at different detention times and loading rates that would represent the potential range for full-scale application at Arcata. These data are summarized in Table 10.8. The BOD and TSS in the pond effluent varied considerably during this period, and not all of the cells were uniformly vegetated. Seasonal variations in performance were observed, but Table 10.8 presents only the average effluent characteristics for each of the cells over the entire study period. It is apparent from the data that the wetlands were able to produce excellent effluent quality over the full range of loadings and detention times used.

The long-term average performance of the Arcata system is summarized in Table 10.9. It is clear that both the treatment and enhancement marshes provide significant treatment for BOD and TSS. The long-term removals follow the pilot project results. Most of the nitrogen is removed during the final stage in the enhancement marshes. This is because of the long hydraulic detention time (HRT = 9 d), the availability of oxygen and nitrifying organisms in the open water zones, and anoxic conditions for denitrification in the areas with emergent vegetation.

Table 10.8
Summary of results, phase 1 pilot testing, Arcata, CA (11)

Item	HRT (d)	HLR (gal/ft^{2}d)	BOD (mg/L)	TSS (mg/L)	Fecal coliform (CFU/100 mL)
Influent		26	37	3,183	
Effluent					
Cell 1	2.1/10.7	5.89/1.22	11	6.8	317
Cell 2	1.5/17	5.89/0.5	14.1	4.3	272
Cell 3	2.7/29	4.66/0.5	13.3	4.7	419
Cell 4	1.5/15	5.39/0.5	12.7	5.6	549
Cell 5	3.7	2.94	14.0	4.3	493
Cell 6	5.2	2.4	10.7	4.0	345
Cell 7	5.2	4.4	13.3	7.3	785
Cell 8	5.2	2.4	15.3	7.2	713
Cell 9	6.6	1.71	11.9	9.4	318
Cell 10	3.8	1.71	12.6	4.9	367
Cell 11	7.6	1.47	9.4	5.7	288
Cell 12	5.5	1.47	9.0	4.3	421

Table 10.9
Long term average performance, Arcata (11)

Location	BOD (mg/L)	TSS (mg/L)	TN (mg/L)
Raw influent	174	214	40
Primary effluent	102	70	40
Pond effluent	53	58	40
Wetlands	28	21	30
Enhancement marshes	3.3	3	3

The treatment wetlands (7.5 acres), with nominal HRTs of 3 days, met weekly limits of 30-mg/L BOD and TSS 90% of the time. The enhancement wetlands (28 acres), with a nominal HRT of 11 days, met weekly limits of less than 5-mg/L BOD/TSS 90% of the time. Performance of both wetlands results primarily from proper operation and appropriate design that involves a combination of emergent vegetation and open water zones. TSS levels are higher in cell effluents where outlets are located in open water zones. Recent advances in wetland waste treatment can be found from the literature (18–20).

NOMENCLATURE

Symbol Definition Units (SI)

AN = Ammoniacal nitrogen
A_s = Surface area of wetland, m^2
BOD = Biochemical oxygen demand, mg/L

C = Carbon
C_e = Effluent pollutant concentration, mg/L
CH_4 = Methane
C_o = Influent pollutant concentration, mg/L
CO_2 = Carbon dioxide
COD = Chemical oxygen demand
°C = Degree Celsius (centigrade), °C
d_m = Depth of media, m
d_w = Depth of water from media surface, m
EPA = Environmental protection agency
FWS = Free water surface
HFS = Horizontal flow system
HLR = Hydraulic loading rate, m/d
HRT = Hydraulic retention time
k_T = Temperature dependent first-order reaction rate constant, d^{-1}
k_{20} = Rate constant at 20°C
L = Length of the wetland cell, m
n = Porosity, or the space available for water to flow through the wetland decimal
N = Nitrogen
NH_4^+ = Ammonium ion
NO_2 = Nitrogen dioxide
N_2 = Nitrogen
N_2O = Nitrous oxide
NO_2^- = Nitrite
NO_3^- = Nitrate
NH_3 = Free ammonia, mg/L
NH_{4eff} = Effluent ammonia, mg/L
$NO_{3\,inf}$ = Influent nitrate, mg/L
NO_{4eff} = Effluent nitrate, mg/L
O_2 = Oxygen
P = Phosphorus
Q = The average flow through the wetland, m^3/d
RZM = Root zone method
RRF = Rock–reed filter
rz = The percent of wetland bed depth occupied by root zone decimal
SF = Surface flow
SSF = Subsurface flow
SS = Suspended solids, mg/L
SS_e = Effluent SS, mg/L
SS_o = Influent SS, mg/L
spp. = Species
t = Hydraulic retention time, d
T = Temperature

TKN = Total Kjeldahl nitrogen, mg/L
TSS = Total suspended solids
TN = Total nitrogen
US = United States
V = Volume of water in the system, m^3
VFS = Vertical flow system
VSB = Vegetated submerged bed
W = Width of the wetland cell, m
θ = Temperature coefficient

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11
Modeling of Biosorption Processes

Khim Hoong Chu and Yung-Tse Hung

CONTENTS

Abstract Biosorption entails the use of microbial or plant biomass, usually inactivated, to remove toxic metal ions in aqueous solutions. It is particularly effective in dealing with low concentration, high volume metal waste streams. Although biosorption processes have not yet been commercialized to any significant extent, they offer a promising area for future developments. This chapter presents several process models that can facilitate the design and analysis of batch and fixed bed biosorption systems.

1. INTRODUCTION

The pollution and health problems that are caused by toxic metals are of increasing concern to the general public. In addition to anthropogenic sources, metal pollution may be attributed to natural processes. For example, arsenic is a naturally occurring element in the earth's crust. Its presence in ground and surface waters in many parts of the world is believed to originate from geological reactions. Arsenic is considered as a soft metal and its toxicity effects are similar to those of lead and mercury (1). Ingesting high levels of arsenic over many years can cause cancers of the skin, liver, lung, kidney, and bladder as well as neurological and cardiovascular problems.

It has been well documented that arsenic contamination in drinking water has created a serious health crisis in countries like India and Bangladesh, where millions of people already show symptoms of arsenic poisoning. The United States Geological Survey reported that more than 10% of tested groundwater samples had arsenic concentrations exceeding 10 μg/L in 24% of the US counties surveyed (2). Regulations in many parts of the world stipulate that metals such as mercury, copper, cadmium, lead, chromium, and arsenic be removed from

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_11,

potable water supplies and waste streams down to parts per billion levels. For instance, the World Health Organization's recommended guideline for arsenic in drinking water is 10 μg/L, and public water systems in the USA must comply with a new EPA standard of 10 μg/L for arsenic in drinking water beginning January 2006. The increasingly stringent regulations are posing formidable challenges to the scientific community involved in developing highly efficient metal removal technologies while keeping costs to a minimum. One potential metal removal technology, which may satisfy the dual requirement of high efficiency and low cost, is biosorption. The cost of biomass can be kept to a minimum through the use of industrial biomass byproducts generated by the fermentation industry, biomass propagated through inexpensive means, or biomass harvested from nature.

Biosorption entails the use of microbial or plant biomass, usually inactivated, to remove metal ions in aqueous solutions. It is particularly effective in dealing with low concentration, high volume metal waste streams. Over the last 20 years, numerous biomass types including bacteria, yeasts, fungi, microalgae, and macroalgae as well as heterogeneous biomass such as activated sludge have been tested for their ability to treat water contaminated with trace quantities of metal ions (3). The metal sequestration ability of biological materials is attributed to the presence of a myriad of functional groups or ligands on the biomass surface, which are able to interact with metal ions. The interactions of metal ions with these functional groups are various and, for the most part, not well understood. Results reported to date indicate that most biomass species are capable of interacting with a wide range of heavy metal ions. Efforts have been made to impart specificity through chemical modification of the ligands of biomass. Nevertheless, given the numerous species of biomass, it is not inconceivable that a natural biomass may be found that can remove a specifically targeted metal ion from complex mixtures. Consequently, biosorption may have potential use not only for the remediation of metal-contaminated waste streams, but also for the recovery of metals for recycling. Although biosorption processes have not yet been commercialized to any significant extent, they offer a promising area for future developments.

Because most natural biomass is soft and fragile, the use of biomass on a large scale causes troublesome solids handling problems. Commercial applications of biosorbents will most likely be conducted using fixed bed columns that are widely used in conventional activated carbon and ion exchange systems. Such applications require that the mechanical strength of biomass be enhanced in order to avoid operational problems such as clogging or pressure drop fluctuations. Indeed, three commercial biosorbents developed so far (Bio-Fix™, AMT-BIOCLAIM™, and AlgaSORB™) are produced in the form of porous beads, which can be packed into fixed bed columns. A large body of knowledge exists in the adsorption literature that is applicable to the design and analysis of biosorption processes based on spherical, porous beads (4, 5). This chapter presents several process models that can facilitate the design and analysis of biosorption systems. Mathematical models for predicting the performance of batch and fixed bed biosorption processes are included. Because of mathematical and numerical complexities associated with rigorous adsorption process models, which are usually cast in the form of partial differential equations, this chapter places emphasis on models that can be solved analytically or simplified to yield analytical approximations. Modern high-speed computers coupled with the availability of user-friendly software packages for solving sets of

partial differential equations have greatly reduced the need for analytic solutions. Nonetheless, from the perspective of preliminary process design, analytic expressions are more convenient to use, computationally simpler, and could have immediate practical benefits. Moreover, more rigorous mathematical models than those discussed here generally require inordinate effort to generate substantial data for model validation and parameter estimation.

2. BATCH OPERATION

2.1. Batch Process Models

Batch biosorption processes are relatively simple to operate, requiring easily available equipment such as vessels and stirrers. Batch operation is especially suited for treating low concentration, high volume waste streams containing toxic metal contaminants. A typical batch operation comprises a series of steps. First, a vessel containing a metal-laden solution in contact with a biosorbent is agitated for a period of time to allow the wastewater to reach the discharge limits. Second, the treated solution is withdrawn for discharge. Third, a small amount of an eluant is added to the vessel which is agitated for a period of time to elute the adsorbed metal. Fourth, the spent eluant containing the eluted metal is withdrawn. Fifth, a wash step may be used to condition the biosorbent for reuse in the next cycle of treatment.

A typical design problem entails estimating the quantity of biosorbent needed to process a given volume of a metal-contaminated waste solution. The design procedures are fairly simple for well mixed batch systems, where equilibrium is achieved. However, biosorption may be slow in cases where immobilized biomass beads are used owing to intrabead mass transfer resistance. If sufficient time is allowed for equilibrium to be reached, the design of single-stage batch systems is based on mass balances and thermodynamic equilibrium relationships. The mass balance is given by:

$$V(c_o - c_e) = V_m(q_e - q_o), \tag{1}$$

where c_o and c_e are the initial and final metal concentration in the bulk solution, q_o and q_e are the initial and final metal concentration in the biosorbent, V is the amount of solution, and V_m is the amount of biosorbent. q_o is of course equal to zero when a biosorbent initially free from the metal contaminant is used. When the properties of the waste solution to be treated (c_o and V) and the discharge limit (c_e) are specified, it is still not possible to estimate the amount of biosorbent required (V_m) from Eq. (1) because q_e is unknown. Equation (1) must be solved in conjunction with the equilibrium isotherm, which relates q_e to c_e at constant temperature. Unlike gas-phase isotherms, liquid-phase isotherms are generally a weak function of temperature, but they are strongly affected by factors such as solution pH and ionic strength. In general, the equilibrium isotherm for a given metal–biosorbent system cannot be predicted from theory and experiments are imperative. In Sect. 2, we consider how biosorption equilibrium data are generated and modeled.

2.2. Equilibrium Isotherms

An equilibrium isotherm defines the equilibrium distribution of a metal contaminant between the solution and the biosorbent at a fixed temperature. Biosorption equilibrium data

can virtually never be predicted, but must be measured. Batch experiments are often used to generate equilibrium data owing to their simplicity. When equilibrium is established in a single-metal batch biosorption system, from the mass balance given in Eq. (1) we can write:

$$q_e = q_o + \frac{V}{V_m}(c_o - c_e). \tag{2}$$

It is generally difficult to measure q_e directly. The standard approach is to measure c_e; q_e can then be calculated from Eq. (2) for batch experiments with known initial solution concentration (c_o), amount of solution (V), amount of biosorbent (V_m), and initial metal concentration on the biosorbent (q_o). A series of batch experiments is conducted by varying either the initial metal concentration or amount of biosorbent to generate pairs of q_e vs. c_e data. These experimentally generated q_e vs. c_e equilibrium data are used to construct the equilibrium isotherm graphically. For biosorption systems containing a single metal contaminant, the equilibrium isotherm is a function of environmental factors such as pH, ionic strength, and temperature. Once these factors are fixed, the equilibrium isotherm is, in principle, independent of the experimental conditions employed to measure it. In other words, a unique isotherm can be generated by using any convenient measurement method (batch or continuous-flow) and by varying either c_o or V/V_m.

Biosorption equilibria can be expressed in mathematical form by fitting the data on q_e vs. c_e to isotherm equations that are commonly used in the gas adsorption literature (4). Because most biosorption data on q_e vs. c_e over a wider range of solution concentration concave toward the abscissa, they are described as "favorable." A typical favorable isotherm is shown in Fig. 11.1. Such favorable shape can be described in mathematical form by the well-known Langmuir equation (6), which is given by:

$$q_e = \frac{q_m b c_e}{1 + b c_e}, \tag{3}$$

where q_m is the maximum or saturation uptake capacity and b is an affinity constant. The two adjustable parameters q_m and b provide good flexibility in correlating the favorable isotherm commonly observed in biosorption. It should be noted that metal biosorption isotherms

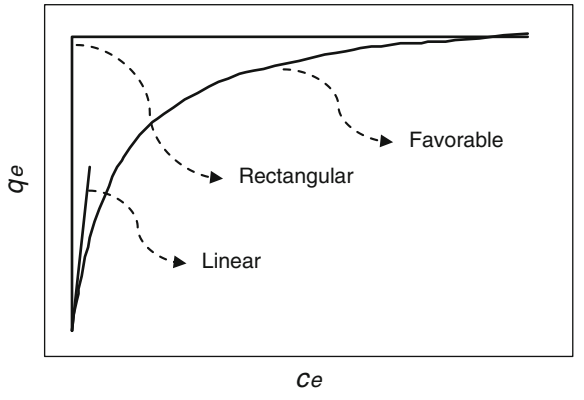

Fig. 11.1. Shapes of favorable, linear, and rectangular isotherms.

frequently resemble Langmuirian isotherms without actually obeying the thermodynamics of the Langmuir model. Nevertheless, the Langmuir model has been used successfully in numerous biosorption studies to correlate biosorption equilibrium data. Consequently, the Langmuir equation is essentially an empirical fitting tool and its parameters have only qualitative mechanistic relevance. It is reasonable to assume that any isotherm equation involving more than two parameters will offer greater flexibility than the Langmuir equation for capturing the shape of an experimentally observed isotherm. A variety of different isotherm equations, which have been developed for gas-phase adsorption, may be used in a purely empirical way to describe biosorption equilibrium data (7).

Once the Langmuir isotherm parameters have been determined from a nonlinear least-squares fit of experimental equilibrium data, the Langmuir equation [Eq. (3)] can be combined with the mass balance equation [Eqs. (1) or (2)] to yield the following design equation which predicts the amount of biosorbent per unit volume of solution (V_m/V) required to reduce the metal contaminant concentration from c_o to c_e:

$$\frac{V_m}{V} = \frac{(c_o - c_e)(1 + bc_e)}{q_m b c_e}. \tag{4}$$

Alternatively, the extent of biosorption can readily be calculated from Eqs. (2) and (3) once c_o, V_m/V, q_m, and b are known. Simultaneous solution of Eqs. (2) and (3) yields the following equations for c_e and q_e:

$$c_e = \frac{\sqrt{h^2 + 4c_o/b} - h}{2} \tag{5a}$$

$$q_e = \frac{V}{V_m}\left(c_o - \frac{\sqrt{h^2 + 4c_o/b} - h}{2}\right), \tag{5b}$$

where

$$h = \frac{1}{b} + \frac{V_m}{V} q_m - c_o. \tag{5c}$$

It may not be possible to find an analytical solution when other nonlinear isotherm equations are used. In that case, the solution may be found graphically. Once c_o and V/V_m are chosen, Eq. (2) indicates that plotting q_e against c_e will give a linear line with a negative slope. This is known as the operating line. The intersection of the operating line with the isotherm plot gives the equilibrium concentrations q_e and c_e. Examples 1 and 2 illustrate the use of the analytical and graphical solution methods.

When the metal concentration in solution is sufficiently small, the equilibrium may be modeled by a linear isotherm:

$$q_e = K c_e, \tag{6}$$

where K is an equilibrium constant. On the other hand, when the isotherm is highly favorable, it may be approximated as a rectangular or irreversible isotherm:

$$q_e = q_m. \tag{7}$$

Equation (7) implies that q_e is independent of the solution concentration and goes straight up from the origin to q_m and then extends horizontally from that value. Many biosorption isotherms published in the literature can indeed be approximated as rectangular with negligible error. As will be discussed later, from a mathematical viewpoint these two limiting forms of a favorable isotherm, sketched in Fig. 11.1, are very useful as they allow derivation of analytical approximations from rigorous process models.

2.3. Rate Models

As mentioned earlier, batch biosorption may be slow in cases where immobilized biomass beads are used owing to slow intrabead diffusion. A related design problem would be to estimate the time needed to process a given waste stream in a batch contactor. Various rate models have been developed for spherical, porous adsorbents such as activated carbon and ion-exchange resins. In principle, these models can be applied directly to describe the kinetic behavior of biomass immobilized in porous support. Three of these literature models, pore diffusion, homogeneous surface diffusion, and second-order reversible reaction, are described here. These models are selected because either asymptotic solutions exist for limiting cases or they can be solved analytically which facilitate greatly the design of batch systems or analysis of experimental data.

2.4. Pore Diffusion Model

Within the context of this model it is assumed that intrabead mass transfer occurs by diffusion in liquid-filled pores with a driving force expressed in terms of the pore liquid concentration gradient. In addition, external boundary layer mass transfer resistance is included in this model. Accordingly, the following conservation equations and initial and boundary conditions describe the biosorption kinetics for spherical biomass beads of radius R in a closed batch system:

For the biomass bead:

$$(1-\varepsilon_p)\frac{\partial q_s}{\partial t}+\varepsilon_p\frac{\partial c_p}{\partial t}=\frac{D_e}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_p}{\partial r}\right) \tag{8a}$$

with initial and boundary conditions:

$$t=0,\ c_p=0,\ q_s=0, \tag{8b}$$

$$r=0,\ \frac{\partial c_p}{\partial r}=0, \tag{8c}$$

$$r=R,\ D_e\frac{\partial c_p}{\partial r}=k_f(c-c_{pi}) \tag{8d}$$

and for the bulk liquid:

$$\frac{dc}{dt}=-\frac{3k_f}{R}\frac{V_m}{V}(c-c_{pi})=-\frac{V_m}{V}\frac{d\bar{q}}{dt} \tag{9a}$$

with initial condition:

$$t=0,\ c=c_o. \tag{9b}$$

In these equations, c_p is the metal concentration in the pore liquid, c_{pi} is the metal concentration in the pore liquid adjacent to the bead surface, c is the metal concentration in the bulk liquid, q_s is the metal concentration in the bead's solid expressed on a pore-free volume basis, $\bar{q}$ is the metal concentration in the bead averaged over the bead volume, t and r are the time and bead radial coordinate, ε_p is the bead porosity, D_e is the effective pore diffusivity which is assumed constant, and k_f is the external boundary layer mass transfer coefficient.

Assuming that the adsorbed metal is in equilibrium with the pore liquid at each radial position within the bead, Eq. (8a) can be written as:

$$\left[(1-\varepsilon_p)\frac{dq_s}{dc_p}+\varepsilon_p\right]\frac{\partial c_p}{\partial t}=\frac{D_e}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c_p}{\partial r}\right). \tag{10}$$

The quantity dq_s/dc_p is the slope of the equilibrium isotherm. When the isotherm is nonlinear, a numerical solution of Eqs. (8a) and (10) is required. However, when the isotherm is very favorable it may be assumed to be rectangular. For a rectangular isotherm the following analytical solution is found (8):

$$\frac{D_e c_o}{R^2 q_m}t=\left(1-\frac{1}{\mathrm{Bi}}\right)I_2-I_1, \tag{11a}$$

where

$$I_1=\frac{1}{6\lambda\Lambda}\ln\left[\frac{\lambda^3+\eta^3}{\lambda^3+1}\left(\frac{\lambda+1}{\lambda+\eta}\right)^3\right]+\frac{1}{\lambda\Lambda\sqrt{3}}\left[\tan^{-1}\left(\frac{2\eta-\lambda}{\lambda\sqrt{3}}\right)-\tan^{-1}\left(\frac{2-\lambda}{\lambda\sqrt{3}}\right)\right], \tag{11b}$$

$$I_2=\frac{1}{3\Lambda}\ln\left(\frac{\lambda^3+\eta^3}{\lambda^3+1}\right) \tag{11c}$$

with

$$\eta=\left(1-\frac{\bar{q}}{q_m}\right)^{1/3} \tag{11d}$$

$$\Lambda=\frac{V_m q_m}{V c_o} \tag{11e}$$

$$\lambda=\left(\frac{1}{\Lambda}-1\right)^{1/3} \tag{11f}$$

$$\mathrm{Bi}=\frac{k_f R}{D_e}. \tag{11g}$$

The above asymptotic solution can be used to estimate the two mass transfer coefficients k_f and D_e by fitting Eq. (11a) to the experimental uptake curves of batch biosorption systems with known c_o, V_m, V, R, and q_m. We illustrate the use of this asymptotic expression in Example 3.

2.5. *Homogeneous Surface Diffusion Model*

In this model it is assumed that the rate of metal uptake is controlled by the boundary layer and intrabead mass transfer resistances. The model assumes intrabead diffusion occurs as a function of the concentration gradient of adsorbed metal. In addition, the following assumptions are made: uniform spherical biomass bead, local equilibrium within biomass bead, and constant diffusivity. The batch uptake kinetics described in terms of the two mass transfer resistances are given by the following set of conservation equations and initial and boundary conditions.

For the biomass bead:

$$\frac{\partial q}{\partial t} = \frac{D_s}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial q}{\partial r}\right) \tag{12a}$$

with initial and boundary conditions:

$$t = 0,\ q = 0, \tag{12b}$$

$$r = 0,\ \frac{\partial q}{\partial r} = 0, \tag{12c}$$

$$r = R,\ D_s\frac{\partial q}{\partial r} = k_f(c - c_i) \tag{12d}$$

and for the bulk liquid:

$$\frac{dc}{dt} = -\frac{3k_f}{R}\frac{V_m}{V}(c - c_i) = -\frac{V_m}{V}\frac{d\bar{q}}{dt} \tag{13a}$$

with initial condition:

$$t = 0,\ c = c_o. \tag{13b}$$

In these equations, q is the adsorbed metal concentration, c_i is the metal concentration in the bulk liquid adjacent to the bead surface, and D_s is the effective diffusivity. Local equilibrium is assumed to exist at the bead–liquid interface, where c_i varies with time and is related to $q_i(t, r = R)$ through the equilibrium isotherm.

When the isotherm is nonlinear, a numerical solution of Eqs. (12a) and (13a) is generally required. The model, however, can be solved analytically for certain limiting cases. When the isotherm is very favorable, the metal concentration in the biomass bead is nearly constant and essentially independent of the metal concentration in the bulk liquid. For these conditions, Helfferich and Hwang (9) have shown that a simple criterion is available to determine the relative importance of external and intrabead mass transfer resistances in terms of the dimensionless group δ which is given as:

$$\delta = \frac{1}{5}\frac{k_f R}{D_s}\frac{c_o}{q_m}. \tag{14}$$

When $\delta < 1$ the external film resistance is dominant. In that case, metal concentration at the bead surface is negligibly small ($c_i \sim 0$) and the uptake rate is proportional to the bulk liquid

phase concentration (c). Thus, Eq. (13a) can be integrated directly yielding the following limiting solution (10):

$$\frac{c}{c_o} = \exp\left(-\frac{3k_f}{R}\frac{V_m}{V}t\right). \tag{15}$$

Conversely, when $\delta > 1$ intrabead diffusion is dominant. Under these conditions, the metal concentration adjacent to the bead surface is nearly constant and equal to the saturation capacity ($q_i \sim q_m$) when the isotherm is rectangular. The uptake rate is then essentially independent of the bulk liquid phase concentration (c), and the solution can be approximated by integrating Eq. (12a) directly. The limiting solution under intrabead mass transfer control is given as (10):

$$\frac{\bar{q}}{q_m} = 1 - \frac{6}{\pi^2}\sum_{k=1}^{\infty}\frac{1}{k^2}\exp\left(-\frac{k^2\pi^2 D_s t}{R^2}\right). \tag{16}$$

Because δ is directly proportional to c_o, its magnitude may be adjusted in stirred batch experiments by operating with very low and very high solution concentrations. The two mass transfer coefficients k_f and D_s can, therefore, be determined independently by fitting Eq. (15) to the concentration-time data obtained at low metal concentration (external mass transfer control) and by fitting Eq. (16) to the concentration-time data obtained at high metal concentration (intrabead mass transfer control) for a given batch biosorption system. It should be noted that, in principle, both k_f and D_s are concentration dependent. However, in practice k_f is mainly dependent on hydrodynamics, while D_s has been found to increase with the adsorbed solute concentration. In addition, k_f may be estimated from well-established correlations, while it is difficult to make a priori prediction of D_s. Example 4 illustrates how D_s may be obtained from an analysis of transient batch uptake data. Once k_f and D_s are known, a full solution of the homogeneous surface diffusion model [Eqs. (12a) and (13a)] allows one to predict the kinetic behavior of agitated batch contactors once c_o, V_m, V, and R are known and the isotherm is defined.

When the isotherm is linear and surface diffusion controls, an analytical solution is available for the case of negligible external resistance (11). The expression for the fractional uptake curve for biosorption from a well-mixed solution of finite volume is:

$$\frac{\bar{q}}{q_\infty} = 1 - \sum_{k=1}^{\infty}\frac{6\alpha(\alpha+1)\exp\left(-D_s\beta_k^2 t/R^2\right)}{9 + 9\alpha + \beta_k^2\alpha^2}, \tag{17a}$$

where q_∞ is the final metal uptake when equilibrium is established in the batch contactor. α is given by:

$$\alpha = \frac{V}{V_m K} \tag{17b}$$

while β_k is given by the nonzero roots of the equation:

$$\tan\beta_k = \frac{3\beta_k}{3 + \alpha\beta_k^2} \tag{17c}$$

Once V, V_m, and R are specified, and the equilibrium constant K estimated from batch equilibrium experiments, the diffusion coefficient D_s may be estimated by fitting Eq. (17a) to the fractional uptake curves of batch kinetic experiments.

2.6. Second-Order Reversible Reaction Model

The differential material balance for a batch biosorption system is given by

$$V_m \frac{d\bar{q}}{dt} = -V \frac{dc}{dt}. \tag{18}$$

In the second-order reversible reaction model, the biosorption of a metal contaminant to biomass bead is assumed to be monovalent and homogeneous, according to the following reversible reaction:

$$M + A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} M \cdot A, \tag{19}$$

where M represents the metal contaminant, A represents an adsorption site on the biomass, $M \cdot A$ is the metal–biomass complex, k_1 is the second-order forward rate constant, and k_2 is the first-order reverse rate constant. The rate of metal biosorption for the above reaction scheme can be expressed as:

$$\frac{d\bar{q}}{dt} = k_1 c(q_m - \bar{q}) - k_2 \bar{q}. \tag{20}$$

At equilibrium ($d\bar{q}/dt = 0$), Eq. (20) results in the familiar Langmuir isotherm model:

$$\bar{q} = q_e = \frac{q_m b c_e}{1 + b c_e}, \tag{21}$$

where b is provided by the following equation:

$$b = \frac{k_1}{k_2}. \tag{22}$$

Substituting Eq. (22) into Eq. (20) gives:

$$\frac{d\bar{q}}{dt} = k_1 \left[c(q_m - \bar{q}) - \frac{1}{b}\bar{q} \right]. \tag{23}$$

The integration of Eqs. (18) and (23) with the appropriate initial conditions yields the following analytical solution (12):

$$\frac{c}{c_o} = 1 - \frac{1}{c_o}\frac{V_m}{V} \frac{(\mu + \omega)\left[1 - \exp\left(-2\mu \frac{V_m}{V} k_1 t\right)\right]}{\left[\frac{(\mu+\omega)}{(\omega-\mu)} - \exp\left(-2\mu \frac{V_m}{V} k_1 t\right)\right]}, \tag{24a}$$

in which μ and ω are defined as:

$$\mu^2 = \omega^2 - c_o q_m \frac{V}{V_m} \tag{24b}$$

$$\omega = \frac{1}{2}\left(c_o \frac{V}{V_m} + q_m + \frac{1}{b}\frac{V}{V_m} \right). \tag{24c}$$

Equation (24a) is the solution of the rate model predicated on the kinetic form of the Langmuir isotherm from which the concentration–time profile for a given batch system can be calculated. When the experimental conditions are specified (c_o, V, and V_m) and the equilibrium parameters (q_m and b) determined from batch equilibrium experiments, Eq. (24a) can be fitted to the batch concentration–time data, in order to identify the rate constant k_1. Once k_1 is known, k_2 can be calculated from Eq. (22). Example 5 illustrates the use of Eq. (24a). It should be noted that this model assumes that both external film and intrabead diffusion steps are instantaneous and that the surface interaction step is rate-limiting. For diffusion-controlled biomass beads, the rate constants k_1 and k_2 are thus not the intrinsic rate constants reflecting metal interaction with the biomass bead, but rather are lumped coefficients that reflect the contributions of mass transfer as well.

3. COLUMN OPERATION

3.1. Fixed Bed Process Models

The limited number of commercial biosorbents developed so far (Bio-Fix™, AMT-BIOCLAIM™, and AlgaSORB™) are produced in the form of porous beads, which possess strong mechanical strength. These biomass beads can be used in continuous-flow fixed bed columns. In such systems, the concentration profiles in the liquid and biosorbent phases vary in both space and time. As a result, the design and optimization of fixed bed columns are difficult to carry out a priori without a quantitative modeling approach. From the perspective of process modeling, the dynamic behavior of a fixed bed column is described in terms of the effluent concentration–time profile, i.e., the breakthrough curve.

A typical breakthrough curve for a contaminant is shown in Fig. 11.2 as the ratio of the effluent concentration (c) to the influent concentration (c_F) vs. time or throughput volume. The shape of this curve is determined by the shape of the equilibrium isotherm and is influenced by the individual transport processes in the column and in the biomass bead. The most efficient biosorption performance will be obtained when the shape of the breakthrough curve is as sharp as possible. Figure. 11.2 shows that for short times the contaminant in the feed is

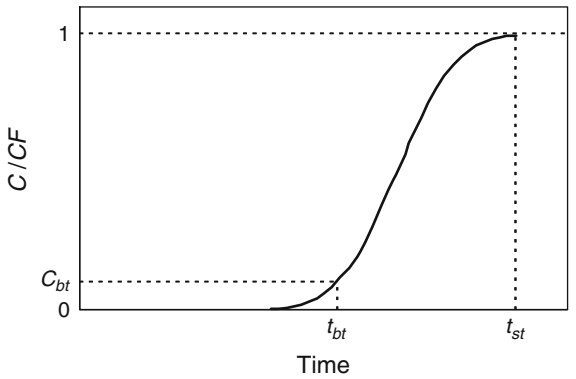

Fig. 11.2. A typical breakthrough curve.

taken up completely by the column. After a while, contaminant breakthrough occurs and the effluent concentration increases with time. It is normal practice in single column operations to terminate the influent flow at the breakthrough time (t_{bt}) at which the contaminant reaches a specified concentration, c_{bt}. For multiple columns operated in series, loading of the columns continues until the saturation point (t_{st}) is reached at which the effluent concentration becomes equal to the feed concentration. The variation of the breakthrough and saturation points with respect to operating variables such as the influent flow and feed concentration is, therefore, of great practical interest. The general position of the breakthrough curve along the time or effluent volume axis depends on the capacity of the column with respect to the amount of contaminant applied to the column. The actual size of a biosorption column is thus determined from the capacity at breakpoint.

We will begin our study of single-column biosorption with a description of the differential mass balance equation for a continuous-flow fixed bed column. The model is predicated on isothermal biosorption of a single metal contaminant and constant linear velocity for the liquid. The differential mass balance for the column is given by:

$$\nu \frac{\partial c}{\partial \mathrm{z}} + \frac{\partial c}{\partial t} + \frac{(1-\varepsilon)}{\varepsilon} \frac{\partial \bar{q}}{\partial t} = D_{\mathrm{L}} \frac{\partial^2 c}{\partial z^2} \tag{25a}$$

with initial and boundary conditions:

$$t = 0, \quad c = \bar{q} = 0, \tag{25b}$$

$$z = 0, \quad \frac{D_{\mathrm{L}}}{\nu} \frac{\partial c}{\partial z} = c - c_{\mathrm{F}}, \tag{25c}$$

$$z = L, \quad \frac{\partial c}{\partial z} = 0, \tag{25d}$$

where ν is the interstitial velocity, D_{L} is the axial dispersion coefficient, ε is column void fraction, L is the column length, and z is the column length coordinate. Cooney (13) shows that the effect of axial dispersion can be neglected in liquid-phase systems with negligible error. The D_{L} term in Eqs. (25a) and (25c) is thus set to zero.

3.2. *Rate Models*

Modeling the dynamic behavior of a fixed bed column depends on finding the solution to Eq. (25a) with a suitable rate expression, which relates the rate of metal uptake, $\partial \bar{q}/\partial t$, to c or $\bar{q}$. Various fixed bed process models have been formulated at different levels of complexity, which differ mainly in the choice of rate expression. An excellent account of such models has been given by Ruthven (4). The aim of this section is to present a concise summary of fixed bed process models based on the three rate models described in Sect. 2.3 (pore diffusion, homogeneous surface diffusion, and second-order reversible reaction) and an additional rate model predicated on quasichemical reaction kinetics.

3.3. *Pore Diffusion Model*

A complete fixed bed process model may be constructed by combining the pore diffusion model described by Eq. (8a) and the fixed bed continuity equation described by Eq. (25a).

The solution of the two sets of equations has to be obtained numerically when the equilibrium isotherm is nonlinear. However, for certain limiting forms of the isotherm analytical expressions exist. Weber and Chakravorti (14) gave the following analytical solution assuming a rectangular isotherm and neglecting axial dispersion:

$$(\sigma - 1)N_{\mathrm{p}} = \frac{15}{\sqrt{3}} \tan^{-1}\left[\frac{2\psi + 1}{\sqrt{3}}\right] - \frac{15}{2}\left[\ln(1 + \psi + \psi^2) - \frac{1}{3}\right] + \frac{5}{\mathrm{Bi}}\left[\ln(1 - \psi^3) + 1\right] - \frac{5\pi}{2\sqrt{3}}, \tag{26a}$$

where

$$\sigma = \frac{c_{\mathrm{F}}}{q_{\mathrm{m}}} \frac{\left(\frac{\varepsilon v t}{L} - \varepsilon\right)}{(1 - \varepsilon)}, \tag{26b}$$

$$N_{\mathrm{p}} = \frac{15 D_{\mathrm{e}} L (1 - \varepsilon)}{\varepsilon v R^2}, \tag{26c}$$

$$\psi = \left(1 - \frac{c}{c_{\mathrm{F}}}\right)^{1/3}. \tag{26d}$$

A priori prediction of breakthrough curves (c/c_{F} vs. t) can be obtained from Eq. (26a) provided that the feed concentration (c_{F}), interstitial velocity (v), bead radius (R), column length (L), column void fraction (ε), and bead porosity (ε_{p}) are known. The remaining parameters in Eq. (26a) comprise the equilibrium parameter q_{m} and the two mass transfer parameters k_{f} and D_{e}, which can be estimated from batch experiments, as described in Sects. 2.2 and 2.4. Note that it is possible to estimate k_{f} from engineering correlations. We give an example of how one calculates a breakthrough curve from Eq. (26a) in Example 6.

3.4. Homogeneous Surface Diffusion Model

The same homogeneous surface diffusion model described previously [Eq. (12a)] applies to column operation. In general, a numerical solution is needed because of the nonlinearity of the equilibrium isotherm. An analytical solution of Eqs. (12a) and (25a) assuming a rectangular isotherm and negligible axial dispersion has been obtained by Yoshida et al. (15). Under constant pattern conditions, this solution is given by:

$$\frac{c}{c_{\mathrm{F}}} = \frac{1}{\delta} \exp\left(\tau - \xi + \delta - 1 - \frac{1}{\delta}\right) \quad \text{for } \tau - \xi \leq -\delta + 1 + \frac{1}{\delta} - \ln\left(\frac{1 + \delta}{\delta}\right), \tag{27a}$$

$$\frac{c}{c_{\mathrm{F}}} = 1 - \frac{\delta}{1+\delta} \exp\left\{\frac{1}{\delta}\left[-\tau + \xi - \delta + 1 + \frac{1}{\delta} - \ln\left(\frac{1 + \delta}{\delta}\right)\right]\right\} \quad \text{for } \tau - \xi \geq -\delta + 1 + \frac{1}{\delta} - \ln\left(\frac{1 + \delta}{\delta}\right) \tag{27b}$$

when $\delta \geq 1$, and by

$$\frac{c}{c_F} = \exp(\tau - \xi - 1)$$
$$\text{for } \tau - \xi \leq 1 - \ln(1+\delta), \tag{27c}$$
$$\frac{c}{c_F} = 1 - \frac{\delta}{1+\delta} \exp\left\{\frac{1}{\delta}[-\tau + \xi + 1 - \ln(1+\delta)]\right\}$$
$$\text{for } \tau - \xi \geq 1 - \ln(1+\delta) \tag{27d}$$

when $\delta \leq 1$. δ, τ, and ξ in Eqs. (27a)–(27d) are defined as:

$$\delta = \frac{1}{5}\frac{k_f R}{D_s}\frac{c_F}{q_m}, \tag{27e}$$
$$\tau = \frac{3k_f}{R}\frac{c_F}{q_m}\left(t - \frac{L}{v}\right), \tag{27f}$$
$$\xi = \frac{3k_f}{R}\frac{(1-\varepsilon)}{\varepsilon}\frac{L}{v}. \tag{27g}$$

Note that for the sake of convenience, the above analytical expressions have been derived by replacing Eq. (12a) with a linear driving force approximation (15). Since the equilibrium capacity, q_m, and the diffusion coefficient, D_s, may be estimated from laboratory-scale batch experiments while the external mass transfer coefficient, k_f, may be estimated from established correlations, Eqs. (27a)–(27d) provide a priori prediction of breakthrough curves once the feed concentration (c_F), interstitial velocity (v), bead radius (R), column length (L), and column void fraction (ε) are specified. Example 7 illustrates how a breakthrough curve may be calculated from Eqs. (27a)–(27d).

Fixed bed models that consider intrabead diffusion such as the pore diffusion and homogeneous diffusion models described above require a numerical solution of the governing equations when the isotherm is nonlinear. With present-day computing facilities this is no longer an intractable problem. However, semiempirical or short-cut methods are still used extensively for the initial design and analysis of fixed bed adsorption columns. Some of the widely used semiempirical models for simulating breakthrough curves of activated carbon columns are well covered in the recent book by Cooney (5). In general, these semiempirical models are easier to use and more efficient from a computational point of view than rigorous mechanistic models, which are much more complicated mathematically. The semiempirical approach, however, requires breakthrough information gained from extensive pilot-scale tests, which is used in model calibration and verification in order to ensure that the effects of various operating conditions and design parameters are properly accounted for. We describe here two such semiempirical fixed bed models that are predicated on the chemical reaction type of rate equations: a second-order reversible reaction model and a quasichemical kinetic model.

3.5. Second-Order Reversible Reaction Model

The rate equation for this model is given by Eqs. (20) or (23). The analytical solution to Eqs. (20) and (25a) neglecting axial dispersion, first obtained by Thomas (16), is given by:

$$\frac{c}{c_F} = \frac{J(n/r_T, nT)}{J(n/r_T, nT) + \left[1 - J(n, nT/r_T)\right] \exp\left[(1 - 1/r_T)(n - nT)\right]} \tag{28a}$$

with

$$r_T = 1 + bc_F, \tag{28b}$$

$$n = \frac{(1-\varepsilon)}{\varepsilon} \frac{q_m k_1 L}{v}, \tag{28c}$$

$$T = \frac{\varepsilon}{(1-\varepsilon)} \frac{(1/b + c_F)}{q_m} \left(\frac{vt}{L} - 1\right). \tag{28d}$$

The function J is given by (17):

$$J(x, y) = 1 - \int_0^x \exp(-y - \theta) I_o(2\sqrt{y\theta}) d\theta, \tag{28e}$$

where I_o refers to a zero-order modified Bessel function of the first kind.

Although mathematically elegant, this solution is too complex and thus of little practical use. When the product of x and y [from Eq. (28e)] is greater than 36, the following approximation can be used, within 1% accuracy, to calculate the value of J:

$$J(x, y) = \frac{1}{2} \left\{ 1 - \text{erf}(\sqrt{x} - \sqrt{y}) + \frac{\exp\left[-(\sqrt{x} - \sqrt{y})^2\right]}{\sqrt{\pi}\left[\sqrt{y} + (xy)^{1/4}\right]} \right\}, \tag{28f}$$

where erf(m) is the error function of m.

Unlike the asymptotic solutions of the pore diffusion and homogeneous surface diffusion models which have been derived by assuming a rectangular isotherm, the Langmuir isotherm is embedded in the analytical solution of the second-order reversible reaction model. However, a major drawback of Eq. (28a) is that the rate constant k_1 is a lumped parameter. As mentioned earlier, k_1 contains the effects of both intrinsic kinetics and mass transfer and its value is, thus, dependent upon the relative magnitudes of these processes which are in turn affected by the operating and system variables of a given fixed bed column. Breakthrough curves computed from Eq. (28a) based on k_1 values estimated from batch experiments (see Sect. 2.6) may not provide good agreement with experimental breakthrough curves if the dominant rate processes are different in the batch and fixed bed systems. A more common approach is to extract k_1 from breakthrough curves obtained from pilot-scale fixed bed tests and determine how k_1 varies with operating variables such as the flow rate and feed concentration. Example 8 illustrates the procedures involved in calculating a breakthrough curve from Eq. (28a), which is commonly referred to as the Thomas model in the adsorption literature.

3.6. *Quasichemical Kinetic Model*

In this model, it is assumed that the metal–biomass interaction can be represented by the following quasichemical kinetic rate expression:

$$\frac{\partial \bar{q}}{\partial t} = k_3 c(q_\mathrm{m} - \bar{q}), \tag{29}$$

where k_3 is a rate constant. This rate equation implies that at equilibrium ($\partial \bar{q}/\partial t = 0$) Eq. (29) reduces to a rectangular equilibrium relationship between the bulk liquid and the biomass bead ($\bar{q} = q_\mathrm{e} = q_\mathrm{m}$).

The analytical solution to Eqs. (29) and (25a) neglecting axial dispersion, first obtained by Bohart and Adams (18), is given by:

$$\frac{c}{c_\mathrm{F}} = \frac{\exp(u)}{\exp(u) + \exp(w) - 1} \tag{30a}$$

with

$$u = k_3 c_\mathrm{F} \left(t - \frac{L}{v} \right), \tag{30b}$$

$$w = \frac{k_3 q_\mathrm{m} L}{v} \left(\frac{1 - \varepsilon}{\varepsilon} \right). \tag{30c}$$

The well-known "bed depth service time" (BDST) approach to fixed bed column design is based on this solution, commonly referred to as the Bohart–Adams model. The rate constant k_3 is a lumped parameter and is often treated as an adjustable parameter, which can be estimated by fitting Eq. (30a) to the experimental breakthrough curves of pilot-scale fixed bed tests. It is likely to be a function of operating variables such as the feed flow rate. Example 9 demonstrates the use of this solution.

Note that the Bohart–Adams model is sometimes inadvertently referred to as the Thomas model in the biosorption literature. The rate expression of the Thomas model, Eq. (20), reduces to the following form when $k_2 \ll k_1$:

$$\begin{aligned} &k_2 \ll k_1 \rightarrow k_2 \bar{q} \ll k_1 c(q_\mathrm{m} - \bar{q}) \\ &\frac{\partial \bar{q}}{\partial t} = k_1 c(q_\mathrm{m} - \bar{q}) - k_2 \bar{q} \rightarrow \frac{\partial \bar{q}}{\partial t} \sim k_1 c(q_\mathrm{m} - \bar{q}). \end{aligned} \tag{31}$$

Comparison with the rate equation of the quasichemical kinetic model [Eq. (29)] shows that the two expressions are equivalent provided that we set $k_1 = k_3$. Likewise, the Langmuir equation employed by the Thomas model reduces to a rectangular isotherm under the same condition:

$$\begin{aligned} &k_2 \ll k_1 \rightarrow \ bc_\mathrm{e} = \frac{k_1}{k_2} c_\mathrm{e} \gg 1, \\ &q_\mathrm{e} = \frac{q_\mathrm{m} b c_\mathrm{e}}{1 + b c_\mathrm{e}} \rightarrow \ q_\mathrm{e} \sim q_\mathrm{m}. \end{aligned} \tag{32}$$

The Bohart–Adams model can therefore be regarded as a limiting form of the Thomas model.

4. EXAMPLES

Example 1

This example describes a batch system where a volume of a metal-contaminated waste solution (V) having an initial metal concentration c_o is placed in an agitated vessel and a quantity of biomass bead (V_m) is added to effect a specified reduction in metal concentration from c_o to c_e. Biosorption is allowed to continue for a time sufficient to essentially achieve equilibrium.

A wastewater containing 50 mg/L cadmium at pH 5.5 and 20°C is to be treated with a certain type of biomass immobilized in polyvinyl alcohol gel to reduce the cadmium level to 5 mg/L. This biosorption is described by the following Langmuir isotherm:

$$q_e = \frac{10{,}000 c_e}{1 + 0.2 c_e},$$

where q_e is in mg/L bead and c_e is in mg/L. Determine the biosorbent dose (liters of biomass bead per liter of solution) needed in a one-stage process.

Solution

Since the system follows a Langmuir isotherm, how much biomass bead needs to be added can be calculated from Eq. (4):

$$\frac{V_m}{V} = \frac{(c_o - c_e)(1 + bc_e)}{q_m b c_e}$$

$$c_o = 50\,\text{mg/L};\ c_e = 5\,\text{mg/L}$$

$$q_m b = 10{,}000\ \text{L solution/L bead};\ b = 0.2\,\text{L/mg}$$

$$\frac{V_m}{V} = \frac{(50 - 5)\,[1 + (0.2)(5)]}{(10{,}000)(5)},$$

$$\frac{V_m}{V} = 1.8 \times 10^{-3}\ \text{L bead/L solution.}$$

Note that it is usually more convenient to express the biosorbent dose in units of mass of biomass bead per unit volume of solution. The unit conversion can be done by multiplying the calculated biosorbent dose by the density of the biomass bead.

Example 2

We plan to add 1×10^{-2} L of the biomass bead described in Example 1 to 5 L of a waste solution containing 40 mg/L cadmium. What final cadmium concentration can we expect for a single-stage batch system? Do this analytically and graphically.

Analytical solution

Since the biosorption system follows a Langmuir isotherm, the final cadmium concentration can be calculated from Eq. (5a):

$$c_e = \frac{\sqrt{h^2 + 4c_o/b} - h}{2},$$

where

$$h = \frac{1}{b} + \frac{V_m}{V} q_m - c_o$$

$$q_m b = 10{,}000$$

$$q_m = \frac{10{,}000}{0.2} = 50{,}000\,\text{mg/L}$$

$$h = \frac{1}{0.2} + \frac{1 \times 10^{-2}}{5}(50{,}000) - 40$$

$$= 65\,\text{mg/L}$$

$$c_e = \frac{\sqrt{65^2 + \frac{4(40)}{0.2}} - 65}{2}$$

$$= 2.94\,\text{mg/L}.$$

Graphical solution

The operating line is easily calculated from Eq. (2):

$$q_e = q_o + \frac{V}{V_m}(c_o - c_e)$$

$$= 0 + \frac{5}{1 \times 10^{-2}}(40 - c_e)$$

$$= 20{,}000 - 500 c_e.$$

Both the operating line and the equilibrium isotherm are plotted in Fig. 11.3. The intersection gives $c_e = 3\,\text{mg/L}$.

Example 3

Estimation of D_e. Although batch systems are not the preferred method of full scale operation, batch equilibrium and kinetic studies are frequently performed to determine the equilibrium and mass transfer parameters as part of model development for fixed bed column applications. This example and Example 4 illustrate, respectively, how batch kinetic data may be used to estimate the effective diffusivity of the pore diffusion and homogeneous surface diffusion models while Example 5 shows how the rate constant of the second-order reversible reaction model may be estimated from the same batch kinetic data.

Typical batch concentration–time data are shown in Fig. 11.4. The measured c vs. time data can be converted to $\bar{q}$ vs. time data using the following mass balance equation with known c_o,

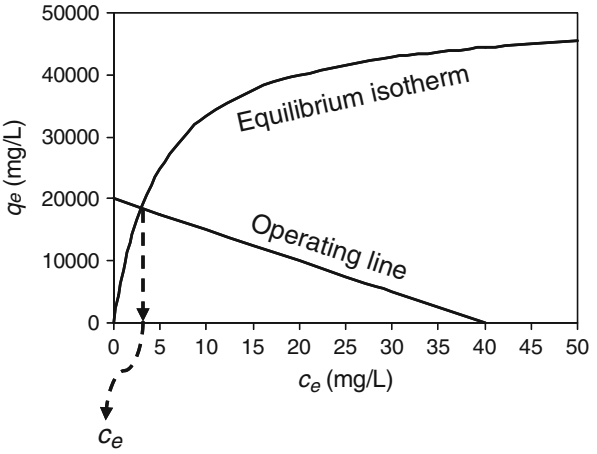

Fig. 11.3. Graphical solution to Example 2.

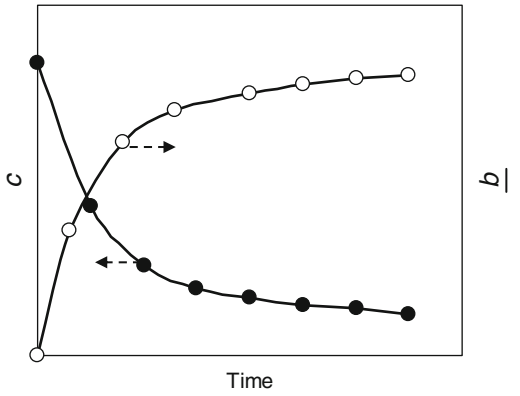

Fig. 11.4. Batch concentration–time and uptake-time profiles.

q_o, V, and V_m, (see Fig. 11.4):

$$\bar{q} = q_o + \frac{V}{V_m}(c_o - c).$$

The asymptotic solution to the pore diffusion model is given by Eq. (11a) which can be linearized as follows:

$$\frac{I_1}{I_2} = \left(1 - \frac{1}{\mathrm{Bi}}\right) - \frac{D_e c_o}{R^2 q_m}\frac{t}{I_2}.$$

This equation suggests that a plot of I_1/I_2 vs. t/I_2 should give a straight line with intercept $(1 - 1/\mathrm{Bi})$ and slope $(D_e c_o/R^2 q_m)$. D_e can, therefore, be estimated provided that the following parameters are known: c_o, V, V_m, R, and q_m.

Example 4

Estimation of D_s. The same batch uptake data shown in Fig. 11.4 may be used to estimate the effective diffusivity (D_s) of the homogeneous surface diffusion model. It should be noted that the batch transient data must be measured under intrabead diffusion control by using relatively high initial metal concentration. D_s may be estimated by fitting Eq. (16) to the experimental $\bar{q}/q_m$ vs. t data by nonlinear regression provided that R and q_m are known. A useful approximation of Eq. (16) which is a series function is given as (19):

$$\frac{\bar{q}}{q_m} \sim \sqrt{1 - \exp\left[\pi^2(-\gamma + 0.96\gamma^2 - 2.92\gamma^3)\right]},$$

where

$$\gamma = \frac{D_s t}{R^2}.$$

Example 5

Estimation of k_1. The rate constant of the second-order reversible reaction model k_1 can be estimated from the c vs. t data shown in Fig. 11.4 by nonlinear regression of Eq. (24a). When c_o, V, V_m, q_m, and b are known, a unique value of k_1 can be estimated from Eq. (24a) since k_1 is the only adjustable parameter.

Example 6

Predict the breakthrough curve of a fixed bed column for the biosorption of a metal from a wastewater using the pore diffusion model.

Equation (29a) can be used to make a priori prediction of breakthrough curves provided that the following sets of parameters are known:

(a) Column parameters: L, ε
(b) Biomass bead parameters: R, ε_p
(c) Feed solution parameters: c_F, v
(d) Equilibrium parameter: q_m
(e) Rate parameters: k_f, D_e

As mentioned previously, D_e can be estimated from small-scale batch kinetic experiments (see Example 3) while k_f may be estimated from engineering correlations (5).

Example 7

Predict the breakthrough curve of a fixed bed column for the biosorption of a metal from a wastewater using the homogeneous surface diffusion model.

Depending on the value of δ, either Eqs. (27a) and (27b) or Eqs. (27c) and (27d) can be used to make a priori prediction of breakthrough curves provided that the following sets of parameters are known:

(a) Column parameters: L, ε
(b) Biomass bead parameter: R
(c) Feed solution parameters: c_F, v

(d) Equilibrium parameter: q_m
(e) Rate parameters: k_f, D_s

The effective diffusivity D_s can be estimated from small-scale batch kinetic experiments under intrabead diffusion control (see Example 4) while k_f may be estimated from engineering correlations (5).

Example 8

Predict the breakthrough curve of a fixed bed column for the biosorption of a metal from a wastewater using the second-order reversible reaction model.

Breakthrough curves can be calculated from Eq. (28a) provided that the following sets of parameters are known:

(a) Column parameters: L, ε
(b) Feed solution parameters: c_F, v
(c) Equilibrium parameters: q_m, b
(d) Rate parameter: k_1

The rate constant k_1 can be estimated from small-scale batch kinetic experiments (see Example 5). Because k_1 is a lumped parameter, caution is advised when using k_1 estimated from batch experiments to predict the breakthrough behavior of fixed bed columns.

Example 9

Predict the breakthrough curve of a fixed bed column for the biosorption of a metal from a wastewater using the quasichemical kinetic model.

The quasichemical kinetic or Bohart–Adams model is rarely used to make a priori prediction of breakthrough curves. Instead, the model equation is often fit to the breakthrough curves of pilot-scale column tests to determine its parameters. A useful approximation is to assume $t \gg L/v$ and to disregard the "1" term in the denominator since $\exp(w)$ is usually $\gg 1$ and write the model equation [Eq. (30a)] as:

$$\frac{c}{c_F} = \frac{\exp(k_3 c_F t)}{\exp(k_3 c_F t) + \exp\left[\frac{k_3 q_m L(1-\varepsilon)}{\varepsilon v}\right]}$$

$$\exp(k_3 c_F t) + \exp\left[\frac{k_3 q_m L(1-\varepsilon)}{\varepsilon v}\right] = \frac{c_F}{c}\exp(k_3 c_F t).$$

If we divide each term by $\exp(k_3 c_F t)$, take the natural logarithm of each side, and rearrange the equation, we get:

$$\frac{k_3 q_m L(1-\varepsilon)}{\varepsilon v} - k_3 c_F t = \ln\left(\frac{c_F}{c} - 1\right)$$

$$t = \frac{q_m L(1-\varepsilon)}{\varepsilon v c_F} - \frac{\ln\left(\frac{c_F}{c} - 1\right)}{k_3 c_F}.$$

If we define $N = q_m(1 - \varepsilon)$, which is the uptake capacity of the biomass bead per unit volume of the fixed bed, and $u_s = \varepsilon v$, which is the superficial velocity, we get:

$$t = \frac{NL}{u_s c_F} - \frac{\ln\left(\frac{c_F}{c} - 1\right)}{k_3 c_F}.$$

This equation suggests that a plot of t vs. $\ln(c_F/c - 1)$ should be linear with intercept $(NL/u_s c_F)$ and slope $(1/k_3 c_F)$. The two parameters N and k_3 can, therefore, be estimated by fitting the above equation to the measured breakthrough data provided that the following parameters are known: c_F, L, and u_s.

NOMENCLATURE

b = Langmuir affinity constant, m^3/kg
Bi = Biot number defined by Eq. (11g)
c = Metal concentration in bulk solution, kg/m^3
c_{bt} = Metal concentration at breakthrough point, kg/m^3
c_e = Equilibrium metal concentration in solution, kg/m^3
c_F = Feed metal concentration, kg/m^3
c_i = metal concentration in solution adjacent to bead surface, kg/m^3
c_p = Metal concentration in pore liquid, kg/m^3
c_{pi} = Metal concentration in pore liquid adjacent to bead surface, kg/m^3
c_o = Initial metal concentration, kg/m^3
D_e = Effective pore diffusivity, m^2/s
D_L = Axial dispersion coefficient, m^2/s
D_s = Effective diffusivity for homogeneous diffusion model, m^2/s
h = Parameter defined by Eq. (5c), kg/m^3
I_o = Modified zero-order Bessel function of the first kind
I_1 = Parameter defined by Eq. (11b)
I_2 = Parameter defined by Eq. (11c)
J = Function defined by Eq. (28e)
k = Index in Eqs. (16) and (17a)
k_1 = Forward rate constant for second-order reversible reaction model, $m^3/(kg\ s)$
k_2 = Backward rate constant for second-order reversible reaction model, s^{-1}
k_3 = Rate constant for quasichemical kinetic model, $m^3/kg.s$
k_f = External film mass transfer coefficient, m/s
K = Equilibrium constant
L = Column length, m
n = Parameter defined by Eq. (28c)
N = Uptake capacity of biomass bead/bed volume
N_p = Parameter defined by Eq. (26c)
q = Metal concentration in biomass bead, kg/m^3
q_e = Equilibrium metal concentration in bead, kg/m^3
q_i = Metal concentration in bead adjacent to bead surface, kg/m^3

q_m = Maximum uptake capacity, kg/m^3
q_o = Initial metal concentration in bead, kg/m^3
q_s = Metal concentration in bead on a pore-free basis, kg/m^3
$\bar{q}$ = Bead-average metal concentration, kg/m^3
q_∞ = Final metal concentration in bead, kg/m^3
r = Bead radial coordinate, m
r_T = Parameter defined by Eq. (28b)
R = Bead radius, m
t = Time, s
t_{bt} = Breakthrough point, s
t_{st} = Saturation point, s
T = Parameter defined by Eq. (28d)
u = Parameter defined by Eq. (30b)
u_s = Superficial velocity, m/s
v = Interstitial velocity, m/s
V = Bulk solution volume, m^3
V_m = Volume of biomass bead, m^3
w = Parameter defined by Eq. (30e)
x = Parameter defined by Eq. (28e)
y = Parameter defined by Eq. (28e)
z = Column axial coordinate, m

Greek symbols

α = Parameter defined by Eq. (17b)
β = Parameter defined by Eq. (17c)
δ = Parameter defined by Eqs. (14) or (27e)
ε = Column void fraction
ε_p = Bead porosity
$\gamma = D_s t/R^2$
η = Parameter defined by Eq. (11d)
λ = Parameter defined by Eq. (11f)
Λ = Parameter defined by Eq. (11e)
μ = Parameter defined by Eq. (24b), kg/m^3
θ = Parameter defined by Eq. (28e)
σ = Parameter defined by Eq. (26b)
τ = Parameter defined by Eq. (27f)
ω = Parameter defined by Eq. (24c), kg/m^3
ξ = Parameter defined by Eq. (27g)
ψ = Parameter defined by Eq. (26d)

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Heavy Metal Removal by Microbial Biosorbents

Dae Haeng Cho, Eui Yong Kim, and Yung-Tse Hung

CONTENTS

Abstract Conventional methods for heavy metal removal are precipitation, coagulation, reduction, ion exchange, evaporation, and membrane processes. This chapter describes the use of microbial biosorbents in removing heavy metals. Environmental factors, mechanisms, and isotherms of biosorption were discussed. Biosorption kinetics includes pseudo-first-order, pseudo-second-order, and Elovich kinetics model.

1. INTRODUCTION

Due to the rapid industrialization, an alarming amount of toxic heavy metals has been released into the environment endangering natural ecosystems and public human health. Also, due to their mobility in natural water ecosystems and their toxicity to higher life forms, heavy metal ions in waste water and ground supplies have been regarded as major inorganic contaminants in the environment. Hundreds and thousands of tons of heavy metals are discharged from electric battery manufacturing, electroplating, refining process, internal-combustion engines fueled with leaded petroleum, mill tailings, landfill run off, and mining activities. Even if they are present in dilute, undetectable quantities, they are hazardous through natural processes such as biomagnification, concentrations may become elevated to such an extent that they begin exhibiting toxic characteristics. Heavy metals act on the central nervous system, kidney and liver damage, renal disturbances, lung insufficiency, bone lesions, cancer, and hypertension in humans. Elements such as lead and cadmium exhibit human toxicity at extremely low concentrations. The elements silver, chromium, copper, and zinc

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_12,

also exhibit toxic properties to human although the concentrations are orders of magnitude higher than that required for Cd or Hg toxicity.

Conventional methods for heavy metal removal are precipitation, coagulation, reduction, ion exchange, evaporation, and membrane processes. These methods have several disadvantages such as less effective removal of metal ion, high reagent requirements, high costs, the generation of toxic sludges, and the problem of the safe disposal of the materials (1). Biosorption (biological metal removal) process has distinct advantages over conventional methods, for example, highly selective, more efficient, easy to operate, and cost effective.

The potential for using microorganism in the treatment of metal-bearing wastewater has been studied intensively and many microorganisms including bacteria, fungi, and algae have been found to remove metals from solutions (2, 3). The biosorption of heavy metal ions by microorganisms may be placed into two categories: (a) metabolism-independent entrapment in the cellular structure and subsequent sorption on to the binding sites present in the cellular structure and (b) metabolism-dependent transport across the cell membrane through the cell metabolic cycle (4). The metal-sorption mechanisms including complexation, ion exchange, coordination, adsorption, chelation, and microprecipitation are complex and dependent on the chemistry of the metal ions, surface properties of the microorganisms, and cell physiology (5, 6). The biosorption process is affected by physico-chemical influence of the environment, such as pH, temperature, biomass concentration, initial metal concentration, and competing ion (7).

Biosorption of heavy metals is affected by many experimental factors such as pH, ionic strength, biomass concentration, temperature, and presence of different metallic ions in solution. The variability of these factors in real wastewaters makes it necessary to know how they influence biosorption performance. As a consequence of these possible multiple interactions the comprehension of biosorption phenomena is very complex and requires a study of both the solution chemistry of metal ions (depending on pH, anions and/or ligands in solution) and the mechanisms of passive metal uptake (ion exchange, complexation, microprecipitation, etc.) (7).

In order to develop an effective and accurate design model for adsorption systems, adsorption kinetics and equilibrium isotherm data are two of the most important parameters to understand. Kinetic analyses not only allow estimation of sorption rates but also lead to suitable rate expressions characteristic of possible reaction mechanisms. The calculated kinetic parameters can be of a great practical value for technological applications since kinetic modeling successfully replaces time and material consuming experiments A majority of research for sorption rate model has been based on a reaction kinetic sorption process in which reaction rate constants are determined as the key parameters describing the process (8, 9).

Biosorption phenomena occur as a result of metal ion interactions with functional groups in various functional groups on the cell surface. It is believed that phosphate, carboxyl, amine, and amide groups found in carbohydrates, lipids, proteins, and other biopolymers of the microbial cell envelope represent the main sites for metal adsorption (10). The charge distribution and geometry of these binding sites may vary with the composition of the cell envelope of each microorganism, resulting in markedly different metal-binding affinities.

2. CONVENTIONAL TECHNOLOGIES FOR HEAVY METAL REMOVAL

Metal removal or recovery processes are carefully considered not only toxic heavy metal removal in environmental aspects, but also precious metal recovery in industrial aspects. Those metals considered environmentally hazardous, or which are of technological importance, strategic significance or economic value must be removed or recovered at their source using appropriate treatment systems. Although many processes for heavy metal removal/recovery have been studied, more efficient process are needed for recycle of water, strict regulation for the effluent concentration of heavy metals, and the reduction of operating cost. Each treatment process has their own advantages and disadvantages and to know these factors is useful for selection and application to the specific case. Brief considerations of conventional metal treatment processes are as follows.

2.1. Chemical Precipitation

The most widely used process for removal of heavy metals from solution is chemical precipitation. The conventional process of heavy metal removal from industrial wastewater involves chemical precipitation of metals usually by lime, followed by settling of the metal precipitates in a pond and/or a clarifier. The most commonly used precipitation technique is hydroxide treatment due to its relative simplicity, low cost of precipitant, and ease of automatic pH control. Hydroxide precipitates tend to resolubilize if the solution pH is changed, but the removal of mixed metal wastes may not be effective because the minimum solubilities for different metals occur at different pH condition. Carbonate precipitation and sulfide precipitation has also been used for the treatment of metal containing waste water. Generally, precipitation has been widely used for its simplicity, but has two drawbacks: it usually results in a net increase in the total dissolved solids of the wastewater being treated, and large amount of sludge requiring treatment, which, in turn, may contain toxic compounds that may be difficult to treat (11).

2.2. Ion Exchange

Ion-exchange resins have recently found a niche in the market of water and waste-water treatment. Also, they are an effective means of removing heavy metals from wastewater. When the resins are saturated, they must be regenerated with an acid or alkaline medium to remove the metal ions from the resin bed. Due to the fact that ion exchange is efficient in removal of dissolved solids from normally dilute spent rinse waters, it is well suited for use in water purification and recycles. Ion exchange may be capable of treating for high purity heavy metal solution and sequential operation. However, it requires pretreatment process to reduce suspended solid concentration in solution to prevent fouling or channeling. However, apart from their cost, which can be prohibitive especially to smaller processing plants, resins are vulnerable to oxidation by chemicals, are affected by the presence of magnesium or calcium ions in solution, and are prone to fouling by precipitates and organics (12).

2.3. Membrane Technology

The use of membrane technology for valuable metal removal is gaining considerable attention in many industries. The ultrafiltration can be used to remove water from wastewater containing emulsified oil, and exclude the metal particles. However, ultrafiltration membranes need to be cleaned and backflushed regularly to operate efficiently and replaced periodically. Reverse osmosis (RO) may be applied in plating processes removing sodium chloride. RO system requires high-quality feed for efficient operation, thus wastewater must be treated to remove solids prior to RO treatment. Application of membrane technology to metal-bearing waste streams has several major drawbacks. Apart from the expense, membranes are also unable to resist certain types of chemicals and pH values and are prone to deterioration in the presence of microorganisms. Membrane fouling, compaction, scaling, limited life of membranes, dissolution of the membrane by oxidized agents, solvents and other organic compounds, and applicability only to feed streams with low concentrations of metal ions are major limitations associated with the use of membrane technologies.

2.4. Flocculation and Coagulation

The coagulation–flocculation processes facilitate the removal of suspended solids, colloidal particles. It is used in the final stage of solids–liquids separation. Coagulation is the destabilization of colloidal particles brought about by the addition of a chemical reagent called coagulant. Flocculation is the agglomeration of destabilized particles into microfloc and after into bulky floccules that can be settled called floc. The addition of another reagent called flocculant or a flocculant aid may promote the formation of the floc. Flocculation is the slow stirring or gentle agitation to aggregate the destabilized particles and form a rapid settling floc. This technique has been known to be capable of removing heavy metals from solution. EPA investigated the use of lime softening and coagulation (using ferric sulfate or alum) for removal of heavy metals as Pb^{2+}, Cd^{2+}, Cr^{3+}, Cr^{6+}, etc (13).

2.5. Flotation

Flotation, nowadays, is considered a well-established unit operation in the field of mineral and environmental technology. It also has been practiced for the separation of biological materials, such as algae from drinking water sources, mainly due to their small size and density. Flotation, following metal biosorption, was proved to be a useful and effective separation method of metal-loaded biomass, producing efficient removals, usually over 95%. The main critical parameters are solution pH and ionic strength. The different techniques, such as foam or bubble fractionation, foam separation or froth flotation, were examined for the separation of metal-loaded baker's yeast *Saccharomyces cerevisiae* (14).

2.6. Electrodialysis

Electrolytic metal recovery is one of a number of technologies capable of removing metals from wastewater. Electrolytic industrial processes for metals include the production of metals themselves from their compounds, which is called the electrowinning of metals; the electrolytic purification of metals; and the deposition or electroplating of metals on conducting

surfaces. In all three types of electrolytic process, the reactions are reduction of ions of the metal in solution in some carefully selected electrolyte. This process is a highly energy-dependent and labor-intensive process. Electrodialysis is a process that efficiently maintains a low metal ion concentration in the anodizing bath solution by transporting metal ions from the bath solution through a selective membrane into a capture media using an electrical current to induce flow. In the electrodialysis process, ionic components of a solution are separated through the use of semipermeable ion-selective membranes. However, this process is moderately high capital cost, increase in the number of possible exposures with regard to the handling of hazardous waste, and must be able to locate company that will recover and reclaim metals from the sludge.

The conventional approaches to heavy metal removal mentioned above are summarized in Table 12.1.

Table 12.1
Conventional metal removal technologies

Method	Disadvantage	Advantage
Chemical precipitation	pH dependence Difficult separation Adverse effect by complexing agent Resulting sludges Chemicals required	Simple and chip
Ion exchange	Sensitive to particles High operational cost No selectivity to alkaline metals Metallic fouling	No sludge generation Pure effluent metal recovery possible
Membrane	Membrane fouling Limited life of membrane Expensive High pressure	Pure effluent
Flocculation Coagulation	Chemicals required (electrolytes) Depend on basin design	Generate very fine particles of precipitates
Flotation	Less selective for heavy metals	Cost competitive to precipitation
Electrodialysis	Takes time Large electrode surface area required Fouling Expensive	Metal Selective

3. HEAVY METAL REMOVAL BY MICROBIAL BIOSORBENTS

3.1. Biosorption

The conventional heavy metal removal processes have several disadvantages such as less effective removal of metal ion, high reagent requirements, high costs, the generation of toxic sludges, and the problem of the safe disposal of the materials (1). Compared with conventional methods for removal of toxic heavy metals, biosorption process offers the advantages of low cost, minimization of the volume of chemical and/or biological sludge to be disposed of, high efficiency in detoxifying very dilute effluents, and high metal selectivity. These advantages have served as the primary incentives for developing biosorption processes to treat waste water contaminated by toxic heavy metals. Also the increasing demand for eco-friendly and economical technologies has led to the search of low-cost alternatives for heavy metal treatment. In this light, biological materials have emerged as an eco-friendly and economic option. The advantages of biosorption are as follows.

- *Cost effective*. The cost for biosorbents is low since often they are made from abundant natural source or waste biomass from industry.
- *Metal selective*. The metal sorption capacity of different types of biomass can be more or less selective on different metals. This depends on various factors, such as type of biomass, mixture in the solution, type of biomass preparation, and physico-chemical environment.
- *Regenerative*. Biosorbents can be reused after the metal is recycled. Some types of biomass are immobilized in a synthetic polymer matrix to obtain the required mechanical propertied for repeated reuse.
- *Minimization of sludge generation*. No secondary problems with sludge occur with biosorption, as is the case with many other techniques such as precipitation.
- *Metal recovery possible*. Metal can be recovered after being sorbed from the solution by desorbing solutions such as acid and chelate agents.
- *Competitive performance*. Biosorption is capable of a performance comparable to the most similar technique, ion exchange treatment.

Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals. Biosorbents are prepared from the naturally abundant and/or waste biomass from industrial use. The potential for using microorganism in the treatment of metal-bearing wastewater has been studied intensively and many microorganisms including bacteria, fungi, and algae have been found to remove metals from solutions (2, 3). Microbial biomass can passively bind large amounts of metals, a phenomenon commonly referred to as biosorption, thus providing a cost-effective solution for industrial wastewater management.

The biosorption of heavy metal ions by microorganisms may be placed into two categories: (a) metabolism-independent entrapment in the cellular structure and subsequent sorption on to the binding sites present in the cellular structure (biosorption) and (b) metabolism-dependent transport across the cell membrane through the cell metabolic cycle (bioaccumulation) (4). However, bioaccumulation is mediated only by living biomass. Further, bioaccumulation is a growth-dependent process and it is difficult to define a variety of effluents in contrast to biosorption which is growth independent. Thus, microbial biomass can be used and exploited more effectively as biosorption rather than bioaccumulation.

Table 12.2
Microbial biosorbents for the removal of heavy metals

Yeast & Fungi	Bacteria	Algae
Aspergillus niger	*Arthrobacter globiformis*	*Ascophyllum nodosum*
Aureobasidium pullulans	*Arthrobacter simplex*	*Chlorella vulgaris*
Cladosporium resinae	*Arthrobacter viscosus*	*Clodophara crispata*
Ganodoma lucidum	*Bacillus subtilis*	*Durvillea potatorum*
Penicillium chrysogenum	*Escherichia coli*	*Ecklonia maxima*
Penicillium digitatum	*Micrococcus luteus*	*Fucus vesiculosus*
Phanerochaete chrysoporium	*Pseudomonas aeruginosa*	*Lessonia flavicans*
Rhizopus arrhizus		
Rhodotorula aurantiaca	*Pseudomonas fluorescens*	*Sargassum filipendula*
Rhodotorula glutinis	*Pseudomonas syringae*	*Sagassum fluitans*
Rhodotorula rubra	*Streptomyces longwoodensis*	*Sargassum natans*
Saccharomyces cerevisiae	*Streptomyces niveus*	*Sargassum vulgare*
	Streptomyces noursei	
	Zoogloea ramigera	

Biosorption is a rapid phenomenon of passive metal sequestration by the nongrowing biomass (15). The binding capacities of certain biomass are comparable with the commercial synthetic cation exchange resins. Biosorption mainly involves cell surface complexation, ion exchange, and microprecipitation. Different microbes have been found to vary in their affinity for different heavy metals and, hence, differ in their metal-binding capacities. Some biomass exhibit preference for certain heavy metals, whereas others do not show any specific binding and are broad range.

3.2. Microbial Biosorbents

Microbial biomass types have been investigated for their biosorptive potential that include bacteria, yeasts, filamentous fungi, and marine algal (12, 16–20). The reported microbial biosorbents are listed in Table 12.2.

Certain biomass types are evidently more suitable than others to a specific application. The affinity that a biosorbent material exhibits for a specific metal cation will dictate the practicality of its implementation for remediation of a particular waste stream.

Among micro-organisms, fungal biomass offers the advantage of having a high percentage of cell wall material, which shows excellent metal-binding properties. Many filamentous fungi and yeast have shown an excellent potential of metal biosorption, particularly the genera *Rhizopus*, *Aspergillus*, *Streptoverticillum, Penicillium, Rhodotorula*, and *Saccharomyces* (21–26).

Of the species studied, fungi have been studied extensively, partly because of the wide range of morphological types they possess and availability of large amounts of fungal biomass and products derived from industrial processes and fermentations (27). Fungi are able to remove heavy metals from waste water in rather substantial quantities. In certain instances, biosorption of heavy metals by fungal cells has been observed to be more than that of conventional

adsorbents such as activated carbon and ion-exchange resins. Among fungi, *Rhizopus* sp. and *Aspergillus* sp. have been studied extensively as biosorbent for a variety of heavy metals. *Penicillium chrysogenum* showed the ability of gold biosorption from a cyanide solution although the capacity was not encouraging.

Yeasts possess an acknowledged potential for removal of heavy metal cations (29, 30). Yeasts are used in a variety of industrial fermentation processes and can be easily cultivated using unsophisticated fermentation techniques and inexpensive growth media. Yeasts cultures are also amenable to genetic and morphological manipulations, which may result in better raw biosorbent material. Among yeasts, heavy metal biosorption by *Saccharomyces cerevisiae* has been most studied (31, 32). In particular, this yeast is a reasonably potent biosorbent material for cadmium. It was recently reported that some soil yeasts including *Rhodotorula* sp. were resistant to heavy metal toxicity and have shown to play a role in processes of mineral cycling (26, 32, 33). Cho et al. reported that *R. glutinis* and *R. aurantiaca* showed the high capacity of biosorption for lead (23, 24). *Rhodotorula* sp. also has an aptitude for degradation of cyano-metals and bioleaching of mineral-containing metals (34, 35).

There are reports on the biosorption of metal using bacteria such as *Pseudomonas* sp., *Zoogloea ramigera*, *Streptomyces* sp., and *Arthrobacter* sp. (7, 17, 36). Among bacteria, *Bacillus* sp. has been identified as having a high potential for metal sequestration and has been used in commercial biosorbent preparation (37). The members of this genus are easy to culture and have shown high tolerance to heavy metal toxicity. *Zoogloea ramigera* has long been considered the typical activated sludge bacterium responsible for the formation of activated sludge flocs. Immobilized *Zoogloea* was shown to have a high adsorption capacity for Cu and Cd ions.

There are many reports on the biosorption of heavy metals by marine algae such as *Sargassum* sp., *Ascophyllum* sp., and *Chlorella* sp. (6, 38). Marin algae offer advantages for biosorption due to bulk availability of their biomass from water bodies and their macroscopic structures. Thus, marine algae became the candidate for the alternative biosorbents. *Sargassum* seaweed in this group has shown very high biosorptive capacities for various metals (39). In brown algae *Sargassum* biomass, alginate in the cell wall is the main component responsible for the heavy metal sorption.

3.3. Environmental Factors for Biosorption

In metabolism-dependent biosorption, cell wall structure and the metabolic state of the cell depend on substrate composition, thus growth in different media should influence the capacity and selectivity of metal uptake by creating other binding sites or diverse enzymatic system within the cell. The use of living cells for the biosorption of heavy metals has the disadvantage in nutrient requirements, metal toxicity, and cell death system failure. Thus, the control of environmental factors affecting the biosorption of living cell is a more complicated and tedious procedure.

It was reported that dead microbial cells are able to remove heavy metal ions from metal-laden wastewater. The biosorption technology is the passive method of metal removal by dead biomass. The dead (metabolically inactive) biomass of a variety of microorganism have been shown to produce effective biosorbents. The use of dried biomass as biosorbents mainly

depends on chemical mechanisms involving the interactions of metal ions with functional groups that are native to the proteins, lipids, and carbohydrates (especially polysaccharides) associated with the cell wall surface.

Some methods of killing cells (physical methods such as drying, heat treatment and chemical methods such as acidic, caustic, organic treatments) may actually improve the biosorption properties of the biomass. Aksu and dönmez (40) reported that the heat treatment method (drying) increased the biosorption capacity of the *Candida* biomass by 91.9% as compared with that of untreated biomass. They suggested that the enhanced sorption capacity could be attributed to more complex actions taking place on the surface, such as the formation of electrostatic bonds, change in the overall surface charge, and modification of binding sites.

Biosorption of heavy metals is affected by many experimental factors such as pH, ionic strength, biomass concentration, temperature, and presence of different metallic ions in solution. The variability of these factors in real wastewaters makes it necessary to know how they influence biosorption performance. As a consequence of these possible multiple interactions the comprehension of biosorption phenomena is very complex and requires a study of both the solution chemistry of metal ions (depending on pH, anions, and/or ligands in solution) and the mechanisms of passive metal uptake (ion exchange, complexation, microprecipitation etc.) (7).

A very rapid biosorption suggests that biosorption is typical for sorption of metals involving no energy-mediated cell surface binding. Rapid sorption of metal by the biosorbent is desirable providing for a short solution-biosorbent contact time in the actual process (41).

The ability of microbial biomass to bind metals in solution has been shown to be a function of pH. For example, change of less than 1 pH unit results in an increase in the amount of metal adsorbed from almost 0 to 100% (42, 43). The solution pH affects both the solubility of metals and the ligands responsible for binding of metal ions at the cell wall (44). The metal biosorption depends on the protonation or deprotonation of the cell wall functional groups. At low pH, the concentration of protons is so high that metal binding sites become positively charged and metal cations and protons compete for binding sites, which results in lower sorption of metal. With an increase in pH, the functional groups on the cell wall with negative charge increase due to deprotonation of the metal binding sites, which promote the metal sorption. The optimal pH value for adsorption of metal ions varies with the type of biomass and metal ions. pH between 4.0 and 8.0 is widely accepted as being optimal for metal sorption for almost all types of biomass (30, 45).

The biomass concentration is an important factor that determines the extent of metal biosorption from solution. It was reported that higher specific sorption at lower biomass concentrations could be due to an increased metal to biosorbent ratio (46). It was suggested that with increasing biomass concentration there is an increase in electrostatic interactions between cells and this causes the cells to agglomerate, which contribute to a decrease in the amount of binding sites available. However, Fourest and Roux (44) reported that the reduction in metal sorption with increasing biomass concentration is due to an insufficiency of metal ions in solution with respect to available binding sites.

Temperature changes in biosorption of metal affects the stability of the metal species initially placed in solution and microorganism–metal complex (47). In the case of metabolically inactive biosorbents, the dependence of capacity on temperature change can be negligible (48, 49). In contrast, biosorption of Cr(VI) by *Rhizopus niglicans* and lead (II) removal by *Zoogloea ramigera* showed endothermic nature (7, 50).

It is well known that the presence of some competing ions such as calcium, magnesium, sodium, and potassium can affect the sorption of heavy metal ions to biomass and reduce the binding capacity to some extent (49, 51). Schiewer (52) was reported that the electrostatic attraction only influenced the binding of light metal on biomass. According to his report, when heavy metal cation binding by marine algae *Sargassum* biomass is tested under the presence of Na^+ ion, Na^+ binding can be neglected unless present at high concentrations since it only binds weakly through electrostatic attraction and does not compete significantly with the binding of metal and proton. Since Na^+ is only bound electrostatically, it can only compete or interfere with the electrostatic (not covalent) binding of protons and divalent metal ions.

3.4. Biosorption Mechanisms

The complexity of the cell wall structure implies that there are many ways for the biosorption of heavy metals by microorganisms. Therefore, biosorption mechanisms are various and in some cases they are composed of more than one mechanism. However, the biosorption mechanisms are not completely understood. The biosorption mechanisms are summarized in Figs. 12.1 and 12.2.

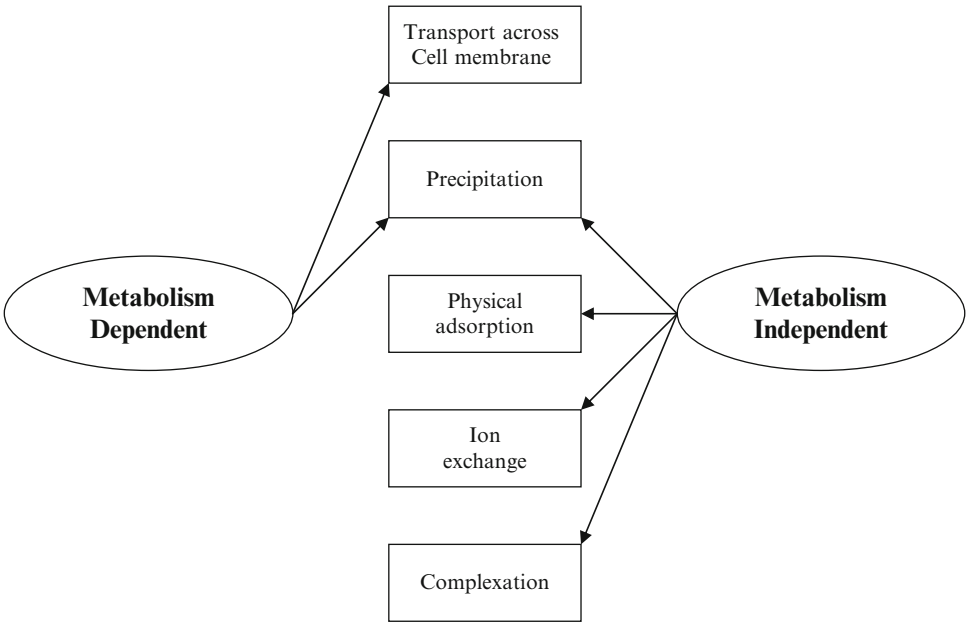

Fig. 12.1. Biosorption mechanisms according to the dependence on the metabolism of cells.

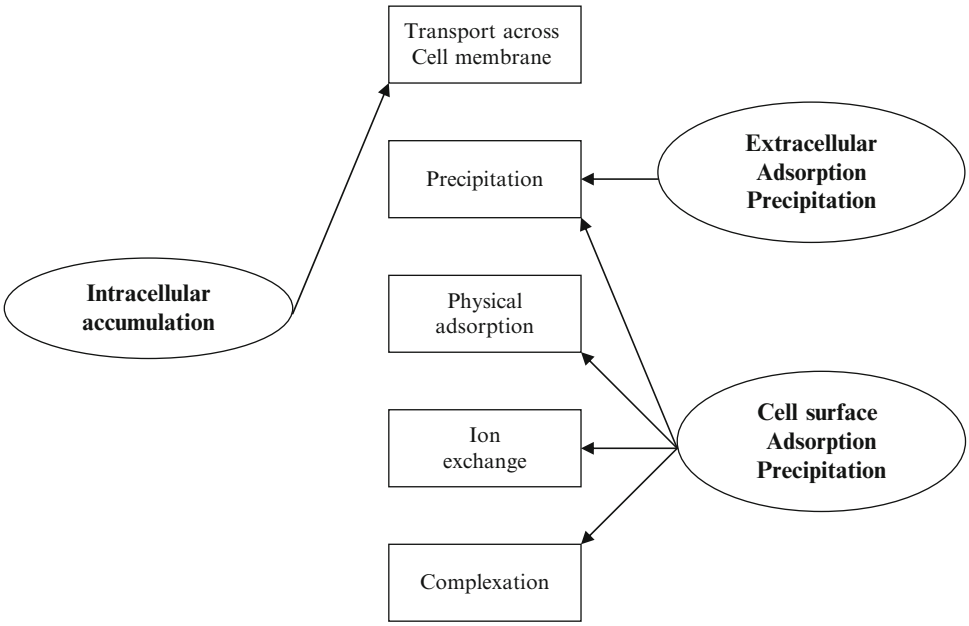

Fig. 12.2. Biosorption mechanisms according to the location where the metal removed is found.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into two categories (53):

1. *Metabolism dependent* (*active metal uptake, bioaccumulation*). Transport across cell membrane, precipitation. It is an energy-driven process.
2. *Metabolism independent* (*passive metal uptake, biosorption*). Precipitation, physical adsorption, ion exchange, complexation.

Dead cells sequester metals through chemical functional groups of the material comprising the cell and in particular the cell wall, which constitutes a large percentage of the cellular dry weight. Passive metal uptake is relatively rapid and can be reversible.

According to the location where the metal removed from the solution is found, biosorption may also be classified as follows (4):

1. Extracellular accumulation/precipitation may be facilitated by using viable microorganisms
2. *Cell surface sorption/precipitation*. Ion exchange, complexation, physical adsorption, precipitation can occur with alive or dead microorganisms
3. *Intracellular accumulation*. Transport across cell membrane requires microbial activity

The mechanism of biosorption is summarized as follows (41):

1. *Transport across the cell membrane*. This phenomenon is associated with cell metabolism by living biomass. This process may be mediated by the same mechanism used to convey metabolically essential ions, such as potassium, magnesium, and sodium. The metal transport system may become confused by the presence of heavy metal ions of the same charge and ionic radius (37).

2. *Complexation*. The metal biosorption from solution may take place through complex formation on the cell surface after interaction between the metal and active sites. Metal ions can bind to single ligand or through chelation. The cell surface complexation is on the concept of surface charge generated from the amphoteric surface sites, which are capable of reaction with sorbing cationic or anionic species to form surface complexes.
3. *Coordination*. The binding of metals to ligands is based on the formation of coordination compounds. The metal acts as a Lewis acid, i.e., tends to acquire enough electrons to reach an inert state, and the ligand acts as a Lewis base, i.e., has electron pairs that can be shared with the metal. Coordination, then, is a Lewis acid–Lewis base neutralization process.
4. *Ion exchange*. Ion exchange plays an important role in sorption by algal biomass and modeled the binding of heavy metal ions and protons as a function of metal concentration and equilibrium pH (52). The light metal ions presence in cell wall and membrane, such as K^+, Na^+, Ca^{2+}, and Mg^{2+} can also exchange with the metal cations.
5. *Chelation*. Chelation takes place when a ligand forms coordinate bonds with a metal through more than one pair of shared electrons, thus forming a ring structure. Depending on the requirement for electrons of the metal and the construction of the ligand, there can be a sharing of up to eight electron pairs between a single metal ion and ligand.
6. *Microprecipitation*. (Micro) precipitation may be either dependent on the cellular metabolism or independent of it. In the former case, the metal biosorption from solution is often associated with an active defense system of microorganisms. They react in the presence of a toxic metal, producing compounds which favor the precipitation process. In the case of (micro) precipitation not dependent on the cellular metabolism, it may be a consequence of the chemical interaction between the metal and the cell surface.

The physiological state of the organism, the age of the cells, the availability of micronutrients during their growth, and the environmental conditions during the biosorption process (such as pH, temperature, and presence of certain coions), are important parameters that affect the performance of a biosorbent.

3.5. Biosorption Sites

A variety of ligands located on the cell wall is known to be involved in metal biosorption (10). The main chemical groups of biomass surfaces that are capable of participating in sorption and chelation of a number of bivalent metal cations are polar or anionic in nature, such as hydroxyl, sulfhydryl, carboxyl, and phosphate, mainly those from polysaccharidic materials, which constitute most of the cell wall. The nature of the specific interactions between metal ions and biomass is quite controversial due to their complex nature and the significant number of different available binding sites for metal ions. However, the exact nature of functional groups and mechanisms responsible for heavy metal biosorption on microorganisms are not clear. The cell wall composition of various microorganisms is as follows.

Like algae, fungi also contain rigid cell walls. Although cellulose is present in the walls of certain fungi, many fungi have noncellulosic walls. The fungal cell wall presents a multilaminate, microfibrillar structure, an outer layer of glucans, mannans, or galactans and an inner microfibrillar layer, the crystalline properties of which are conferred by the parallel arrangement of chains of chitin or cellulose or noncellulosic glucan, with a continuous transition between both layers (10). The wall of a yeast cell is a remarkably thick (100–200 nm) envelope, which contains some 15–25% of the dry mass of the cell. Major structural constituents

of the cell wall are polysaccharides (80–90%), mainly glucans and mannans, with a minor percentage of chitin. Glucans (both β-2,6 and β-1,3-linked glucans are represented) provide strength to the cell wall, forming a microfibrillar network. Mannans are present as an α-1, 6-linked inner core with α-1,2- and α-1,3 side chains. Other components of the cell wall are variable quantities of proteins, lipids, and inorganic phosphate, polyphosphate, and pigments.

Fungal cell wall is composed of several layers bearing anionic groups to which metal cations bind. The adsorptive capacity of the fungal cell wall for heavy metals is determined by the structural organization of the entire protein–carbohydrate complex and by the degree of dissociation of the negatively charged functional groups and their accessibility to the metals (54).

The algal cell wall is structurally similar to the fungal cell wall. In many cases the cell wall is composed of a network generally consisting of cellulose and interspersed with amorphous materials. But it is usually modified by the addition of other polysaccharides such as pectin (highly hydrated polygalacturonic acid containing small amounts of the hexose rhamnose), xylan, mannans, alginic acids, or fucinic acid. Most of the algal cells are often covered by mucilaginous layers characterized by a significant metal sorption capacity due to the presence of uronic acids. In particular, alginic acid (linear, binary copolymer of 1,4-linked α-L-glucuronic acid and β-D-mannuronic acid) contained in brown algae shows high metal sorption capacity. Commercially important brown algae generally contain alginic acid in the range of 13–40 wt% on a dry weight basis, as a structural component of the cell wall in the form of alginates. The ability of alginate to form gels by ion exchange reaction with multivalent metal ions is a suitable property as a sorbent of heavy metals.

The functional groups responsible for the biosorption of heavy metals in the cell wall are mainly carboxyl, phosphoryl, and amine group. These functional groups provide the available binding sites of heavy metals on microorganism.

Carboxyl groups are found in abundance in cell wall attributed to organic acids, lipids, and polysaccharides. Uronic acids confer a net negative charge to the polymer and play an important role in the binding capacities of the polymer. The acidic (carboxylic) groups of uronic acid are partially ionized (carboxylate ion) in aqueous solution and these could attract and sequester metals.

Phospholipids present in the cell wall may exhibit phosphoryl groups, such as phosphatidylcholine and phosphatidylethanolamine, with minor proportions of phosphaditylinositol, phosphatidylserine, or phosphaditylglycerole, as well as sterols, mainly ergosterol and zymosterol. The yeast periplasm is a thin (35–45 Å), cell wall associated region external to the plasma membrane and internal to the cell wall. It mainly contains secreted proteins (mannoproteins) that are unable to permeate the cell wall, but fulfill essential functions in hydrolyzing substrates that do not cross the plasma membrane: invertase converts sucrose into glucose and fructose; acid phosphatase catalyzes the liberation of free phosphate from organic compounds. It was reported that the phospholipids mainly composed of phosphatidylcholine and phosphatidylethanolamine were found in the cell wall of the *R. glutinis* R-1 (55). The role of phosphomannans and carboxyl groups of cell wall protein of *Saccharomyces serevisiae* for metal binding has been identified (17). Reidl et al. (56) reported the orthophosphate extrusion in syringomycin-treated cells of *Rhodotorula pilimanae*. Polyphosphates have been

known to occur in numerous filamentous fungi and in the yeasts. In microbial cells, inorganic polyphosphate (polyP) plays a significant role in increasing cell resistance to unfavorable environmental conditions and in regulating different biochemical processes (57, 58).

Amino group is abundant in cell wall in the form of protein–peptide, protein–polysaccharide, and enzymes. Chitin and chitosan also exhibited amine group as yeast cell wall component. Chitin is a polymer of *N*-acetylglucosamine residues linked by β(1–4) glycosidic links and associated with protein in the cell walls to which it is linked via nonaromatic amino acid residues. Chitosan is produced by the deacetylation of chitin found in fungal cell walls. Chitin is found as microfibrils in the inner layer of the cell wall in the glucan matrix and mainly located in bud scars. It was reported that *Rhodotorula* sp. contained a chitin as a cell wall polysaccharide (59, 60). Kapoor and Viraraghavan (3) reported that chemically treated *Aspergillus niger* to prevent the participation of amine group in metal biosorption showed the dramatically reduction of Pb^{2+} biosorption capacity.

4. BIOSORPTION ISOTHERMS

Adsorption equilibrium may be expressed in the form of (a) a graphical or tabular record based on measurements, (b) an empirical algebraic expression fitted to the data and usually selected for its generality and simplicity of calculational use, or (c) equations based on the molecular statistics of the underlying process. Any such relationship may apply at only one temperature and is thus known as an equilibrium isotherm.

Once the adsorption process starts, it continues until equilibrium is approached between the sorbate concentrations on the solid phase and in solution. Equilibrium summons the end of the process and hence reflects the sorption capacity or affinity for a given solute.

4.1. The Langmuir Isotherm

This is proposed by Langmuir (61) for homogeneous adsorption. It assumes a uniform adsorbent surface with energetically identical sorption sites. The Langmuir formula is proposed as follows:

$$q_{eq} = q_{max} b C_{eq} / (1 + b C_{eq}), \tag{1}$$

where q_{max} is the maximum metal sorption (mg metal/g of biomass) and b is the Langmuir isotherm constant (l/mg metal). q_{max} and b can be obtained from the linear plot of $1/q_{eq}$ vs. $1/C_{eq}$.

$$1 / q_{eq} = (1 / q_{max} b C_{eq}) + (1 / q_{max}). \tag{2}$$

The Langmuir isotherm considers sorption as a chemical phenomenon. It was first theoretically examined in the adsorption of gases on solid surfaces. Langmuir constant b is related to the energy of adsorption through the Arrhenius equation. The higher the b, the higher is the affinity of the sorbent for the sorbate. A q_{max} can also be interpreted as the total number of binding sites that are available for biosorption, and q_{eq} as the number of binding sites that are in fact occupied by the sorbate at the concentration C_{eq}. Although the Langmuir model sheds no light on the mechanistic aspects of sorption, it provides information on uptake

capabilities and is capable of reflecting the usual equilibrium sorption process behavior. Langmuir assumed that the forces that are exerted by chemically unsaturated surface atoms (total number of binding sites) do not extend further than the diameter of one sorbed molecule and, therefore, sorption is restricted to a monolayer. In the simplest case the following *assumptions* were made:

(a) Fixed number of adsorption sites: at equilibrium, at any temperature, and gas pressure a fraction of the surface sites θ is occupied by adsorbed molecules, and the fraction $1–\theta$ is free
(b) All sorption sites are uniform (i.e., constant heat of adsorption)
(c) Only one sorbate
(d) One sorbate molecule reacts with one active site
(e) No interaction between sorbed species

Assumption of a value for the surface area covered per molecule then could allow computation of the active specific surface area of the sorbent using Avogadro's number. However, the concept of "*surface area*" cannot be used in gel-like sorbents that most biosorbents may be. As long as its restrictions and limitations are clearly recognized, the Langmuir equation can be used for describing equilibrium conditions for sorption behavior in different sorbate-sorbent systems, or for varied conditions within any given system.

Generally, the Langmuir isotherm does not describe equilibrium behavior accurately, especially with heterogeneous adsorption systems where adsorption continued beyond a monolayer. However, it is of practical importance because it is mathematically convenient and easily integrable.

4.2. The Freundlich Isotherm

The Freundlich (62) isotherm describes equilibrium on heterogeneous surfaces and, hence, does not assume monolayer capacity and takes the following form for a single component adsorption:

$$q_{eq} = K_F C_{eq}^{1/n}, \tag{3}$$

where K and n are the Freundlich constants. K related to the adsorption capacity; the larger its value, the higher the capacity. n is the adsorption intensity or the heterogeneity of the sorbent; the more heterogeneous the surface, the larger its value. Equation (3) can be linearized in logarithmic form and the Freundlich constants can be determined.

$$\log q_{eq} = (1/n) \log C_{eq} + \log K_F. \tag{4}$$

This isotherm is widely recommended due to its accuracy. It gives more accurate results than the Langmuir isotherm for a wide variety of heterogeneous adsorption systems. Though accurate and mathematically convenient, one drawback is that Freumdlich isotherm does not converge to Henry's law at low surface coverage and, therefore, fails to describe equilibria as $q \to 0$ and is thermodynamically inconsistent.

4.3. The Redlich–Peterson Isotherm

This is a more general formula than both Langmuir and Freundlich isotherms. The Redlich–Peterson isotherm formula is expressed by:

$$q_{\mathrm{eq}} = K_{\mathrm{R}} C_{\mathrm{eq}} / \left(1 + a_{\mathrm{R}} C_{\mathrm{eq}}^{\beta}\right), \tag{5}$$

where K_{R}, a_{R}, and β are the Redlich–Peterson constants (63). This equation can be converted to a linear form following:

$$\ln\left[(a_{\mathrm{R}} C_{\mathrm{eq}} / q_{\mathrm{eq}}) - 1\right] = \ln K_{\mathrm{R}} + \beta \ln C_{\mathrm{eq}}. \tag{6}$$

A graphical plot of the Redlich–Peterson isotherm shows that a "plateau" is reached after a continual rise in the curve, i.e., several layers of adsorption occurs first. This isotherm describes equilibrium for heterogeneous surfaces as it contains the heterogeneity factor β. It also converges to Henry's law at low surface coverage and is, therefore, thermodynamically consistent. However, it does not have as wide a practical application as the Langmuir and the Freundlich isotherms due to the inconvenience of evaluating three isotherm constants.

The illustration of the equilibrium adsorption plots and the summarized isotherm models are shown in Figs. 12.3, 12.4 and Table 12.3.

5. BIOSORPTION KINETICS

The study of sorption kinetics in heavy metal removal from wastewater is significant as it provides valuable insights into the reaction pathways and into the mechanism of sorption reactions. Monitoring a kinetic experiment enables us to see how the sorption system is

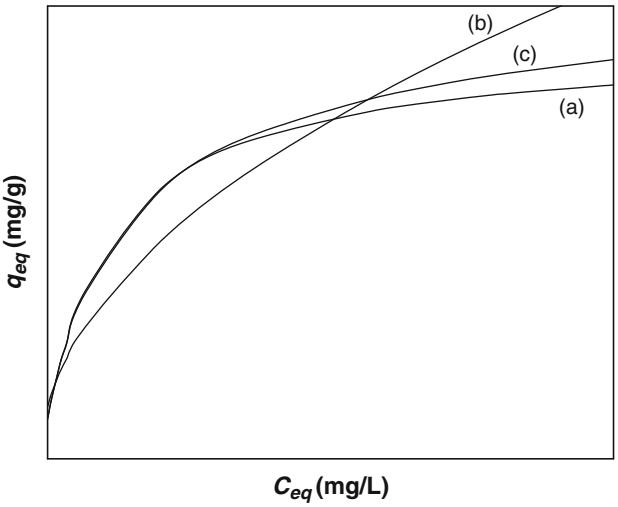

Fig. 12.3. Illustration of the adsorption equilibrium plots (**a**) Langmuir isotherm, (**b**) Freundlich isotherm, and (**c**) Redlich–Peterson isotherm.

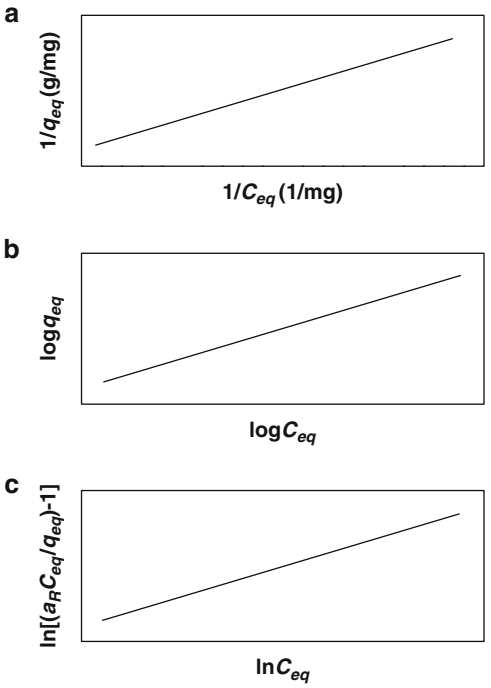

Fig. 12.4. Linearlized equilibrium isotherm equations (**a**) Langmuir isotherm, (**b**) Freundlich isotherm, and (**c**) Redlich–Peterson isotherm.

Table 12.3
Frequently used adsorption isotherm models

Isotherm	Equation	Linearized form	Description
Langmuir	$q_{eq} = q_{max} b C_{eq}/(1 + bC_{eq})$ q_{max}: maximum metal sorption b: affinity	$1/q_{eq} = (1/q_{max} bC_{eq}) + (1/q_{max})$	Monolayer surface adsorption system
Freundlich	$q_{eq} = K_F C_{eq}^{1/n}$ K_F: adsorption capacity n: intensity of heterogeneity	$\log q_{eq} = (1/n) \log C_{eq} + \log K_F$	Heterogeneous surface adsorption system
Redlich–Peterson	$q_{eq} = K_R C_{eq}/(1 + a_R C_{eq}^{\beta})$ β: heterogeneity factor	$\ln[(a_R C_{eq}/q_{eq}) - 1] = \ln K_R + \beta \ln C_{eq}$	Heterogeneous surface adsorption system

affected by process variables and to understand the steps which limit sorption. In addition, the kinetics describes the solute uptake rate which in turn controls the residence time of sorbate uptake at the solid–solution interface. Therefore, it is important to be able to predict the rate at which sorbate is removed from aqueous solutions in order to design appropriate sorption treatment processes. The sorption kinetics, thus, constitute a major criterion in the determination of the interest of sorption processes.

Numerous sorption kinetics have been studied in order to investigate the adsorption phenomena. These kinetic models included the pseudo-first-order kinetic model, the pseudo-second-order kinetic model, and the Elovich kinetic model.

5.1. Pseudo-First-Order Kinetic Model

The Lagergren rate equation (64) was the first rate equation for the sorption of liquid/solid system based on solid capacity. The Lagergren rate equation is one of the most widely used sorption rate equations for the sorption of a solute from a liquid solution. It may be represented as

$$\mathrm{d}q / \mathrm{d}t = k_1(q_{\mathrm{eq}} - q_t). \tag{7}$$

Integrating Eq. (7) for the boundary conditions $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$, gives:

$$\log(q_{\mathrm{eq}} / (q_{\mathrm{eq}} - q_t)) = k_1 t / 2.303 \tag{8}$$

which is the integrated rate law for a pseudo-first-order reaction, where q_{eq} is the amount of metal sorbed at equilibrium (mg/g); q_t is the amount of metal sorbed at time t (mg/g); and k is the equilibrium rate constant of pseudofirst sorption (1/min). Equation (8) can be rearranged to obtain a linear form

$$\log(q_{\mathrm{eq}} - q_t) = \log q_{\mathrm{eq}} - (k_1 t / 2.303). \tag{9}$$

The equation applicable to experimental results generally differs from a true first-order equation in two ways (65).

1. The parameter $k_1(q_{\mathrm{eq}} - q_t)$ does not represent the number of available sites.
2. The parameter $\log(q_{\mathrm{eq}})$ is an adjustable parameter. Often it is found not equal to the intercept of a plot of $\log(q_{\mathrm{eq}} - q_t)$ against t, whereas in a true first order process, $\log(q_{\mathrm{eq}})$ should be equal to the intercept of a plot of $\log(q_{\mathrm{eq}} - q_t)$ against t.

In order to fit Eq. (9) to experimental data, the equilibrium sorption capacity, q_{eq}, must be known. In most cases in the literature, the pseudo-first-order equation of Lagergren does not fit well for the whole range of contact time. In Eq. (9), one has to find some means of extrapolating the experimental data to $t = 1$, or treat q_{eq} as an adjustable parameter to be determined by trial and error. For this reason, it is necessary to use a trial and error method to obtain the equilibrium sorption capacity, q_{eq}.

5.2. Pseudo-Second-Order Kinetic Model

If the sorption rate of system is a pseudo-second-order mechanism, the rate-limiting step may be chemical sorption or chemisorption involving valency forces through sharing or the exchange of electrons between sorbent and sorbate as covalent forces. There are certain assumptions in description of this kinetic model (66).

1. There is a monolayer of metal ion on the surface of sorbent
2. The energy of sorption for each ion is the same and independent of surface coverage
3. The sorption occurs only on localized sites and involves no interactions between sorbed ions
4. The rate of sorption is almost negligible in comparison with the initial rate of sorption

The kinetic rate equation can be written as follows:

$$\mathrm{d}q_t \,/\, \mathrm{d}t = k_2(q_{\mathrm{eq}} - q_t)^2, \tag{10}$$

where k is the rate constant of sorption, (g/mg min), q_{eq} is the amount of divalent metal ion sorbed at equilibrium, (mg/g), q_t is amount of divalent metal ion on the surface of the sorbent at any time, t, (mg/g).

Separating the variables in Eq. (10) gives:

$$\mathrm{d}q_t \,/\, (q_{\mathrm{eq}} - q_t)^2 = k\mathrm{d}t. \tag{11}$$

For the boundary conditions $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$; the integrated form of Eq. (11) becomes:

$$1 \,/\, (q_{\mathrm{eq}} - q_t) = 1 \,/\, q_{\mathrm{eq}} + kt \tag{12}$$

which is the integrated rate law for a pseudo-second-order reaction.

Equation (12) can be rearranged to obtain:

$$q_t = t \,/\, \left(1 \,/\, kq_{\mathrm{eq}}^2 + t \,/\, q_{\mathrm{eq}}\right) \tag{13}$$

which has a linear form of

$$t/q_t = 1 \,/\, \left(k_2 q_{\mathrm{eq}}^2\right) + (1 \,/\, q_{\mathrm{eq}})t. \tag{14}$$

If the initial sorption rate is

$$h = kq_{\mathrm{eq}}^2, \tag{15}$$

then Eqs. (13) and (14) become:

$$q_t = t \,/\, (1 \,/\, h + t \,/\, q_{\mathrm{eq}}) \tag{16}$$

and

$$t \,/\, q_{\mathrm{eq}} = 1 \,/\, h + t \,/\, q_{\mathrm{eq}}. \tag{17}$$

The constants can be determined experimentally by plotting of t/q_t against t.

5.3. Elovich Kinetic Model

A widely used equation to describe the kinetics of chemisorption is the Elovich equation

$$\mathrm{d}q \,/\, \mathrm{d}t = a \exp(-\mathrm{b}q_t), \tag{18}$$

where a and b are parameters of the equation. The parameter a is regarded as the initial rate because $\mathrm{d}q/\mathrm{d}t \to a$ as $q \to 0$ and parameter b is related to the extent of surface coverage and activation energy for chemisorption.

Given that $q = 0$ at $t = 0$, the integrated form of Eq. (18) becomes:

$$q_t = (1 \,/\, b) \ln(t + t_0) - (1 \,/\, b) \ln t_0, \tag{19}$$

where $t_0 = 1/ab$. If $t \gg t_0$, Eq. (19) is simplified as:

$$q_t = (1 / b) \ln(ab) + (1 / b) \ln t. \tag{20}$$

The application of the Elovich equation in liquid phase sorption is gaining in popularity. The Elovich equation was also successfully used to describe the sorption kinetics of ion-exchange system (8).

The three kinetic models are summarized in Table 12.4 and Fig. 12.5.

Table 12.4
Frequently used sorption kinetic models

Kinetic model	Equation	Linearized form	Description
Pseudo-first-order	$dq/dt = k_1(q_{eq} - q_t)$	$\log(q_{eq} - q_t) = \log q_{eq} - (k_1 t/2.303)$	Trial and error method was required to obtain q_{eq} value
Pseudosecond order	$dq_t/dt = k_2(q_{eq} - q_t)^2$	$t/q_t = 1/(k_2 q_{eq}^2) + (1/q_{eq})t$ $t/q_{eq} = 1/h + t/q_{eq}$ $h = kq_{eq}^2$: initial rate	It must be assumed that the sorption follows the Langmuir equation
Elovich	$dq/dt = a \exp(-bq_t)$	$q_t = (1/b) \ln(ab) + (1/b) \ln t$ A: initial rate B: extent of surface coverage	Successfully used to describe the chemisorption kinetics

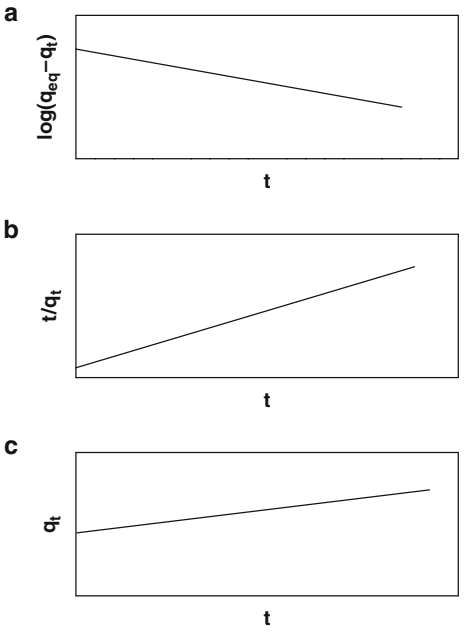

Fig. 12.5. Linearlized kinetic model equations (**a**) pseudo-first-order kinetic, (**b**) pseudo-second-order kinetic, and (**c**) Elovich kinetic.

6. EXAMPLES

Example 1

The biosorption experiment was done using 250-mL Erlenmeyer flask with 50 mL of metal-bearing solution of initial metal concentration of 100 mg/L. The quantity of used biomass was 0.1 g and final equilibrium concentration of metal was 25 mg/L after allowing enough time for developing the sorption equilibrium. Calculate the specific sorption value (mg/g).

Solution

The specific metal sorption value was calculated using the following equation:

$$q_{\mathrm{eq}} = V(C_{\mathrm{i}} - C_{\mathrm{eq}})/1{,}000M,$$

where q is the specific metal sorption (mg metal/g of biomass), V is the volume of metal solution (mL), C_{i} and C_{eq} are the initial and equilibrium concentration of metal (mg metal/L) respectively, M is the dry weight of the biomass (g).

Thus, $q_{\mathrm{eq}} = 50(100 - 25)/1{,}000 \times 0.1 = 37.5$

Therefore, the specific metal sorption value is 37.5 (mg metal/g of biomass).

Example 2

The M^{2+} biosorption experiments by biomass A were done under different initial metal concentrations. 0.1 g of biomass was added to 50 mL of solution of M^{2+} in 250-mL Erlenmeyer flasks shaken at fixed rpm in an orbital shaker at constant temperature for enough time to obtain equilibrium. The results obtained at different initial metal concentrations are shown in Table 12.5.

(a) Draw the linear plot of Langmuir isotherm for biosorption of M^{2+} by biomass A.
(b) Draw the linear plot of Freundlich isotherm for biosorption of M^{2+} by biomass A.
(c) Find the Langmuir and Freundlich isotherm parameters and correlation coefficient of each isotherm for biosorption of M^{2+} by biomass A.
(d) Determine the more suitable isotherm model to explain this biosorption system. Explain the meaning of this result.

Solution

(a) To draw the linealized equation of Langmuir isotherm, the parameters of Eq. (2) can be calculated from Table 12.5 and shown in Table 12.6. The linear plot of Langmuir isotherm can then be drawn in Fig. 12.6.
(b) To draw the linealized equation of Freundlich isotherm, the parameters of Eq. (4) can be calculated from Table 12.5 and shown in Table 12.7. The linear plot of Freundlich isotherm can then be drawn in Fig. 12.7.
(c) The calculated isotherm parameters are presented in Table 12.8.
(d) The Langmuir isotherm gives a good fit for all experimental data than Freundlich isotherm. Conformity of these data to the Langmuir model indicated that this biosorption system could be characterized as a monolayer, single site type phenomenon with no interaction between ions adsorbed in neighboring sites.

Table 12.5
The results obtained at different initial metal concentrations

Initial M^{2+} concentration (mg/L), C_i	Equilibrium M^{2+} concentration (mg/L), C_{eq}
20	5.6
25	7.4
30	9.4
50	16.6
70	22.7
100	34.5
200	98.9
300	180.5
400	279.7
500	375.1
600	470.0

Table 12.6
The equation parameters of Langmuir isotherm for linear plot

Initial M^{2+} concentration (mg/L), C_i	Equilibrium M^{2+} Concentration (mg/L), C_{eq}	q_{eq} (mg/g)	$1/q_{eq}$ (g/mg)	$1/C_{eq}$ (1/mg)
20	5.6	7.22	0.139	0.180
25	7.4	8.79	0.114	0.135
30	9.4	10.30	0.097	0.106
50	16.6	16.70	0.060	0.060
70	22.7	23.65	0.042	0.044
100	34.5	32.75	0.029	0.030
200	98.9	50.55	0.010	0.010
300	180.5	59.75	0.006	0.006
400	279.7	60.17	0.004	0.004
500	375.1	62.50	0.003	0.003
600	470.0	65.00	0.002	0.002

Example 3

The biosorption of M^{2+} by biomass B were carried out under initial metal concentrations of 50, 100, 200, and 300 mg/L. 0.1 g of biomass was added to 50 mL of solution of M^{2+} in 250-mL Erlenmeyer flasks shaken at fixed rpm in an orbital shaker at constant temperature. The q_{eq} values with time at different initial metal concentrations are shown in Table 12.9. If the rate of sorption of M^{2+}biosorption by biomass B is pseudo-second-order kinetic, find the second-order rate constants for this biosorption system.

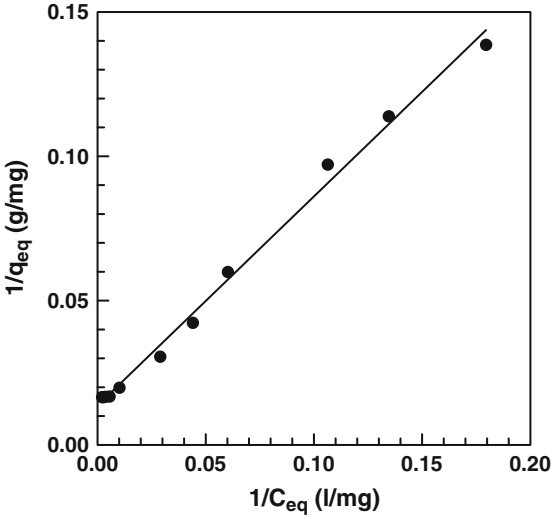

Fig. 12.6. Linear plot of Langmuir isotherm.

Table 12.7
The equation parameters of Freundlich isotherm for linear plot

Initial M^{2+} concentration (mg/L), C_i	Equilibrium M^{2+} concentration (mg/L), C_{eq}	q_{eq} (mg/g)	$\log q_{eq}$	$\log C_{eq}$
20	5.6	7.22	0.858	0.746
25	7.4	8.79	0.944	0.871
30	9.4	10.30	1.013	0.973
50	16.6	16.70	1.223	1.220
70	22.7	23.65	1.374	1.356
100	34.5	32.75	1.515	1.538
200	98.9	50.55	1.704	1.995
300	180.5	59.75	1.776	2.256
400	279.7	60.17	1.779	2.447
500	375.1	62.50	1.795	2.574
600	470.0	65.00	1.813	2.672

Solution

The second-order rate constants can be determined by plotting of t/q_t against t from Eq. (14). The plot of t/q_t against t is shown in Fig. 12.8.

The slopes and intercepts of the straight line from Fig. 12.8 and second-order rate constants determined from this data are shown in Table 12.10. The slopes and intercepts of Fig. 12.8 are $1/q_{eq}$ and $1/(k_2 q_{eq}^2)$ in Eq. (14), respectively.

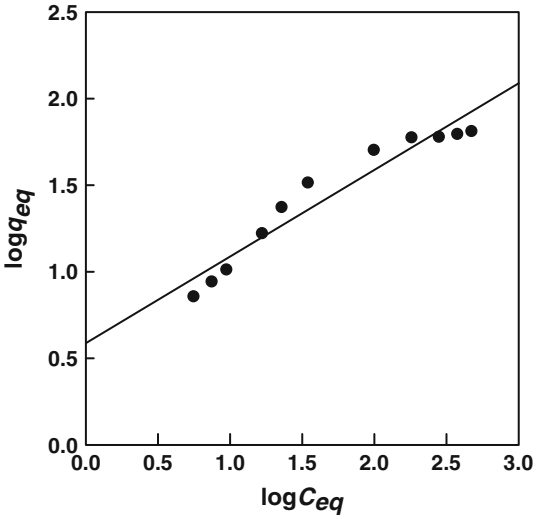

Fig. 12.7. Linear plot of Freundlich isotherm.

Table 12.8
Langmuir and Freundlich isotherm parameters

Isotherm model	Parameters	Value	R^2
Langmuir	q_{max} (mg/g)	73.5	0.994
	b (1/mg)	0.02	
Freudlich	K_F	3.9	0.931
	N	2.0	

Table 12.9
The q_{eq} values with time at different initial metal concentrations

t (min)	C_i (mg/L)			
	50	100	200	300
	q_t (mg/g)			
1	16.1	25.1	27.0	28.9
5	16.9	26.8	28.0	30.0
10	17.2	28.6	30.5	31.6
30	17.8	29.1	32.2	33.4
60	18.5	30.9	33.9	35.6
90	19.0	31.1	34.1	36.2
120	19.4	31.9	34.3	36.5
150	19.3	31.9	35.0	36.8
180	19.3	31.9	35.0	36.9

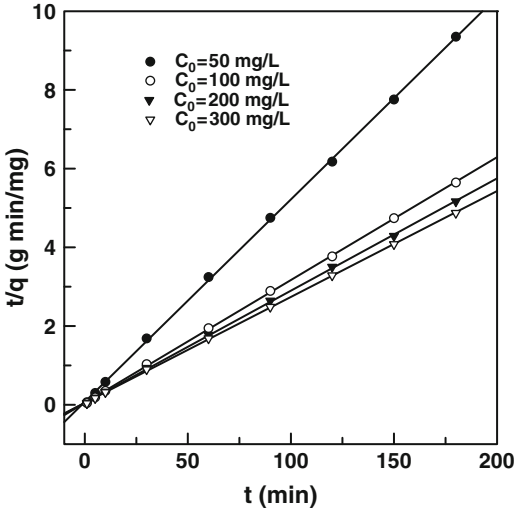

Fig. 12.8. The plot of t/q_t against t.

Table 12.10
The slope and intercept of the straight line from Fig. 12.8 and second order rate constants determined from this data

C_i (mg/L)	Slope	Intercept	$k_2 \times 10^2$ (g/mg min)	R^2
50	0.0514	0.0737	3.58	0.999
100	0.0312	0.0471	2.07	0.999
200	0.0285	0.0468	1.74	0.999
300	0.0269	0.0487	1.49	0.999

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13

Simultaneous Removal of Carbon and Nitrogen from Domestic Wastewater in an Aerobic RBC

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CONTENTS

Abstract Carbon and nitrogen are the major pollution sources that contribute to environmental quality problems. This chapter describes sources of carbon and nitrogen in wastewaters, bioreactors for carbon and nitrogen removal, and processes for simultaneous removal of carbon and nitrogen. Application of various Rotating Biological Contactors (RBC) processes for simultaneous removal of carbon and nitrogen is discussed. Nitrification and denitrification process, and design of RBC are covered in this chapter.

1. INTRODUCTION

Increasing urbanization coupled with economic liberalization has led to the accumulation of pollutants in the environment. Increased density of population and industrialization leads to rapidly rising municipal and industrial water requirements. At the same time, increased pollution of channels, lakes, rivers, and ground water sources occur as a result of the discharge of large quantities of polluted water, industrial chemicals, and other toxic substances. There is

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_13

an ardent need for innovative technologies for pollution-management. Technologies should be not only innovative but also sustainable. A sustainable technology with environmental protection as its back-up is the need of the hour. Recent environmental management practice has shifted its focus from the end of the pipe treatment to the pollution reduction at source. The concept of Best Management Practice (BMP) is emphasized in most of the industrial processes today. Aquatic environment warrants immediate action against accumulation of organic and inorganic toxicants. Although, the removal of organic constituents of wastewater is still a concern, inorganic nutrient removal and/or oxidation receives considerable attention.

Carbon and nitrogen are the major pollution sources that contribute to environmental quality problems. Nitrogen enters the aquatic environment from both natural and anthropogenic sources. Natural sources include precipitation, dust fall, nonurban runoff and biological fixation. As a result of activities of human beings, the quantities of nitrogen contained in precipitation, dust fall and nonurban runoff have all increased. Other sources deriving from human activities include runoff from urban areas, municipal wastewaters, drainage from agricultural lands and feedlots, and septic tank leachate. Many industrial wastewaters contain high ammonium concentrations generated from coking (450–4,100 mg/L), fertilizer (200–1,000 mg/L), synthetic rubber (800 mg/L), hydrometallurgy (500–9,500 mg/L), animal husbandry (500–2,300 mg/L) and carcass selling (100–1,400 mg/L) industries. All of the pollution sources, i.e., municipal, industrial, and agricultural, must be managed in order to reduce the carbon and nitrogen concentration within a certain level to improve the quality of the environment. Problems that are associated with carbon and nitrogen are (a) imbalance of natural ecological systems and increase of eutrophication; (b) depletion of dissolved oxygen in surface waters, which kills fish and create septic conditions; (c) odor problems; (d) contaminants that complicate water treatment, such as ammonia used for water supplies that require an increase of chlorine dosage to achieve a free chlorine residual in the process of disinfection; and (e) increase risks to human health, such as NO_3-N concentration in the groundwater for potable use.

1.1. Characteristics of Domestic Wastewaters

Nitrate is regarded as an undesirable substance in public water. Although it occurs naturally in water, elevated levels of nitrate in groundwater usually result from human activities, such as over use of chemical fertilizers in agriculture and improper disposal of human and animal wastes (1). High nitrate concentration in drinking water may cause serious problems in humans and animals (1–3). In order to protect against this effort, the United States Environmental Protection Agency (4) has established the maximum contamination level of nitrate in drinking water at 10 mg NO_3-N/L, which corresponds to the maximum allowance recommended by the World Health Organization (WHO) and maximum acceptable limit in Canada (5). Typical concentrations of the various forms of nitrogen (organic, ammonia, nitrate, and nitrite) along with some other constituents in domestic sewage, upon classification of the same as strong, medium and weak, shows that in the organic nitrogen, the concentrations are 35, 15, and 8 mg/L, while in ammonia nitrogen, the concentrations are 50, 25, and 12 mg/L. Nitrate and Nitrite nitrogen concentrations should be nil for discharge. These concentrations are subjected to wide seasonal and diurnal variations and are average figures only (6).

1.2. Adverse Effects of Nitrogenous Discharges

The impact of the discharge of domestic wastewater into rivers, lakes, estuaries and the sea is a matter of great concern in most countries. High strength domestic wastewater discharges in certain arid areas of the world may cause alarming increase in groundwater nitrate levels. Acute water scarcity in such areas results in the generation of a highly concentrated sewage, which may be finally discharged on soil after partial treatment centrally or through individual units. Not many of the treatment plants are designed to remove nitrogen from sewage, and the effluent is normally utilized for surface irrigation. The disposal of domestic wastewaters in areas not served by sewer systems is almost exclusively by the use of septic tank and seepage fields. Effluents from septic tanks generally contain 50–70 mg N/L with about 75% of the nitrogen present as ammonium and 25% as organic nitrogen. The effluent from septic tank is usually discharged to aerobic seepage fields, where ammonium and organic nitrogen are transformed to nitrate, which may be transported to ground water (7, 8). Abeliovich (9) indicated high COD ($\sim$1,000 mg/L) and high NH_4^+-N ($\sim$75 mg/L) concentrations in raw sewage and in the stored domestic wastewater reservoirs in Israel used for summer irrigation. In Jaipur (Rajasthan, India), the raw sewage received at the activated sludge plant has a BOD of 600–800 mg/L and $NH_4{}^+$-N concentration of 80–110 mg/L during summers when the water shortage is acute. The treated wastewater having high nitrogen concentration is discharged into a lake resulting in a steep rise in groundwater nitrates in the vicinity. In a study, over 40% well-water samples were found to contain more than 11.3 mg/L NO_3-N, a limit set by the WHO for drinking water (10).

The situation in the whole arid and semi-arid region of Rajasthan is alarming. The Central Ground water Board published a nitrate contour map of Rajasthan which indicates many belts encompassed by 750, 600 and 500 mg/L nitrate concentration contours (11). Because nitrate contamination of groundwater is generally pandemic, the costs associated with remediating groundwater are high and the risk to human health especially of infant methaemoglobinaemia is involved, the matter has to be looked in to seriously (12, 13).

1.3. Nitrogen Forms and Transformation in Wastewater Treatment

Classically, sewage treatment is directed toward the removal of suspended solids, organics, BOD, and bacterial contaminants. The behavior of nitrogenous materials during treatment and their presence in effluents generally has been ignored. Nitrification of secondary effluents has been traditionally practiced to serve as an indicator of a well oxidized effluent. By contrast, nitrification has also been minimized in certain cases to save on capital and operating costs. However, evidence on the adverse effects of nitrogen has led to the formulation of standards to limit the discharge of nitrogen compounds. A summary of the nitrogen removals obtained during both primary and secondary treatment shows that total nitrogen removal of 5–25% and 25% could be attained in these two stages (14).

In the past, domestic wastewater treatment was confined to organic carbon removal. In recent years, increasing pollution levels in the receiving waters and more stringent effluent limitations for discharges to sensitive zones have been the driving force in developing and

implementing new treatment techniques to control in addition to carbon, other significant parameters such as nitrogen, phosphorus, and priority pollutants.

2. CARBON AND NITROGEN REMOVAL FROM DOMESTIC WASTEWATERS

The general treatment alternatives available for the treatment of wastewater can be divided into two major categories: (a) physical/chemical treatment systems and (b) biological treatment systems. Physical treatments include screening, sedimentation, filtration, and flotation. Chemical treatments include disinfection, adsorption, and precipitation. The major biological processes used for wastewater treatment can be separated into five major groups: aerobic process, anoxic process, anaerobic process, combined aerobic-anoxic-anaerobic processes, and pond processes. The principal application of the processes is for (a) the removal of the carbonaceous organic matter in wastewater, (b) nitrification, (c) denitrification, (d) phosphorus removal, and (e) waste stabilization. The biological processes are considered the most effective and economic process in the field of wastewater treatment (15).

The removal of soluble organic matter (SOM) from wastewater streams has been the major application of biochemical operations for many years. For typical domestic waste streams, which have a biodegradable chemical oxygen demand (COD) range between 50–4,000 mg/L, aerobic cultures of microorganisms are especially suitable. Removal occurs as microorganisms use a portion of the carbon in the waste stream as a food source, converting it to new biomass and converting the remaining into carbon dioxide (CO_2). The CO_2 is released as a gas, and the biomass is removed by sedimentation. To accomplish the removal of soluble organic, a culture of heterotrophic bacteria must be maintained in suitable environmental conditions. The microorganisms are classified as heterotrophic because they derive their carbon from an organic source, such as the incoming waste stream, methanol, or ethanol.

In domestic wastewater, nitrogen is present as ammonia (NH_3) and as organic nitrogen (NH_2^-) in the form of amino groups. In the process of ammonification, the organic nitrogen is released as ammonia, as the organic matter containing it undergoes biodegradation. Two groups of bacteria are responsible for converting ammonia to the innocuous form, nitrogen (N_2). The completion of this process occurs in two steps by completely different bacteria and in very different environments. In the first step, nitrifying bacteria oxidize ammonia to nitrate (NO_3^-) in a process called nitrification. Nitrification is the biological process by which ammonia is first converted to nitrite and then to nitrate. Nitrification can be achieved in any aerobic-biological process at low organic loadings and where suitable environmental conditions are provided. Nitrifying bacteria are slower growing than the heterotrophic bacteria, which comprises the greater proportion of the biomass in both fixed film and suspended growth systems. The key requirement for nitrification to occur, therefore, is that the process should be so controlled that the net rate of accumulation of biomass, and hence, the net rate of withdrawal of biomass from the system, is less than the growth rate of the nitrifying bacteria (14). The bacteria responsible for nitrification are chemolithotrophic autotrophs that are also obligate aerobes requiring an aerobic environment. Chemolithotrophic bacteria obtain energy from the oxidation of inorganic compounds, which in the nitrogen cycle are ammonia (NH_3)

and nitrate (NO_3^-). Autotrophic bacteria obtain their carbon source from inorganic carbon such as carbon dioxide. In the second step (denitrification), facultative heterotrophic bacteria convert nitrate to nitrogen gas which is released to the atmosphere. This is accomplished only in an anoxic environment in which the bacteria use NO_3^- as an electron acceptor. The ultimate electron acceptor is nitrogen, which undergoes a stepwise conversion from an oxidation state of +5 in NO_3^- to 0 in N_2. This process may be carried on by some of the same facultative heterotrophic bacteria that oxidize the soluble organic matter under aerobic conditions. The requirements for the denitrification process are: (a) nitrogen present in the form of nitrates; (b) an organic carbon source, and (c) an anaerobic environment. However, the presence of any dissolved oxygen will inhibit denitrification since the preferential path for electron transfer is to oxygen instead of to nitrate.

2.1. Biochemical Reactions

The following equations describe the biochemical reactions that are occurring simultaneously governing the removal of soluble matter and ammonification are as follows:

$$\underset{\text{(organic matter)}}{\text{COHNS}} + \text{O}_2 + \text{nutrients} \approx \text{CO}_2 + \text{NH}_3 + \underset{\text{(new cells)}}{\text{C}_5\text{H}_7\text{O}_2\text{N}} + \text{other end products} \tag{1}$$

$$\text{C}_5\text{H}_7\text{NO}_2 + 5\text{O}_2 \approx 5\text{CO}_2 + 2\text{H}_2\text{O} + \text{NH}_3 + \text{energy} \tag{2}$$

Equation (1) gives the biodegradation of organic material, including ammonification, and cell synthesis. Equation (2) represents the endogenous respiration of the biomass. The carbon source for cell synthesis is provided from an organic compound; therefore, the bacteria are heterotrophic. The equations also indicate that oxygen is required for both reactions to occur.

Nitrifying bacteria are chemolithotrophic autotrophic microorganisms that obtain their energy from the oxidation of ammonia and nitrite and their carbon source from carbon dioxide. Below are the two equations for nitrification.

$$55\text{NH}_4^+ + 76\text{O}_2 + 109\text{HCO}_3^- \Rightarrow \text{C}_5\text{H}_7\text{O}_2\text{N} + 54\text{NO}_2^- + 57\text{H}_2\text{O} + 104\text{H}_2\text{CO}_3 \tag{3}$$

$$400\text{NO}_2^- + \text{NH}_4^+ + 4\text{H}_2\text{CO}_3 + \text{HCO}_3^- + 195\text{O}_2 \Rightarrow \text{C}_5\text{H}_7\text{O}_2\text{N} + 3\text{H}_2\text{O} + 400\text{NO}_3^- \tag{4}$$

Equation (3) describes the oxidation of ammonia to nitrite by the bacteria Nitrosomonas. Equation (4) describes the oxidation of nitrite to nitrate by the bacteria Nitrobacter. Both steps must occur in an aerobic environment.

The final step in the removal of nitrogen from the waste stream occurs when the nitrates produced in the nitrification process are converted to nitrogen gas by the process of denitrification, described below:

$$\text{NO}_3^- + 2\text{CH}_3\text{OH} \Rightarrow 6\text{NO}_2^- + 2\text{CO}_2 + 4\text{H}_2\text{O} \tag{5}$$

$$6NO_2^- + 3CH_3OH \Rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^- \quad (6)$$

$$6NO_3^- + 5CH_3OH \Rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^- \quad \text{(Overall Reaction)} \quad (7)$$

The above equations show methanol as the organic carbon source; however, any organic carbon source could be used. Organic carbon in the waste stream is used by returning nitrified effluent back to the anoxic/equalization tank, to mix with the influent. Equation (5) is an energy reaction in which nitrate is converted to nitrite. Equation (6) is also an energy equation for which nitrite is converted to nitrogen gas. The overall reaction is shown in Equation (7).

This process must be designed to achieve the above reactions simultaneously within one reactor. While maintaining an aerobic environment within the filter, reactions (1)–(4) are promoted. The purpose of returning nitrified effluent back to the anoxic/equalization tank is to mix the nitrates with both the raw organic carbon in the influent, and any organic carbon that has been released from the stored sludge as solute. Allowing the filter environment to become anoxic will promote the reactions of Equation (7) (denitrification).

Since biological removal of nitrogen is both possible and economically viable, many of today's wastewater treatment plants require the removal of both soluble organic matter and nitrogen. To achieve this requires: a heterotrophic population of bacteria operating in an aerobic environment to remove the SOM; a chemolithotrophic autotrophic population of bacteria also operating in an aerobic environment to convert the ammonia to nitrate, and finally a facultative heterotrophic population of bacteria to convert nitrate to nitrogen gas but in an anoxic environment. Therefore, typical treatment plant designs approach the removal of organics and nutrients in one of two ways. The first method is to combine the aerobic steps (SOM removal and nitrification) into one operation and design the anoxic denitrification process as a separate unit operation. The second method is to design three separate unit operations for each step. The type of technology utilized greatly influences the number of unit operations required to reach the desired effluent treatment level (18).

3. BIO-REACTORS EMPLOYED FOR CARBON AND NITROGEN REMOVAL

Biochemical operations have been classified according to the bioreactor type because the completeness of the biochemical transformation is influenced by the physical configuration of the reactor. Bioreactors fall into two categories depending on how the biological culture is maintained within suspended growth or attached growth (also called fixed film). In a suspended growth reactor, the biomass is suspended in the liquid being treated. In a fixed film reactor, the biomass attaches itself to a fixed media in the reactor, and the wastewater flows over it. Examples of suspended growth reactors include activated sludge and lagoons. Examples of attached growth include rotating biological contactors (RBCs), trickling filters, and submerged attached growth bioreactors (SAGBs), also called biological aerated filters (BAFs). Extensive research has been conducted on both the activated sludge process and the RBC process, but to a lesser degree on the other types.

During the last 20 years, different configurations of SAGBs have been conceived, and modest advances in the understanding of the systems have been made. The advantages of SAGBs or BAFs are that they may operate without a solids separation unit process after biological

treatment, and they operate with high concentrations of viable biomass. Removal of sludge is usually achieved by backwashing the filter. In such bioreactors, the hydraulic retention time (HRT) is less than the minimum solids retention time (SRT) required for microbial growth on the substrates provided. This means that the growth of suspended microorganisms is minimized, and the growth of attached microorganisms is maximized. The low hydraulic retention time results in a significantly smaller required volume to treat a given waste stream than would be achieved with either a different fixed film reactor or a suspended growth reactor for the same waste stream.

3.1. Trickling Filters

Trickling filters have been widely employed for nitrification, and the extent of nitrification in trickling filters depended on a variety of factors; including temperature, dissolved oxygen, pH, presence of inhibitors, filter depth and media type, loading rate, and wastewater BOD (16, 17). Low-rate trickling filters allowed the development of a high-nitrifying population. For rock media filters, organic loading should not exceed 0.16 kg BOD_5/m^3/day (19). Higher loading rates (0.36 kg BOD_5/m^3/day) were allowable in plastic media trickling filters because of the higher surface area of the plastic media (20). If two filters were used, heterotrophic growth occurs in the first filter and nitrification in the second filter (21) conducted a pilot plant study of tertiary trickling filters, recommending a media surface loading rate of 0.4 g NH_3-N/m^2/day for complete nitrification (effluent NH_3-N < 2.0 mg/L) at a water temperature of 10°C.

3.2. Rotating Biological Contactor

Rotating biological contactor (RBCs) is an aerobic, attached growth or hybrid, two phase contactor unit which finds use in the treatment of biodegradable waste of liquid origin. In its physical design, a series of closely spaced discs is mounted on a shaft which rotates at very low speeds of 2 to 5 rpm. The discs are partially submerged in a trough carrying wastewater, and provide substratum for biofilm attachment and enhanced physiological activity. The biofilm is alternately submerged to absorb substrate from the wastewater and raised out of the liquid to oxidize the absorbed substrate. The succession of biofilm and liquid film over the media surface controls oxygen transfer efficiency and substrate transfer efficiency. The thickness of liquid film and its retention time above the wastewater line decide the oxygen transfer efficiency. The first stages of an RBC mostly removed organic materials, whereas subsequent stages removed NH_3-N as a result of nitrification, when the BOD_5 was low enough. Ammonia oxidizers could not effectively compete with the faster-growing heterotrophs that oxidize organic matter. Nitrification occurs only when the BOD was reduced to approximately 14 mg/L, and increases with rotation speed (22, 23). RBC performance was negatively affected by low dissolved oxygen in the first stages and by low pH in the later stages where nitrification occurred (24). Degradable organic carbon inhibits nitrification at concentrations greater than 15–20 mg/L BOD_5; extremely low concentrations of influent BOD_5 (less than 10 mg/L) did not improve nitrification (25). The inhibition of nitrification by particulate BOD suggested that clarified influent should be used for nitrifying the biofilm process.

3.3. Conventional Activated Sludge Processes at Low Loadings

Nitrification in a conventional activated sludge system was found was relatively low for carbon removal and nitrification of sewage because carbon removal and nitrification occurred in the same reactor with an activated sludge system. This resulted in a population mixture of mainly heterotrophs and few autotrophs. In this kind of treatment system, it was not possible to enrich the autotrophic bacteria because the slower growing autotrophs were removed with the surplus sludge. It was necessary to separate the autotrophic from the heterotrophic biomass in order to increase the specific nitrification rate. Extended aeration activated sludge process showed very high efficiencies for nitrification, whose aeration was controlled by means of DO and redox potential measurements (26).

Simultaneous organic carbon removal-nitrification by an activated sludge process with cross-flow filtration made the sludge retention time very long; simultaneous carbon removal-nitrification was achieved quite well under the loading rate of about 0.10 g BOD/g VSS/day. The efficiency of dissolved organic carbon removal was more than 95%, and nitrification was sufficient (NH_3-N was not detected in the effluent) (27).

3.4. Two-Stage Activated Sludge Systems with Separate Carbonaceous Oxidation and Nitrification Systems

The nitrification process requires nitrifying bacteria with sludge that has been aged for a long time and high dissolved oxygen concentration. In addition, they were susceptible to inhibition by a wide range of compounds at concentrations so low as not to affect the heterotrophic bacteria. For these reasons, it would seem sensible to separate the processes of carbonaceous removal and nitrogen removal into separate reactors (28). Separate carbonaceous oxidation and nitrification systems were found to show minimized sludge washout with the nitrification stage, and the process could be operated successfully at a shorter detention time, lower MLSS, and solid retention time (29).

Denitrification using two zone activated sludge systems and showed to be capable of removing 75% of the total N from about 30 mg TN/L in the feed to <10 mg TN/L in the effluent. The multiple anoxic zones with a step feed process appeared to be the most cost effective denitrification option because it made the fullest use of the carbon that was present in the feed as the carbon source for step feed denitrification.

4. PROCESSES EMPLOYED FOR SIMULTANEOUS CARBON AND NITROGEN REMOVAL

Currently, the processes used for carbon and nitrogen removal can be divided into two major groups: separated stage and single stage processes. For multiple stages of carbon and nitrogen removal, there is a disadvantage for denitrification which occurs either in the addition of external carbon or the recycle part of the effluent of nitrifying bacteria. Carbon and nitrogen removal occurring in a single unit is a possibility to overcome these disadvantages. Multiple and single processes for the removal of carbon and nitrogen are presented as follows.

4.1. Separated Stage Process

A two-stage nitrification and denitrification process in an activated sludge system with recycle of mixed liquid from the nitrification stage to the denitrification stage could be employed. The carbon removal and nitrification stages could be performed in an aerobic activated sludge tank, and the denitrification in an anaerobic stirred tank reactor or an anaerobic plug-flow reactor (30). To optimize the removal of nitrogen by the denitrification process, an external source of carbon and energy was normally required. suggesting that an extra carbon source, molasses, must be used.

Upflow Submerged Filters could be tried for multistage carbon and nitrogen removal. Two upflow submerged filters could be employed with the nitrification in the first filter followed by denitrification in the latter. The nitrified effluent of the nitrification filter could be combined with organic carbon source (molasses) and fed into the submerged denitrification filter. Conversion of ammonium nitrogen to oxidized nitrogen requires about 4–5 mg/L. Research on submerged filters have been conducted by Cecen et al. (31).

Submerged biofilm columns attached to fibrous carries with effluent recycle in subsequent anaerobic and aerobic processes in series could be employed. An essential condition is that in the aerated columns, sufficient air flow rate should be maintained, so that the DO is about 3–5 mg/L.

4.2. Single Stage Process

Experiments on an Attached-Growth Circulating Reactor (AGCR) were conducted to investigate its efficiencies on organic carbon and nitrogen removal (32). The optimal COD loading rate was found to be $5\,g/m^2/day$ corresponding to the TN loading rate of $0.54\,g/m^2/day$. At this loading rate, the removal rates of COD and TN of 4.8 and $0.43\,g/m^2/day$, respectively (or 96% COD removal and 79% TN removal efficiencies), could be achieved. The overall AGCR performance was limited by the nitrification efficiency at the high TN loading rates of $0.54\,g/m^2/day$.

A combined anaerobic–aerobic system with internal recirculation of effluent in a single fluidized bed reactor had demonstrated simultaneous removal of organic carbon and nitrogen. With the loading rate of organic carbon $<1.2\,kg/m^3/day$ and nitrogen $<0.2\,kg/m^3/day$ and HRT of 24 h, the levels of purification could reach COD removals of >80% and the effluent concentration of $(BOD_5)S < 10\,mg/L$, $NO_x\text{-}N < 5\,mg/L$, $NH_3\text{-}N < 1\,mg/L$ (33). Single continuous flow fluidized bed reactor consisting of porous packing materials was constructed by Xin-Hui et al. (34), for simultaneous removal of carbon and nitrogenous substances under different C/N (mass ratio values). A TOC removal of >91% and a maximum total nitrogen removal of 85% were achieved under a moderate C/N value.

Aerated lagoon could provide effective organic carbon and nitrogen removal for domestic wastewater. Intermittent aeration of an aerated lagoon resulted BOD removal ranged from 69 to 86% at a detention time of 2–6 days. (35).

Simultaneous Nitrification and Denitrification (SND) in RBC showed that SND was strongly influenced by C/N ratio of wastewater, hydraulic loading, and oxygen partial-pressure ($P7070_o$) in the phase. The maximum nitrogen removal efficiency was achieved at C/N ratio

of 6.0, HRT of 5.5 h, and a P_o of 0.10 atm. Nitrification mainly occurred in a biofilm rotating through the air phase, while denitrification mainly occurred in a biofilm rotating through the water phase. The removal efficiency, due to the SND, increased with a decrease in the disk rotating speed up to the optimum speed of around 3 rpm (36).

The sequencing batch reactor (37) and the intermittent operation of the extended aeration process (38) had exhibited their ability of accommodating nitrification, denitrification, biological oxidation, sedimentation, and flocculation all in a single unit for domestic sewage.

Kondo et al. (39) developed simultaneous removal of BOD and nitrogen with an anoxic/oxic porous biomass support system. In the full-scale field test, the system was used for the treatment of sewage and operated with a schedule of 1.5 h aeration and 0.5 h agitation. Less than 20 mg/L of the effluent BOD (93% BOD removal) and 15 mg/L of total nitrogen could be achieved at the organic loading rate of 0.8 kg-BOD/m^3/day. At the low temperature of 13°C, the nitrification rate was slow and the removal efficiency of nitrogen fell to 65%. Total nitrogen removal efficiency could reach as much as 75% with an annual average temperature of 13–31°C.

An entrapped mixed microbial cell process (EMMC) process was investigated for its simultaneous removal of carbon and nitrogen in a single reactor under alternate schedules of aerobic, anoxic, and anaerobic conditions (40). The process proved to be economically and technically feasible for simultaneous carbon and nitrogen removal with 96% of organic removal and 73–76% of nitrogen removal. Comparison of process performance with other biological treatment processes revealed that the EMMC process is comparable to other advanced biological processes for simultaneous carbon and nitrogen removal in a single bioreactor.

5. DEVELOPMENT OF RBCs

The use of RBC for wastewater treatment was started in the year 1950 in West Germany in order to improve secondary treatment process (41). The investigators used wooden and plastic flat discs rotating in wastewater. Stenglin (42) began to manufacture 2 and 3 m diameter expanded polystyrene discs in West Germany. Hartmann (Water Services, 1979) investigated the use of aluminum and its alloys as media surface and many full-scale treatment plants were installed using duralumin media. The year 1970 was the turning point for the rapid development of RBC. The pioneer, Antonie (43), changed the conventional flat disc module to a more cost effective concentrated corrugated and honeycomb module units using HDPE. Various media configurations such as Bio-surf (44), active RBC surface module (45), lattice configuration (46, 47), rotating cage (48), Bio-spiral unit (49), Aero-surf unit (50), etc. have been tried by different researchers to bring down the cost of the rotating units, which represent 65–70% of the total cost of the system (51).

RBCs have been tried extensively for single stage carbon removal and nitrification as well as for separate stages in series for BOD removal and nitrification from municipal wastewaters (23, 30, 52–54). Effects of various operating parameters like turbulence (55), disc rotation speed (56), hydraulic conditions (55), organic particulate matter (57, 58), and recirculation (25) on nitrifying biofilms have been studied in detail. Some literature is also available on simultaneous nitrification and denitrification in micro-aerobic films (36, 59), but there

have been no reports on simultaneous organics and nitrogen removal in a fully aerobic biofilm. McCann and Sullivan (60) reported the advantages of an aerated RBC included thinner biofilms and higher dissolved oxygen concentration. The thinner biofilm permits closer spacing of media and therefore more total surface area. The system may be able to treat higher loading rates with increased efficiency and safeguard against shock loads. Crawford (61) reported that an RBC process was chosen by the city of Guelph, Ontario, because of its low energy requirement, its small land requirement, and the fact that it did not require a final settling tank if followed by filtration. Banerji (62) discussed the many factors affecting the performance of RBCs. Included among these were influent substrate concentration, residence time, surface hydraulic loading rate, wastewater temperature, and media rotational speed. Kincannon and Groves (63) determined that suspended solids affect RBC kinetics. At low hydraulic loadings ($0.061–0.122\,m^3/day/m^2$, $1.5–3.0\,gpd/ft^2$), microbial suspended solids are not washed out of the system and are capable of oxidizing substrate. Food to microorganisms ratios were similar to those of conventional activated sludge systems. A treatment efficiency of better than 95% was obtained with an F/M ratio of less than one. Suspended solids, although dependent upon individual situations, should be considered in the economical design of RBC units. Grady and Lim (64) have presented the fundamental concepts of RBC performance from the standpoint of environmental engineering education. The model of RBC process involves effectiveness factors. Among the factors included are influent flow rate, concentration, disk rotational speed, degree of submergence, oxygen transfer rate and dissolved oxygen levels. Regent (65) determined that RBCs were able to achieve good quality effluent while treating domestic wastewater for small communities in Yugoslavia. Three years of operating experiences were summarized. Influent BOD_5 concentrations of 288.4 mg/L yielded an average effluent concentration of 23.0 mg/L BOD_5 (92% removal), and influent concentrations averaging 108.0 mg/L corresponded to effluent concentrations of 18.0 mg/L BOD_5 (84% removal). The suspended solids reductions averaged 96.5%. The system also showed good removal of *Escherichia coli* and effluent disinfection was necessary. Performance data for an RBC plant serving a population of 20,000 in Kirkville, Missourie showed that the units were efficient under normal organic and hydraulic loads (66). An average removal efficiency of 88% and average BOD_5 concentration of 24 mg/L were obtained. Efficiency was adversely affected at high organic and hydraulic loadings. The soluble BOD loading rate seemed to be the best indicator for the operation of the process. Pano et al. (67) presented preliminary results of a study of the kinetic constants of a RBC treating domestic wastewater at 15°C. The yield coefficient, Y ranged from 0.81 to 1.44 and the decay co-efficient, K_d ranged from 0 to 0.44/days. The maximum specific growth rate was 1.47–2.92/day, and the half velocity constant, K_s, was 6.0–67.4 mg COD/L.

5.1. Application of Rotating Biological Contactors for Domestic Wastewater Treatment

Rotating Biological Contactor consists of segmented corrugated polymer discs attached together to form a media pack. The polymer discs, also referred to media panels, are held within an enclosed basin, submerged by approximately 40% of the surface area. Wastewater passes through the basin as the disks slowly rotate, at approximately 1 rev./min, exposing

the biological growth (biomass) alternately to the wastewater, and to the surrounding air. Typically, a media pack consisting of a collection of media panels, represents one stage of the treatment process. The RBC can consist of up to four or even five separate media packs depending upon the population served. The characteristic features of RBC medium are:

(a) It provides support for the biomass
(b) Contacts the biomass with the waste
(c) Contacts the biomass with air
(d) Creates shear force to cause biomass slough-off continuously
(e) Mixes the liquid waste in a contoured tank and
(f) Keeps the sloughed biomass in suspension in the tank.

5.1.1. Components of Rotating Biological Contactor

5.1.1.1. Shaft

The shaft is the central structure of an RBC unit. Fracture of shaft will result in the complete collapse of the unit. Once shaft failure occurs, the entire unit RBC unit has to be replaced.

5.1.1.2. Media Support Structure

The media support structure consists of 4–12 equal media segments per disc, dependent on the size of the RBC. Each individual segment is usually supported by three through rods, attached at their ends to a supporting structure. Once part of the structure has fractured, additional stresses will be redistributed across the entire media structure resulting in sudden collapse of the unit.

5.1.1.3. RBC Bearings

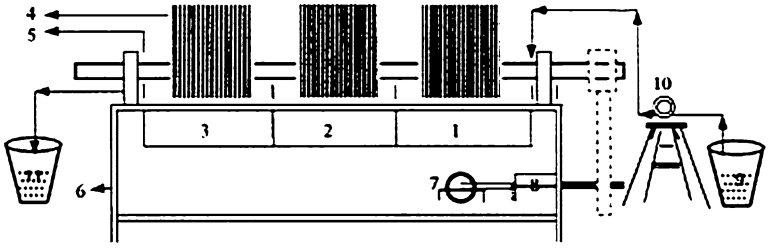
Bearings are very important for the operation of RBC. For proper operation of bearing good quality of grease must be used. It is most important for RBC user and manufacturer to recognize that bearings of RBCs are highly loaded and operate at extremely low rotational speeds. Because of low rotational speeds, the effect of grease churning is no longer present, normally to be found on machines operating at high rotational speeds. Since the elastohydrodynamic film thickness is a function of viscosity, a low viscosity will result in metal to metal contact within a bearing accelerating bearing failure. Therefore, a grease type with minimum viscosity of $1{,}000\,mm^2/s$ at 40°C should be employed on all RBC bearings.

5.1.1.4. Drive Motor

Drive motor is the heart of RBC, and it helps in driving the media support structure over the through-rods which support the media panels. It must be selected to account for the unbalance in the biomass after long periods of standstill, as could be experienced with the loss of electric power.

5.1.1.5. Through Rods

Through rods support the media pack and load distribution on them within a segment is dependent on the angular position of corrugations formed within the media pack. Maximum biomass growth of 5 mm on the inlet media pack should be used as the minimum loading criteria for new generation RBCs.



1. Stage 1
2. Stage 2
3. Stage 3
4. Discs
5. Shaft
6. Stand
7. Motor
8. Gear Box
9. Influent synthetic Wastewater
10. Peristaltic Pump
11. Effluent

Fig. 13.1. Schematic representation of an experimental RBC unit.

5.1.1.6. Nuts and Bolts

Nuts and bolts are required to fasten other components and they should be locked by positive locking systems such as locking plates, to ensure that the initial tightening torques are maintained.

The schematic of an experimental representation of RBC is presented in Fig. 13.1.

5.1.2. Mechanism of Substrate Removal in RBC

Major constituent of the RBC process is the biofilm. Biofilms are multiphase systems that consist of solids and of a liquid phase in the void space between the solids. An RBC biofilm is composed mainly of microorganisms, extracellular polymers which hold the cells together and to the surface of the discs (68, 69), water and sometimes, inorganic matter retained from the bulk liquid. Removal of soluble substrates inside biofilms includes three basic phenomena

1. transportation (diffusion) of substrates
2. substrate reactions and
3. transportation of the reaction products

Diffusion of the soluble substrate into the biofilm is a prerequisite for bacterial reactions. This transportation is governed by the biofilm thickness and the structure of the film formed by the bacteria. Substrate reactions inside the biofilm results in the formation of end products which have to be transported out of the biofilm, as their accumulation inside the biofilm is detrimental to the bacteria. Build-up of reaction products inside the biofilm may strongly influence the transportation of substrates in the film. For instance, outbreak of nitrogen bubbles produced as a result of denitrification may result in sloughing off of the biofilm or change the transport mechanism. Further, reaction kinetics cause inevitable changes in the biofilm structure. Precipitation of reaction products (e.g., calcium phosphate) turns parts of the biofilm into coral-like areas without bacterial activity (70).

5.1.3. Development of Microbial Communities in Aerobic RBC

The microbial communities in RBCs consist of primarily bacteria, protozoa, and metazoa. The community structure is largely influenced by the environmental parameters to which it is exposed to (71). The succession of these organisms from start up progresses from zoo flagellates and small amoebae to free swimming bacteriovorus ciliates and then to peritrichous and carnivrourous ciliates, rotifers, and large amoebae. Few organisms have been associated with mixed liquor, the reasons for it reported to be low dissolved oxygen concentrations, short residence time, and lack of sludge recycle (72). Moreover, the rate of growth of heterotrophic micro organisms is significantly higher than the nitrifying biofilms. Nitrifiers not only have a low growth rate but also a lower rate of attachment to disks. Furthermore, detachment of biomass, due to shear, took place at a significantly higher rate in the nitrifying biofilms. Thus, it is seen that the rate of attachment or affinity for attachment to disks is lower in the nitrifying organisms. Biofilm growth and biomass attachment to disk surface in the RBC system is governed by many factors such as environmental conditions, disk rotating speed, roughness of disk surface, substrate type and loading, and nature of seed organisms. Biofilm development is influenced by three phenomena: attachment of bacteria from bulk liquid to disk surface, multiplication of attached bacteria, and detachment of bacteria from biofilm by decay and shear. Studies on microbial communities in aerobic RBC have revealed that nitrifying biofilms are more influenced by shear stress than the heterotrophic biofilms (72).

5.2. Importance of Aerobic RBC

Fixed Film systems have been used for and organic matter stabilization and nutrient removal. Rotating Biological Contactors (RBCs) have been employed in recent years for the treatment of various types of substrates, including municipal wastewaters. RBC system has been used for biological nutrient removal involving aerobic and anoxic conditions, and various schemes have been developed for nutrient control involving suspended or attached growth process. Some applications of Aerobic RBC include

- Carbon removal
- Ammonia nitrification
- Denitrification
- Predenitrification
- Postdenitrification
- Simultaneous nitrification and denitrification accompanying carbon removal.

Some of these applications are discussed in the section.

5.2.1. Nitrification in RBCs

RBC when used for nitrification, results in a very low sludge yield from the attached culture and, as such, a treatment plant requiring carbon oxidation, nitrification, and denitrification steps need not have clarifiers between these steps. RBCs have been used extensively for combined carbon removal and nitrification as well as for separate stage carbon oxidation and nitrification. These have been found extremely useful to upgrade existing treatment plants to incorporate nitrification. Pretorius (73) conducted an exhaustive study on secondary

effluent from sewage treatment works for nitrification in RBC and obtained a nitrification rate of 2.4 g N/m^2/day after 30 days of operation which decreased to 1.86 N/m^2/day on prolonged run. Microscopic observation of scrapings from discs revealed that this decrease was associated with protozoa and other grazing organisms appearing on the films. pH had a marked effect on the rate of nitrification which dropped significantly below a neutral pH. The optimum pH was between 7.0 and 8.0 and nitrification occurred below pH 5.0. Above pH 9.0, nitrite concentration increased significantly indicating that the nitrite oxidizers were affected at high pH more adversely than ammonia oxidizers. The nitrification rate increased by 1.25 times when temperature was increased from 10 to 20°C. Average dry weight of solids on discs was 0.812 g/m^2, of which 89.2% was volatile and nitrification rate improved continuously when DO was raised from 0.5 to 2 mg/L. A further increase in DO concentration from 2 to 8 mg/L did not improve the rate significantly. An increase in concentration of oxygen from 21% (in air) to 50% doubled the nitrification rate.

Boller et al. (22, 74) worked on secondary effluent from sewage treatment plant and described the influence of various design and operational parameters, which affect the resulting substance fluxes in to and out of the biofilm and the biomass activity regarding nitrification in TFs, RBCs and different aerated biofilters. From experiments with RBCs under different organic loads, it was concluded that nitrification starts to take place below organic loads of 15 g COD/m^2/day, and it is fully developed at about 8 g COD/m^2/day only. In a series of RBCs separated by walls, a strong decrease in biofilm thickness and nitrifying activity was observed in subsequent stages. The nitrification activity dropped dramatically below pH 7.0 and came to a complete halt in a pH range of 6.5–6.7. Under non NH_4^+-N and nonalkalinity limited conditions, the maximum nitrification rates of the order of 1.5 g NH_4^+-N/m^2/day at 10°C were obtained. The maximum nitrification rates were strongly dependent on the rotational speed increasing almost linearly from 1 to 2.5 g N/m^2/day for speeds of 1.5–6 rpm. Particles in the secondary effluent adsorbed preferentially on first RBC units affecting the nitrification rate adversely. With optimized conditions of flow reversal and prefiltration, high specific nitrification rates upto 3.0 g NH_4^+-N/m^2/day could be achieved.

Free ammonia concentration greater than 0.1 mg N/L significantly inhibited the oxidative activity of *Nitrobacter* resulting in a transient accumulation of nitrite ions (75). The *Nitrobacter* population rapidly recovered its lost metabolic activity once the free ammonia concentration became less than 0.1 mg N/L. The EPA's manual 4 on nitrogen control describes that the rate of nitrification approaches a first-order relationship at effluent ammonium–nitrogen levels less than 5 mg/L; the maximum zero order removal rate occurs at levels above 5 mg/L. For a multistage RBC system, an empirical design approach is recommended for the treatment of municipal wastewaters for carbon and nitrogen removal. Substrate loading rates control oxygen demand and biofilm thickness. Substrate loading parameters for carbon oxidation systems include soluble BOD or COD, while loading parameters for nitrification systems include NH_4^+-N, TKN and soluble organic nitrogen.

Yamagiwa et al. (76) formed a biofilm on an oxygen enrichment tube support that consisted of a poly dimethylsiloxane hollow fiber membrane. Simultaneous organic carbon removal and nitrification were carried out in a single step treatment of domestic sewage. The nitrification rate in the biofilm was about 2.2 g/m^2 day at an air pressure of 19.6 kPa and was comparable to

that in conventional biofilm process designed specially for nitrification. Klees and Silverstein (25) examined the effect of recirculation on biological nitrification in a pilot scale RBC. Recirculation improved nitrification at all HRTs, but the effects were marginal. Recommendations for designing RBCs for nitrification were presented by O'Shaughnessy et al. (77) on the basis of their operational data. A minimum of two stages were recommended. The major design parameters included ammonia loading rate and volume to surface area ratio. The maximum volume to surface area ratio evaluated was $0.0163\,m^2/m^2$ ($0.4\,gal/ft^2$). Loading rates up to $0.977\,g\,NH_3\text{-}N/m^2$ day provided 94% removal of ammonia. Ito and Matsuo (78) conducted studies on nitrification using three different types of RBC units. Their data showed that the process is sensitive to organic loading, and that both nitrification and denitrification could proceed if methanol is added as a carbon source for denitrification.

5.2.2. Denitrification in RBC

Submerged RBC have been tried by many researchers for nitrification, while some have preferred using micro-aerobic films for combined nitrification–denitrification in a single unit. RBCs, as such, are not very popular for carrying out denitrification of wastewater.

Murphy et al (30) used a submerged RBC in series and obtained denitrification rates of $0.7–18\,g\,N/m^2/day$ at various feeding rates. Temperature had a strong influence on the rate of reaction. At low temperature of 15°C, the presence of DO in the first stage inhibited denitrification. At higher temperature, additional inputs of $(NO_3^- + NO_2^-)$-N were needed for rate determinations.

Blanc (42) presented the results of laboratory and pilot-scale investigations to evaluate design parameters for denitrifying RBCs. They gave a relation:

$$NR = 0.59NA + 18.8 \tag{8}$$

where,

NR = nitrogen removed, mg N/m^2 h
NA = nitrogen applied, mg N/m^2 h

Removals increased with increasing detention times. The methanol requirement for optimum removal was estimated as

$$CH_3OH\,mg/L = 2.6NO_3^- - N\,mg/L + 0.91\,DO\,mg/L \tag{9}$$

Cheung and Keuth (79) presented data that indicated that denitrification is first order. This was contrary to reports by other investigators that it has zero order or half first order kinetics. The first order kinetics were explained on the basis of nitrate loading (up to $18.85\,g/m^2/day$). It was suggested that zero order kinetics were obtained when the disk could not support increased growth with increased substrate, and denitrification became independent of substrate loading. The solids retention period was also found to be a linear function of substrate loading and a hydraulic detention time of 1.5 h was shown to be the preferred hydraulic detention time.

Rusten and Odegaard (80) reported that denitrification followed a zero order rate in an RBC system with respect to oxidized nitrogen concentration and a first order reaction with respect to

BOD. Denitrification was optimum at pH values of 7–8.5; about 2.4 mg BOD was consumed per mg of oxidized nitrogen removed; and an alkalinity of 0.0713 meq. was produced per mg of oxidized nitrogen removed in the system.

5.2.3. Combined Nitrification–Denitrification in RBC

Among nitrogen-removal process, the biological nitrification–denitrification process has become widely accepted for its reliability and low cost. In this process, nitrogenous pollutants are first converted into nitrate by the autotrophic nitrification bacteria under aerobic conditions. The nitrate is subsequently converted to nitrogen gas by the heterotrophic denitrification, the carbonaceous pollutants are also converted to carbon dioxide by the aerobic heterotrophic bacteria, leaving very little organic matter behind for the subsequent denitrification. Thus, the lack of organic carbons often limits the denitrification efficiency. To over come the carbon deficiency, an external organic carbon source has to be added, which results in an extra operating cost. Many researchers have utilized the anoxic layers in RBC biofilms, where the D.O was controlled by various means for simultaneous nitrification and denitrification.

Odegaard and Rusten (81) used Bardenpho process in a biodisk system to remove nitrogen without the addition of methanol. Ammonium rich influent passed through an anoxic submerged biodisk tank into an aerobic biodisk unit where nitrification occurred. Nitrified water was recycled to anoxic tank where denitrification took place using raw water as the carbon source. The anoxic–anaerobic system with recirculation, without the use of an external carbon source, was shown to give nitrogen removals in wastewater corresponding to:

$$R_n = 100\, r/(r + 1) \tag{10}$$

where,

R_n = percent nitrogen removed
r = recirculation ratio.

Masuda et al. (59) used a covered RBC in which the oxygen pressure was controlled for studying simultaneous nitrification and denitrification in biofilm. NH_4^+-N loading was fixed at 1 g/m^2 day. Nitrification efficiencies of 40–90% were obtained at various HRTs between 1.4 and 1.3 h. A maximum nitrogen removal efficiency of about 40% was obtained at oxygen partial pressure of approximately 0.07 atms. Increasing sewage C:N ratio from 1.5 to 2.5 and 3.5 resulted in increased nitrogen removals.

Watanabe et al. (61) studied simultaneous nitrification and denitrification in micro-aerobic biofilm using a partially submerged RBC whose air phase oxygen partial pressure was controlled. The maximum nitrogen removal was achieved at about 0.1 atm oxygen partial pressure and was found to be strongly influenced by C/N ratio, hydraulic loading besides the oxygen partial pressure in air phase.

Watanabe et al. (82) studied simultaneous nitrification and denitrification in partially and fully submerged RBCs. In municipal wastewater treatment where influent had a C:N ratio around 3.5, the maximum nitrogen removal efficiency was about 60%. With a synthetic influent having about 25 mg/L NH_4^+-N and 70 mg/L TOC, they reported that nitrogen removal at higher organic loading, was limited by nitrification, while at lower organic loading it was

limited by denitrification. At HRT 5 h and TOC loadings of 3.2 g/m^2 day, high nitrification and removal efficiencies (over 90% and 65% respectively) were obtained. Despite a high DO (2–4 mg/L), denitrification was quite significant.

Siegfred et al. (83) assessed the technical feasibility to treat digested black water from vacuum toilets (>1,000 mg NH_4^+-N L^{-1}) in a lab-scale oxygen-limited autotrophic nitrification/denitrification (OLAND) rotating biological contactor. After an adaptation period that lasted for 2.5 months, a stable nitrogen removal rate of 700 mg N L^{-1} d^{-1} was recorded in subsequent 5 months. It was found that suppression of nitrite-oxidizing bacteria at free ammonia levels above 3 mg L^{-1} resulted in nitrogen removal efficiency of 76%. The favorable ratios of both organic and inorganic carbon to nitrogen guaranteed endured annamox activity and sufficient buffering capacity. Results of Flourescent In Situ Hybridization (FISH) revealed that aerobic and anoxic ammonium-oxidizing bacteria (Aer AOB and AnAOB) made up 43 and 8% of the biofilm, respectively. Since a part of Aer AOB was probably present in anoxic biofilm zones, their specific ammonium conversion was very low, in contrast to the high specific AnAOB activity. Density Gradient Gel Electrophoresis (DGGE) analysis revealed that the dominant species (Aer AOB and AnAOB) were resistant to transition from synthetic medium to digested black water. This study demonstrates high rate of nitrogen removal from digested black water by one stage partial nitrification and anammox, which will allow significant decrease in operational costs compared to conventional nitrification/denitrification.

5.2.4. Single Stage Carbon Removal, Nitrification, and Denitrification in an Aerobic RBC System

Domestic wastewater treatment is mandatory due to the release of nitrogen in various forms in surface and ground waters which can pose certain health hazards on human beings and cattle which consume such contaminated water. It can also have wide ranging effects on the flora and fauna of the aquatic ecosystem receiving high nitrogenous wastewaters. Concentration of ammonia as low as 0.2 mg/L have been reported as harmful, while concentrations as high as 1.5 mg/L can lead to the death of all types of fish although lower organisms can survive concentrations of up to 9 mg/L (84). Nitrification in the receiving waters may exert a significant oxygen demand resulting in the depletion of dissolved oxygen. High ammonia containing wastewaters when discharged on land may result in building up ground water nitrates. The costs associated with remediating groundwater nitrates are high. Increased nitrogen loads can stimulate the growth of lower aquatic plants such as algae and phytoplankton which may lead to eutrophication of lakes with a consequent sharp reduction in the oxygen content in the water and removal of vital base of higher organisms. This may lead to taste and odor problems and aesthetically displeasing conditions (85). Ammonia in waters used for drinking purposes increases the chlorine dosage required to achieve free chlorine residual in disinfection. In view of above problems, it becomes necessary to evolve an economically feasible treatment technology which could upgrade the existing treatment plants with minimal alterations and least possible addition to the capital and operating costs.

The use of *Thiosphera pantotropha*, a heterotrophic nitrifier and aerobic nitrifier, can effectively combine all the steps needed for organics and nitrogen removal. The available literature on *T. pantotropha*, one of such organisms, describes in details its particular properties

of heterotrophic nitrification and autotrophic denitrification. A close analysis of the above mentioned properties of *T. pantotropha* indicates a great promise for its use in a mixed culture biofilm system to combine carbon oxidation, nitrification, and denitrification together without encountering problems of acclimation, poor settling characteristics of the biomass, and low removal efficiencies. Another benefit could be a lesser overall sludge production. In the conventional approach for nitrogen removal, the denitrification unit needs an additional carbon source as electron donor thus resulting in overall higher sludge production than in a single step process with no external organic input. A careful selection of operating parameters such as, loading rates for organics and nitrogen and the retention time in the reactor to bring DO in the desired range may help in evolving a highly efficient and compact system for carbon and nitrogen removal at very low costs.

5.3. Advantages of Aerobic RBC

Rotatory Biological Contactors have proved to be a viable means for the secondary treatment of wastewaters originating from both industrial and municipal sources. They have been successfully used for organic matter stabilization and nutrient removal. RBC offers the following characteristics and distinct advantages as compared to suspended growth system (86, 87)

- *The films in the system possess high ability of nitrogen removal* because it contains some long generation time bacteria that grow very slowly like nitrifying bacteria, which is created by the fixed growth of film in a stable eco-system.
- *Wide spectrum pollutant removal* can be achieved because of the existence of more species of organisms in the film when compared with the activated sludge process. The organisms include aerobic, facultative and anaerobic bacteria and fungi, algae, and zoo planktons, which are rarely found in activated sludge.
- *The treatment capacity per unit volume of the process is remarkably larger than activated sludge process.* A huge amount of biomass grows and occupies the whole space of the treatment facility with vast surface area, and the biomass amount per unit volume is larger than that of the suspended activated sludge process. Unit treatment capacity is greater than that of the activated sludge process.
- *Less surplus sludge*, Organisms of higher trophic levels exist in the film, which means that the food chain in the film is longer than in the activated sludge process. As a result, more sludge produced is consumed by organisms in the film, and there is surplus sludge produced when compared with the activated sludge process.
- *Energy saving and convenient in operation/maintenance*. The system is simple and convenient in operation and maintenance for no returning of sludge and effluent is required.
- *Stable operation efficiency*. The process can sustain and adapt fluctuation of hydraulic and organic loading, since it possess larger amount of biomass and longer food chain when compared with the activated sludge process, i.e., it possesses a more complex and stable eco-system, and our previous experiments showed that it recovered very quickly even after being destroyed by shock loading.
- *Reduced washout of nitrifying organisms*. On numerous occasions, nitrifying organisms are washed out in settling basins of ASP because of hydraulic surges or poor settling characteristics (88, 89). The attachment to a surface can be considered an integral step to the overall microbial flocculation phenomenon. It was found that the nitrifying organisms formed denser and

thinner biofilm. Though they show poorer attachment properties as compared to heterotrophs, the chances of washout are much reduced.

5.4. Demerits of RBC

Mechanical failures are common and unpredictable during the operation of an RBC. Typical failures reported in an in-depth investigation by the US Environmental Protection Agency (90) into the design, operation, and maintenance of RBCs included shaft breakage, stub shaft damage, media degradation and damage, and bearing failure.

- *Shaft failure*: Fracture of shafts have been experienced on several RBCs which has resulted in the complete breakdown of the unit. It is the most severe form of mechanical breakdown requiring total replacement of the unit as the media panels are damaged beyond repair.
- *Bearing failure*: Bearing failure can impair the operation of RBCs and is the result of incorrect grade of grease being used, grease starvation or in some instances grease contamination. This can in turn result in cage failure, severe pitting of rolling elements, wear of the elements and in severe instances scoring of stub shaft.
- *Media support structure failure*: The bolts or straps that clamp the media supporting rods to the supporting structure suffer from fatigue fracture within 10 years as a direct result of large bending movements being reacted back on to the supporting frame. Any stresses induced during the manufacture of the bolts or straps which are normally "U" shaped also account for this fracture. This type of fracture is believed to cause a domino effect, where the load is transferred on to adjacent end clamps/straps thereby reducing the service life of the unit.
- *Galvanic corrosion*, associated with the use of mixture of materials for the manufacture of the units results in a reduction in the operational life of components. Use of two different materials with some form of electrolyte, like sewage wastewater induces bimetallic corrosion.
- *Microbiologically induced corrosion (MIC)*: Large amounts of biomass exist in the first stage of a highly loaded RBC contributing to the removal of organic materials in the influent. Sulfide present in the influent or produced deep within the biofilm induces the growth of sulfide oxidizing bacteria (SOB) such as Beggiatoa to grow on the biofilm surface. Production of sulfide due to oxygen depletion causes Beggiatoa to compete with other heterotrophic organisms for oxygen and in extreme cases, it will take over the first stage of an overloaded RBC and progressively will dominate the entire system. Microbially induced corrosion leads to rapid material deterioration. Micro organisms play a role in inducing and accelerating corrosion by several mechanisms including formation of aggressive metabolites such as organic and inorganic acids and sulfides. Sulfate reducing bacterium (SRB), which are anaerobic organisms that have a restricted nutritional spectrum, are instrumental in causing microbial corrosion. They tend to grow with other micro and macro organisms and deprive the system of oxygen and generate nutrients at the expense of their own metabolism (91, 92).

Beggiatoa provides nutrients for SRB and promotes their growth. Formation of thick biofilm occurs as a result of which diffusion of oxygen into the biofilm is hindered, and also the out ward diffusion of metabolites and corrosion products can be impeded, thus allowing the areas within the biofilm to become anaerobic. MIC is a localized corrosion that can appear as pitting, crevice corrosion, under-deposit corrosion, or stress corrosion cracking. Hydrogen sulfide production as a result of SRB enhances corrosion and causes hydrogen embrittlement. Here, hydrogen sulfide is believed to play a mechanistic role in promoting the entry of hydrogen into iron based alloys. Many structural and operational problems on RBCs with heavy biomass

growth of Beggiatoa have been cited including failures of shaft and media panels (90, 93–95). Use of supplemental air is believed to eliminate Beggiatoa growth and establish thinner active biomass implying less stress on load carrying structural members of an RBC. Increase of rotational speed is also an alternative method to reduce biomass growth. Increase in rotational speed increases the shearing force acting to strip excessive biomass growth.

5.5. Major Design Criteria for New Generation RBCs

A UK water company issues mechanical specification to all RBC manufacturers, whereby manufacturer has to comply with the following design specifications (96)

- Stainless steel should not be used on future RBCs, or materials having different galvanic constants.
- Polymers must not be subject to compression or tension strengths over 4 MPa.
- All stresses within the frame and shaft must be subject to a limiting bending stress of 20 MPa, unless a full finite element analysis is undertaken which considers fatigue stress concentration factors, etc. In designing the frames, consideration must be given to the flexural centre used when determining the torsion due to twisting, more-over, the calculations must consider unsymmetric bending if a closed form of solution is required. Where sections are subject to twisting, the 'wrapping' function must be carefully accounted for. Also, steps must be taken to reduce the fatigue strength on all galvanized components.
- In the design of the through-rods which support the media panels, nonsymmetric loading of biomass must be considered whilst accessing the dynamic loading acting on these rods. Moreover, consideration must be given to the draining of liquid as the RBC emerges from the sewage. For example, the unbalance caused by the simultaneous lifting and draining of sewage water.
- All bolts and screws must have pretightening torques such that they overcome friction factors of 0.18 under the bolt head, and, 0.2 in the screw thread. Moreover, the design must demonstrate that the cyclic loading in the bolt, combined with any direct stresses does not exceed the pretightening tension.
- The drive motor must be selected to take into account the unbalance of the biomass after long periods of standstill, as could be experienced with loss of electric power
- A novel design approach for shaftless RBCs has been proposed. This design reduces the occurrence of loss operation of RBC units and ensures that the effluent continually meets discharge standards. The added significance of the design is a 50% reduction in costs (97).

5.6. Recent Developments

T. pantotropha – a sulfur bacterium capable of heterotrophic nitrification and autotrophic denitrification was identified during studies on desulfurizing, denitrifying effluent treatment system. Robertson and Kunen (98) isolated a bacterium which was able to grow aerobically as well as anaerobically on reduced sulfur compounds and hydrogen while fixing carbon dioxide. This isolate was also found capable of mixotrophic and heterotrophic growth on a wide range of substrates, thereby proving itself to be a facultative anaerobe and facultative autotroph. In view of its ability to oxidize reduced sulfur compounds and because it is a chain-forming coccus, the isolate was given the generic name *Thiosphaera* and the species name *pantotropha* in recognition of its wide range of potential substrates. The organism is capable of simultaneous heterotrophic nitrification and aerobic denitrification. Ample literature has been published, after the isolation of this bacterium by Robertson and Kunen (98), on its

peculiar enzyme system to explain the properties of heterotrophic nitrification (99–101) and aerobic denitrification (99, 102–107). Reports on the use of this bacterium in suspended and immobilized forms in axenic cultures for carbon removal, nitrification and denitrification are available (100, 108, 109). Till date, only one report on a simultaneous organics and nitrogen removal in fully aerobic RBC is available (110). A single stage aerobic RBC system has been employed using *T. pantotropha* for simultaneous carbon removal, nitrification, and denitrification (111).

T. pantotropha is a nonmotile, Gram-negative coccus ($0.7 \times 0.9\,\mu m$) which is frequently seen in pairs or long chains. It has a %Guanine–Cytosine ratio of 65.8–66.0 which is in the same range as that of other chemolithotrophs such as *Thiobacillus* A2 (65–68%) and *Paracoccus denitrificans* (66.5%). On plates of acetate or thiosulfate containing solid medium, it grows as off-white, translucent, round colonies. It can grow over a pH range of 6.5–10.5 with an optimum at 8.0; the temperature range permitting growth lies between 15 and 42°C with an optimum at 37°C. It is both catalase and oxidase positive.

Promotion of *T. pantotropha* needs the mention of the following important reasons: (1) The specific nitrifying activity of the heterotrophs is said to be 10^3–10^4 times lower than that of autotrophs. However, the ammonia oxidizing rates of *T. pantotropha* are only 10–10^3 times lower than the autotrophs (108). While growing as heterotroph, the growth rates of it tend to be much higher than those for the autotrophs (the μ_{max} for *Nitrosomonas europea* is about 0.03–0.05/h, and that of *T. pantotropha* can be as high as 0.4/h under same growth conditions), giving it a competitive advantage (100); (2) The aerobic denitrification rates were much higher than heterotrophic nitrification rates in chemostat studies with axenic cultures of *T. pantotropha* at all dilution rates indicating extra capacity of this bacterium to take nitrate or nitrite coming from other routes apart from its nitrification path (100). The above considerations indicate the possibility of using a mixed culture having autotrophic nitrifiers and *T. pantotropha* along with other heterotrophs to evolve an optimum carbon and nitrogen removal system.

In wastewater treatment, the nitrification step is often a bottle neck. The residence time in nitrification unit is mainly determined by slow growing nitrifiers. In view of its higher growth rate and ability to convert ammonia to nitrogen gas, the use of *T. pantotropha* can provide an attractive alternative to wastewater treatment for simultaneously removing two priority pollutants viz. carbon and nitrogen. A few advantages that can be accured by such a system over conventional ones are: (1) No prior carbon removal step required before nitrification, (2) No external carbon source for denitrification, (3) Lesser buffer needed as alkalinity generated during denitrification can partly compensate for the alkalinity destroyed in nitrification, (4) No acclimation problems as faced in a single stage oxic–anoxic system (108, 111). Before employing such a treatment scheme, it would be essential to study the interaction of *T. pantotropha* with other heterotrophs and autotrophic nitrifiers in mixed bacterial biomass.

Robertson et al. (100) studied simultaneous nitrification and denitrification in aerobic chemostat cultures of *T. pantotropha*. The maximum rate of nitrification obtained in these experiments was 93.9 nmol ammonia/min/mg protein. The nitrification rate was found to reduce by the provision of nitrate, nitrite, or thiosulfate to the culture medium. Both

nitrification and denitrification increased as DO fell, until a critical level of approximately 25% of air saturation was reached. At this point, the rate of aerobic denitrification was equivalent to the anaerobic rate.

Nitrification appeared to oxygen limited at this DO concentration and nitrification rate started falling. The nitrification rates ranged from 7.9 to 93.9 nmol ammonia/min/mg protein, while denitrification rates varied from 12.7 to 506.9 nmol/min/mg protein for dilution rates of 0.02–0.17/h and various combinations of ammonia, nitrate, and nitrite nitrogens.

Van Neil et al. (112) studied the competition between a heterotrophic nitrifier *T. pantotropha* and an autotrophic nitrifier *N. europea* for ammonia in chemostat cultures. They varied the C:N ratio between 1.9 and 10.4 and found that a higher C:N ratio favored the growth of *T. pantotropha*, but it was able to outcompete *N. europea* for ammonia only at a value of 10.4. At DO below 10, the autotroph became oxygen limited and the heterotroph dominated in the culture. When the dilution rate increased from 0.04 to 0.067/h, *N. europea* could not maintain itself in the chemostat. Nitrification by *T. pantotropha* was equivalent to that of *N. europea* when the cell ratio of heterotrophic to autotrophic nitrifiers was 250. These parameters may prove to be of great importance for understanding the behavior of an environmental system working on simultaneous carbon and nitrogen removal.

In a study (113, 114), the ability of *T. pantotropha* mixed with activated sludge was confirmed to aerobically denitrify synthetic wastewater having NO_3-N concentrations upto 425 mg/L. The hydraulic retention times were 0.5 and 1 day and solids retention time varied between 2 and 8 days. An NO_3-N removal efficiency of 75–85% was obtained. DO in the reactors was always more than 2.5 mg/L. The COD removal rates varied from 0.53 to 1.06 g COD/g VSS/day, while the nitrate removal rates varied from 0.176–0.355 g N/g VSS/day. The nitrate removal rate increased with an increase in COD loading rate upto 1.5 g COD/g VSS/day after which it attained a constant value.

Another study by Gupta et al. (115) involved the growth of a mixed biofilm in a rotating biological contactor containing *T. pantotropha*, autotrophic nitrifiers, and other heterotrophs for treating a synthetic fertilizer industry wastewater. The influent had a high TKN upto 1,386 mg/L and nitrate–nitrogen of 400 mg/L. TKN removal of 44–95% and nitrate removal of 97–98% were achieved simultaneously at different hydraulic retention times and nitrogen loadings. The overall TKN removal rates varied between 6.37–7.98 g/m^2 day. An overall nitrogen loading of 9.36 g/m^2 day at 2-day HRT was found to yield the best results. The first stage showed an extremely high ammonia oxidation rate of 19.15 g N/m^2 day and denitrification rate of 20.93 g N/m^2 day under optimum conditions. Nitrite accumulation was a major problem in the process.

Geraats et al. (108) gave a metabolically structured model for the study of growth, nitrification, and denitrification by *T. pantotropha*. They used the results of aerobic and anaerobic continuous culture experiments supplied with ammonia and nitrate. Hooijmans et al. (108) described a model to determine the growth and coupled nitrification/denitrification by immobilized *T. pantotropha* using measurements and modeling of oxygen profiles. The average value for the maximum specific growth rate was 0.52/h and the maximum oxygen conversion rate was 1.0 mol/Cmol/h. The maximum specific acetate uptake rate was 2.0 mol/Cmol/h and the Monod constant for acetate was 2.9×10^{-2} mol/m^3. The maximum specific nitrification

rate was 11% of the total oxygen uptake rate. Both the models, put together, could describe successfully the kinetic behavior of the chemostat cultures as well as immobilized oxygen-consuming cells.

It is now clear that the conventional approach to remove organics and nitrogen from municipal wastewaters may involve the following variations: a) Separate units for organics removal, nitrification, and denitrification. This may result in high efficiencies of the individual units but would require a high capital investment and elaborate maintenance problems. The need for an external carbon source will further add to the cost of treatment; b) Single unit for organics removal and nitrification and a separate unit for denitrification. The design of the first unit will be governed by the autotrophic nitrifiers requiring extended aeration and high SRTs. The second unit will require an external carbon source making the overall process expensive; c) All the three reactions may be combined in a single unit having alternate oxic/anoxic zones. This will pose acclimation problems and may result in poor settling of biomass. The process would require skilled supervision and the removal efficiencies would be low.

The use of simultaneous heterotrophic nitrifier and aerobic denitrifier can efficiently combine all the steps needed for organics and nitrogen removal. The available literature on *T. pantotropha*, one of such organisms, describes its peculiar properties of heterotrophic nitrification and aerobic denitrification. All these studies were made either on axenic cultures or a coculture with *Nitrosomonas*. The potential of this bacterium for wastewater treatment has not yet been exploited. A close analysis of the above mentioned properties of *T. pantotropha* indicates a great promise for its use in a mixed culture biofilm system to combine carbon oxidation, nitrification, and denitrification together without encountering the problems of acclimation, poor settling characteristics of the biomass, and low removal efficiencies. Another benefit could be a lesser overall sludge production. In the conventional approach for nitrogen removal, the denitrification unit needs an additional carbon source as electron donor thus resulting in overall higher sludge production than in a single step process with no external inorganic input. A careful selection of operating parameters such as, loading rates for organics and nitrogen and the retention time in the reactor to bring DO in the desired range may help in evolving a highly efficient and compact system for carbon and nitrogen removal at very low costs.

In this direction, a mixed culture bacterial film containing *T. pantotropha* in a RBC for the treatment of different strengths of simulated domestic wastewater by Gupta (116). High nitrification rates (0.47–1.85 $g/Nm^2/day$) were obtained for corresponding loading rates of 0.69–3.35 $g/Nm^2/day$ despite concurrent high organic loadings of 6.9–32 $g\,COD/m^2/day$. There was a simultaneous nitrogen removal of 18–72%. The system was able to achieve effluent standards for both organics and nitrogen under such high loading rates which are conventionally used to design treatment facilities for only organics removal. Step-feeding resulted in achieving the effluent at much higher loading rates. The biofilm showed better shock loading characteristics than autotrophic biofilms used for nitrification. A vast superiority of the present system over the conventional ones has been established because of the introduction of *T. pantotropha*.

In a conventional RBC described by Gonenc and Harremoes (117) designed for carbon removal and nitrification, the nitrogen balances indicated that an average of 8% of the total influent nitrogen did not appear in the effluent. Gupta and Gupta (110) reported that 18–49%

nitrogen was unaccounted in the first stage effluent which could primarily be attributed to aerobic denitrification by *T. pantotropha.* There was little loss (<5%) in the subsequent stages except for the last two observations. The increase in unaccounted nitrogen in the second and third stages for these observations may be due to the leakage of carbon from the first stage at high loading rates thereby helping *T. pantotropha* to survive and denitrify. The amount of ammonium-nitrogen removed by assimilation was not accounted for as the maximum nitrogen removal by assimilation of coculture of *T. pantotropha* and *Nitrosomonas* in a chemostat experiment was reported to be 15% as a C:N ratio of 3.8 (118). At a lower C:N ratio of 3.2 used in our system and considering the low suspended solids in the effluent, the nitrogen lost in assimilation can be neglected. The overall nitrogen removals of 44–63% due to a simultaneous aerobic denitrification in the present study further establish its superiority over conventional ones. The net alkalinity consumed per unit of ammoniacal nitrogen removed varied between 3.62 and 5.97. If the generation of alkalinity during denitrification is considered equal to half of that destroyed during nitrification, then the above ratio assumes values between 4.4 and 7.14. This reflects a saving in terms of chemicals required for maintaining the desired alkalinity levels in a separate nitrification systems.

Some of the salient points to be noted from the study of Gupta and Gupta (119) were

1. The calculated dry biomass of *T. pantotropha* increased from 5.1 to 19.5 g as the organic loading rate increased from 10 to 26.6 g COD/m^2 day, while that of *Nitrosomonas* fluctuated in a close range of 0.027–0.060 g.
2. As the organic loading rate increased from 10 to 32 g COD/m^2 day, the ratio of *T. pantotropha* biomass to *Nitrosomonas* biomass increased from 106 to 363.
3. The percent heterotrophic nitrification contribution increased from 40 to 65% as the organic loading rate increased from 10 to 32 g COD/m^2 day. This was due to an increase in both *T. pantotropha* biomass and the ratio of *T. pantotropha* biomass to *Nitrosomonas* biomass as the organic loading increased.
4. Very high organic removal rates (8.7–25.9 g COD/m^2 day) and high nitrification rates (1.53–1.85 g NH_4^+-N/m^2 day) in the first stage area can result in a highly improved performance of the aerobic RBC for a simultaneous carbon removal, nitrification, and denitrification by introducing *T. pantotropha* in fixed biofilm.

The study of treatment feasibility of domestic wastewater in an aerobic RBC using a mixed culture biofilm showed that simultaneous carbon removal and nitrification can be achieved at high rates (116).

6. SUMMARY AND CONCLUSIONS

Domestic wastewater treatment has received considerable attention in the recent times. Nutrient removal has become mandatory because of the establishment of adequate legislative measures in different countries. Physico-chemical treatment, biological processes and treatments, and land treatment have been approached for nitrogen removal with adequate success. A single stage aerobic RBC system can be successfully employed for simultaneous carbon removal, nitrification, and denitrification.

Aerobic RBC can be recommended for simultaneous removal of carbon and nitrogen. RBCs are the best choice for domestic wastewater treatment and the following points highlight their efficiency and acknowledge their growing importance in wastewater treatment.

- A huge saving in chemicals as the buffer requirements would be reduced substantially using aerobic RBC.
- Complete elimination of a separate denitrification unit, thus major savings in the cost of external carbon source could be effected; when aerobic RBC is depended on.
- Nitrification rates were not affected despite a high carbon loading rate when aerobic RBC is employed.
- Ease of handling as neither separate units would be required to handle; no provision would be needed to develop anoxic/oxic as adopted presently in a single sludge system.
- Very high organic removal (25.9 g COD/m^2 day) and high nitrification rates (1.85 g NH_4^+-N/m^2 day) can be achieved with the aerobic RBC for simultaneous carbon removal, and nitrification.
- Aerobic RBC shows good response to both continuous and sudden shocks and restored to normal performance quickly.
- A rotating biological contactor can be successfully employed to treat different concentrations of simulated domestic wastewater for simultaneous carbon oxidation, nitrification, and denitrification in a mixed culture biofilm containing *T. pantotropha*, which is a heterotrophic nitrifier and an aerobic denitrifier.
- Focus on good design, providing equipment requiring little maintenance at a competitive cost and maintaining stringent manufacturing standards, will encourage the guaranteed application of RBCs for pollution prevention.
- Reduction in disc diameter of RBC brings about an increase in power economy, but results in a considerable reduction in the removal rate of BOD per unit floor area of the RBC.
- Selection of the lowest peripheral speed of the disc guarantees power economy of the system. Operation of RBCs with peripheral speed close to its minimum renders the aerobic RBC system most economical for domestic wastewater treatment.
- Aerobic RBC systems could be successfully operated, provided the units are designed for daily average effluent BOD concentration.
- Adaptation of flow balancing tank prior to RBC unit can greatly reduce the required floor area of the aerobic RBC system.
- Choice of best materials for construction of RBC units based on stress levels will help in eliminating operational failures.
- Improved RBC design could revolutionize applicability of RBCs to high flow/highly populated regions (120–122).

7. DESIGN EXAMPLES

Example 1

Determine the specific TKN removal rate for an effluent with BOD 140 g/m^3 and TKN = 20 g/m^3

$$\text{TKN removal rate } (R_n) = 0.82(\text{BOD/TKN})^{-0.44}$$

$$R_n = 0.82(140/20)^{-0.44}$$

$$= 0.38 g/m^2/day$$

Solution

$$\text{TKN removal rate } (R_n) = 0.38\,\text{g/m}^2\text{/day}$$

Example 2
Determine the number of RBC shafts for the first stage of a 3 stage RBC. Also determine the flow rate per train

$$\begin{aligned}\text{Conditions:}\quad &\text{Ist stage soluble BOD}(S_{BOD}) = 13\,\text{g/m}^2\text{day}\\ &\text{Effluent soluble BOD}(S_{BOD}) = 70\,\text{g/m}^3\\ &\text{Use } 9{,}300\ \text{m}^2\text{/shaft, flow rate} = 4{,}500\,\text{m}^3\text{/day}\end{aligned}$$

Solution

$$\begin{aligned}\text{Ist stage soluble BOD}(S_{BOD}) &= 13\,\text{g/m}^2\text{day}\\ S_{BOD}\text{ loading} &= 70\,\text{g/m}^3 \times 4{,}500\,\text{m}^3\text{/day}\\ &= 315{,}000\,\text{g/day}\\ \text{Disk area required} &= \frac{315{,}000\,\text{g/day}}{13\,\text{g/m}^2\text{day}}\\ &= 24{,}230.76\,\text{m}^2\end{aligned}$$

Use $9{,}300\,\text{m}^2$/shaft

$$\begin{aligned}\text{Number of shafts} &= \frac{24{,}230.76\,\text{m}^2}{9{,}300\,\text{m}^2\text{/shaft}}\\ &= 2.6\end{aligned}$$

Use 3 shafts for first stage at $9{,}300\,\text{m}^2$/shaft

$$\begin{aligned}\text{Flow rate/train} &= \frac{4{,}500\,\text{m}^3\text{/day}}{2\text{ trains}}\\ &= 2{,}250\,\text{m}^3\text{/day}\end{aligned}$$

(Assume 2 trains with 3 shafts)

Solution
3 RBC Shafts are required for the first stage of a 3 stage RBC with an effluent BOD of $70\,\text{g/m}^3$ and flow rate of $4{,}500\,\text{m}^3\text{/day}$.

Example 3
Determine the number of RBC shafts for the second stage of a 3 stage RBC. Also determine the flow rate per train

$$\text{Conditions: IInd stage soluble BOD}(S_{\text{BOD}}) = 12\,\text{g/m}^2\text{day}$$
$$\text{Effluent soluble BOD}(S_{\text{BOD}}) = 75\,\text{g/m}^3$$
$$\text{Use } 9{,}300\text{m}^2\text{/shaft, flow rate} = 4{,}000\,\text{m}^3\text{/day}$$

Solution

$$S_{\text{BOD}}\text{ loading} = 75\,\text{g/m}^3 \times 4000\,\text{m}^3\text{/day}$$
$$= 300{,}000\,\text{g/day}$$

$$\text{Disk area required} = \frac{300{,}000\,\text{g/day}}{12\,\text{g/m}^2\text{day}}$$
$$= 25{,}000\,\text{m}^2$$

Use $9{,}300\,\text{m}^2\text{/shaft}$

$$\text{Number of shafts} = \frac{25{,}000\,\text{m}^2}{9{,}300\,\text{m}^2\text{/shaft}}$$
$$= 2.6$$

Use 3 shafts for the 2nd stage
(Assume 3 trains with 3 shafts)

Solution
3 RBC Shafts are required for the second stage of a 3 stage RBC with an effluent BOD of $75\,\text{g/m}^2$ and flow rate of $4{,}000\,\text{m}^3\text{/day}$.

Example 4
Determine the number of RBC shafts for the third stage of a 3 stage RBC. Also determine the flow rate per train

$$\text{Conditions: IIIrd stage soluble BOD}(S_{\text{BOD}}) = 10\,\text{g/m}^2\text{day}$$
$$\text{Effluent soluble BOD}(S_{\text{BOD}}) = 80\,\text{g/m}^3$$
$$\text{Use } 9{,}300\text{m}^2\text{/shaft, flow rate} = 4{,}000\,\text{m}^3\text{/day}$$

Solution

$$S_{\text{BOD}}\text{ loading} = 80\,\text{g/m}^3 \times 4{,}000\,\text{m}^3\text{/day}$$
$$= 320{,}000\,\text{g/day}$$

$$\text{Disk area required} = \frac{320{,}000\,\text{g/day}}{10\,\text{g/m}^2\text{day}}$$
$$= 32{,}000\,\text{m}^2$$

Use $9{,}300\,\mathrm{m}^2/\mathrm{shaft}$

$$\text{Number of shafts} = \frac{32{,}000\,\mathrm{m}^2}{9{,}300\,\mathrm{m}^2/\mathrm{shaft}}$$
$$= 3.4$$

Use 3 shafts for the 2nd stage
(Assume 3 trains with 3 shafts)

Solution
3 RBC Shafts are required for the third stage of a 3 stage RBC with an effluent BOD of $10\,\mathrm{g}/\mathrm{m}^2$ and flow rate of $4{,}000\,\mathrm{m}^3/\mathrm{day}$.

Example 5
Calculate the soluble BOD concentration of a first stage of a 3 stage RBC using the following data:

Parameter	Unit	Primary effluent	Target effluent
Flow rate	$\mathrm{m}^3/\mathrm{day}$	4, 200	
BOD	g/m^3	140	20
S_{BOD}	g/m^3	90	10
TSS	g/m^3	70	20

Assume area of RBC $= 9{,}300\,\mathrm{m}^2$

Solution

$$\text{Soluble BOD concentration}\,(S_1) = \frac{-1+\sqrt{1+4(0.00974)(A_\mathrm{s}/Q)S_0}}{(2)(0.00974)(A_\mathrm{s}/Q)}$$
$$S_0 = 90\,\mathrm{g}/\mathrm{m}^3$$
$$A_\mathrm{s}/Q = \frac{\text{Area of shaft}}{\text{Flow rate/ train}},$$
$$\text{Flow rate/train} = \frac{4{,}200\mathrm{m}^3/\mathrm{day}}{3\text{ trains}}$$
$$= 1{,}400\,\mathrm{m}^3/\mathrm{day}.$$
$$A_\mathrm{s}/Q = \frac{9{,}300\,\mathrm{m}^2}{1{,}400\,\mathrm{m}^3/\mathrm{day}}$$
$$= 6.6421.$$
$$S_1 = \frac{-1+\sqrt{1+4(0.00974)(6.9751)(90)}}{(2)(0.00974)(6.642)},$$
$$S_1 = 30.45.$$

Solution

$$\text{First stage soluble BOD}(S_1) = 30.45\,\text{g/m}^3$$

Example 6
Determine the organic and hydraulic loading rate of a 3 stage RBC with the following data:

$$\begin{aligned}
\text{BOD}\,(\text{g/m}^3) &= 140,\\
S_{\text{BOD}}(\text{g/m}^3) &= 95,\\
\text{Flow rate} &= 3{,}500\,\text{m}^3/\text{day},\\
A_{\text{S}} &= 9{,}300\,\text{m}^2,\\
L_{\text{org}} &= \frac{(3{,}500\,\text{m}^3/\text{day})(95\,\text{g}\,S_{\text{BOD}}/\text{m}^3)}{(3)(9{,}300\,\text{m}^2)}\\
&= 11.91\,\text{g}\,S_{\text{BOD}}/\text{m}^2/\text{day},\\
\text{HLR} &= \frac{3{,}500\,\text{m}^3/\text{day}}{(3\,\text{stage})(3\,\text{shaft/stage})(9{,}300\,\text{m}^2/\text{shaft})}\\
&= 0.04\,\text{m}^3/\text{m}^2/\text{day}.
\end{aligned}$$

Solution

$$\text{Organic loading rate} = 11.91\,\text{g}\,S_{\text{BOD}}/\text{m}^2/\text{day}$$

$$\text{Hydraulic loading rate} = 0.04\,\text{m}^3/\text{m}^2/\text{day}.$$

Example 7
Determine the overall organic loading for a 3 stage RBC and 2 stage RBC with a flow rate of $3{,}750\,\text{m}^3/\text{day}$ and BOD of $120\,\text{g/m}^3$

Solution

$$\begin{aligned}
L_{\text{org}} &= \frac{(3{,}750\,\text{m}^3/\text{day})(120\,\text{g/m}^3)}{(3\text{stage})(3\text{shaft/stage})(9{,}300\,\text{m}^2/\text{shaft})}\\
&= 5.3\,\text{g BOD/m}^2/\text{day}
\end{aligned}$$

$$\begin{aligned}
L_{\text{org}} &= \frac{(3{,}750\text{m}^3/\text{day})(120\,\text{g/m}^3)}{(2\,\text{stage})(2\,\text{shaft/stage})(9{,}300\,\text{m}^2/\text{shaft})}\\
&= 12.09\,\text{g BOD/m}^2/\text{day}.
\end{aligned}$$

Solution

$$\text{Overall organic loading of 3 stage RBC} = 5.3\,\text{g BOD/m}^2/\text{day}.$$

$$\text{Overall organic loading of 2 stage RBC} = 12.09\,\text{g BOD/m}^2/\text{day}.$$

Example 8
Calculate the soluble BOD concentration of a 2nd stage of a 3 stage RBC with following data:

Parameter	Unit	Primary effluent	Target effluent
Flow rate	m^3/day	4, 300	
BOD	g/m^3	140	20
S_{BOD}	g/m^3	90	10
TSS	g/m^3	70	20

Assume area of RBC $= 9{,}300\,m^2$

$$\text{Soluble BOD concentration}(S_2) = \frac{-1+\sqrt{1+4(0.00974)(A_s/Q)S_1}}{(2)(0.00974)(A_s/Q)},$$

$$S_1 = 30.45\,g/m^3,$$

$$A_s/Q = 6.4\,d/m,$$

$$S_2 = \frac{-1+\sqrt{1+4(0.00974)(6.4\,d/m)30.45\,g/m^3}}{(2)(0.00974)(6.4\,d/m)}$$

$$= 15.5\,g/m^3$$

Solution

$$\text{IInd stage soluble BOD}(S_2) = 15.5\,g/m^3$$

Example 9
Calculate the soluble BOD concentration of a 3rd stage of a 3 stage RBC with following data:

Parameter	Unit	Primary effluent	Target effluent
Flow rate	m^3/day	4, 250	
BOD	g/m^3	100	20
S_{BOD}	g/m^3	90	10
TSS	g/m^3	70	20

Assume area of RBC $= 9{,}300\,m^2$

$$\text{Soluble BOD concentration}(S_3) = \frac{-1+\sqrt{1+4(0.00974)(A_s/Q)S_2}}{(2)(0.00974)(A_s/Q)},$$

$$S_2 = 15.5\,g/m^3,$$

$$A_s/Q = 6.97\,d/m,$$

$$S_3 = \frac{-1 + \sqrt{\sqrt{1 + 4(0.00974)(6.5\,\text{d/m})15.5\,\text{g/m}^3}}}{(2)(0.00974)(6.5\,\text{d/m})}$$
$$= 10.15\,\text{g/m}^3.$$

Solution

Third stage soluble BOD(S_2) = 10.15 g/m^3.

Staged RBC Design for BOD and nitrate removal (nitrification)

Computation Procedure for the design of an RBC Process

1. Determine the influent and effluent S_{BOD} concentrations and wastewater flow rate.
2. Determine the TKN removal rate using the equation below:
   $R_n = 0.82(\text{BOD/TKN})^{-0.44}$.
3. Determine the TKN removal.
4. Determine the RBC disc area for the first stage based on a maximum Soluble BOD of 12–15 g S BOD/m^3/day.
5. Determine the number of RBC shafts using a standard disk density of 9, 300 m^2/shaft.
6. Select the number of trains for the design, flow per train, number of stages, and disk area/shaft in each stage. For the lower loaded stages, a higher disk density may be used.
7. Based on the design assumptions made in step 4, calculate S_{BOD} and TKN Concentration in each stage. Determine if the S_{BOD} concentration which will be achieved. If not, modify the number of stages per stage and/or disk area per stage. If effluent S_{BOD} concentration is met, evaluate alternatives to further optimize the design. Note that the procedure lends itself to spread sheet calculation.
8. Develop secondary clarifier design.

Example 10

Following design conditions are given:

Develop a process design for a staged RBC system

Parameter	Unit	Primary effluent	Target effluent
Flow rate	m^3/day	4, 600	
BOD	g/m^3	125	20
S_{BOD}	g/m^3	75	10
TSS	g/m^3	70	20
TKN	g/m^3	90% removal	5

Solution

1. Determine the number of RBC shafts for the first stage

$$\text{Assume first stage} S_{BOD} = 15\,\text{g/m}^2\text{/day},$$
$$S_{BOD}\text{ loading} = 75\,\text{g/m}^3 \times 4{,}600\,\text{m}^3\text{/day}$$
$$= 345{,}000\,\text{g/day},$$
$$\text{Disk area required} = \frac{345{,}000\,\text{g/day}}{15\,\text{g/m}^2\text{/day}}$$
$$= 23{,}000\,\text{m}^2.$$

Use $9{,}200\,\text{m}^2$/shaft

$$\text{No. of shafts} = \frac{23{,}000\,\text{m}^2}{9{,}300\,\text{m}^2/\text{shaft}}$$
$$= 2.5$$

Use 3 shafts for first stage at $9{,}300\,\text{m}^2$/shaft

2. Select number of train and number of stages Assume 3 trains with 3 stages/train

$$\text{Flow rate/train} = \frac{4{,}300\,\text{m}^3/\text{day}}{3\text{trains}}$$
$$= 1{,}433.3\,\text{m}^3/\text{day}.$$

3. Calculate S_{BOD} concentration in each stage using shaft area and flow to each train:
   (a) Stage 1:

$$S_1 = \frac{-1 + \sqrt{1 + 4(0.00974)(A_\text{s}/Q)S_0}}{(2)(0.00974)(A_\text{s}/Q)},$$
$$S_0 = 75\,\text{g/m}^3,$$
$$A_\text{s}/Q = \frac{\text{Area of shaft}}{\text{Flow rate/train}},$$
$$\text{Flow rate/train} = \frac{4{,}300\,\text{m}^3/\text{day}}{2\text{ trains}}$$
$$= 1{,}433.3\,\text{m}^3/\text{day},$$
$$A_s/Q = \frac{9,300\,\text{m}^2}{1{,}433\,\text{m}^3/\text{day}}$$
$$= 6.41.$$
$$S_1 = \frac{-1 + \sqrt{1 + 4(0.00974)(6.41)(75)}}{(2)(0.00974)(6.41)}$$
$$S_1 = 27.56\,\text{g/m}^3$$
$$\text{First stage soluble BOD}(S_1) = 27.56\,\text{g/m}^3$$

   (b) Repeat calculate similar to (a) above solving for S_2 and S_3

   For S_2 and S_3 yields

   $S_2 = 14.4\,\text{g/m}^3$

   $S_3 = 9.13\,\text{g/m}^3$

   Because goal was $10\,\text{g/m}^3$ for S_3, the proposed design is satisfactory for BOD removal

4. Determine TKN removal rate, the specific TKN removal rate using the equation:

$$(R_n) = 0.82(\text{BOD/TKN})^{-0.44}$$

$$\text{BOD/TKN} = 125/20 = 6.25,$$

$$R_n = 0.82(6.25)^{-0.44}$$

$$= 0.35\,\text{g/m}^2\text{/day}.$$

Determine the TKN removal

$$Q = 100L/S.$$

90% removal is to be achieved at first stage

$$\text{TKN removal} = 0.90(4{,}000\,\text{m}^3\text{/day})\,20\text{g/m}^3$$

$$= 720{,}000\,\text{g/day}.$$

5. Determine the organic and hydraulic loadings:
   (a) First stage organic loading

$$L_{org} = \frac{(4{,}600\,\text{m}^3\text{/day})(75\,\text{g/m}^3)}{(3\text{ stage})(9{,}300\,\text{m}^2\text{/shaft})}$$

$$= 12.36\,\text{g BOD/m}^2\text{/day}.$$

   (b) Overall organic loading

$$L_{org} = \frac{(4{,}600\,\text{m}^3\text{/day})(75\,\text{g/m}^3)}{(3\text{stage})(3\text{shaft/stage})(9{,}300\,\text{m}^2\text{/shaft})}$$

$$= 4.12\,\text{g BOD/m}^2\text{/day}.$$

   (c) Hydraulic Loading

$$\text{HLR} = \frac{4{,}600\,\text{m}^3\text{/day}}{(3\text{ stage})(3\text{ shaft/stage})(9{,}300\,\text{m}^2\text{/shaft})}$$

$$= 0.05\,\text{m}^3\text{/m}^2\text{/day}.$$

Summary:

Parameter	Unit	Value
No. of trains	No.	3
Flow rate/train	m^3/day	1,333.3
No. of stages	no.	3
Total discharge/stage	m^2	9,300
First stage S_{BOD} loading	g BOD/$m^{2/}$day	12.36
Total no. of Shafts	no.	3
Overall organic loading	g BOD/$m^{2/}$day	4.12
Hydraulic loading/shaft	m^3/m^2/day	0.05

NOMENCLATURE

A_s = Area of shaft, m^2
Aer AOB = Aerobic ammonium oxidizing bacteria
AnAOB = Anaerobic ammonium oxidizing bacteria
BAF = Biological aerated filters
BOD = Biochemical oxygen demand, g/m^3
COD = Chemical oxygen demand, mg/L
DGGE = Density gradient gel electrophoresis
DO = Dissolved oxygen, mg/L
EMMC = Entrapped mixed microbial cell
EPA = Environmental protection agency
FISH = Flourescent in situ hybridization
HDPE = High density poly ethylene
HLR = Hydraulic loading rate, $m^3/m^2/day$
HRT = Hydraulic retention time, days
K_d = decay co-efficient, days
K_s = half velocity constant, mg COD/L
L_{org} = Organic Loading rate, g $BOD/m^{2/}day$
MIC = Microbiologically Induced Corrosion
NA = Nitrogen applied, mg, N/m^2 h
NR = Nitrogen removed, mg, N/m^2 h
P_0 = Partial pressure of oxygen, atm
P_a = Partial pressure of air, atm
Q = Flow rate, m^3/day
r = Recirculation ratio, %
RBC = Rotating biological contactor
R_n = Total Kjeldhal Nitrogen removal rate, $g/m^2/day$
SAGB = Submerged attached growth bioreactor
SND = Simultaneous nitrification and denitrification
SOB = Sulfide oxidizing bacterium
SOM = Soluble organic matter
SRB = Sulfate reducing bacterium
SRT = Solids retention time, days
S_0 = Soluble BOD, g/m^3
S_1 = First stage Soluble BOD, g/m^3
S_2 = Second stage Soluble BOD, g/m^3
S_3 = Third stage Soluble BOD, g/m^3
TOC = Total organic carbon, mg/L
TSS = Total suspended Solids, g/m^3
TKN = Total Kjeldhal Nitrogen, g/m^3
rpm = Revolutions per minute
y = Yield coefficient
μ = Maximum specific growth rate

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14
Anaerobic Treatment of Low-Strength Wastewater by a Biofilm Reactor

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CONTENTS

Abstract Anaerobic systems are gaining application for the direct treatment of low-strength wastewater, and compared to aerobic methods, offer lower operation cost but reduced removal efficiency. This chapter discusses generally the anaerobic process and technology and is concerned primarily with the use of the anaerobic filter (AF) system, emphasizing the design, operation, and performance characteristics of the reactor, the modeling of the process, the accumulation of biosolids during operation, and the posttreatment of the anaerobically treated wastewater to improve effluent quality.

1. ANAEROBIC PROCESS

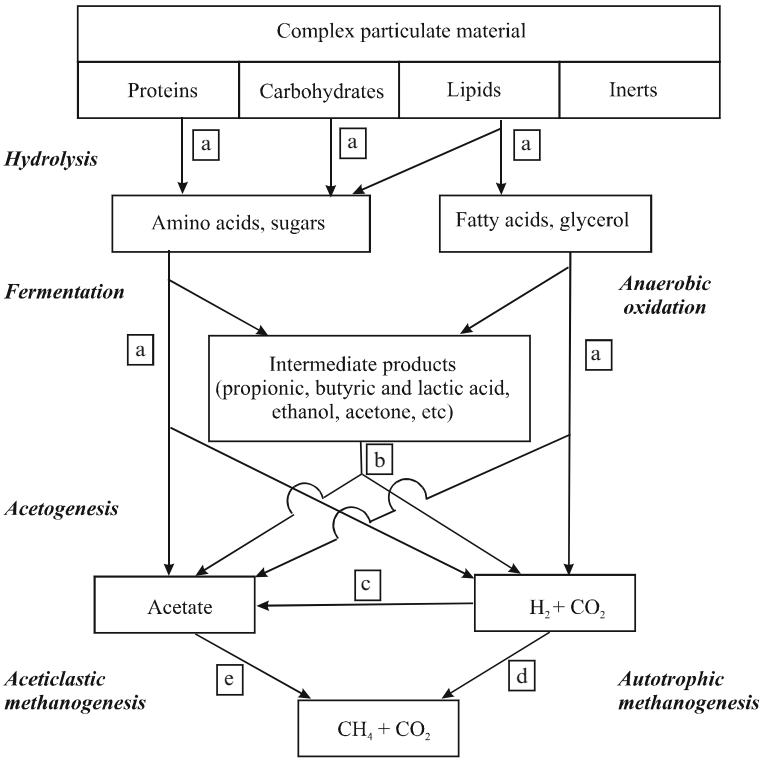
1.1. Anaerobic Metabolism

The anaerobic process is comprised of the sum of biological phenomena, which split organic matter to CH_4 (methane) and CO_2 (carbon dioxide). The release of CH_4 from silt in marshes was first described in 1776 by Volta, while in 1856, Reiset reported that CH_4 was liberated from the decomposition of manure piles; about 20 years later, Bechamp proposed that CH_4 resulted from microbiological action (1, 2). The first "clean" culture of a methanogenic microorganism was isolated in 1936 by Barker, who named it *Methanobacillus*

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_14,

omelianskii; however, in 1967, Bryant and coworkers demonstrated that actually two microorganisms coexisted in this culture, one non-methanogenic, "S", which oxidized ethanol to acetate and H_2 (hydrogen) and the other methanogenic, *Methanobacterium bryantii*, which reduced bicarbonate and H_2 to CH_4; in addition, Schnellen in 1947 had isolated two clean methanogenic cultures, *Methanosarkina barkeri* and *Methanobacterium formicium* (1, 3).

The anaerobic metabolism is carried out by a group of microorganisms, which acting symbiotically degrade complex organic compounds to the final products CH_4 and CO_2, and the process may be separated into the four main stages given in Fig. 14.1 (4–8). Complex organic materials (such as proteins, carbohydrates, and lipids) are first hydrolyzed by exoenzymes to simple soluble compounds, making possible their passage through the cellular membrane. The simple organic compounds are converted to short-chain fatty acids (acetic, propionic, butyric, lactic), alcohols, ketones, H_2, CO_2, NH_3 (ammonia), and the short-chain fatty acids (except acetic) are then degraded to acetic acid, H_2, and CO_2. Finally, acetate is converted to CH_4 and CO_2, while CO_2 is reduced by H_2 to CH_4.



a. Hydrolytic and fermentative bacteria
b. Hydrogen producing acetogenic bacteria
c. Hydrogen consuming acetogenic bacteria
d. Carbon dioxide reducing bacteria
e. Aceticlastic methanogens

Fig. 14.1. Anaerobic metabolism of complex organic materials (4–8).

Hydrolysis of complex organics refers to the sum of individual compounds, which have different hydrolysis rates, and is considered to follow first-order kinetics (9, 10). Fermentation is the process in which the substrate acts as both electron donor and acceptor, and soluble carbohydrates and amino acids are degraded to simpler products (4). The main products of carbohydrate fermentation are ethanol, acetate, H_2, and CO_2; a large number of amino acids and other nitrogenous organic matter serve as an energy source for anaerobic bacteria, and the degradation of amino acids includes oxidation-reduction reactions between one or more amino acids or nonnitrogenous compounds, which originate from amino acids (11). The rate-limiting step in the anaerobic degradation of proteins has been reported to be hydrolysis (10).

Molecular H_2 is the main electron acceptor in the anaerobic oxidation of long-chain fatty acids (4). Free fatty acids and those produced from the hydrolysis of lipids are degraded by anaerobic bacteria to give short-chain fatty acids, mainly acetic or both acetic and propionic, which are subsequently oxidized to acetate and H_2; this stage could be named acetogenesis, as acetate is the main product. Two types of mechanisms are involved, acetogenic dehydrogenation and acetogenic hydrogenation; in the first type, hydrogen-producing and fermentative bacteria are involved and utilize protons as electron acceptors, and in the second type (also termed homoacetogenic metabolism), acetate is the sole end product (12).

About 70% of the CH_4 produced is derived from the decarboxylation of acetate, the most important substrate for aceticlastic methanogens (4, 13), and two genera of bacteria utilize acetate to produce CH_4, *Methanosarcina* and *Methanosaeta* (8). Most methanogenic bacteria utilize H_2 and CO_2 for growth, and the syntrophic existence of hydrogen-producing acetogenesis (H_2 production during the fermentation of carbohydrates and proteins and the anaerobic oxidation of fatty acids) and hydrogen-utilizing methanogenesis (removal of the H_2 produced) allows the completion of the anaerobic process.

1.2. Anaerobic Process Dependence

Methane-producing bacteria require a minimum substrate concentration in order to function properly. This threshold level is related mainly to undissociated acetic acid and depends on the species of bacteria involved; values of 130 and 2.6 mg/L total acetate have been reported for *Methanosarcina barkeri* and acclimated sludge (*Methanotrix sp.*) near pH 7 (14), and the minimum concentration of acetic acid, which would enable steady-state biofilm kinetics for the development of *Methanotrix*, has been estimated to be 3.7 mg total biochemical oxygen demand (BOD_L)/L (15). The presence of suspended organic matter would affect the microbial growth rate because of the need for hydrolysis of this matter.

Temperature has a considerable effect on the bacterial growth rate and process efficiency. The optimum temperature range for the development of methanogenic bacteria is reported to be between 35 and 40°C, and the optimum temperature for *Methanotrix soehngenii* is 37°C (16, 17). Methane production follows the Arrhenius equation in the range of 10–37°C (16), and temperature reduction causes a decrease in the microbial maximum specific growth and specific utilization rates and an increase in the net biomass yield (18).

The presence of high levels of SO_4^{2-} (sulfate) can cause reduced stabilization of organic matter and production of CH_4, as well as odor problems. The optimum temperature range for the development of sulfate-reducing bacteria is 30–35°C, somewhat lower than the range for

methanogens (35–40°C), and the half-velocity substrate concentration constants of methane producers and sulfate reducers in a biofilm with acetate as the substrate have been estimated to be 32.8 and 9.5 mg/L, respectively, indicating that sulfate reducers compete well with methanogens, especially at low acetate ion concentrations (19). It should be noted that the maximum specific growth rate of sulfate-reducing bacteria in pure cultures is higher than that of methanogens, while in biofilm, the opposite holds true; this has been attributed to the increased ability of methane producers to attach on the packing media (20).

Methane-producing bacteria are sensitive to low dissolved oxygen (DO) concentrations, and exposure to O_2 (oxygen) lowers their adenylate charge resulting in their death. Oxygen effects an irreversible disassociation of the F_{420}-hydrogenase enzyme complex, and in cells which have developed under Fe^{2+} (ferrous iron) limitation, the F_{420} is rapidly reduced with exposure to O_2; this may be due to the lack of the protective superoxide dismutase (SOD) enzyme (3). The survival time of anaerobic microorganisms under the effect of atmospheric O_2 ranges from less than 45 min (*Peptostreptococcus anaerobicus*) to more than 72 h (two strains of *Clostridium perfringens*), and depending on this time anaerobic bacteria have been classified in three categories: intolerant (<2 h), moderately tolerant (4–8 h), and tolerant (>72 h) (21). A correlation has been found to generally hold between SOD activity and O_2 tolerance; the most tolerant anaerobic bacteria demonstrate little SOD activity and reduce the O_2 level at a much lower rate than other tolerant bacteria, thus surviving for a longer time in its presence (21). The effect of O_2 on granular anaerobic sludge has been examined by several researchers (22–24), and the occurrence of methanogenesis under excessive O_2 levels would suggest either that methanogenic bacteria are tolerant to this condition, or that they are protected inside the sludge granules; facultative bacteria consume O_2, thereby creating microniches inside the granules where there is also development of methanotrophic bacteria (22). The level of O_2 causing 50% inhibition after a 3-day exposure was found to be 7–41% O_2 in the head space, and the final DO concentration at the end of exposure was 0.05–6.10 mg/L; absence of substrate during the exposure period, as well as mixing, negatively affected the O_2 tolerance of methanogens (23). It has also been demonstrated that anaerobic-aerobic-coupled reactors could be operated to concurrently maintain anaerobic and aerobic bacteria when DO is present in the recirculating liquid (24). Finally, it has been reported that O_2 penetrates the layer of an aerobic biofilm by 100–300 μm (25, 26).

1.3. Direct Anaerobic Treatment of Wastewater

The anaerobic process has been traditionally employed for the stabilization of sludge in municipal and industrial wastewater handling facilities and for the treatment of domestic wastewater with onsite septic tanks or other systems (27–30). The process is recently gaining increased application for the direct treatment of low and medium-strength wastewater, including municipal wastewater, especially in areas with high ambient temperature; in addition, upgrading of organically overloaded systems may be accomplished through the anaerobic pretreatment of the wastewater (28, 31–33). A high hydraulic loading rate (HLR) is required when low-strength wastewater is treated to compensate for the reduced concentration of organic matter, and systems capable of retaining biomass need to be used.

Table 14.1
Comparative evaluation of direct anaerobic treatment of wastewater (28, 34–38)

Advantages	Disadvantages
• Lower operation cost; no energy required for aeration	• Lower removal efficiency
• Lower nutrient requirements with resulting conservation of nutrients; increased potential for effluent reuse	• Need for additional posttreatment depending on effluent discharge limits for organic matter, suspended solids, and nutrients
• Lower excess sludge production due to low microbial growth rate; stabilized sludge with improved dewatering characteristics	• Limited design and operation experience with pilot and full-scale application
• Technology with simpler construction, operation and maintenance requirements	• Produced CH_4 is not utilized for energy purposes; significant amounts of CH_4 are lost in the liquid effluent
• Long idle periods possible; suitable for treatment of wastewater from seasonally operating facilities	• Sensitive to low ambient temperatures
• Wide range of wastewater (strength, composition, flow) that can be treated	• Long startup period, if appropriate inoculum is not available
• Core technology for decentralized sustainable wastewater treatment systems	• Potential bad odor due to formation of H_2S (hydrogen sulfide); proper handling of biogas needed to minimize odor effects and CH_4 emission to the atmosphere

The direct anaerobic treatment of low-strength wastewater offers significant advantages when compared to common aerobic systems, but also has several disadvantages; these are summarized in Table 14.1 (28, 34–38). The low concentration and characteristics of the substrate treated and the high hydraulic loading required are the controlling factors for the anaerobic process.

The increased fraction of suspended organic matter, especially when raw municipal wastewater is handled, and the related low hydrolysis rate affect the bacterial growth rate. It has been estimated that in order to achieve a sufficiently active anaerobic biomass in a upflow anaerobic sludge blanket (UASB) reactor, the volatile suspended solids to chemical oxygen demand (VSS/COD) ratio in the influent wastewater should not exceed the value of 0.1 (39). The removal of suspended solids in primary sedimentation, or the use of a two-step treatment train where the solids are retained in first step, have also been proposed as means to overcome this problem (40). The interaction between the sulfate reducers and methane producers is a consideration, and is affected by the SO_4^{2-} levels present. Treatment of low-strength synthetic wastewater containing different SO_4^{2-} concentrations (30, 150, and 600 mg/L) has shown

that although the COD removal efficiency was unaffected (86–88%), COD reduction via the sulfate-reducing bacteria increased as the SO_4^{2-} level increased (41). The high hydraulic loading of the reactor causes loss of biomass and dissolution of a significant quantity of CH_4 in the liquid effluent phase, while the variation in wastewater strength and flow rate affect the process. The required minimum solids retention time (SRT) in the anaerobic process is high because of the low development rate of anaerobic microorganisms, and for effective treatment the SRT should be 2–10 times the minimum (42); it should be mentioned that the SRT in an attached growth anaerobic reactor (containing 30,000 mg VSS/L) is 60 times greater than the corresponding value in an activated sludge system (3,000 mg VSS/L) (43).

Cost estimates for the direct treatment of low-strength wastewater by different aerobic systems and a UASB reactor have been made by several researchers (39, 44–46), and comparative data based on the related cost of the aerobic process (considered as 1) are presented in Table 14.2. Evaluation of these data would show that compared to activated sludge systems, the UASB reactor offers substantial cost reduction when posttreatment is not considered, which ranges from 50 to 80% for capital and from 50 to 74% for operational costs. However, because posttreatment is needed to achieve secondary effluent discharge limits, incorporation in the treatment train of a low-tech system to polish the anaerobic effluent would reduce the gain in capital and operational costs by 16–25% and by 65–74%, respectively.

Table 14.2
Comparative costs of anaerobic and aerobic wastewater treatment systems (39, 44–46)

Treatment system	Relative cost (capital/operational)[a]				
	Mergaert et al.	Vieira	van Velsen and Wildschut[b]		Schellinkhout and Collazos
	Population equivalent				
	100,000	Not given	16,000	135,000	50,000
Complete activated sludge		1/1			
Activated sludge	1/1				
Oxidation ditch	1/1		1/1		1/1
Trickling filter				1/1	1.06/0.47
Stabilization pond			0.53/–	0.39/–	0.95/0.26
UASB reactor	0.5/0.5	0.2/–	0.30/–	0.32/–	0.48/0.26
Screens + UASB + drying beds		0.6/0.4–0.5			
UASB + trickling filter					0.84/0.35
UASB + stabilization pond					0.75/0.26

[a]Estimated assuming that the corresponding aerobic process cost (capital or operational) is equal to 1.
[b]Reported as total treatment cost/kg 5-day biochemical oxygen demand (BOD_5) removed.

Genung and coworkers (47) have compared an anaerobic filter (AF) reactor to an activated sludge system, considering the treatment of low and medium-strength wastewaters (250 and 1,000 mg COD/L) at two levels of flow (190 and 3,800 m^3/day). The complete AF system at the lower flow rate and for the stronger and weaker wastewaters used 47 and 55%, respectively, of the energy needed by the activated sludge system; the corresponding values at the higher flow were 30 and 60%. The energy used by the AF stage alone was correspondingly 8 and 6% and 12 and 15% of the energy used by the activated sludge stage. Approximately, 36 and 60% of the capital cost of the AF system was associated with the column and packing material for the 190 and 3,800 m^3/day systems; and the total capital cost of the AF system was lower than that of the aerobic system at the flow of 190 m^3/day and greater at the flow of 3,800 m^3/day.

The anaerobic process could be a viable alternative for the treatment of low-strength wastewater, and when combined with a low-cost aerobic posttreatment system could yield an acceptable quality effluent. However, the low CH_4 release level and wide fluctuation in production rate and composition do not facilitate the utilization of biogas as an energy resource, as it is widely practiced when high-strength wastewater and sludge are treated anaerobically.

2. ANAEROBIC TREATMENT SYSTEMS

2.1. Historical Development

A historical review of the evolvement of the anaerobic process in the treatment of wastewater has been presented by McCarty (2, 27). The first application of this process was made by Mouras and was reported in a French journal in 1881; Mouras used an airtight chamber (the Mouras' automatic scavenger) for the treatment of suspended material and found that the particulate organic matter was subjected to liquefaction. Ten years later, Scott-Moncrieff constructed a tank with an empty bed in the lower part and a stone bed in the upper part (perhaps the first hybrid reactor) for the treatment of wastewater from a group of 10 people; the sludge which remained in the lower section was stabilized after 7 years and was readily available for disposal. Houston confirmed Scott-Moncrieff's findings and reported that there was a great reduction in sludge volume. Cameron in 1895 constructed in Exeter, England, a tank similar to the Mouras's scavenger, which he named a septic tank, and successfully treated the wastewater of the city using this system; CH_4 gas produced in several septic tanks was collected and used for heating and lighting at the disposal works. A similar system, which incorporated vertical baffles reaching 0.6–1.0 m below the surface of the wastewater in the tank, was designed at about the same time by Talbot in Illinois. Septic tanks began to be used widely, but their effluent was often black and offensive and contained undigested material. Clark in Massachusetts proposed in 1899 that this problem could be alleviated by fermenting the sludge in a separate tank. Subsequently, Travis presented a two-stage process, where the suspended matter was separated from the wastewater and directed to a digestion chamber (a hydrolyzing chamber); however, wastewater was also passed through this chamber causing increased total suspended solids (TSS) levels and septic conditions in the effluent. Imhoff in 1905 modified Travis' tank by preventing wastewater to flow through the hydrolyzing chamber, where only the settled solids were treated anaerobically; this system, named an

Imhoff tank, significantly reduced the cost of sludge disposal and was accepted rapidly, and by the end of 1914, about 75 locations in the United States had been given a license to use the Imhoff tank.

Shortly thereafter, interest in the anaerobic process moved from the treatment of wastewater to the treatment of settled sludge. The first separate heated anaerobic sludge tank was operated in 1927 in Essen-Rellinghausen, Germany, and the favorable results it achieved great popularity to separate digestion, especially in larger cities. The significance of the CH_4 produced from sludge digestion was also recognized, and since 1923 efforts were undertaken in Germany for its utilization for heating and other energy applications (2).

Winslow and Phelps (48) in 1909 studied the preliminary treatment of municipal sewage in a biolytic tank with additional treatment in a sand filter. The tank had the shape of an inverted pyramid in the lower part and a cylinder in the upper, and flow was in an upward direction; this unit was similar to the UASB reactor which was developed much later. At a hydraulic retention time (HRT) of 8.5 h, the TSS and VSS removal (50 and 47%) was similar to the efficiency obtained in septic tanks, while the solubilization of solids was better (72%). The effluent from the biolytic tank was directed for additional treatment to an intermittent-flow sand filter at an HLR of 207–320 L/m^2 day. According to Coulter and coworkers (49), Frank and Rhynus continued the study of the biolytic tank some years later and concluded that its behavior was not satisfactory, and this may have delayed the development of the direct anaerobic wastewater treatment process.

2.2. *Anaerobic Reactors*

High-rate anaerobic systems have been recently developed for the treatment of strong agroindustrial wastewater as well as weak municipal-type wastewater. These systems achieve separation of the HRT and SRT, and their operation is based on the following three principles (50):

(a) Accumulation of an increased quantity of biomass in the reactor (via sedimentation, floc agglomeration, attachment on media, or recycling), thus effecting a much greater SRT than the corresponding HRT.
(b) Better contact between biomass and wastewater.
(c) More active biomass because of acclimation and the development mode.

The general types of anaerobic reactors which have been employed are presented in Fig. 14.2 and include the contact (anaerobic contact process) unit (49), the AF reactor (51), the anaerobic expanded or fluidized bed (AEB or AFB) reactors (28), the UASB reactor (31), the anaerobic baffled reactor (ABR) unit (52), the anaerobic sequencing batch (ASB) reactor (53), and the anaerobic migrating blanket (AMB) reactor (54). These are mostly continuous feed reactors and can be classified in three main categories based on the criteria of morphology and support of biomass: attached biomass, nonattached biomass, and hybrid systems. In addition, batch feeding systems with sequencing operating conditions have been used (55), and modified systems have been employed.

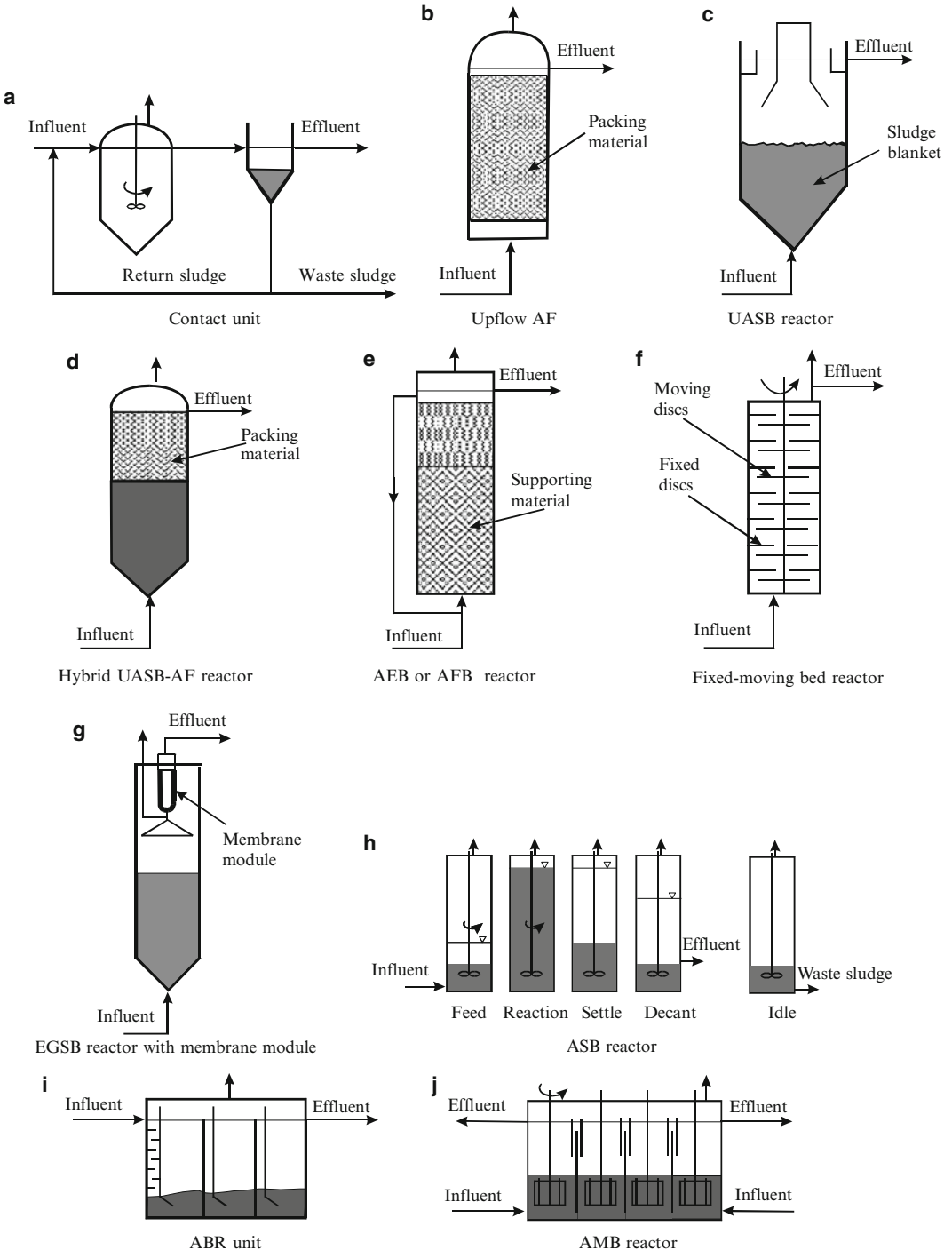


Fig. 14.2. Schematics of high-rate anaerobic reactors for wastewater treatment (28, 31, 49, 51–55, 68, 70, 120).

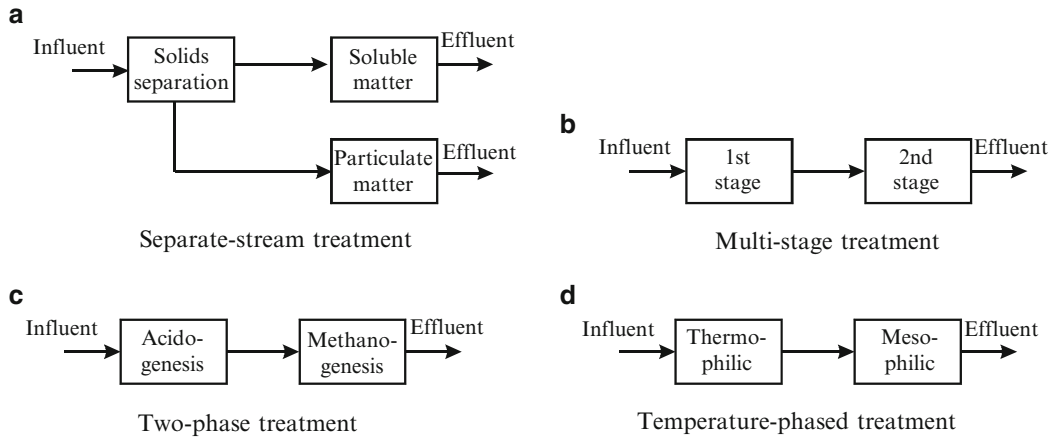


Fig. 14.3. Anaerobic process separation into dedicated functions (56–61).

Several methods have also been identified for the separation and enhancement of the processes which take place in the anaerobic reactors; these are presented in Fig. 14.3 and include the following systems:

(a) Parallel systems, where the soluble and particulate substrates are treated separately, but there is no processes separation (56).
(b) Stage systems, with two or more reactors in series and without any process separation (57, 58).
(c) Two-phase systems, where acidogenesis and methanogenesis are separated (59, 60).
(d) Systems with different operating temperatures, where the microorganisms are separated depending on their optimum growth temperature (61).

The contact unit was developed in the mid 1950s, and was an attempt to increase the presence of microorganisms following the activated sludge principle of recycling sludge. The need for increased retention of microorganisms also led to reactors using packing media in order to enhance the attachment and growth of biomass. The AF was developed in the mid 1960s and was initially an upflow reactor using gravel as packing material (42, 62), however, a downflow AF was also used later-on (63). The main differences between the two modes, except for the direction of flow, concern the type and orientation-placement of the packing media; in the upflow AF packing is randomly placed, while in the downflow AF it is placed with vertical orientation. The downflow AF can treat wastewater with a high TSS concentration (64, 65) and can handle better the accumulation of biomass and inert solids, which is a potential factor for filter clogging (66). The AFB or AEB reactors offer high media specific surface, enabling an increased quantity of active biomass to be attached on the packing material and the elimination of clogging, canalization and biogas entrapment (43, 50).

The UASB reactor was developed in the late 1970s, although treatment schemes such as the biolytic tank and the contact unit should be considered its precursors (43, 67). Operation of this reactor is based on the development of granular or flocculent sludge, which enables the

biomass to exist at high concentration and have good settling properties; the biogas–liquid–solids separation is accomplished in the upper part of the unit and has a significant role in its proper functioning. Hybrid systems have been developed to improve liquid–solids separation, including the UASB-AF reactor, which involves placement of a filter in the upper part of the unit (68, 69) and the expanded granular sludge bed (EGSB) reactor, which is a UASB unit with an expandable bed (70); a high upflow velocity is employed in the EGSB unit to expand the sludge bed and enable better biomass-substrate contact and is achieved by increasing the height to diameter ratio of the reactor and by providing recycle.

The ABR unit was developed as a system of UASB reactors in series where, however, the presence of granular biomass is not necessary. The reactor initially operated in both downflow and upflow modes, although recently the upflow mode is mainly employed and recycling may be applied. The ABR unit combines the advantages of the AF and UASB reactors and offers operation stability, increased biomass concentration, good biomass-substrate contact, while it eliminates problems associated with clogging and biomass loss (71, 72). Several other anaerobic systems have been proposed, but have not been widely used, and include the ASB (73), reversing anaerobic upflow system (RAUS) (74), and AMB (54) reactors; their operation is based on the fundamental principles of the previous systems with minor modifications. The ASB reactor is a batch-type system that involves four sequencing phases of operation, feed, reaction, settle and decant, and provides mixing during the feed and reaction cycles. The RAUS unit consists of two reactors connected to each other; at any time, one unit is fed upward, while the other acts as a settling tank; then the flow is reversed, and the function of the reactors changes. The AMB reactor consists of continuously fed compartments in series with mixing and the biomass tends to migrate to the final compartment; after a given time interval, the flow is reversed in order to prevent biomass wash-out from the lead compartment. Membrane modules have also been incorporated to EGSB reactors in order to minimize sludge loss in the effluent and achieve better quality treated wastewater (75).

3. ANAEROBIC BIOFILM REACTORS

3.1. Reactor Configuration and Hydraulic Characteristics

The operation of the AF reactors, the configuration of biofilm reactors most frequently employed, is affected by factors related to the packing material provided (type, size, specific surface area, void ratio, microporosity, pore size) and the hydraulic mixing effected (which is dependent on the packing material and reactor configuration). Initially, best packing material was considered to be that which offered high specific surface for the attachment of biomass and increased void ratio for the minimization of clogging and short circuiting; however, several other factors have also been found to be important.

The effect of the reactor configuration and biomass activity on the performance of AF units has been examined using filters, which had the same volume but different height to diameter ratios, were packed with flex rings and were fed with synthetic substrate (nonfat dry milk) at 35°C; the flow in the reactors was completely mixed at organic loading rates (OLR) 6–8 kg COD/m^3 day or higher because of high biogas production and little difference was found in

performance, indicating that reactor configuration was not a major factor in the design of fully packed AF units (76). However, increased biogas production would result in better mixing, yielding a completely mixed flow mode in upflow AF reactors (77, 78).

Use of packing material with large pore size and high void ratio would reduce the short circuiting caused by dead zones because of accumulated biomass, which decrease the active reactor volume, or by the biogas produced, which results in higher upflow superficial velocities. The flow regime in a "clean bed" (a bed without biomass) approaches plug flow with a high degree of dispersion, while in a "dirty bed" (a bed with biomass) it follows a completely mixed pattern reflecting significant short circuiting (79–81).

Hydraulic flow simulation studies conducted with tracers in upflow AF units packed with cross-flow media having different specific surface values and operating at Reynolds numbers under 25, demonstrated that four main regions existed: the inlet zone where completely mixed flow occurred, the plug flow zone which included a dead zone, and the completely mixed zone. The presence of packing material resulted in increased plug flow compared to an unpacked column, and an increase in the media specific surface also resulted in plug flow and hydraulic dead zones in the reactor (77). Other studies performed on AF units partially packed with Pall rings and capable of recirculation demonstrated that the decrease in packing volume increased the mixed zone for a given flow velocity and biogas production per unit of packing specific surface; the optimum operation strategy for this hybrid AF system would involve a well mixed biomass at the reactor bottom in order to facilitate better contact with the substrate, and movement with the biogas of the partially treated wastewater through the mixed zone to the packing material where a plug flow regime prevails (78).

Solids accumulation influences the hydraulic behavior of the AF reactor. The average HRT measured in a clogged AF treating glucose was much lower than the theoretical value computed before seeding the reactor, while the axial dispersion coefficient was substantially increased, indicating that the flow was not plug type but approached the completely mixed region. Head loss distribution analysis has shown that there is considerable hydraulic loss in the reactor bottom, but limited change is observed in the middle column section indicating the existence of channels (82).

3.2. Packing Media

The packing material which has been used in upflow AF reactors of pilot or full-scale in Europe and North America is mostly plastic (randomly placed or in prefabricated cubes) (83). However, many different materials have been employed in numerous research investigations, and in a study of the specific surface of packing materials 29 representative media were examined; these had been reported in the literature to have been used for the immobilization of microorganisms, and on the basis of their surface microtopography was classified in the following three categories (84):

(a) Smooth media (polypropylene bead, glass bead, peristaltic tube, porcelain, powdered activated carbon, perspex, polyvinyl chloride, glass).
(b) Uneven media (straw, paddy stem, nylon, sand, gravel, stone).

(c) Porous media (jute fibers, gravel, soil, granulated clay, limestone, ceramic, oyster shell, refractory brick, diatomaceous earth, casuarina seed, granular activated carbon, thermocol, sponge, pumice stone, polyurethane foam).

The effect of the packing material characteristics on the behavior of AF reactors has been examined by several researchers. Mueller and Mancini used polypropylene rings in an upflow AF to treat medium-strength wastewater and concluded that plastic material with low weight and high porosity was advantageous compared to gravel because its higher void volume enabled greater biomass accumulation (85). A similar conclusion was reached by Hudson and coworkers who studied the treatment of shellfish processing wastewater using AF units packed with gravel and oyster shells; the packing material with the higher void ratio and specific surface (the shells) resulted in higher COD removal (86). Murray and Van Den Berg reported that microporosity played a significant role in the attachment of microorganisms, with the optimum biomass accumulation observed when the micropore diameter was 1–5 times the main dimension of the microorganisms (87). The release of inorganic nutrients from clay media (mainly Fe^{2+}) was found by Wilkie and Colleran to enhance the development and attachment of methane bacteria (88).

Young and Dahab examined the effect of the type, size, and shape of packing media and concluded that prefabricated media with larger pore size gave best results and behaved better in terms of short circuiting; this indicated that the effectiveness of the packing material in retaining biomass was a more crucial factor than its specific surface area (89). Song and Young have also reported that specific surface did not influence significantly the efficiency of upflow AF units; they found that cross-flow media gave better COD removal and increased solids accumulation, and attributed this to improved flow redistribution in the media channels (90). Oleszkiewicz and Thadani studied the effect of the presence or absence of packing material and its type on anaerobic hybrid reactors (packed bed 40% of total volume) and concluded that ceramic rings, which had a lower void ratio and higher specific surface than vertically placed PVC (polyvinyl chloride) tubes, resulted in greater COD removal and biogas production, lower biomass loss, and less short circuiting (91). Huysman and coworkers have reported that surface roughness, porosity and pore size were the most important factors for the development of biofilm in porous media, while surface roughness was a crucial factor in nonporous materials, and stated that microorganisms which develop in the inner sections of packing may confront problems with substrate diffusion (92). Anderson and coworkers comparing the use of porous (Siran® sintered glass rings) and nonporous (PVC rings) media in upflow AF reactors, showed that glass offered better stability and efficiency at high organic loading and that surface roughness increased the attachment and accumulation of biomass because microorganisms developed inside the pores and were protected from flow shear stresses and changes in the surrounding environment (93). Tay and coworkers examined the effect of specific surface area, void ratio, pore size, and OLR in upflow AF units treating synthetic wastewater at 35°C and found that packing material characteristics did not influence the behavior of the units at OLR up to 4 kg COD/m^3 day, with COD removal remaining better than 90%. However, at OLR in the range of 8–16 kg COD/m^3 day, pore size and void ratio,

but not specific surface, affected reactor performance; high pore size reduced clogging with solids and the creation of dead spaces, enabling better biomass-substrate contact (79, 80).

3.3. *Biomass Development and Time of Operation*

Clogging may be observed in AF units after a prolonged period of operation, depending on the characteristics of the packing material (void ratio, pore size) and the quality of the wastewater (organic substrate, TSS content); in addition, the length of filter operation may affect reactor performance. Several researchers have investigated these problems and have examined the form of biomass developed (attached or suspended) and its periodic removal.

Hall using upflow AF reactors to treat liquor resulting from heat treatment of sludge and diluted brewery wastewater at 35°C has found that 60% of the retained solids were in a nonattached form. The accumulation of solids (both attached and unattached) after 440 days of operation reduced mixing efficiency, resulting in short circuiting and dead spaces; however, this situation could be improved with the removal of suspended solids by draining, which helped improve flow distribution and did not adversely affect COD removal efficiency (94). Hanaki and coworkers have noted that biomass accumulation was greater in the bottom than in the upper part of upflow AF units packed with plastic rings which treated synthetic wastewater; however, in the upper column, higher methanogenic activity was observed (95).

Ehlinger and coworkers have reported that the type of substrate treated had a significant role in AF clogging. Using two upflow AF units packed with clay which treated glucose and volatile acids-based substrates, they observed signs of clogging after 6 months of operation in the reactor treating glucose; this was attributed to acid-forming microorganisms in the glucose-fed unit, which were characterized by greater cell synthesis and polysaccharide secretion (82). Jhung and Choi have found that the development of granular biomass in an AF reactor resulted in improved organic matter removal, and reported that the filter reached steady-state conditions faster than a UASB reactor run in parallel and treating the same wastewater and had better behavior when the characteristics of the feed wastewater were varied; a carbohydrate substrate with a greater COD to volatile acids ratio enhanced the development of filamentous microorganisms and consequently of granular sludge, and this permitted higher organic loadings (96).

Manariotis and Grigoropoulos have reported that long-term operation seemed to adversely affect filter behavior, as treatment efficiency and biogas release at the same level of hydraulic loading generally deteriorated with the time of operation, and cautioned that short-term experimental findings may not be duplicated in actual field application. They examined the direct treatment of raw municipal wastewater by three upflow AF units containing ceramic saddle, plastic ring, and gravel packing, which were run mostly at 25°C under a wide range of hydraulic and organic loadings over a 3-year period. Plugging was not experienced during this extended period, although periodic column draining was only practiced in two of the reactors (97). Solids production is generally limited with low-strength wastewater; however, removal of accumulated material would be required at intervals in order to restore the AF hydraulic loading capacity.

4. LOW-STRENGTH WASTEWATER TREATMENT

4.1. Anaerobic Filters

The first studies which examined the direct treatment of wastewater by AF reactors were reported in the late 1960s and concerned medium-strength synthetic wastewater with a low TSS content (42, 62); however, a decade earlier, the use of an AF following a contact unit had been proposed, in a effort to reduce solids loss (49, 98). Treatment of various low-strength wastewaters has been undertaken in the ensuing years, including raw or settled domestic and municipal wastewaters, synthetic substrates, and food processing wastes. Several studies reported in the literature during the past 30 years are chronologically presented in Table 14.3 (47, 86, 97, 99–116), where the type and size of the reactor used, the characteristics of the packing media employed, the hydraulic and organic loadings applied, the type and strength of wastewaters treated, and the removal efficiencies obtained are presented. As can be seen, the AF reactors used varied considerably in size, from small (1–2 L) laboratory units to large (up to 75 m^3) pilot systems, many different packing materials and wastewaters were tested, and the duration of the studies ranged from a few months to several years.

While evaluating the work summarized in Table 14.3, it should be kept in mind that laboratory reactors may exhibit a greater removal efficiency than pilot or plant-scale units, that short-term findings may not be fully duplicated in actual field installations, and that synthetic wastewater is usually an easier substrate to treat than municipal wastewater. Field application of the AF process is lagging and additional large-scale, long-term studies are needed in order to improve process understanding, develop widely accepted design criteria, and encourage use of this technology and utilization of its benefits (72, 97, 110). In the following sections, key aspects concerning the AF reactor design, operation and performance are discussed.

4.1.1. Startup

The required AF reactor startup period depends on the seeding strategy selected and the type of influent substrate used, and generally varies from a few weeks to a few months. Digested sewage sludge has often been employed as an inoculum, and use of seed quantities 7–50% of the reactor volume has been reported (104, 105, 116). Bodik and coworkers (114) seeded an AF which treated municipal wastewater and synthetic substrate with primary anaerobic digester sludge from a municipal wastewater treatment plant with a TSS content of 13.0–14.0 g/L (VSS 6.5–8.6 g/L). Startup was quick, and in two weeks COD removal was greater than 80%, when the reactor operated at a temperature of 23°C and an HRT of 20 h.

Startup has also been accomplished without the introduction of seed solids using effluent from other anaerobic units; however, this procedure requires a lengthier period (97, 117). Manariotis and Grigoropoulos (97) filled and fed upflow AF units with effluent from conventional batch anaerobic reactors which treated domestic wastewater fortified with dogfood, and over a period of 50 days gradually replaced the effluent with fortified wastewater. Startup was slow, and during the initial 8 months with the units operating at a temperature of about 25°C and under low hydraulic loading conditions (HRT 12.5 to 4.2 days), the soluble chemical oxygen demand (SCOD) values were higher in the effluents than in the influent, indicating lagging

Table 14.3
Anaerobic filter treatment of low-strength wastewater (47, 86, 97, 99–116)

Study		Reactor characteristics							Experimental study characteristics											
Authors and publication year	Ref. no.	Column			Packing material				Duration (months)	T (°C)	HRT		OLR		Influent wastewater			Removal efficiency		
		Type	Volume (L)	Height/ diameter (m)	Type	Size (cm)	Void ratio	Specific surface (m^2/m^3)			Empty bed (h)	Void volume (h)	COD (kg/m^3 day)	BOD_5 (kg/m^3 day)	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	COD (%)	BOD_5 (%)	TSS (%)
Raw municipal wastewater																				
Raman and Khan (1978)	(99)	Upflow	3,630	1.4/1.6[a]	Stones	2.5–3.5			6	28	16[b]					175	125		72	57
									5	31	22[b]					210	172		80	88
Genung et al. (1980)	(47)	Upflow	5,700[c]	5.6/1.5	Ceramic rings	2.5	0.7		24	10–25	5–36[c]	2.5–10.5[d]		0.05–0.61		60–220	60–250		55	75
Genung et al. (1982)	(100)	Upflow	75,000	5.4/4.9	Pall rings	7.5	0.93	105	9	15–24	17–95				132–390	42–106	62–114	28–64	40–78	60–86
Genung et al. (1985)	(101)	Upflow	75,000	5.4/4.9	Pall rings	7.5	0.93	105	4.5	12–18	9.5		0.35	0.13				50	63	80
									7	20–25	13.1		0.30	0.14–0.23				45–57	50–69	80
Kobayashi et al. (1983)	(102)	Upflow	17	0.94/0.15	PVC		0.97	144	2	20–35		24	0.32	0.05–0.54	288	163	118	73	75	73
Abramson (1986–1987)	(103)	Upflow	667	2.44/0.61	Corrugated PVC				20	27	6–60				67[e]		100	72[e]		73
Sanchez et al. (1997)	(104)	Downflow	2		Ceramic rings	1.6 × 1.5[f]	0.7	191		30–35	4–72				913	220	254			
Wilson et al. (1998)	(105)	Upflow	11.3	1.00/0.12	Plastic rings	1.5 × 1.5[f]		220		17–28	5.0–10.1		0.96–2.0		359–465			52–76		

Camargo and Nour (2001)	(106)	Upflow	750	0.8[c]/–	Bamboo rings	(3.5–4.0) × (3.5–4.0)[f]			48			2–9[g]			996	620	317	60–80		
Elmitwalli et al. (2002)	(107)	Upflow + hybrid	60 + 65	2.1/0.19 + 2.3/0.19	Reticulated polyurethane foam					13 13 13		4+8[g] 2+4[g] 3+6[g]			461–528			71 59 63		
Manariotis and Grigoropoulos (2006)	(97)	Upflow	12.5	0.82/0.14	Ceramic saddles	1.6 × 1.6 × 2.0[h]	0.59	308	20	23–29	7.2–74	4.3–44	0.12–1.8	0.04–0.86	323–546	111–258	190–327	52–85	55–94	55–97
									2	15	24	14	0.49	0.24	488	244	227	41	46	42
		Upflow	12.5	0.82/0.14	Plastic rings	2.5 × 2.6[f]	0.67	186	21	23–29	12–74	8.0–50	0.12–0.95	0.04–0.46	323–507	111–244	193–327	55–83	56–92	69–96
		Upflow	12.5	0.91/0.14	Crushed stones	1.9–2.5[f]	0.46	186	21	23–29	12–74	5.5–34	0.12–0.92	0.04–0.46	323–534	111–288	171–396	50–80	50–83	70–96
									2	15–16	24	11	0.38–0.55	0.15–0.27	379–548	152–269	236–259	50–54	46–60	64–79
									Settled municipal wastewater											
Raman and Khan (1978)	(99)	Upflow	15	1.65/0.11	Broken stones	2.0–2.5			7 8	25–32 25–33	12 12	5 5			267 256	120 198	104 120	71	80 84	79 86

(Continued)

Table 14.3 (Continued)

Study		Reactor characteristics							Experimental study characteristics									
Authors and publication year	Ref. no.	Column			Packing material				Duration (months)	T (°C)	HRT		OLR		Influent wastewater			Removal efficiency		
		Type	Volume (L)	Height/ diameter (m)	Type	Size (cm)	Void ratio	Specific surface (m^2/m^3)			Empty bed (h)	Void volume (h)	COD (kg/m^3 day)	BOD_5 (kg/m^3 day)	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	COD (%)	BOD_5 (%)	TSS (%)
Viraraghavan et al. (1989)	(108)	Upflow	5.9		Rock	2.5–5.0	0.47	95	2.5	23–27		9[g]		0.12		119	111		76	85
		Downflow	5.9		Rock	2.5–5.0	0.47	95	11	23–38		7–15[g]		0.05–0.11		90–94	79–169		78–82	86
		Downflow	5.9		Rock	2.5–5.0	0.47	95	14	23–38		7–15[g]		0.05–0.11		90–127	81–169		78–83	78–86
		Downflow	6.1		Wood	2.5 × 10.0	0.61		12	23–38		9–22[g]		0.05–0.14		84–106	74–169		78–83	85–94
		Horizontal	8.2		Rock	2.5–5.0	0.42	95	14	23–38		8–30[g]		0.03–0.10		90–127	77–169		79–87	88–96
		Downflow	1,190	4.3/0.6	Rock	10.0–15.0	0.45	30	4	24–28		25–28[g]		0.20		96–105	83–89		41–50	65–81
		Upflow	790	4.3/0.6	Rock	10.0–15.0	0.46	30	1	18–20		17[g]		0.46		149	169		32	56
Tanaka et al. (1991)	(109)	Upflow	0.94		Plastic	1.0 × 1.0	0.83			37	8–24				242–272		107–121	38–54		78–90
										Septic tank effluent										
Viraraghavan and Dickenson (1991)	(110)	Upflow	9.5	1.2/0.10	Plastic ballast rings	5.0	0.96	114		5		29–114			207–286	122–188		5–57	42–75	
										10						126–188		25–62	57–86	
										20						126–188		49–65	64–86	

Synthetic wastewater																			
Frostell (1979)	(111)	Upflow	2 × 0.85	0.48/(0.08–0.11)[i]	Activated carbon	0.1 × 0.3	0.61	2,900	22–26		3.7–15	0.52–2.1		400–430		71–83			
Lindgren (1983)	(112)	Upflow	18	1.85/0.11	Polyurethane		0.95		20–35					150–600					
Matsushige et al. (1990)	(113)	Upflow	4		Vinylidene chloride				5–10	7.5–30[g]			66[j]	200	33–71	27–48			
									20–30	7.5–30[g]			66[j]	200	70–85	46–95			
Bodik et al. (2002)	(114)	Upflow	1.5	0.5/0.07	Cut insulating tubes	(1.5–2.0) × 2.0[f]	0.88		8	10–20[g]			490–540	250–280	230	46–63	41–71	72–80	
									15	10–20[g]			570–690	290–310	70–200	84–87	70–91	90–93	
									20	6–20[g]			450–780	320–440	170–390	77–92	81–96	81–95	
Manariotis and Grigoropoulos (2003)	(115)	Upflow	3.9	0.7/0.09	Corrugated plastic rings	1.5 × 2.4[f]	0.90	308	23–29	12–74	11–67			312–457	142–281	51–144	56–79	57–89	55–92
									15–16	12–24	11–22			331–347	209–230	62–64	83–89	92	83–86

(Continued)

Table 14.3
(Continued)

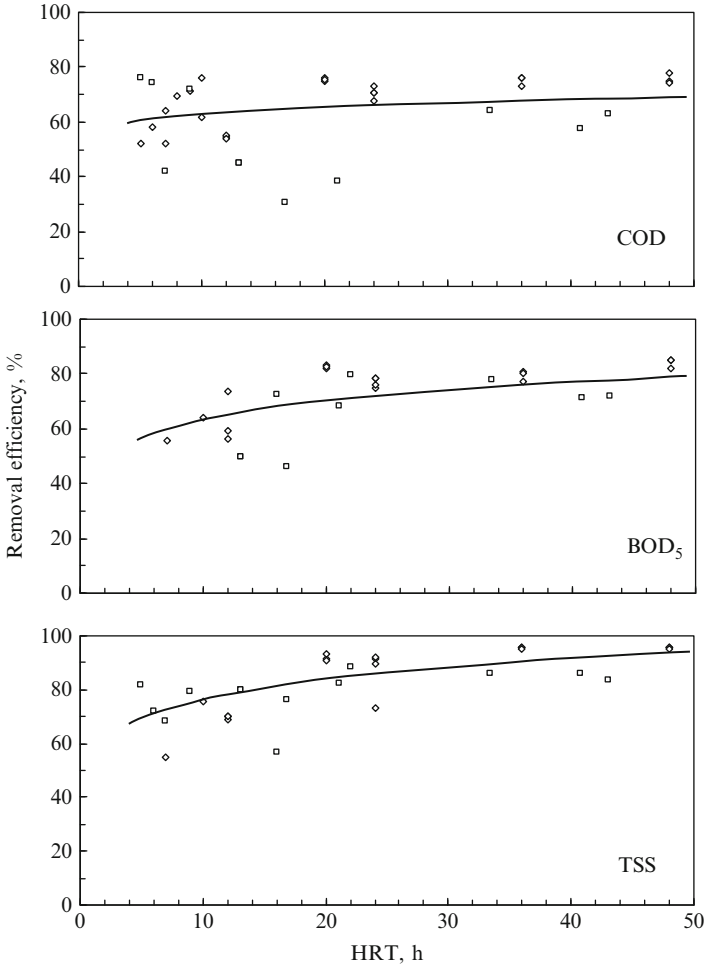
Study		Reactor characteristics							Experimental study characteristics										
Authors and publication year	Ref. no.	Column			Packing material				Duration (months)	T (°C)	HRT		OLR		Influent wastewater			Removal efficiency		
		Type	Volume (L)	Height/ diameter (m)	Type	Size (cm)	Void ratio	Specific surface (m^2/m^3)			Empty bed (h)	Void volume (h)	COD (kg/m^3 day)	BOD_5 (kg/m^3 day)	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	COD (%)	BOD_5 (%)	TSS (%)
Food processing wastewater																				
Hudson et al. (1978)[k]	(86)	Upflow	28		Granite stone	2.5–3.8	0.53	130		18–26		40–60			407–466	310	12–18	33–55	78	25–50
										10–24		8.4			121		39	38		62
		Upflow	28		Oyster shells		0.82	656–984		18–26		38–74			407–466	310	12–18	74–81	88	25–50
										10–22		7.9			121		39	45		59
Reyes et al. (1999)[l]	(116)	Multistage (upflow and downflow)	9		Waste tyre rubber	1.0 mL[m]	0.66					8–96			941	210	888	55–70[n]	63–82[n]	72–90[n]

[a] Width of square-shaped column.
[b] Reactors fed for 9.5 h/day.
[c] Based on packed-bed zone.
[d] Values determined experimentally.
[e] Total organic carbon (TOC) value reported.
[f] Length × diameter.
[g] HRT basis not given.
[h] Length × width × height.
[i] Diameter range of cone-shaped column.
[j] COD_{Mn} values reported ($KMnO_4$ oxidation).
[k] Shellfish wastewater treated.
[l] Diluted piggery wastewater treated.
[m] Volume of spherical medium 1.0 mL.
[n] Values estimated from graphs.

development of methanogenic biomass; however, significant COD reduction was observed during this period and the effluent TSS remained at a very low level.

4.1.2. Performance

The performance of AF reactors is affected by the operating conditions, mainly the HRT, OLR and temperature, and the type of substrate treated. Removal efficiencies reported in numerous experimental studies are given in Table 14.3, together with the characteristics of the wastewaters and the operating conditions of the test units. In addition, the effect of the HRT on upflow AF performance, in terms of COD, BOD_5, and TSS removal, is shown in Fig. 14.4 (97, 99–102, 105, 106). The data used to construct this figure reflect treatment



Note: Data from: ▫ pilot plant studies, ◇ laboratory studies

Fig. 14.4. Effect of hydraulic retention time on anaerobic filter performance (97, 99–102, 105, 106).

of municipal wastewater and reactor operation at temperatures higher than 15°C, and were obtained in both laboratory and pilot-plant studies. Removal curves have been developed for each characteristic using logarithmic fitting; although some individual values exhibit considerable spread, especially in the case of COD, the curves are acceptable, considering that the data employed were derived in a series of studies conducted under varying experimental conditions, and useful.

A decrease in HRT, or correspondingly increase in OLR, and operation at lower temperature levels would cause a reduction in the reactor removal efficiency for organics and solids. Manariotis and Grigoropoulos (97) have found that at 25°C and with municipal wastewater, the reduction in COD removal was more intense at HRT values less than 10 h and OLR levels more than 0.5 kg COD/m^3 day; and reported that a 10°C temperature drop (from 25 to 15°C) in AF units operating at a 24-h HRT significantly affected the COD and TSS removals (with maximum decreases from 53 to 41% and from 73 to 42%, respectively). Frostell (111), who treated synthetic wastewater at 22–26°C, also found that the reduction of HRT from 15 to 4 h with a subsequent increase in OLR from 0.5 to 2 kg COD/m^3 day decreased the COD and SCOD removals by about 10% (from 83 to 71% and from 90 to 81%, respectively). Genung and coworkers (118), however, have reported that in an upflow AF treating municipal wastewater at 10–25°C and a HRT of 6–35 h, temperature variation did not influence the removal efficiency, when the OLR remained constant. Kobayashi and coworkers (102) have also noted that a drop in temperature from 35 to 25°C did not significantly influence the reactor removal efficiency or the biogas production rate, but a further reduction to 20°C resulted in decreased BOD_5 and TSS removals. Viraraghavan and Dickenson (110), who used an upflow AF to treat septic tank effluent, found that a reduction in the HRT level affected the organic material removal more at a temperature of 5°C than at higher temperatures (10 or 20°C).

It should be noted that the behavior of AF reactors may change after prolonged operation, especially if operational conditions have been widely varied in the interim period. It has been reported that when laboratory upflow AF units treating municipal wastewater functioned again under similar conditions (HRT, temperature) after they had been in operation for extended periods, their performance (removal efficiency, biogas release rate) at the same level of hydraulic loading generally deteriorated with time, and long-term operation seemed to adversely affect filter behavior (97). Laboratory findings always need to be carefully evaluated before being projected to full-scale application.

4.1.3. Biogas Production

The release of biogas from AF units treating low-strength wastewater shows a significant variation in production rate and composition, is generally associated with a high N_2 (nitrogen) fraction, and is influenced by the loss of soluble CH_4 in the liquid effluent and the accumulation of suspended organic matter in the reactor. The escape of CH_4 in the treated effluent and release of N_2 from the untreated influent to the biogas, which are controlled by Henry's law and the related partial pressures of the gases, are intensified because of the large volumes of wastewater passed daily through the reactor (72). The solubility of CH_4 in water at equilibrium conditions and 101.3 kPa CH_4 partial pressure varies with temperature, and values of 36.9 and

30.1 mL/L have been given at 15 and 25°C, respectively (119); assuming an average of 70% CH_4 content of the biogas, the CH_4 lost in the liquid effluent would be 25.8 and 21.1 mL/L at the two temperatures. More than the 50% of the CH_4 produced in the reactor has been reported to leave in the effluent, which can be oversaturated with CH_4 (97, 120).

Limited information is available relative to biogas production in AF reactors treating municipal wastewater. Biogas release rate and composition showed a significant variation, 5–120 L/day and 0–80% respectively, and CH_4 production corresponded to 33% of the theoretical level in a pilot upflow AF (5.7-m^3 packed section volume) with a design flow of 19.0 m^3/day (47, 121); this would correspond to a maximum release rate of 6.8 mL/L of influent wastewater at design flow. Methane production averaged 0.05, 0.11, and 0.16 m^3/kg COD removed at temperature levels of 12–18, 22, and, 20–25°C, respectively, and the corresponding CH_4 content of the biogas was 3–9, 60–80, and 63–76% in another pilot upflow AF (75 m^3) which was operated at HRT values of 17 h to 4 days (101). A higher maximum biogas release, ranging from 59 to 64 mL/L of influent, was observed in laboratory upflow AF reactors (12.5 L) which also treated municipal wastewater at HRT values of 30–48 h. The conversion of COD to biogas in these units operating at an HRT of 30 h and 23–29°C was 0.17–0.18 m^3/kg COD removed, and the biogas contained 69–70% CH_4 (97). A similar biogas production level of 0.16 m^3/kg COD removed was determined for an upflow AF (17 L) treating municipal wastewater at an HRT of 24 h and 20–35°C, and the biogas contained 65% CH_4 (102). It should be noted that the stabilization of retained and accumulated organic material would affect the amount of biogas released and that a higher rate of daily COD uptake does not always correspond to a larger daily gas release rate. The conversion of organic matter to biogas is at a level much lower than the theoretical value of 0.35 m^3 CH_4 standard temperature and pressure (STP)/kg COD removed, reflecting the substantial loss of CH_4 in the effluent; consequently, the term "apparent conversion" may be more appropriately used (72, 97).

Biogas production is generally higher when low-strength synthetic wastewater is treated. A two-stage upflow AF arrangement (0.85 L each stage) running at HRT levels of 3.7–15 h and 22–26°C, achieved a conversion rate ranging from 0.11 to 0.18 m^3 CH_4 STP/kg COD removed (111). Similarly, an upflow AF (3.9 L) operating at a 24-h HRT and 25°C gave a maximum biogas yield of about 0.20 m^3/kg COD removed, which assuming a 68% CH_4 content corresponded to 0.14 m^3 CH_4/kg COD removed (115).

Reactor operation at decreased temperature levels would result in lower organic removal, biogas release and COD conversion to biogas, and a higher CH_4 escape in the effluent. Lowering the temperature of operation by 8–10°C (to around 15°C) reduced COD conversion to biogas by 48–69% when municipal wastewater was treated, and the reduction was greater when synthetic wastewater was processed, reaching up to 96% (97, 101, 115).

4.1.4. Packing Material

The void ratio of the packing material has been reported to control the accumulation of biomass in the interstitial space, affecting the efficiency of the reactor (86), and the density of the media to influence the relative presence of acidogenic and methanogenic bacteria, with lower numbers of methanogens observed in sparsely packed than in closely packed AF units

(122). Miyahara and coworkers have observed that suspended acidogenic bacteria were more than those attached to the filter media, and comparing the operation of an unpacked upflow reactor and a partially packed AF concluded that the use of packing promoted the accumulation of lipolytic and methanogenic bacteria, facilitated the degradation of both insoluble and soluble matter and hydrolysis of cellulose, and enhanced effluent quality (122).

The quantity of solids held and removed with drainage is also a factor to be considered in selecting packing media, since draining of the AF reactor constitutes a simple method for discharging unattached biosolids, thereby delaying clogging of the filter. Manariotis and Grigoropoulos have found that the morphology of the packing material used significantly affected the removal of retained solids and the volume of drainage recovered; this volume was substantially smaller than the available void space and increased with column height. The total liquid recovery was found to range from 22 to 36% of column voids and was lower in an upflow AF packed with ceramic saddles (relatively dense packing, void ratio 0.59) than a reactor packed with gravel (less dense packing, void ratio 0.46); as a consequence, the quantity of biomass removed from the first unit with drainage was also much less (123).

4.1.5. Biomass Accumulation and Disposal

Sludge accumulation has been observed in AF reactors after a period of operation and clogging may be encountered. Initially, most of the organic material is stabilized and solids are removed in the lower section of an upflow AF; however, gradually the concentration of biosolids increases within the reactor, and the solids shift to upper column sections (123, 124). The COD/BOD_5 ratio of samples taken inside the AF was also found to increase with time reaching values as high as 8 or 9, and the VSS/TSS ratio to decrease to a level of 0.5 or less (the corresponding ratios in untreated municipal wastewater were in the area of 2.1 and 0.7); these changes would indicate that in a one-step configuration, the AF reactor is able to both reduce the organics and solids in the liquid stream and to stabilize the retained biosolids (123).

A carbon balance, based on data from a pilot upflow AF (5.7 m^3) which treated municipal wastewater at OLR of 0.024–0.19 kg COD/m^3 day, led to the estimation that after 16 months of operation, the accumulated biomass should be expected to occupy 40% of the reactor void volume assuming that the treated effluent did not contain any dissolved CH_4 and 21% of the volume considering that it was saturated with CH_4. These estimates were low, and when the reactor was drained and opened at the end of 2 years, only 43% of the initial void volume was found to remain free; the drained sludge contained 60% volatile solids (VS) while the liquid in the interstitial voids had 50% VSS. Tracer studies conducted before opening the reactor had indicated a plug flow mode and formation of channels, possibly resulting from solids accumulation and poor influent wastewater distribution (47, 118, 121).

Draining of the AF column helps remove unattached biomass, and effluent recycle applied prior to draining facilitates the removal of attached solids and settled sludge (125). The total biosolids accumulated in laboratory and pilot-scale upflow AF reactors fed with municipal wastewater for an extended period (34 and 24 months, respectively) were measured and found to correspond to about to 33 and 45 kg of solids/1,000 m^3 of wastewater treated (101, 123). The quality of sludge removed from the laboratory reactor was evaluated and found to satisfy the volatile solids reduction (VSR) criterion, one of the parameters employed to ascertain

compliance with the US Environmental Protection Agency (USEPA) Part 503 rule for the beneficial use or disposal of biosolids (123). A minimum 38% VSR is required by the USEPA regulations in order to meet the vector attraction reduction requirement (126); a simple means for estimating the VSR achieved is the Van Kleek relationship described by Eq. (1) (127).

$$\mathrm{VSR} = \frac{\mathrm{VS_F} - \mathrm{VS_W}}{\mathrm{VS_F} - (\mathrm{VS_F VS_W})} \times 100 \tag{1}$$

where VSR, volatile solids reduction, %; $\mathrm{VS_F}$, fraction of volatile solids in feed on solids-only basis; $\mathrm{VS_W}$, fraction of volatile solids in digestion residue on solids-only basis.

Although the AF system is not a typical facility considered under the USEPA rule, biosolids withdrawn from the reactor should meet the VSR requirement prior to further processing.

4.2. Modified Systems

Various configurations of reactor setup and operational mode have been examined and are presented in this section. These include partially packed AF and moving bed reactors, coupling AF and UASB reactors, and application of recycle to AF units.

A reactor arrangement consisting of a UASB section in the lower part and moving packing media which floated in the upper part (Fig. 14.2), was employed for the treatment of medium-strength synthetic wastewater (COD of 1,000–2,000 mg/L) at 37°C, and the objective of the study was to evaluate the effect of the HRT (in the range of 2.8 h to 7 days) and corresponding organic loading (128). The effluent TSS concentration was low, even for an HRT as low as 3.0 h (except during the startup period). The accumulation of biomass on the packing material was limited, compared to the biomass held in the interstitial voids and the sludge blanket, and the maximum quantity held was 0.5 mg VS/piece of packing. The attached biomass did not seem to significantly affect the removal efficiency, but the moving packing material had a vital role in the entrapment of solids even at high hydraulic loadings. At an HRT of 5 days, a reduction in temperature from 37 to 32°C and 27°C did not influence the COD removal efficiency, which remained at 94–96%. Upflow reactors combining a UASB section in the lower part and a packed-bed in the upper part (33% of the reactor volume) were also studied for the treatment of low-strength synthetic wastewater (129). Recycling had little effect on COD removal when the influent COD ranged from 300 to 1,000 mg/L and the OLR was 13 kg $\mathrm{COD/m^3}$ day. An HRT lower than 1.0 h did not yield adequate COD removal; however, levels from 1.2 to 2.0 h gave removals of 75–80%, when influent COD values ranged from 750 to 1,000 mg/L.

A pilot-scale reactor (180 L) packed with polyurethane in 40% of its volume was operated with municipal wastewater at ambient temperature (10–20°C) and HRT values of 1.6 and 6 h for 3 months (130). Removals of COD and SCOD 33–55% were obtained at temperatures between 13 and 20°C; however, to achieve a higher efficiency, the reactor had to operate at an increased temperature. The results achieved by the modified unit were comparable to those obtained with UASB and AFB reactors, except for the necessity to remove the accumulated sludge. Another pilot system (160 L) using inclined parallel discs of polyurethane was also employed for the treatment of municipal wastewater at an HRT of 1.2 h and 20°C for 1 year. This system, combining physicochemical action due to the parallel discs and microbial

degradation of the soluble organic matter by the immobilized bacteria, obtained better treatment efficiency (COD and TSS removals of 57 and 44%), without experiencing clogging problems or need for removal of sludge (131).

A fixed-moving bed reactor (28 L) incorporating stationary and rotating discs (Fig. 14.2) was employed for the treatment of settled municipal wastewater at 16°C and an HRT of 12 h (120). The mixing rate influenced efficiency, and mixing at 5 rpm increased the removal of organic matter; however, rotation speeds higher than 15 rpm did not improve efficiency. Biogas production and composition showed significant variation, and the CH_4 lost in the liquid effluent was estimated to be more than 50% of the amount produced.

A five-stage AF system (9 L total volume) was operated in series in upflow and downflow modes for the treatment of low-strength (diluted) piggery wastewater (116). Most of the organic material was removed in the first stage, which at higher HRT values (24 h or more) was capable of achieving the targeted final removal level; at lower HRT levels (8 and 12 h), however, the removal of COD was better distributed through the five stages although it occurred mainly in the first three, and the removal of BOD_5 took place mostly in the first stage.

4.3. Process Modeling

Models have been developed on the basis of material balances to describe the mass rate change of the substrate or biomass around the AF reactor; these do not consider recycling and assume complete mixing, and are expressed by Eqs. (2) and (3).

$$V\left(\frac{\mathrm{d}S}{\mathrm{d}t}\right) = QS_\mathrm{i} - QS_\mathrm{e} - VR_\mathrm{s} \tag{2}$$

$$V\left(\frac{\mathrm{d}X}{\mathrm{d}t}\right) = VR_\mathrm{x} - QX_\mathrm{e} \tag{3}$$

where V, reactor volume, L; $\mathrm{d}S/\mathrm{d}t$, substrate concentration change rate, mg/L day; $\mathrm{d}X/\mathrm{d}t$, biomass concentration change rate, mg/L day; Q, wastewater flow rate, L/day; S_i, S_e, influent and effluent substrate concentration, mg/L; R_s, substrate utilization rate, mg/L day; X, biomass concentration, mg/L; R_x, net biomass growth rate, mg/L day; X_e, effluent biomass concentration, mg/L.

Matsushighe and coworkers (113) applied biomass and substrate balances to a laboratory-scale AF treating low-strength synthetic wastewater assuming a completely mixed flow pattern, and expressed the $S_\mathrm{e}/S_\mathrm{i}$ ratio as a relationship between the biomass concentration inside the AF and the HRT:

$$\frac{S_\mathrm{e}}{S_\mathrm{i}} = \frac{1}{1 + kX\theta_\mathrm{e}} \tag{4}$$

where k, first-order constant, dependent on temperature (°K) as follows: $k = \mathrm{e}^{\frac{-11{,}810}{T}+33.54}$ for BOD and $k = \mathrm{e}^{\frac{-8.358}{T}+21.05}$ for TOC; θ_e, hydraulic retention time based on column empty volume, h.

Mass balance and film penetration have also been used to describe the performance of AF reactors treating low-strength wastewater, including municipal wastewater, and the following simplified form has been employed (101, 124):

$$S_e = S_i e^{-KH/q^n} \tag{5}$$

where K, treatability factor, 1/min; H, packing bed height, feet (1 foot = 0.3048 m); q, hydraulic loading, gpm/foot^2 (1 gpm/foot^2 = 58.674 m^3/m^2 day); n, constant dependent on the packing material characteristics.

Empirical relationships to describe the removal efficiency for organic matter of AF units based on experimental results have also been proposed (86, 102, 132) and take the following expressions:

$$E = 100\left(1 - \frac{\alpha}{\theta_e}\right) \tag{6}$$

$$E = E_m\left(1 - \frac{\alpha}{\theta_e}\right) \tag{7}$$

where E, organic matter removal efficiency, %; E_m, maximum organic matter removal efficiency, %; α, experimentally determined constant, h.

Equations (6) and (7) show the significant effect of the HRT on the performance of AF reactors and are applicable over a wide range of organic loading and wastewater strength. Kobayashi and coworkers (102), using data from the treatment of municipal wastewater in a laboratory upflow AF estimated the value of constant α to be 2.0 h at 25 and 35°C and 4.0 h at 20°C; obviously, the constant is dependent on the experimental setup and data, and values obtained from test AF should not be directly transferred to performance predictions for full-scale AF.

Regression analysis of experimental data from AF units treating municipal wastewater is a valuable tool for expressing AF performance. A general relationship correlating effluent quality in terms of COD with influent COD and HRT is given by Eq. (8):

$$S_e = a S_i^x \theta_e^y \tag{8}$$

where a, x, y, regression analysis constants.

It should be noted that the nonbiodegradable fraction of COD is not reflected in this relationship, and that this general form of equation could be used to describe the operation of AF units with different packing materials treating various substrates. Wilson and coworkers (105) on the basis of experimental results proposed the following specific equation for the estimation of the effluent COD concentration of an upflow AF packed with highly porous media:

$$S_e = 0.084\, S_i^{0.73}\, \theta_e^{0.97} \quad R^2 = 0.924 \tag{9}$$

As can be seen, S_e is affected by both the HTR and S_i; however, it is more dependent on the hydraulic loading. Manariotis and Grigoropoulos (97) modified Eq. (8) for use with AF reactors containing low porosity media to reflect the significant reduction of the HRT when

void volume ratio is considered, and proposed the following form:

$$S_e = aS_i^x \theta_v^y \tag{10}$$

where θ_v, hydraulic retention time based on packed column void volume, h.

Equation (10) can be written in a more general form to account for the characteristics of the packing media,

$$S_e = aS_i^x \theta_v^y \varepsilon^z \tag{11}$$

where ε, packing media void ratio; z, regression analysis constant. Based on experimental findings from laboratory upflow AF units treating municipal wastewater, Manariotis and Grigoropoulos (97) proposed the following general equation:

$$S_e = 0.7390 S_i^{0.824} \theta_e^{-0.374} \varepsilon^{-0.213} \quad R^2 = 0.862 \tag{12}$$

Other empirical relationships can be found in the literature (133). Efforts have also been undertaken to develop more complex mathematical models in order to describe the performance of AF reactors used for the treatment of municipal or low-strength wastewater and predict treatment efficiency and gas production rate and composition (103, 112, 134). Such models are simplified by limiting consideration to hydrolysis and methanogenesis (the fermentation step is overlooked) (134) or to acid formation and methanogenesis (112), and substrate utilization is described by Monod kinetics. It is interesting to note that based on model predictions, Cakir and Stenstrom (134) suggested that a 24-h HRT is required to achieve greater than 60% removal of COD.

4.4. Seasonal Operation

An interesting characteristic of anaerobic reactors is the ability of their biomass to withstand long periods without feeding and return to an active condition quickly, making these units suitable for treating wastewater generated in tourist areas or produced by seasonally operating agroindustrial activities; however, limited attention has been given to this important aspect. Rapid reactivation of laboratory AF units treating milk or potato-based synthetic wastes after periods of inoperation up to 6 months has been reported (135), and the same was found true when anaerobic granules obtained from UASB reactors fed with a synthetic substrate and stored at temperatures up to 22°C for 10–18 months were used again at 35°C (136, 137).

The restarting process was recently studied using four upflow AF reactors after a 2-year inactive period. The reactors had been run for almost 3 years before their operation was interrupted, and during this period, three units (12.5 L) were fed mostly with municipal-type wastewater and one (3.9 L) with synthetic wastewater (Table 14.3); however, after restarting, all AF were fed with synthetic wastewater (97, 115, 138). The response of the units which had treated municipal wastewater was not very rapid, although much faster than startup, and after 8 days COD removal was only about 30%, while 50 days were needed for effluent COD to drop below 100 mg/L; on the contrary, the response of the unit which has treated synthetic wastewater was immediate and effluent COD was from the beginning of the restart effort below 100 mg/L, reaching after 30 days values below 50 mg/L (a removal in excess of 80%).

The difference in behavior was attributed to the use of the same type of substrate before and after the inactive period in the smaller reactor.

These findings are supported by studies conducted with other types of anaerobic reactors. These include AFB and UASB reactors treating raw municipal wastewater which were fed again after 4 months of inoperation (139) and an ABR unit treating low-strength synthetic wastewater which was placed in operation after a 2-year inactive period (72). Although additional work is needed in this area, it would seem that the AF reactors can be restarted after a prolonged idle period within a short time and function effectively soon thereafter; this is important, especially when the lengthy reactor startup period is considered.

4.5. Reactor Design Recommendations

Specific aspects that should be considered in the design of upflow AF reactors to be used for the direct treatment of low-strength wastewaters are highlighted in this section. In the absence of full-scale AF application or even more large pilot-scale findings, which would have yielded valuable information, these recommendations are based primarily on experience derived from laboratory and limited pilot plant-scale studies.

The feed inlet structure at the bottom of the reactor is a crucial feature, as it helps to provide improved distribution of the wastewater, prevent channeling through the packed bed, and reduce dead spaces in the AF; it should be noted that when low-strength wastewater is treated, biogas production is low, and consequently mixing inside the reactor is not intense. A single perforated distribution plate or multiple nozzles at the bottom of the AF could serve for this purpose, and the lower reactor section (10 to 20% of column height) should not be packed with media to facilitate influent solids accumulation and removal. Adequate pretreatment of the influent wastewater (screening, grit removal) should be applied to effect the retention of inert materials present which might plug and unnecessarily load the filter.

Packing material with an open structure yielding a void ratio of more than 0.90, would enable easier removal of accumulated solids during draining. Materials which could be used for the construction of AF reactors include stainless steel, steel, plastic, and concrete; because of potential corrosion problems caused by the oxidation of H_2S and the presence of dissolved CO_2, concrete and steel reactors should be properly coated.

Accumulation of sludge in the AF reactor is an important operational aspect and a sludge management program should be provided in order to establish the procedure and frequency of actions to be taken for removing entrapped solids and ensuring that the biosolids withdrawn meet applicable disposal regulations. In addition to solids retained in the unpacked bottom section, sludge can be removed by physical draining of the entire column, and solids removal can be assisted by recirculating effluent before draining at a high flow rate.

Operation temperature should be above 15°C in order for the reactors to attain adequate performance; the released biogas could be utilized to heat the reactor, although this would not be practical in smaller installations. Because secondary effluent discharge limits may not be sustainably achieved and a polishing step might have to be added to improve effluent quality and satisfy related requirements, the AF reactors would not need to function at top efficiency conditions, and consequently could be operated at lower temperatures.

Proposed operating parameters for the AF process are an HRT from 6 to 24 h and an OLR up to 2 kg COD/m^3 day, depending on influent wastewater strength, ambient temperature, and quality of treated effluent desired. Under these conditions, removal of COD, BOD_5, and TSS of 60 to 68%, 60 to 73%, and 73 to 85%, respectively, may be anticipated (Fig. 14.4), however; lower (by about 5 to 10%) efficiencies should be accepted. In sizing the AF column, additional work is needed to define the diameter and height relationship for full-scale installations; it is noted that in laboratory-scale upflow AF reactors, a height of 0.8 to 1.0 m (with a diameter of about 0.15 m) was found adequate for the treatment of municipal wastewater, although a diameter of 4.9 m and a height of 5.4 m (with the packed bed 3.0-m high) were selected for a pilot-scale system (Table 14.3). The packing material employed would also influence the diameter of the column and consequently its height; a column to packing particle diameter ratio in excess of 30 has been reported to be necessary in order to minimize voidage variation at different regions of cylindrical beds packed with spheres and prevent wall effects (140).

4.6. Posttreatment

The decreased removal efficiency of the anaerobic process and inability to satisfy stringent secondary effluent regulations in effect in the European Union (EU) and the United States of America (USA) (Table 14.4 (141, 142)), make necessary that additional treatment be provided to improve (polish) the AF effluent quality. In the past 15 to 20 years, attention has been directed to reducing a major part of the organic and suspended solids loads in the anaerobic stage (usually a UASB or AF reactor) with additional removal of organics, solids, and nutrients in a subsequent aerobic stage [an aerobic filter (AerF), rotating biological contactor (RBC) unit, natural treatment system (stabilization pond, duckweed pond, constructed wetland) or solids-separation facility]; effluent recycling may also be practiced to enhance the reduction of nutrients (especially Total-N).

Posttreatment schemes which have been studied with anaerobic biofilm reactors are summarized in Fig. 14.5 (143–151), and are briefly discussed in the following paragraphs. Additional work conducted using a UASB reactor or an anaerobic lagoon as the first stage can be found in the literature (152–160).

A pilot multistage system which consisted of three AF in series (5, 4, and 4 m^3, respectively), an AerF (3 m^3), a sedimentation tank (3.7 m^3 with 2.0 m^2 surface), and a filtration unit (downflow mode with 60-cm bed height) (Fig. 14.5a) was employed for the treatment of municipal wastewater, addressing the removal of both organic matter and nutrients (143). When the stabilization of organic matter was studied (phase 1), all AF units operated in series and recycle was not employed; the HRT (based on total anaerobic-aerobic volume) was 46 h during the initial 200 days and 31 h thereafter, corresponding to OLR of 0.13 and 0.19 kg BOD_5/m^3 day. During the first 100 days, about 50% of the total BOD_5 was removed by the first AF while the second and third AF had no contribution to treatment. When nutrient removal was examined (phase 2), the first AF was not used and effluent from the sedimentation unit was recycled to the second AF at ratios of 1 and 3; the HRT was 51 h (based on total volume), the OLR 0.082 kg BOD_5/m^3 day, and the nitrogen loading rate (NLR) 0.023 kg N/m^3 day. At a recycle ratio of 1 total removals were in the range of 90 to 98% for COD, BOD_5 and TSS, 95 to 98% for ammonia nitrogen (NH_4-N) and total kjeldahl nitrogen (TKN),

Table 14.4
Effluent regulations for discharge of biologically-treated wastewater (141, 142)

Characteristic[a]	Treatment						
	EU			USA			
	Secondary-advanced[b]		Lagoons	Secondary		Equivalent to secondary[c]	
	Concentration[d] (mg/L)	Removal[d] (%)	Concentration[d] (mg/L)	Concentration[e] (mg/L)	Removal[f] (%)	Concentration[e] (mg/L)	Removal[f] (%)
BOD_5	25	70–90		30 (45)	85	45 (65)	65
$SBOD_5$			25				
$CBOD_5$				25 (40)	85	30 (65)	65
COD	125	75					
SCOD			125				
TSS	35	90	150	30 (45)	85	45 (65)	65
Total-P	2 (1)	80					
Total-N	15 (10)	70–80					
pH				6–9		6–9	

[a] $SBOD_5$, soluble 5-day biochemical oxygen demand; $CBOD_5$, carbonaceous 5-day biochemical oxygen demand; Total-P, total phosphorus; Total-N, total nitrogen.

[b] Discharge to sensitive areas subject to eutrophication; for nutrients, the first value reflects a PE (population equivalent) of 10,000–100,000 and the value in parenthesis a PE $>$ 100,000.

[c] Use of a trickling filter or a waste stabilization pond as the principal process, with proper operation and maintenance of the treatment works to consistently achieve effluent quality.

[d] Based on a 24-h composite (time or flow) sample.

[e] The first value reflects a 30-day average and the value in parenthesis a 7-day average.

[f] Based on a 30-day average.

and 65% for Total-N; and the removal of BOD_5 and Total-N improved at a recycle ratio of 3. It was reported that more than 90% of the nitrogen was removed in the second AF (50% of this as a result of denitrification) and the remaining by bacterial uptake and sedimentation; and the rates of overall nitrogen reduction, denitrification (anaerobic stage), and nitrification (aerobic stage) were given as 0.021, 0.010, and 0.05 kg N/m^3 day, respectively. Solids production was estimated to be 0.32 and 0.45 kg TSS/kg BOD_5 removed in phases 1 and 2.

Treatment of municipal wastewater has also been undertaken using a similar system which consisted of a two-stage AF (4 m^3 each stage), an AerF (3 m^3), and a sedimentation tank (Fig. 14.5b) (161, as reported by 146). An HRT of 50 h (based on the total filter volume) was applied and sedimentation tank supernatant was recycled to the first AF in order to provide two denitrification stages and one nitrification stage. This system also performed well and at a recycle ratio of 1.06 the average removals of BOD_5, TSS and Total-N attained were approximately 98, 98 and 65%, respectively; increasing the recycle ratio to 3, raised the BOD_5 and Total-N removals.

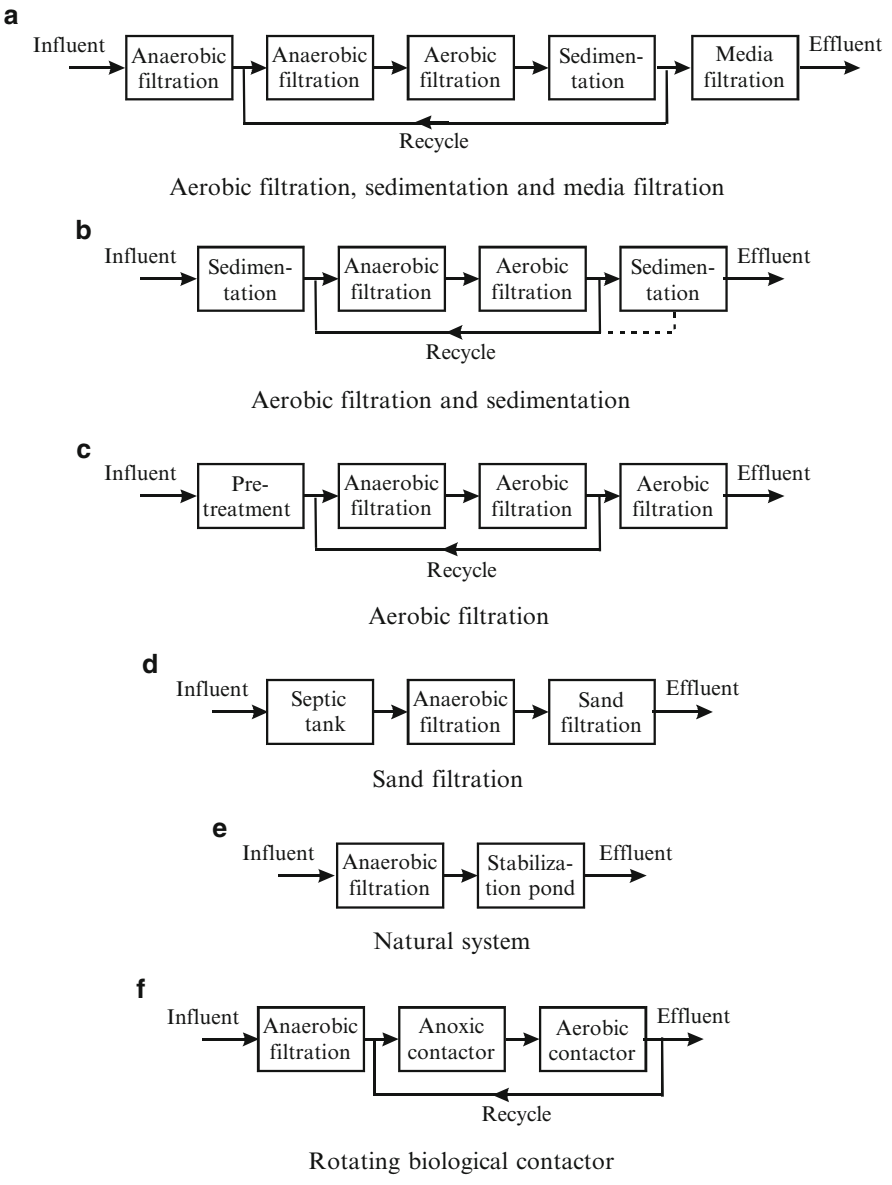


Fig. 14.5. Posttreatment schemes for anaerobic filtration (143–151).

The effect of recycle on nitrogen removal was studied in a small laboratory system consisting of an upflow AF (4.0 L) and an AerF (1.0 L with an attached 0.5-L sedimentation unit) in series (Fig. 14.5b), which treated low-strength synthetic wastewater at 20°C and a steady influent flow corresponding to HRT values of 30 and 7.5 h in the two filters (144). A recycle ratio varying from 0 to 4 was employed, yielding HRT values for the combined flow which

ranged from 30 to 3.8 h in the AF and from 7.5 to 0.8 h in the AerF; and the NLR applied to the AerF varied from 0.013 to 0.018 kg NH_4-N/m^3 day. Organic matter was mainly removed in the AF and effluent levels improved with recycling; the TOC concentration in the AF and AerF effluents ranged from 8.3 to 4.4 mg/L and from 4.0 to 3.0 mg/L, respectively. Also, an increase in the recycle ratio resulted in decreased methanogenesis (CH_4 in the biogas was decreased and N_2 was increased) and effected a reduction of Total-N (from 19.5 to 7.0 mg/L) and NH_4-N (from 17.2 to 3.7 mg/L) and an increase of oxidized nitrogen (Oxid-N) (from 0.1 to 1.9 mg/L) in the AF effluent; however, at the same time, the concentrations of the Total-N and the Oxid-N dropped in the AerF effluent (from 17.2 to 6.9 mg/L and from 16.7 to 6.6 mg/L), while NH_4-N remained at a low level (0.1 to 0.6 mg/L). The nitrification rate was higher than 90% at all recycle levels and was independent of the ratio applied.

An AF-AerF configuration, which did not provide final sedimentation (Fig. 14.5b) was examined for the treatment of medium-strength synthetic wastewater. The laboratory filters (10.5 L each) operated at 37°C and 20 to 22°C, respectively; final loading conditions of 23 h HRT, 4.4 kg COD/m^3 day OLR, and 0.25 kg NH_4-N/m^3 day NLR were applied to the AF and air was supplied to the AerF at a rate of 10 to 120 L/h (145). The study involved two phases, nitrification of the anaerobic effluent and recycling of the AerF effluent to the AF; and although the strength of the wastewater treated was beyond the scope of this chapter, some of the findings of the study are worth mentioning. Nitrification was dependent on the influent COD concentration and some of the influent NH_4-N was assimilated by heterotrophic bacteria during COD removal; NH_4-N removal through denitrification reached a level of 70% at recycle ratios of 4 and 5; COD removal in the AF reactor decreased from 77 to 66% at recycle ratios from 0 to 5, however, overall system efficiency remained constant at 99%, as a result of the heterotrophic COD reduction in the AerF.

A reactor (4.22 m^3), separated by baffles into a sedimentation–solids separation chamber, a submerged downflow AF, a downflow AerF, a final sedimentation unit and a disinfection chamber (Fig. 14.5b) was used for the treatment of low-strength hospital wastewater in order to simulate domestic wastewater treatment for several people (146). The unit was tested over an 11-month period at temperatures in the range of 16 to 27°C and under different operating conditions, which included: constant flow (1.76 m^3/day) with recycle (at a 3.7 ratio) (phase 1), intermittent flow simulating domestic wastewater release (1.86 m^3/day) without recycle (phase 2), and intermittent flow (1.76 m^3/day) with recycle (3.1 ratio) (phase 3). The overall removals of BOD_5, TSS, Total-N, and Total-P were 92 to 95%, 91 to 94%, 21 to 61%, and 17 to 21%, respectively, depending on the operating conditions; a high level of Total-N reduction (58 and 61%) occurred during phases 1 and 3, when the denitrification rate was 91 and 87% and the nitrification rate was 33 and 40%, respectively. Solids production was low, 0.15 to 0.34 kg TSS/kg BOD_5 removed (the high value was determined in phase 2 which did not employ recycling).

An onsite system consisting of two downflow AF, a downflow AerF, a sedimentation tank, and a disinfection chamber (Fig. 14.5b) was employed for the treatment of household wastewater (black water) from a 5-member family (147). The average BOD_5 removal obtained was higher than 90% (the BOD_5 in the effluent satisfied a 20 mg/L concentration), and the corresponding TSS and Total-N values were 90 and 40%. In an effort to improve the removal

of nitrogen, the system was modified by adding sedimentation and equalization tanks before the AF and applying recycle from the AerF to the AF; both the effluent BOD_5 and Total-N concentrations obtained by the modified system satisfied the 20-mg/L value.

A pilot plant system consisting of an upflow hybrid AF reactor (238 L), a nitrification tank (233 or 119 L) containing immobilized nitrifier pellets and a downflow AerF (58 L), with recycling of nitrification tank effluent to the AF (Fig. 14.5c), was tested for the removal of organics, solids, and nitrogen from municipal wastewater (148). At an HRT of 5.5 h for the total system, a recycle ratio of 3 and 25 to 250% variation in load, complete nitrification was achieved and overall removal efficiencies of 96 to 97% for BOD_5 and TSS and 74 to 75% for Total-N were obtained; and the effluent met target quality (BOD_5, TSS and Total-N concentrations of 10, 5 and 10 mg/L, respectively). A maximum denitrification rate of 0.28 kg N/m^3 day was effected in the AF, and the DO present in the influent and recycle streams did not adversely affect this process.

Another onsite system consisting of two AF (104.8 m^3 total volume) and two sand filters (66.9 m^2) was employed for the treatment of wastewater generated by a 1,000-student school (Fig. 14.5d); the wastewater was pretreated in a septic tank and recycling was not applied (149). Over a period of 1 year, the flow averaged 18.9 m^3/day (one-half of the design flow) and the AF and sand filter removed about 44 and 75% of their influent organic matter. The total COD and BOD_5 removals obtained by this system averaged 86 and 98%, with effluent COD ranging from 8 to 120 mg/L; the sand filter under aerobic conditions achieved nitrification, and the effluent NO_3-N (nitrate nitrogen) concentration ranged from 7 to 10 mg/L.

A two-stage biological system consisting of a laboratory AF and a facultative waste stabilization pond (WSP) (Fig. 14.5e) was investigated over a period of 8.5 months for the treatment of municipal wastewater (150). The upflow (12.5 L) AF operated as the first stage at 23–29°C and 15°C, an HRT of 0.5 to 3.0 days and an OLR of 0.13 to 0.96 kg COD/m^3 day and yielded COD, BOD_5, and TSS removals ranging from 50 to 82%, 50 to 84%, and 70 to 91%, respectively. The WSP (195 L with 0.36 m^2 surface) operated at ambient temperature, an HRT of 4.9 to 9.8 days and an OLR of 19 to 75 kg BOD_5/ha day and average overall system reductions of 82% (68–90%) COD and 90% (52–99%) TSS were determined. The nitrogen and phosphorus removals showed a wide fluctuation and were limited, especially in the anaerobic step; additional nitrogen reduction was obtained in the stabilization pond where nitrification was evident at different periods. It was proposed that by appropriate selection of operational parameters, depending on anticipated ambient temperature conditions, the AF-WSP combination would sustainably achieve effluent discharge values conforming to secondary treatment of municipal wastewater; however, additional treatment will be needed to effect significant nutrient reductions. The use of a natural system for posttreatment constitutes an acceptable low-cost, low-energy addition, especially in small population areas.

The AF and anoxic–aerobic RBC configuration (Fig. 14.5f) was also tested for a short period, when effluent from an upflow AF (3.9 L) was used in place of settled municipal wastewater to feed a small two-stage (anoxic-aerobic) RBC system (1.0 and 2.75 L, with 0.034 and 0.297 m^2 contact area, respectively) which was employed for organic and nitrogen removal (162). The AF reactor run at an HRT of 23 h, and during the time, the reactors were connected in series effluent from the aerobic RBC unit was recycled to the anoxic RBC unit

at a ratio of 3. Limited data indicated improved overall organic removal efficiency, and the use of the AF reduced the organic load to the two RBC units by 33 and 35%; an increased NH_4-N concentration in the anaerobic effluent impaired the removal of nitrogen in the RBC units, however, the overall NH_4-N reduction was around 95%. These preliminary findings are promising, but need to be confirmed under more vigorous test conditions.

Posttreatment is a necessary polishing step if secondary effluent regulations (Table 14.4) are to be sustainably met, and enables AF reactor operation at higher loadings or lower temperatures, since the filter would not need to function at maximum treatment efficiency. It is also feasible to develop an anaerobic–anoxic–aerobic treatment configuration based on the AF, which could achieve improved nitrogen and phosphorus removals, satisfying advanced treatment requirements. Several posttreatment schemes are presented in this section and the trend seems to be toward the use of low-tech or natural systems and the reduction of the stages incorporated in the treatment train. The AF reactor constitutes valuable technology which could serve well the needs of relatively small residential or industrial entities, especially when variable loads or interrupted operation must be accommodated.

5. DESIGN EXAMPLES

Several example problems and questions are presented in this section to help clarify aspects discussed in the preceding sections and demonstrate procedures that lead to the design of AF reactors.

Example 1

Explain how the specific surface area of packing materials may be computed; use as examples (a) gravel and (b) plastic ring-type media.

Solution

1. The surface area of large media, such as crushed stone or ceramic saddles, can be estimated by carefully folding thin paper over individual pieces making sure that all surfaces are covered, cutting the edges that are loose, unfolding the paper and measuring its area (use can be made of calibrated graph paper or a planimeter); cavities or other surface anomalies would not be accounted in this rough approximation.
2. The surface area of ring-type media may be geometrically-computed; assuming that d, l, and w are the external diameter, length and wall thickness of the ring, its surface area s can be obtained from the following relationship,

$$s = \frac{\pi d^2}{4}1 + \frac{\pi(d-2w)^2}{4}1 + 2\left[\frac{\pi d^2}{4} - \frac{\pi(d-2w)^2}{4}\right]$$

   where conversion factors need to be applied if dimensions are not given in uniform units.
3. The average surface area s_{avg} for a representative sample (about 5%) of the packing material is determined and the specific surface area is computed using the relationship:

$$\text{specific surface area, } \mathrm{m^2/m^3} = \frac{(\text{number of media pieces in column})(\mathrm{s_{avg}, cm^2})}{(\text{column volume, L})} \times \frac{(10^{-4}\,\mathrm{m^2/cm^2})}{(10^{-3}\,\mathrm{m^3/L})}$$

Example 2
Explain how the void ratio of a packed AF column may be determined.

Solution

1. The packed column is filled with water and drained; the column is allowed to drain for an additional time (usually about 6 h).
2. The column is again filled with water and the volume of water required is carefully measured; the void ratio is obtained using the following relationship:

$$\text{void ratio} = \frac{\text{volume of water, L}}{\text{volume of column, L}}$$

Example 3
Discuss how the hydraulic behavior of an upflow AF reactor treating low-strength wastewater may be studied and evaluated.

Solution

1. The hydraulic behavior of the AF reactor may be evaluated through mixing studies using tracers. Factors such as the interstitial superficial upflow velocity, biogas mixing, and biomass accumulation significantly affect the reactor performance (81), and the flow behavior in a packed bed changes with solids accumulation.
2. Several materials have been used as tracers, including Rhodamine B (a fluorescent substance that can be measured using a fluorometer) (81) and lithium (a metal added as lithium chloride that can be determined using an atomic absorption spectrometer) (78). An important characteristic of a tracer is its ability to be easily measured at very small concentrations.
3. A small quantity of the tracer is injected instantaneously or for a defined period of time into the reactor influent, and effluent samples are collected for a period of time (at least twice as long as HRT θ_e, or until the tracer concentration falls below the detection limit); the sampling interval depends on the HRT employed (for $\theta_e < 12$ h samples may be taken every 20 to 30 min, while for longer θ_e, the interval may be increased to 60 min after the initial 12 h). The tracer concentration C_e in each effluent sample collected is determined using an appropriate method and recorded with the corresponding time after tracer injection t_e the sample was taken.
4. The time and concentration values are normalized in order to enable the comparison of the mixing pattern at different HRT levels using the following relationships,

$$\text{normalized time } (t_{en}) = \frac{\text{time after injection } t_e}{\text{hydraulic retention time } \theta_e}$$

$$\text{normalized concentration } C_{en} = \frac{\text{tracer effluent concentration } C_e}{\text{(tracer mass injected)/(reactor empty bed volume)}}$$

using uniform units. A tracer recovery check and duplicate or triplicate tests should be performed to assure the validity and reproducibility of the data.
5. The normalized tracer concentration C_{en} is plotted against the normalized time after injection t_{en} and the resulting curve is evaluated to ascertain the flow pattern through the reactor. Typically, a plug flow mode will yield a bell-shaped curve peaking near the HRT, while a complete mixed mode will produce a curve rising to its highest level soon after tracer injection and then slowly

declining for a period longer than the HRT; and the area under the curve reflects the mass of tracer injected.

Example 4

An AF reactor packed with corrugated plastic rings (0.90 void ratio) has a volume of 5.0 m^3 and treats 7.0 m^3/day of domestic wastewater with an average COD and BOD_5 content of 440 and 210 mg/L. Compute the HRT employed and the OLR applied to the filter.

Solution

The HRT values on an empty bed and void volume basis are:

$$\theta_e = \frac{V(m^3)}{Q(m^3/day)} \times (24\,h/day) = \frac{5.0 \times 24}{7.0} = 17.1\,h$$

$$\theta_v = \frac{\varepsilon V(m^3)}{Q(m^3/day)} \times (24\,h/day) = \frac{0.90 \times 5.0 \times 24}{7.0} = 15.4\,h$$

Because this packing material has a high void ratio, the difference between the HRT values computed on the two bases is small; however, the difference would be substantial if low porosity media is used (e.g., for crushed rock with $\varepsilon = 0.47$, $\theta_v = 8.0$ h).

The organic load applied daily to the AF is

$$\text{organic load } 440\,mg\,COD/L \times (10^{-6}\,kg/mg)\,(10^3\,L/m^3) \times 7.0\,m^3/day = 3.08\,kg\,COD/day$$

or

$$210\,mg\,BOD_5/L \times (10^{-6}\,kg/mg)\,(10^3\,L/m^3) \times 7.0\,m^3/day = 1.47\,kg\,BOD_5/day$$

and the OLR is:

$$OLR = \frac{\text{organic load (kg/day)}}{V\,(m^3)} = \frac{3.08}{5.0} = 0.62\,kg\,COD/m^3\,day$$

or

$$\frac{1.47}{5.0} = 0.29\,kg\,BOD_5/m^3\,day$$

The COD/BOD_5 ratio ($440/210 = 2.1$) is typical and the average OLR (0.62 kg COD/m^3 day) is relatively low; however, considerable fluctuation in values may be anticipated. On the basis of a typical BOD_5 load of 60 g/capita day (141), the PE served by this AF unit is:

$$PE = \frac{BOD_5\text{ load (kg/day)}}{60\,(g/capita\,day)}\,(10^3\,g/kg) = \frac{1.47 \times 10^3}{60} = 24.5 \text{ or } 25 \text{ people}$$

Example 5

Estimate the theoretical conversion rate of organic matter to CH_4 (m^3 CH_4 STP/kg COD) and show how it can be determined.

Solution

1. Assuming that the organic matter is represented by $C_6H_{12}O_6$ (glucose), the following reaction may be used to estimate COD,

$$\underset{180\,g}{C_6H_{12}O_6} + \underset{6\times32\,g}{6O_2} \rightarrow 6CO_2 + 6H_2O$$

yielding a value of 1.067 [(6 × 32)/180] g COD/g $C_6H_{12}O_6$.

2. The anaerobic conversion of $C_6H_{12}O_6$ to CH_4 and CO_2 is shown by the following reactions,

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

$$2CH_3COOH \rightarrow 2CH_4 + 2CO_2$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

and the net overall reaction is:

$$\underset{1\,mol}{C_6H_{12}O_6} \rightarrow \underset{3\,mol}{3CH_4} + 3CO_2$$

Considering that 1 mol of $C_6H_{12}O_6$ is equivalent to 180 g and corresponds to 192 g COD (180 × 1.067 g/g) and that the total volume of CH_4 (at STP conditions) is 67.2 L (3 mol × 22.4 L/mol), the theoretical conversion rate is:

$$\frac{67.2\,L\,CH_4}{192\,g\,COD\,converted} \times \frac{(10^{-3}\,m^3/L)}{(10^{-3}\,kg/g)} = 0.35\,m^3\,CH_4\,(STP)/kg\,COD\,converted$$

3. Alternatively, as it is shown by the following reaction,

$$\underset{16\,g}{CH_4} + \underset{2\times32\,g}{2O_2} \rightarrow CO_2 + 2H_2O$$

the COD of 1 mol CH_4 is 64 (2 × 32) g and the amount of CH_4 (STP) produced from complete metabolism under anaerobic conditions is:

$$\frac{22.4\,L\,CH_4/mol\,CH_4}{64\,g\,COD/mol\,CH_4} = 0.35\,L\,CH_4\,(STP)/g\,COD\,converted$$

$$\text{or } 0.35\,m^3\,CH_4\,(STP)/kg\,COD\,converted$$

Example 6

Estimate the volume of biogas produced when the AF reactor in Example 4 is operated at 25°C and an HRT of 17 h.

Solution

1. Under these operating conditions, the following values may be selected: 65% COD removal in the reactor, 0.17 m^3 biogas produced/kg COD removed, and 65% CH_4 content of the biogas.
2. Considering that the COD load applied to the reactor is 3.08 kg/day, the COD removed is,

$$0.65 \times 3.08\,kg/day = 2.00\,kg/day$$

and the biogas produced is,

$$0.17\,\mathrm{m^3\,biogas/kg\,COD\,removed} \times 2.00\,\mathrm{kg\,COD\,removed/day} = 0.34\,\mathrm{m^3/day}$$

and corresponds to:

$$0.34\,\mathrm{m^3\,biogas/day} \times 0.65 = 0.22\,\mathrm{m^3\,CH_4/day}$$

This value is substantially lower than the theoretical value (Example 5),

$$0.35\,\mathrm{m^3 CH_4\,STP/kg\,COD\,removed} \times 2.00\,\mathrm{kg\,COD\,removed/day} = 0.70\,\mathrm{m^3\,CH_4(STP)/day}$$

partly as a result of increased CH_4 loss in the effluent when low-strength wastewater treated.

Example 7

Estimate the anticipated removal efficiency and effluent quality obtained by an AF reactor which has plastic ring packing with a 0.85 void ratio, operates at 25°C and HRT of 12 and 24 h, and treats municipal wastewater; if needed, use commonly accepted values for wastewater flow and strength.

Solution

1. Considering a wastewater flow rate of 250 L/capita day and BOD_5 and TSS values of 60 and 65 g/capita day, and assuming a COD/BOD_5 ratio of 2.1, the wastewater influent concentrations are:

$$\mathrm{BOD_5} = \frac{60\,\mathrm{g/capita\,day}}{250\,\mathrm{L/capita\,day}} \times (10^3\,\mathrm{mg/g}) = 240\,\mathrm{mg/L}$$

$$\mathrm{TSS} = \frac{65\,\mathrm{g/capita\,day}}{250\,\mathrm{L/capita\,day}} \times (10^3\,\mathrm{mg/g}) = 260\,\mathrm{mg/L}$$

$$\mathrm{COD} = 240\,\mathrm{mg\,BOD_5/L} \times 2.1 = 504\,\mathrm{mg/L}$$

2. Solving Eq. (12),

$$S_e = 0.7390 S_i^{0.824} \theta_e^{-0.374} \varepsilon^{-0.213}$$

for θ_e 0.5 and 1.0 day (12 and 24 h), S_i 504 mg/L and ε 0.85, and considering that,

$$\text{COD removal, } \% = \left(\frac{S_i - S_e}{S_e}\right) \times 100$$

COD removal efficiencies of 67 and 74% are determined for HRT of 12 and 24 h, respectively.

3. Alternatively, Fig. 14.4 may be used to read from the curves the corresponding removal efficiencies for COD, BOD_5, and TSS; these are 63, 64, and 78%, respectively, for a 12-h HRT and 68, 73, and 85% for a 24-h HRT. The COD removals estimated using Eq. (12) and Fig. 14.4 are close and average values of 65 and 71% may be accepted.
4. The treated effluent concentrations computed on the basis of the estimated removal efficiencies would be at HRT of 12 and 24 h: BOD_5 86 and 65 mg/L, COD 176, and 146 mg/L and TSS 57 and 39 mg/L; these values do not satisfy effluent guidelines for biologically-treated wastewater (Table 14.4). Operation of the AF at a 24-h HRT would yield removal efficiencies (estimated at

73% BOD_5, 71% COD and 85% TSS) which are close to the recommended values for secondary treatment, however, posttreatment should be considered.

Example 8

A laboratory AF reactor (12.5 L volume, 80 cm height) treating municipal wastewater at HRT values which were step-wise decreased from 3.1 d to 20 h was drained after running for a period of 18 months. During this period 4,700 L of wastewater were passed through the reactor, and a material balance on influent solids indicated that 1,203 g TSS and 848 g VSS were fed. The following data were collected during draining of the column:

	Column section, cm			
	0–20	20–40	40–60	60–80
VSS/TSS ratio	0.417	0.436	0.474	0.548
Drainage volume, mL	350	498	500	748

Determine the VSR obtained in the drained sludge.

Solution

The VSR may be computed using Eq. (1),

$$\mathrm{VSR} = \frac{\mathrm{VS_F} - \mathrm{VS_W}}{\mathrm{VS_F} - (\mathrm{VS_F S_W})} \times 100$$

and considering that the fractions of VS in the feed and digested sludge (VS_F and VS_W) correspond to the VSS/TSS ratios in the influent and drained solids. On this basis, VS_F is equal to 0.705 (848/1,203) and VS_W to 0.482 [$(350 \times 0.417 + 498 \times 0.436 + 500 \times 0.474 + 748 \times 0.548)/(350 + 498 + 500 + 748)$] (assuming a constant density for the drainage); consequently,

$$\mathrm{VSR} = \frac{0.705 - 0.482}{0.705 - (0.705 \times 0.482)} \times 100 = 61.1\%$$

a value well above the required 38% minimum.

Example 9

The laboratory AF reactor of Example 8 over a 2-year period treated municipal wastewater at an average temperature of 24.5°C (16.0–26.8°C) and the HRT was step-wise decreased from 3.1 d to 7 h. During this period, 8,000 L of wastewater were passed through the reactor, and considering data from the individual operation intervals, it was established that 3,600 g COD were fed to the AF and 1,300 g COD left in the effluent. Also, the total biogas released over this time was 260 L with an average CH_4 content of 70%, corresponding to 160 L CH_4 (STP). Finally, sporadic measurements indicated that the SO_4^{2-} levels in the raw wastewater and the AF effluent were in the area of 70 and 22 mg/L, respectively. Prepare a COD mass balance around the reactor for the 2-year period.

Solution

1. The average COD concentrations in the raw and treated wastewater over the entire period were,

$$S_i = \frac{\text{COD in feed, g}}{\text{wastewater treated, L}} \times (10^3\,\text{mg/g}) = \frac{3{,}600\,\text{g}}{8{,}000\,\text{L}} \times 10^3 = 450\,\text{mg/L}$$

$$\text{and} \quad S_e = \frac{\text{COD in effluent, g}}{\text{wasteweater treated, L}} \times (10^3\,\text{mg/g}) = \frac{1{,}300\,\text{g}}{8{,}000\,\text{L}} \times 10^3 = 162\,\text{mg/L}$$

   and of the influent wastewater COD 36.1% [(1,300/3,600) × 100 g] remained in the treated effluent.
2. The 260 L of the biogas given for the 2-year period were computed as the sum of the volumes released for each operational interval, and the 160 L CH_4 (STP) were obtained from the corresponding CH_4 volumes for each individual interval considering the average temperature for the period. For example, during an interval when the AF operated at an HRT of 1.0 day and at an average temperature of 25.2°C, 1,100 L of wastewater were treated and 60 L of biogas were released; assuming constant temperature and pressure conditions throughout this period, the biogas released converted to STP conditions would be,

$$60\,\text{L} \times \frac{273^\circ\text{K}}{(273 + 25.2)^\circ\text{K}} = 54.93\,\text{L biogas (STP)}$$

   and the corresponding CH_4 in the biogas on the basis of 70% CH_4 content is 54.93 L × 0.70 = 38.45 L CH_4 (STP).
3. A significant amount of the CH_4 produced is dissolved in the liquid effluent and this has been reported to be about 50% of the total; consequently, 160 L CH_4 (STP) produced can be considered to have been released in the treated wastewater. It should be noted that a more accurate value could have been computed using data for each operational interval, however, these data were not given.
4. The total CH_4 (STP) produced (released in the biogas and dissolved in the liquid effluent) in the entire period is estimated to be 320 L, and considering a value of 0.35 m^3 CH_4 (STP)/kg COD removed (0.35 L/g), it corresponds to 914 g COD (320/0.35) or 25.4% [(914/3,600) × 100] of the influent COD.
5. The amount of COD converted to biomass can be estimated from the amount of biosolids accumulated in the reactor during the 2-year period. Using an average value of 39[(33 + 45)/2] kg solids/1,000 m^3 wastewater treated, the sludge totals,

$$39\,\text{kg}/1{,}000\,\text{m}^3 \times 8{,}000\,\text{L} \times (10^{-3}\,\text{m}^3/\text{L}) \times (10^3\,\text{g/kg}) = 312\,\text{g}$$

   which, assuming a value of 0.8 g COD/g TSS (123), corresponds to 250 g COD (312 g solids × 0.8 g COD/g TSS) or 6.9% [(250/3,600) × 100] of the influent COD.
6. The amount of COD used by sulfate-reducing bacteria should also be considered. Assuming that the reduction of SO_4^{2-} is described by the following reaction, where the organic substrate is again represented by $C_6H_{12}O_6$,

$$\underset{180\,\text{g}}{C_6H_{12}O_6} + \underset{3\times 96\,\text{g}}{3SO_4^{2-}} \rightarrow 6HCO_3^- + 3H_2S$$

   a value of 0.625 [(3 × 96)/180] g $C_6H_{12}O_6$/g SO_4^{2-} is computed, and considering a value of 1.067 g COD/g $C_6H_{12}O_6$ (Example 5) corresponds to 0.67 (1.067 × 0.625) g COD/g SO_4^{2-} reduced. Based on the available data, the reduction of SO_4^{2-} is 48 mg/L (70–22); consequently,

32 mg/L COD (0.67 × 48) or 256 g COD (32 mg/L × 8,000 L × 10^{-3}) or 7.1% [(256/3,600) × 100] of the influent COD is utilized by sulfate reducing bacteria.

7. The raw wastewater COD which can be accounted for by this mass balance totals 75.5%, including: (a) 25.4% converted to CH_4, (b) 6.9% converted to biomass, (c) 7.1% used by sulfate reducers, and (d) 36.1% remaining in the AF effluent. Although 24.5% of the influent COD is not reflected, it must be recognized that in conducting the balance, it was necessary to make several assumptions and use estimated average values.

Example 10

An AF reactor-submerged wetland system is designed for the treatment of the wastewater from a small hotel and the final effluent will be discharged into the soil. The hotel has a capacity of 90 beds and a staff of 10 persons, and is located in a coastal area where the average daily temperature ranges from 12 to 35°C during the year. For the wastewater, consider an average flow rate of 300 L/capita day and a BOD_5 load of 60 g/capita day; and for the AF, a design HRT of 24 h and packing material consisting of randomly placed plastic rings, 2.5 × 2.5 cm, with a void ratio of 0.85. Determine the following: the size and dimensions of the AF reactor, the estimated effluent quality, and the required surface area for the submerged wetland.

Solution

1. The design loads are,
   population served: 90 + 10 = 100 capita
   wastewater flow: 100 capita × 300L/capita day × ($10^{-3}m^3/L$) = 30.0m^3/day
   organic load: 100 capita × 60 g BOD_5/capita day × (10^{-3}kg/g) = 6.0 kg BOD_5/day and on the basis of the design HRT of 1.0 day (24 h), the required working volume of the AF is:

$$V = \theta_e Q = 1.0\,\text{day} \times 30.0\,\text{m}^3/\text{day} = 30.0\,\text{m}^3$$

2. The height of the AF should not be above 3.0–5.0 m, and the lower section should not be packed, providing space for sludge accumulation. Selecting a net height of 4.6 m, the required internal diameter is 2.9 m; about 10–15% of the AF height (50–70 cm) should be left unpacked, and additional height for the inlet and outlet structures should be considered.
3. Alternatively, if two parallel AF reactors are chosen, each unit should have a volume of 15.0 m^3, and selecting a height of 3.7 m the diameter would be 2.3 m.
4. The influent BOD_5 concentration is 200 mg/L [(6.0 kg/day/30.0 m^3/day) × 10^3], and assuming a 68% reduction in the reactor, the effluent BOD_5 concentration would be 64 mg/L [200 × (1–0.68)].
5. The organic load which is applied to the wetland by the AF effluent is,

$$64\,\text{mg/L} \times 30.0\,\text{m}^3/\text{day} \times 10^{-3} = 1.92\,\text{kg}\,BOD_5/\text{day}$$

   and assuming a moderate OLR on the submerged wetland of 60 kg BOD_5/ha day, based on a recommended area of 5–10 m^2/PE or OLR 6–12 (60/10 to 60/5) g BOD_5/m^2 day and depth of 60 cm (163), the required wetland surface area is:

$$\text{wetland area} = \frac{\text{organic load}}{\text{OLR}} = \frac{1.92\,\text{kg}\,BOD_5/\text{day}}{60\,\text{kg}\,BOD_5/\text{ha day}} \times (10^4\,\text{m}^2/\text{ha}) = 320\,\text{m}^2$$

NOMENCLATURE

α = Experimentally determined constant, h [Eqs. (6) and (7)]
a = Regression analysis constant [Eqs. (8), (10), and (11)]
ABR = Anaerobic baffled reactor
AEB = Anaerobic expanded bed (reactor)
AerF = Aerobic filter
AF = Anaerobic filter
AFB = Anaerobic fluidized bed (reactor)
AMB = Anaerobic migrating blanket (reactor)
ASB = Anaerobic sequencing batch (reactor)
BOD_5 = 5-day biochemical oxygen demand, mg/L
BOD_L = Total biochemical oxygen demand, mg/L
$CBOD_5$ = Carbonaceous 5 day biochemical oxygen demand, mg/L
COD = Chemical oxygen demand, mg/L
DO = Dissolved oxygen, mg/L
dS/dt = Substrate concentration change rate, mg/L day
dX/dt = Biomass concentration change rate, mg/L day
ε = Packing media void ratio
E = Organic matter removal efficiency, %
EGSB = Expanded granular sludge bed (reactor)
E_m = Maximum organic matter removal efficiency, %
H = Packed-bed height, feet or m
HLR = Hydraulic loading rate, m^3/m^2 day or L/m^2 day
HRT = Hydraulic retention time, h or day
θ_e = Hydraulic retention time based on column empty volume, h
θ_v = Hydraulic retention time based on packed column void volume, h
k = Temperature dependent first order constant [Eq. (4)]
K = Experimentally determined treatability factor, 1/min [Eq. (5)]
n = Packing media characteristics-dependent constant [Eq. (5)]
NH_4-N = Ammonia nitrogen, mg/L
NLR = Nitrogen loading rate, kg/m^3 day
NO_3-N = Nitrate nitrogen, mg/L
OLR = Organic loading rate, kg/m^3 day or kg/ha day
Oxid-N = Oxidized nitrogen, mg/L
PE = Population equivalent (based on 60 g BOD_5/capita day)
q = Hydraulic loading, gpm/foot^2 or m^3/m^2 day
Q = Wastewater flow rate, L/day or m^3/day
RAUS = Reversing anaerobic upflow system
RBC = Rotating biological contactor
R_s = Substrate utilization rate, mg/L day
R_x = Net biomass growth rate, mg/L day
R^2 = Regression analysis correlation coefficient

$SBOD_5$ = Soluble 5-day biochemical oxygen demand, mg/L
SCOD = Soluble chemical oxygen demand, mg/L
S_e = Effluent substrate concentration, mg/L
S_i = Influent substrate concentration, mg/L
SRT = Solids retention time, days
STP = Standard temperature and pressure (0°C, 101.3 kPa)
T = Temperature, °C or °K
TKN = Total kjeldahl nitrogen, mg/L
TSS = Total suspended solids, mg/L
TOC = Total organic carbon, mg/L
Total-N = Total nitrogen, mg/L
Total-P = Total phosphorus, mg/L
UASB = Upflow anaerobic sludge blanket (reactor)
V = Reactor volume, L or m^3
VS = Volatile solids, mg/L
VS_F = Fraction of volatile solids in feed on solids-only basis
VSR = Volatile solids reduction, %
VSS = Volatile suspended solids, mg/L
VS_W = Fraction of volatile solids in digestion residue on solids-only basis
WSP = Waste stabilization pond
x = Regression analysis constant [Eqs. (8), (10), and (11)]
X = Biomass concentration, mg/L
X_e = Effluent biomass concentration, mg/L
y = Regression analysis constant [Eqs. (8), (10), and (11)]
z = Regression analysis constant [Eq. (11)]

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Biological Phosphorus Removal Processes

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CONTENTS

Abstract Enhanced biological phosphorus removal (EBPR) processes developed for wastewater treatment are mainly based on the enrichment of activated sludge with phosphorus-accumulating organisms under alternative anaerobic–aerobic conditions. According to the literature information of the EBPR processes, this chapter attempts to review the biochemical models, microbiology of the EBPR processes, and the main operating parameters that may influence the performance of the EBPR processes.

1. INTRODUCTION

It is well known that excess nutrients such as nitrogen (N) and phosphorus (P) in water bodies result in serious eutrophication, which is currently a global problem. Eutrophication may affect the general aspects of water bodies, decreasing its aesthetic appeal and making necessary treatment for drinking water more difficult and expensive. Aquatic life is also adversely affected by this excess vegetable matter due to its depletion of oxygen, slowing down the currents and sometimes producing toxic matters. In response to the harmful effects of nutrients, more and more stringent regulations for controlling nutrient discharge in receiving waters have been implemented in many countries, e.g., typical effluent standards that require nitrogen and phosphorus concentrations in effluent must be less than 3 mg total nitrogen/l and 1 mg P/l, respectively. However, it should be noted that the phosphorus levels vary by region and water body. In some regions, more stringent phosphorus discharge limit was set. The

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_15

Spokane River in Washington, U.S., is a prime example. The regulators there set a phosphorus discharge limit for treatment plants of 50 μg/L (1).

N and P are two necessary elements for the growth of algae, while P input is considered more critical since many of the cyanobacteria are diazotrophic, capable of satisfying their N requirements from the fixation of atmospheric nitrogen (2, 3). Chemical precipitation with alum, ferric chloride, and lime has been widely used as a proven technology for phosphorus removal (4). However, chemical treatment for P removal has the disadvantages of high chemical costs, chemical handling and storage requirements, increased chemical slurry production, and subsequent slurry handling and disposal costs. Biological processes are cost-effective and environmentally sound alternatives to the chemical treatment of nutrient-containing wastewater (5). Biological phosphorus removal (BPR) from wastewaters is based on the enrichment of activated sludge with phosphate accumulating organisms, namely PAOs (6, 7). These PAOs are able to accumulate P in bacterial cells in the form of polyphosphate (polyP) granules in excess levels normally required to satisfy the metabolic demands of growth, such a storage process is commonly referred to as enhanced biological phosphorus removal or EBPR in short (8, 9). In past decades, many treatment plants had been designed and built to deliberately reduce not only organic carbon and nitrogen but also phosphorus by EBPR process. The advantages of EBPR over the chemical precipitation include reduced sludge production, obviation of effluent salinity problems experienced with the chemical process, easier management, and significantly higher reuse potential of produced sludge.

2. BIOCHEMICAL MODELS FOR ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL

In 1955, Greenburg et al. (10) proposed that activated sludge could take up phosphorus at a level beyond its normal microbial growth requirements. Subsequently, Srinath et al. (11) reported in 1959 that soluble phosphorus in mixed liquor decreased rapidly to below 1 mg/L under varying conditions of aeration. In 1965, Levin and Shapiro (12) further proposed the concept of excess biological phosphorus removal, i.e., "luxury uptake" with alternating anaerobic/aerobic sequence of biological treatment systems, which is the accepted mechanism of P removal. The EBPR process operates on the basis of alternating anaerobic and aerobic conditions with substrates being supplied in the anaerobic stage, while a specific group of bacteria capable of accumulating extra phosphates beyond the anabolic needs, namely P-accumulating organisms (PAOs), can be selected ecologically by this way. Under anaerobic conditions, PAOs tend to release phosphorus and uptake simple organic carbon. In the subsequent aerobic phase, PAOs prefer to generate energy by metabolizing the previously stored organic carbon. As a result, the cell polyphosphate pools are replenished. In such an anaerobic–aerobic alternative process, both phosphorus and organic carbon present in the wastewater stream are considerably reduced.

The EBPR is mainly based on a series of biochemical reactions involved in anaerobic and aerobic phases. So far, two main biochemical models for EBPR have been commonly accepted, i.e., the Comeau/Wentzel model and the Mino model for convenience. The common assumption of these two models is that alternative anaerobic/aerobic phases are essential for the growth of bacteria that can accumulate phosphate in the form of intracellular polyP

granules and perform EBPR; short chain fatty acids are taken up and stored in the form of polyhydroxyalkanoates (PHA) as typical internal carbon source in the anaerobic phase, and production of polyP and glycogen at the expense of the stored PHA during the subsequent aerobic phase without extracellular and easily biodegradable carbon left over.

2.1. The Comeau/Wentzel Model

The Comeau/Wentzel model was initially developed in 1985 (9, 13). The salient points of this model are that (1) the model accepts the genus *Acinetobacter* as typical of the PAO group, and the carbon and phosphorus biochemical pathways specific to *Acinetobacter* spp. are recognized in this model; (2) the ATP/ADP and the NADH/NAD ratios are identified as the key parameters that may regulate these pathways.

2.1.1. Under Anaerobic Conditions

The high extracellular acetate concentration allows passive diffusion of acetate into the cell. In the Comeau/Wentzel model, the intracellular acetate is activated to acetyl-CoA by coupled ATP hydrolysis, while the ATP hydrolysis releases cations (e.g., K^+ or Mg^{2+}) and the anion $H_2PO_4^-$. The cations are released to the bulk solution via a proton mediated antiport protein carrier, and the phosphorus is released via a hydroxyl mediated antiport protein carrier. Two acetyl-CoA molecules condense to form acetoacetyl-CoA, which is further reduced by $NAD(P)H_2$ to form hydroxybutyryl-CoA, which then is polymerized to form poly-β-hydroxybutyrate (PHB). Conversion of intracellular acetate to PHB maintains a favorable concentration gradient for further diffusion of acetate into the cell. Organisms with stored PHB are able to use these as carbon and energy sources to grow and to assimilate phosphate to synthesize polyP under aerobic conditions.

To supply the reducing power ($NAD(P)H_2$) needed to convert acetoacetyl-CoA to hydroxybutyryl-CoA, part of acetate is metabolized via the tricarboxylic acid (TCA) cycle. As a result, partial acetate is oxidized to carbon dioxide by TCA cycle for providing reducing power; meanwhile partial acetate is used for formation of PHB, which was proposed by Matsuo (14), Comeau et al. (13), and Wentzel et al. (9). The ATP required in the process is regenerated from ADP by transfer of an energy-rich phosphoryl group from polyphosphate (polyP) to the ADP. Originally, this transfer was proposed to be direct, catalyzed by the enzyme ATP, e.g., polyphosphate phosphotransferase according to the following reaction:

$$(\text{PolyP})_n + \text{ADP} \leftrightarrow (\text{PolyP})_{n-1} + \text{ATP} \tag{1}$$

However, evidence shows that there is an intermediate step in the ATP generation mediated by the combined action of the enzymes and AMP, i.e., polyphosphate phosphotransferase and adenylate kinase according to the following reactions (15):

$$(\text{PolyP})_n + \text{AMP} \leftrightarrow (\text{PolyP})_{n-1} + \text{ATP} \tag{2}$$

$$\text{ADP} + \text{ADP} \leftrightarrow \text{ATP} + \text{AMP} \tag{3}$$

Whichever pathway is operative, the net result is a decrease in the stored polyP concentration and a generation of ATP. Conversion of acetate (Ac) to PHB $(C_4H_6O_2)_n$ can be summarized

as follows:

$$2n\text{Ac} + 2n\text{ATP} + n\text{NADH}_2 + \text{CoASH} \rightarrow (\text{C}_4\text{H}_6\text{O}_2)_n\text{CoA} + n\text{NAD} + 2n\text{ADP} + 2n\text{Pi} \quad (4)$$

Metabolism of acetate via the TCA cycle for production of reducing power can be written as:

$$n\text{Ac} + n\text{ATP} + 4n\text{NAD} \rightarrow 4n\text{NADH}_2 + n\text{ADP} + n\text{Pi} + 2n\text{CO}_2 \quad (5)$$

The net result of these processes can be expressed as:

$$9n\text{Ac} + 9n\text{ATP} + \text{CoASH} \rightarrow (\text{C}_4\text{H}_6\text{O}_2)_{4n}\text{CoA} + 9n\text{ADP} + 9n\text{Pi} + 2n\text{CO}_2 \quad (6)$$

It appears from Eq. (6) that for every acetate utilized, one ATP is required, and one ADP and one Pi are generated. This gives a theoretical molar ratio of acetate uptake to P release of 1:1.

2.1.2. Under Aerobic Conditions

In the Comeau/Wentzel model, PHB is broken down and used for either anabolic or catabolic metabolism. In anabolism, carbon skeletons generated from PHB are incorporated into cell mass. In catabolism, the PHB is broken down to acetyl-CoA, which enters the TCA and associated glyoxylate cycles. Reducing equivalents ($NADH_2$) generated in these cycles are subsequently oxidized via the electron transfer pathway, and simultaneous oxidative phosphorylation generates ATP. The ATP generated is further used for cell energy requirements (e.g., biosynthesis) and synthesis of polyP. Phosphate uptake for polyP synthesis occurs via the hydroxyl mediated antiport, and cation uptake via the proton mediated antiport. However, it should be pointed out that the model does not explain the increase in intracellular carbohydrate (16) and increase in extracellular carbohydrate (17).

2.2. The Mino Model

The Mino model was developed to explain observations on a laboratory-scale anaerobic/aerobic system receiving an artificial substrate of acetate, propionate, glucose, and peptone and observations on batch tests conducted using sludge from the laboratory-scale system. In the laboratory-scale anaerobic/aerobic system, Mino et al. (16) measured the changes in soluble P, polyP, PHB, acetate, and intracellular carbohydrate. They observed a decrease of intracellular carbohydrates in the anaerobic phase and an increase in the subsequent aerobic phase. Evaluation of these results is hampered by uncertainty as to whether the analytical methodology used to determine carbohydrate adequately differentiated between extracellular and intracellular carbohydrates, while in some methods, extracellular carbohydrates are not separated from intracellular carbohydrates. For this reason, in describing the Mino model, no differentiation is made between extracellular and intracellular carbohydrates. Another point that requires clarification is whether the changes in PHB and carbohydrates are mediated by the same organism type, or by different organism types that may present in the mixed culture systems. To explain their results, Mino et al. (16) assumed that a single organism type would cause the observed changes in both carbohydrates and PHB. Obviously, this point requires further experimental clarification. Below is a brief description of the Mino model.

2.2.1. Under Anaerobic Conditions

Acetate is first taken up by the organism, and intracellular acetate is activated to acetyl-CoA by coupled hydrolysis of ATP (P released to the bulk solution). The ATP required in Eq. (2) is supplied by the accumulated polyP. PHB is synthesized from acetyl-CoA (AcCoA) according to the following reaction:

$$2n\text{AcCoA} + n\text{NADH}_2 \rightarrow (\text{C}_4\text{H}_6\text{O}_2)n + n\text{NAD} + 2n\text{CoASH} \tag{7}$$

Up to this stage the Mino model is in agreement with the Comeau/Wentzel model. The main difference between the Comeau/Wentzel and the Mino model is the production of reducing equivalents required for the conversion of acetyl-CoA to PHB. The Mino model suggests that reducing equivalents is produced by the conversion of glycogen to acetyl-CoA via pyruvate, and not by oxidation for acetyl-CoA via TCA cycle. Under anaerobic conditions, intracellularly stored glycogen $(\text{C}_6\text{H}_{10}\text{O}_5)_n$ is converted to pyruvic acid via the Embden-Meyerhof-Panas (EMP) pathway with the production of reducing equivalents (NADH_2). The pyruvic acid is further converted to acetyl-CoA with the production of carbon dioxide. The overall reaction for the breakdown of carbohydrate to acetyl-CoA can be expressed as follows:

$$\begin{aligned}(\text{C}_6\text{H}_{10}\text{O}_5)_n + 3\text{ADP} + 3n\text{Pi} + 4n\text{NAD} + 2n\text{CoASH} \\ \rightarrow 2n\text{AcCoA} + 4n\text{NADH} + 3n\text{ATP} + 2n\text{CO}_2\end{aligned} \tag{8}$$

Thus, the reducing equivalents (NADH_2) required in the reduction of acetate to PHB under the anaerobic conditions are supplied by the consumption of carbohydrate via the EMP pathway. By combining the reaction for the consumption of glycogen with that of the activation and conversion of acetate to PHB, the following net reaction for changes in intracellular carbon is obtained:

$$(\text{C}_6\text{H}_{10}\text{O}_5)_n + 6n\text{Ac} + 3n\text{ATP} \rightarrow (\text{C}_4\text{H}_6\text{O}_2)_n + 3n\text{ADP} + 3n\text{Pi} + 2n\text{CO}_2 \tag{9}$$

This reaction is in agreement with the observation by Bordacs and Chiesa (18), i.e., almost no $^{14}\text{CO}_2$ is produced from [^{14}C]-acetate during the anaerobic period, which indicates that the acetate taken up anaerobically is not oxidized to CO_2, and thus not metabolized through the TCA cycle. Other experimental evidence shows that glycogen is involved in the anaerobic metabolisms of EBPR sludges (19, 20). With the increase of evidence favoring a key role of glycogen in EBPR, the Mino model is now widely accepted. However, the possibility of partial functioning of the TCA cycle cannot be totally excluded. The experimental results by using ^{13}C labeled acetate as substrate showed that a small fraction of acetate was metabolized through the TCA cycle under anaerobic conditions supplying 30% of the reducing power for PHA formation (21). Thus, it seems that the oxidation of acetyl-CoA via TCA cycle can meet the demands of reducing power of PHA synthesis, and the oxidation of glycogen to acetyl-CoA will provide the remainder (21, 22).

For the bioenergetics of anaerobic substrate assimilation and PHA synthesis by PAOs, glycogen catabolism is thought to provide ATP for PHA production besides ATP from polyP degradation, and the amount of energy produced by glycogen depends on the pathway for glycogen catabolism (20, 22, 23). In the study by Mino et al. (16), nitrate concentrations in

the laboratory-scale systems and batch tests were not reported. In this case, if nitrate was discharged to the anaerobic phase or was present at the beginning of the batch tests, an uptake of acetate for denitrification would take place without concomitant P release. This would partially explain the high ratio of acetate uptake to P release, i.e., 3.4:1 as reported by Mino et al. (16).

2.2.2. Under Aerobic Conditions

The anaerobically stored PHA is further utilized as the energy and carbon source to recover the glycogen and polyP levels. As a result, the stored PHA decreases and soluble orthophosphate is taken up by the sludge with the increase in intracellular glycogen and polyP.

2.3. The Adapted Mino Model

2.3.1. Under Anaerobic Conditions

Compared to the Mino model, the reducing equivalents in the adapted Mino model that convert acetate to PHB are supplied by consuming carbohydrates through the Entner–Doudoroff (ED) pathway. In fact, this has a significant influence on the stoichiometry of P release and acetate uptake because consumption of carbohydrates through the ED pathway produces markedly less energy than that produced through the EMP pathway, thus more energy production via polyP breakdown will be necessary to convert acetate to acetyl-CoA. Consumption of carbohydrates via the ED pathway can be written as follows:

$$(C_6H_{10}O_5)_n + 3n\text{NAD} + n\text{NADP} + 2n\text{ADP} + 2n\text{CoASH} + 2n\text{Pi} \rightarrow 2n\text{AcCoA} + 3n\text{NADH} + n\text{NADPH}_2 + 2n\text{ATP} + 2n\text{CO}_2 \quad (10)$$

Equation (10) shows that only 2 ATPs are produced, while Eq. (8) indicates that in the EMP pathway, 3 ATPs are generated per carbohydrate consumed. Acetyl-CoA produced by the consumption of carbohydrates is further converted to PHB according to Eq. (7). Combining Eqs. (10) and (7) gives the overall equation for the consumption of carbohydrates:

$$(C_6H_{10}O_5)_n + 2n\text{NAD} + n\text{NADP} + 2n\text{ADP} + 2n\text{Pi} \rightarrow (C_4H_6O_2)_n + 2n\text{NADH}_2 + n\text{NADPH}_2 + 2n\text{ATP} + 2n\text{CO}_2 \quad (11)$$

Assume that Eq. (4) is acceptable for the production of PHB from acetate; the overall process can be summarized as:

$$(C_6H_{10}O_5)_n + 6n\text{Ac} + 4n\text{ATP} \rightarrow (C_4H_6O_2)_n + 4n\text{ADP} + 4n\text{Pi} + 2n\text{CO}_2 \quad (12)$$

Note that NAD and NADP are used interchangeably, i.e., either form can be used in PHB synthesis. Comparison of Eq. (12) with Eq. (9) for the EMP pathway shows that in the ED pathway 4Ps are released for every 6Ac taken up, i.e., molar ratio of Ac taken up to P released is about 1.5:1; however, in the EMP pathway 6 moles of Ac are taken up for every 3 moles of P released, i.e., molar ratio of Ac taken up to P released is 2:1.

2.3.2. Under Aerobic Conditions

The adapted model follows the Mino model for PHB utilization, P uptake and polyP formation, cell synthesis, and carbohydrate regeneration from the PHB. The regeneration of carbohydrates from PHB assumes that the organisms possess the required biochemical pathways as discussed above. Necessarily, the regeneration must involve the formation of glucose from acetyl-CoA. In organisms where this conversion occurs, acetate undergoes an anabolic sequence known as the glyoxylate cycle. A mechanism for carbohydrate regeneration has been proposed as follows: PHB is broken down to acetyl-CoA via normally accepted biochemical pathways (9, 24). Acetyl-CoA is further converted to phosphoenolpyruvate via malate and oxaloacetate. In fact, phosphoenolpyruvate is an intermediate in the ED pathway and can be converted to carbohydrate by a reversal of the ED pathway. In both the Mino and the adapted Mino models, the formation of carbohydrates under aerobic conditions is essential for PHB formation under the subsequent anaerobic condition. Conceptually, the formation of a carbon storage sink under the carbon limiting conditions may present difficulties, and merits further study.

There is apparent consensus regarding many key features of the organism behaviors mediating EBPR and the biochemical pathways involved. However, little experimental biochemical data are yet available to validate any of these empirical models, and even NMR data are unable to fully explain the behaviors of the different communities since the structure/function relationships of the populations involved are completely unknown (25). In addition, many aspects of the biochemical models are still not fully understood, e.g., (1) the source of reducing equivalents (NADH required in the reduction step for converting acetate to PHB) remains to be unclearly defined; (2) experiments carried out to verify biochemical models in terms of the key features (PHB/P and acetate/P ratios) could not offer quantitative consistency with predictions, etc (26). Therefore, in order to successfully design and manage the EBPR process, a sound understanding of the energetic metabolism and biochemical pathways of this process is essential.

3. MICROBIOLOGY OF THE EBPR PROCESSES

The biochemical models developed for EBPR are mainly based on the assumption that there is a typical group of microorganisms dominating the EBPR process. So far, phosphorus accumulating organisms (PAOs) and their competitors, the non-polyphosphate glycogen accumulating organisms (GAOs) have been identified. Evidence shows that the EBPR communities are very diverse phylogenetically, as are the non-EBPR activated sludge communities.

3.1. Phosphorus Accumulating Organisms

Acinetobacter spp. was the first group of phosphorus accumulating organisms (PAOs) isolated and identified for EBPR (8). The PAOs are clusters of coccobacillus-shaped microorganisms containing polyphosphate, and are dominant in the EBPR process (27–32). A number of the organisms associated with phosphorus removal have been isolated from the EBPR processes, including *Lampropedia* (33), *Microlunatus phosphovorus* (34), *Micropruina glycogenica* (35) and *Tetrasphaera* spp. (36). However, none of these isolates exhibits all the

characteristics that EBPR sludge should possess, e.g., some isolates lack the anaerobic acetate metabolisms (acetate uptake and its conversion to PHA for storage coupled with hydrolysis of stored polyP and consequent release of orthophosphate under anaerobic conditions) (37).

The disadvantages associated with analyzing natural microbial communities using culture-dependent methods have been discussed extensively in the literature. Thus, culture-independent approaches, including chemotaxonomic methods such as quinone profiling (38) and molecular methods, e.g., the fluorescence in situ hybridization (FISH) (39), the clone library approach, denaturing gradient gel electrophoresis (DGGE) (40), and terminal restriction fragment length polymorphisms (T-RFLP) (41), have been used for studying the microbiology of EBPR.

Chemotaxonomic markers have been used for the analysis of microbial community composition, in which the presence of certain cell components may indicate the presence and relative abundances of particular bacterial populations (38). For example, diaminopropane was used as a marker for members of the genus *Acinetobacter* present in full-scale plants with high P removal (42). The type of respiratory quinone in biological samples can be quantitatively determined, and the quinone patterns should explicitly reflect the chemotaxonomic composition of the examined samples, e.g., ubiquinone Q-8, diagnostic of the β-*Proteobacteria* was the most abundant quinone in both EBPR and non-EBPR biomass samples, and not Q-9 associated with the γ-*Proteobacteria*, including *Acinetobacter* spp (43). Menaquinone profiles may also change during the EBPR, and had been suggested as possible useful chemical indicators for monitoring P removal (44).

A fluorescent antibody staining technique developed for the identification of *Acinetobacter* revealed that the number of *Acinetobacter* in the EBPR processes studied was less than 10% of total bacteria and could not account for the EBPR observed (45). So far, more and more evidence shows that *Acinetobacter* spp. would not be real PAOs (46, 47). The FISH with the group-specific oligonucleotide probes targeting rRNA further revealed that there was an underestimation of bacteria belonging to the β-subclass of *Proteobacteria* and an overestimation of bacteria in the γ-subclass of *Proteobacteria* with culture-dependent method (47). Therefore, it appears that the culture-dependent enumerations of the γ-subclass bacteria of the genus *Acinetobacter* in the EBPR plants would result in significant overestimation. FISH using a probe specific for *Acinetobacter* also showed that the number of *Acinetobacter* was very small, i.e., the role of *Acinetobacter* in the biological P removal process might be insignificant (48). In addition, the number of DNA sequences found for the *Actinobacteria* were much lower than the number of these bacteria detected by the other methods (e.g., FISH); these may suggest that their DNA is not as readily obtained as that from other bacteria (49, 50).

Bacterial community structures of P-removing and non-P-removing sludges have been studied and further compared by 16S rDNA clone library analysis (49). It was found that in both sludges, the predominant bacterial group represented in the clones was the β-*Proteobacteria* at a level of 28%, while the *Rhodocyclus* group within the β-*Proteobacteria* was represented more in the reactor with greater P removal (49). However, it should be realized that determination of the microbial community structure using this method may not be representative because of a relatively low number of clones examined, which would not represent the full species abundance in the sludges.

So far, *β-Proteobacteria* has been reported to be the most abundant bacteria in different activated sludge processes when FISH was used as the method of analysis (47, 51, 52). Using FISH technique, Bond et al. (53) found that two subgroups of the *β-Proteobacteria* comprised 55% of all bacteria in an efficiently operating laboratory-scale EBPR reactor. Olsen et al. (54) described the lab-scale PAOs-enriched, high-performing EBPR cultures by analyzing the full-cycle rRNA, while Hesselmann et al. (55) firstly reported the definitive phylogenetic placement of the *β-Proteobacteria*-2 subgroup PAO as a close relative of *Rhodocyclus* spp. and named the organism "*Candidatus Accumulibacter phosphates*" or *Accumulibacter* for short. Using FISH and post-FISH chemical staining techniques, Crocetti et al. (56) further demonstrated that the *Accumulibacter* cells were able to cycle polyP according to EBPR. This in turn provides support to the finding by Hesselmann et al. (55).

Single strand conformational polymorphism (SSCP) and 16S rDNA clone library analysis have been used to study the microbial ecology of efficient and deteriorated EBPR (57, 58). SSCP showed an abundance of *Accumulibacter* in the microbial ecosystem as well as the prominent appearance of other bacteria, notably some *γ-Proteobacteria* and microorganisms closely related to *Haliscomenobacter* in the *Bacteroidetes phylum* (58). Increasing evidence shows that *Accumulibacter* is a PAO in both laboratory- and full-scale EBPR processes (59, 60). Thus, *Accumulibacter* has been presumed to be the first of many confirmed PAOs. Additional PAO candidates may include *Actinobacteria* (47), *α-Proteobacteria* (61), and *γ-Proteobacteria* (62).

3.2. Non-polyphosphate Glycogen Accumulating Organisms

Tetrad-arranged cocci had been found in glucose-fed EBPR process with a poor phosphorus removal, and they were called glycogen-accumulating organisms (GAOs), but these GAOs could grow well when acetate was the carbon source in anaerobic–aerobic reactors (63, 64). GAOs are often described as large oval cells ($2-3\,\mu m$ in diameter) that form compact aggregates, and apparently attach together with extracellular slime (64). So far it is known that the GAOs may out-compete the PAOs in anaerobic–aerobic EBPR systems under some conditions. It has been hypothesized that the GAOs could assimilate glucose anaerobically better than the PAOs, and eventually used it for the production of PHA, which could be further metabolized under subsequent aerobic conditions for glycogen formation. These seem to imply that the GAOs are selectively favored and become predominant populations under certain conditions. Since the GAOs are unable to synthesize polyP under aerobic condition, this leads to the failure of the EBPR in the GAOs-dominant process (23). As noted by Mino et al. (23), a deeper insight into the biodiversity of PAOs and GAOs is strongly needed for optimizing the EBPR under different operating conditions, which may in turn select and enrich different PAOs and GAOs.

4. BIOLOGICAL PHOSPHORUS REMOVAL PROCESSES

To date, all biological phosphorus removal processes developed are based on alternative aerobic and anaerobic cycle operation.

4.1. Process Description

4.1.1. PhoStrip Process

The PhoStrip process was first reported in 1965 (12, 65), which is a combination of both biological and chemical phosphorus removal processes. The PhoStrip process has been referred to as a side-stream process since a portion of the return activated sludge flow is diverted for phosphorus stripping and subsequent precipitation with lime. Because a percentage of the return sludge that is subjected to anaerobic conditions for different detention times in the stripper tank is adjustable, a wide range of phosphorus removal can be achieved. Control of the side-stream permits phosphorus removal to be divided between supernatant from the stripper and waste activated sludge, i.e., phosphorus removal is carried out by chemical precipitation or in the waste biological sludge. In this process, an effluent concentration less than 1 mg/L total phosphorus can be achieved with less dependence on the BOD strength of the influent wastewater. A large percentage of the phosphorus removal is tied up as lime sludge, which causes less concern than handling a phosphorus-rich waste biological sludge. Compared with the direct chemical addition to an activated sludge aeration basin for phosphorus precipitation, the PhoStrip process may require a lower chemical dosage, and is cost-effective because the lime dosage is a function of the alkalinity and not the amount of phosphorus to be removed, as is the case for alum and iron salts. This potential advantage is dependent on wastewater alkalinity, phosphorus concentration, and relative chemical costs.

4.1.2. The Bardenpho Process

The Bardenpho process is an activated sludge process specially designed to accomplish biological phosphorus and nitrogen removal. The original process developed by Barnard (66) is a single-sludge, four-stage (anoxic–aeration–anoxic–aeration) system intended for nitrogen removal through nitrification and denitrification. For the purpose of P removal, the Bardenpho process has been modified by adding an anaerobic stage ahead of the original four-stage Bardenpho nitrogen removal system. Such a modification allows for the creation of an anaerobic–aerobic contacting condition necessary for biological phosphorus uptake. In the modified Bardenpho process (67), the recycled activated sludge separated from the clarifier is mixed with the influent wastewater prior to the anaerobic contactor. Such a mixing strategy can initiate luxury phosphorus uptake by releasing phosphates first. The mixed liquor from the anaerobic contactor then flows into the first anoxic denitrification stage in which it is further mixed with the internally recycled mixed liquor from the aerobic nitrification zone. In the first anoxic stage, nitrate is denitrified to nitrogen gas using the influent BOD as carbon source. About 70% of the nitrate–nitrogen produced in the system can be removed in the first anoxic stage. Then, the mixed liquor flows into the aerobic nitrification zone in which luxury phosphorus uptake, ammonium oxidation, and additional BOD removal occur. Following the aerobic nitrification stage, a second anoxic stage can further provide the possibility to enhance additional denitrification, which is designed to remove additional nitrate in order to minimize nitrate fed back to the anaerobic contactor. The final aerobic stage provides a short time period of mixed liquor aeration prior to clarification to minimize anaerobic conditions and phosphorus release in the second clarifier.

4.1.3. Anaerobic/Oxic Process

The Anaerobic/Oxic process, namely A/O process, was initially developed for the removal of phosphorus and/or nitrogen from wastewater (68, 69). The A/O is a single-sludge suspended growth system that combines anaerobic, anoxic, and aerobic zones in sequence. The anaerobic and the aerobic stages are divided into a number of equal size complete-mixed compartments. Partition of several compartments makes the hydraulic flow approaching plug-flow and prevents backmixing. For the removal of phosphorus, three compartments are commonly used for the anaerobic stage and three or more for the aerobic stage. Recycled sludge from the clarifier is mixed with influent wastewater in the anaerobic section so that there is sorption of BOD by biomass, with accompanying phosphorus release necessary for biological phosphorus removal. The anaerobic section is covered and equipped with mechanical mixers for mixing but not aeration. The oxic stage required for the oxidation of BOD and uptake of the phosphorus released in the anaerobic stage, is aerated with air or pure oxygen. Phosphorus is removed by the discharge of the waste sludge from the system, which may contain 4–6% P by dry weight. The achievable phosphorus concentration in effluent is dependent on the rate of sludge wasting, which is controlled by the operating solids residence time (SRT). Relatively short SRTs and high organic loading rates are the key features of the A/O process (67). Compared to the Bardenpho process, this results in greater sludge production and more phosphorus removal per unit of BOD removal in the system. However, the choice of further sludge stabilization methods (anaerobic or aerobic digestion) must account for the amount of phosphorus released during stabilization as well as the effect of recycle streams from the stabilization units on facility performance.

When necessary, nitrification can be accomplished in the oxic section operated at a properly selected SRT and organic loading suitable for the growth of nitrifying bacteria. When denitrification is required, the anoxic section is included between the anaerobic and oxic sections, namely A^2/O process. The anoxic section is deficient in dissolved oxygen, but chemically bound oxygen in the form of nitrate or nitrite is introduced by recycling nitrified mixed liquor from the oxic section back to the anoxic section. Internal recycle flow of 100–300% has been used, and consequently 40–70% of nitrate–nitrogen removal can be achieved by this way.

4.1.4. The UCT Process

The UCT process for biological phosphorus removal is the modification of the Bardenpho process. In the UCT process, the recycled activated sludge is directed to the anoxic stage instead of the anaerobic stage as in the Bardenpho process (67, 70). In fact, the UCT process is based on the finding that initial phosphorus removal efficiency could be negatively affected by nitrate–nitrogen entering the anaerobic stage. Nitrate may serve as an electron acceptor during the biological oxidation of BOD entering the anaerobic stage. This in turn results in competition for the soluble, readily biodegradable BOD that would normally be converted to fermentation products for subsequent use by the biological phosphorus-removing bacteria in the anaerobic zone in the absence of nitrate–nitrogen. In the Bardenpho or A/O process, the ratio of the nitrate–nitrogen in the sludge recycled to the anaerobic stage and the soluble BOD available in the influent to that zone determines if sufficient BOD will remain after denitrification to produce a necessary concentration of the fermentation products for biological

phosphorus removal. For wastewaters with a relatively high ratio of TKN to BOD, the effect of nitrate–nitrogen in the recycled sludge on anaerobic zone fermentation may be significant for these two processes.

4.1.5. The Modified Activated Sludge Process

In practice, the existing activated sludge systems can be changed operationally to create an anaerobic fermentation zone ahead of the aeration zone for biological phosphorus removal. This modified activated sludge process typically involves turning off air flow or aerators in the front of the activated sludge basin.

4.1.6. Combined Process for Biological Phosphorus Removal

A stripper is added in a combined biological system for phosphorous removal. The stripper consists of a complete mix tank for anaerobic contact of a side-stream of return activated sludge followed by a clarifier for separation of the stripped sludge. This combination allows for more than 97% total phosphorus removal compared to 40–50% removal for the anaerobic–aerobic sequence without the stripper (67). This process is operated with a relatively low organic loading, and nitrification can also be realized. High nitrate production may have effect on the phosphorus removal efficiency in the anaerobic–aerobic system without the stripper.

4.1.7. SBR Process

Sequencing batch reactor (SBR) has been widely applied for biological phosphorus removal. The SBR is a fill-and-draw activated sludge system. The operation steps of a SBR basically consist of a fill period in which flow is diverted to one of the SBR tanks while the other tank(s) operates in the reaction, settle, effluent withdrawal, or idle operation sequences (70). After the fill period, the reactor contents are mixed, but not aerated, to provide the anaerobic period for phosphorus release and uptake of soluble fermentation products. The next step is the aeration period followed by a settling period without aeration and mixing. The effluent is then withdrawn at the end of the settling period. After the effluent withdrawal, a variable length of idle time may occur, which depends on the influent flow rate (70). Combined with the step-feed strategy, a high degree removal of total phosphorus (>98%), total nitrogen (>97%), and total COD (>95%) was consistently and reliably achieved after a 3-month start-up period in SBR (71).

4.1.8. Granular Sludge Process

During the past few years, aerobic granules for organic carbon removal and for simultaneous carbon and nitrogen removal have been developed in aerobic sequencing batch reactors (SBR) (72–74). Compared to conventional activated sludge flocs, aerobic granular sludge has a regular, dense and strong physical structure, good settling ability, high biomass retention, and the ability to withstand shock-loading rate. So far, almost all biological phosphorus removal is carried out by activated sludge. With the development of granular sludge technology, Lin et al. (75) first produced phosphorus-accumulating microbial granules in alternative anaerobic and aerobic sequencing batch reactors at different substrate P/COD ratios for biological phosphorus removal. The structure of the granules became more compact and dense as the substrate P/COD ratio increased and the P uptake by granules fell within the range of 1.9–9.3%

by weight, which is comparable with the uptake obtained in conventional enhanced biological phosphorus removal processes.

A recent study showed that the chemical precipitation-related P in granules only accounted for less than 10% of the total P accumulation, i.e., biological storage was mainly responsible for the observed P accumulation in microbial granules (76). According to the elemental analyses (76), the empirical formulas of P-accumulating granules developed at different substrate P/COD ratios were generated, and significant difference in the elemental compositions between P-accumulating and non P-accumulating granules was observed, indicating a shift in microbial association. The substantial accumulation of calcium and magnesium ions was also found in the P-accumulating granules, and was closely related to the polyphosphate accumulated in granules. The granular sludge technology for biological phosphorus removal is expected to overcome problems encountered in the suspended growth P removal process, such as sludge bulking, large treatment plant space, secondary P release in a clarifier, higher production of waste sludge.

The application of this granular technology to an abattoir wastewater in a sequencing batch reactor showed that P removal was over 98% when influent total P was 217 mg/L (77). Meanwhile, the results showed that single granular SBR could realize the simultaneous COD, N, and P removal in abattoir wastewater (77). In addition, P-removal efficiency by steady-state aerobic granules at temperatures of 20, 15, and 8°C were over 95% (78). All these results showed that aerobic granular sludge technology offers a possibility to design compact wastewater treatment plants based on simultaneous COD, N and P removal in one sequencing batch reactor (78–80). In addition, it has been proven that selection for slow-growing organisms PAO improved the granule stability, particularly at low oxygen concentrations (80). Therefore, the simultaneous removal of COD, N, and P is favorable to the long-term operation stability of aerobic granular sludge.

4.2. Process Applications and Limitations

The PhoStrip, Bardenpho, and A/O processes are capable of removing total phosphorus from 4 to 12 mg/L normally found in municipal wastewaters to 1–2 mg/L (67). Industrial practice shows that the PhoStrip process can consistently produce effluent with a soluble phosphorus concentration less than 1.0 mg/L as PO_4-P. However, due to variability in flow and wastewater characteristics as well as other operation reasons, excursions above 1 mg total phosphorous/l in treated effluent are often encountered (70). The PhoStrip process is particularly applicable to cases where only phosphorus removal is required, i.e., with no requirement on nitrification. Basically, the PhoStrip process is not applicable when hydraulic retention time in the aeration unit exceeds 10 h, or when significant nitrification occurs in the system. If nitrification is necessary, PhoStrip can be used in conjunction with the first stage of a two-stage activated sludge process, or a single-stage activated sludge system, while modifications, e.g., increased hydraulic retention time in the anaerobic unit, would be required in order to compensate for the effect of nitrate.

The A/O process is often applied for phosphorus removal with or without nitrification. Concentration of total phosphorus in the treated effluent from the A/O process is usually in the range of 1.5–3.0 mg/L. Since significant amounts of effluent phosphorus are associated

with the suspended solids, filtration of the effluent from the A/O process would be necessary in order to produce water with total phosphorus less than 1 mg/L. It is also possible to design the A/O process for denitrification. However, the capability of the A/O process for the phosphorus removal and complete nitrification–denitrification remains undemonstrated. It should be noted that phosphorus-rich waste sludge is generated from the A/O process. Aerobic or anaerobic digestion has been commonly employed to stabilize this sludge through removing degradable portions of volatile solids. In particular, when anaerobic digestion is chosen, the digested liquor contains high concentration of phosphorus and should be treated by chemical precipitation of phosphorus before returning to the front end of the A/O system (67).

The Bardenpho process is an effective process designed for the removal of both the phosphorus and total nitrogen, while it is rarely used when only phosphorus removal is desired. It seems hard for the Bardenpho process to produce an effluent with total phosphorus less than 2 mg/L or soluble phosphate less than 1 mg P/l without supplemental mineral addition. If the total phosphorus in the effluent below 1 mg/L is required, filters are needed for further polishing the effluent from the Bardenpho process. Since SRT in the Bardenpho process is typically maintained at about 20 days and can be as high as 40 days, the excess sludge generated from the system is well stabilized. Regarding the UCT process, it is generally suitable for the treatment of wastewaters with influent TKN to COD ratios greater than 0.08 or influent COD to TKN ratios less than 12.0 (81).

5. FACTORS AFFECTING EBPR

In the operation of full-scale EBPR, difficulties in assuring stable and reliable system performance have been recognized. Failure of EBPR process has occurred in the laboratory as well as in full-scale wastewater treatment plants without a clear cause (82–84). It has been believed that a possible reason may be due to the competition between PAOs and GAOs induced by the operational conditions, such as substrate, sludge age, anaerobic/oxic time ratio, and so on.

5.1. Type of Substrate

The success of activated sludge plants for EBPR depends on the composition of wastewater to a certain extent (13, 85). Tam et al. (86) showed that the addition of readily biodegradable carbon significantly enhanced the nutrient removal while fructose or starch supplementation was detrimental to P-removal. Glucose, propionate, and amino acid rich synthetic wastewater are also extremely detrimental to P-removal (87). Glucose, which may induce accumulation of the GAOs, is the least effective carbon source and is not recommended for biological phosphorous removal (88). In the study of the influence of wastewater biodegradability on EBPR in batch tests with different kinds of carbohydrates, e.g., saccharose, cellobiose, starch, and cellulose, Martinez et al. (89) reported that soluble carbohydrates allowed an EBPR mechanism, but particulate carbohydrates seemed to cause non-biological P-removal. Among all kinds of substrates tested, volatile fatty acids are the most effective substrate for EBPR since those non volatile fatty acid substrate need to be converted to acetic acid first for further take-up by bacteria (84, 90). It has been proved in batch tests with prefermentation of glucose

that the addition of VFA of two to five carbon chain lengths with the exception of propionate would result in greater P-removal (91, 92). As carbon source, acetate, and butyrate were equally good for P-removal whereas propionate was the least efficient VFA for EBPR (93). In addition, branched VFA have been shown to be superior to their linear counterparts for more efficient P-removal. Basically, 1 mg of phosphorus removal requires about 7–9 mg of VFAs (94). Besides direct addition of VFAs as substrate for P-removal, VFAs can also be produced on-site by other wastewater (95).

5.2. Organic Loading

Influent organic loading is an important parameter which determines the extent of excess phosphorus removal in a biological process since low COD loading results in poor P-removal, and excessive COD loading can lead to deterioration of the BPR process. High influent acetate concentrations would negatively affect BPR (96), e.g., increasing the influent acetate concentration to 400 mg/L led to efficient anaerobic P-removal in a biofilm system; however, further increasing the influent acetate concentration to above 600 mg/L resulted in cessation of anaerobic P-release and subsequently a deterioration of the P-removal capability (97). At higher COD loading rates, the sludge appears to convert the influent organics first to a storage product, namely 3-hydroxyvalerate, which is mainly utilized by the GAOs. This would result in a failure of phosphorus removal process (98). The ratio of influent acetate, or BOD, or COD to P also exerts an influence on the BPR removal capability. The influent to the anaerobic zone of the BPR system should have a ratio of BOD_5 to the total P higher than 20:1 or COD:P ratio greater than 40:1 for achieving an effluent P concentration of less than 1.0 mg/L (99).

5.3. Magnesium and Potassium

In the EBPR process, evidence shows that Mg^{2+} and K^+ concentrations in bulk solution tend to increase in anaerobic phase and decrease in subsequent aerobic phase. Furthermore, the charge ratio of cation decrease to phosphate decrease in the bulk solution is one mole positive charge decrease per mole phosphate decrease (13). This is mainly due to the fact that one positive charge is required to stabilize one phosphate group in any polyP chain and the expulsion of each phosphate molecule from the cell needs one cationic charge from K^+ or Mg^{2+} (100). The cation limitation would have negative effect on anaerobic P-release and acetate uptake, leading to P-removal decrease. It is likely that the magnesium and potassium concentrations would play an important role in maintaining the stability of the EBPR process. So far, there is no evidence to show that calcium is involved in EBPR. In general, there are excessive K^+ and Mg^{2+} in municipal wastewater, i.e., no cation limitation could be assumed in the EBPR process (99). However, full-scale sewage treatment plants designed for EBPR may periodically experience short- or long-term shortage of potassium in the influent, while excess potassium strongly influences the properties of activated sludge, and results in the poor dewatering property and effluent quality (101).

5.4. Nitrate Content in the Influent

Evidence shows that the presence of NO_x-N can disturb the release of phosphorus in the anaerobic zone and further reduce the uptake of phosphorus in the aerobic zone (67, 102, 103).

The possible reason behind is that in the presence of nitrate–nitrogen in the anaerobic zone, denitrifier will consume the organic carbon readily available for PAOs. Generally, 5 g COD is consumed in denitrifying 1 g of nitrate–nitrogen in the anaerobic zone. These imply that the presence of nitrate would reduce the net influent BOD/P ratio to the system. For wastewater with a low BOD concentration, nitrate entering the anaerobic zone can significantly deplete the BOD available for conversion to anaerobic fermentation products. Depending on the amount of nitrate received, this will lead to a lowered phosphorus removal efficiency, or even prevent biological phosphorus removal. For wastewater with high soluble organic concentration, the effect of nitrate may not be significant. If soluble organic concentration is high enough, VFA reduction and phosphorus release can occur simultaneously (104). In addition, the recirculation ratio of the return activated sludge also plays an important role as it affects the amount of inflow nitrate–nitrogen to the anaerobic zone. The redox potential is another key factor determining the rate of anaerobic P-release. In general, a lower redox potential would favor the phosphate release in the anaerobic phase (105). The presence of low-concentration oxygen or other oxidizing agents, e.g., nitrate, may alter the redox potential, and thus may negatively impact the rate of phosphate release.

5.5. Phosphorus Loading

So far, research attention has been given to EBPR of low-phosphate wastewaters, while there are few attempts to apply biological systems to treat influents with phosphorus concentration higher than 20 mg P/l. The ratio of phosphorus to total organic carbon (P/TOC) in a system is crucial in effectively selecting PAOs as well as in giving them a competitive advantage (106). Kinetically, low phosphate loadings may suppress the growth of PAOs. This would eventually lead to the establishment of GAOs over PAOs, i.e., GAOs dominate at low P loadings (107, 108).

5.6. Temperature

Although EBPR processes have been applied successfully for both cold and warm wastewater, it is clear that low temperatures would pose a negative effect on biological P-removal. An incomplete P-uptake was observed in the aerobic phase at 5 and 10°C, while at 20 and 37°C, a complete P-uptake was achievable (109, 110). In contrast, good or even comparatively better P-removal efficiency at lower temperatures of 5–15°C was reported by Barnard et al. (82). These inconsistent results may be due to a poor understanding of PAOs. Panswad et al. (111) reported that the PAOs would belong to lower-range mesophiles, or perhaps psychrophiles and predominated only at 20°C or possibly lower, while the GAOs would be classified, somewhat, as mid-range mesophilic organisms with optimum temperature between 25.0 and 32.5°C.

5.7. pH

More efficient biological phosphorus removal normally occurs at pH values of 7.5–8.0. The maximum specific growth rate of *Acinetobactor* at a pH 8.5 was 42% higher than that at a pH of 7.0 (112). The pH between 6.5 and 7.0 has an insignificant effect on the specific phosphorus uptake rate in the aerobic zone; however, once the pH drops below 6.5, the PAO activity steadily declines, and 100% of the activity would be lost at a pH 5.2 (113). A negative

effect of acidic pH on both acetate uptake and P-release in the anaerobic stage had been observed, whereas a more alkaline pH inhibited the uptake of acetate and stimulated more P-release than at acidic pH (98). The pH of a combined EBPR and BNR system requires careful monitoring since the various processes, such as nitrification, denitrification, P-release and P-uptake, all have specific pH ranges within which they can be optimized. Nitrification, in particular, appears to be sensitive to the changes in pH, e.g., the optimum pH is in the range of 7.9–8.2 for *Nitrosomonas*, whereas it ranges from 7.2 to 7.6 for *Nitrobacter* (114). The optimum pH for denitrification appears to be in between 7.0 and 8.0 (70). As discussed above, maintenance of a stable, neutral pH is essential for the stability of the EBPR process. When the influent pH was reduced from 7.2 to a weakly acidic value of 6.3, P-removal efficiency was adversely affected and 15 days were required to reestablish steady-state conditions (109).

5.8. Dissolved Oxygen

A combined EBPR and BNR process must satisfy many different oxygen demands from the bacterial populations present in the system. Activated sludge systems designed for carbon oxidation and nitrification typically require DO levels greater than 2 mg/L (70, 115). If the DO is too low, phosphorus removal may be reduced, incomplete nitrification will result, and a poor settling sludge may be developed. In the EBPR process, the anaerobic zone must be kept nearly free of oxygen (0.0–0.2 mg/L DO) as the presence of oxidizing substances will interfere with the EBPR process, while an oxygen concentration of 3.0–4.0 mg/L in the oxic zone has been recommended with BNR (116). In practice, the maintenance of oxygen concentrations above 4 mg/L will cause a waste of energy for aeration. Meanwhile, excessive aeration may negatively affect the EBPR process as cessation of P-uptake occurs due to the depletion of poly-hydroxy-butyrate (PHB) in an over-aerated process (6). In addition, denitrification performance could be limited due to the increase in DO recycled to the first anoxic zone.

5.9. Lengths of Anaerobic and Aerobic Phases

EBPR is realized through an alternative anaerobic–aerobic cycle operation; thus, the relative lengths of anaerobic and aerobic phases would have a profound effect on the performance of the EBPR process. A long anaerobic phase would aid the polyP accumulators in the competition for food against other heterotrophs capable of anaerobic substrate uptake (117, 118). The necessity and the success of longer anaerobic contact times depend on the strength and nature of the wastewater. It should be noted that unnecessary PHA oxidation in the absence of extracellular P would occur if the aerobic reaction time is longer than that required for P-uptake (119). In practice, by adjusting the aeration time to that required for P-uptake, residual PHA is sustained in the SBR and excess phosphate-uptake reaction potential (PRP) is generated during transient influent excursions in P. Consequently, a shorter anaerobic contact time would result in insufficient phosphorus release. However, at a longer anaerobic contact time, polyP microbial bacteria would be inactivated. It appears that anaerobic contact time must be optimized together with aerobic contact time in the EBPR process. An/Ox contact time ratio of 1/2 has been recommended for efficient EBPR (120).

5.10. Solid Retention Time

Phosphorus removal is strongly dependent on solid retention time (STR). The different populations involved in the combined nutrient removal processes have different requirements in relation to SRT (67, 70). In general, slow-growing organisms, such as nitrifying bacteria, require longer SRTs, e.g., the Bardenpho process is often operated at longer STRs in order to accomplish nitrification and denitrification. However, lower sludge yields associated with the longer SRTs would hinder the phosphorus removal capacity because a decrease in the polyP-microbial fraction in the mixed culture would occur with the increase in SRT (121). Another problem associated with a shorter SRT is poor sludge settleability. Evidence shows that the optimum SRT for EBPR should be around 10 days, leading to the maximum nutrient removal efficiencies and minimum SVI (67, 103, 122–125). It is clear that biological system should not be operated at SRT in excess of that required for overall COD, N, and P removal.

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16
Total Treatment of Black and Grey Water for Rural Communities

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CONTENTS

Abstract Decentralized or on-site treatment systems for domestic waste and wastewater treatment can be the answer to many of the world's environmental health problems. Poor or remote communities need technologies developed for the application, and an economically viable whole-of-waste approach is necessary. Vermicomposting offers a natural option to treat domestic waste and wastewater into reusable products. The extent of treatment can be incorporated into the design, providing flexibility and scalability required for the user community.

1. INTRODUCTION

Of all the world's water, only 2.5% is freshwater, which is suitable for consumption and industrial and agricultural uses. The remaining 97.5% is oceans and seas (1). 87.3% of the freshwater is in polar ice caps and glaciers, 12.3% is stored underground and only 0.4% water is available on the surface and atmosphere of earth. Thus, less than 0.01% of all water is suitable to sustain life on earth (Fig. 16.1). Domestic water usage is only a very small part of the total freshwater demand. Major demands are in agriculture followed by industry.

Per capita freshwater use is less in less developed countries than in the more developed countries. The world's water consumption has increased with time but faster than the increase

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_16,

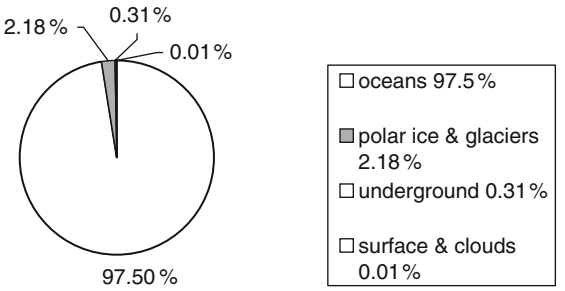

Fig. 16.1. The amount of water available for sustaining life (Source: (1)).

in population. Consumption increased from 580 km^3/year in 1900 to 3,700 km^3/year in 2000 (2), which is more than a six-fold increase. During the 1900–2000 period, the world's population increased four-fold (3). This per capita increase is mainly due to the changes in lifestyle and increased industrialization.

Despite decades of planning and investing in urban and rural water supply systems, the World Health Organization (WHO) estimates that by the end of the decade, more than 1.1 billion people lacked access to safe water and 2.4 billion were without adequate sanitation (4). Regions most affected are Africa, Asia and the Pacific. In Asia, where 85% of the population has access to drinking water, only 20% of the available drinking water meets health and safety standards (5).

One of the contributing causes to the problem is the reliance on centralized systems for water management. Providing safe piped water to dispersed populations in rural areas has proved to be costly for governments, donors and private utilities. Many water supplies in developing countries are intermittent and characterized by a poorly maintained infrastructure that is inadequate and overloaded. In Africa, several cities such as Johannesburg, Dakar and Nairobi have outgrown the capacity of local sources and are forced to carry water from a distance of 200–600 km (6). A good part of the population in these countries depends on water vendors for small volumes of costly water of unsure quality (7).

Many environmental problems come from different forms of waste created by our lifestyle and economic development. Industrial and automotive emissions create acid rains and breathing problems, while industrial and commercial liquid effluents create groundwater and surface water pollution. Solid wastes create problems in terms of demands for disposal of space and water pollution through leaching. Technologies exist and are continuously developed for managing waste, but at times, it seems that the magnitude and evolution of waste outrun the solutions. The fast pace of population growth, change in lifestyles and increased use of resources have magnified waste generation. Many of the issues of waste generation are localized and need localized treatment options, as centralized treatment of waste is not always the most efficient approach. Sustainable development and appropriate technology have become primary parameters for choosing and developing technologies (8). What works for cities may not work for rural areas, and what works for the developed world may not be appropriate for developing or under-developed nations. Research is necessary to find ways to deal with specific problems in specific regions (9–13). Localized integrated systems of water

supply and wastewater management have a great appeal in areas where water is scarce, where infrastructure costs for the supply and treatment are relatively high for the local population and where the environmental and public health concerns of water management are significant.

Due to the environmental, financial and social constraints, both urban and rural water planners in developing countries are opting for simple, sustainable, low-cost solutions that allow overall water that needs to be achieved with fewer resources, less disruption of ecosystems and lower costs (2). Examples of such solutions include integrating water, wastewater and household waste management systems, separate collection and treatment of the various categories of waste streams created in the household; and the recovery of valuable substances for reuse, for example, water, compost, biogas and fertilizer (14). Treatment and reuse of wastewater (greywater and blackwater) has been gaining importance in recent years across the world.

Greywater is composed of all domestic household wastewater that does not come from toilets or does not include sewage. This includes wastewater that flows from baths/showers, clothes washing, and dishwashers and kitchen sinks. Toilet wastewater, often garbage disposal waste, is called blackwater. Treated greywater reuse for non-potable purposes such as irrigation, laundry and toilet flushing is being adopted in Australia and worldwide. Greywater contains far less nitrogen, fewer pathogens and breaks down much faster than blackwater (15). Thus, simpler treatment may be applied to purify the water to reusable standard.

Human waste is a major source of nutrients and energy that can be tapped, as demonstrated in some ancient cultures. Solid waste is as big a problem in the developing world as is wastewater (118). In the modern world, flushing toilets are the norm, which adds significant quantities of wastewater (blackwater) to the waste stream. Studies have progressed in the direction of extracting fuel out of waste treatment technologies, mostly using anaerobic methods (16–18). The process of composting, which is an aerobic process, creates heat and converts solid wastes into compost that can be used as a fertilizer (19, 20). Whether done with microbes and/or worms, the process goes through similar stages and the end results are mostly the same (21).

Technology that integrates the technology of wastewater management and solid waste management in order to provide a treatment option for 'blackwater' can be devised. The processes of composting, microbial and worm action, as well as generated heat provide an opportunity to convert biodegradable household wastes and blackwater to compost and usable water. Blackwater that has been well treated by the composting technology should be safe enough to mix with greywater (all domestic wastewater excluding blackwater) and then produce good quality water after further treatment. If the entire biodegradable waste and wastewater at houses can be converted into reusable compost fertilizer and good quality water, then a total waste management system has been developed. Such a technology can be adapted to small commercial establishments and residential complexes (22–24).

The history of sewage treatment systems dates back to 1700 BC, in palaces where treated wastewater was used in irrigation (25). Technologies have changed with our development into a modern society and different preferences, and there are now many treatment systems. Many approaches have been tried and tested for the purification of wastewater and studies have been reported for decades (26). As Gleick (2) describes, a new way of thinking is unavoidable in managing our water resources and the way we use water.

Water demands, utilization and availability differ among regions and communities depending on the geography and lifestyles. It is not possible and realistic to create a common water management program for all walks of life, but appropriate technology water management programs need to be developed for different circumstances. Generally, residential wastewater output can be differentiated into greywater and blackwater, and include ANS (Anthropogenic Nutrient Solutions).

The major source of wastewater from residential and commercial complexes and institutions is greywater, which is the effluent from washbasins, laundries, bathrooms and kitchens. Some reports can be found that exclude kitchen sink effluent from the definition, owing to the high content of nutrients and suspended solids and defined as blackwater or even termed "brown water" (27). Greywater with heavy contamination or suspended solids has been termed 'dark grey water' for identification purposes in some scientific studies (28). Blackwater is the effluent from toilets and has high amounts of suspended solids and a very high pathogen concentration. Laundry effluent from houses and institutions with infants and ill people can be considered to have higher than normal pathogen levels, but under normal conditions, only toilet effluent is termed "blackwater."

Blackwater is a major problem, as it has to be collected and treated lest it becomes a health hazard. The quantities of blackwater created per capita vary between various cultures and places, depending on a particular lifestyle. In most urban areas, a combined sewer is used to carry away the residential greywater and blackwater together for the treatment at centralized treatment facilities (29, 30).

The differences between greywater and blackwater are well documented (Table 16.1). The amount of nitrogen, pathogens and other pollutants are far less present in greywater compared to blackwater. The BOD_5 (5-day Biochemical Oxygen Demand – oxygen required for the decomposition of the organic content in greywater during the first 5 days, determined as BOD after a 5 day period of incubation under standard conditions) for greywater is 90% of UOD (Total or Ultimate Oxygen Demand) compared with 40% of blackwater (31). This means that greywater is far less polluting compared to blackwater in the long run, as the BOD of greywater more quickly depletes compared to blackwater. Kitchen sink water contains more nutrients and possibly more suspended solids than other forms of greywater. In terms of pathogens and other specific constituents, kitchen sink water can only be defined as greywater, not blackwater. Greywater allows easy and faster treatment compared to blackwater, which needs more intense treatment because of its high COD (Chemical Oxygen Demand – all chemical (organic and inorganic) activities give a measure of organics) and microbial content (32). The COD of domestic wastewater could be as high as 5,000–6,000 mg/L (19, 33).

2. DOMESTIC WASTEWATER CHARACTERISTICS

Greywater has been estimated to account for about 75%-v/v of the combined residential sewage worldwide (34). Water usage surveys carried out in capital cities have identified an average wastewater flow of 586 L per day per household (35) with greywater representing 68% of the total household wastewater (Table 16.2).

Table 16.1
A comparison of greywater and blackwater (Sources: (19, 31–33, 110, 111))

Parameter	Greywater	Blackwater	Grey ± black
BOD_5 (g/p/d & mg/L)	25 & 150–300	20 & 2,000–3,000	71
BOD_5 (% of UOD)	90	40	–
COD (g/p/d & mg/L)	48 & 300	72 & 2,000–6,000	–
Total P (g/p/d & mg/L)	2 & 4–35	1.6	4.6
Total N (g/p/d)	1 (0.6–5 mg/L)	11 (main source urine)	13.2
TSS (g/p/d)	18	> 50	70
Pathogens	Low	Very high	Very high
Main characteristic	Inorganic chemicals	Organics, pathogens	Inorganics, organics and pathogens

$g/p/d$ gram/person/day.

Table 16.2
Approximate percentage of wastewater generated in domestic premises (Source: (35))

Wastewater type	Total wastewater		Total greywater	
	% Total	(L/day)	% Total	(L/day)
Toilet	32	186	–	–
Hand basin	5	28	7	28
Bath/shower	33	193	48	193
Kitchen	7	44	11	44
Laundry	23	135	34	135
Total	100	586	100	400

The characteristics of greywater depend on the quality of the water supply, the type of distribution net for both drinking water and greywater (leaching from piping, chemical and biological processes in the biofilm on the piping walls) and thirdly from the activities in the household (36).

The compounds present in greywater vary, reflecting different lifestyles, customs, installations and use of chemical household products. Variations in water consumption affect the composition and volume. Chemical and biological degradation of greywater, within the transportation network and during storage also complicates evaluations of the composition of greywater (35) (Fig. 16.2).

2.1. Physical Parameters

Physical parameters of relevance in assessing and monitoring greywater are temperature, colour, turbidity and suspended solids (37). The temperature of greywater has been found

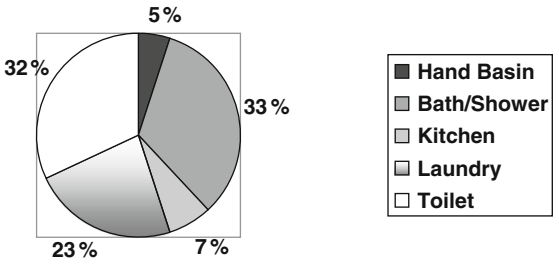

Fig. 16.2. Percentage values of wastewater generated in domestic premises (Source: (35)).

to vary within the range 18–38°C with high temperatures due to the use of warm water for personal hygiene in developed countries. Eriksson et al. (34) noted that this may be a problem as it favours microbiological growth and may also result in $CaCO_3$ precipitation, the solubility of which and that of other inorganic salts decrease at increased temperatures.

Sources of suspended solids in the greywater include food particles, soil and sand particles, hair, fibres and zeolites (from laundry wastewater). Suspended solids may induce clogging of piping or sand filters used for treatment. Stabilization of the solid phase due to the combination of colloids and surfactants can act to decrease agglomeration of colloidal matter (38), which may reduce the efficiency of pretreatment based on settling.

The wash cycle in domestic automatic washing machines has significantly higher turbidity values compared to the rinse cycle, 39–296 and 14–29 Nephelometric Turbidity Units (NTU), respectively (37). For other greywaters, the turbidity was found to be in the range 15.3–240 NTU (39). There are no known values for turbidity of greywater from kitchen sinks reported in the literature. Eriksson et al. (34) observed Total Suspended Solids (TSS) and Total Solids (TS) to be in the ranges 17–330 mg/L and 113–2,410 mg/L, respectively with the highest values originating from laundry and kitchen (Table 16.3).

2.2. Chemical Parameters

The key chemical parameters in assessing and monitoring greywater quality are alkalinity, hardness and pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), nitrogen and phosphorus concentrations. Alkalinity and hardness contribute to assessing the risk of clogging and are largely determined by the quality of the drinking water. Chemicals added during the use of water have a limited impact on these parameters (but not always). Greywater that originates from the laundry is alkaline and has pH values in the range 8–10, while the other types of greywater generally have somewhat lower pH values (range 5–8.7) (36). Kitchen wastewater would be more acidic.

BOD and COD are parameters that indicate the risk of oxygen depletion due to degradation of organic matter (40). COD:BOD ratios have been found to be as high as 4:1 (37). Most COD derives are from household chemicals like dishwashing and laundry detergents (41).

The bathroom fraction contains 184–633 mg/L COD and 7–300 mg/L BOD; the laundry fraction 725–1,815 mg/L COD and 48–472 mg/L BOD, while the kitchen fraction contains 26–1,380 mg/L COD and 5–1,460 mg/L BOD. Mixed grey wastewater values range from

Table 16.3
Characteristics of different types of greywater (Source: (37))

	Laundry	Bathroom	Kitchen sink
Physical properties	in mg/L	in mg/L	in mg/L
Colour (Pt/Co units)	50–70[a]	60–100[a]	
Suspended solids	79–280[a,c,g]	48–120[a,g]	134–1, 300[f,g]
TDS		126–175[e]	
Turbidity, Nephelometric Turbidity Units	14–296[a,b,c]	20–370[a,b,e]	
Temperature in °C	28–32°C (83–90°F)	18–38[d]	
Chemical properties	in mg/L	in mg/L	in mg/L
pH	9.3–10[a]	5–8.1[a,b,d,e]	6.3–7.4[f]
Electrical conductivity	190–1, 400[a]	82–20, 000[a,d]	
Alkalinity	83–200 as ($CaCO_3$)[a]	24–136 (as $CaCO_3$)[a,e]	20.0–340.0[f]
Hardness		18–52 (as $CaCO_3$)[e]	
BOD_5	48–380[a,c]	76–200[a]	
BOD_7	150[g]	170[g]	387–1, 000[g]
COD	375[g]	280[g] up to 800 COD_{Cr-}	26–1, 600[f,g]
TOC	100–280[c]	15–225[e]	
Dissolved oxygen		0.4–4.6[d]	2.2–5.8[f]
Sulphate		12–40[b]	
Chloride (as Cl)	9.0–88[a]	3.1–18[a,b]	
Oil and grease	8.0–35[a]	37–78[a]	
Nutrients			
Ammonia (NH_3-N)	$< 0.1 -$ 3.47[a,b,c,g]	$< 0.1 - 25$[a,b,d,g]	0.2–23.0[f,g]
Nitrate and nitrite[h] as N	0.10–0.31[a]	$< 0.05 - 0.20$[a]	
Nitrate (NO_3-N)	0.4–0.6[c]	0–4.9[b]	
Phosphorus as PO_4	4.0–15[c]	4–35[b,d]	0.4–4.7[f]
Nitrogen as total	1.0–40[a]	4.6–20[a]	15.4–42.8[f]
Tot-N	6–21[c,g]	0.6–7.3[b,g]	13–60[g]
Tot-P	0.062–57[a,c,g]	0.11–2.2[a,g]	3.1–10[g]
Ground elements	in μg/L	in μg/L	in μg/L
Aluminium (Al)	$< 0.1 - 21$[a]	< 0.1[a]–1.7[g]	0.67–1.8[g]
Barium (Ba)	0.019[g]	0.032[g]	0.018–0.028[g]
Boron (B)	$< 0.1 - 0.5$[a]	< 0.1[a]	
Calcium (Ca)	3.9–14[a,g]	3.5–21[a,g]	13–30[g]
Magnesium (Mg)	1.1–3.1[a,g]	1.4–6.6[a,g]	3.3–7.3[g]

(*Continued*)

Table 16.3
(Continued)

	Laundry	Bathroom	Kitchen sink
Potassium (K)	1.1–17[a,g]	1.5–6.6[a,g]	19–59[g]
Selenium (Se)	< 0.001[a]	< 0.001[a]	
Silicon (Si)	3.8–49[a]	3.2–4.1[a]	
Sodium (Na)	44–480[a,g]	7.4–21[a,g]	29–180[g]
Sulphur (S)	9.5–40[a]	0.14–3.3[a,g]	0.12[g]
Heavy metals			
Arsenic (As)	0.001–< 0.038[a]	0.001A–< 0.038[g]	< 0.038[g]
Cadmium (Cd)	< 0.01 – < 0.038[a,g]	< 0.01[a,g]	< 0.007[g]
Chromium (Cr)	< 0.025[g]	0.036[g]	< 0.025 – 0.072[g]
Cobalt (Co)	< 0.012[g]	< 0.012[g]	< 0.013[g]
Copper (Cu)	< 0.05 – 0.27[a,g]	0.06–0.12[a,g]	0.068–0.26[g]
Iron (Fe)	0.29–1.0[a,g]	0.34–1.4[a,g]	0.6–1.2[g]
Lead (Pb)	< 0.063[g]	< 0.063[g]	< 0.062 – 0.14[g]
Manganese (Mn)	0.029[g]	0.061[g]	0.031–0.075[g]
Mercury (Hg)	0.0029[g]	< 0.0003[g]	< 0.0003 – 0.00047[g]
Nickel (Ni)	< 0.025[g]	< 0.025[g]	< 0.025[g]
Silver (Ag)	0.002[g]	< 0.002[g]	< 0.002 – 0.013[g]
Zinc (Zn)	0.09–0.44[a,g]	0.01–6.3[a,g]	0.0007–1.8[g]
Xenobiotic organic compounds			
Detergents	Identified[d]		
Long chained fatty acids	Identified[e]		
Microbiological parameters			
Campylobacter spp.	n.d[a]	n.d[a]	
Candida albicans		n.d[e]	
Colifager PFU/mL	10^2–10^3[g]	388×10^3[g]	< 3[g]
Crytosporidia	n.d[a]	n.d[a]	
Escherichia coli[h]	8.3×10^6[g]	3.2×10^7[g]	1.3×10^5–2.5×10^8[g]
Faecal coliforms	9–1.6 × 10^4[a,b,c]	1–8 × 10^6[a,b,c]	
Faecal streptococci	23–1.3 × 10^6[a,b,c,g]	1–5.4 × 10^6[a,c,g]	5.15 × 10^3–5.5 × 10^8[g]
Giardia	n.d[a]	n.d[a]	
Heterotrophic bacteria[h]		up to 1.8 × 10^6[d]	

(*Continued*)

Table 16.3
(Continued)

	Laundry	Bathroom	Kitchen sink
Pseudomonas aeruginosa		n.d[e]	
Salmonella spp.	n.d[a]	n.d[a]	
Staphylococcus aureus[i]		$1–5 \times 10^{5e}$	
Thermotolerant coli[h]	8.4×10^{6g}	up to $10^{6d,g}$	$0.2 \times 10^{6}–3.75 \times 10^{8g}$
Total coliform[h]	$56–8.9 \times 10^{5a,b,c}$	$70–2.8 \times 10^{7a,b,c,e}$	
Total bacterial population (cfu/100 mL)		$300–6.4 \times 10^{8e,b}$	

[a](39); [b](112); [c](113); [d](43); [e](114); [f](75); [g](41); [h]per 100mL; [i]per mL
Identified: only qualitative analyses, no quantifications were performed.

210–740 mg/L COD and 150–530 mg/L BOD (42). Dissolved oxygen concentrations in grey wastewater have been found to be in the ranges between 2.2–5.8 mg/L (75) and 0.4–4.6 mg/L (43).

The total nitrogen (TN) concentration in greywater is given as 0.6–74.6 mg/L (42). Kitchen wastewater contributes the highest levels of TN with values ranging from 40 to 74 mg/L (Table 16.3). Corresponding values for ammonium are $< 0.05–25$ mg/L. Owing to the fact that faecal matter is seldom present in greywater, the lowest levels of TN are found in the bathroom and laundry wastewater (41).

Total phosphorus concentrations in greywater vary depending on the washing detergent (primary source of P) used (44). In areas where phosphorus detergents are used, concentrations range between 6 and 23 mg/L Tot-P compared to regions where non-phosphorus detergents are used (4 and 14 mg/L) (38).

Other pollutants of importance to be considered when planning the reuse of greywater are heavy metals, particularly Al, Fe, Mn, Cd, Cu, Pb, Hg, Zn, Ni and Cr (39). Laundry wastewater has been found to contain elevated sodium levels compared to other types of greywater due to the use of sodium as counter ion to several anionic surfactants used in powder laundry detergent or the use of sodium chloride in ion exchanges (44). Only relatively low concentrations of heavy metals have been reported in literature (36).

The major organic components in the influent to a wastewater treatment plant have been reported as long-chain fatty acids and their esters (45). The main sources of these compounds are soap, edible oils and fat. A screening method showed that the majority of xenobiotic organic compounds (XOCs) consist of detergents. By-products can be formed when different chemicals in the greywater react with each other. Oxidation and microbiological activity may also lead to the production of degradation products that have other properties than the parent compounds (37).

The characteristics of the fresh greywater can change significantly during storage – it has been found that storage for 24 h improves the quality of greywater, but storage for more than 48 h enhances the conversion of greywater to blackwater due to oxygen depletion (46).

2.3. Microorganisms

The important parameters for assessing and monitoring the biological contamination of greywater are pathogenic viruses, bacteria, protozoa and helminths. These are introduced into greywater by hand washing after toilet use, washing of babies and small children, diaper washing, and washing uncooked vegetables and raw meat.

Some viruses, e.g. enteroviruses, can be spread in faecally contaminated waters. Organisms that are relatively resistant to disinfection and are of major concern include *Cryptosporidium* and *Giardia* (protozoa) (36). The coliform group of organisms (*Escherichia coli* and thermotolerant coliforms such as *Citrobacter* and *Klebsiella*) is generally accepted as the most suitable indicator of faecal contamination since the organisms are relatively easy and inexpensive to detect and have similar survival time of pathogenic enterobacteria. Thermotolerant coliforms are the most sensitive but least specific indicator group for faecal contamination as these coliforms may also occur naturally in soil and vegetation. *E. coli* is the most specific indicator of faecal contamination.

Laundry wastewater was found to contain 9×10^4–1.6×10^4 per 100 mL faecal coliforms, 5.6×10^5–8.9×10^5 per 100 mL of total coliforms and faecal streptococci in the range 1×10^6–1.3×10^6 per 100 mL. Bathroom wastewater contains up to 3×10^3 per 100 mL faecal coliforms, 2.0–2.4×10^7 per 100 mL of total coliforms and 1–7×10^4 per 100 mL of faecal streptococci. *E. coli* in kitchen water has been observed in the range of 0.1×10^6–2.5×10^8 per 100 mL and thermotolerant coli in the range of 0.2×10^6–3.8×10^8 per 100 mL (41).

3. GUIDELINES FOR WATER TREATMENT AND TESTING

Though an indication of some level of faecal indicator bacteria cannot be taken as final pathogenic quality criteria, enumeration of *E. coli* as the most commonly found indicator organism in human excreta has been accepted by WHO guidelines (4, 47). Colony counts (Colony Forming Units – CFU) have been accepted for routine monitoring of thermotolerant coliforms and *E. coli* (47, 48). Counts of less than 100 CFU/100 mL for disinfected water supply and less than 500 CFU/100 mL for un-disinfected supply have been prescribed. Tests for the presence of specific pathogenic organisms are appropriate for special investigations but are not recommended for routine monitoring of water supplies, due to the complexity of testing, associated cost and unreliability of detection (47).

Total dissolved solids (TDS) values of more than 600 mg/L has been mentioned in WHO guidelines (4, 47) as affecting the palatability of drinking water. A turbidity of less than 5 NTU has been given to be acceptable for consumption, but turbidity less than 1 NTU is required for effective disinfection. Australian standards have prescribed less than 100 mg/L of nitrate and 0.5 mg/L for ammonia in drinking water for safe consumption (47).

There are differences between standards due to the basis of calculations done to formulate the standards and guidelines. For example, the average body weight of a person is different between Australian and WHO drinking water guidelines (47, 49). Generally, international standards have to take into account the existing conditions in developed as well as developing and poor countries whereas standards in specific countries need only to account for the specific conditions.

4. TRADITIONAL WASTEWATER TREATMENT

Sewage treatment plants (STPs) are the conventional management systems for wastewater in many parts of the world. Gunther gave the typical composition of STP effluent as follows: a 5-day biochemical oxygen demand (BOD_5) $<$20 mg/L, 30 mg/L suspended solids (SS), 25 mg/L total nitrogen (TN), 10 mg/L total phosphorus (TP) and 200 *E. Coli* counts per 100 mL (50).

Lower levels of contamination can be achieved with enhanced treatment in tertiary treatment systems. In most instances, chlorination or ultraviolet (UV) radiation before discharge disinfects the effluent (51). Natural, physical, chemical and biological processes in water bodies are often relied upon in many parts of the world for the final polishing of the wastewater.

STP effluent is a resource, and in many treatment systems, it is being reused for applications such as irrigation and landscaping. However, a major study of Perth's wastewater management (52) revealed that reuse of all the effluent from that city was not possible as there was simply not enough land for nearby broad acre application. Sometimes, treated effluent is stored for peak demand during summer months, but this storage imposes extra infrastructure costs.

There are difficulties in locating sufficient land within a reasonable distance of STPs in high population areas for both storage and application (53, 54). Public health is a major concern, particularly the pathogen level of the effluent from parks, gardens and playing fields. There are strict guidelines on the use of treated effluent in agriculture (55).

In some situations, onsite sanitation systems are easier to plan and finance than centralized STP units. Whilst they have their problems, as discussed in the following, onsite treatment and reuse can be implemented based on specific site conditions. Onsite systems have been used for many centuries, most commonly found today in communities and sites where connection to centralized systems would not be feasible. The cost of a centralized sewage system is usually more than four times that of onsite alternatives with septic tanks being the most expensive of these alternatives (56).

The most commonly used "wet" onsite sanitation facility is the septic tank (Fig. 16.3). Through settling of solid material and biodegradation of organics, passive anaerobic treatment of wastewater is achieved. Approximate removal rates are 50% BOD_5, 75% suspended solids, 10% TN and 15% TP before disposal (51). Minimum maintenance is required; beyond periodically pumping out the sludge to ensure adequate treatment of the effluent and to avoid clogging. Advantages of anaerobic treatment include the production of methane as a source of energy, low energy requirements and low sludge growths.

Problems associated with the use of septic tanks in developing countries include poor design and a total lack of maintenance. Building regulations are commonly disregarded in

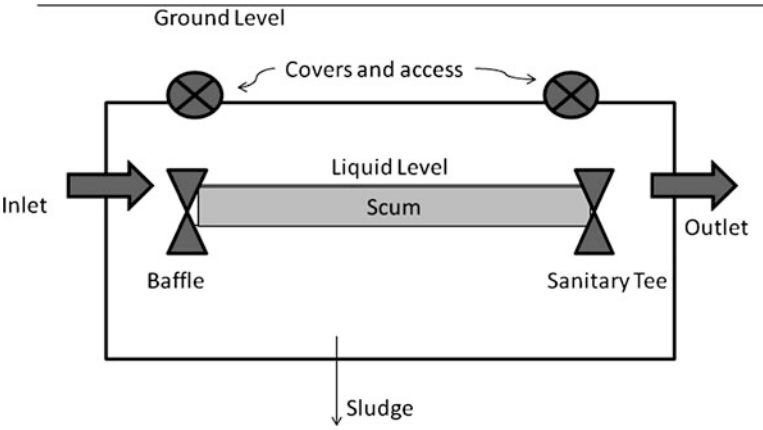

Fig. 16.3. Cross-section of a septic tank (Adapted from: (115)).

the design of septic tanks (57). Septic tanks are not emptied regularly as required. Insufficient tank capacity, a common practice to reduce capital costs, results in premature disposal of septic tank effluent into leaching fields. In systems where the septic tank is part of a flow scheme, the carryover of solids and grease to the subsequent treatment processes results in clogging of pipes and reduction in treatment efficiency of the other process units.

High quantities of BOD_5 in septic tank effluent result in the release of pathogens into disposal fields while large amounts of nitrogen and phosphorus enhance eutrophication in unsaturated aerobic zones in disposal fields (58). In areas where water is drawn as a potable source, there are potential problems of nitrate toxicity as well as pathogen contamination. Some communities build septic tanks in low-lying areas where the subsoil structure is too impermeable for leaching of the septic tank effluent, while others directly pump their effluent into inland water bodies through storm water drainage systems. Where drainage fields are too small, leachate pollutes piped water, well water, canals and rivers (57).

Other onsite wastewater treatment systems include aqua privies (Fig. 16.4) and cesspools. Both consist of two interconnected tanks; the first tank is for solid settlement, and the second for soakage where purified effluent flows. These systems are inexpensive to construct and operate, and typical water consumptions are 0.6 kL per household per month compared to septic tank consumptions of 5 kL per household per month (56). However, both need frequent desludging. The digesters are small and effluent seeping into the ground can result in pollution.

Aerated wastewater treatment systems (AWTs) are small, self-contained biological treatment systems, which use mechanical devices to provide mixing, aeration and pumping of effluent (59). After chlorination, effluent from these systems is typically applied on land using surface or subsurface irrigation (Fig. 16.5).

Aerobic treatment units are often used in areas where septic tank leach fields are unsuitable and/or where there is not enough land available for disposal. They have been shown (Table 16.4) to produce an effluent with lower BOD_5, SS and faecal coliform concentrations compared to septic tank effluent and are thus used where higher levels of wastewater treatment

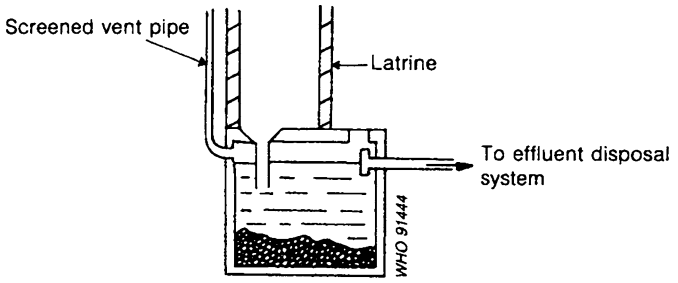

Fig. 16.4. Aqua-privy (Source: (56)).

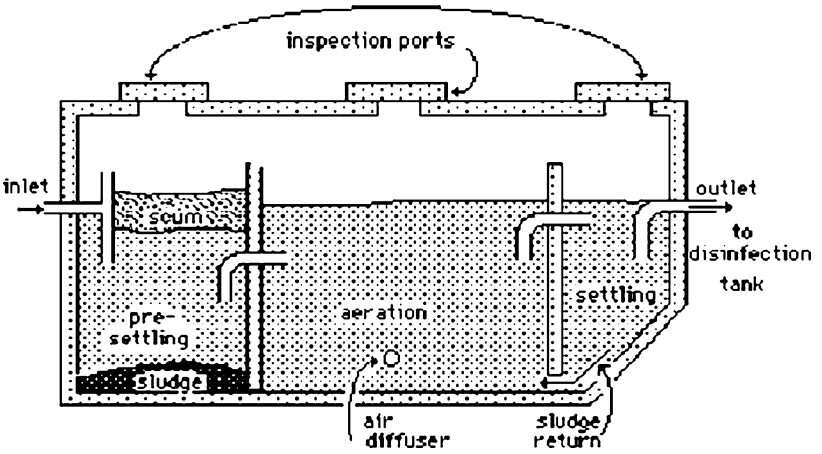

Fig. 16.5. Aerated wastewater treatment system (Source: (115)).

Table 16.4
Comparison of raw effluent quality with effluent from septic tank and aerated wastewater treatment system (Source: (51))

Parameter (mg/L)	Raw effluent	Septic tank effluent	AWT effluent
BOD_5	300–400	120–150	5–80
SS	260–300	40–190	5–100
TN	50–60	40–50	25–50
NO_3-N (% of TN)	(0%)	(0%)	(80%)
TP	10–15	10–15	7–12
PO_4-P (% of TP)	(45%)	(90%)	(85%)
Faecal Coliforms org/100 mL	10^5–10^7	10^5–10^7	10–10^3

BOD_5 biochemical oxygen demand, *SS* suspended solids, *$NO_3 - N$* nitrate nitrogen, *TN* total nitrogen, *TP* total phosphorus, *$PO_4 - P$* orthophosphate phosphorus.

are required (60–63). AWTs are of limited use in developing countries due to energy requirements and the need for frequent maintenance.

The above systems are unsustainable where public health, environmental impacts, maintenance and energy issues are considered. The use of soil-based disposal methods for the effluent acts to waste water instead of reclaiming it and can have significant public health and environmental impacts. Wastewater is a resource and should be treated as such. Systems, which emphasize the resource of wastewater, are likely to be sustainable. They may also offer cheaper solutions and lesser demands on potable water through the use of the reclaimed water (water reuse).

4.1. Wastewater Treatment and Reuse

Water reuse is gaining importance not only amongst professionals, but also amongst the general population. Potable reuse of treated greywater has been reported from Namibia, Pretoria and the USA (64). On the other hand, direct recycling of domestic blackwater in agriculture and aquaculture has been practiced in many countries with tremendous risk to human health (65–70).

In India, except the big city centres where space is limited, houses, small residential units, institutions and most commercial centres have separate plumbing for greywater and toilets. The blackwater goes to septic tanks and greywater goes to pits from where the water irrigates the plants through natural percolation into the ground. There appears to be no adverse health reports on this separate treatment. To be on the safe side, there is an argument that proper (or approved) treatment of the separate waste streams should be made mandatory.

The choice of technology that is appropriate for the particular implementation is important in terms of maintenance and cost. Some modern technologies, such as reverse osmosis, have significant cost implications and give rise to problems of implementation in developing economies. The emphasis in this review is on small-scale local systems appropriate to residential areas.

The simplest method for reuse of greywater, the "Mexican Drain" (71), is a basic system where greywater is collected and fed directly to plants using a bucket or hose and no treatment occurs. Other greywater systems are being developed around the world. It should be noted that greywater treatment is an emerging technology and most of the systems mentioned below are still under research.

As an example, in Australia, a greywater treatment system, which utilized a Biomax aerobic treatment unit, was recently approved and installed (72). Modifications to the unit included additional baffles in the aerobic and anaerobic chambers for more effectiveness in the treatment of low biomass effluent input. Small-scale irrigation using subsurface tubing was the only reuse system currently in practice for this effluent. Comparisons of the performance of the AWT treating combined wastewater factions (blackwater + greywater) and greywater alone showed no significant variations in BOD_5, SS and nutrient levels in both effluents.

Fremantle Inner City Agriculture (FINCA) used a concept of amended soil filtration to treat and reuse greywater for the irrigation of a 800 m^2 community garden and is using the greywater from two adjacent houses to irrigate it (72). Greywater was collected by gravity from

two adjacent households into a collection tank. This was then let into plastic lined trenches filled with a mix of 85% red sand and 15% red mud. Phosphorus was found to be absorbed into the clay material, while nitrification–denitrification processes are responsible for nitrogen removal. Since the garden was heavily vegetated, plant uptake acted as another form of nutrient removal. Pathogen reduction was achieved through filtration. Nitrogen was removed from the system by intermittent drying and wetting causing nitrification–denitrification.

Near-potable standards for greywater recycled through biological processes have been reported in an experiment at Loughborough University (73). Hammes et al. (30) reported on a 'mix-first-and-separate-later' approach experiment, which produced very safe recycled water. The authors claim that by this method, more nutrients were made available from the combined household sewage by removing urine and faeces from greywater by *ecotechnological* methods. The different components of wastewater were treated according to their individual qualities. There are problems in treating combined blackwater and greywater effluent, and advantages in treating them as separate waste streams. In smaller systems it may be better to treat the blackwater to greywater quality, and then treat all the greywater together.

Lodge et al. (36) reported that the technology employed at the largest water recycling treatment plant in Europe, at the Millennium dome, involved a Biological Aerated Filter (BAF) for greywater treatment, which removes suspended solids (SS) and carbonaceous organics with microorganisms. After treatment, the water from wash areas, rainwater from the roof, and groundwater was further treated through ultrafiltration and reverse osmosis. The 50–240 mg/L BOD of greywater was reduced to 1–15 mg/L and 48–124 mg/L of SS are reduced to 2–5 mg/L by BAF. The millennium project uses only greywater, and excludes blackwater (higher BOD and SS)- discarded into the sewer (74).

Jefferson et al. reported the highest efficacy of treatment for membrane bioreactors (MBR), above the performance of membrane aerated bioreactors (MABR) and BAFs (18). MBR proved to be very effective in stabilizing influent water quality variations. Shin et al. experimented on a sequencing batch reactor (SBR) with microfiltration techniques for greywater reuse at an office building in Japan. The effluent had 20 mg/L SCOD (Soluble Chemical Oxygen Demand, COD of the filtered effluent from which all particulate matter have been removed), 5 mg/L BOD and 0.5 mg/L ammonia (75). The SBR was superior compared to other mentioned technologies (MBR, MABR, BAF, etc.,) and the cyclic operation mode proved better than the conventional activated sludge processes. SS concentration was one handicap and microfiltration reduced this to very low levels. SBR technology is good enough for applications, such as gardening and toilet flushing, as per current standards (49).

Anda et al. (73) reviewed different technologies in greywater treatment currently under research in Western Australia. In amended soil filters, 90-mm diameter perforated HDPE pipes are used for subsurface irrigation in prepared ground where a thick vegetation of vegetables and herbs are grown. Aerobic biological activity and the presence of earthworms are promoted. The ground is prepared with red mud, sand and a thick layer of wood chip mulch. System performance is currently being monitored (76). Separately in sand filtration, greywater is filtered through two deep bed sand filters and then applied to an irrigation field.

In another study reported by Anda et al. (73), the combined effluent of treated blackwater and greywater was aerated to achieve secondary treatment standards, and then disinfected before irrigation of constructed wetlands. *Phragmites australis* was used as macrophyte for nutrient stripping. Emergent macrophytes and submergent macrophytes were used for better performance across various seasons. Details of the process or long-term performance data were not available (76).

The 'Aquarius' aerobic treatment unit is reported to remove nutrients to below 1 mg/L. The technology involves primary sedimentation and aerobic digestion, anoxic denitrification and chemical phosphorous removal, aerobic biological oxidation, including nitrification in subsurface biofilter and denitrification in a submerged filter, secondary clarification and sludge recycling and finally chlorination. For treating greywater alone, the first stage can be avoided because of low SS levels. Treated water is used in toilet cisterns after disinfection (76). Any excess effluent can be used in the garden.

Of all the above five technologies reported by Anda et al. (73), aerobic treatment and irrigation is the most commended, due to the good nutrient removal and safety of aerobic treatment. Though nutrients are good for irrigation, these could be problematic when the treated greywater is to be used for other purposes such as non-potable residential use. The associated costs were not available from the paper, but the Aquarius technology may be best suited for places without much space such as big cities with a high population and massive residential complexes (e.g. Singapore, Bombay and Tokyo).

For households without a garden/lawn/agriculture land, irrigation will not be useful. An alternative would be centralized collection and storage for irrigation, i.e., to collect treated greywater through pipes that lead to a location that needs to be irrigated. Constructed wetlands can be considered where adequate space is available. There are other uses for treated greywater, such as flushing toilets, car washing, construction works, fire hydrants, etc.

Hammes et al. (30) experimented with anaerobic digestion (AD) for treating biowastes with blackwater treatment at thermophilic conditions, with options of partial energy recovery as biogas containing methane (1 m^3 methane gives 35 MJ energy) and water reuse. Their report pointed out that between 70% and 90% of annual expenses are related to waste transport to centralized treatment plants. The authors suggested co-digestion (AD) of dry black waste (solid part of blackwater) with grey waste (biowaste) (32). Thermophilic anaerobic reactions are complex, and odorous gases are generated. The technology is unlikely to be marketable for household use. The system is not totally accessible and accidental input of any material could disrupt anaerobic reactions by creating an organic shock load. Their technology requires that only dry toilets are used and this is not very acceptable amongst the wider population.

Dixon et al. (22, 31, 44) demonstrated the water saving potential of a combination of wastewater reuse and rainwater harvesting. The basis of their analysis was the data from a small-scale study of domestic water appliance usage, from which cumulative frequency distributions were derived for each hour of the day and for occupancy. Their study concentrated on an urban housing environment.

The application of natural and artificial wetlands is becoming increasingly popular. Examples included an artificial three root-zone treatment, gravel-based wetland system developed by (77). These systems were planted with the aquatic plants *Schoenoplectus validus* and

Phragmites australis for nutrient removal purposes. Monitoring suggested the effectiveness of plant uptake for nutrient removal (94.9% TN removal and 98.7% TP removal). However, there was a slight reduction in the content of organics' (50% BOD_5 and 59.5% COD).

Many types of aquatic macrophytes have been used in domestic greywater treatment, traditionally in reed bed or pond systems. Submergent macrophytes such as *Schoenoplectus Validus* and *Triglochlin huegelii* were examined by Mars et al. (78) in Western Australia. *T. huegelii* proved, in this test, very useful in removing nitrogen and phosphorous. The authors suggest lagoons, wetlands and constructed basins filled with plants like this for nutrient stripping. Though this can be cost effective and environmentally friendly, it needs space and the applicability of this technology in residential areas would not be attractive.

Mander and Mauring (76) designed a greywater purification plant that simulates plant and microorganism interactions occurring in a normal riparian ecotone. Water is led in under the roots of the planted vegetation and stored in a pond to be fed into consecutive ponds. This is repeated three times to increase turnover rate, and by that attain a large reduction of incoming pathogenic bacteria, BOD_5 and nutrients. After the last pond, the water is let into a sand filter system and is collected in a well. Before entering the pond system, the water passes through a section filled with lime-gravel to increase the surface for organic material reduction by aerobic bacteria and to buffer pH. *Alnus spp.,* a nitrogen fixing plant is grown in this wet park system because of its capacity to extract phosphorus from waters with a low N/P ratio. Also, some fishes and crayfishes are introduced into the ponds to control insect larvae and digest leaf litter and other organic matter. Due to the long turnover time, the slow flow and the long underground passage, the reduction of bacteria and viruses emitted with the greywater would be almost complete and the study showed that the treated greywater is fully appropriate for reuse in the building, even as drinking water (79).

There is limited information on treatment systems currently in use in Africa, Asia and the Pacific. Most of the systems under trial use biological processes for the treatment of wastewater. Examples include constructed wetlands and peat filtration (80–82). For blackwater treatment, emphasis is being placed on safe dry sanitation methods (for example, ventilated pit latrines and compost latrines), as the supply of piped water is still non-existent in many regions.

Issues of environmental health demand that we utilize our resources wisely. Discharging effluent from centralized wastewater treatment facilities into rivers, lakes and oceans can be viewed as losing resources. Health issues regarding residential wastewater reuse require careful analysis. The degree of exposure and physical conditions of the persons affect the chances of infection. Many reports speak of people engaged in illegal reuse of greywater, such as in Western Australia (24, 76). Public health authorities have to develop appropriate guidelines on water reuse for each area. Proper risk analysis is a must with reference to the particular area – the perception of risk can change dramatically depending on location and lifestyle. While many technologies are available for wastewater treatment, they all need careful evaluation of their advantages and disadvantages. Some are costly, while others are useful in different scales.

5. ECOLOGICALLY SUSTAINABLE WASTEWATER MANAGEMENT SYSTEM: A CASE STUDY

The University of Western Sydney Research Group for Sustainable Engineering and Technology has developed a treatment flow scheme based on ecotechnologies for wastewater treatment. The viability of a low-cost appropriate technology was tested using a holistic approach on whole-of-waste treatment using biological/ecological methods (83–85). The technique of vermicomposting that has been in use for centuries around the world for solid waste treatment was put to test. Some of the studies reported in literature, such as Dowmus and Biolytix, could be compared to the technology researched but on a different scale. Mostly, vermicomposting has been applied to sludge treatment (86). The aims of the study reported in this article focused on a low-cost technology for wastewater treatment, with the added benefit of solid waste treatment into a useable product (vermicompost) that is scaleable for different user-levels and waste input. This would also be an appropriate technology for wastewater management in arid areas and developing world (87).

5.1. Background

Advantages of the application of ecosystems in water purification include low construction and maintenance costs, low energy requirements, flexibility where design is concerned, efficiency and the ability to reuse wastewater and nutrients. Factors that are deterrent to the use of natural ecosystems include land space requirements, high levels of organics and the microbiological risk associated with wastewater reuse for irrigation and potable reuse. Treatment of blackwater and putrescible solids are done in a vermicomposting unit, while the essential elements of the greywater treatment system, at a concept level, are aerobic grease trap, slow sand filter, evaporation and treatment bed and UV disinfection. The overall design concept is provided in Fig. 16.6.

5.2. Design Parameters and Considerations

The vermicomposting unit that treats the solid waste and blackwater was designed for a single-person waste generation and constructed out of stainless steel to avoid corrosion problems. The average organic waste generation per person per day is a minimum of 700 g dry weight of solid waste from all sources. Excreta waste is estimated at 400 g wet weight (88, 89), with the output of a normal toilet per flush at 6 L. The volume of the flush may decrease as the results of on-going research on optimizing the ultra-low dual-flush toilets continues, with the volume possibly reducing to less than 4 L per full flush. Solid wastes mainly consist of kitchen waste and other food scraps, garden waste, paper shreds and miscellaneous organics such as manure. Seasonal variations can affect the quantities of garden waste added to the organic waste component.

Settled pig slurry was used in this experimental run as blackwater. Similarities between human and pig sewage with respect to nutrients have been documented (90, 91). Blackwater passes through the vermicomposting medium made up of solid wastes slowly through the interstitial spaces. A high humidity will saturate the substrate mass; thus, the passage of the liquid will be faster. Drier substrate will delay the HRT (hydraulic retention time). This will

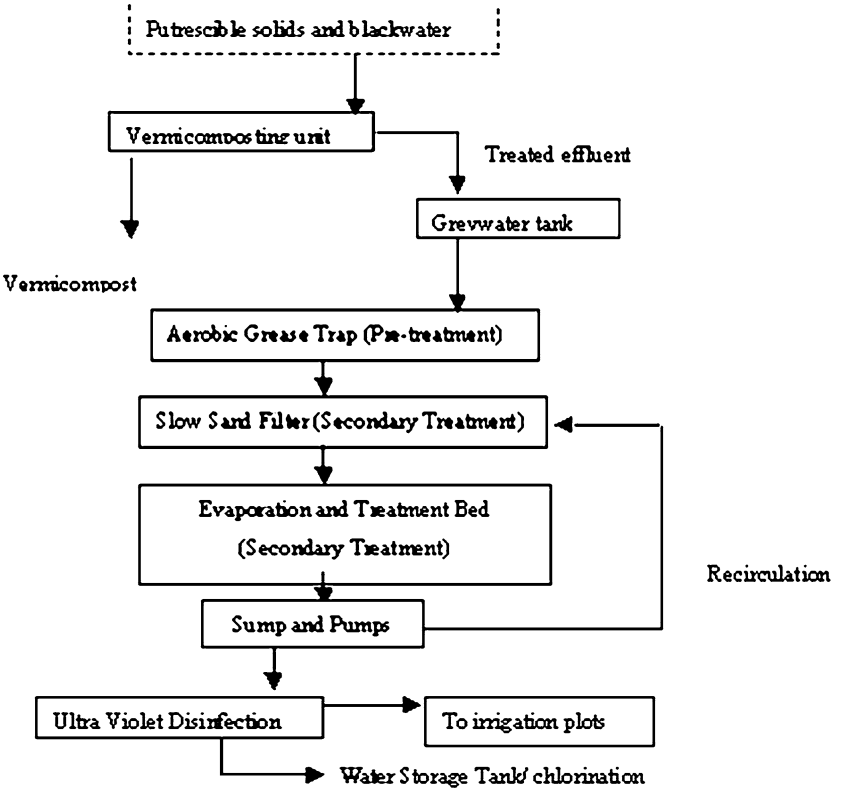

Fig. 16.6. UWS treatment flow scheme (Source: (83, 84)).

not affect the decomposing process adversely, but the wastewater treatment may not meet the expected targets of effluent quality and quantity. A thorough risk analysis was conducted, the results of which had been presented elsewhere (92).

Keeping a steady liquid flow, proper HRT and reducing moisture removal by aeration are critical in the successful operation of a vermicomposting system. The extent of treatment received by the liquid waste depends in part on the duration the liquid has been in the system, the retention time. Composting worms can survive in a high humidity environment. The higher the HRT, the better the treatment. But, as the liquid waste is added regularly, a high HRT will cause a build-up of liquid waste that will block the inter-particle spaces in the composting mass, thereby creating anaerobic conditions and foul odours, which leads to process failure. Therefore, keeping the correct HRT is crucial. The method of adding the liquid waste depends on the design HRT, which in turn is somewhat dictated by the amount and nature of solid materials in the substrate and the solids retention time.

The effluent from the unit, comparable in pollutant levels to greywater, was added to the greywater tank where synthetic greywater was kept. Synthetic greywater was constructed as part of the experimental design (Table 16.5) (93). It was realized at the start of the experiment

Table 16.5
Experimental synthetic greywater (Source:(83))

Substance	Concentration (mg/L)
Dextrin	85
Ammonium chloride	75
Yeast extract	70
Soluble starch	55
Sodium carbonate	55
Washing powder (automatic non-enzyme)	30
Sodium dihydrogen phosphate	11.5
Potassium sulphate	4.5
Substance	Concentration (mL/L)
Settled sewage	10
Shampoo	0.1
Cooking oil	0.1
BOD (approx)	200

that there had to be a tight control on the inputs to the greywater system, otherwise it would be impossible to assess its performance. It can be argued that the synthetic greywater is not as variable as that found in a household greywater or that it is not entirely compatible with greywater chemistry of any particular region. However, experimental design and system definition dictates a uniform greywater at this stage of development of the UWS system. When the operational characteristics of the treatment plant are better understood then trials will be conducted using greywater from a sample of households in different regions.

The liquid from the above tank flowed under gravity to the greywater treatment system. The design developed at the University of Western Sydney (80, 81) placed an aerobic grease trap in the form of a vermicomposting treatment system prior to the sand filter (Fig. 16.7). This grease trap is designed to trap grease and other particulate matter (e.g. consider the material washed out of kitchen sinks). It also treats this solid material and degrades it to a usable product (compost). At the same time, the wastewater passing through the system is treated to reduce its pollutant load, particularly the suspended solids and oxygen demand.

A slow sand filter follows the grease trap in the treatment train. The intermittent sand filter utilizes fine media, in the range 0.2–0.4 mm (94–96). It has been noticed that all other factors remaining equal, splitting the flow into doses increases removal efficiencies and allows for higher hydraulic loading rates (97). A salient characteristic of individual and other small-scale systems is the great variability in the daily flow-rates in wastewater collection systems. Recirculation provides a means of enhancing the temporal stability of the wastewater. It increases the hydraulic loading without increasing the organic loading (97). Recirculation ratios range from 3.4:1 to 7:1 (recycled flow: forward flow) (98). Sand filters are effective in the removal of suspended particles with effluent turbidity consistently below 1.0 NTU (99–101); and 90 to 99+ percent reductions in bacteria and viruses (99, 101–104).

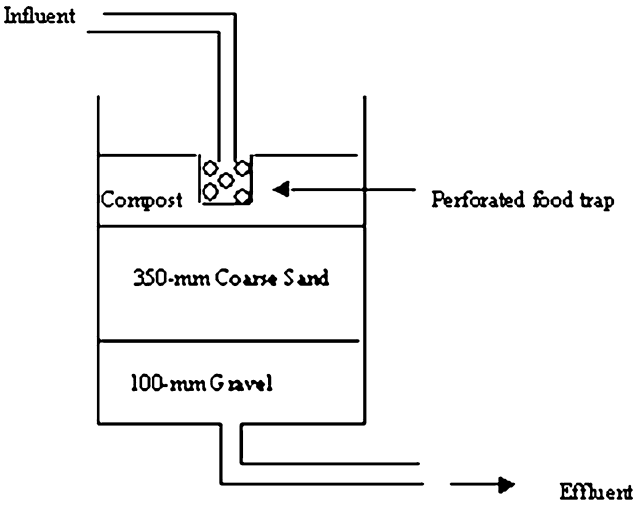

Fig. 16.7. Aerobic grease trap employing vermicomposting (Source: 83).

A multipass (recirculating) evaporation and treatment bed was designed for this experiment. A 0.6 m deep cell was constructed using concrete blocks to hold the filter media. An impermeable geomembrane liner was installed at the bottom and sides of the cell to prevent seepage out of the cell and infiltration into the cell. An overlap about 50 cm wide was provided to keep the lining in place while laying the media. Immediately above the geomembrane liner was given a layer of support gravel around the drainage pipe. Coarse sand is the main substrate and is 350 mm thick. The top surface was covered with 25 mm of mulch – critical for plant survival especially during the dry periods. It is necessary to keep the top surface flat to facilitate water level control, vegetation planting and growth and prevent the formation of stagnant pools.

The treatment bed was designed for a hydraulic loading rate of 200 mm/day. A recirculation ratio of 3:1 (recirculated flow from evaporation and treatment bed: slow sand filter effluent) was employed. Agapanthus, *Fescue Demeter* grass, white clover and corn were the plants grown in the evaporation and treatment bed. Attempts to grow tomatoes and pansies failed showing the inability of coarse sand to support the growth of such crops. Wastewater (recirculated flow and slow sand effluent) into the treatment bed was uniformly distributed over the design area using pressure distribution, as the level topography of the experimental site did not allow for gravity flow. Header pipes that include overhead sprinklers and a manifold were used to provide uniform wastewater distribution over the surface area of the evaporation and treatment bed.

The evaporation and treatment bed and disinfection components were not assessed in detail at this stage in the program. Initial assessment involved monitoring, reconfiguring and studying the performance of the aerobic grease trap and slow sand filter.

5.3. Sampling and Testing

In order to assess the system performance in terms of its applicability in the real world, the main parameters of interest focused on the pathogen reduction, nutrient conversion and pollutant removal. The main parameters of interest in this study, from a water treatment viewpoint, were the pathogenic content, dissolved and suspended solids, turbidity, dissolved oxygen, pollution potency due to oxygen demand, conductivity, pH, nitrogen and phosphorous content. For solids, material conversion and reduction in particle size and volume were of importance.

Raw blackwater samples and filtered blackwater from the VU were tested for different physical, chemical and microbiological parameters. The parameters tested at the UWS facilities on regular basis were TSS, TDS, turbidity, electrical conductivity, pH, DO, BOD_5, COD, ammonia -N, nitrate –N and phosphate. Procedures for sampling, sample handling and testing were followed as given in the Standard Methods and Procedures for Water and Wastewater Analysis (105). Where spectroscopic instruments used, the manufacturer's manual was followed. Microbiological analysis was done at the NATA accredited Australian Government Analytical Laboratory for *E. coli* and total coliform.

The same sampling and testing procedures were followed for greywater treatment as well. Samples were taken at the greywater source tank, effluent of AGT and SSF. Detailed testing of effluents of the remaining units in the system is scheduled for the next phase of the project and will be presented in the future.

5.4. Treatment Performance

The method of analyzing and describing the processes in the vermicomposting system can be complex and very time consuming. The approach adopted in this pilot scale study related to a simple input–output model, with less attention paid to the dynamics and processes within the treatment system. The advantage of this approach was that it reduced the complexity of the analyses and brought the project within an achievable timeline.

It was noted that irrespective of the weather conditions that covered all the seasons, the temperature within both VU and AGT, the temperature remained within habitable conditions for the worms. This is inferred to be the result of the channels created by burrowing worms through the solid matrix. This indicated a stable worm population in both vermicomposting units. The carbonaceous material in the matrix and the compost itself acted as insulating material in cold season, retaining the heat generated by the decomposition process.

The wooden material (mulch) added to VU to increase the bulk in assisting water drainage remained unprocessed by worms. In the AGT, an accumulation of unprocessed food organics reached an average depth of 60 mm by the end of the first week, because of high loading rate of $3.3\,kg/m^2$/week per person. An acclimatization period was introduced with a reduced loading rate ($0.88\,kg/m^2$/week), which gave satisfactory results. In both the VU and the AGT, the worm population soon established well, and the biodegradable materials, including kitchen waste and garden organics, were converted into vermicasts by the end of the composting period of 2 months.

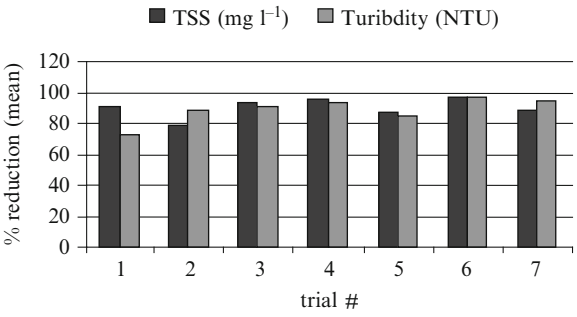

Fig. 16.8. TSS and turbidity reductions in VU during different trial runs (Source: (84)). *VU* vermicomposting unit.

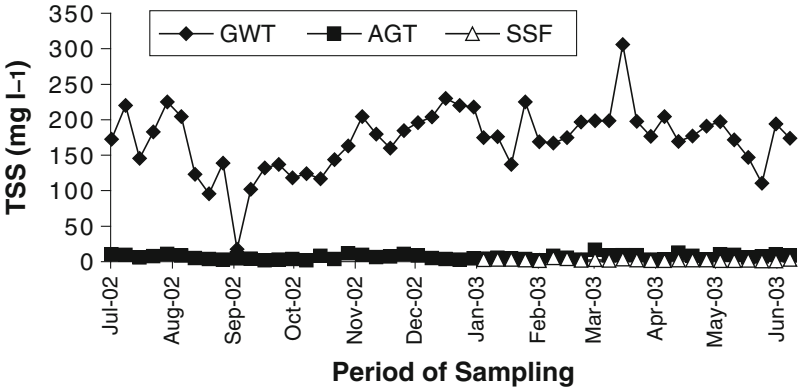

Fig. 16.9. TSS removal with time across the greywater treatment system (Source: (83)). *GWT* grey water tank, *AGT* aerobic grease trap, *SSF* slow sand filter.

An overall mean reduction of 89.32% in TSS was observed between raw blackwater and the effluent of the VU (Fig. 16.8). The TSS reductions reported from the AGT averaged at 96.2%, while SSF attained a further 98.7% reduction in TSS (Fig. 16.9). Values of the parameters in raw greywater tank (GWT) are also shown in the graphs.

The VU unit attained average 88.7% reduction in turbidity, the corresponding figures for AGT was 86.5% and for SSF 97.2%. The mean final values for TSS and turbidity, in the effluent from SSF, were 2.31 mg/L and 3.60 NTU. This was a reduction from initial mean values of 4,030 mg/L and 3,264 NTU, respectively, in the raw blackwater. The turbidity reductions were due partly to treatment in the three units as well as due to dilution in the greywater stream. Figure 16.8 shows the trend in reductions of TSS and turbidity in VU.

Most TDS readings concerning the VU increased between the raw blackwater and the treated effluent at an average 74.77% over the entire testing period. Conductivity showed similar trends to TDS, as expected. The increased TDS probably accounts for most of the change in conductivity. The mean conductivity value increased over the entire testing period

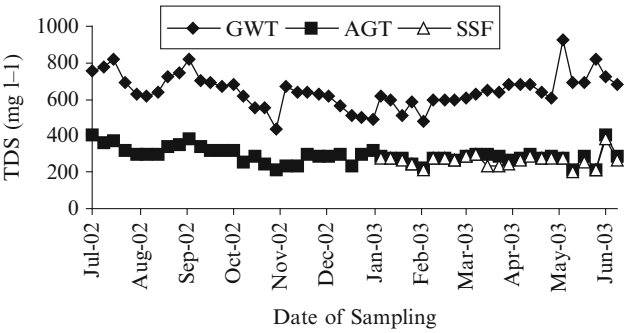

Fig. 16.10. TDS reduction in the greywater treatment system (Source: (83)). *GWT* grey water tank, *AGT* aerobic grease trap, *SSF* slow sand filter.

was 69.77%. The process of composting converts nutrients, such as nitrogen in the solid waste materials, into more soluble form (19). This could be one reason for the increased TDS. Nitrification of ammonia into nitrate also increases dissolved solids. Analysis of the influent and effluent gave an average reduction of 88.82% in ammonia levels (NH_3-N) and an increase of 636% in nitrate levels (NO_3-N) was noted. Phosphorous content, as reactive phosphate, also increased averaging at 182.58% over the entire testing period.

On the contrary, AGT achieved 54.8% reduction in TDS readings and SSF further reduced it by 59% (Fig. 16.10). Release of nutrients from AGT could be lower, compared to VU, due to smaller loading of putrescible organics. Also, the nitrogen content of material input to the VU is far higher than that of AGT.

The high increase in nitrate levels pointed to high nitrification rates promoted by aerobic conditions in the VU. This was confirmed by an average increase of 81% in DO readings across VU. Increase in the DO somewhat corresponded in terms of variational trends to reductions in BOD_5 values (Fig. 16.11). An overall average reduction of 97.49% in BOD_5 was reported between raw and final effluent across the VU, with a reduction of 70% in COD. Reductions in the organic pollutant content were consistent and gave results comparable between all the trial runs and to available data on greywater (31, 36, 73, 75, 106, 116). Increase in nitrate and phosphate levels is in agreement with studies elsewhere (116).

The greywater treatment units of AGT and SSF provided a COD reduction of 89.45% (Fig. 16.12) and BOD_5 reduction of 98.1% (Fig. 16.13) The ratio between COD and BOD_5 gives an indication whether or not the organic matter present in wastewater is readily biodegradable. The mean COD/BOD ratio of raw wastewater was approximately 2.85:1, which is well within the reported range (88), while that of treated effluent was 26.89:1. The ratio for AGT and SSF together was 1.7:1. This clearly means that the processes undergoing in the treatment unit conform to natural processes.

It has been observed that only 25–30% of organic matter is truly soluble, and its removal is through oxidation into CO_2 and H_2O. The remaining 75% of organic matter in wastewater is present in suspended form (107). It is argued that most biological treatment systems depend on gravity settling. In the vermicomposting matrix of VU and AGT, these suspended organics

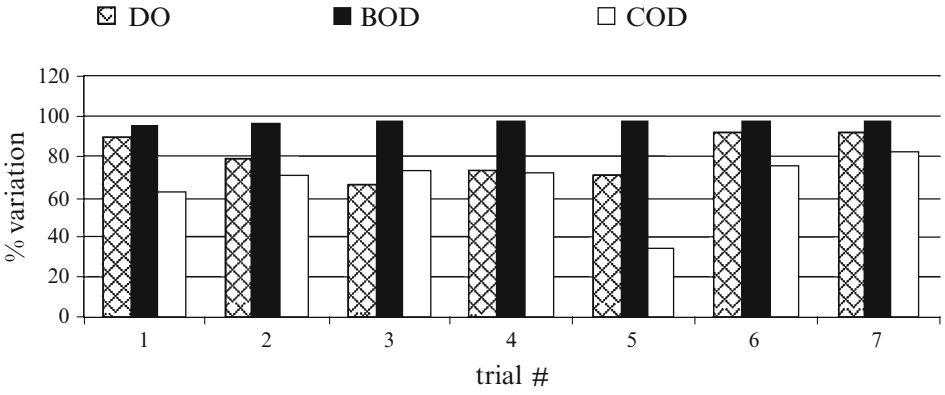

Fig. 16.11. Variations in DO, BOD_5 and COD across VU (Source: (84)). *VU* vermicomposting unit.

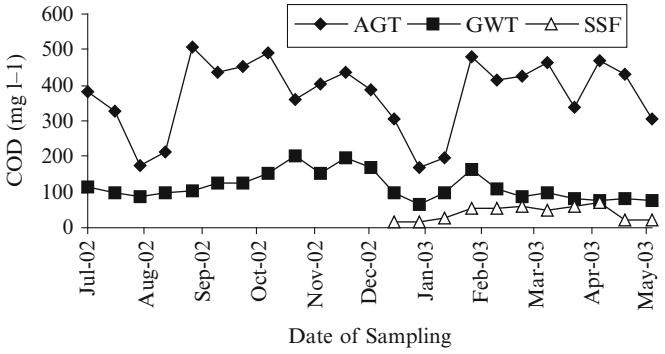

Fig. 16.12. COD reduction across the greywater treatment system (Source: (83)). *GWT* grey water tank, *AGT* aerobic grease trap, *SSF* slow sand filter.

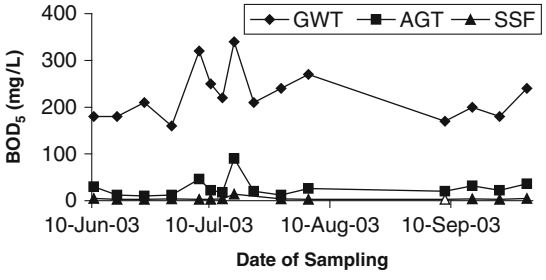

Fig. 16.13. BOD_5 reduction across the greywater treatment system (Source: (83)). *GWT* grey water tank, *AGT* aerobic grease trap, *SSF* slow sand filter.

become entrapped within the matrix. This compost possesses significant biosorptive and bioflocculative properties, which increases compaction and enhances the removal of solids and colloidal BOD. It supports a diversity of microorganisms, the biopolymer production of which is responsible for the above properties.

As for the SSF, data from literature points out that purification of wastewater occurs within the 20–30 cm media depth (108, 109). A filter bed 35 cm deep ensured a more consistent DO concentration throughout this unit.

Microbiological analysis for faecal coliform and indicator organism *E. coli* showed the most important aspects of the treatment of wastewater by vermicomposting. The VU reported an average of two magnitudes of reduction in both parameters, while this improved to four magnitudes of reduction in the effluent from SSF. An average initial reading of 1.8×10^7 CFU per 100 mL in faecal coliform was reduced to an average of 30 CFU per 100 mL reading with similar readings for *E. coli*. Microbiological spike tests with high pathogen content also produced consistent results. Other studies have also shown of reduction in coliform numbers by vermicomposting (117, 119, 120).

5.5. Conclusions

Tests on the working prototype system for 'whole-of-waste' approach in domestic waste and wastewater treatment revealed interesting and encouraging results for the wastewater stream considered. Blackwater and greywater at residential level received excellent treatment with vermicomposting technology in terms of physical and biological pollution. Further treatment with slow sand filter yielded better quality effluent. The design and construction of a low-cost aerobic greywater treatment system was intended to investigate new and innovative ways to treat and recycle wastewater at a low cost.

The purpose of this study was to provide a system that would be easy to maintain, flexible and be affordable for households and small communities in developing countries. The capacity of the aerobic grease trap and slow sand filter to remove the common pollutants present in domestic greywater, namely organic matter, suspended solids, dissolved solids, and nutrients were investigated under controlled conditions. The goal of wastewater treatment was to provide effluent that met certain criteria. These criteria provided a measure against which the results of the monitoring program could be compared.

Further research with remaining units of evaporation and treatment bed and subsequent disinfection should remove remaining chemical pollutants as well as making it safer in terms of biological pollution. The end product is expected to be potable and results of further tests will be presented in the future.

ACKNOWLEDGEMENT

The authors express their gratitude to the Microbiology laboratory at the Australian Government Analytical Laboratories (AGAL), Pymble, NSW for the microbiological analysis of the liquid and solid samples, the results of which formed, partly, the basis of this paper.

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17
Anaerobic Treatment of Milk Processing Wastewater

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CONTENTS

Abstract Anaerobic processes are widely used for the treatment of milk and dairy effluents. This technology has been subjected to significant development and real-scale application in the last few decades and offers highly favorable perspectives to accomplish a complete biodegradation of the components present in milk processing wastewaters such as sugars, proteins, and fats. Nowadays, anaerobic systems for the treatment of milk wastes can be operated successfully constituting an important contribution for the preservation of environmental quality.

1. INTRODUCTION

The sustainable development of a society requires a reduction of the dependency on fossil energy sources and a decrease in the amount of pollution discharged to the environment (1). Presently, there is a growing interest in alternative energy sources as a result of increased demand for energy coupled with a rise in the cost of available fuel. The needs and priorities of a sustainable society will lead to a situation in which, concerning the treatment of wastes, the possibilities of energy production will be as important as pollution control (1). The rapid industrialization observed in the last century has resulted in the generation of large quantities

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_17

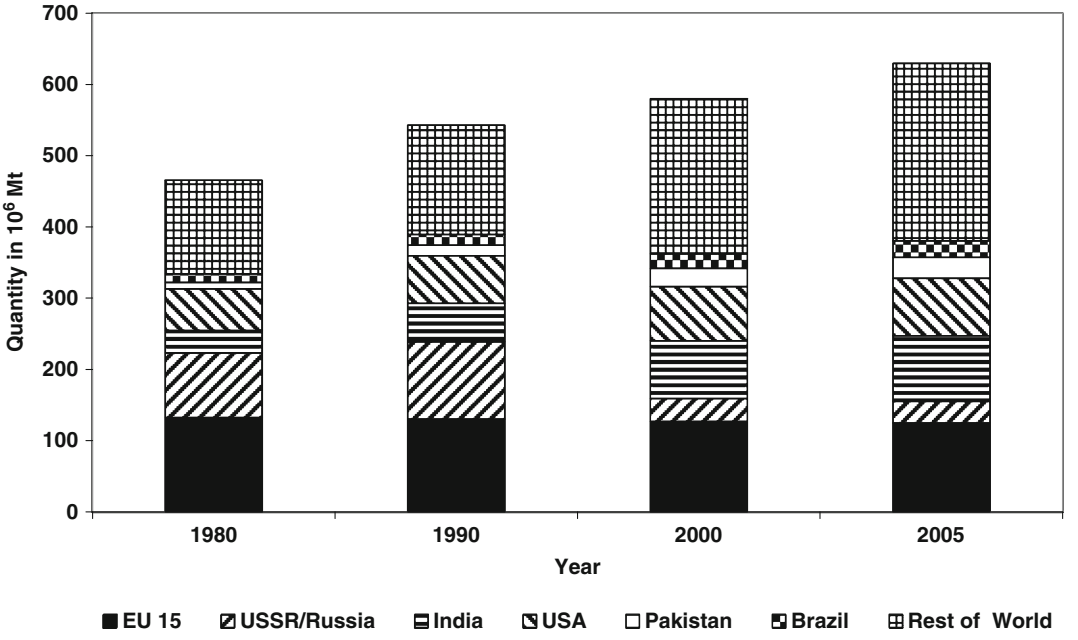


Fig. 17.1. Evolution of milk production in the world (4).

of effluents with high organic contents, which, if treated suitably, can result in a perpetual source of energy (2). Specifically, milk processing effluents have the potential to provide a carbon source in a form that may be converted to methane by anaerobic microorganisms, opening a possibility for a clean energy source together with pollution control (3).

1.1. The Milk Processing Industry

Milk processing industry has grown steadily in most of the countries of the world because of the continuous growth in the demand of milk and milk products. The world milk production has a growing rate around 2.8% per annum (4), see Fig. 17.1. The general tendency in milk processing industry in developed countries is toward the construction of fewer but larger installations and toward higher automation and process efficiency (5). Although there is a negative environmental impact associated with industrialization, this effect may be minimized and energy may be tapped by means of anaerobic treatment of the liquid effluents.

1.2. Major Environmental Problems Caused by Milk Processing Effluents

The environmental impact of industrial milk processing plants can be very severe, especially due to the discharge of large volumes of liquid effluents containing high concentrations of organic matter, nutrients and acid or alkaline products. Although most of these components are biodegradable, some of them such as milk sugars (mainly lactose) are readily consumed in the receiving medium, while some others such as proteins and, especially, fats are quite difficult to degrade (5). The substrates present in milk processing effluents feed algal blooms

that deplete dissolved oxygen, damage habitats for fish nurseries, and threaten leisure activities (6). Furthermore, the discharge of the untreated effluent directly onto land or a water body will not use the effluent's potential application as a source of clean energy (methane). The main problems caused by the liquid wastes from milk processing industries are summarized below.

1.2.1. Direct Discharge into a Water Body

The decomposition of the organic substrates will cause a severe depletion on the dissolved oxygen of the receiving waters, and it may lead to several important consequences such as anaerobic conditions and bad odor, death of certain branches of a water body, and consequent loss of original biodiversity. The effluents from milk processing industries exert a Chemical Oxygen Demand (COD) in the receiving media that is very high and also very rapid; about 50% of the COD is exerted within 24 h of discharge causing serious problems in the receiving water bodies.

The presence of proteins, phosphorus, and nitrogen based compounds will rise the nutrient level in the receiving water and potentially provide conditions for eutrophication. Quite often, the wastewater temperature is much higher than that of the receiving water medium, and this might cause significant alterations on the life conditions of certain species, not only because of the consequent decrease in oxygen solubility but also because some biological species are sensitive to temperature changes. The pH peaks, typical of milk processing effluents, may also alter the pH of the medium with consequences on the balance of chemical components in the water.

1.2.2. Direct Discharge onto Land

The use of milk processing effluents for irrigation is widely spread in underdeveloped countries, but this practice has many environmental disadvantages, e. g., the need for large areas and the effects on water resources due to run-off to water bodies and/or infiltration to groundwater reservations. If the fats content of the rejected effluent is high, then the effects of changing the characteristics of the soil most frequently increasing soil impermeabilization together with excessive organic and nutrient loading also need to be considered. Many small dairy factories dispose of their effluents by irrigation onto lands or pastures. Surface and ground water pollution is therefore a potential threat posed by these practices.

1.2.3. Treatment in Lagoons

The treatment of milk processing effluents in lagoons is also widely spread among third world countries. This treatment system requires the use of large areas although not as large as for irrigation. If the bottom and side surfaces of the lagoon are not conveniently impermeabilized, contamination of groundwater by infiltration may occur. On the other hand, if the lagoon is not covered, bad odors may rise, and the methane produced by anaerobic processes may escape to the atmosphere exerting a green house effect.

The effluents from milk processing industries contain predominantly milk and milk products riginated from process losses. Milk losses in an industrial milk processing plant may attain about 0.5–2.5% of the incoming milk, but in some cases, they might reach 3–4% (5). Although the correct action upon the process and the implementation of good management practices may decrease substantially the amount of milk losses to the effluent, there is a lower

limit of about 2.5 kg of milk lost per 1,000 kg of processed milk (7). Because of high water consumption, it is estimated that the volume of the discharged effluents is around 2.5 times the volume of the processed milk (3). Taking into consideration that the volume of world milk production in 2005 was about 630×10^6 mton (Fig. 17.1), these data indicate that large amounts of milk are lost and large volumes of milk processing effluents that require adequate treatment are generated.

The conventional methods for the disposal of milk processing effluents include the reutilization of certain fractions present in the effluent, for example, milk whey and lactose (8, 9). Coupled with recovery of some waste constituents or when this is not an economic alternative, several wastewater treatment processes may be used, mainly biological processes. Presently, in milk processing industries, a great percentage of wastewater treatment systems are aerobic, although in the last two decades, there has been a steady growth of anaerobic treatment applications (5).

2. THE EFFLUENTS FROM MILK PROCESSING INDUSTRIES

In order to understand the environmental issues of milk processing effluents, it is necessar to consider although briefly the nature of milk and the main characteristics of milk processing industries.

As a consequence of the development of milk preserving techniques, there was a rise in the production of milk products, which was not accompanied by the modernization of production processes and equipment. This caused a higher volume of product losses, spillages, frequent unbalancing in effluent treatment plants, and the surge of a severe problem in effluent treatment. It is not yet quite clear if the degree of modernization of the production processes and installations is related to the volumes of effluent produced (10–13).

2.1. Origins of Liquid Pollution in the Milk Processing Industry

Although the consumption of fresh milk has grown following economic development, a great part of milk utilization occurs after milk has been processed in several operations (heating, transformation in butter, cheese, yogurt, desserts, etc). Figure 17.2 presents the main operations involved in the production of several milk products. Within a milk processing plant, there may coexist the productions of several categories of products, such as, milk, cheese, yogurt, cream, ice-cream, frozen products, food ingredients, whey solids, lactose, etc. Typically, a conventional installation of a milk processing industry is involved simultaneously in the production of several of these products with significant seasonal fluctuations.

As can be seen in Fig. 17.2, the main operations in milk processing are clarification, pasteurization, and homogenization. Pasteurization and clarification do not affect the composition and the characteristics of the effluents that are relevant for effluent treatment processes. On the other hand, through homogenization, the fat globules are reduced from 1–15 μm, as present in raw milk, to 1–2 μm, in homogenized milk, thus forming a stable emulsion. This process is important in terms of effluent characteristics since it implies that a major percentage of milk processing effluents have their fat components in a form that is difficult to separate from the matrix (a stable emulsion) hindering the efficiency of the physical separation systems.

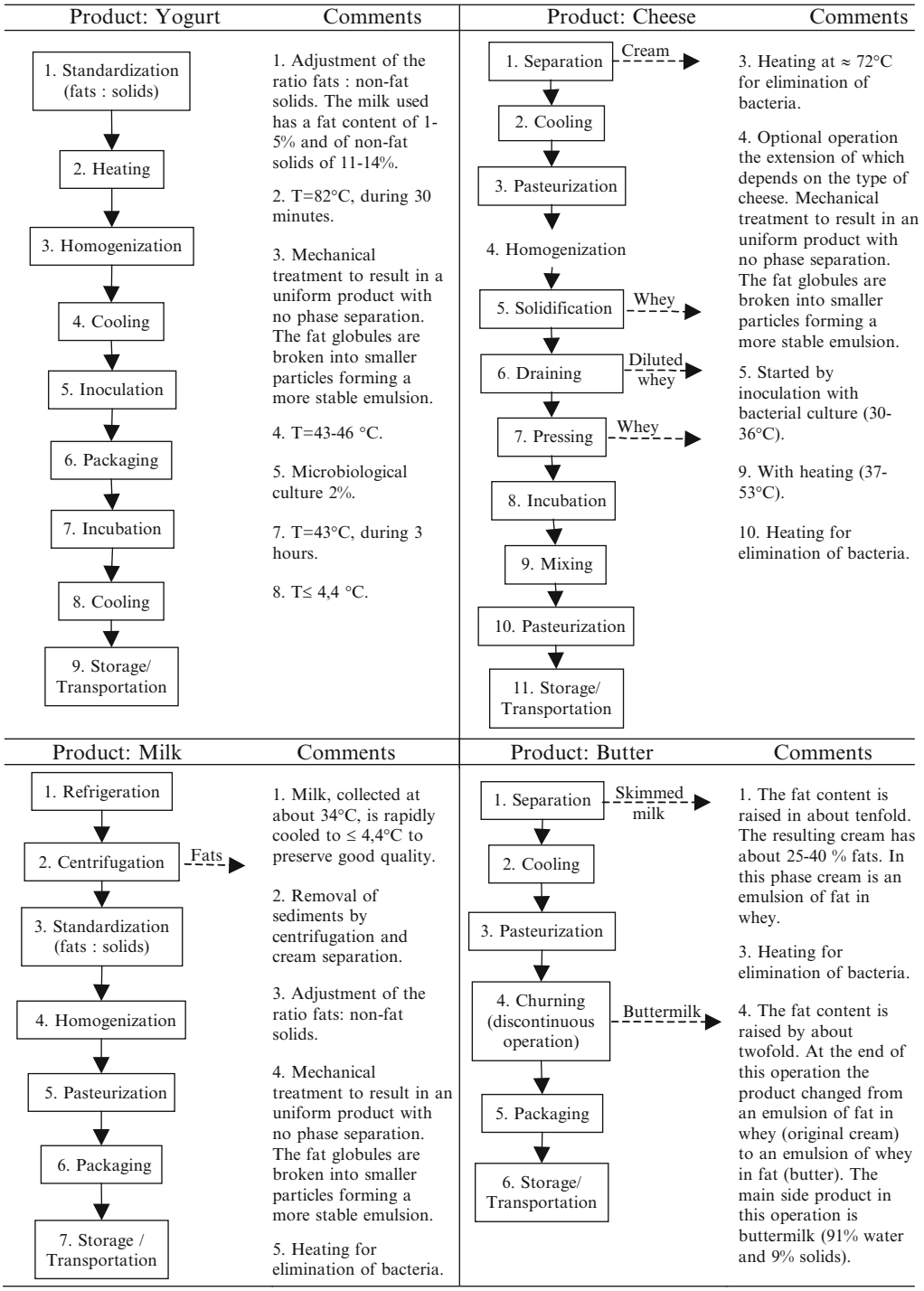


Fig. 17.2. Main operations in milk industries (adapted from ref. (13)).

As was referred above, an industrial plant for milk processing is involved in the simultaneous production of various products. The effluent streams originated from the different production lines are discharged in different moments often not coinciding with each other. This results in a final effluent that varies widely both in volume and in composition. Superimposed on these daily variations, there are also some weekly, monthly, and seasonal variations (12).

The effluents from milk processing industries may be divided in five main categories:

- Clean water from heating and cooling operations
- Wastewater with low pollution concentration, originated from the end of cleaning cycles
- Heavily polluted wastewater, contaminated with milk or milk products, originated from the beginning of cleaning cycles or from discharges of raw milk or milk products
- Domestic wastewater
- Rainfall wastewater

Due to the different characteristics of these effluents it is most convenient, for treatment facilitation, to segregate these effluents as some of them can be reutilized or discharged in water bodies, or in a municipal collector, after being subject to a low cost treatment.

The nature of the effluents generated in a milk processing industry is in general very similar reflecting the overwhelming influence of the loss of milk and milk products. Yet, each process generates a wastewater with a specific volume and composition. Table 17.1 presents typical origins of liquid pollution in milk processing industries.

2.2. Characterization of Effluents from Milk Processing Industry

The diversity of products and production techniques does not allow a formulation of the characteristics of a typical milk processing effluent. Nevertheless, some general characteristics may be identified (12, 16):

1. Presence of high concentrations of COD, biological oxygen demand (BOD), oils and fats as well as proteins and calcium.
2. Presence of bacterial cultures used in many production processes.
3. Great variability of flow and effluent characteristics as a result of discontinuous production and cleaning in all production processes.
4. Presence of acids, bases, and disinfectants from cleaning process used to inhibit bacterial activity in the production process.
5. Temperature above the normal ambient temperature due to use of hot water for cleaning.
6. Frequent concentration peaks due to the discharge or spillage of raw milk, intermediate or final products and chemicals.
7. High variability in all the above factors.

Table 17.2 presents ranges for some components of milk processing effluents. Milk contains a wide variety of proteins and sugars, casein and lactose being respectively the most important protein and sugar in milk. Fat is present in milk mainly as an emulsion of lipids, which are esters (i.e., triglycerides) of glycerol and long chain fatty acids – LCFA (Fig. 17.3). Long chain fatty acids are carboxylic acids with a hydrophilic acid group in one end and an alkyl hydrophobic group in the other end. The most important LCFAs in milk fat are oleic, myristic, palmytic, and stearic acids (Table 17.3).

Table 17.1
Typical origins of liquid pollution in milk processing industries (adapted from refs. (14, 15))

Operation	Pollution (kg BOD_5/m^3 processed milk)			
	(a)		(b)	
	Average	Range	Average	Range
Milk reception, churn washing, cleaning	0.26	0.11–0.66	0.31	0.13–0.80
Cooling raw milk, storage, washing tanks and pipelines	0.19	0.07–0.31	0.23	0.08–0.37
Washing tankers	0.25	0.10–0.40	0.30	0.14–0.48
Skimming, storing skimmed milk and cream plus cream pasteurizing	0.66	0.46–1.20	–	–
Churning and washing butter	0.46	0.25–0.80	0.55	0.30–0.96
Evaporating skimmed milk to low total solids	0.23	0.16–0.30	–	–
Evaporating skimmed milk to high total solids and spray drying	0.74	0.14–1.50	–	–
Roller drying	0.53	0.25–1.30	–	–
Pasteurization of milk and storage	0.29	0.10–0.54	0.35	–
Bottling pasteurized milk	0.11	–	–	–
Bottle washing	0.23	0.05–0.37	–	–
Pasteurizing milk, storage, bottling and bottle washing	0.85	0.49–1.70	–	0.12–0.65
Clotted cream	1.20	–	1.44	–
Cream pasteurizing and packing	0.79	–	–	–
Cheese making (hard pressed)	0.89	0.23–2.00	1.07	0.28–2.40
Cottage cheese (washed curd)	15.00	–		
Condensing fresh whey to low total solids	0.25	–	–	–
Condensing stale whey to high total solids	–	–	–	–
Condensate	0.25	–	–	–
Plant washing	0.75	–	–	–
Condensing sweetened separated condensed milk	1.40	–	1.68	1.44–2.04
Full-cream evaporated milk and canning	0.75	1.20–1.70	0.89	0.17–1.80
Ice-cream making and packing (estimated)	1.60	0.50–1.00	–	–

References: (a) (14); (b) (15).

Table 17.2
Characterization of milk processing effluents (adapted from refs. (2, 5))

	Units	Dairy factory	Dairy factory	Cheese factory	Yoghurt and buttermilk factory
pH	–	5.6–8	5–11	7.32	–
COD	mg/L	1,120–3,360	633–4,500	4,430	1,500
BOD	mg/L	320–1,750	241–2,600	3,000	1,000
Suspended solids	mg/L	28–1,900	–	–	–
Total solids	mg/L	–	710–5,100	–	–
VSS	mg/L	–	250–804	–	–
TSS	mg/L	–	240–943	1,100	191
Fats, oil and grease	mg/L	68–240	60–690	754	–

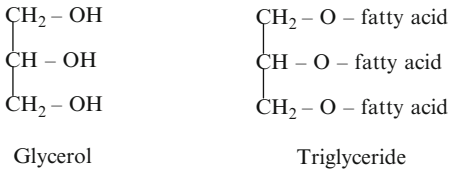


Fig. 17.3. Glycerol and triglycerides.

Table 17.3
Composition of cow milk (data from refs. (17, 18))

Component	% w/w	
Water	85.6–88.1	
Proteins	3.11–3.7	
Lactose	4.48–4.79	
Ash	0.71–0.75	
Non-fat solids	8.43–9.19	
Total solids	11.87–14.34	
Fats	3.44–5.15	
Main LCFAs in milk fats (% of total LCFAs)		
	Range	Average
Oleic (18:1)	25.27–40.31	31.90
Myristic (14:0)	15.56–22.62	19.78
Palmitic (16:0)	5.78–29.0	15.17
Stearic (18:0)	7.80–20.37	14.91

(n:m)n number of carbon atoms in acid chain; m number of double bonds.

For a given industrial plant, the concentration of milk and milk products in the effluent depends on the specific process, on the volume of processed milk, on the conditions and characteristics of the equipment, on the loss and waste reduction procedures, on the attitude of management and employees toward environmental problems and on water management practices.

Nowadays, in milk processing industries, the final destiny of liquid wastes is the area in the domain of water management that requires more improvements (12). Although there is in fact a tendency for a shift from many small plants toward fewer and larger installations, there are still many industries located in small rural areas where the access to adequate treatment systems is still a problem (19). In 1979, Brown and Pico (10), made a survey on the characteristics of the milk processing effluents in the USA and concluded that they could be treated in municipal plants. In the last 20 years, this perspective has changed considerably because of the raise in the costs of discharge imposed by the municipal authorities, and presently, the major part of the milk processing industries have on-site treatment installations for total or partial treatment (12).

Besides diluted milk and milk products, dairy processing effluents may also contain variable amounts of cleaning products. The biological oxygen demand exerted by these cleaning chemicals is typically under 200 mg/L, so it is not meaningful as compared to the organic load from milk and milk products in the wastewater. Although not significant in terms of organic load, these cleaning compounds contribute significantly to the refractory COD in the effluent and to the toxicity phenomena and low performance observed in some biological treatment systems. In cleaning procedures (presently CIP – Clean in Place systems are widely applied), there is a need for using disinfection and detergent compounds to inhibit biological growth in the production systems. A large variety of cleaning solutions may be used depending on the equipment, water hardness, and other factors. The most used chemicals for this purpose are nitric acid, phosphoric acid, caustic soda, and sodium hypochlorite, but in some processes, iodide acids and ammonium quaternarium compounds are also used (Table 17.4). Nowadays, due to environmental problems, the trend in the cleaning procedures is toward using more nitric acid and less of the preferable, phosphoric acid. Yet, since from a cleaning process point of view, phosphoric acid is preferable and it is not probable that its utilization will diminish further (12). Notwithstanding its chemical composition, the temperature of the cleaning solution is around 64–82°C, and thus most of the effluents have temperatures higher than normal temperature.

2.3. The Specific Problems of Cheese Whey

Whey is a liquid waste or a subproduct generated in the cheese making process by the precipitation of casein from milk using acid (resulting in acid whey) or rennet (resulting in sweet whey). Cheese whey represents about 80–90% of the volume of milk used in cheese production with the making of 1 mton of cheese resulting in about 8 mton of liquid whey. Whey contains more than 50% of the milk solids including 20% of the proteins and most of the lactose. The precise composition varies with the different manufacturing methods of casein and cheese products and with milk production season. Because of its very specific characteristics, cheese whey must be regarded on its own in what concerns the wastewater

Table 17.4
Main chemicals used in milk processing industries[a] (adapted from refs. (12, 16))

Compound	Main use	Effects on biological treatment systems
Caustic soda	Alkaline cleaner	FOG emulsification, pH raise, inhibition
Soda ash	Alkaline cleaner	FOG emulsification, pH raise, inhibition
Polyphosphates	Alkaline cleaner	pH raise, inhibition
Sulfated alcohols	Wetting agent, antiseptic, germicide	Inhibition
Alkyl aryl sulfonates	Wetting agent	Inhibition, foaming
Quaternarium ammonium surfactants	Wetting agent, sanitizers, foot washers	Inhibition, foaming
Complex phosphates	Emulsification, protein peptization, dispersion	Raise in P concentration, inhibition
Organic acids (acetic, propionic, citric, lactic, tartaric acids)	High temperature acid cleaning	Inhibition, pH drop
Inorganic acids (phosphoric, nitric, sulfuric acids)	High temperature acid cleaning	Raise in nutrient concentration, inhibition, pH drop
Acid salts	High temperature acid cleaning	Inhibition, pH drop
Sodium hypochlorite	Sanitizer	Inhibition
Iodine compounds	Sanitizers	Inhibition

[a]Other chemicals used in minor doses include: ammonia, trisodium phosphate, hydrochloric acid, hydroxyacetic acid, sodium metasilicate, hydraulic oils, propylene glycol, emulsifiers, antifoaming agents.

treatment or especially in what concerns its recovery potentialities. In case of small-scale cheese production plants, the problem arises of choosing between treatment in dedicated plant or the investment in modern technologies for recovery of valuable whey components (e.g., recovery of lactose and proteins, or spray-drying, bioconversion of lactose to ethanol or yeast biomass, among others). When analyzing the various options for the destination of cheese whey, it is important to consider that a plant for the recovery of whey or of whey products also generates effluents that require treatment before discharge (20, 21). Although these effluents are much less concentrated than whey, they have an organic content that is significant in comparison with other milk processing effluents (Table 17.5). In the circumstance were no recovery solution may be adopted, it is necessary to find a solution for the final destiny of cheese whey. Whey represents a potential energy source and presents several advantages if it is subject to anaerobic digestion because this solution offers an excellent approach from both energy/resource conservation and pollution control considerations. In general, from an economical point of view, the most convenient treatment solution is anaerobic digestion followed by an aerobic posttreatment in combination with the effluent from the main process. Cheese whey is highly concentrated, highly biodegradable, and has a low bicarbonate alkalinity (Table 17.5). These characteristics make it very difficult to treat whey in high-rate

Table 17.5
Characterization of whey and whey processing effluents (adapted from refs. (20–22, 26, 28))

Parameter	Units	Value (SD)
Concentrated whey		
COD total	mg/L	68,814 (11,518)
COD soluble	mg/L	57,876 (11,272)
TSS	g/kg	1.3 (1.14)
VSS	g/kg	0.94 (0.74)
TKN	mg/L	1,462 (263)
NH_4^+-N	mg/L	64 (31)
P total	mg/L	379 (49)
PO_4-P	mg/L	326 (64)
Effluent from whey processing plant		
T	°C	25.5 (2.5)
pH	–	7.0 (2.0)
BOD_5	mg/L	896 (310)
COD	mg/L	1,624 (556)
TOC	mg/L	546 (167)
TKN	mg/L	109 (80)
NH_4^+-N	mg/L	8.5 (6.3)
TSS	mg/L	261 (180)
VSS	mg/L	188 (149)
Whole whey		
Parameter	Units	Value
COD	g/L	60–70
BOD	g/L	35–45
Deproteinated whey		
COD	g/L	50–60
BOD	g/L	30–40
Acid whey (average composition)		
Humidity	%	94–95
Grease	%	0.3–0.6
Protein	%	0.8–1.0
Lactose	%	3.8–4.2
Minerals	%	0.7–0.8
Lactic acid and other products	%	0.1–0.8
Sweet whey (average composition)		
Humidity	%	93–94
Grease	%	0.3–0.5
Protein	%	0.8–1.0
Lactose	%	4.5–5.0

(Continued)

Table 17.5
(Continued)

Parameter	Units	Value (SD)
Minerals	%	0.5–0.7
Lactic acid and other products	%	0.1–0.4
Raw whey		
COD total	mg/L	57,010–66,040
COD soluble	mg/L	45,800–55,730
SS	mg/L	4,000–6,160
VSS	mg/L	3,840–5,960
NH_4^+-N	mg/L	30–120
PO_4^{3-}-P	mg/L	210–950
Protein	mg/L	4,000–7,000
pH	–	3.0–6.3

biological systems because of the formation of exopolymeric materials that are responsible for low sludge settleability and biomass wash-out (22). It is known that the use of up-flow anaerobic sludge blanket (UASB) reactors for the treatment of milk processing effluents and especially for cheese whey is severely limited by the difficulty in obtaining or keeping a good granulation in the anaerobic sludge (21, 23, 24). It has also been reported that the high level of carbohydrates in whey promotes the growth of acid forming bacteria but is detrimental to methane producing bacteria (25). Because of the rapid acidification of the whey, the treatment in anaerobic systems requires a two-phase process since the addition of extra alkalinity would represent an economical limitation for a one-phase process (26, 27).

Data from anaerobic large-scale installations for whey treatment are sparse, but it appears that loading rates of up to 10 kg COD/m^3-day are applied and COD reductions in long term operation may reach 75–85% (21). Gas yield varies from 35 to 38 m^3 gas with methane content of 60–62% per m^3 of treated whey (21), with an energy value of about 21 MJ/m^3 (29). Most of the reactors are operated at mesophilic temperature although thermophilic operation is also possible.

A number of operational problems have been noted for large-scale whey digesters including pH variations due to deficient equalization or production variation, odor, and detrimental effects of fat and calcium (21). Some strategies to overcome these difficulties were the addition of surfactants or nutrients to improve the performance of the anaerobic digestion (30, 31), the use of a two phase system to improve the stability of the methanogenic phase (26), the combination of thermophilic and mesophilic temperature in different phases of the anaerobic process, and the combination of anaerobic and physical–chemical process (32). Despite the problems, the success of the application of anaerobic technology for methane production from whey or whey processing wastes has been demonstrated (21). Although the major reactor configurations and operation strategies referred above have been evaluated at laboratory, pilot and full-scales, it is still not clear if any of these should be preferred over the others. In any case, the effluent from this anaerobic digestion process is generally not suitable for disposal in

natural water bodies, requiring some form of aerobic posttreatment to polish the wastewater before ultimate disposal (29).

2.4. Good Management Practices and Benchmarking

The analysis of the data in Table 17.1 suggests the classification of the discharges from milk processing industries in two main groups:

(i) Intentional discharges not avoidable because they are related with the process itself; and
(ii) Accidental discharges that can be avoided, which occur because of operating errors, and that are not indispensable for good production.

It is important to note that the largest volumes of liquid pollution from milk processing industries originate from intentional discharges, which are cleaning operations of transportation tanks and pipes, and cleaning of equipment whenever there is a halt in production. The large water volumes needed for these cleaning operations result in the volumes of the effluent being higher than the volumes of the processed milk (33). Other effluent sources (accidental discharges) are related to deficiencies in the functioning of the equipment or to operation mistakes that may cause discharges of milk or milk products. The losses from intentional discharges may be minimized with good management practices, while the accidental discharges should be eliminated. As an example of a good practice, the data from de Haast et al. (8) show that increasing the draining time of a 45 L vessel from 3 to 90 s will diminish the volume of milk loss in about 88%.

The progressive adoption of good management practices by milk processing industries will lead to a decrease in the loss of raw materials and to the decrease in water consumption. Consequently, the liquid wastes will become increasingly more concentrated, although with lesser volume, and for this reason even more adequate for anaerobic treatment. According to Bickers and Bhamidimarri (33) the application of good managing practices may decrease the average world value of 0.5–37 m^3 of effluent per m^3 of processed milk to 0.5–2 m^3/m^3. Assuming that all precautions have been taken to minimize the volume of the effluent to the lower possible limit, the load discharged to the receiving medium can only be reduced by an adequate treatment of the effluent (15).

The operators of milk processing industries should strive to reach the benchmarking values for the rejected effluents. Presently, the benchmarking volume for liquid effluent is around 1 m^3/mton of processed milk and a BOD load of under 2.5 kg BOD/mton of processed milk, the optimum being 1–1.5 kg BOD/mton processed milk. In the case of the effluents from cheese or butter production, the BOD concentration in the effluent should be lower than 2 kg BOD/mton of product (34). Table 17.6 presents some values for product losses in a well-run installation.

In order to reach benchmarking values, process control in terms of key parameters will allow identification of opportunities for reducing the product losses and improve process performance. Pollution prevention and control practices for liquid effluents in the milk processing industry include (34):

- Reduction of product losses by means of better production control
- Reutilization of product losses in lower grade products like cattle feed

Table 17.6
Benchmarking values for milk industries (adapted from ref. (34))

Operation	Product losses		
	Milk	Fat	Whey
Butter/transport of skimmed milk	0.17	0.14	n.a.
Butter and skimmed milk powder	0.60	0.20	n.a.
Cheese	0.20	0.10	1.6
Cheese and whey evaporation	0.20	0.10	2.2
Cheese and whey powder	0.20	0.10	2.3
Consumer milk	1.9	0.7	n.a.
Full-cream milk powder	0.64	0.22	n.a.

n.a. not applicable.
Note: data expressed as percentage of the volume of milk, fat or whey processed.

- Optimization of water use and of chemicals for cleaning
- Recirculation of cooling water
- Improvement of water recycling opportunities by segregation of the effluents from sanitary installations, process, cooling operations and condensation, cleaning of transport tanks, and rainwater
- Reuse of condensates for cleaning instead of fresh water
- Use of high-pressure nozzles to minimize water consumption
- Reduction of phosphorus based cleaning products

Additional good pollution prevention practices that can be suggested for milk processing industries are the use of filtration technology and separation of effluent streams (e.g., cheese whey streams).

As with other wastes, the logical way to deal with milk processing effluents is to include the following steps in a waste reduction program (35): (a) prevention, (b) minimization, (c) recycling, and (d) treatment. In the cases were the first three steps have been widely explored and applied, so as to avoid the double cost associated with loss of raw materials and waste treatment, the forth step is the one where more developments are to be expected. Within this framework, anaerobic digestion has a major role.

3. THE ANAEROBIC TREATMENT PROCESS

When considering the pollution of water bodies, there are several wastewater treatment options depending mainly on the type of industry, the effluent characteristics, geographical situation, land availability, and economic factors. In most of the common situations, a complete treatment system for milk processing effluents involves physical and chemical operations as well as biological processes. Biological processes are mostly used for removal of organic matter and since they are based on the maintenance of biological activity, the control of environmental and operating conditions assumes a primordial role. The microorganisms are used to convert the organic matter present in the effluent into several gaseous and dissolved products and new cell material. Since cellular tissues have a specific gravity higher than

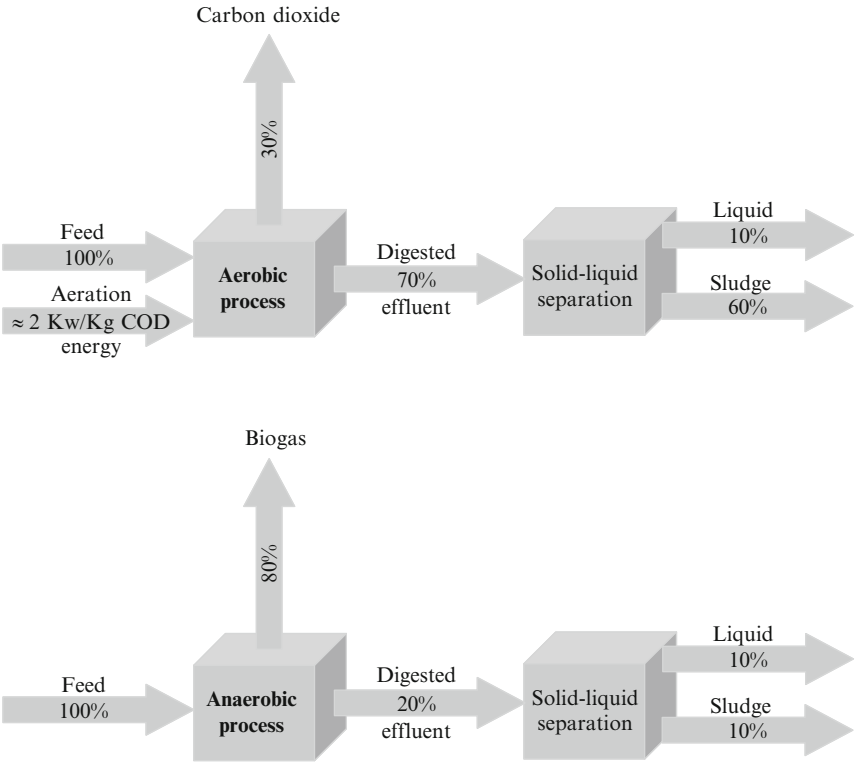


Fig. 17.4. COD mass balances for aerobic and anaerobic processes.

water, the resulting biomass may be removed by gravity settling and organic matter removal is effective only when this separation is achieved.

Biological effluent treatment processes may be classified as aerobic and anaerobic. Aerobic processes use oxygen to digest organic matter, while in anaerobic processes, organic matter is transformed in carbon dioxide and methane in the absence of free oxygen. Figure 17.4 presents the typical mass balances for both processes.

3.1. Description of Anaerobic Process

Anaerobic digestion is the biological degradation of organic or inorganic matter performed by a complex microbiological ecosystem in the absence of a free oxygen source. During the degradation process, the organic matter is converted mainly to methane, carbon dioxide, and biomass.

The compounds involved in anaerobic digestion can be classified as primary substrates present in the wastewater, as intermediate substrates and as final products. For a complex effluent like milk processing wastewater, the primary substrates can be grouped into oils and fats, proteins and hydrocarbons, with each of these substrates being present in the solid, colloidal, or soluble form. Hydrocarbons are easier to degrade than proteins, which in turn are easier than oils and fats (Fig. 17.5).

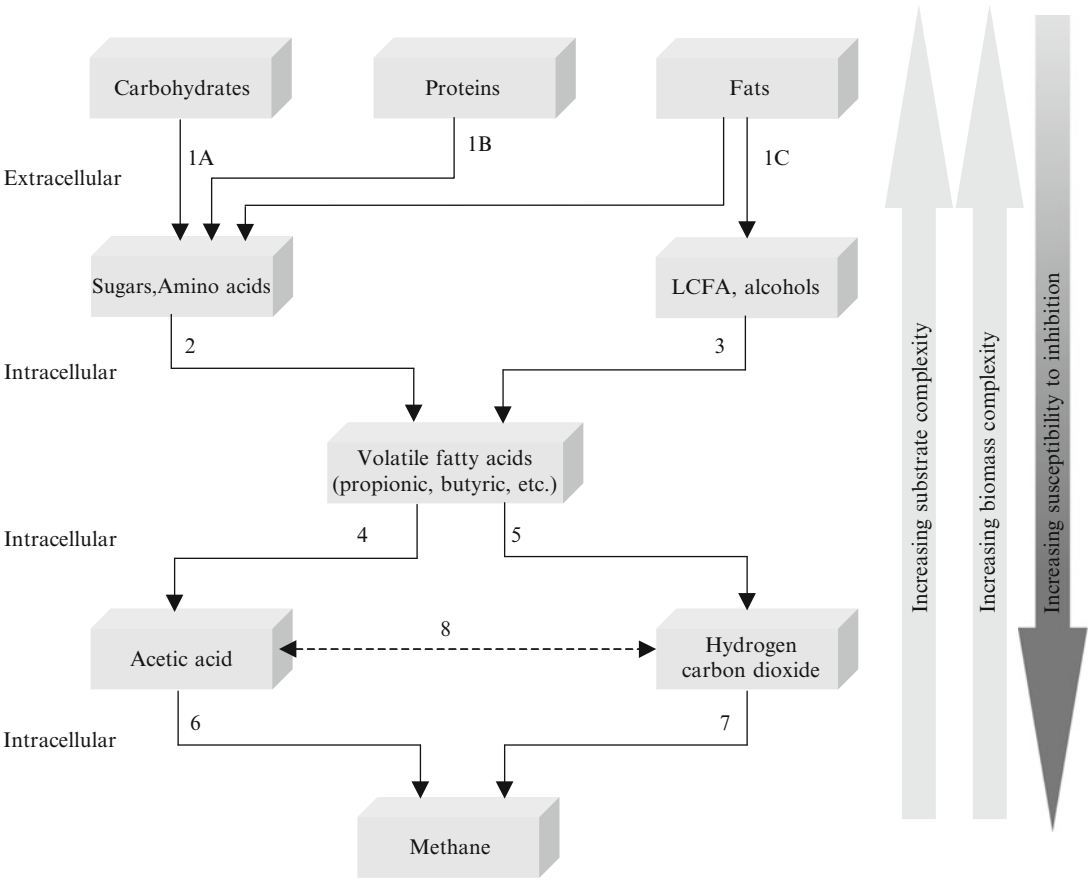


Fig. 17.5. Anaerobic process (see text for meaning of numbers).

The intermediary substrates may be a wide range of gaseous and soluble compounds and the final products are normally gases (methane and carbon dioxide) and bacterial cells.

Anaerobic degradation proceeds by way of a series of parallel and sequential processes performed by a variety of microbial consortia. The process may be divided schematically in four main steps performed by five distinct bacterial populations (Fig. 17.5, Tables 17.7 and 17.8).

The following explanations refer to the reaction steps in Fig. 17.5.

(i) Hydrolysis steps (1A, 1B, 1C): generally the anaerobic digestion of complex substrates starts with the hydrolysis, that is the liquefaction of complex organic compounds (lipids, proteins and polysaccharides) into simpler monomers such as soluble sugars, amino acids, peptides, and long chain fatty acids (LCFA). This is performed by means of extracellular enzymes (exoenzymes) secreted by a complex consortia of hydrolytic and acidogenic bacteria. Hydrolysis is necessary because microorganisms are not able to consume particulate or nonsoluble substrates since these are too large to cross the cell membrane. Therefore, the enzymes are released to the cell environment to break down these insoluble molecules into smaller units that can be processed

Table 17.7
Description of anaerobic process

Step (Fig. 17.5)	Description	Microorganisms	Specific features and environmental conditions
1A	Hydrolysis of carbohydrates	Acidogenic fermentative	Particulate carbohydrates are hydrolyzed to simple sugars
1B	Hydrolysis of proteins	Acidogenic fermentative	Proteins are hydrolyzed to amino acids Need for acclimation of biomass Production of ammonia which may inhibit the process Bacteria (*Clostridium*, *Streptococcus*, *Bacteroides*, *Selenomonas*, *Butyrivibrio*, *Fusobacterium*)
1C	Hydrolysis of fats	Acidogenic fermentative	Lipids are converted to long chain fatty acids (LCFA) Slower hydrolysis step which may avoid VFA accumulation Lipolytic bacteria (*Clostridia* and the *Micrococci*)
2	Fermentation of sugars and amino-acids	Acidogenic fermentative	Simple sugars and amino acids are degraded to volatile fatty acids (VFA) such as acetate, propionate and butyrate
3	β-oxidation of LCFA	Obligate hydrogen producing acetogenic	LCFA are converted to acetate and hydrogen Inhibited by LCFA themselves Need for acclimation of biomass Generally is the rate limiting step (slower than methanogenesis) Hindered by low solubility of LCFA H_2 produced severely inhibits the growth of these strains Only grow in the presence of a hydrogen-consuming partner *Mesophilic bacteria* *Syntrophomonas sapovorans* (*Methanospirillum hungatei* as syntrophic partner) *Syntrophomonas wolfei* (*Desulfovibrio* sp. as syntrophic partner) *Thermophilic bacteria* *Thermosyntropha lipolytica* (*Methanobacterium* sp. as syntrophic partner)

(Continued)

Table 17.7
(Continued)

Step (Fig. 17.5)	Description	Microorganisms	Specific features and environmental conditions
			Short rod thermophile (*Methanobacterium thermoautotrophicum* as syntrophic partner) LCFA is not degraded without the supplement of a exergonic cosubstrate (it is a energy-required process)
4 and 5	OHPA Oxidation of VFA (except acetic) to acetic acid and H_2	Obligate hydrogen producing acetogenic	Higher fatty acids are converted to acetate and hydrogen Only grow in the presence of a hydrogen- or formate-consuming partner *Syntrophobacter wolinii* (propionate decomposer) *Syntrophomonas wolfei* (butyrate decomposer) H_2 produced severely inhibits the growth of these strains Sensitive to pH drop
6	Acetoclastic methanogenesis	Acetoclastic methanogenic	Acetate is converted to CH_4 These bacteria do not adapt to LCFA LCFA inhibition Inhibition by ammonia (hydrolysis product of proteins) *Methanothrix* spp. (now, *Methanosaeta*) and *Methanosarcina* spp. Sensitive to pH drop
7	Hydrogenotrophic methanogenesis	Hydrogenotrophic methanogenic	H_2 and CO_2 are converted to CH_4 Free LCFA inhibition The bacteria play an important role in completion of anaerobic digestion, in accumulating H_2 and in maintaining low levels of H_2 Inhibition by ammonia (hydrolysis product of proteins) *Methanospirillum hungatei* and *Desulfovibrio* sp.
8	Conversion of CO_2 and H_2 into acetic acid and vice-versa	Homoacetogenic	

Table 17.8
Some reactions in anaerobic degradation of milk processing effluents (adapted from refs. (36, 37))

Substrate	Reaction	ΔG^0 (KJ)	Comments
Propionic acid	$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$	+76	Acetogenesis
Butyric acid	$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$	+48	Acetogenesis
Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COOH + 2H_2$	+9.7	Acetogenesis
Palmitic acid	$CH_3(CH_2)_{14}COOH + 14H_2O \rightarrow 8CH_3COOH + 14H_2$	+402.4	Acetogenesis by β-oxidation cycle
$CO_2 + H_2$	$CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$	−95	Homoacetogenesis, maintenance of low H_2 partial pressure
Acetic acid	$CH_3COOH \rightarrow CH_4 + CO_2$	−31	Acetoclastic methanogenesis
Hydrogen	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−131	Hydrogenotrophic methanogenesis, maintenance of low H_2 partial pressure
Methanol	$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	−312	Methanogenesis

inside the living cell. Hydrolysis is considered the rate limiting step in the degradation of particulate substrates (38), but the hydrolysis of soluble hydrocarbons, globular proteins (as present in milk), and lipids is quite fast.

Several different factors influence the production of the extracellular enzymes responsible for the hydrolysis of lipids, proteins, and sugars. It is known that the production of proteases, the enzymes responsible for protein hydrolysis, may be suppressed when easily degradable substrates are present in the reaction medium (39). The production of lipases, the enzymes responsible for lipid hydrolysis, may be stimulated by the presence of triglycerides or fatty acids (40). Some proteins are known to affect superficial tension and thus inhibit the bonding (adsorption) between lipases and the fat material subject to hydrolysis (41). It has been observed that in the absence of methane production, no lipid hydrolysis occurs (42, 43). At a pH lower than 6, the methanogenic bacteria are inhibited and no methane production takes place, and as a consequence, lipids will not be hydrolyzed. These findings are of special importance when considering the anaerobic treatment of wastewaters containing readily acidified/degradable substrates, such as sugars, together with complex substrates, such as proteins and fats, as it is the case of milk processing effluents.

(ii) Acidogenesis or fermentation steps (2, 3): this is the step where the dissolved compounds resulting from hydrolysis are converted to simple compounds. The substrates are mainly soluble amino acids and sugars, and the products are organic acids and alcohols, among other minor products (lactate, succinate, pyruvate, propionate, butyrate, valerate, acetate, ethanol, ammonia, H_2 and CO_2). In this process, organic compounds serve both as electron donors and acceptors (the process does not need an external electron acceptor), and this is the first step in anaerobic degradation resulting in energy production.

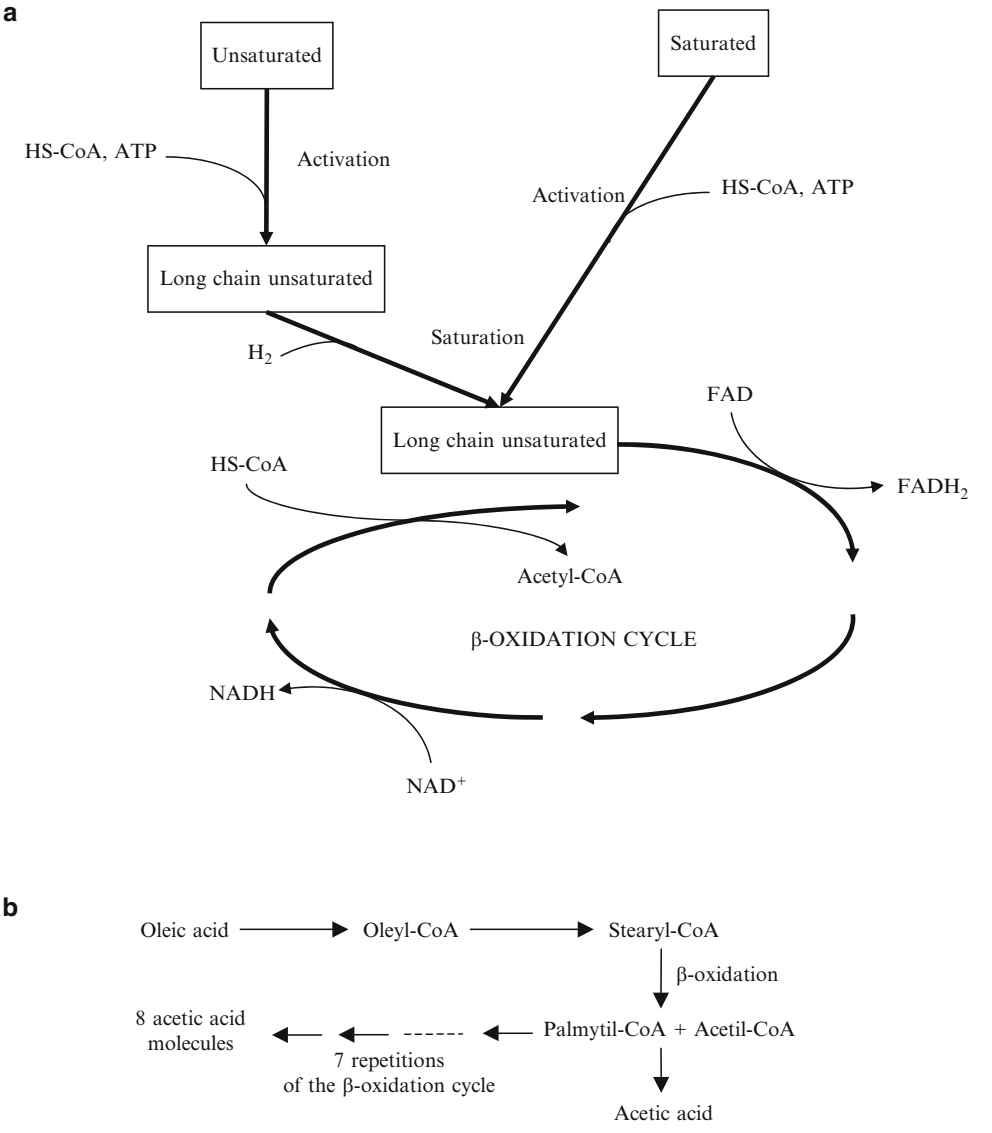


Fig. 17.6. Obligate Hydrogen Producing Acetogenesis (OHPA) (**a**) degradation pathway of LCFA present in milk effluents (**b**) example of β-oxidation cycle for oleic acid (C18:1).

Most generally, the acidogenesis of amino acids is performed via Strickland reactions, and acidogenesis of soluble sugars is performed via the Embden–Meyerhof pathway. The degradation of LCFA requires an external electron acceptor, and their degradation is closely linked to acetogenesis with obligatory hydrogen production (OHPA), see Fig. 17.6.

An important aspect of the degradation of amino acids is the production of NH_3 that affects the buffer capacity of the media and constitutes an essential nutrient (N). On the other hand,

NH_3 is toxic at high concentrations, but generally the level of proteins in milk processing effluents does not give rise to toxicity by this component. Furthermore, in the acidogenesis step, a sensible drop in buffer capacity may occur. Since many of the methanogenic bacteria, responsible for methane production from hydrogen and carbon dioxide, are very susceptible to low pH values, a drop in pH will result in a decrease in the consumption of hydrogen leading to a shift in the products of acidogenic bacteria. Some of the products (e. g. propionate) will be formed in higher quantities. In these cases, the acidogenic bacterial population may not be able to accommodate the amount of acids produced, and the process will deteriorate; the pH will decrease because of accumulation of volatile fatty acids (VFA), and ultimately methane production will cease. This process is called reactor acidification and should be avoided at all cost namely by the presence of sufficient buffer capacity.

(iii) Syntrophic acetogenesis and hydrogenotrophic methanogenesis steps (4, 5, 7): Syntrophic acetogenesis is the degradation of the fermentation products to acetate using bicarbonate or hydrogen ions as external electron acceptors. Syntrophic acetogenesis is a path for acetate production, in which substrate oxidation is made possible only by the simultaneous reduction of hydrogen ions or by the reduction of bicarbonate to formate. This process is coupled with methanogenesis from hydrogen (hydrogenotrophic methanogenesis) which keeps a low hydrogen concentration as required by the reaction thermodynamics (Table 17.7). The production of hydrogen during the oxidative reactions of acetate production is referred to as acetogenesis with obligate hydrogen production (OHPA) and the hydrogen depletion to produce methane is referred to as hydrogenotrophic methanogenesis. Hydrogenotrophic methanogenesis keeps the hydrogen partial pressure low enough for the thermodynamic conditions need in the acetogenesis to be accomplished (this process is also named interspecies hydrogen transfer).

From the products that result from hydrolysis of milk lipids, that is saturated and unsaturated LCFA and glycerol, it is the LCFA that cause most of the problems in anaerobic digestion. From a biochemical standpoint, glycerol causes no significant problems (44). All the LCFA are degraded via OHPA. The most important mechanism for this degradation is the β-oxidation (42, 45, 46), see Fig. 17.6.

In the β-oxidation mechanism (Fig. 17.6), carbon chain fragments with two carbon atoms are successively removed from the LCFA carbon chain in the form of acetyl-CoA which is then converted to acetate. According to Novak and Carlson (46), depending on the LCFA being saturated or unsaturated, the limiting step of their degradation is the activation by an enzyme molecule or the β-oxidation, respectively (Fig. 17.6). During β-oxidation, the LCFA are degraded by OHPA bacteria to VFA and hydrogen; even numbered LCFA are degraded to acetic acid and hydrogen and odd numbered LCFA are degraded to acetic acid, propionic acid, and hydrogen (45, 47). Since most of the naturally occurring LCFAs are even numbered, the main product of β-oxidation is acetate. Due to the hydrogen production associated with β-oxidation it is clear that the hydrogen consuming bacteria must be sufficiently active in order to keep a low hydrogen partial pressure. This step of the degradation of LCFA through β-oxidation is considered the most problematic in the anaerobic degradation of milk processing effluents. It has been reported that LCFA are not degraded unless this degradation is accompanied by methane formation (42). Thermodynamically, the reactions of the β-oxidation of LCFA are possible only if the hydrogen partial pressure is kept below

approximately 10^{-4} atm. Novak and Carlson (46) reported that the hydrogen produced during the degradation of LCFA inhibits this reaction (β-oxidation). The hydrogen depletion for methane production will lower the pH_2 allowing for LCFA degradation. Other authors have also reported that LCFA are inhibitory of their own degradation (48–50).

(iv) Methanogenesis steps (6, 8): acetoclastic methanogenesis is the breaking of acetate into methane and carbon dioxide by highly specialized microorganisms (e. g., *Methanosaeta genus* and *Methanosarcina genus*). About 70% of the methane produced in the anaerobic process results directly from the degradation of acetic acid (45). Some authors (51) have reported that reactors operating in extreme conditions may use an alternative pathway for methane production that is the syntrophic acetate oxidation to hydrogen and carbon dioxide by acetogenic or homoacetogenic bacteria in parallel with hydrogenotrophic methanogenesis. Methanogenic bacteria are the most sensitive bacterial group in what concerns the inhibition by LCFA (50, 52).

4. THE ANAEROBIC TREATMENT OF MILK PROCESSING EFFLUENTS

In the last years, there had been a growing interest on anaerobic systems for the treatment of milk processing effluents due to the known advantages of these processes for treating wastewater with high organic loads.

The adoption of high rate anaerobic technology by the dairy industries has faced several difficulties resulting from the complexity of biological degradation of some compounds present in the wastewater.

Since the main role of anaerobic process is the removal of organic matter and it does not remove significant amounts of nutrients, it is worthy to emphasize that anaerobic treatment is only a pretreatment and must be integrated in a sequence of treatment steps. The anaerobic treatment step is generally followed by an aerobic polishing step before discharge. This may be attained by installing a local aerobic activated sludge unit or in some cases, by the discharge to municipal sewers for later treatment in a municipal plant. In a broader view, anaerobic treatment should be regarded as forming a central part in the concept of Environmental Protection & Resource Conservation, EP&RC (53–55).

4.1. Benefits of Anaerobic Process for Milk Processing Effluents

The advantages and disadvantages of anaerobic vs. aerobic treatment have been widely discussed in literature (54, 56–60), but particularly for the case of milk processing wastewater, there are some specific considerations that deserve to be pointed out:

(a) Large quantities of energy are necessary for aeration (generally the oxygen consumption is over 3.0 kg/kg BOD_5) since milk processing effluents are very concentrated (frequently more than 2,000 mg COD/L, see Table 17.2), and also because they are rejected at high temperature and are readily biodegradable. In the anaerobic process, more than 90% of the substrate energy is retained in the produced biogas being easily recovered onsite and used as a fuel source.

(b) In case of milk processing effluents, which contain appreciable amounts of lipids, the difference in the maximum loads applicable with aerobic and anaerobic systems is of paramount importance. In conventional activated sludge systems (aerobic systems), only low loads can be processed and high biomass concentrations cannot be attained, e. g., about 0.1 kg BOD/kg TSS-day and about 5 kg TSS/m^3, respectively. In contrast, modern high rate anaerobic systems can

accommodate loads ten times as higher allowing for a reduction in reactor size of 60%. This result is a significant benefit considering that the higher costs in a wastewater treatment plant are construction costs.

(c) In aerobic systems, the bulky sludge that is frequently formed with complex fat containing effluents, such as milk processing effluents, is difficult to separate in the final clarifier (6, 14); also, the readily degradable sugars promote the growth of the less dense microorganisms. This leads to biomass loss, clogging of percolating filters (14), and loss of efficiency.

(d) In the case of purely aerobic systems, as is the case of activated sludge systems, it is necessary for the substrate to be in solubilized form so as to be assimilated through the bacterial cell wall. It is known that the anaerobic bacteria have a hydrolytic activity higher than aerobic bacteria. Milk being highly colloidal is not readily degraded by aerobic bacteria as it is by anaerobic microorganisms.

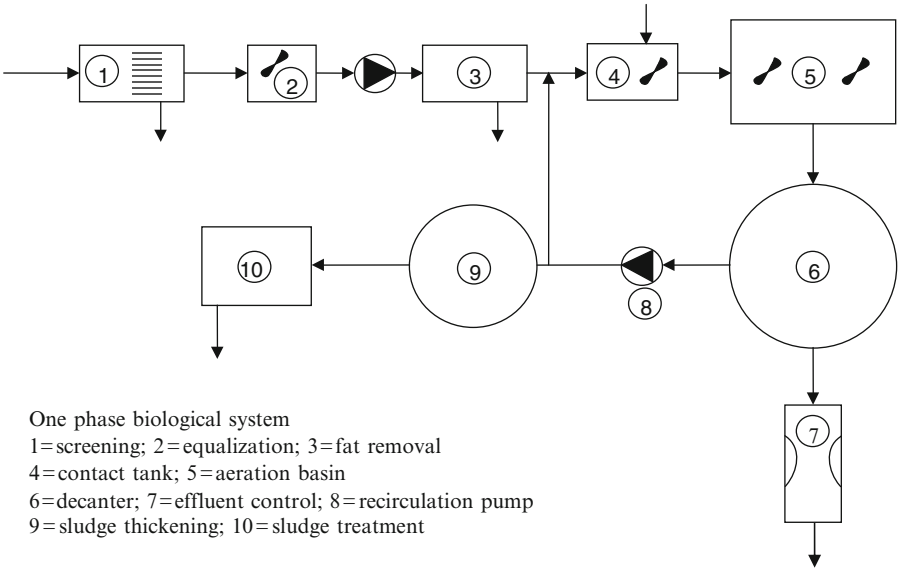
(e) One of the principal advantages pointed to the aerobic treatment systems is the fact that they are able to remove nutrients (phosphorus and nitrogen) coupled to the fact that presently large quantities of phosphoric acid are used as cleaning agents in milk processing industries. In general, the phosphorus content in milk processing effluents is very high and superior to what is necessary for an aerobic process. For this reason, even for aerobic systems, it will be necessary to have an additional polishing step for phosphorus removal, and this need will be more stringent if the legislation for phosphorus discharge will become more restrictive as is the present tendency. These considerations blur a significant difference between anaerobic and aerobic systems which in the past favored heavily the aerobic technology.

4.2. The Role of Anaerobic Systems in a Treatment Plant for Milk Processing Effluents

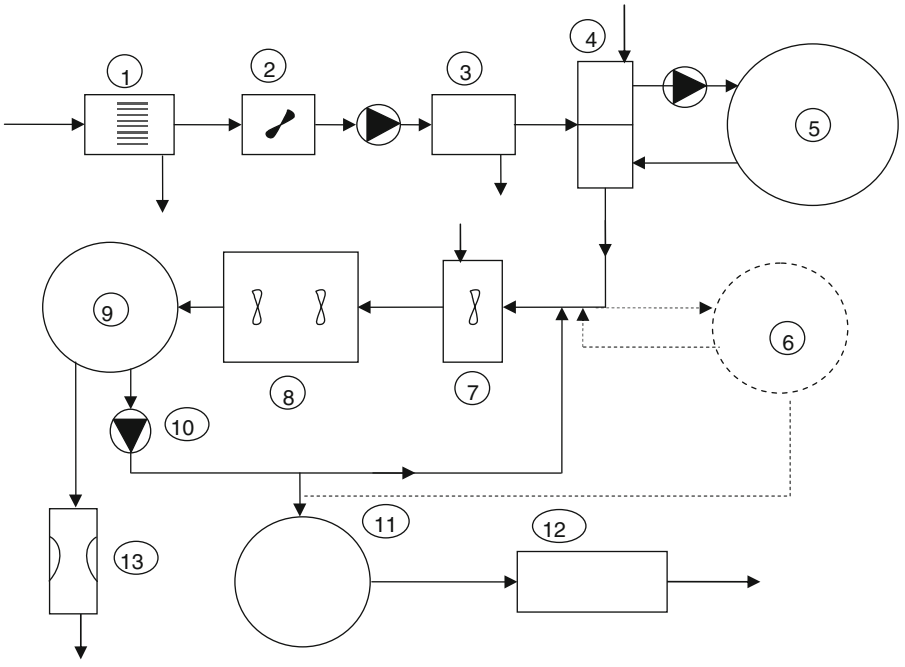
Nowadays in what concerns milk processing industries, the final destination of the liquid effluents is still the area in water management where more improvement is necessary (12). An on-site installation for the treatment of milk processing effluents may be designed to meet the specific demands of a particular wastewater and so provide economic benefits to the industrial plant as well as a reliable protection against organic overload in the municipal treatment plant. Basically, the methods for anaerobic treatment of milk processing effluents are similar to those used for domestic wastewater. Yet, the industrial application of anaerobic systems is more developed than municipal application, since the treatment of industrial effluents is mainly local as opposed to what happens with domestic wastewater. This allowed for the industry to develop and apply especially tailored systems for each case.

Current practice for the treatment of milk processing effluents varies considerably, since each plant rejects a different wastewater depending on the products and processes used. A treatment scheme applicable to a specific case might not be useful for another one and each particular situation calls for a treatability study.

The treatment system used for treating milk processing wastewaters depends on the degree of purification required and on the localization of the discharge point (direct or indirect discharge), but it is generally considered as having three phases: pretreatment, removal of organic matter, and final polishing. The most common configuration for a treatment plant in dairy industries includes gritting for removal of cheese clots and other solids, fat removal, equalization, biological treatment (in one or two phases), and final effluent decanting before discharge (Fig. 17.7). Parallel to this, some sludge handling system must be defined.



One phase biological system
1=screening; 2=equalization; 3=fat removal
4=contact tank; 5=aeration basin
6=decanter; 7=effluent control; 8=recirculation pump
9=sludge thickening; 10=sludge treatment



Two phase biological system
1=screening; 2=equalization; 3=fat removal
4=contact tank; 5=biological filter; 6=decanter 1; 7=contact tank; 8=aeration basin; 9=decanter 2;
10=recirculation pump; 11=sludge thickening; 12=sludge treatment; 13=effluent control

Fig. 17.7. Scheme of typical treatment plants for milk processing effluents (from (13)).

The nature of the preliminary treatment depends on the type of products of the milk processing industry. In general, the pretreatment of milk processing effluents consists of screening, equalization, neutralization and air flotation (for solids and fat removal). An important characteristic of milk solids is that they are not easily settleable, that is solids removal by sedimentation is not very efficient (61). Of the possible air flotation systems, CAF-Coarse Air Flotation or DAF-Dissolved Air Flotation, the DAF system is most convenient because the size of the fat globules after homogenization (around 1–2 μm) makes it difficult to separate the fat from the liquid matrix. In DAF systems, the air bubble has a diameter around 30–50 μm, and this smaller dimension as compared to CAF bubble size is essential for a good efficiency of flotation systems, since the smaller the air bubbles the easier their adherence to fat and/or solid particles. Furthermore, with the addition of coagulants and flocculants, very efficient separation is achievable. For these reasons, DAF has become the standard for fat oil and grease (FOG) removal in the milk processing industry. However, the coagulants and flocculants are fairly expensive and alter the composition of the retained fats and solids, so that reuse as animal feed is sometimes not possible. In what concerns the effects of pretreatment an important remark is that proteins are the only nitrogen source in milk processing effluents. If the pretreatment system employed removes most of the solids (mainly fats and proteins precipitated by acidification of lactose) the remaining wastewater may be nutrient deficient with regards to subsequent biological treatment (16).

Generally, it is more convenient to treat the effluents before they acidify (fresh effluents). Yet, most of the milk processing plants discharge their effluents for short periods of time in each day and in these circumstances, there is probably more advantage in equalizing the effluent, thus damping variations in flow and concentration and diluting harmful substances, than there is disadvantage in treating the acidified effluent. In what concerns cleaning solutions, it is convenient to store them and discharge them throughout the whole period of operation of the treatment plant.

As a general rule, equalization has always to be considered in a system for the treatment of milk processing effluents. The equalization basin smoothes the flow rate, the load, the temperature, and pH variations. If the equalization basin is too small, some fluctuations may occur in the anaerobic reactor. On the other hand, if the hydraulic retention time (HRT) in the equalization basin is too long, the prevailing anaerobic conditions will give rise to odors and also to acidification of some hydrocarbons and precipitation of organic matter resulting in a raise of the solids content fed to the reactor. In practice, the hydraulic retention time varies between 0.5 and 72 h, although the most used range is from 6 to 12 h implying a dimensioning of the basin from 1/4 to 1/2 of the total volume of the daily effluent. The tank should be well mixed and isolated and it should be covered to avoid odors. Generally, the effluent is discharged by means of an overflow or by means of a floating arm take off by gravity or bombing.

Equalization also permits some reaction time for hydrolysis and acidification of the wastewater and for neutralization of residual oxidants from cleaning operations. Through equalization, it is possible to reach a significant reduction in pH allowing economies in terms of chemicals for neutralizing purposes. The need for equalization varies with the type of anaerobic treatment system used. For low load anaerobic systems (e. g., anaerobic lagoons),

the need for equalization is minimal, since the high HRT in the treatment systems allows the smoothing of the variations in the characteristics of the wastewater. The average load systems (e. g., contact process) can endure some variations in temperature and pH, but they can suffer a decrease in their performance as a consequence of flow or load variations. The high rate systems (e. g., filters, UASB, EGSB, fluidized beds) are very sensible to variations in the feed, and thus equalization is a must, although some high rate anaerobic systems also use high recirculation ratios (3:1 or higher) in order to minimize the feed variations.

Although the maintenance of a neutral pH as well as the removal of solids before admission to the bioreactor are important, the separation of fats may not be indispensable if a complete biodegradation of these components may be attained inside the system. Anaerobic digestion offers highly favorable perspectives to the accomplishment of this objective. The main problem to be considered is the need of a long residence time for the fat particles so that they may be degraded, and also some care in avoiding the toxicity caused by the LCFA and the accumulation of fats in some parts of the equipment (especially in filters). Milk fat is very difficult to degrade in biological systems and special devices or operating schemes are necessary to favor the contact between biomass and fatty matter in order to attain a complete as possible degradation.

In the great majority of treatment plants, the posttreatment of milk processing effluents includes an aerobic reactor. In case this is an activated sludge system, it should be adapted for nitrification and denitrification of the effluent; that is, it must be adapted to remove nitrogen compounds. The dimensioning of any aerobic posttreatment step receiving the effluent from a first anaerobic reactor must include a nitrification phase and an anoxic zone for denitrification which is generally located at the head of the system.

4.3. Anaerobic Digestion of Effluent Components

Due to the effort made in the last decades to gain insight on the microbiological aspects of anaerobic digestion, nowadays sugars and proteins on their own are no longer problematic in the degradation of milk processing effluents. The careful application of the recommendations found in the literature (37) based on research and industrial application in the last years makes it possible to operate reliably any anaerobic reactor treating sugar and/or protein wastewaters. The major problem in anaerobic treatment of milk processing effluents lies in the degradation of the fats and/or their hydrolysis products, i.e., LCFA.

The problems found in the anaerobic treatment of milk processing effluents may be divided in two main classes (52):

1. Loss of biomass due to sludge wash-out; and
2. Inhibition of the microbiological activity of the biomass.

Within this framework, the role of each class of substrates present in milk processing effluents (sugars, proteins and fats) is discussed below.

4.3.1. Sugars

The hydrocarbons present in milk (mainly lactose) are the major source of the high organic load exerted by the effluents from milk processing industries. Lactose is a substrate that is

readily available to be degraded by the bacterial populations that are typical of high rate anaerobic reactors (62–66). Special care must be taken in case of very strong effluents, in which the rapid acidification of sugars coupled with eventual lack of alkalinity (e.g., whey or whey processing effluents) may cause reactor instability. In general, the following of the recommendations published in literature about the start-up of high rate reactors will ensure the success of these treatment systems in case of simple soluble substrates like milk sugars.

Although the degradation of milk sugars is relatively rapid and does not present problems (depending obviously of the following of the recommendations mentioned above), the truth is that the presence of sugars will aggravate the problems caused by other components of the effluent (proteins and fats). Due to the wide variations in flow typical of milk processing effluents these streams are directed to equalization basins having hydraulic retention times of 12–24 h. During this retention period in the equalization basin, there is an acidification of the effluent that may cause a decrease in pH below the isoelectronic point of casein (pH about 4.6). This will originate the coagulation and precipitation of the proteins and also the precipitation of fats by entrainment and adsorption to the protein particles. So, due to the conjugation of the high concentration of easily degradable sugars and the presence of proteins and fats, the effluents from milk processing have a high content of solids that may cause problems in their anaerobic degradation because, as it is well known, the lower the degree of substrate solubilization the lower its biological degradation rate. Furthermore, according to some authors (39, 67–71), the production of enzymes capable of degrading complex substrates, e.g., proteinaceous and/or fatty matter, may be hindered by the presence of easily degradable substrates such as glucose, amino-acids, or lactose. Contrary to these verifications, Hwu (48) referred that the degradation of oleic acid (the most common LCFA in milk effluents) was significantly enhanced by the addition of an easily degradable cosubstrate, e.g., butyrate or glucose.

Another problem enhanced, although indirectly, by the existence of high concentrations of sugars in the effluent is sludge flotation, due to the high biogas production coupled with the presence of complex substrates. The tendency of complex substrates, especially fats and LCFA, to adsorb onto the surface of the biomass strongly favors flotation and biomass wash-out by entrainment with gas flow. Petruy (72) reported that in spite of an extensive adsorption of milk fats onto the surface of biomass particles, no significant biomass flotation was observed because of the absence of biogas production.

4.3.2. Proteins

When high rate anaerobic systems were applied to the treatment of effluents with significant amount of proteins, the results obtained were not as good as for the case of effluents containing only simple and soluble hydrocarbons (54). Proteins are an important fraction of the polluting load exerted by the wastewater from many milk processing industries, and they may be degraded to VFA and subsequently to methane in anaerobic treatment systems. In these systems and generally in all biological systems, the presence of proteins in the wastewater has been linked with various problems:

– Scum forming leading to accumulation of organic matter (proteins and fats) inside the reactors and to the formation of floating layers on the upper part of the reactors (73–76) that lead to the

loss of biomass in the out flow stream. The forming of this scum layer and the accumulation of proteins and fats causes clogging and forces the system to frequent stops for cleaning (77)
- The growth of filamentous organisms that have a tendency to aggregate forming a bulky biomass with very poor sedimentation characteristics also leads to sludge flotation and biomass loss. van Andel and Breure (78) reported that anaerobic biomass fed with a protein rich effluent had a quite viscous appearance and very poor sedimentation behavior. These authors also noted that proteins would easily adsorb onto the biomass particles without being degraded
- Protein mineralization when in high amounts can originate levels of ammonia that are toxic to the anaerobic organisms. Yet in the case of milk processing effluents, the inhibition by ammonia has not been a relevant problem so far (12)
- In what concerns the anaerobic degradation of proteinaceous wastewater a problem of importance is the fact that proteins may eventually not degrade completely and will produce amines that give rise to bad odors. Some suggestions about this problem were given by Lettinga et al. (37) and by Verstraete and Vandevivere (79)
- As was mentioned above, the precipitation of milk proteins (casein) will lead to the formation of aggregates of solid material that are difficult to degrade (80). Protein denaturation (loss of tertiary structure) is a main mechanism of hindering their decomposition
- Several authors have reported that the presence of easily degradable substrates (sugars and hydrocarbons) will hinder the degradation of more complex substrates like proteins and fats (71). This result was observed even with bacterial populations previously adapted to protein degradation

The previous adaptation of the biomass to protein degradation seems a very important parameter for the anaerobic degradation of these substrates. Perle et al. (44) observed that anaerobic non-adapted cultures would not degrade milk protein (casein) but within 3 months of adaptation, the proteolytic activity (mainly extracellular) would rise significantly and protein degradation was very efficient. Apparently, this adaptation is needed only for the hydrolytic step since the nonadapted cultures were able to degrade amino acids resulting from protein hydrolysis (44). In principle, all bacterial groups are able to degrade substrates as simple as sugars and amino acids and hydrolysis is the step requiring a more specialized biomass. Some authors (72, 81) reported that protein degradation by granular biomass presented serious problems due to a large disparity between the removal from the liquid medium and the biological degradation. Even for removal efficiencies of 90%, the protein conversion to methane would not rise above 65% (72). This confirmed that the main initial mechanism of protein (and other complex substrates) removal is mainly from a physical–chemical nature (entrapment and/or adsorption) and not biological (82, 83).

It has been frequently observed that the adsorption of complex substrates onto the granular biomass will lead to the deterioration of granules characteristics (77, 84). The use of granular sludge UASB reactors for the treatment of wastewaters containing milk proteins requires special configurations of GSL separators; even then success is not fully guaranteed.

4.3.3. Fats

Neutral fats, that is, fats before the hydrolytic step, are prejudicial to anaerobic treatment mainly because they originate the flotation of the biological sludge and consequent loss of active biomass (48, 52, 74, 77, 82). It is known that fat has a tendency to ascend to the top of the reactors by flotation and also by entrainment with the liquid flow and/or the released

biogas (77). Even in low or medium load reactors and with different configurations of up-flow systems, the accumulation of fat layers in the top of reactors and in the biogas lines has been reported (16, 29, 77).

Since the first investigations reported on the effects of fats in anaerobic digestion (42, 85, 86), it has been clearly established that the LCFA resulting from lipid hydrolysis are responsible for inhibition of various microorganisms even when in millimolar concentrations. These effects have been largely reported in several works on anaerobic treatment applications for complex effluents (48, 52) and specifically for milk fat or milk fat components (44, 72, 83). Unsaturated LCFA are more toxic than saturated LCFA (46). Oleic (C18:1) is the LCFA present in larger quantity (25–40%) in the LCFA mixture resulting from milk fat hydrolysis (14).

Many studies have been made with the purpose of analyzing the effects of LCFA in the anaerobic process. One of the first studies was performed by Hanaki and coworkers (85) and lead to the conclusion that LCFA affect the obligate hydrogen producing bacteria and also the acetoclastic and hydrogenotrophic methanogens that are responsible for the conversion of the products from the β-oxydation of LCFA. As a consequence, the LCFA inhibit their own degradation causing a potential serious instability in anaerobic reactors treating fat containing effluents. Hanaki et al. (85) also observed that the LCFA would disappear from solution very rapidly and would accumulate in the solid phase (biomass) without being degraded. These observations have been confirmed by numerous investigations ever since (48, 52, 72, 82, 87, 88). In fact, these inhibition phenomena of the LCFA are closely related to adsorption of the substrate onto the surface of the biological sludge (48). Some authors (89, 90) attributed the LCFA inhibition to the physical interaction between the acids and the cell membrane of the microorganisms. This suggests that the biomass concentration in a reactor has a very important role, since inhibition will be dependent to some extent on the biomass/substrate ratio (91), contrary to what was reported by Koster and Cramer (86) and by Rinzema et al. (49). Some investigations on anaerobic digestion of substrates containing LCFA (52, 86) or containing milk fat (44, 72) have shown that these substrates are extremely toxic to the anaerobic bacteria leading to an immediate decrease in the methanogenic activity of the biomass to which they are added. It has been reported that once their toxic limit (MIC, see Table 17.9 is exceeded, these substrates lead to the death of almost the whole of the acetogenic

Table 17.9
Toxic thresholds for some LCFA present in milk processing effluents (adapted from ref. (86))

LCFA	T (°C)	MIC (mM)	MIC_{50} (mM)
Caprylic $C_{8:0}$	30	6.75	>10
Capric $C_{10:0}$	30	2.6	5.9
Lauric $C_{12:0}$	30	1.6	4.3
Mystiric $C_{14:0}$	30	2.6	4.8
Oleic $C_{18:0}$	30	2.4	4.35

$C_{x:y}$ means carbon chain with x carbon chain length and y double bonds, respectively. MIC_{50} means MIC at which 50% of methanogenic activity remains.

and methanogenic populations (49) and to a significant loss of the physiological activity of the cultures (44). The toxic threshold values reported in Table 17.9 may vary, depending on the operating conditions and especially on the type of sludge and the presence or absence of some metal nutrients, as for example calcium, which has the capacity to precipitate the LCFA, thus lowering their inhibitory action (52). Notwithstanding these variations, it is worthy to refer that any value of MIC or MIC_{50} published in the literature for a specific LCFA will serve merely as a rough indication, since there is a strong synergy effect that turns an LCFA mixture (as present in milk processing wastewaters) much more toxic than the individual acids per se (48, 86). On the other hand, it is also important to note that the bacteria do not respond to the bulk liquid concentration of LCFA, but that they respond to the concentration at the interface between the liquid and the biomass particle (52). The interface concentration is influenced by the mass transfer rate and so also by the biomass concentration in the reactor, and especially by the hydrodynamic characteristics of the system. This relation between the toxicity level of the LCFA and the reactors hydrodynamic conditions means that the response of continuous reactors to a LCFA load may not be estimated from results obtained from batch essays. On the other hand, it also means that the capacities of different reactor configurations to endure LCFA loads may vary substantially.

Since hydrolysis is not the rate limiting step in anaerobic digestion of complex fat containing effluents (42, 44, 85), it would be expected that results obtained for LCFA would apply to emulsified triglycerides as present in milk effluents. However, it is not possible to extrapolate the results obtained for individual LCFA or for LCFA mixtures to the emulsified fats present in milk. The fats, especially in the form of triglyceride emulsions, cause more severe problems than LCFA in the hydraulic functioning of biological reactors, namely sludge flotation and loss of active biomass through wash-out. Laboratory experiments indicated that triglyceride emulsions or milk fats severely impair the stability of anaerobic high-rate sludge bed reactors (52, 72, 92). The main problem detected was the strong wash-out (52) or the flotation of granular sludge (72) or of the flocculent sludge (92). It is noteworthy that sludge flotation and wash-out only occurred after serious overloading during the treatment of LCFA solutions (48, 52) whilst with milk fats or triglyceride emulsions it occurred for very low loads viz. 1–3 $kgCOD/m^3$-day (52, 72, 92). Sludge flotation due to adsorption of fats or LCFA onto the biomass particles is enhanced by the biogas bubbles adhered to the biomass (48, 52). In case of milk processing effluents, it must be stressed that the rapidly acidifying sugars produce high biogas flows in anaerobic systems.

The problem of sludge wash-out so frequently observed and reported for the anaerobic treatment of milk wastes is not adequately solved by the use of a special GSL separator design or by the use of a hybrid configuration like UASB + filter (52, 93) or UASB with several sieve drum separator designs (49, 52, 72). The sieve drum separator requires a brushing device, suffers from severe clogging and does not prevent the loss of small biomass particles. It is known that milk fat tends to cause degranulation of the granular biomass in UASB reactors (48). It has also been reported that the most important bacteria for the LCFA degradation are not amenable to granulation (48), and therefore are easily washed out of the reactor system even with sieve drum separators. The packing layer on the top of hybrid UASB/filter reactors can aggravate the problem of biomass wash-out; great part of the organic matter is retained in

the packing leading to the clogging of the filter and accumulation of biogas under it. On the other hand, the occurrence of channeling through the filter medium has been observed, leading to severe biomass wash-out, but the most significant drawback is that the packing layer acts in retaining the biomass on the top of the reactor and impairs its return to the sludge bed (52).

Toxicity by LCFA during anaerobic treatment of milk processing and dairy effluents is commonly a result of inhibition of acetogenic and/or methanogenic bacteria, this two bacterial groups being the slowest growing members of the anaerobic food chain. When treating complex wastewaters like milk processing effluents, inhibition of the extracellular enzymes responsible for the hydrolysis of polysaccharides, proteins and fats may also occur.

4.4. Special Considerations for Anaerobic Treatment of Milk Processing Effluents

The effluents from milk processing industries, like other complex fat containing effluents, form a particular class of wastewaters when considering anaerobic treatment. This is a consequence of the characteristics of the effluents, that is, the simultaneous presence of sugars, proteins, and fats, as discussed above. In view of the particularities of the wastewater and the research developed in the past few years, it is important to discuss the relations between phenomena like biomass adaptation, adsorption of complex substrates onto biomass surface, mass transfer limitations, and inhibition when assessing the application of anaerobic treatment to milk processing effluents.

Adaptation is the acquisition, by a microbial community, of a capacity to degrade substrates that before that adaptation were toxic or inhibitory. In microbiological populations, adaptation to a specific substrate may be a physiological response of existing bacteria, modifying their cells to better cope with the toxic compounds, or more likely it will result from a shift in the microbial population because of the growth of new bacteria that are more tolerant to the toxicity. Obviously in the absence of adaptation, inhibition of the biological process will be observed.

It is generally accepted that biomass gradually exposed to growing concentrations of a toxic or an inhibiting substrate, will develop a resistance to that toxicity or inhibition. An important condition to be met, in order to achieve a good result in the treatment of milk processing wastewater, is that the viable biomass is sufficiently adapted to the substrate. In this way, adaptation is a key factor in the application of anaerobic treatment to milk processing effluents which are potentially toxic effluents (56). Several studies have supported the notion that previous adaptation of the biomass to the substrate is a beneficial or even essential condition for the well succeeded operation of anaerobic treatment systems applied to milk or fat containing effluents (94, 95). Biomass adaptation allows the attainment of a higher treatment capacity (96) and acts as a defense against inhibition effects (97).

In case of milk processing effluents, the potentially inhibitory substrates are proteins, mainly casein, and LCFA, mainly oleic acid (85). Perle et al. (44) reported that the inhibition problems caused by milk lipids and by casein were the underlying reasons for the low performance of anaerobic systems used in the treatment of milk processing effluents. These authors also observed that casein remained undegraded by anaerobic cultures not adapted to this substrate. On the other hand, adapted cultures were very efficient in degrading casein as well as the amino acids formed in this degradation where amino acids had null inhibitory action. Milk

fats were considered the most important vehicle for the inhibition of methanogenic activity and general physiological activity (as measured by ATP levels) that has an immediate, but lasting, influence in reducing biogas production in the anaerobic cultures to which they were added. Based on these results, Perle et al. (44) recommended that anaerobic systems should be used in the treatment of milk processing effluents only when the lipid level was under 100 mg/L.

Toxicity or inhibitory effects of a certain substrate is generally discussed in terms of the ratio of inhibitory substrate/biomass (98). If the substrate does not adsorb onto the biomass, then the inhibition effects will be independent of the biomass level and will mainly depend on the substrate liquid concentration. On the other hand, if adsorption is determinant in the inhibition process, then inhibition effects will decrease with decreasing biomass level.

Koster and Cramer (86) reported that although adsorption has an important influence in the inhibition mechanism of LCFA in anaerobic treatment, the inhibitory effect of LCFA in granular sludge was more related to the volumetric concentration of the inhibitor (LCFA) than to the amount of inhibitor per unit of biomass. Rinzema et al. (49) confirmed the importance of the volumetric concentration, as compared to the inhibitor/biomass ratio, and concluded that acetotrophic methanogenic bacteria would not adapt to LCFA when exposed either to toxic or lower than toxic concentrations. The recovering of the activity was only possible through the development of a new population with the capacity of degrading the LCFA by the β-oxidation mechanism. In industrial scale reactors, this means that a "poisoned" reactor will need recovery period of about 1–2 months, and that recovery must be initiated with very low LCFA concentrations. The observations of Rinzema et al. (49) are in accordance with results from Yang and Anderson (75) who after 150 days, observed no adaptation effects in a granular biomass fed with ice-cream effluent. Contrary to this, the works from Hwu (48), Nadais et al. (91), and Alves et al. (99) show that adaptation of anaerobic biomass to milk fats and/or LCFA is possible and highly desirable in the operation of high-rate systems. The works from Nadais et al. (91) show that after a 2 weeks period, significant changes may be detected in the anaerobic flocculent sludge capacity to degrade milk components. Morgan et al. (100) also observed that the modifications in the biological population of a flocculent biomass in high-rate anaerobic reactors fed with ice-cream effluents would go on for several weeks. The authors concluded that the complex nature of this substrate with high protein and fat content was more determinant to the natural selection and to the development of microbial ecology than the type of anaerobic reactor (contact, up-flow filter, UASB, fluidized bed).

The length of the adaptation period is still to be determined and certainly depends on a number of factors like

- Initial biomass characteristics and diversity
- Temperature, at thermophilic temperatures biomass has doubling times much higher than at mesophilic temperatures
- Effluent characteristics

When analyzing the recent works on the anaerobic treatment of complex fat containing effluents, it becomes clear that the main goal in the adaptation of a microbial population to milk processing effluents will be the development of a steady population of syntrophic β-oxidizers capable of overcoming the problems associated with milk fat/LCFA.

Hwu (48) observed that such syntrophic β-oxidizers where not present in granular aggregates and were present in the fine particles washed out in high-rate granular sludge bed systems. For this reason, the use of biomass recirculation was suggested in order to enhance the β-oxydation of LCFA in anaerobic granular sludge systems.

Keeping in view the initial physical–chemical removal mechanism observed in the anaerobic treatment of milk effluents and also the surface mechanism of the inhibition by LCFA, it is obviously important to consider the role of adsorption in anaerobic treatment of milk processing effluents.

The biosorption process is defined as the uptake or accumulation of particles and/or chemical substances by biomass. Acknowledgement of the importance of adsorption phenomena in the anaerobic treatment of complex fat containing substrates has increased parallel to the understanding of the removal mechanisms in wastewater treatment systems. Nowadays, it is well established that adsorption of specific compounds (e.g., proteins, lipids, and long chain fatty acids-LCFA) to bacteria and sludge is a phenomenon that can strongly affect or even completely control the performance of an anaerobic reactor (48, 81, 82).

The adsorption phenomena in the anaerobic treatment of milk processing effluents has been extensively reported in the literature both on flocculent (85, 87, 101, 102) and on granular biomass (48, 81, 82, 87, 103, 104). It was observed that the initial removal of organic matter (about 50% of the initial concentration) from the liquid medium by the biomass was very fast and that pseudo-equilibrium was reached within a short period (1/4 to 24 h) of contact time. An eventual subsequent stabilization reaction would proceed much slower and uniformly. Contrary to what has been reported for other substrates (105, 106), the adsorption of LCFA and milk fats onto anaerobic biomass appears to be nonspecific (89) that is not dependent on bacterial species. Also, in contradiction to what was observed with other substrates (107, 108) the adsorption of fats/LCFA onto anaerobic biomass is influenced by the size of the biomass aggregates being higher for the smaller particles (87, 104). Finally, the adsorption uptakes of fats and LCFA onto anaerobic sludge are higher for higher initial concentration of these complex substrates (48, 101) which was not verified for other substrates (108). So the adsorption of milk fats and/or LCFA onto anaerobic biomass (flocculent or granular) seems to be governed by mechanisms that are somewhat different from those that determine the adsorption of other substrates (mainly non-fatty substances). In a study of anaerobic treatment of milk effluents, Schoepfer and Ziemke (102) found that sludge that had not been fed for several months appeared to loose most of its adsorption capacity. Hwu (48) found a higher adsorption of a mixture of LCFA onto granular sludge adapted to fat containing wastewater in comparison with the adsorption of oleate onto nonadapted granular sludge and ascribed this difference to a synergistic effect of LCFA, but also considered that the sludge adaptation might be of influence. According to Hwu (109), the adsorption of LCFA onto anaerobic sludge is a biologically mediated phenomenon.

This adsorption of milk fats or their hydrolysis products (LCFA) onto the anaerobic granular sludge has been reported to cause disintegration of the granules (23, 72) and to bestow a gelatinous appearance on the sludge (23). Most of the acetogens are hydrophobic (110) and since LCFA act as surfactants in the pH range prevailing in anaerobic reactors, they lower the liquid surface tension thus impairing aggregation of hydrophobic bacteria.

According to Lettinga et al. (37), sludge bed reactors do not necessarily require the use of granular sludge. It is possible to operate these reactors using flocculent sludge provided that it has good settleability. It is known that in the case of milk processing effluents, the limiting step is the hydrolysis of particulate substrates (38) or the degradation of LCFA to short chain acids (85). For these reasons, it is natural that flocculent sludge being predominantly acidogenic (54) will result in a better degradation of complex substrates as compared to granular biomass which is predominantly methanogenic (54). It has been verified (48) that the bacteria that degrade oleic acid in granular sludge bed reactors were found in the fine particles and would not form granules. The reported order in oleate conversion rates for anaerobic biomass was (48): granular < dispersed < washed out. This may be a reason for the poor results published in literature for the anaerobic degradation of milk fats in granular sludge reactors (75, 81). It is also known that UASB reactors inoculated with granular sludge bare lower solids concentration in the feed than flocculent sludge reactors. In view of the high solids content that may be present in acidified milk processing effluents, flocculent biomass appears as the most adequate for the use in anaerobic treatment of these wastewaters.

4.5. Application of Anaerobic Technology to Milk Processing Effluents

As it is well known, an important class of complex wastewater is those effluents containing appreciable amounts of solids and fats/LCFA as it is the case of milk processing effluents. Extensive research has been performed on the anaerobic treatment of these effluents in laboratory, pilot and full-scale installations (48, 52, 72, 82, 92, 111–113). The similarities and differences between the main types of high-rate reactors were extensively covered by Hickey et al. (24) and by Weiland and Rozzi (114). Anaerobic digestion is the most suitable option for the treatment of milk processing effluents. The presence of biodegradable compounds coupled with the advantages of anaerobic process over other treatment methods makes it an attractive option (2).

4.5.1. Types of Anaerobic Systems Used for Milk Processing Effluents

The full-scale application of anaerobic technology to high-strength and high-volume liquid effluents such as milk processing effluents requires the development of reactors, in which the microorganisms converting the waste to methane could be retained in the reactor. Many of the bacteria involved in the process grow very slowly requiring a long solids retention time (SRT) to avoid wash-out. In contrast, the large volumes of liquid wastes to be processed impose a relatively short hydraulic retention time. The most representative anaerobic systems used for the treatment of milk processing effluents are the following: anaerobic lagoons, contact process, anaerobic filter, up-flow anaerobic sludge bed, fluidized/expanded bed, and hybrid configurations. Figure 17.8 shows some of the high-rate anaerobic concepts with actual or predictable application to milk processing effluents. Figure 17.9 illustrates the distribution of real scale high-rate anaerobic systems for treating effluents from dairy and milk processing industries. Table 17.10 presents an overview of world anaerobic full-scale installations in the dairy and milk processing industries.

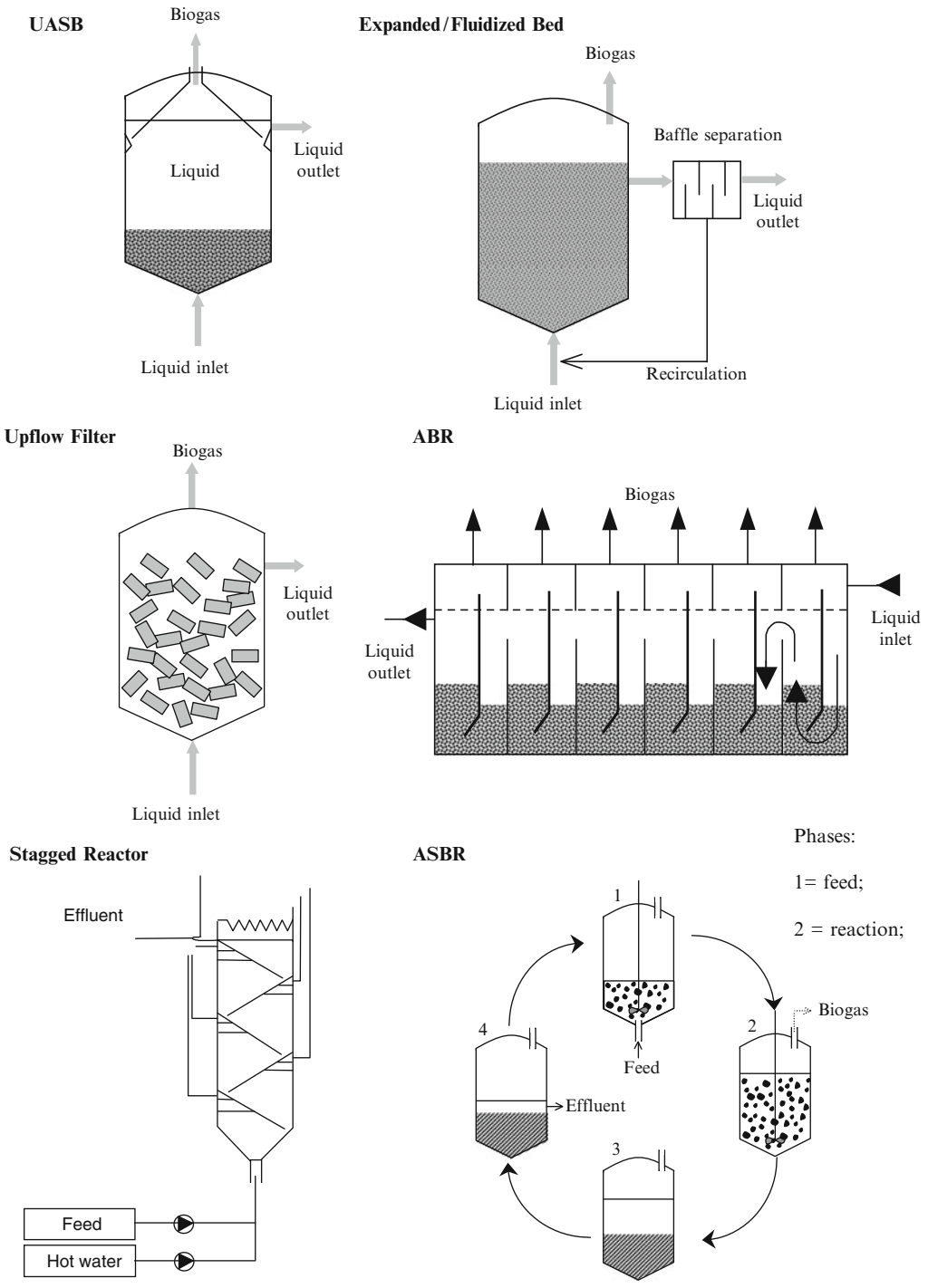
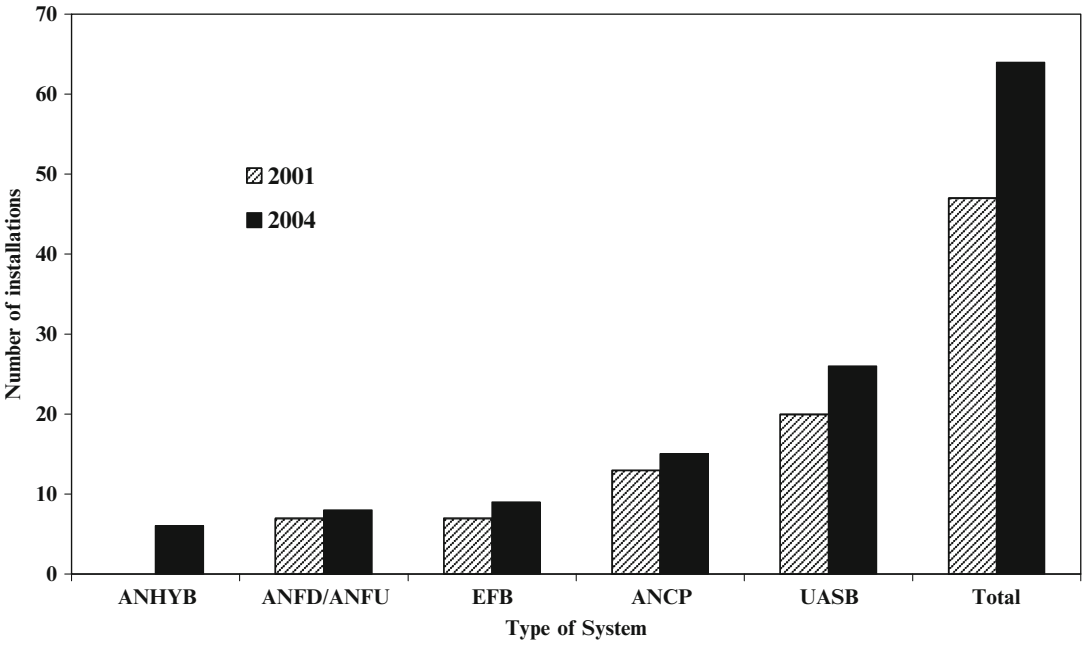


Fig. 17.8. High-rate anaerobic systems used for milk processing effluents.



ANYB = Hybrid systems
ANFD = Anaerobic Filter (Downflow)
ANFU = Anaerobic Filter (Upflow)
EFB = Expanded/Fluidized bed
ANCP = Anaerobic Contact Process
UASB = Upflow Anaerobic Sludge Bed

Fig. 17.9. Distribution of real-scale anaerobic treatment systems for milk processing effluents (data from ref. (115)).

4.5.1.1. ANAEROBIC LAGOON

The anaerobic lagoon is the most used system world wide for the treatment of milk processing and other food industry wastes. Their use is common in the third world countries because they need little or no technology and know-how. The construction of anaerobic lagoons is very simple and the process is usually built in one cell, but many combinations can be used both in parallel or sequential arrangement. The high residence times allow for the sedimentation and anaerobic degradation of the organic matter. Occasionally, sludge recirculation and mechanical agitation are used for improvement of contact between substrate and biomass.

In some industries (including milk processing installations), anaerobic lagoons may be present in a natural cover caused by the ascending solids, fats, and oils under quiescent conditions, forming a thick layer. A more modern variation is the synthetic cover to avoid odors and biogas escaping.

Table 17.10
Anaerobic full-scale installations used in the milk processing effluents (data from refs. (16, 115))

Installation	Flow (m^3/h)	Load COD total (kg/day)	Reactor volume (m^3)	Removal COD total (%)	HRT (h)	Load COD total (kg/m^3-day)
Low rate						
Alto Dairy	15	4,100	6,050	85	403	0.68
Arizona Dairies	–	–	–	–	–	–
Luis Farms	–	–	–	–	–	–
M&M Dairies	–	–	–	–	–	–
Bancroft Dairy	38	15,600	10, 400	75	274	1.50
Mid-America Dairy	48	4,560	11,400	85	238	0.40
Tulare, Cidade de	695	59,280	114,000	80	164	0.52
Turkey Hill Dairy	12	2,130	2,840	80	237	0.75
Average rate						
Carbery Milk Products	40	9,600	2,544	95	14	17.65
CCPL – Rio de Janeiro	25	1,980	300	75	12	6.60
Foremost/Leprino	120	25,500	12,500	80	104	2.04
Gold Bond Ice Cream	11	3,300	1,600	85	55	5.50
Haagen Daz Ice Cream	14	4,900	2,300	80	164	2.13
Kerry Ingredients	10	2,343	1,300	85	130	1.80
Lacto-Lima	21	12,640	2,528	87	120	5.00
Lacto-Lusa	21	12,640	2,528	87	120	5.00
Cidade de Madison	–	8,500	–	–	–	–
Mikkeli Dairy	25	1,320	350	75	14	3.77
Millbank Cheese	2	3,245	1,100	85	550	2.95
West Lynn Creamery	47	12, 000	5, 683	85	121	2.11
High rate						
Dunkirk Ice Cream	61	10, 227	1, 350	80	22	7.58
Fermiers Savoyards	5	1, 300	110	90	22	11.82
Kerry Co-op	200	43, 000	4, 150	80	21	10.36
Saint Hubert	42	3, 000	254	80	6	11.81
Agropur	65	6, 500	900	80	14	7.22
Boruclo Whey	146	10, 000	950	75	7	10.53
CCPL	50	3, 000	300	75	6	10.00
Colombo Yogurth	16	1, 818	200	82	13	9.09
Kaserei	67	4, 500	450	80	7	10.00
Kraft	58	3, 274	400	82	7	8.19
So. Caernarvon	5	6, 000	2, 200	85	440	2.73
Sylvester Whey Products	40	6, 100	500	85	13	12.20
Borden/Meadow Gold	32	8, 727	2, 652	80	83	3.29
EDC	6	13, 300	760	75	127	17.50

4.5.1.2. Contact Process

The contact process is a perfectly mixed tank (CSTR) in which the biomass in the effluent stream is separated and recycled to the reactor to keep a high concentration of microorganisms in the reactor and a high SRT. The key components for this process are the mixed tank, the effluent degasification unit, and the biomass separation system. Degasification of the effluent is crucial because the biogas entrapped within the biological particles hinders the separation of the biomass from the liquid. Due to the characteristics of the anaerobic flocs, the sedimentation unit should be of parallel plate type. The anaerobic sludge has the same applications as the aerobic sludge (land fertilizer, etc.), but it has the advantage of being more stabilized chemically and producing less odors. This process is still widely used because it is the one that has a higher capacity to degrade solids and fats with no accumulation inside the reactor. But in spite of being used world-wide to the present day, anaerobic CSTRs are increasingly giving way to faster and more efficient higher-rate anaerobic digesters, notably anaerobic filters, UASB reactors, and expanded/fluidized bed reactors.

4.5.1.3. Anaerobic Filter

The anaerobic filter was developed as one of the first anaerobic systems with biomass retention by attachment on a supporting media resulting in high concentrations of biomass and high SRT inside the reactor. The choice of the support media varies from activated carbon, rock, pall rings, PVC supports, and reticulated polystyrene. These systems may be operated in an up-flow or in a down-flow configuration. The wastewater is distributed from above (down-flow configuration) or below (up-flow configuration) the support media. The down-flow systems are much less used than the up-flow systems. In the up-flow systems, wastewater to be treated flows from the reactor bottom upward. The methane forming bacteria stick to the surface of the support medium and also exist in the space between the media in the lower section of the reactor. According to some authors, the biological conversion capacity of the up-flow anaerobic filter reactors is mainly associated with the suspended biomass aggregates in the lower part of the reactor, being the attachment of biofilms to the packing only of marginal importance (116, 117). In what concerns the true fixed film systems, although some modern support materials have been developed with high specific surface areas, the biomass concentration in these reactors is considerably lower than in anaerobic systems with mobile biomass aggregates. Consequently, the maximum conversion rate is lower implying a lower design capacity or a lower safety factor against overloading by the LCFA present in milk wastes. On the other hand, although providing a real safeguard against sludge wash-out, the packing material gives a high risk of clogging and channeling that must be considered in the case of milk processing effluents.

Fixed film reactors offer the advantages of simplicity of construction, elimination of mechanical mixing, better stability at higher organic loads, the capability to withstand large toxic and organic shock loads, and quick recovery after a starvation period (2). The main limitation of this design is that the reactor volume is relatively high compared to other high rate processes due to the large volume occupied by the support media. Another important constraint when considering the treatment of complex substrates like milk processing effluents

is clogging of the reactor due to the increase in biofilm thickness and/or to the high suspended solids concentration present in the wastewater (2).

4.5.1.4. Up-Flow Anaerobic Sludge Bed Reactor

The development of up-flow anaerobic sludge bed reactors (UASB) was based on the possibility of forming granular biomass aggregates that can be retained inside the reactor without the need of a support medium. In the UASB system, the reactor consists of an up-flow tank with a feed inlet distribution system at the bottom and a three phase separator at the top (Gas–Solids–Liquid separator). The wastewater is evenly distributed over the reactor bottom through inlet pipes and flows upward through a bed of anaerobic sludge at the lower part of the reactor (sludge bed). During the passage through the sludge bed, particulate matter is entrapped and the biodegradable matter is removed from solution by the anaerobic bacteria and converted into biogas and a small fraction of anaerobic biomass. The ascending biogas provides gentle mixing of the sludge bed and is collected at the top of the reactor in a gas collector system from where it is withdrawn. The remaining water–sludge mixture enters a settling compartment, where the sludge can settle and flow back to the sludge bed. The water is collected in effluent gutters and discharged out of the reactor. Effluent recycle (to fluidize the sludge bed) is not required as sufficient contact between the wastewater, and the sludge is guaranteed even at low organic loads because of the influent distribution system. The critical point in UASB systems is the gas–solids–liquid separator. Usually, this is built on a proprietary basis and specific designs have been conceived for specific types of wastes. UASB systems have few mechanical components and so operation and maintenance are easy.

Although initially the granular biomass was considered indispensable for the functioning of the system, some authors suggested the utilization of flocculent sludge when treating complex fat containing wastes (37, 82, 92, 113). The principal reasons for the comparatively large success of the UASB system are its simple and inexpensive construction and its ability to retain very high amounts of high quality biomass and thus accommodate high organic space loads and provide ample safety against shock loads.

A major advantage of UASB reactors is that the technology has comparatively less investment when compared to anaerobic filters or fluidized bed systems. Among notable disadvantages are the somewhat long start-up period, the requirement of a biological sludge with good settling properties, and the need for skilled operation.

UASB systems in particular seem sensitive to pH and load variations, and to high fat and calcium concentrations, all of which were considered to disrupt the settleability of the sludge and sludge granule formation (21).

A very popular technology derived from the initial UASB concept is the Internal Circulation reactor (IC) considered an ultra-high-rate anaerobic system. This system features a two-stage separation/collection of biogas within a tall cylindrical vessel and uses the gas-lift principle to induce internal circulation of treated effluent. The tall cylindrical design of this reactor makes it very suitable for applications where land is at a premium.

4.5.1.5. Expanded/Fluidized Bed Reactors

The distinction between expanded and fluidized beds is not clearly defined. In general, it is considered that expanded beds are those subject to an increase in bed volume from 5 to 25%

over the initial (rested state) bed volume. On the other hand, fluidized bed systems have been tested or operated with bed volumes 25–50% higher than initial bed volumes. Contrary to fluidized bed, in expanded bed technology, granular sludge biomass is more used rather than inert support media.

In fluidized reactors (and eventually in expanded bed reactors), the biomass is fixed on small support particles that are retained inside the reactor. The media used are small particle size sand, activated carbon, etc. for fluidized beds and slightly larger particles like sand, gravel, or plastics etc. in expanded beds. Particle bed fluidization occurs beyond a certain up-flow liquid velocity depending on the particle density and other factors such as the pressure loss in the bed. Under fluidized state, each media provides a large surface area for biofilm development. Fluidized bed technology is more effective than anaerobic filter technology as it favors the transport from the bulk to the surface of the aggregates and thus enhances the contact between the microorganisms and the substrate. In relation to the filter process, the fluidized bed system also presents the main advantage of avoiding clogging, yet its capacity for the removal and degradation of suspended solids is almost null. The moving bed systems do not result in higher safety against sludge wash-out. The sludge fluctuation will also occur in these systems when the balance between the liquid up-flow velocity and the biomass/support sedimentation velocity is disturbed because of effects of ascending lipids adsorbed onto the particles. These problems are of special concern in the treatment of milk effluents due to their high content of fats and solids.

In relation to anaerobic filters, these systems have several advantages such as the elimination of bed clogging, lower hydraulic head loss combined with better hydraulic circulation, and a greater surface area per unit of reactor volume and consequent lower capital costs. However, the need for effluent recycling in order to attain bed expansion or fluidization may increase operating costs.

In this system, performance is critically dependent on the efficient distribution of the influent/recycle stream to ensure a rapid, uniform flow through the reactor bed and adequate biomass growth. Significant drawbacks of this configuration range from the relatively high capital and operation/maintenance costs due to the complexity of operation.

The expanded granular sludge bed reactor (EGSB) is a modified form of UASB system, in which a slightly higher superficial liquid velocity is applied (5–10 m/h) as compared to 3 m/h for soluble wastewater and 1–1.25 m/h for partially soluble wastewater in an UASB (37). As a result of bed expansion, the contact between substrate and biomass is very good and the transport of substrate into the sludge aggregates is much better as compared to systems where the mixing is much lower (UASB). Benefits of EGSB reactor over UASB systems are valid especially for low strength VFA containing wastewaters (2).

4.5.1.6. Hybrid Configurations

The hybrid configurations result from a combination of two or more principles of operation of other existing configurations. The most frequent examples are the combination of UASB and filter or UASB and contact process with the objective of raising the sludge inventory in the sludge bed. Such hybrid configurations are designed to take advantage of the beneficial features of several anaerobic processes without realizing the high cost of employing multiple

separate processes. On the other hand, the growing interest in these hybrid systems also stems from the fact that none of the simpler initial concepts is fully adequate for some specific effluents.

4.5.1.7. Two-Phase Systems

These processes are based in the assumption that the anaerobic degradation of organic compounds is performed by two main groups of microorganisms that have distinct metabolic characteristics. These systems consist of two reactors in series in which acidogenic and methanogenic phases take place separately. This separation is possible in the circumstances where the acidogenic phase is faster than the methanogenic step, since if the opposite occurs, methanogenic populations will start to grow in the first phase. A major inconvenient pointed to the two-phase processes is the fact that the coordinated activity of the several bacterial groups is essential for the process stability since the phase separation will alter the concentrations of the intermediate species in a way that might turn unfavorable to methanogenic bacterial growth (37).

Specifically for effluents from milk processing and dairy industries, another concept of two-phase systems was presented by Zeeman et al. (118): the up-flow acidifying sludge reactor (UASR), in which the first phase was used to remove proteins and lipids by acidification of easily degradable sugars. The pH drop reaching the isoelectronic pH point of casein (around pH = 4.6) causes precipitation of protein and fats. The acidified effluent from this first phase is treated in an EGSB reactor whilst the protein/fat sludge is treated in a thermophilic reactor.

A recent example of a modification of the conventional UASB reactor is the anaerobic staged reactor developed by van Lier et al. (119) also shown in Fig. 17.8. Basically, in each module of the staged reactor, all of the anaerobic degradation phases occur simultaneously. Consequently, for a nonsoluble and non acidified feed a mainly acidogenic flora will develop in the first stage(s), but also some acetogenic and methanogenic organisms will probably be present. When treating a partially soluble feed, the first stage will serve primarily for hydrolysis and also partially for acidification of the substrate. With the development of the degradation processes in the subsequent stages of the system, a biomass with higher methanogenic activity will develop. The biological sludge will be different in each compartment depending on the prevailing environmental conditions and on the intermediary substrates remaining for degradation. Since the mixture of the whole reactor biomass is avoided, in principle each stage develops a specific type of biomass. In case phase separation occurs in a staged reactor, this would be the consequence of a natural selection. This kind of reactor is especially indicated for thermophilic operation (120) since at thermophilic temperatures, there is a higher inhibition by reaction products or by substrates.

A further example of an anaerobic reactor using the concept of biomass segregation is the anaerobic baffled reactor (ABR) developed by Bachmann et al. (121) from the rotating biological contactor. According to Nachaiyasit and Stuckey (122), the ABR can be considered as a series of UASB reactors, and it was called initially "modified sludge bed reactor" (123) that does not require granular sludge for operation. This reactor concept consists of a series of vertical baffles that force the liquid to flow under or over them from the feed inlet toward the outlet (see Fig. 17.8). The baffles fixed either at the top or at he bottom of the reactor divide

the reactor in a number of compartments that cause segregation of biomass and biogas. In this way, the liquid flow and the substrate degradation cause a selection of the trophic groups along the reactor length. Among the new designs of anaerobic high-load reactors, the ABR is quite promising as a new and flexible concept for application to a wide variety of domestic and industrial wastewaters including complex effluents (124, 125). According to Nachaiyasit and Stuckey (123), this reactor design is especially applicable in situations when the wastewater flow to be treated is intermittent and the reactor receives low maintenance and care.

4.5.2. Design Considerations for Anaerobic Systems in Milk Processing Industry

General design considerations common to all anaerobic treatment applications include (115):

- Equalization requirements, volume/time.
- Pretreatment requirements (Total Suspended Solids (TSS)/FOG removal).
- Need for wastewater heating/cooling and type of heat exchanger.
- Nutrient (micro and/or macro) requirements – N, P, S, Fe, Cu, etc.
- Need for pH and/or alkalinity adjustment.
- Odor and corrosion control concerns.
- Handling of biogas, excess sludge and anaerobic effluent.
- Process control requirements – degree of monitoring and control.
- Staffing and training requirements.

Specific design considerations for each particular anaerobic system are presented below (115).

Anaerobic lagoon:

- Availability of space.
- Proximity to subdivisions, commercial areas, and individual residences.
- Hydrogeological and geotechnical constraints (e.g., groundwater level, soil permeability).
- Frequency and magnitude of high winds.
- Duration and intensity of freezing weather.
- Natural cover or synthetic cover.
- Cover resistance to ultraviolet (UV) degradation.
- Sludge recycle, gas collection and reuse, mechanical mixing, and other special features.
- Eventual need to remove settled solids.
- Rainwater/snowmelt removal from cover.

Anaerobic contact process:

- Mesophilic or thermophilic process operation.
- Steel, reinforced concrete, or prestressed concrete reactor construction.
- Side-entering or top-entering mixing.
- Atmospheric versus vacuum degasification.
- Solids removal via sedimentation or gas flotation.
- Lamella or conventional clarifier sedimentation.
- Flow-type versus suction-type conventional clarifier solids removal.
- Special features such as membrane separation and degasifier odor control.

UASB reactor:

- Flow/load equalization.
- Preconditioning (partial acidification) of the wastewater.

- Limitation of influent TSS to 10–20% of influent COD.
- Limitation of FOG levels to < 100 mg/L.
- Steel or concrete reactor construction.
- Corrosion resistant material selection for cover and proprietary settler.
- Uniform, steady distribution of influent within sludge bed.
- Adequate storage volume for backup sludge supply.
- Specific needs for minimum levels of calcium and micronutrients.

Anaerobic filter system (up-flow or down-flow):

- Flow/load equalization.
- Provisions for wastewater pretreatment to limit TSS and FOG in the feed.
- Preconditioning of the wastewater.
- Steel, reinforced concrete or prestressed concrete reactor construction.
- Internal media material, type and configuration.
- Uniform, steady distribution of influent within sludge bed.
- Provision for removal of solids from the support media.
- Identification of the method for measuring biomass levels in the reactor.

Expanded/fluidized bed reactor system:

- Flow/load equalization.
- Preconditioning (partial acidification) of the wastewater.
- Limitation of influent TSS to 10–20% of influent COD.
- Limitation of FOG levels to < 100 mg/L.
- Steel or fiberglass reactor construction.
- Corrosion resistant material selection for cover and internals.
- Carrier material selection for systems with a carrier media.
- Carrier cleaning and solids removal system.

4.5.3. Loads and Operating Parameters in Anaerobic Systems for Milk Processing Effluents

Table 17.11 presents some data on the operation of high-rate anaerobic systems used for milk effluents. Information on low and medium rate systems may be found in literature (115, 126).

4.5.4. Summary of Results for Anaerobic Treatment of Milk Processing Effluents

Tables 17.10 and 17.12 present data on industrial-scale, lab-scale, and pilot-scale anaerobic systems used for milk effluents.

4.5.5. Choice of Anaerobic System for Treatment of Milk Processing Wastewater

In general, technologies for wastewater treatment are evaluated based on factors such as sludge management, capital costs, operator requirements, and operating and maintenance costs. A technology is acceptable to an industry if it requires less capital, less land area, and is more reliable when compared to other well-established options. For an anaerobic system, this translates into the process being able to run at high organic and hydraulic loading rates with minimum operating and maintenance requirements.

In the choice of the adequate anaerobic system, the most important factor is the nature of the wastewater to be treated, since not all systems are adequate for some complex substrates. In the

Table 17.11
Loads and operating parameters in anaerobic systems (26, 57, 58, 126)

Anaerobic filter			
Organic load (kg COD/m^3-day)	0.1–30[a]	5–20[b]	2–10[c]
HRT (h)	$\sim$ 24[d]		10–15[c]
COD removal (%)	65–75[a]		70–80[c]
Critical solids concentration in feed (mg/L)			450–1,050[c]
UASB			
Organic load (kg COD/m^3-day)		5–15[b]	2–15[c]
HRT (h)	< 24[d]		10–50[c]
COD removal (%)			70–90[c]
Critical solids concentration in feed (mg/L)		10–20% of feed COD[b]	
Expanded bed			
Organic load (kg COD/m^3-day)			2–50[c]
HRT (h)	$\ll$ 24[b]		0.5–24[c]
COD removal (%)			70–80[c]
Critical solids concentration in feed (mg/L)	Not critical but solids are not removed[a]		

[a]Ref. (26).
[b]Ref. (126).
[c]Ref. (58).
[d]Ref. (57).

case of milk processing wastewater, the selection of reactor type must take into consideration the main problems discussed above and caused by the simultaneous presence of sugars, proteins, and fats. Nevertheless, milk processing effluents are an application particularly adequate for anaerobic treatment because they have above ambient temperature and high concentrations of organic substrates. In fact, the higher the flow and the concentration of organic matter the higher the economic advantage in the use of anaerobic technology (127).

Apart from the characteristics of the effluent to be treated, the main factors to consider in the selection of anaerobic technology for treatment of milk processing effluents are:

- Lower investment costs (land and technology)
- Higher reliability and flexibility in relation to other well established treatment options
- Lower operation costs
- Absence of environmental emissions, especially odor
- Automated operation
- Maintenance and control costs

A careful analysis of the characteristics of each system must be performed to choose the most adequate technology (Table 17.13).

In order to elect with reliability the most appropriate system, it is necessary to perform a systematic evaluation of the different configurations with the specific wastewater to be treated and if possible with a sample of the biomass that will be available to inoculate the reactor. The choice of the system must be supported by its capacity of being operated at high hydraulic and organic loads with low operation and maintenance costs. Concerning treatability studies, it is

Table 17.12
Summary of the operational conditions for the anaerobic treatment of milk processing effluents (adapted from refs. (2, 5))

Reactor	Volume (L)	T (°C)	HRT (day)	Influent concentration (g COD/L)	COD reduction (%)	OLR (kg CODb/m^3-day)
AF	2	37	5.9	60.7	98.3	9.4
AF	–	40	1	2.9	93.8	2.9
DSFF	0.7	30	3.3	66	96.0	20
DSFF	0.7	30	0.3	4.1	75.0	15
UASB	1.2	30	0.3	4.1	78.0	15
UASB	4	35	2	29.4	97.5	14.7
UASB	1	35	0.12	2.8	91.1	23.8
UASB	4	35	0.22	2.3	96.0	10.4
UASB	8	30	0.21	1.8	87.0	8.5
FB	0.6	35	0.33	0.34	80.0	1.0
FB	2.5	35	1.33	5.0	92.0	3.8
ASBR	3.5	35	3.2	4.3	96.0	6.25
UASB	4×10^6	35	8	4.4	63.0	0.55
AF	14.2	35	1.9	6	98.0	6.29
SAF	17.7	35	2.05	6	98.0	5.92
TF	40.5	35	0.11	0.333	81.0	4.45
UASB	–	–	2.3–11.6	5–77	95–99	1–28.5
UASB	–	–	5.4–6.8	47–55	90–94	7–9.5
UASB	–	–	3.3–12.8	16–50	90–95	1–6.7
UASB (dairy)	–	–	0.07	2.05	90	31
UASB (cheese whey)	–	–	5	4.5–38.1	–	–
2-stage (cheese whey)	–	–	10–20	72.2	36	–
UFFLR	–	–	5	79	95	14
DSFFR	–	–	5	13	88	2.6
FBR	–	–	0.4	7	90	7.7
FBR	–	–	0.1–0.4	0.8–10	63–87	6–40
AAFEB	–	–	0.6–0.7	5–15	61–92	8.2–22
AnRBC	–	–	5	64	76	10.2
SDFA	–	–	–	69.8	99	16.1
UASB	–	–	1.5	11	94	7.1
UASB	–	–	5	5–28.7	97–99	0.9–6
DUHR	–	–	7	68	97	10
UASB (whey permeate)	–	–	5–0.4	10.4	–	–

Table 17.13
Choice of anaerobic systems for milk processing effluents (adapted from refs. (2, 128, 129))

	CSTR	Contact	Anaerobic filter	Fluidized bed	UASB	EGSB
Start-up	Excellent	Bad	Very good	Good	Acceptable	Acceptable
Start-up period (weeks)	2–4	2–4	3–4	3–4	4–16	4–16
Operation	Acceptable	Acceptable	Excellent	Good	Good	Good
Control	Excellent	Bad	Good	Good	Good	Good
Shock resistance						
Temperature	Good	Bad	Excellent	Excellent	Excellent	Excellent
Toxic	Good	Bad	Excellent	Excellent	Very good	Very good
Organic load	Very good	Bad	Excellent	Excellent	Excellent	Excellent
TSS load	Good	Bad	Good	Acceptable	Acceptable	Acceptable
Channeling effect	Not present	Non-existent	High	Non-existent	Low	Very low
Effluent recycle	Not required	Not required	Not required	Required	Not required	Required
GSL separation	Not required	Not required	Beneficial	Beneficial	Essential	Essential
Carrier packing	Not essential	Not essential	Essential	Essential	Not essential	Not essential
Loading rates (kg COD/ m^3-day)	0.25–3	0.25–4	1–40[a]	1–100[a]	10–30[a]	10–30[a]
HRT (day)	10–60	10–60	0.5–12	0.2–5	0.5–7	0.5–7
Main advantages	Simple technology	Long SRT and relatively short HRT	Simplicity of construction	Resistance to inhibitors	Lower investment relatively to filter and fluidized bed	Excellent contact between biomass and substrate
	Adequate mixing	Good contact between biomass and substrate	No mechanical mixing	Good contact between biomass and substrate	Low loss of solids	
	Good contact between biomass and substrate	Efficiently retained biomass system	Good stability at high loads	No bed clogging	Well settling sludge	
	Eliminates scum and thermal stratification		Good resistance to organic or toxic shocks			
Main disadvantages	Washout of the active biomass	Need for a biomass separation system	Relatively high volume	Need for effluent recycle	Long start-up	Long start-up

(*Continued*)

Table 17.13
(Continued)

	CSTR	Contact	Anaerobic filter	Fluidized bed	UASB	EGSB
	Long retention times	Need for effective mixing	Clogging of the media	Need for skilled operation	Need for sufficient amount of granular sludge	Need for sufficient amount of granular sludge
	High cost of installation and mixing	Limited tolerance to hydraulic loading and biomass retention	Significant pressure loss		Need for skilled operation	Need for skilled operation
	Biomass growth based system and not a efficiently retained biomass system					
Tolerance to fats and solids in the higher loads	Good	Fair – good	Poor	Poor	Flocculent sludge: fair –good Granular sludge: poor	Poor

[a]The higher loads are achievable only with prior fats removal.

important to remember that the results obtained in laboratory or pilot scale are usually from a short term operation and that in the case of milk processing effluents, the long term behavior of the system may differ significantly from this. In general, when performing laboratory or pilot experiments to access the behavior of a system in the treatment of a certain effluent, the factors that are monitored and used for this evaluation are the higher attainable organic and hydraulic loads, the COD or BOD removal efficiencies and the biogas production. In a preliminary study of the applicability of a certain anaerobic system to milk processing effluents, the optimization of these factors is not enough or, in other words, might lead to the need for high safety factors. This is because the main factor that governs the performance of a reactor treating complex fat containing effluents is the accumulation of the removed organic matter and not the removal efficiency or the production of biogas on their own (113). It is necessary to use these factors in complement with each other so as to calculate the efficiency of methanization of the removed organic material. In this way, a correct evaluation of the reactor performance can be made.

4.5.6. Control of Anaerobic Processes Applied to Milk Processing Effluents

Anaerobic digestion is a process that is significantly affected by the operating conditions. Since the process depends on the formation of several intermediate products that are toxic or

inhibitory to the anaerobic biomass, it is important that the rates of reaction are high enough to avoid accumulation of these intermediate compounds inside the system which would result in reactor imbalance and failure.

The good performance of an anaerobic reactor for milk processing effluents relies on the equilibrium of the reaction rates and is determined mainly by both the applied load and the influent concentration. Besides the fulfillment of the specific requirements for these particular effluents, other general parameters that are determinant due to the sensitivity of the bacterial populations are the following:

1. Temperature – this is the environmental factor with most influence upon the behavior of the system. The optimum temperature for growth of most of the anaerobic species is around 35°C. The mesophilic range (25–45°C) is most commonly used in anaerobic systems and the number of known thermophilic species (>45°C) is still small. Temperature shocks may negatively affect the performance of any anaerobic reactor. Concerning the rates of biochemical reactions, thermophilic temperatures are favorable as compared to mesophilic. Specifically for LCFA, the higher temperature increases the solubility diminishing negative hydraulic effects (flotation and wash-out). On the other hand, higher LCFA solubilization enhances their inhibitory/toxic action by means of enhanced bioavailability. Thus, thermophilic degradation of LCFA proceeds at higher rates than mesophilic degradation, but the former present higher sensibility to LCFA inhibition/toxicity (48, 130). Nevertheless, reactor recovery after LCFA overload is faster for thermophilic reactors as compared to mesophilic because thermophilic bacteria have higher doubling times. So, the high temperature shocks may be detrimental to anaerobic mesophilic reactors treating milk processing effluents, not only because of direct effect on bacterial populations but also due to the enhanced bioavailability of LCFA caused by higher solubility. The thermophilic anaerobic application at industrial scale is not well documented in the literature.
2. pH – this is also a very important parameter in microbiological metabolism. The major part of the bacterial populations (with the exception of most of the methanogenic species) is capable of growing in a pH range spreading through 3 pH units. Usually, the maximum growth is observed for a pH around 6.5–7.5. One phase systems are operated at a pH of 7.0–7.5 and generally they have a good buffer capacity in pH near neutral values. In two-phase reactors, the first stage (acidogenic) proceeds over a range of pH values but the second step (methanogenic) is most sensible to pH variations, the optimum pH being 6.8–7.2. The most problematic steps of anaerobic digestion in relation to pH changes are the steps in which H^+ is formed (Fig. 17.5). A pH drop may cause a shift in chemical equilibrium and may lead to back reactions and accumulation of toxic intermediate compounds. Milk processing effluents are readily acidifying effluents with generally low alkalinity levels. In the operation of anaerobic systems for these effluents, care must be taken to ensure that enough alkalinity is present or added to avoid sharp pH drops. Special care must be taken upon start-up or overload periods, since in those circumstances, the sequential reactions of anaerobic digestion may shift from balance and lead to accumulation of inhibitory acids.
3. Mixing – in some reactor configurations, mixing is crucial for eliminating gradients in the parameters that exert the most influence upon the kinetics of the process. Some of the ways to improve the mixing characteristics of a reactor are effluent recirculation, biogas recirculation, and mechanical agitation. Presently, the most used form of mixing is the recirculation of part of the produced biogas which also serves as a form of pH control. The mixing in an anaerobic reactor treating milk processing effluents is also important to enhance the contact between biomass and substrate, to decrease mass transfer limitations and to eliminate channeling and

Table 17.14
Monitoring and control of anaerobic reactors for milk processing effluents (adapted from ref. (29))

Parameter	Frequency
Feed	
Flow	Daily
COD, BOD, VFA/alkalinity	Daily
SS, toxic substances	Daily/continuous
Reactor	
Temperature, pH	Daily/continuous
Sludge content	When needed (once per month)
Effluent	
COD, BOD, VFA/alkalinity	Daily
SS	Daily/continuous
N, P	When needed (once per week)
Biogas	
Amount	Continuous
Composition	Daily/continuous
Excess sludge	
Amount	Continuous
Dry matter	When needed

dead volumes, thus avoiding gradients in kinetic rate parameters and minimizing local inhibition effects. Mixing may also have significant benefits in reducing inhibition effects of isolated inputs of toxic substrates.

Table 17.14 presents a summary of other parameters to be monitored and controlled in anaerobic reactors for milk processing effluents.

5. CASE STUDIES

The main operational problems occurring in milk processing wastewater treatment plants are related with process discharges (raw materials and products) or discharges of chemical products. With respect to the process discharges, the most common are the discharges of raw materials like fresh milk and products like whey, which increase sharply the organic load applied to the treatment plants and consequently to the anaerobic reactors. Concerning the use of chemicals, it may occur discharges of sodium hydroxide from CIP units, of soda lime, which is the main neutralizing agent used in the wastewater treatment plants, or cleaning products used for mill disinfection, which have a toxic effect on the anaerobic bacteria, with a consequent potential decrease on their activity, until eventually their complete inactivation.

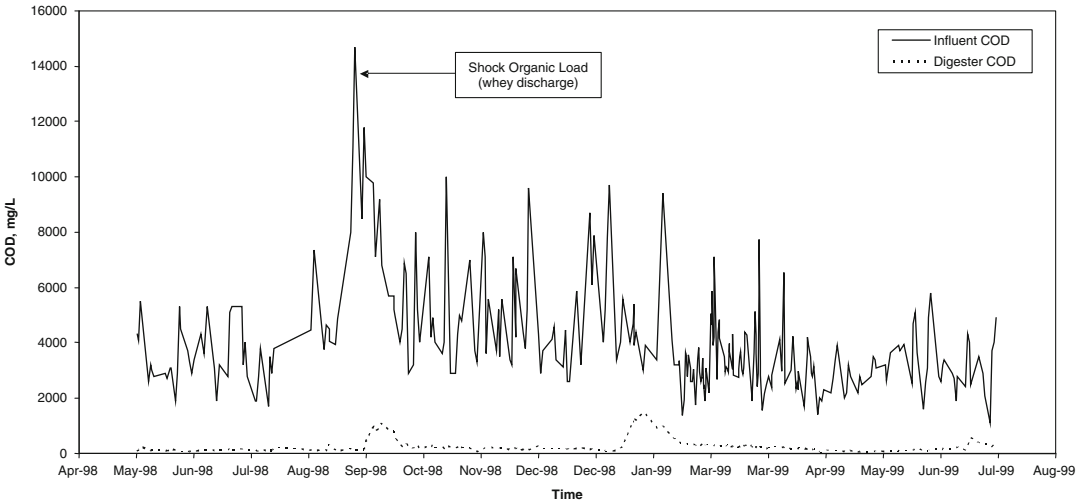


Fig. 17.10. Evolution of COD values (case studies 1 and 2).

In this section, four of these typical situations are described, which had occurred in the treatment plants existing in two milk processing industries producing cheese in Portugal, both using anaerobic contact reactors.

5.1. Case Study 1: Organic Shock Load (Whey Discharge)

Figure 17.10 presents the evolution of the influent COD as well as the COD existing inside the anaerobic contact reactor installed in a wastewater treatment plant treating an effluent from a milk processing mill producing cheese, which also incorporates a whey drying tower. As can be seen from the graph, by mid August 98 occurred a whey discharge because of the malfunctioning of the whey refrigeration system, which resulted in the increase of the organic load applied to the anaerobic reactor to around four times the normal operational value.

The reactor response to the sudden increase in the applied organic load was an increase on the reactor COD content (Figs. 17.10 and 17.12), as well as a sharp increase on the VFA concentration (achieving values higher than 600 mg/L as acetic acid) and a slight decrease in the pH, as can be seen in Fig. 17.11. Simultaneously, it also occurred a very high loss of the total suspended solids (TSS) inside the reactor, which varied from 6,000 mg TSS/L to less than 2,000 mg TSS/L (Fig. 17.12), with the corresponding loss of biomass.

The reactor recovery was attempted by a drastic decrease in the applied organic load. This decrease was obtained by lowering the inlet flow rate to the digester and by-passing the excess flow to the aerobic system, until the achievement inside the reactor of the normal values for the operational and control parameters (VFA, pH and COD concentrations). After this, the inlet flow rate was increased step by step until the total flow rate was achieved. This strategy allowed a gradual recovery of the process although with a slow increase on the TSS concentration inside the reactor, as can be seen in Fig. 17.12. The duration of the recovery time of the process after this accidental discharge was around 5 months. However, in the end of this

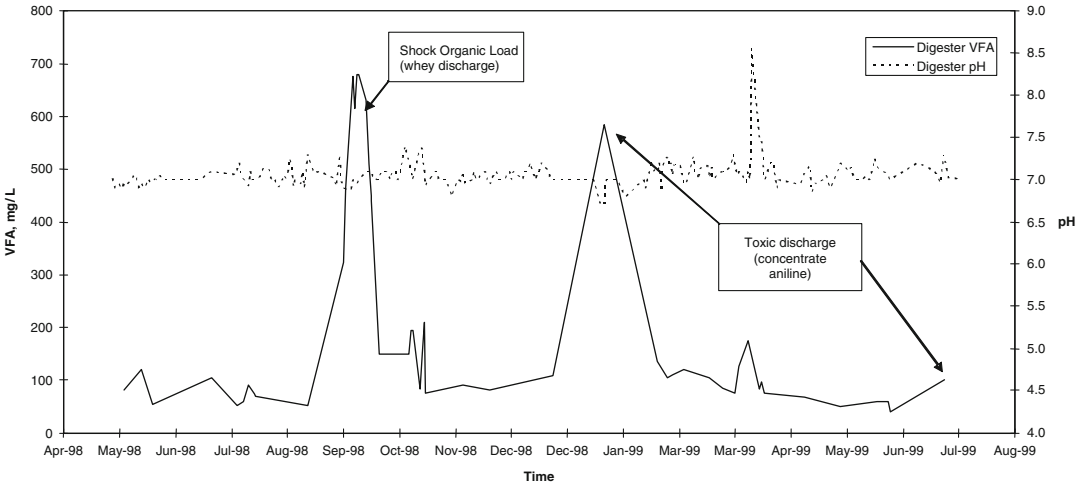


Fig. 17.11. Digester VFA and pH evolution (case studies 1 and 2).

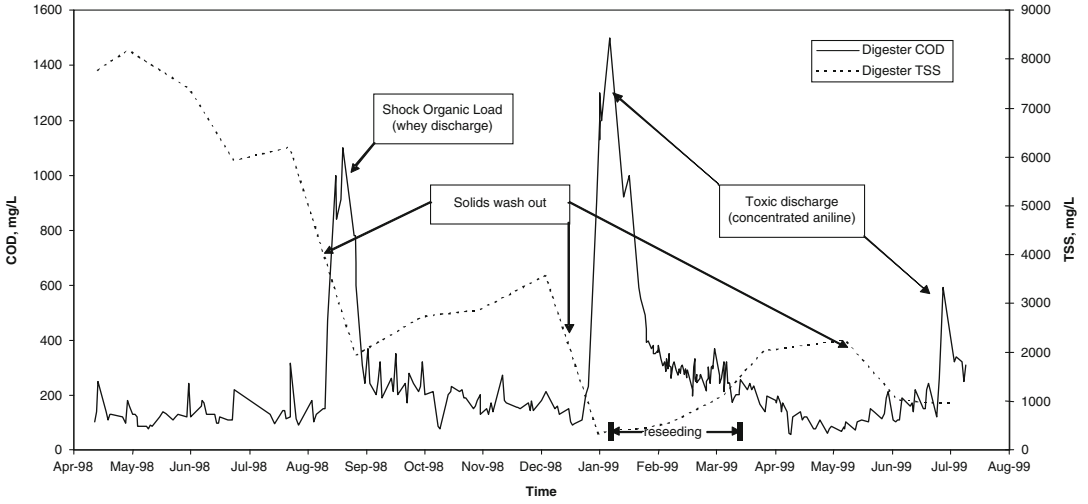


Fig. 17.12. Digester COD and TSS evolution (case studies 1 and 2).

recover period, the TSS inside the reactor was only 3,500 mg TSS/L, lower than the amount before this discharge, which showed that the reactor had not achieved a complete recovery.

5.2. Case Study 2: Toxic Discharge (Concentrated Aniline)

In the same milk processing wastewater treatment plant of Case Study 1, and after 5 months trying to recover the reactor after the occurrence of the organic shock load, by the beginning of January 99, an accidental discharge of a toxic product (concentrated aniline used for cheese cover) occurred before the achievement of total recovery of the anaerobic process (reactor

TSS concentration was lower than 4,000 mg TSS/L). This discharge was not reported to the treatment plant manager, so it was not possible to act immediately in the operation of the anaerobic process before the reactor failure. Hence, it was observed biomass inhibition and sludge wash-out. As a consequence, a total loss of the reactor solids content was observed together with a high increase on VFA and COD reactor content concentrations, respectively 600 mg VFA/L and 1400 mg COD/L, as a result of bacteria inhibition and death (Figs. 17.11 and 17.12).

At this moment, due to the very low amount of TSS inside the reactor (less than 500 mg TSS/L), the reactor recovery was only possible through a reactor reseeding and start-up procedure performed from the middle of January 99 until March 99, as can be seen in Fig. 17.12. By May 99, when the reactor was almost recovered and achieving full capacity, 100 mg COD/L, 60 mg VFA/L, and 2,000 mg TSS/L, there was again another accidental discharge of aniline, although in less quantity, which caused again the failure of the reactor. It was necessary to start again the recovery of the reactor through a new reseeding and start-up period (data not presented).

As a conclusion on this type of accidental discharge (toxic compound), it can be stated that the inhibition of the anaerobic bacteria was very severe, causing the failure of the process, and the recovery was possible only through a reseeding and start-up of the anaerobic reactor.

5.3. Case Study 3: Chemical Discharge (Soda Lime)

The chemical discharge (soda lime) happened in August 03 in another milk processing mill (also cheese making installation) wastewater treatment plant because of a control valve malfunction in the neutralizing unit. Around 5 m^3 of concentrated soda lime had been added to the anaerobic reactor and pH values higher than 10 had been reached (Fig. 17.13). The COD concentration inside the anaerobic reactor increased almost ten times the normal operational value achieving values higher than 1,200 mg COD/L, as can be seen in Fig. 17.13. To restore rapidly the operational pH values (6.8–7.2), the anaerobic reactor content was neutralized with sulfuric acid. At the same time, and in order to avoid an organic shock load, the inlet flow rate was decreased. After some period, a stepwise increase of the organic loading rate (through the increase of the flow rate) was applied. This procedure had occurred between the middle of August 03 until the end of September 03. However, these actions were not sufficient to prevent biomass wash-out.

From Fig. 17.14, it can be observed that the total suspended solids concentration inside the anaerobic reactor dropped sharply by the end of September 03, as consequence of the biomass wash-out. Hence, a reseeding was planned and was initiated in October 03. Due to the lack of seeding material at this time, the reseed period lasted for 3 months (until December 03) and a new start up procedure was initiated. By the end of January 04, the reactor was working properly at full capacity with a good efficiency, achieving a COD of 150 mg COD/L and a solids content of 12,000 mg TSS/L inside the digester.

As a conclusion on this type of accidental discharge (chemical compound), it can be stated that the inhibition of the anaerobic bacteria was very severe, also causing process failure and the need for a re-seeding and start-up of the anaerobic reactor (recovery time of around 5 months).

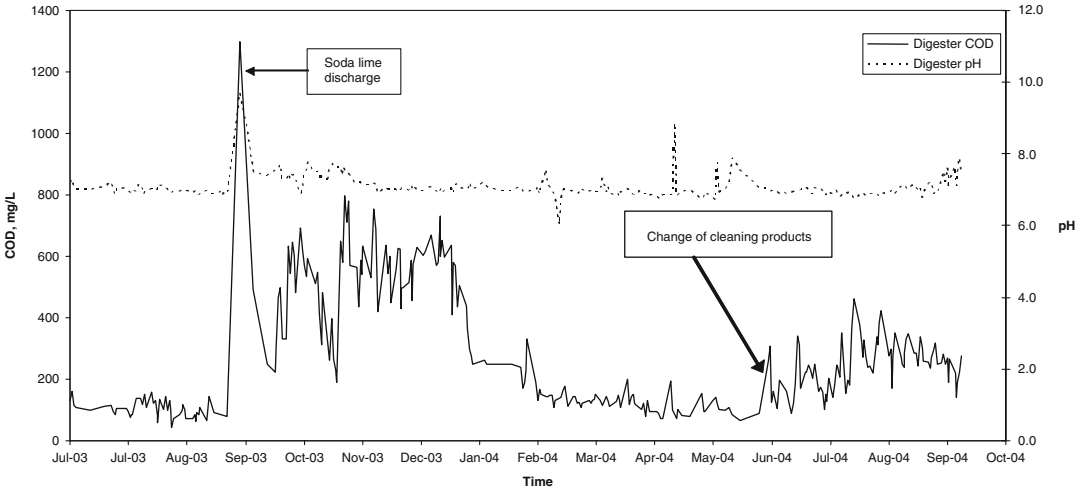


Fig. 17.13. Digester COD and pH evolution (case studies 3 and 4).

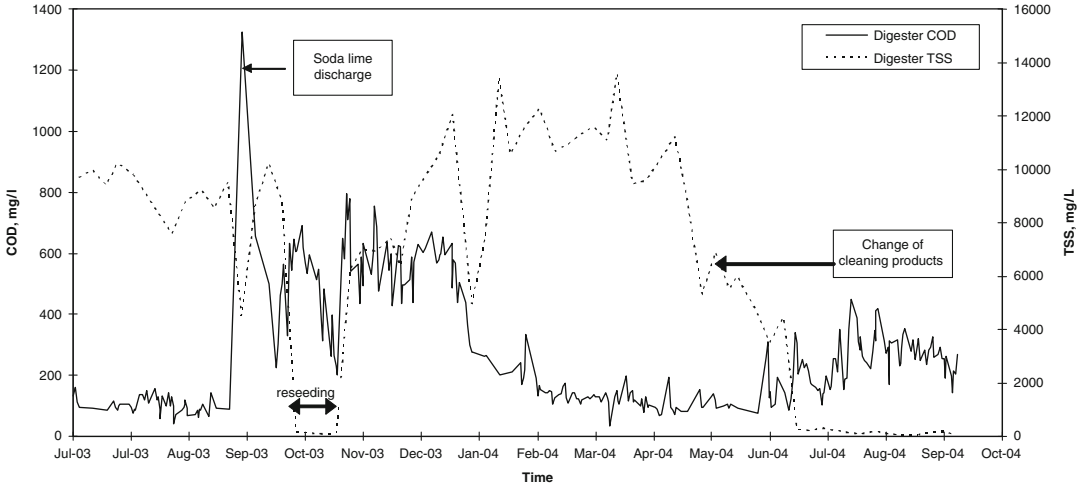


Fig. 17.14. Digester COD and TSS evolution (case studies 3 and 4).

5.4. Case Study 4: Change in Cleaning Products

From Figs. 17.13 and 17.14, it can be observed that, by May 04, something had caused the COD concentration to increase and a very high loss of the total suspended solids inside the anaerobic reactor. After an investigation on the cause of these phenomena, a correlation was established between these facts and a change in the cleaning products (disinfectants) used in the mill during this period. In order to avoid higher organic shock loads, the inlet flow was decreased. By October 04, the anaerobic reactor was working at a low flow rate (25% of the

total flow rate) and was still functioning very unstably. Although it was known which cleaning product caused this behavior, the toxic agent was not identified. However, due to process and sanitary reasons, it was not possible to change immediately the cleaning products, so the anaerobic reactor had to work at these unstable conditions during a large period, which caused biomass wash-out. Only after the changing of the cleaning products, it was possible to do a new reseed of the digester and apply the start-up procedure. After this action it was observed a gradual recovery of the reactor (data not shown).

As a conclusion on this type of discharge (change of cleaning products used in the mill), it can be stated that care must be taken wherever there is a need to change the disinfectants used in the process, due to its potential inhibitory effect on the anaerobic bacteria.

6. DESIGN EXAMPLES AND QUESTIONS

In this section, some design examples will be presented for different types of anaerobic reactors treating milk processing effluents from industrial mills. The anaerobic reactors under analysis are: anaerobic contact reactor, anaerobic up-flow filter, and IC reactor (modified UASB reactor).

The anaerobic reactors under study are included in wastewater treatment plants designed to treat industrial effluents from milk processing industries in order to meet legal discharge requirements, with the lowest operation costs.

In general, milk processing wastewater treatment plants using an anaerobic technology have the configuration as presented on the block diagram of Fig. 17.15:

1. Pretreatment consisting of screening, grit removal, flow measurement, oil and grease removal, and equalization.
2. Anaerobic treatment.
3. Aerobic treatment in an activated sludge system designed for the following processes: nitrification/denitrification, aeration and sludge settling and recycle.

6.1. Design Example 1: Anaerobic Contact Reactor (Cheese Mill)

The anaerobic contact reactor is installed in an industrial wastewater treatment plant with the total configuration presented in Fig. 17.15, receiving liquid effluents from a milk processing industry producing cheese. In this mill, there are three different wastewaters to be treated: domestic wastewater, industrial wastewater, and cheese whey (35% of the total daily volume).

The equalization tank was designed for a hydraulic retention time of 15 h, and the tank is provided with a mixing system (propeller type) to avoid solids settling and milk fat flotation. This wastewater component (fat) is separated in a flotation unit (DAF type) to prevent operational problems at the anaerobic reactor, namely sludge bulking and sludge wash-out due to the presence of the milk fat. The flow rate from the equalization tank is constant ($20\,m^3/h$), and the treatment plant operates 24 h/day on a 7 days a week basis.

The anaerobic reactor (Fig. 17.16) consists basically on a cylindrical concrete tank with an inlet distribution system at the reactor bottom. Sludge mixing is provided by sludge recycling at a sufficient flow rate ($150\,m^3/h$) to keep the sludge on the bottom slightly expanded and

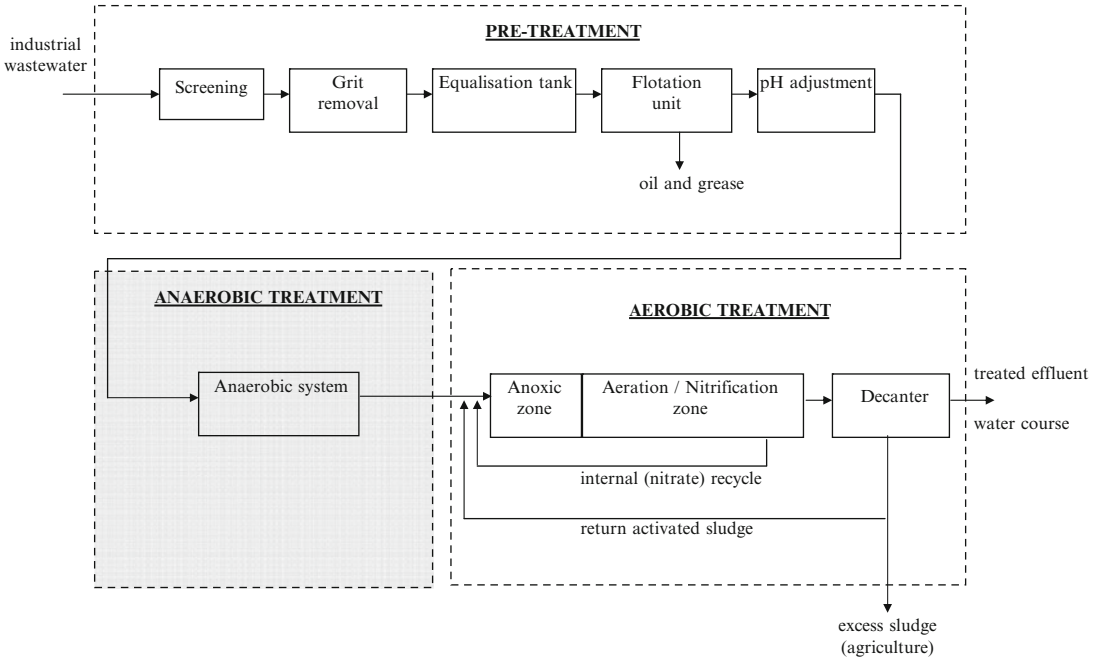
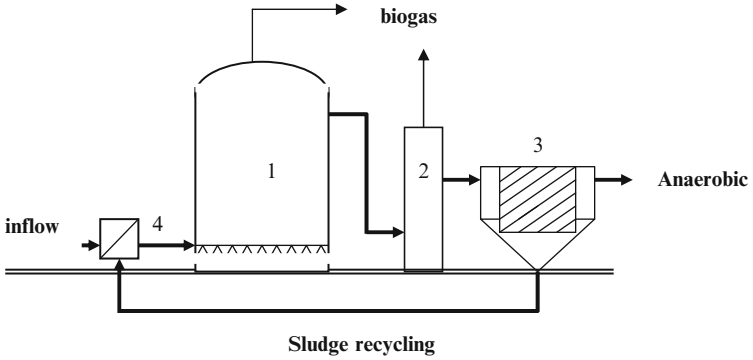


Fig. 17.15. Block diagram for milk wastewater treatment plants.



1 = CSTR anaerobic digester; 2 = degasification tower; 3 = lamella separator; 4 = heat exchanger

Fig. 17.16. Anaerobic reactor (design example 1).

allowing also a good influent distribution. To reach a mesophilic temperature inside the reactor (35°C), the influent is heated by direct steam injection prior to entering the reactor.

The effluent leaving the anaerobic reactor contains a considerable amount of sludge which has to be separated and recycled back to the reactor. Due to the solids settling characteristics,

Table 17.15
Main characteristics of the combined industrial effluent (design example 1)

Parameter	Unit	Value
Flow rate	m^3/day	480
COD	mg/L	25, 800
BOD_5	mg/L	13, 600
TSS	mg/L	1, 400
Total N	mg/L	300
Total P	mg/L	130
Sulphate	mg/L	600
Temperature	°C	24

the separation process is performed in a parallel plate separator (cross flow type) denominated "SEPAFLOC."

The design of the anaerobic contact reactor was based on the main characteristics of the combined industrial wastewater presented in Table 17.15, assuming no COD removal in the pretreatment units.

The total COD load to be treated was 12,640 kg/day, and the maximum design volumetric organic load rate was 5 kgCOD/day-m^3 of reactor volume. The corresponding active volume of the reactor is 2,500 m^3.

The total reactor volume is 2,800 m^3 with a cylindrical shape form. The corresponding reactor diameter is 18.9 m, and the total height is 10.1 m, with a water height of 9.5 m approximately. The reactor is operated at 32–37°C and in a pH range of 6.8–7.5 with an expected control point of 7.2. The estimated biogas production was 234 m^3/day.

The anaerobic contact reactor efficiency was estimated as 87% for COD and as 90% for BOD_5. The assumed effluent characteristics after anaerobic treatment were 4,650 mg/L for total COD, 1,775 mg/L for total BOD_5, and 1,500 mg/L for TSS.

The aerobic activated sludge treatment consists of a tank with two reactors in series: anoxic basin and aeration basin. The anoxic basin, also called denitrification basin, has 330 m^3 of volume and is located in front and adjacent to the aeration basin. This last basin has a volume of 2,250 m^3.

6.2. Design Example 2: UASB Reactor IC Type (Milk Processing Mill)

The IC reactor was installed in a wastewater treatment plant designed to treat the liquid effluents from a milk industry producing UHT milk, skimmed and semi-skimmed.

The main characteristics of the combined industrial effluent are described in Table 17.16.

The treatment plant also has a configuration similar to the block diagram presented in Fig. 17.15. In this treatment plant, the equalization tank was designed for a hydraulic retention time of 8 h, and the flotation unit (DAF type) is provided with a coagulation/flocculation piping system for chemical precipitation, for the anaerobic reactor protection in case of organic overloading.

Table 17.16
Main characteristics of the combined industrial effluent (design example 2)

Parameter	Unit	Value
Flow rate	m^3/day	3,000
COD	mg/L	2,000
BOD_5	mg/L	1,500
TSS	mg/L	600
Total N	mg/L	100
Total P	mg/L	30
FOG	mg/L	350
Temperature	°C	25–35

The anaerobic reactor, called IC reactor, is a technology based on the UASB process and is essentially an up-flow granular sludge bed system. The IC reactor consists of two UASB compartments on top of each other. The produced biogas is separated in two stages within the reactor, and the biogas collected in the first stage drives a gas lift resulting in an internal circulation of wastewater and sludge.

In the IC system there are four sections:

1. Influent feed and mixing compartment
2. Fluidized bed compartment
3. Recirculation system
4. Polishing compartment

The organic load applied to the IC reactor is 5,400 kg COD/day (equivalent to 4,050 kg BOD_5/day). For this organic load, the designed IC anaerobic reactor has a useful volume of 308 m^3 with a total height of 18.5 m and a diameter of 5 m.

The expected efficiency of the IC reactor is 70–80% for BOD removal, working at a temperature between 25 and 35°C.

6.3. Design Example 3: UASB Reactor IC Type (Cheese Mill)

The IC reactor was designed to incorporate a wastewater treatment plant similar to Fig. 17.15, designed to treat the liquid effluents from a milk industry producing cheese, cream, butter, whey, and milk powder.

The equalization tank in this wastewater treatment plant has a hydraulic retention time of 24 h, and the flotation unit is a Plate Water Flotation DAF type.

The liquid effluents from this milk processing industry after passing through the flotation unit have the characteristics described in Table 17.17.

The organic load applied to the IC reactor is 5,600 kg COD/day, and the main dimensions of the reactor are 4 m diameter and 20 m height, with a useful capacity of 250 m^3. The hydraulic retention time is approximately 3.7 h and the average reactor feed flow is 67.5 m^3/h with an average upward velocity of 5.3 m^3/h. Assuming low total suspended solids inlet and

Table 17.17
Main characteristics of the combined industrial effluent (design example 3)

Parameter	Unit	Value
Flow rate	m^3/day	1,600
COD	mg/L	3,500
BOD_5	mg/L	2,800
FOG	mg/L	50–70
Temperature	°C	30

Table 17.18
Main characteristics of the combined industrial effluent (design example 4)

Parameter	Unit	Value
Flow rate	m^3/day	1,100
COD	mg/L	34,900
BOD_5/COD	–	0.53
TSS	mg/L	4,400
Total N	mg/L	500
Total P	mg/L	110
Sulfates	mg/L	460
FOG	mg/L	500
Temperature	°C	30

a temperature above 25°C, the expected removal efficiency is 70–80% for total COD and 80–90% for total BOD with a biogas production of $1,800\,m^3$/day.

The effluent from the IC reactor flows to the activated sludge system with an anoxic tank with a volume of $800\,m^3$ and an extended aeration tank with $1,800\,m^3$.

6.4. Design Example 4: Anaerobic Filter Reactor (Cheese Mill)

This milk processing mill produces cheese, cream, butter, and milk powder. The wastewater treatment plant designed to treat the industrial wastewater is similar to the standard wastewater treatment plant presented on the block diagram of Fig. 17.15. The characteristics of the raw influent are as follow and reflect the presence of a significant amount of whey (Table 17.18)

The anaerobic reactor (Fig. 17.17) is filled with a carrier material considered an ideal growth medium for the anaerobic biomass and equipped with an influent distribution system over the total bottom surface of the reactor. Due to the growth of the biomass on the carrier material, a very stable performance of the treatment plant is obtained. To ensure a sufficient up-flow velocity inside the anaerobic reactor (1 m/h), there is a recycle back flow from the outlet of the reactor, providing a total influent flow rate of the $700\,m^3$/h.

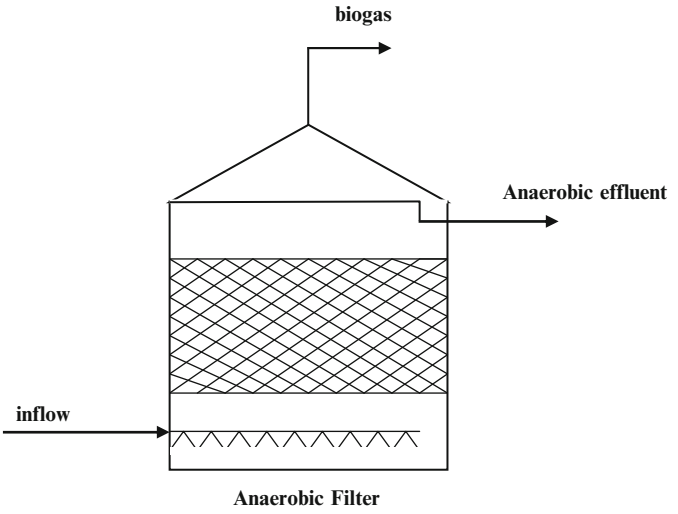


Fig. 17.17. Anaerobic reactor (design example 4).

The anaerobic filter was designed for an organic loading rate of 5 kg COD/m^3-day, and has a hydraulic retention time (HRT) of 7 day (based on the equalized raw influent flow rate) and a useful volume of 7, 680 m^3. The reactor is cylindrical with a total height of 12 m and an internal diameter of 29.8 m.

The COD removal is 80% and the BOD removal efficiency 82%, with a biogas production of 710 m^3/h.

The aerobic treatment is performed in two systems in series, each one comprising an anoxic reactor followed by an aeration tank.

7. TRENDS IN ANAEROBIC TREATMENT OF MILK PROCESSING EFFLUENTS

7.1. Results of Recent Investigations on Anaerobic Treatment of Milk Wastewater

The number and type of anaerobic treatment systems being applied to industrial and agricultural waste streams has grown tremendously since the first technologies were introduced and commercially promoted in the late 1970s and early 1980s. Over the last 30 years, the number of nonlagoon anaerobic installations worldwide has increased by nearly an order-of-magnitude and now probably exceeds 2,200 (115).

Anaerobic treatment technology is being applied more frequently to a variety of unique, high-strength waste streams produced by a wide range of industries and in particular to milk processing wastewaters. Much of the early impetus for such applications was related to complying with discharge regulations. Today, the major impetus for treating such streams is financial, based on the need for a cost-effective, high-performance treatment technology with relatively low operating costs. In addition, the potential economic value of biogas, a by-product of anaerobic treatment, has added a major economic benefit to the picture. Anaerobic

digestion is now widely used to treat high-strength industrial wastewaters with COD levels above 2 g/L, especially in case of carbohydrate-rich effluents (131, 132). The most commonly used reactor type is the UASB. More often than not, however, anaerobic digestion of industrial effluents does not proceed optimally because the composition of these effluents is typically time-variable and nutritionally imbalanced. Also, high liquid surface tensions may lead to granule flotation and, as a consequence, poor effluent quality and wash-out of slow-growing bacteria.

The number of full scale applications to wastewater containing lipids or proteins, such as milk processing, is very limited, mainly because problems were encountered with sludge retention (occurrence of sludge flotation and wash-out) and long-chain fatty acids inhibition (long chain fatty acids, LCFA, production as intermediates during lipids degradation), which is especially threatening in systems operated at a low hydraulic retention time. Therefore, control of sludge wash-out and long chain fatty acids inhibition is a prerequisite for increased application of anaerobic treatment to lipid containing wastewaters. This requires a proper choice between the currently existing high rate reactor types: (a) reactors with mobile biomass aggregates, which can accommodate higher biomass concentrations, and (b) reactors with stationary biofilms with better safety against biomass wash-out.

Biomass retention through adequate granulation is of utmost importance in UASB technology, first in order to obtain a good effluent quality and second in order to ensure a minimal cell residence time of 7–12 days, which is required to avoid the wash-out of the slowest-growing anaerobic bacteria (133). Several studies have indicated that the extend of granulation seems to be largely dependent on the feed composition, such as its mineral composition, its sugar/fatty acids ratio, or its surface tension (110, 134). Therefore it appears worthwhile, in order to make UASB technology more reliable, to develop bio-supportive additives able to maintain the granular sludge in a proper state in periods of start-up or low quality input wastewater. Wirtz and Dague (135) succeeded in shortening the period for sludge granulation by adding a cationic polymer, which allowed the increase on the volumetric load of the reactor much more rapidly.

An improvement in the efficiency of an anaerobic digestion, with respect to biomass wash-out, can be brought about by either suitably modifying the existing digester design or by incorporating appropriate advanced operating techniques. Hence, by suitable modifications in the reactor designs and/or by altering the effluent characteristics, the existing high rate digesters can be accommodated for treatment of organic effluents. Based on the characteristics of the different reactors such as efficiency based on loading rate and COD reduction, biomass retention and other factors like cost, operation, and maintenance requirements, UASB and fixed film configuration appear to be the most suitable.

In the last decade, the emphasis has been on the identification of the critical factors affecting performance, so that the reactor efficiency can be improved by maintaining optimal operating conditions. Furthermore, an assessment of the suitability of specific reactors types for different wastewaters has been performed and the possible modifications in the existing process to enhance the system efficiency were discussed. Leal et al. (136) studied the importance of the use of enzymes for hydrolyzing a wastewater from a dairy industry prior to the biological

anaerobic treatment. In that study, they propose the use of a hybrid technology – enzymatic treatment associated with anaerobic treatment – to enable the reduction in hydraulic retention time and consequently in reactor volume, since it promotes hydrolysis of fats which cause problems of clogging of the sludge bed in anaerobic reactors of the UASB type.

High rate anaerobic digestion of LCFA requires sufficient mixing of the liquid in the digester and sufficient contact between biomass and substrate, and UASB reactors cannot fulfill these requirements. The gas production rate required to achieve sufficient mixing and contact cannot be achieved if lipids contribute 50% or more to the COD of the wastewater, because at high lipid loading rates exceeding 2–3 $kgCQO/m^3$-day, UASB reactors failed completely, despite a high initial concentration of highly active, well settling biomass, and total sludge wash-out occurred (112). EGSB reactors do fulfill the requirements of mixing and contact, and the results obtained with these reactors compare very favorably with those published for more conventional digesters. However, a floating layer of undigested fatty acids and minor amounts of biomass was formed in EGSB reactors. Hence, floating layer formation and mixing characteristics in full-scale EGSB reactors require yet further research.

In case of complex wastewater containing significant amounts of fat (e.g., dairy), the continuous operation has proved to cause problems of scum layer and sludge layers on top of the reactors with subsequent biomass wash-out (52, 137). In some recent works (72, 91), it was shown that the continuous operation of UASB reactors treating dairy wastewater resulted in good COD removals but also high COD accumulation in the sludge bed leading to unstable performance of the reactors on the long run. A high degree of organic matter accumulation in anaerobic reactors treating dairy wastes was also detected by Motta Marques et al. (138) and by Guitonas et al. (139). Anderson et al. (140) reported extensive clogging (accumulation) by fatty matter on the support media of an anaerobic filter treating dairy waste. In an investigation on slaughterhouse wastewater treatment in UASB reactors, Sayed (82) suggested that the prevailing mechanism in the removal of soluble and colloidal COD is adsorption to the surface of biomass particles. This adsorption phenomenon will ultimately result in an enclosure of the sludge particles with a film of increasing thickness, and density, which increasingly will hamper the supply of substrate to the bacteria. A feedless or stabilization period would be important to invert this process and stabilize the accumulated (entrapped and adsorbed) organic matter. As a consequence, Sayed (82) suggested that the most adequate form of treating complex and/or fat containing wastewater would be the use of flocculent sludge and discontinuous feeding. This operating mode was successfully tested by Sayed et al. (141) for slaughterhouse wastewater, by Fergala (142) for domestic wastewater and by Nadais et al. (91) for dairy wastewater. The intermittent feeding operating mode was also recommended by Lettinga and Hulshoff Pol (143) for complex wastewater, namely dairy wastewater. Nadais et al. (113) studied the intermittent operation mode and concluded that the stabilization period has a fundamental importance on the operation of the UASB reactors treating complex fat containing wastewater like milk effluents.

Rinzema et al. (112) developed two modifications of the gas–solids separator for the expanded granular sludge bed (EGSB) reactors to prevent excessive sludge wash-out during anaerobic treatment of lipid emulsions: a hybrid reactor with a layer of floating carrier material

(reticulated polyurethane foam) above the expanded sludge bed, and a novel EGSB reactor equipped with a sieve-drum separator (EGSB-SDS). The first modification showed to be unreliable in the treatment of emulsified lipids, because the floating support material did not prevent strong sludge wash-out. On the other hand, the EGSB reactor equipped with a sieve-drum separator allowed stable anaerobic digestion of emulsified lipids. However, an incomplete conversion to methane of the organic matter removed from the wastewater was obtained, which should be a point for further investigation. The incomplete mineralization was attributed to the accumulation of a large and rather variable amount of lipids in a thick floating layer, which leads to a further modification of the design of the EGSB-SDS system to solve the floating layer problem. Results obtained with the hybrid reactor design showed that recirculation of the floating lipids to the granular sludge bed enhanced their conversion to methane.

An improvement in the efficiency of an anaerobic digestion, with respect to biomass wash-out, can also be brought about by incorporating appropriate advanced operating techniques. This can be addressed, for instance, by the use of membranes coupled with the anaerobic digester for biomass retention. In a membrane bioreactor (MBR) system, membranes are the main solid–liquid separation devices. Two types of MBR have been used according to the location of the membrane unit, i.e., membranes are submerged in the reactor or positioned external to the reactor. The submerged membrane type has attracted great attention in recent years since it is more compact and energy saving (144–146). It has the drawback that control of membrane fouling is more difficult to achieve than external membrane systems.

Interest in anaerobic digestion is increasing because of the well-known advantages for the treatment of high organic concentration wastewaters. Treatment of dairy wastewaters by means of up-flow anaerobic sludge blanket (UASB) reactors (147–149), hybrid UASB reactors (150), expanded granular sludge bed (EGSB) reactors (81), as well as others based on anaerobic filters (28, 151, 152) have been reported in literature. These papers show that anaerobic treatment can be effectively used for these effluents, in spite of the different operational problems quoted in literature, such as sludge flotation or toxicity/inhibition processes.

Today, there are many processes for the treatment of dairy wastewaters. However, two trends are very clear. They are based either on the recovery of valuable components, mainly proteins and lactose, or on the degradation of all substances that can alter negatively the environmental quality of the water courses.

7.2. Future Expected Developments

The bioprocesses that will be used in future for wastewater treatment will still be chosen as they have been in the past, according to technical feasibility, simplicity, and economics. However, the needs and the priorities of a sustainable society will shift the focus on wastewater treatment from pollution control to resource exploitation. In fact, many bioprocesses can provide bioenergy or valuable chemicals while simultaneously achieving the objective of pollution control. Industrial wastewaters from milk processing are ideal candidates for bioprocessing because they contain high levels of biodegradable organic material, which results in a net positive energy or economic balance. Recovery of energy and valuable materials

might reduce the cost of wastewater treatment and somewhat reduce our dependence on fossil fuels (1).

With respect to future developments in the field of anaerobic treatment of milk processing wastewaters, it can be considered:

- Optimization of anaerobic systems through reactor staging, hybridization, thermophilic treatment, accelerated hydrolysis, improved solids retention, and better process control
- Fine-tuning of anaerobic conversions to produce readily disposable effluents
- Utilization of anaerobic treatment processes as a core technology in systems designed to reclaim products from waste streams

Various constructors improved granular sludge bed reactors in recent years aiming at lowering mass transfer resistance and therewith achieving higher organic loading rates. Further improvement might be expected in the field of the treatment of specific wastewaters, so it is foreseen a further development of combination of complementary anaerobic systems, such as hybrid systems. Interesting developments are expected for anaerobic reactors that cannot rely on the development of granular conglomerates or formation of biofilms, for the retention of adequate sludge for successful treatment. This can be achieved by enhanced physical (or physico-chemical) separation of the viable biomass from the treated water. Potential systems are hybrid and/or membrane bioreactors. The major bottle-neck are the relatively high washout of suspended solids and the low rate of hydrolysis in the conventional first generation UASB reactors. Therefore, the improvement of hydrolysis of complex organic matter is of fundamental importance, being the limiting step for the treatment of complex substrates such as the milk processing wastewater. Improved retention of suspended solids in the reactor system will lead to higher sludge retention times, subsequently leading to improved treatment efficiencies. Moreover, a decreased solids load in the effluent will minimize the requirements of the posttreatment step.

Optimization of the reactor configuration can involve staging of the process into separate tanks whereby the conditions for the specific groups of bacteria involved can be optimal. Hydrolysis is greatly improved at high temperatures such as 70°C or more, and a two phase operation scheme whereby the initial treatment occurs at a very high temperature followed by a methanogenic phase at either mesophilic or thermophilic temperatures could be an interesting future development (60).

The breakthroughs dealing with reactor design and operation conditions offer practical solutions to many of the drawbacks that were initially thought to limit the scope of anaerobic digestion, such as instability, temperature requirements, sensitivity to toxicants, shock loads, and feed composition. There remain, however, inherent drawbacks to anaerobic digestion technologies that require further developments in the area of sludge engineering, since sludge adaptation to LCFA may require several weeks to months. Engineered anaerobic consortia therefore are needed to expand the catabolic diversity of sludge and shorten the period of sludge adaptation to toxic substrates. Therefore, it may be advantageous to develop effective and durable anaerobic consortia to inoculate anaerobic reactors treating complex industrial effluents containing lipids and proteins. One option to accelerate the biodegradation of toxic substrates, such as the LCFA, is to inoculate reactors with adequate bacterial strains, so

inoculation of reactors with specific degraders can be an effective means to engineer the consortium needed for degradation. Another option is to seed the reactors with sludge granules whose entire microbial association is already adapted to, or engineered for, the degradation of specific compounds. This opens interesting perspectives for the industrial production of these consortia for bioaugmentation of polluted environments or industrial digesters treating complex wastewaters, as the ones containing fat and proteins (79).

Another potential benefit associated with the large-scale availability of specialized microbial consortia is "biochemical rerouting," that is, the induction of desirable biochemical pathways as, for example, the degradation of malodorous primary amines, anaerobic ammonia oxidation, or homoacetogenesis, and the repression of undesirable pathways, such as the formation of malodorous compounds, which will leave the anaerobic digester and give rise to odor problems (79). Hence, attempts should be made to rechannel anaerobic pathways toward other end-products.

A sustainable society requires a reduction on the dependency on fossil fuels as well as a lowering of the amount of pollution that is generated by different activities. Wastewater treatment is an area in which these two goals can be addressed simultaneously, so as a result, there has been a paradigm shift recently, from disposing of waste to using it (1).

The utilization and acceptability of residuals as resources will progressively become the most appropriate, but not the only strategy for coping with environmental pollution, sustainability and survival within the limits of our ecosystem. Hence, prevention and reduction of dairy wastewater pollution can be achieved by means of direct recycling and reutilization of waste components, such as the use of cheese whey for animal feed (44) or by using different wastewater treatments, such as physical–chemical, aerobic and/or anaerobic biological treatment (153). Physical–chemical treatments allow the partial removal of the organic load by protein and fat precipitation with different chemical compounds such as aluminum sulfate, ferric chloride, and ferrous sulfide (154, 155). However, the reagent cost is high and the removal of soluble chemical oxygen demand (COD) is poor. Therefore, biological processes are often used (156).

New treatment processes are being developed that allow recovery of marketable byproducts together with anaerobic digestion. For example, membrane reactors seeded with Lactobacillus sp. are being designed to recover lactic acid and other acids from agrochemical wastes, before the latter are treated in conventional anaerobic digesters (157). Wastewater treatment for reuse will emphasize the central role of anaerobic digestion as the most sustainable treatment method for mineralizing organic matter. Hence, anaerobic digestion has the potential to play in future a major role in closing water, raw materials, and nutrient cycles in industrial processes (60).

The combination of anaerobic digestion with other biological or physical–chemical processes will lead to the development of optimized processes for the combined removal of organic matter, sulfur, and nutrients in a milk processing wastewater treatment plant. Hence, advanced methods such as coupling of reactors for suitable pretreatment and posttreatment can result in complete treatment of the effluents within the acceptable limits (158–160).

NOMENCLATURE

AAFEB = Anaerobic attached film expanded bed reactor
ABR = Anaerobic baffled reactor
AF = Anaerobic filter
AFB = Anaerobic fluidized bed
ANCP = Anaerobic contact process
ANFD = Anaerobic filter (downflow)
ANFU = Anaerobic filter (upflow)
AnRBC = Anaerobic rotating biological contact reactor
ANYB = Anaerobic hybrid systems
ASBR = Anaerobic sequencing batch reactor
ATP = Adenosine triphosphate
BOD = Biochemical oxygen demand, mg/L
BOD_5 = BOD after 5 days of incubation, mg/L
CAF = Coarse air flotation
CIP = Clean in place systems
COD = Chemical oxygen demand, mg/L
CSTR = Completely stirred tank reactor
DAF = Dissolved air flotation
DSFF = Down-flow stationary fixed film
DSFFR = Down-flow stationary fixed film reactor
DUHR = Down-flow up-flow hybrid reactor
EFB = Expanded/fluidized bed
EGSB = Expanded granular sludge bed reactor
EGSB/SDS = EGSB reactor equipped with a sieve-drum separator
EP&RC = Environmental protection & resource conservation
FAD = Flavin adenine dinucleotide
FADH = Reduced form of FAD
$FADH_2$ = Reduced form of FAD
FB = Fluidized bed
FBR = Fluidized bed reactor
FOG = Fat, oil and grease, mg/L
HRT = Hydraulic retention time, h
IC = Internal circulator reactor
LCFA = Long chain fatty acids, mg/L
MBR = Membrane bioreactor
MIC = Minimum inhibitory concentration, nM
MIC_{50} = MIC at which 50% of methanogenic activity remains, nM
NAD^+ = Nicotinamide adenine dinucleotide
NADH = Reduced form of NAD^+
NH_3 = Free ammonia, mg/L
NH_4^+ = Ammonium, mg/L

N = Nitrogen, mg/L
OHPA = Obligate hydrogen production acetogenic
OLR = Organic loading rate, kg COD/m^3-day
P = Phosphorous, mg/L
PO_4^{3-} = Phosphate, mg/L
PVC = Polyvinyl chloride
SAF = Staged anaerobic filter
SDFA = Semi-continuous digester with flocculant addition
SRT = Solids retention time, h
SS = Suspended solids, mg/L
T = Temperature, °C
TF = Trickling filter
TKN = Total Kjeldahl nitrogen, mg/L
TOC = Total organic carbon, mg/L
UASB = Up-flow anaerobic sludge blanket reactor
UFFLR = Up-flow fixed film loop reactor
UV = Ultraviolet
VFA = Volatile fatty acids, mg/L
VSS = Volatile suspended solids, mg/L

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18

Biological Wastewater Treatment of Nutrient-Deficient Tomato-Processing and Bean-Processing Wastewater

George Nakhla, Zhongda Xu, Alpesh Gohil, Andrew Lugowski, and Yung-Tse Hung

CONTENTS

Abstract A pilot-scale anaerobic/aerobic ultrafiltration system and a bench-scale anaerobic/aerobic system were tested to treat high-strength tomato-processing wastewater and bean-processing wastewater. The anaerobic/aerobic pilot-scale system achieved 99.4% SBOD removal, 91.9% NH_3-N removal, and 100% phosphorus removal at an overall hydraulic retention time (HRT) of 1.5 days and solids retention time (SRT) of 5 days during the tomato canning season. The bench-scale anaerobic/aerobic system was used to confirm the pilot-scale anaerobic/aerobic system performance. Wastewater fractionation and kinetic coefficients were studied using respirometric methods.

1. INTRODUCTION

Food processing industries usually discharge large volumes of wastewater characterized by high chemical oxygen demand (COD) or biological oxygen demand, large amounts of total suspended solids, and various inorganic constituents including nitrogen and phosphorus. The high organic load in the processing wastewater creates a pollution problem to water quality

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_18

when discharged to rivers and lakes (1). In the processing of vegetables, large amounts of waste in the form of peelings and starch as well as some sugars and proteins are released into the waste stream. With the increasing costs of pollution abatement and costly municipal surcharges, food processors are forced to find alternative methods in pretreatment of wastewater prior to discharge for secondary treatment or other treatment systems (2).

In general, the major types of food processing industries associated with environmental objectives may be regarded as (a) the agricultural industry, (b) the meat and fish processing industry, (c) the fruit and vegetable industry, (d) the dairy industry, and (e) the packaging industry (3). Agricultural inputs such as fertilizers, pesticides, feed additives, and irrigation water have been responsible for many of the recent gains in agricultural productivity, but unfortunately, a number also have had, or threaten to have, adverse side-effects on the environment. Wastewater from the meat and fish processing industry, which has a considerable organic load, is strongly polluting the environment and can have adverse effects if discharged into rivers without adequate treatment. Large amounts of fruit and vegetable processing wastes are generated from production processes, i.e., washing, peeling, blanching, transport, instrument washing, and sterilization. These wastes are characterized by chemical constituents, such as carbohydrates, starches, proteins, etc. They are not only several times as strong in terms of biochemical oxygen demand (BOD) as domestic sewage, but also highly variable in strength. Inorganic salts may be present at high concentrations, but nitrogen and phosphorous levels may be low. Wastes from the dairy industry have a high organic strength and a high chemical oxygen demand (COD), and therefore often causes disposal problems. Generally, the wastewaters from dairies form a significant proportion of the sewage, especially from small towns, and may require appropriate pretreatment before discharge to the sewers. Environmental impact is a prime consideration in food packaging and in the food packaging industry. Perhaps, the most adverse effect upon the environment as perceived by the public is the visual effect of packaging litter (3).

This chapter focuses on the treatment of tomato- and bean-processing wastewaters, both of which are nitrogen-limited for aerobic treatment, and presents data from an extensive pilot- and lab-scale study. Sun-Brite Canning Ltd., located at Ruthven, ON, is one of the largest processors of tomato products, i.e., crushed tomatoes, diced tomatoes, tomato sauce, and tomato juice in Canada. During the canning season from the end of August to the middle of October every year, about $1{,}500\,m^3$/day of high strength tomato-processing wastewater is produced from several streams of raw tomato washing, steam peeling, peeled tomato washing, and cooking. Bean-processing wastewater is generated intermittently during the off-canning season. Enforcement of stringent wastewater discharge criteria has forced the food processing industry to look for cost-effective technologies to treat their wastewaters. Historically, food processors located within or adjacent to municipalities have relied on local municipal wastewater treatment plants (WWTPs) for wastewater treatment and disposal. However, increased residential and commercial demand has constrained the ability of municipal WWTPs to accommodate high strength industrial wastewaters, thus forcing industries to use direct discharge to surface water bodies. In this particular case, wastewater needs to be treated to meet the stringent dry-ditch discharge criteria of BOD ≤ 10 mg/L, TSS ≤ 10 mg/L, NH_3-N ≤ 3 mg/L and PO_4^{3-}-P ≤ 0.5 mg/L. Treated wastewater can be used for raw tomato washing and irrigation of nearby farms.

2. WASTEWATER CHARACTERISTICS

Tomatoes are the second-most produced and consumed vegetables in North America (4) as well as the ones that contain some of the most slowly biodegradable products, i.e., seed and skin. Tomato-processing wastewaters are generally high in organic content as well as contain high particulate and colloidal fractions that are not only slowly biodegradable but also exhibit very poor settling characteristics. Furthermore, ammonia concentration in tomato wastewaters is extremely low, potentially limiting treatment efficiency as well as being conducive to filamentous microorganisms proliferation, further hampering efficiency.

Tables 18.1 and 18.2 illustrate the characteristics of tomato-processing wastewaters as obtained from the analysis of fresh wastewater produced during the canning season pilot study as well as the stored tomato wastewater following the canning seasons. It should be asserted that during the pilot study of the canning season, fresh tomato-processing wastewater characteristics were determined on wastewater samples entering the anaerobic/aerobic system from the bottom of the tank serving for storage of the primary wastewater effluent, and thus the high suspended solids. For the lab studies, the average storage time was about 3 months and although storage at $4 \pm 2°C$ is usually adequate to retard biological activity, it is evident that solubilization and hydrolysis of organic nitrogen to ammonia still occurred in light of the long holding times. Table 18.3 demonstrates the characteristics of bean-processing wastewaters as obtained from the analysis of fresh wastewater generated during the off-canning season pilot-study as well as those wastewater stored for less than 1 month.

Because of the intermittent production of bean products, parameters of bean-processing wastewaters fluctuated greatly. Bean wastes were accumulated in a storage tank and then pumped to the system. TBOD:TCOD ratio varied from 0.32 to 0.55 and SBOD:SCOD ratio varied from 0.32 to 0.42. Suspended solids concentrations and influent COD in the

Table 18.1
Fresh tomato wastewater characteristics

Parameters	Values (mg/L)	
	Range	Average
TCOD	3,760–13,770	6,223
SCOD	3,210–5,850	4,273
TBOD	1,350–4,400	2,141
SBOD	1,290–3,000	1,779
Ammonia-N	14.2–44.6	21.5
Nitrate-N	0.3–1.5	0.8
TSS	410–20,020	2,900
VSS	350–5,650	1,380
TP	14.0–100.3	31.1
SP	5.2–30.6	14.1
TKN	91.4–522.2	171.4
STKN	40.2–92.8	60.1
Alkalinity	300–1,100	635

Table 18.2
Stored tomato wastewater characteristics

Parameters	Values (mg/L)	
	Range	Average
TCOD	1,270–9,100	4,347
SCOD	340–6,090	3,439
TBOD	285–4,050	1,850
SBOD	100–2,600	1,341
Ammonia-N	0.0–97.8	33.7
Nitrate-N	0.0–9.3	1.0
TSS	80–3,380	762
VSS	55–2,290	580
TP	2.3–96.3	19.7
SP	0.0–25.6	8.2
TKN	41.3–242.6	107.9
STKN	11.2–128.1	62.6
Alkalinity	200–3,050	1,430

Table 18.3
Bean wastewater characteristics

Parameters	Values (mg/L)	
	Range	Average
TCOD	390–4,376	2,346
SCOD	170–3,260	1,767
TBOD	18–2,370	1,206
SBOD	17–1,980	869
Ammonia-N	0.4–71.2	10.6
Nitrate-N	0.0–5.1	0.8
TSS	120–2,090	481
VSS	90–1,880	382
TP	1.1–40.5	15.9
SP	0.3–21.3	9.9
TKN	12.9–106.8	61.0
STKN	11.9–50.3	32.4
Alkalinity	55–1,200	408.2

bean-processing wastewaters were much lower than the tomato-processing wastewaters. For the bean-processing wastewaters, SBOD:STKN ranged from 12:1 to 35:1 and SBOD:NH_3-N varied from 21:1 to 63:1 during the pseudo-steady state, indicating potential nitrogen limitations. Since different batches of tomato-processing wastewaters were treated in the canning season, solids concentrations varied significantly because of occasional insufficient settling in the primary clarifier during peak production while other parameters varied relatively slightly. For the tomato-processing wastewaters, TBOD to TCOD ratio varied from 0.33 to

0.42 and SBOD to SCOD ratio varied from 0.33 to 0.49, indicating the poor biodegradability of the wastewaters. Tomato-processing wastewaters contained VFAs predominantly acetic acid and propionic acid. Acetic acid and propionic acid accounted for 28.3–37.8% of the wastewater SCOD. The particulate COD of tomato wastewater, varied from 19 to 27% of TCOD, reflecting the high solids concentration even after primary treatment. Furthermore, the SBOD:STKN ratio was from 21:1 to 33:1 while SBOD:NH_3-N ratio was from 62:1 to 104:1, highlighting potentially severe nitrogen limitations.

3. TREATMENT TECHNOLOGIES

The principal biological processes used for the wastewater treatment can be divided into three categories: aerobic process, anoxic processes, and anaerobic processes according to the different operating conditions as shown in Tables 18.4 and 18.5.

4. NOVEL BIOLOGICAL TREATMENT TECHNOLOGIES

Due to the slowly biodegradable characteristics of the wastewater, conventional anaerobic and aerobic technologies are not adequate to remove carbon, nutrients and solids in the

Table 18.4
Major biological treatment processes for food processing wastewater treatment

Type	Common name	Application	Reference
Aerobic processes			
	Activated-sludge processes	Food-processing wastewater	(5, 6)
Suspended growth	Aerated lagoons	Food-processing wastewater	(7)
	Aerobic digestion	Food-processing wastewater	(8)
Attached growth	Trickling filters	Fish-processing wastewater	(9)
	Rotating biological contactors	Tomato-processing wastewater, meat-processing wastewater	(10, 11)
	Packed-bed reactor	Food-processing wastewater	(12)
Hybrid (combined)	Trickling filter/activated sludge	Food-processing wastewater	(13)
Anoxic processes			
Suspended growth	Suspended-growth denitrification	Dairy wastewater	(14)
Attached growth	Attached-growth denitrification	Industrial wastewater	(15)
Anaerobic processes			
Suspended growth	Anaerobic contact processes	Food-processing wastewater	(16)
	Anaerobic digestion	Potato-processing wastewater	(17)
Attached growth	Anaerobic packed and fluidized bed	Food-processing wastewater	(18, 19)
Sludge blanket	Upflow anaerobic sludge blanket	Food-processing wastewater	(20–22)
Hybrid	Upflow sludge blanket/attached growth	Swine wastewater	(23)

Table 18.5
Relative advantages and disadvantages of anaerobic and aerobic processes

Process	Anaerobic	Aerobic
Advantage	Low sludge yield	Good process stability
	Low energy consumption	High effluent quality
	Generation of biogas	Smaller reactor sizes
	Low nutrient requirements	
Disadvantages	Sensitivity to toxicity and influent fluctuation	High energy consumption
	Requires more monitoring	Higher nutrient requirements
	Higher capital costs	High sludge yield
	Usually requires downstream aerobic polishing prior to discharge	High operating costs

high strength tomato-processing wastewater to the standard required for this study. Thus, the suggested solution was to adopt a perfermentation step in the first stage, which was used to hydrolyze the high molecular weight particulate into low molecular weight soluble fraction and make it more biodegradable, and then remove carbon, nutrients and solids in the following aerobic stage to meet the high stringent discharge criteria.

The overall goal of the study was to evaluate treatment alternatives for the nitrogen-deficient tomato-processing wastewater that are capable of meeting very stringent discharge criteria, and to establish the design criteria for the full-scale system. The following two treatment systems were investigated at the laboratory scale level:

1. An anaerobic/aerobic system comprising an anaerobic tank followed by an aeration tank and a clarifier. The effect of the size of the anoxic tank and wastewater temperature was evaluated by operating at anoxic HRTs of 0.25 day and 0.5 day, as well as temperatures of $25 \pm 2°C$ and $32 \pm 2°C$.
2. An upflow anaerobic sludge blanket (UASB) reactor, followed by a polishing system comprising an anoxic tank, an aeration tank, and a clarifier.

In addition, a pilot-scale system, comprising an anoxic tank with an operating volume of 1.0–2.0 m^3, a 4 m^3 aeration tank, and a 1.2 m^3 conical clarifier, was operated during the canning season at a wastewater flowrate of 4.0 m^3/day. Additionally, an ultrafilter was incorporated after secondary clarification to enhance solids removal.

4.1. Pilot-Scale Anaerobic/Aerobic Treatment System

Prefermentation of domestic wastewater to improve biological nitrogen and phosphorus removal was extensively studied (24–28) and sludge prefermentation was evaluated for sludge management (29). Literature is very limited concerning the applications of prefermentation in food-processing wastewater treatment. Merzouki et al. (30) evaluated the effect of prefermentation on a bench-scale anaerobic–anoxic sequencing batch reactor (A2 SBR) for anoxic P removal from slaughterhouse wastewaters and demonstrated that the treatment could not be successfully carried out on the raw wastewater, whereas the process showed very

good nutrient removal performances after prefermentation with removals of COD, NH_3-N and PO_4^{3-}-P achieving 99, 85, and 99%, respectively.

There are no reported cases of the use of prefermentation to overcome nutrient deficiency in food-processing wastewaters, and thus the primary objective of the study was to evaluate the feasibility of using prefermentation to overcome nitrogen deficiency in tomato and bean processing wastewaters.

4.1.1. System Setup

Figure 18.1 shows the schematic diagram of this anaerobic/aerobic biological wastewater treatment system. The system consisted of an anaerobic tank with a mixer, an aeration tank with diffused air system, a 1 m-diameter secondary clarifier, and a membrane ultra-filtration system. The operating volumes of these three aforementioned stainless steel reactors were 1–2 m^3 (by adjustment of valves on the effluent pipes), 4 m^3, and 1.2 m^3, respectively. A top-mounted mixer was used in the anaerobic tank to ensure complete mix in the tank. A peristaltic hose pump accurately controlled the influent flow rate at 4 m^3/day (Q) to the system and two air-driven double diaphragm pumps with adjustable capacity up to 93 m^3/day maintained the internal recirculation ratio R1 (from aeration tank to anaerobic tank) of 2.5Q and R2 (return activated sludge, from the secondary clarifier to the anaerobic tank) of 2.5Q. During the canning season, the solids residence time (SRT) was maintained at 5 days in both anaerobic and aeration tanks by wasting proportional amount of sludges from both bioreactors. pH, ORP, and DO sensors were installed in the bioreactor tanks to monitor real-time operating conditions. DO in the aeration tank was maintained at 2–3 mg/L by adjusting an air control valve.

The feed to the anaerobic/aerobic system was obtained from the primary clarifier effluent on-site. The raw wastewater consisted of several streams of raw tomato washing, steam peeling, peeled tomato washing, and cooking during the tomato-canning season. Lime was

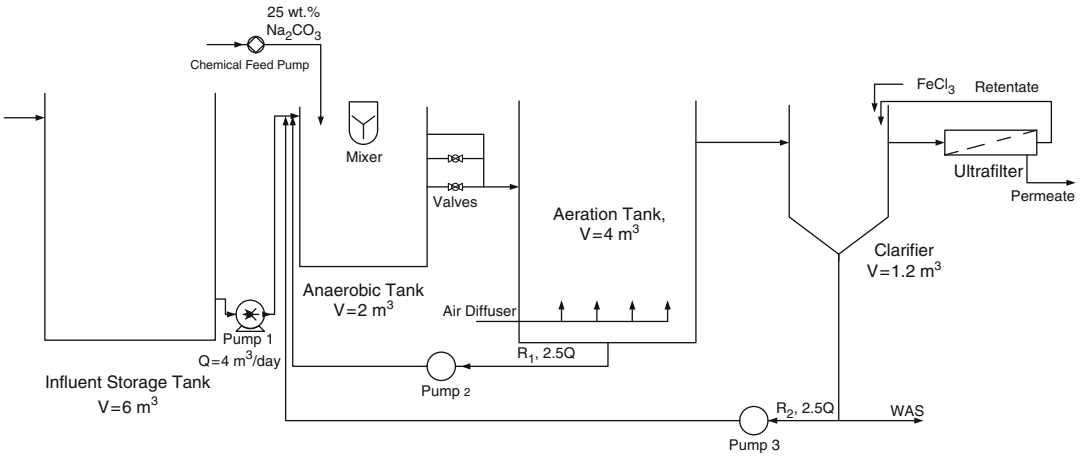

Fig. 18.1. Schematic diagram of anaerobic/aerobic biological wastewater treatment system.

added in the primary clarifier to remove settleable solids. The pH of the influent to the anaerobic/aerobic system was in the range of 4.5–5.5. The operating conditions were maintained at pH of 7–8.5 in the anaerobic tank with continuous addition of 25% (w/w) sodium carbonate solution using a chemical feed pump at 22 L/day.

The membrane module used in the tomato-canning season was a 0.1 m^2, pore size of 0.1 μm cross flow polyvinylidene fluoride (PVDF) module, provided by Pall Canada, Mississauga, ON with a nominal flux of 4.8 m^3/m^2-day. The ultrafiltration system was operated at the vendor-recommended conditions: influent flow rate of 1.2 gallon per minute (GPM) and 15–20 psi of transmembrane pressure (TMP). The timer of the feed pump was set to 0.33 h and that of the backwash pump was set to 0.33 min, i.e., backwash started every 20 min and ran for 20 s.

The system was commissioned and operated for 5 weeks prior to data collection. From day 1 to day 109, the system was processing bean wastewaters and from day 110 to day 161, the system was processing tomato wastewaters. As previously mentioned, due to increased hydraulic and contaminant loadings during the tomato canning season, the focus of this paper is on the tomato wastewaters.

4.1.2. Performance of Anaerobic/Aerobic System

Prior the tomato canning season, from day 1 to day 109, the anaerobic/aerobic system was operated on bean-processing wastewaters without the ultra-filter. Table 18.6 illustrates the pseudo-steady-state performance of anaerobic/aerobic system at an influent flow rate Q of 3 m^3/day corresponding to an overall hydraulic retention time (HRT) of 1.83 days, comprising 0.5 day in the anaerobic tank and 1.33 days in the aeration tank (volume of anaerobic tank was adjusted to 1.5 m^3 during this period), and an SRT of 180 days. Samples were collected and

Table 18.6
Pseudo-steady-state performance of the pilot-scale system treating bean-processing wastewaters at overall HRT = 1.83 days and SRT = 180 days (Unit: mg/L)

	Influent[a]	Anaerobic	Aeration	Clarifier
TCOD	1,452 ± 1,013 (5)			176 ± 53 (5)
SCOD	890 ± 481 (5)			71 ± 6 (4)
TBOD	532 ± 366 (5)			37 ± 24 (5)
SBOD	328 ± 207 (5)			12 ± 7 (5)
NH_3-N	3.0 ± 2.5 (5)			1.9 ± 0.7 (5)
NO_3^--N	0.2 ± 0.2 (5)			0.3 ± 0.3 (5)
TSS	398 ± 252 (5)	5,660 ± 727 (5)	5,526 ± 883 (5)	60 ± 24 (4)
VSS	288 ± 244 (5)	4,958 ± 633 (5)	4,832 ± 721 (5)	48 ± 16 (4)
TP	9.4 ± 8.7 (5)			3.3 ± 1.6 (5)
SP	2.6 ± 2.4 (5)			2.0 ± 1.2 (5)
TKN	35.3 ± 8.9 (4)			17.3 ± 8.5 (4)
STKN	18.6 ± 5.9 (4)			6.6 ± 3.7 (4)
Alkalinity as $CaCO_3$	656 ± 325 (5)			1,400 ± 316 (5)

[a] Number within parenthesis indicates number of samples.

analyzed once a week. The system achieved 96.3% BOD removal, 84.9% TSS removal, 36.7% ammonia removal, and 64.9% phosphorus removal without chemical addition. Pretreatment in the anaerobic tank was effective since the anaerobic tank generated more STKN and more SBOD than originally presented in the effluent. This is evident considering that the data reported for the anaerobic effluent reflected the 6Q flow going through the anaerobic tank. On a mass rate basis, the anaerobic effluent contained about three times as much ammonia and STKN as the influent raw wastewaters. The data of Table 18.6 also clearly indicates excessive effluent TSS exceedance of the 10 mg/L criteria. It should be noted that the system was operated without any coagulant addition.

Table 18.7 summarizes the pseudo-steady state performance of the anaerobic/aerobic system treating tomato-processing wastewaters at a flow rate of 4 m^3/day corresponding to an overall HRT of 1.5 days, comprising 0.5 day in the anaerobic tank and 1 day in the aeration tank (volume of anaerobic tank was adjusted to 2 m^3 in the canning season) and an SRT of 5 days. The system was operated without any nutrient addition during the entire 52-day canning season. The final effluent reached 79 mg/L soluble COD (SCOD), 9 mg/L BOD, 1.2 mg/L ammonia, 1.0 mg/L nitrate, 0.0 mg/L of SP with the addition of ferric chloride at Fe:P = 4.5:1 on a mass basis (Metcalf and Eddy, 2003) in the clarifier effluent, and 1 mg/L TSS/VSS in the permeate. The system achieved 99.4% BOD removal, 99.2% suspended solids removal, 100% phosphorus removal, with low ammonia concentration in the final effluent.

Figures 18.2–18.5 illustrate the SBOD, TSS/VSS, ammonia and nitrate temporal variation profiles during the testing period respectively. The relative stability of the system with respect to SBOD removal is evidenced by the pseudo-steady-state effluent SBOD during the tomato-

Table 18.7
Pseudo-steady-state performance of the pilot-scale system treating tomato-processing wastewaters at HRT = 1.5 days and SRT = 5 days (Unit: mg/L)

	Influent[a]	Anaerobic effluent	Aeration effluent	Clarifier effluent	Permeate
TCOD	4,923 ± 458 (7)			211 ± 42 (6)	82 ± 16 (9)
SCOD	3,874 ± 322 (7)			149 ± 25 (7)	79 ± 14 (9)
TBOD	1,881 ± 192 (7)			38 ± 28 (7)	5 ± 1 (7)
SBOD	1,590 ± 173 (7)			9 ± 5 (7)	5 ± 1 (7)
NH_3-N	19.8 ± 3.3 (7)			1.2 ± 0.5 (6)	
NO_3^--N	1.1 ± 0.3 (7)			1.0 ± 0.3 (7)	
TSS	1,310 ± 338 (7)	9,311 ± 4,606 (7)	8,550 ± 4,514 (7)	95 ± 14 (6)	1 ± 0 (7)
VSS	896 ± 184 (7)	5,253 ± 2,305 (7)	4,507 ± 2,343 (7)	76 ± 17 (6)	1 ± 0 (7)
TP	19.1 ± 4.7 (7)			1.1 ± 2.6 (7)	
SP	11.9 ± 3.7 (7)			0.0 ± 0.0 (7)	
TKN	131.3 ± 13.1 (7)			16.9 ± 13.3 (7)	
STKN	60.2 ± 10.1 (7)			5.0 ± 3.8 (7)	
Alkalinity as $CaCO_3$	568 ± 160 (7)			1,061 ± 251 (7)	

[a] Number within parenthesis indicates number of samples.

canning season from day 139 afterwards. The clarifier effluent TSS hovered around 100 mg/L throughout the canning season as apparent from Fig. 18.3. Notwithstanding the high influent variations as well as the high bioreactor biomass concentrations as high as 14,000 mg/L, the sludge settled very well as reflected by diluted sludge volume index (DSVI) of aerobic mixed

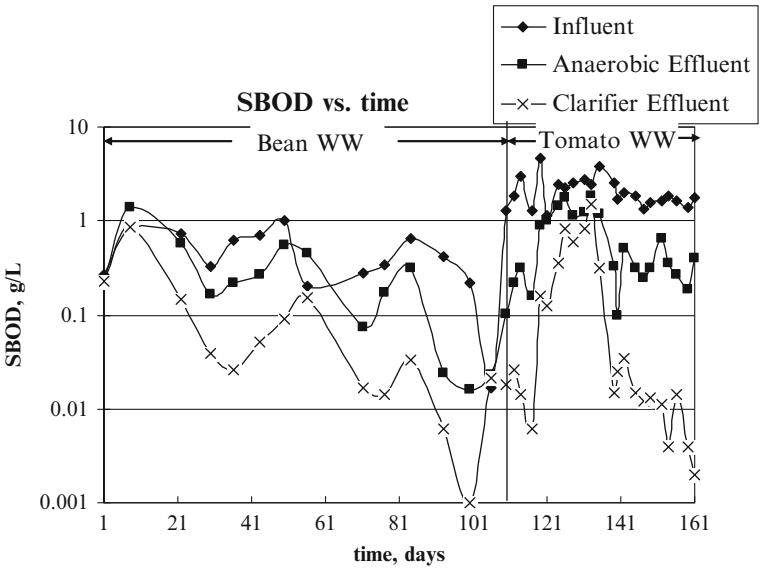

Fig. 18.2. SBOD in pilot-scale system.

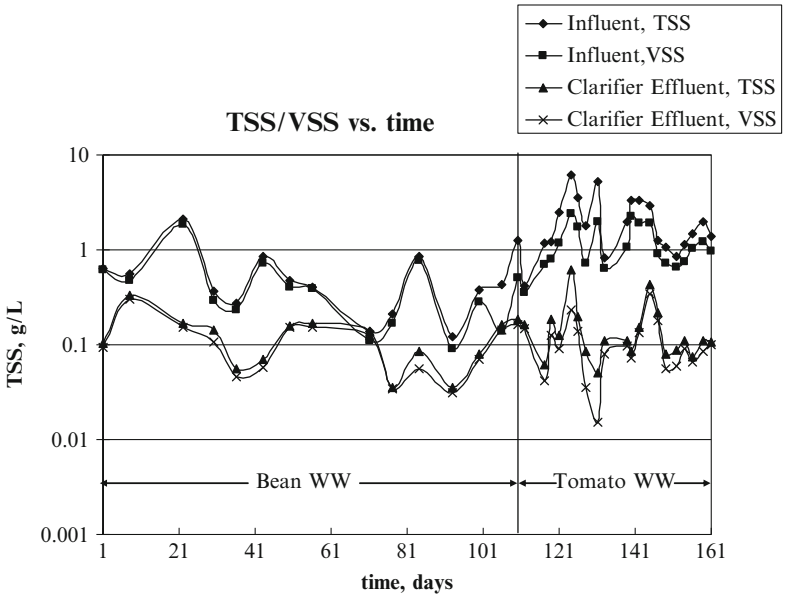

Fig. 18.3. TSS/VSS in pilot-scale system.

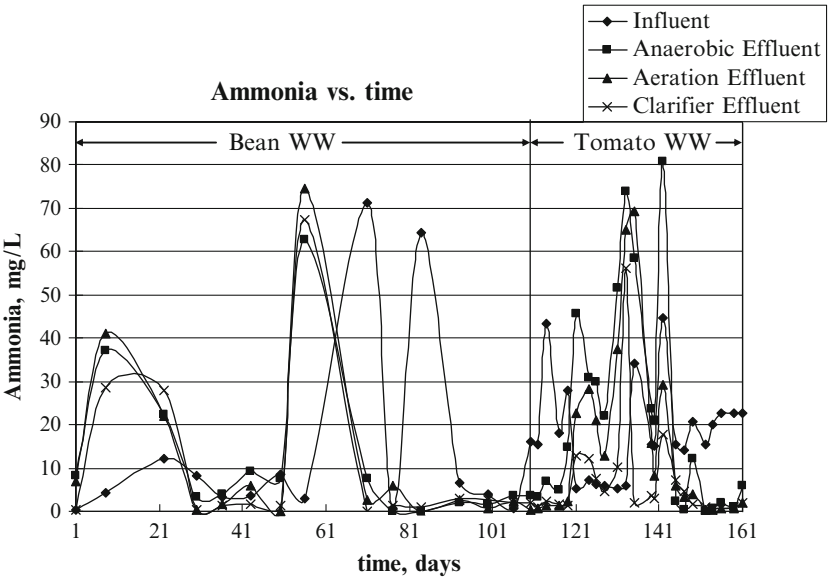

Fig. 18.4. Ammonia in pilot-scale system.

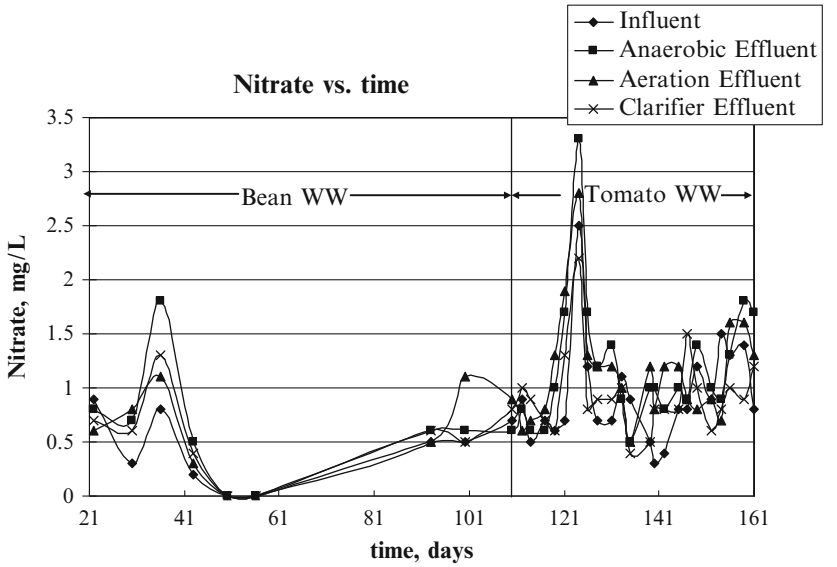

Fig. 18.5. Nitrate in pilot-scale system.

liquor shown in Fig. 18.6 ranging from 22.7 to 116.2 mL/g. However, the demonstrated poor performance of the clarifier was attributed to short circuiting in the 1 m-diameter clarifier and lack of scum collection mechanism and sludge sweeping devices in addition to the high solids loading of up to 219 kg/m^2-day. The clarifier effluent during the pseudo-steady state

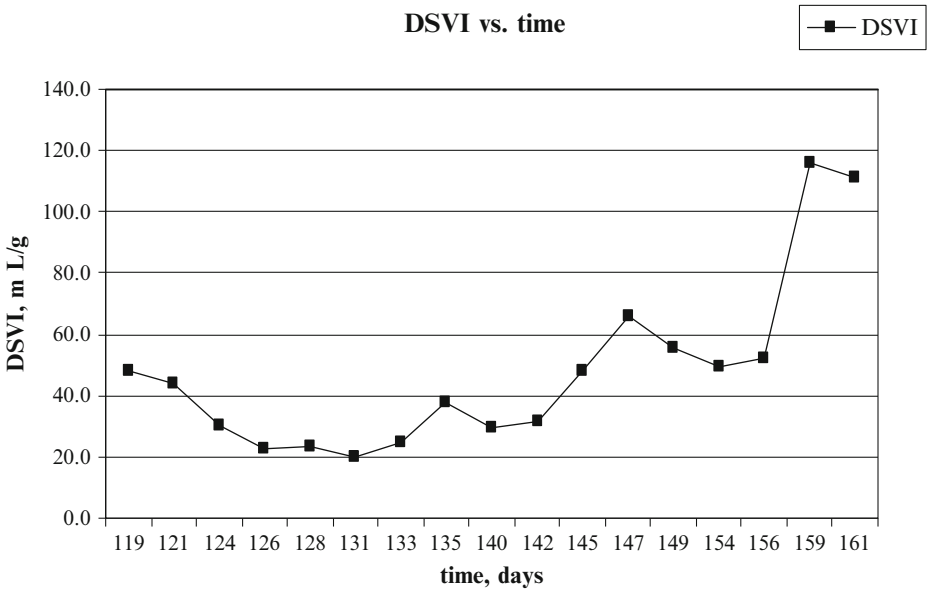

Fig. 18.6. DSVI in pilot-scale system.

performance treating bean-processing wastewaters did not meet the TSS discharge criteria because of operational problems during this period. The system was not adequately attended to during the bean-wastewater run, often resulting in accumulation of sludges on the clarifier surface, as well as stoppage of air-operated recycle pumps. During the pseudo-steady-state treatment of tomato-processing wastewater, the soluble effluent BOD was less than 10 mg/L criterion in 43% of the samples and ranged from 11 to 14 mg/L for the remaining 57% of the samples, while effluent TSS exceeded in all samples. Hence, the portable pilot ultrafiltration membrane system was adopted to further reduce the solids concentration in the final effluent to 1 ± 0 mg/L and meet the TSS discharge criteria. After the incorporation of the ultrafilter, all data points met all the discharge criteria. Figure 18.4 shows that up to 36.7 and 91.9% of ammonia were removed in the system during the bean and tomato waste treatment respectively while no nitrates were generated, as confirmed by Fig. 18.5, clearly corroborating that the nitrogen deficiency was just balanced without nutrient addition. Particulate TKN accounted for 47.3 and 54.2% of total influent TKN in wastewaters. Nitrogen content in the sludge was 7.9 and 7.4% by weight of VSS for bean and tomato wastes, respectively. The biomass yield, calculated from a plot of cumulative VSS produced vs. cumulative COD removed (not shown, $R^2 = 0.92$), was 0.22 kg VSS/kg COD (0.31 kg COD/kg COD, after multiplying the conversion factor of 1.42 mg COD/mg VSS, Metcalf and Eddy, 2003).

4.1.3. Performance of Portable Microza Ultrafiltration System

After the incorporation of the ultrafiltration system during the canning season, effluent suspended solids concentration decreased from more than 100 mg/L in the clarifier effluent to 1 mg/L in the permeate. The permeate quality was quite stable, reflected by the TSS/VSS of

1 ± 0 mg/L and low COD ranging from 65 to 110 mg/L despite wide variations in the influent TCOD (149–628 mg/L) and TSS (80–220 mg/L). Based on the average, the ultrafiltration system achieved 61.1% TCOD removal, 86.8% TBOD removal, and 99.9% TSS removal. Inlet pressure, retentate pressure, and permeate pressure stabilized in the range 12–25, 10–15, and 1–3 psi, respectively. TMP and temperature were 10.5–15.5 psi and 18–25°C, respectively, during the canning season. The permeate flow rate was 0.10–0.12 L/min corresponding to a membrane flux of 1.44–1.73 m^3/m^2-day vis-à-vis the vendor specified clean water flux of 4.8 m^3/m^2-day, due to different suspended solids concentration in the influent.

4.1.4. Impact of Prefermentation in the Anaerobic Tank

4.1.4.1. Operating Conditions

The temporal variation of 2-day average ORP in the anaerobic, and pH in the anaerobic and aerobic bioreactors is depicted in Fig. 18.7. As shown in Fig. 18.7, pH was controlled at around seven and eight, respectively in the anaerobic and aeration tank, which is in optimum range of 7.0–8.5 for anaerobic bioreaction and 7.5–8.6 for aerobic bioreaction. DO was a major factor affecting the performance of the system, and it was controlled in the range from 1.0 to 3.0 mg/L. ORP value fluctuated greatly during bean-processing wastewater run in the −150 to −180 mV, but remained stable at −600 mV during the tomato wastewater run, attesting to the highly anaerobic and fermentative conditions in the bioreactor.

4.1.4.2. Solubilization of COD and BOD

In the anaerobic/aerobic system, the anaerobic tank was adopted to hydrolyze the slowly biodegradable particles and generate volatile fatty acids (VFA). Mass balances of SBOD, SCOD across the anaerobic bioreactor are presented in Figs. 18.8 and 18.9. As shown in the figures, SBOD and SCOD increased by 52.0 and 12.0%, respectively, on average for bean wastes during the off-canning season, and 18.6 and 3.4%, respectively, on the average for tomato wastes during the tomato-canning season across the anaerobic tank.

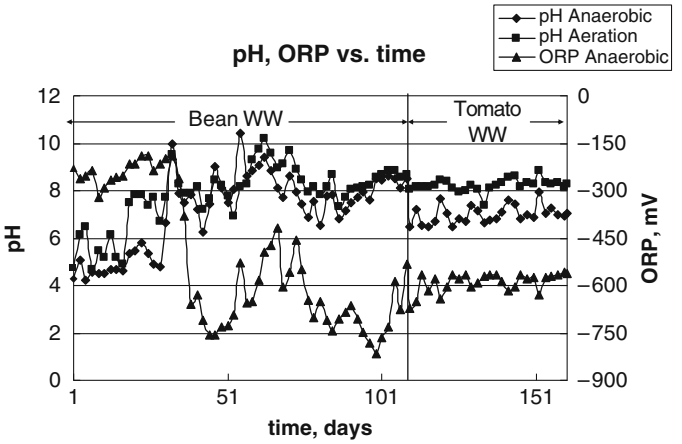

Fig. 18.7. pH and ORP profile of pilot-scale system.

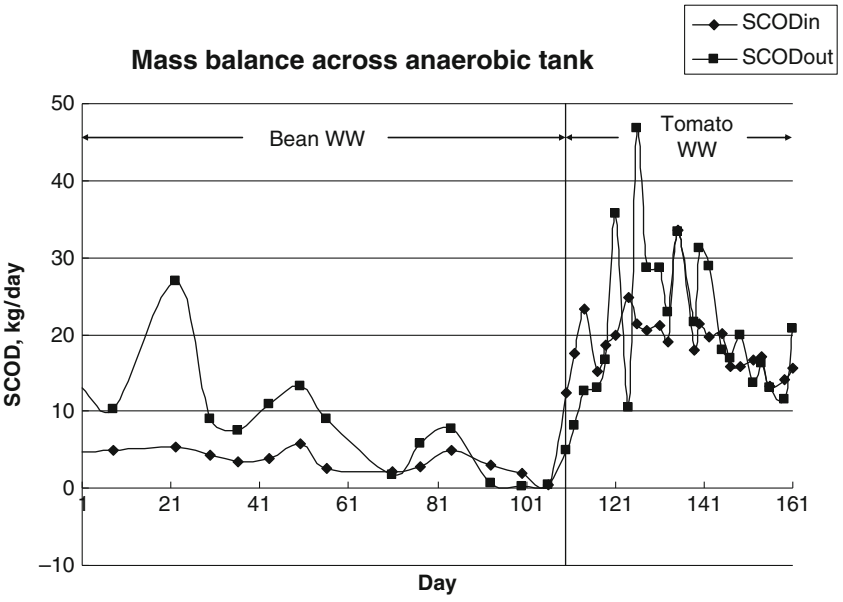

Fig. 18.8. SCOD balance across anaerobic tank.

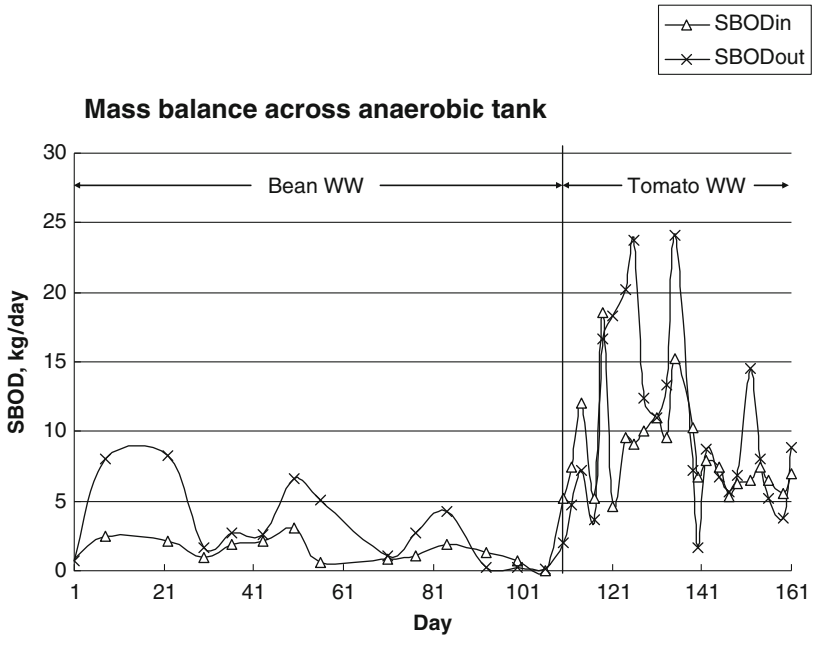

Fig. 18.9. SBOD balance across anaerobic tank.

4.1.4.3. Fate of Nitrogen in the System

STKN mass balance data as shown in Fig. 18.10, prove that the anaerobic tank was effective in hydrolyzing the particulate nitrogen into more soluble forms. On average mass basis, the anaerobic tank generated 143.8 and 19.8% more STKN than the original influent for bean and tomato wastes, respectively, during the entire period.

4.1.4.4. Improvement of Kinetics

Three (3) batches of samples were collected during the tomato canning season from the pilot anaerobic/aerobic system and subjected to a 3-day batch respirometry test on these raw wastewaters and anaerobic effluents. Among these three batches of samples, sample 1, 2, and 3 were collected on day 130, day 144, and day 151, respectively, so sample 3 was in the span of pseudo-steady-state performance of the anaerobic/aerobic system. Respirometric tests of raw wastewater samples with and without ammonia were compared. Table 18.8 lists the results of the respirometric studies. All calculations are expressed per liter of the raw influent wastewater, i.e., after discounting the contribution of recycle streams. From Table 18.8, it is apparent that S_S accounted for 7.7–22.7% of SCOD of raw wastewaters in the runs without ammonia addition, while S_S accounted for 37.5–42.6% of SCOD of raw wastewaters in the runs adding ammonia emphasizing the nitrogen limitation of the raw wastewater. In the runs on anaerobic effluents without ammonia addition, S_S accounted for 23.8–83.5% of SCOD of anaerobic effluent samples. It shows very clearly that nitrogen limitation in the raw wastewater was overcome by prefermentation in the anaerobic tank. More importantly, the implementation of anaerobic prefermentation tank could lead to significant savings in

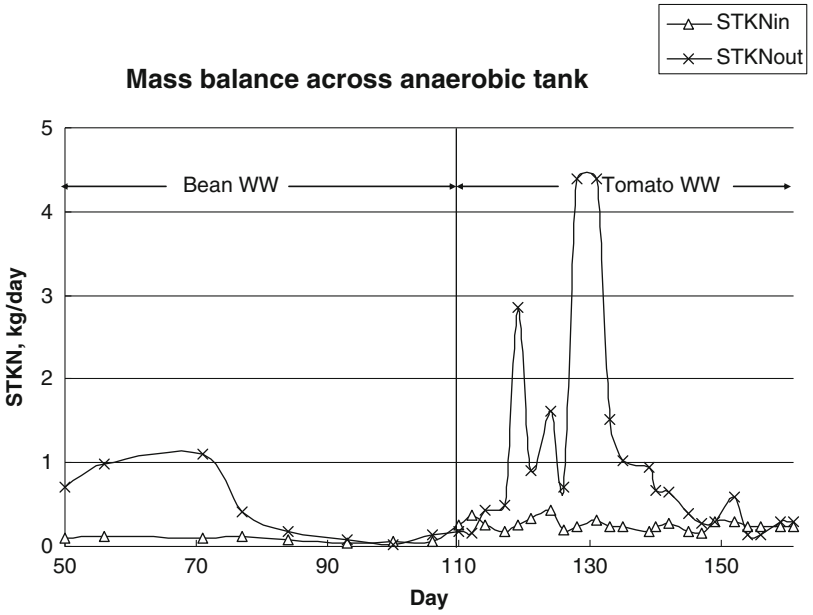

Fig. 18.10. STKN balance across anaerobic tank.

Table 18.8
Comparison of readily biodegradable fractions and kinetics between raw tomato wastewater influent and anaerobic effluent

		Influent (without ammonia addition)	Influent (with ammonia addition)	Anaerobic effluent (without ammonia addition)
Sample 1	S_S, mg/L	446	1,992	3,443
	SCOD, mg/L	5,100	5,150	7,960
	S_S%	8.7	38.7	43.3
	μ_{max}, day^{-1}	2.26	2.05	2.05
	b_H, day^{-1}	0.22	0.27	0.24
	K_S, mg/L	175	175	75
Sample 2	S_S, mg/L	1,195	1,950	2,291
	SCOD, mg/L	5,420	5,200	9,625
	S_S%	22.1	37.5	23.8
	μ_{max}, day^{-1}	0.95	0.95	2.69
	b_H, day^{-1}	0.28	0.27	0.15
	K_S, mg/L	50	50	80
Sample 3	S_S, mg/L	246	1,336	3,043
	SCOD, mg/L	3,100	3,140	3,642
	S_S%	7.7	42.6	83.5
	μ_{max}, day^{-1}	3.04	1.20	5.64
	b_H, day^{-1}	0.26	0.27	0.24
	K_S, mg/L	175	150	100

operating costs for the full-scale treatment system. Based on a 3,000 m^3/day full-scale system, typical wastewater influent COD of 5,000 mg/L and BOD_5 of 2,000 mg/L, and a ratio of BOD:N = 100:5 required for biomass growth, 280 kg N/day in the full-scale system would be required to supplement the raw wastewater nitrogen.

Comparison of raw wastewater and anaerobic effluent also revealed the significant improvement in biodegradation kinetics. We can clearly see that the readily biodegradable fraction increased from 1,992 mg/L (38.7% of influent SCOD) to 3,443 mg/L (43.3% of anaerobic effluent SCOD) in sample 1, increased from 1,950 mg/L (37.5%) to 2,291 mg/L (23.8%) in sample 2 and increased dramatically from 1,336 mg/L (42.6%) to 3,043 mg/L (83.5%) in sample 3. Using the respirometric methods to determine μ_{max} and b_H as above, maximum specific growth rate μ_{max} remained 2.05/day in sample 1 and increased from 0.95 to 2.69/day in sample 2 and increased from 1.20 to 5.64/day in sample 3, which demonstrated that the anaerobic effluent is more readily biodegradable than the raw tomato-processing wastewater.

4.2. Bench-Scale Anaerobic/Aerobic Treatment System

Since the canning season only lasts for 52 days which was not enough for the study of different operating conditions on the performance of wastewater treatment system, a bench-

scale anaerobic/aerobic system was constructed and used to investigate the treatment of high-strength tomato-processing wastewater generated from Sun-Brite Canning Ltd., Ruthven, Ontario as well as for comparison with the pilot-scale anaerobic/aerobic system in the field.

4.2.1. System Setup

The detailed setup of the bench-scale anaerobic/aerobic biological wastewater treatment system is shown in Fig. 18.11. The system was composed of a 75 L influent storage tank with a heavy-duty high-torque mixer, a 5 L plexiglass anaerobic tank with a mixer mounted on the top, a 10 L plexiglass aeration tank with a mixer on the top and copper coil air diffuser immersed in the mixed liquor, and an 8 L plexiglass clarifier. Two (2) immersion heaters with dial temperature controller were put into the anaerobic and aerobic tank to increase the liquid temperature to $32 \pm 2°C$. Internal recirculation ratio R1 (from aeration to anaerobic) of 3Q and R2 (return activated sludge (RAS), from clarifier to anaerobic) of 2Q were employed while influent flow rate (Q) was set at 10 L/day.

The systems were operated at the conditions as shown in Table 18.9. ORP ranged from -200 to -300 mV in the bench-scale anaerobic reactor compared with -500 to -600 mV in the pilot-scale anaerobic reactor. DO was maintained 2.0–3.0 mg/L in the aeration reactor in the pilot-scale and bench-scale system. A detailed discussion of the experimental observation is presented below. Table 18.5 illustrates the performance of pilot-scale anaerobic/aerobic-ultrafiltration system in Period I and Tables 18.10–18.12 demonstrate the pseudo-steady-state performance of bench-scale anaerobic/aerobic system in Period II–IV, respectively.

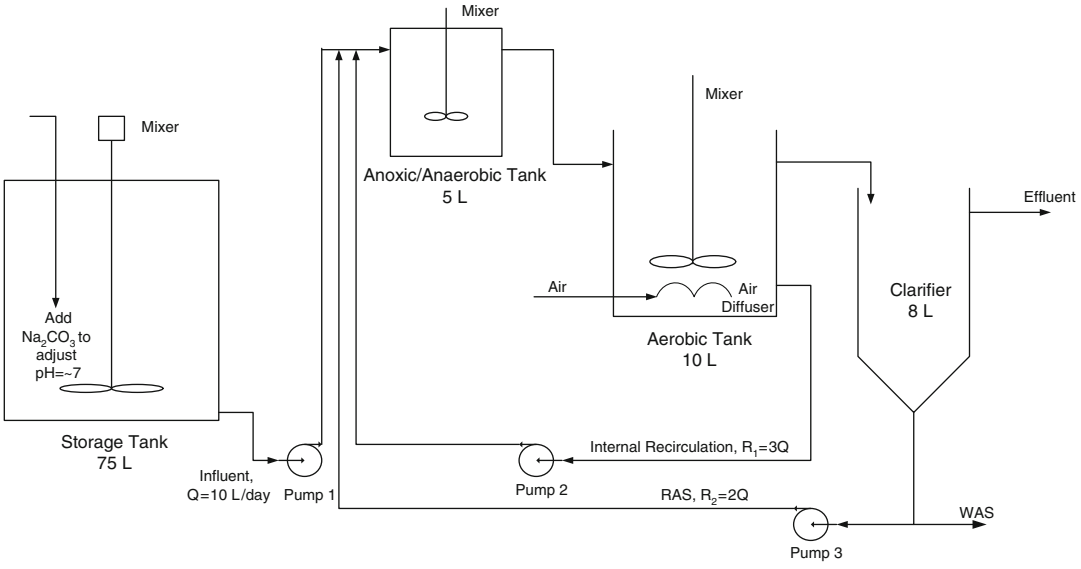

Fig. 18.11. Schematic diagram of bench-scale anaerobic/aerobic biological wastewater treatment system.

Table 18.9
Operating conditions of pilot-scale and bench-scale systems

Period	Day	Scale	HRT, day(s)		SRT, days	Temperature,°C
			Anaerobic	Aerobic		
I	1–52	Pilot	0.5	1	5	25
II	11–112	Bench	0.25	1	10	25
III	113–158		0.5	1	10	25
IV	159–202		0.5	1	10	32

Table 18.10
Pseudo-steady-state performance of the bench-scale system treating tomato-processing wastewater at HRT = 1.25 days and SRT = 10 days, 25°C (Unit: mg/L)

	Influent[a]	Anoxic	Aeration	Clarifier
TCOD	$4{,}610 \pm 432$ (5)			179 ± 25 (5)
SCOD	$3{,}727 \pm 370$ (5)			103 ± 27 (5)
TBOD	$1{,}580 \pm 169$ (5)			17 ± 7 (5)
SBOD	$1{,}285 \pm 234$ (5)			8 ± 5 (5)
Ammonia	6.1 ± 1.3 (5)			1.7 ± 1.1 (4)
Nitrate	1.0 ± 0.5 (5)			1.6 ± 0.5 (2)
TSS	765 ± 109 (4)	$4{,}686 \pm 547$ (5)	$4{,}522 \pm 458$ (5)	66 ± 17 (4)
VSS	633 ± 63 (4)	$3{,}724 \pm 453$ (5)	$3{,}560 \pm 392$ (5)	51 ± 10 (4)
TP	16.9 ± 1.9 (5)			5.7 ± 2.4 (5)
SP	8.6 ± 3.9 (5)			4.8 ± 1.9 (5)
TKN	113.3 ± 9.3 (5)			6.7 ± 2.7 (5)
STKN	62.2 ± 11.6 (5)			3.9 ± 2.6 (5)
Alkalinity as $CaCO_3$	$1,275 \pm 332$ (5)			$1,606 \pm 200$ (5)

[a] Number within parenthesis indicates number of samples.

4.2.2. Effluent Quality in the Anaerobic/Aerobic Systems

Figures 18.12–18.16 illustrate the TCOD, TBOD, total nitrogen (TN), TSS, and VSS temporal variation profiles during the four testing periods, respectively. The influent during pseudo-steady-state performance to anaerobic/aerobic system in Period I had much higher TCOD, TBOD, and TSS concentrations averaging 4,923, 1,881, and 1,310 mg/L, respectively, as compared to averages of 2,788–4,610, 980–1,862, and 442–765 mg/L, respectively, in Periods II–IV, due to the high organic loading and solids loading in the canning season. Because of different batches, systems in Period I and Period II suffered from severe nutrient-deficiency problem revealed by low concentrations of NH_3-N of 19.8 and 6.1 mg/L as shown in Tales 18.7 and 18.8, while higher concentrations of NH_3-N of 50.9 and 34.7 mg/L were observed in Period III and Period IV. The reason for these high ammonia concentrations in the influent is anaerobic degradation as a result of the long storage times of 2–5 months, despite the cold ambient temperature of 4°C.

Table 18.11
Pseudo-steady-state performance of the bench-scale system treating tomato-processing wastewater at HRT = 1.5 days and SRT = 10 days, 25°C (Unit: mg/L)

	Influent[a]	Anoxic	Aeration	Clarifier
TCOD	3, 852 ± 489 (6)			82 ± 8 (6)
SCOD	3, 490 ± 408 (6)			78 ± 8 (6)
TBOD	1, 862 ± 223 (6)			8 ± 4 (6)
SBOD	1, 623 ± 183 (6)			6 ± 3 (6)
Ammonia	50.9 ± 6.4 (6)			0.0 ± 0.0 (6)
Nitrate	0.9 ± 0.4 (6)			1.0 ± 0.4 (6)
TSS	442 ± 94 (6)	4, 293 ± 265 (6)	4, 278 ± 106 (6)	24 ± 8 (6)
VSS	358 ± 72 (6)	3, 355 ± 196 (6)	3, 360 ± 136 (6)	18 ± 6 (6)
TP	16.4 ± 8.4 (6)			4.2 ± 1.9 (6)
SP	8.4 ± 3.5 (6)			3.8 ± 1.9 (6)
TKN	105.6 ± 14.3 (6)			4.8 ± 2.4 (6)
STKN	73.1 ± 15.2 (6)			2.7 ± 1.8 (6)
Alkalinity as $CaCO_3$	2, 117 ± 714 (6)			1, 833 ± 244 (6)

[a]Number within parenthesis indicates number of samples.

Table 18.12
Pseudo-steady-state performance of the bench-scale system treating tomato-processing wastewater at HRT = 1.5 days and SRT = 10 days, 32°C (Unit: mg/L)

	Influent[a]	Anoxic	Aeration	Clarifier
TCOD	2,788 ± 899 (6)			63 ± 9 (6)
SCOD	2,208 ± 769 (6)			52 ± 5 (6)
TBOD	920 ± 397 (6)			8 ± 0 (6)
SBOD	740 ± 324 (6)			6 ± 2 (6)
Ammonia	34.7 ± 17.6 (6)			0.0 ± 0.0 (6)
Nitrate	0.5 ± 0.1 (6)			1.2 ± 0.4 (6)
TSS	445 ± 89 (6)	3,718 ± 509 (5)	3,615 ± 460 (6)	8 ± 4 (6)
VSS	335 ± 67 (6)	3,202 ± 438 (5)	3,090 ± 412 (6)	6 ± 3 (6)
TP	12.8 ± 5.5 (6)			3.3 ± 0.4 (6)
SP	5.8 ± 3.7 (6)			3.0 ± 0.4 (6)
TKN	79.1 ± 23.6 (6)			3.9 ± 1.8 (6)
STKN	50.6 ± 26.1 (6)			2.1 ± 1.1 (6)
Alkalinity as $CaCO_3$	1,325 ± 138 (6)			1,417 ± 75 (6)

[a]Number within parenthesis indicates number of samples.

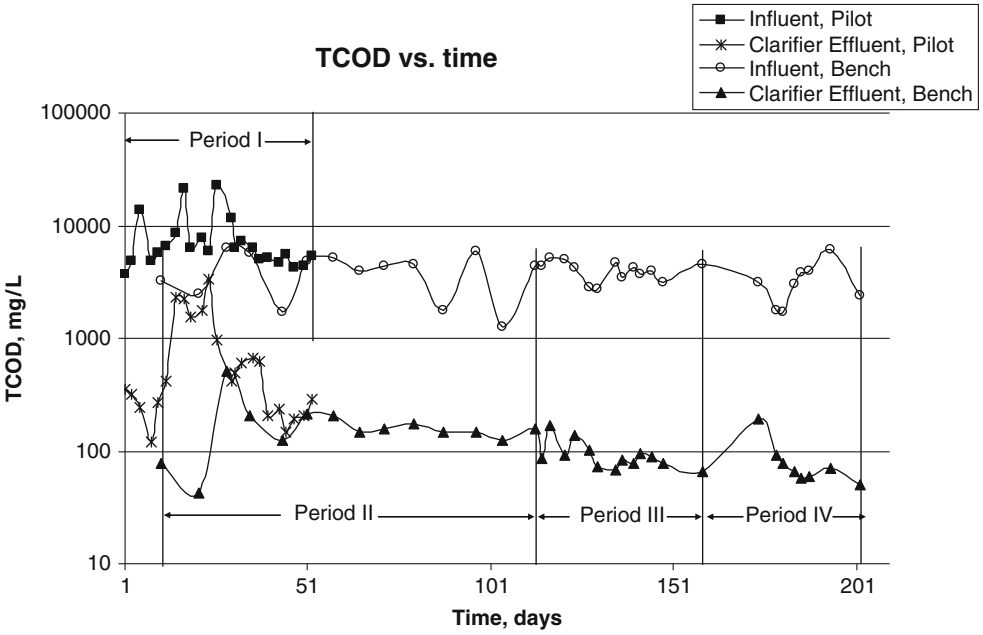

Fig. 18.12. Temporal profile of TCOD in pilot-scale and bench-scale systems.

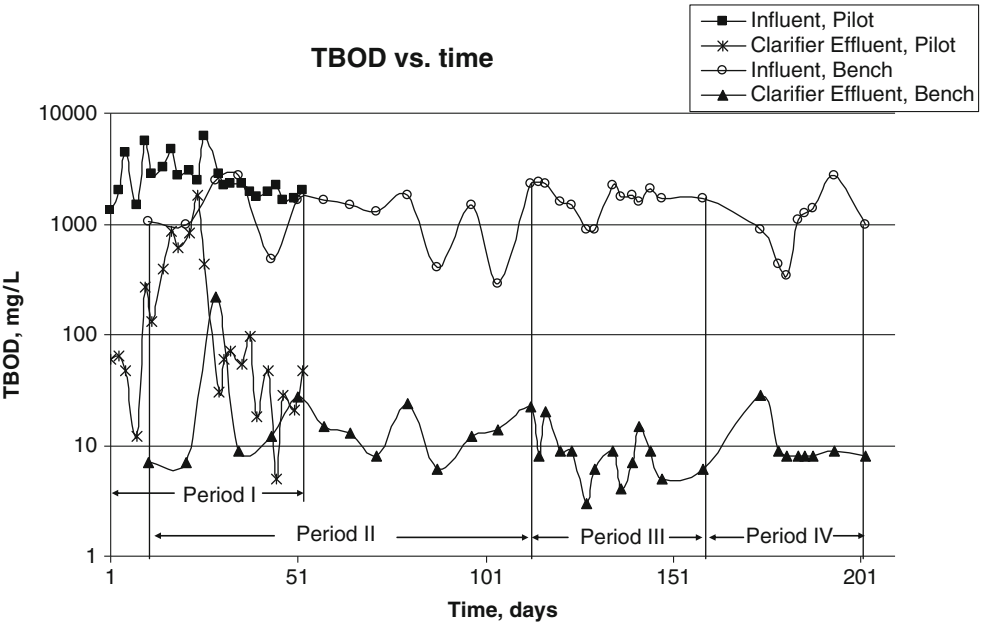

Fig. 18.13. Temporal profile of TBOD in pilot-scale and bench-scale systems.

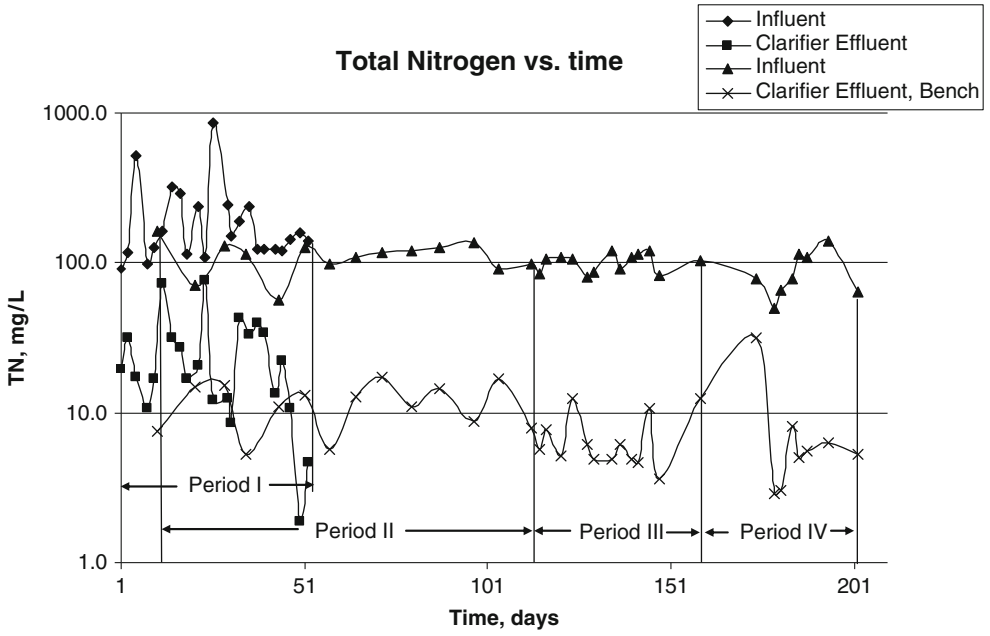

Fig. 18.14. Temporal profile of TN in pilot-scale and bench-scale systems.

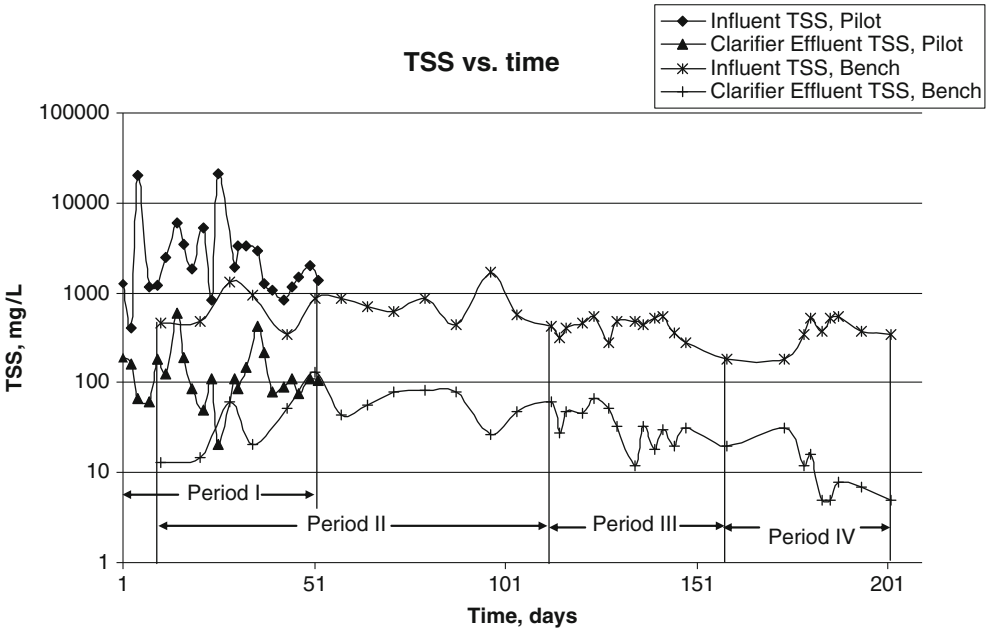

Fig. 18.15. Temporal profile of TSS in pilot-scale and bench-scale systems.

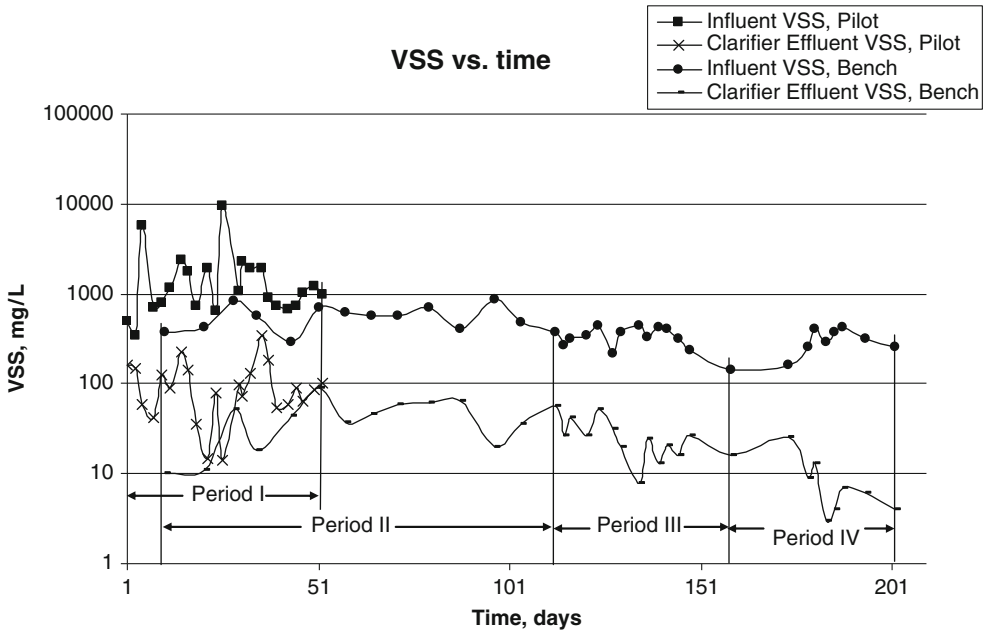

Fig. 18.16. Temporal profile of VSS in pilot-scale and bench-scale systems.

As far as the clarifier effluent quality is concerned, TSS concentrations were 95 and 66 mg/L, respectively, in Periods I and II but declined to 24 and 9 mg/L, respectively, in Periods III and IV as shown in Fig. 18.15. As demonstrated by Figs. 18.12 and 18.13, clarifier effluent TCOD and TBOD concentrations were relatively as high as 211 and 38 mg/L, respectively, in Period I, and 179 and 17 mg/L, respectively, in Period II but declined to 82 and 8 mg/L, respectively, in Period III, and to 67 and 8 mg/L, respectively, in Period IV. A declining trend of clarifier effluent TN concentrations was observed, from 17.9 mg/L in Period I to 3.3 mg/L in Period IV as shown in Fig. 18.14. Low effluent concentrations of ammonia of 0.0–1.7 mg/L and nitrate of 1.0–1.6 mg/L were found during the four operating periods.

Compared with the dry-ditch discharge criteria of BOD < 10 mg/L, TSS < 10 mg/L, NH_3-N < 3 mg/L, and SP < 0.5 mg/L, clarifier effluent quality consistently met the 3.0 mg/L ammonia discharge criterion in all samples from Period I to Period IV.

The pilot-scale and bench-scale system had comparable performance based on the removal efficiencies of SBOD, SCOD, and TSS as shown in Table 18.13. The bench-scale system in period IV achieved the best effluent quality that met the discharge criteria most of the time during the pseudo-steady-state period.

4.2.3. HRT Effect on Anaerobic Tank Performance

The impact of HRT was studied by comparison of the system performance in Period II and III, where half the volume of anaerobic tank was adopted, i.e., 2.5 L in Period II and

Table 18.13
Comparison of removal efficiencies (%)

Period	SCOD	SBOD	Ammonia	TSS
I	96.2	99.4	91.9	92.7[a]
II	97.2	99.4	74.2	91.4
III	97.8	99.6	100	94.6
IV	97.6	99.1	100	98.2

[a] Before incorporation of ultra-filter.

full volume of 5 L was used in Period III. Better effluent quality was achieved in Period III as demonstrated by the effluent SCOD, SBOD, and TSS concentrations. On average, during the pseudo-steady-state performance, SCOD and SBOD were 103 and 8 mg/L, respectively, in Period II as compared to 78 and 6 mg/L, respectively, in Period III. Clarifier effluent TSS averaged 66 mg/L in Period II and declined to 24 mg/L in Period III because of better settling characteristics of the mixed liquor, as confirmed by the SVI and DSVI data in Fig. 18.17. Diluted sludge volume index (DSVI) was in the range of 124.2–189.0 mL/g in period II and 23.7–130.9 mL/g in period III. Thus, sludge settleability was significantly improved by the longer HRT in the anaerobic tank.

Observed yield was calculated based on TCOD according to the following equation for each period in the bench-scale system. As demonstrated in Fig. 18.18, observed yield coefficient was 0.145 mg VSS/mg COD in Period II with a low anaerobic HRT of 0.25 day, which decreased slightly to 0.14 mg VSS/mg COD in Period III.

$$Y_{\text{obs}} = \frac{\text{cumulative VSS generated}}{\text{cumulative COD consumed}} = \frac{\text{cumulative VSS}_{\text{effluent}} + \text{cumulative VSS}_{\text{wasted}} + (\Delta\text{VSS})_{\text{bioreactors}}}{\text{cumulative COD}_{\text{influent}} - \text{cumulative COD}_{\text{effluent}}}. \quad (1)$$

All the above data demonstrate that a higher HRT of 0.5 day favored the performance of the anaerobic prefermentation and achieved better final effluent quality with respect to the discharge criteria, while achieving a similar sludge yield.

4.2.4. Temperature Effect on System Performance

Temperature affects biomass activity and sludge settling characteristics. By comparison of the system performance in Period III and IV, the impact of temperature can be assessed. DSVI of mixed liquor increased from 23.7–130.9 mL/g at 25°C in Period III to 114.9–173.0 mL/g at 32°C in Period IV. On the contrary, pseudo-steady-state TSS of clarifier effluent was 9 mg/L in the Period IV and 24 mg/L in Period III. The DSVI results agree with observations of poor sludge settling at higher temperatures under steady-state conditions (31). It should be asserted that the decrease in clarifier effluent TSS at 32°C despite the relatively poorer sludge settleability is explained by the lower mixed liquor solids observed at the high temperature (3,615 mg/L at 32°C vs. 4,293 mg/L at 25°C). As shown in Tables 18.11 and 18.12, average effluent SCOD decreased by 33% to 52 mg/L in Period IV while no significant difference in

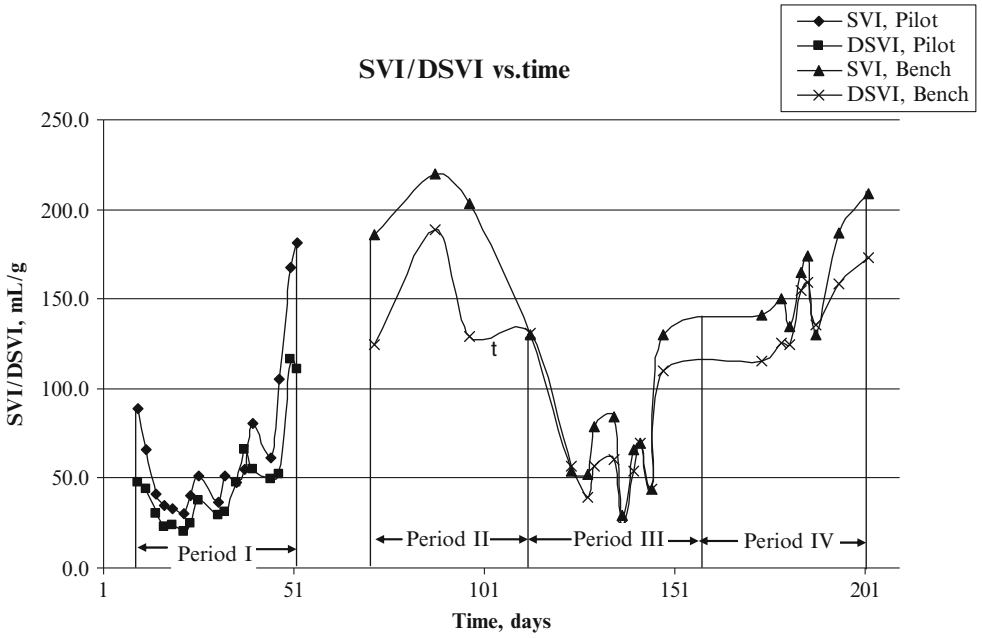

Fig. 18.17. Temporal profile of SVI/DSVI in pilot-scale and bench-scale mixed liquor.

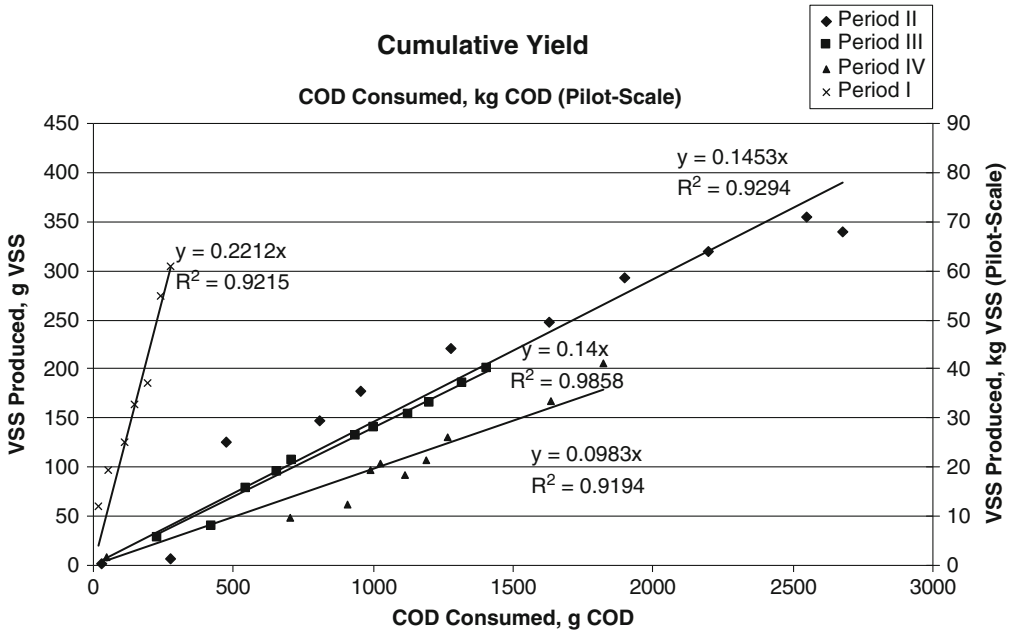

Fig. 18.18. Yield in bench-scale and pilot-scale systems.

SBOD was observed between the two periods. STKN increased by 8.3 and 10.3% across the anaerobic tank in Period III and IV, respectively. The biomass activity, presented as specific oxygen uptake rate (SOUR), was measured in both periods. SOUR increased significantly from 0.15–0.23 mg O_2/mg VSS-day at 25°C in Period III to 0.67–1.24 mg O_2/mg VSS-day at 32°C in Period IV. The biomass yield decreased from 0.14 mg VSS/mg COD in Period III to 0.098 mg VSS/mg COD in Period IV, which agrees with the trend of Krishna et al. (32), where sludge yield decreased from 0.420 g biomass COD/g acetate COD at 15°C to 0.225 g biomass COD/g acetate COD at 35°C.

4.3. Bench-Scale UASB-Anoxic/Oxic System

Anaerobic high rate reactors such as Upflow Anaerobic Sludge Blanket (UASB) reactors have been widely studied and applied to the treatment of food processing wastewaters under mesophilic conditions (20). Several advantages are associated with the application of anaerobic treatment, including (a) low energy requirement, (b) low sludge production, and (c) production of methane gas as a source of energy (33). Generally, anaerobic processes are followed by an aerobic second stage that can employ conventional activated sludge (34), extended aeration (35), and rotating biological contactors (11) to remove NH_4-N and PO_4^{3-}-P.

Elevated upflow velocities have been shown to improve mass transfer and reaction rates, compared to a quiescent UASB (36). While for dilute wastewaters, the flow required for media expansion may be achieved by the raw wastewater flow (34), for high strength wastes such as food industry waste, effluent recycle is necessary to maintain the upflow velocity under the same organic loading conditions. Campos and Anderson (37) achieved higher upflow velocity by reducing both feed concentration and HRT. They observed a methane yield of 0.3 m^3/kg COD removed at an upflow velocity of 0.1 m/h. Effluent recycle flow provides high enough liquid velocity to fluidize the biomass particles, to dilute the influent stream or to return alkalinity (38). By increasing upflow velocity, mixing characteristics can be improved and faster granulation is observed (37). However, increasing up-flow velocity above the design results in a higher biomass concentration in the effluent (38) and a reduction in biogas production because of dissolution of gases in the case of an expanded granular sludge bed reactor (39).

In high-rate anaerobic systems, the addition of neutralizing agents is normally required to maintain the optimal pH of the process. In full-scale anaerobic treatment plants, particularly those handling carbohydrate-based wastes, the addition of caustic soda for maintaining buffer, constitutes a major operational cost (38). The introduction of recycle has been shown to reduce the alkalinity requirements to maintain the pH at its set point (40). For the diluted molasses type of wastewater, Romli et al. (38) achieved reduction in alkalinity consumption by almost half with a recycle/feed flow ratio equal to 2. Due to production of carbon dioxide and release of positively charged ions in anaerobic degradation, bicarbonate alkalinity is generated across UASB reactor, thus providing pH buffer. Tomato-processing wastewater is acidic (pH 5–6) and low in alkalinity (400–600 mg/L), and hence the cost of alkalinity addition may affect the UASB process economics. Thus, the experimental study described here was designed to confirm the effect of effluent recycle on alkalinity.

The accumulation of inert solids in UASB reactors is reported to reduce activity and cause loss of granulation (41). This also adversely impacts effluent quality since these suspended solids are loosely entrapped in biofilm (42), thus necessitating significant sludge wastage quantities. However from a sludge management perspective, lower costs are associated with very long sludge ages that are conducive to low volatile fractions in UASB sludges. Arhan et al. (43) observed the accumulation of inorganic salt at the bottom of a UASB reactor treating baker's yeast production wastewater. Kettunen and Rintala (44) discussed the minimization of the accumulation of inerts in UASB treating leachate by periodically replacing anaerobic sludge. In this work, the feasibility of operating a UASB reactor at long sludge ages and low volatile fractions of granular sludge is demonstrated.

By integrating the anaerobic process with other biological methods and with physical–chemical methods, complete treatment of the wastewater can be accomplished at very low costs, while at the same time, valuable components can be recovered for reuse (33). The main objective of the posttreatment is to enhance the organic matter removal, as well as to promote the removal of components, which are barely affected by the anaerobic treatment, i.e., nutrients and pathogens (45). Processes that have been used for post-treatment include conventional activated sludge (34), extended aeration (35), trickling filters (45), and rotating biological contactors (11). Moreover, these aerobic polishing processes are generally required to meet discharge criteria of 30 mg/L BOD_5 and 30 mg/L of SS. Due to the relatively high nitrogen to COD ratio in the UASB, most aerobic polishing systems are nitrifying systems, which are known for poor sludge settleability that hampers the achievability of stringent effluent SS and BOD_5 criteria. The anaerobic treatment of acidic wastewater such as the tomato processing waste has not been widely reported on the literature. Furthermore, there are no accounts of achievability of stringent effluent criteria BOD_5, TSS, and NH_4-N concentration of less than 10, 10, and 3 mg/L, respectively, with combined anaerobic–anoxic–aerobic systems.

While the overall goal of this study was to establish design criteria for a full scale system, the specific goal was to assess achievability of stringent effluent criteria in treating tomato processing wastewater at different loading rates using a novel UASB-anoxic–aerobic system. UASB effluents rarely meet typical surface discharge criteria of 30 mg/L BOD_5 and 30 mg/L TSS, and hence this study was performed to examine the contaminant removal in the UASB/activated sludge system.

4.3.1. System Setup

A schematic diagram of the laboratory scale UASB-anoxic–aerobic system is shown in Fig. 18.19. It should be noted that the anoxic bioreactor was incorporated in the system on day 190 during the third operational period (OP-3).

The UASB reactor was made of PVC with an internal diameter of 20 cm. The height of the reactor was 59 cm and the working volume of the reactor was 15 L excluding the gas collector. The reactor was fed from the bottom through the inlet distributor in which holes are evenly perforated to avoid blockage because of sludge granules.

Four sampling ports were located across the height of the UASB reactor, including the lowest one at 9 cm, followed by one at 20, 35, and 59 cm from bottom of the UASB reactor.

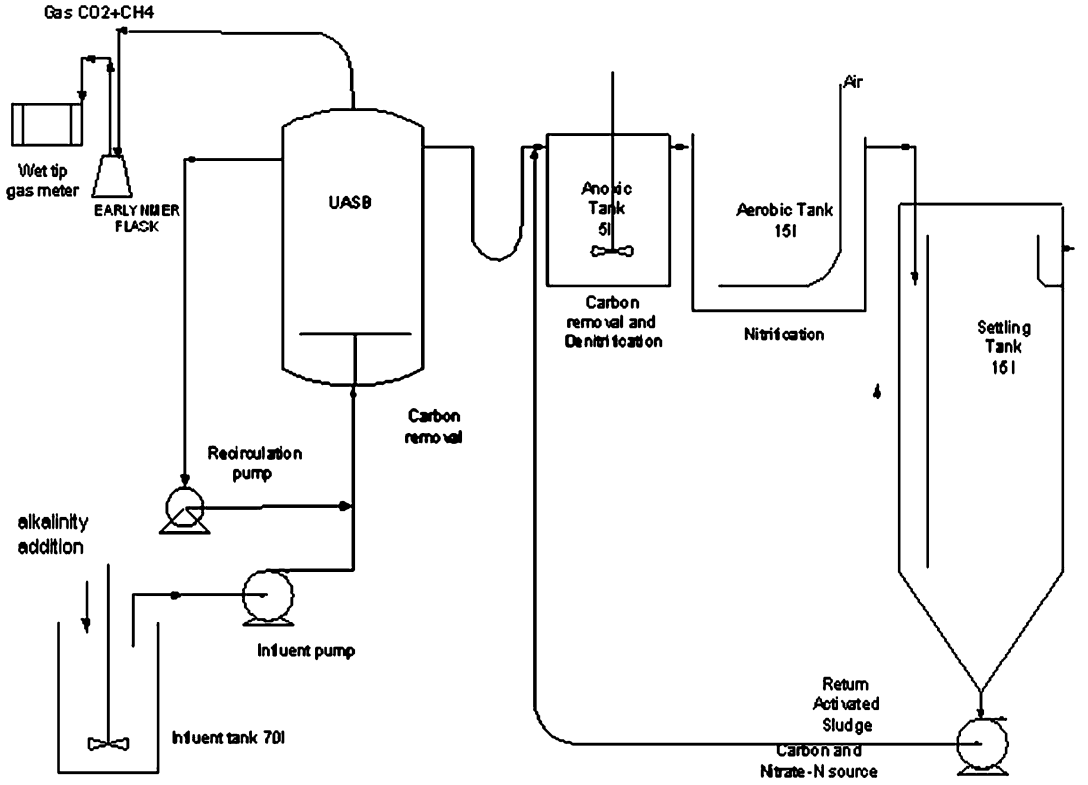

Fig. 18.19. Schematic diagram of UASB-anoxic–aerobic treatment system.

This arrangement was done to determine the sludge concentration profile and VFA/alkalinity ratios. The reactor was operated at HRT of 2.7 days, 1.3 days, and 0.7 day to determine the effect of loading rate on the performance of the system. The pH of the raw wastewater was adjusted between 6.5 and 7.2 using sodium bicarbonate (0.4–0.6 g $NaHCO_3$/L of wastewater). The volume of biogas produced was measured by using a wet tip gas meter. The effluent recycle ratio of 5:1 was employed to fluidize the sludge blanket from day 25 to day 160. The effluent recycle ratio was increased to 7:1 from day 160 to day 250. The effluent recycle flow was maintained by a peristaltic pump. The temperature of the UASB reactor was maintained at $35 \pm 2°C$ using a constant temperature water bath. The effluent from the UASB reactor was fed to the activated sludge system by a peristaltic pump.

The anoxic tank was made of plexiglass with an effective volume 5 L. The contents of the anoxic tank were mixed using an overhead laboratory mixer. The anoxic tank HRT was maintained at 0.3 days during OP-3 when anoxic selector was incorporated.

A 15 L plexiglass square tank was used as the aeration tank to provide an HRT of 0.7–2.7 days. Air was supplied by sparger made of copper tubing with perforations evenly distributed along the entire length. The airflow of 2 L/min was maintained to achieve a uniform dissolved oxygen concentration of 2–3 mg/L in the tank.

4.3.2. System Operation

The 8-month operation of the UASB-anoxic–aerobic system is divided into three operational periods (OP), denoted henceforth as OP-1 to OP-3. During OP-1 which lasted from day 1 to day 37, the overall UASB-oxic system HRT was 5.4 days, anaerobic and aerobic system were operated without sludge wastage. OP-2 which lasted from day 38 to day 200 was characterized by an overall UASB-oxic system HRT of 2.6 days, UASB SRT of 100 days and aerobic SRT of 19.7 days, whereas during OP-3 from day 200 to day 250, the UASB-A/O system HRT was 1.7 days, UASB reactor SRT was 65 days, and the aerobic SRT was 17.8 days.

4.3.3. Performance Analysis

4.3.3.1. START-UP OF UASB REACTOR

The UASB reactor was seeded with digested sludge from an anaerobic digester treating alcohol industry wastewater. Fifteen liter of mixed liquor with TSS and VSS concentrations of 14 and 11.5 g/L, respectively, was inoculated into the UASB. After 4 weeks of start-up, the concentration of sludge reached 60,000 mg/L (6% w/w).

The UASB reactor was fed with synthetic wastewater at 5.5 L/day corresponding to an HRT of 2.7 days for a period of 6 days during OP-1. After about 1 week of running on synthetic waste, tomato waste was fed to the UASB reactor. However, removal efficiency of around 90% was achieved after 3 weeks of continuous operation at the same HRT. After 5 weeks of steady gas generation, the OLR in the UASB reactor was increased from 3.9 to 6.7 kg COD/m^3-day.

4.3.3.2. PERFORMANCE OF UASB REACTOR

It should be noted that steady state conditions were defined as stable effluent quality as well as constant reactor biomass concentrations. For OP-2 and OP-3, the system was run for more than three turnovers of the mean aerobic SRT. A summary of the UASB operating conditions during these three operating periods is shown in Tables 18.14–18.16.

Table 18.14
Summary of bioreactors operating conditions during OP-1: UASB HRT = 2.7 days, aerobic HRT = 2.7 days

Parameters	UASB stage	Aerobic stage
Flow rate (L/day)	5.5	5.5
Influent COD (mg/L)	4,300–7,800	480–3,840
Volumetric loading rate (kg COD/m^3-day)	1.6–2.9	0.17–1.4
F/M (kg COD/kg MLVSS-day)		0.05–0.4
SRT (days)	∼200	∼60
Up-flow velocity (m/h)	0.05	
Biogas yield (m^3/kg COD removed)	0.4	
Biogas production rate (L/day)	4.5–25	
% CH_4 in the gas	78%	
COD removal (%)	75–93%	51%

Table 18.15
Summary of bioreactors operating conditions during OP-2: UASB HRT = 1.3 days, aerobic HRT = 1.3 days

Parameters	UASB stage	Aerobic stage
Flow rate (L/day)	11.5	11.5
Influent COD (mg/L)	2,865–16,000	228–2,200
Volumetric loading rate (kg COD/m^3-day)	2.2–12.3	0.17–1.7
F/M (kg COD/kg MLVSS-day)		0.05–0.5
SRT (days)	100	19.7
Up-flow velocity (m/h)	0.1	
Biogas yield (m^3/kg COD removed)	0.4	
Biogas production rate (L/day)	10–30	
% CH_4 in the gas	78	
COD removal (%)	85–95.6	49

Table 18.16
Summary of bioreactors operating conditions during OP-3: UASB HRT = 0.7 days, anoxic HRT = 0.3 days, aerobic HRT = 0.7 days

Parameters	UASB stage	Anoxic stage	Aerobic stage
Flow rate (L/day)	20	20	20
Influent COD (mg/L)	5,000–7,000	150–250	40–70
Volumetric loading rate (kg COD/m^3-day)	2.5–10	0.6–1	0.05–0.09
F/M (kg COD/kg MLVSS-day)		0.38–0.63	0.04–0.06
SRT, days	65	17.8	17.8
Up-flow velocity in UASB (m/h)	0.2		
Biogas yield (m^3/kg COD removed)	0.43		
Biogas production rate (day^{-1})	20–40		
% CH_4 in the gas	78		

The performance of the UASB reactor and performance of the system is illustrated by the data in Figs. 18.20 and 18.21 for COD removal and organic loading rate (OLR), respectively.

Over the 250-day period summarized by Fig. 18.20, raw wastewater COD concentration averaged $6{,}953 \pm 3{,}278$ mg/L. After anaerobic treatment, an average COD concentration of 925 ± 737 mg/L with an average anaerobic COD removal efficiency of 86.6% was achieved for the complete run, whereas in the OP-3, the anaerobic effluent COD decreased to 300 ± 97 mg/L, reflecting an average anaerobic stage COD removal efficiency of 95.6%.

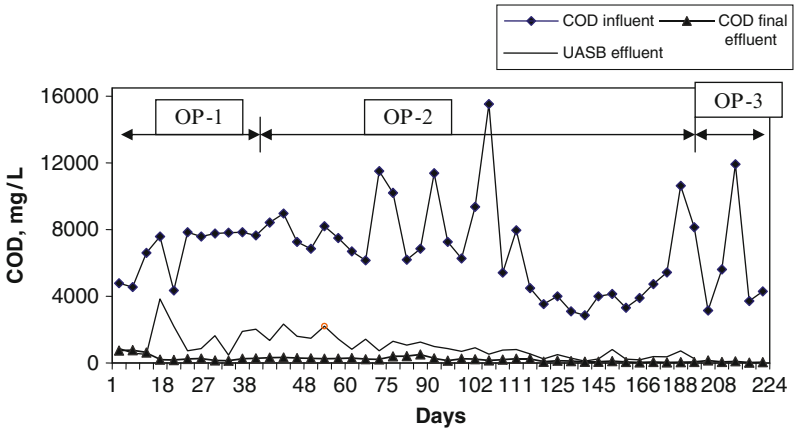

Fig. 18.20. Temporal variations of influent, UASB effluent and final effluent COD.

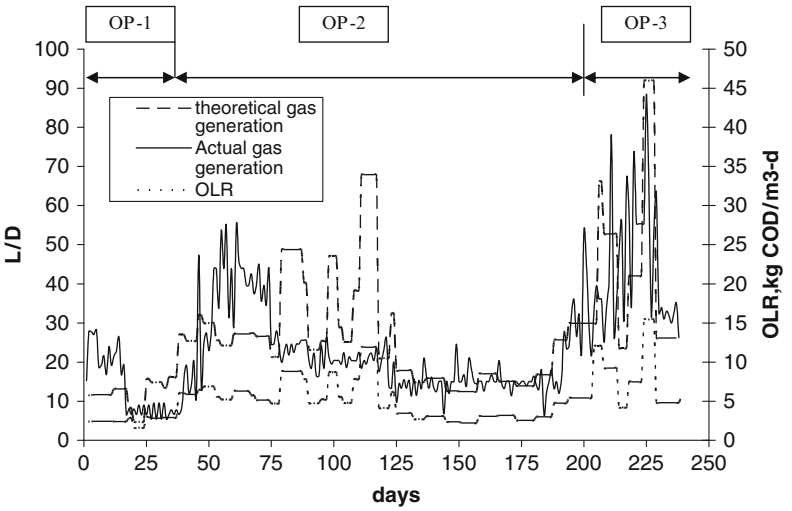

Fig. 18.21. Temporal variations of organic loading rate and biogas production.

Aerobic polishing further reduced the COD concentration to an average value of less than 95 ± 61 mg/L after 125 days of continuous operation with an overall removal efficiency of 98.6%. While influent COD concentration varied widely reaching as high as 16,000 mg/L, the process performance was very good under dynamic loading conditions. This is reflected by the response to the sharp increase in OLR, from 7 kg COD/m^3-day on day 223 to 15.5 kg COD/m^3-day on day 225, during which both gas production increased proportionally from 58 L/day to 88 L/day and effluent COD remained stable at 224 mg/L.

Gas generation, both theoretical and actual, as a function of time, is shown in Fig. 18.21. When comparing the theoretical gas generation with an actual one, for the first 110 days,

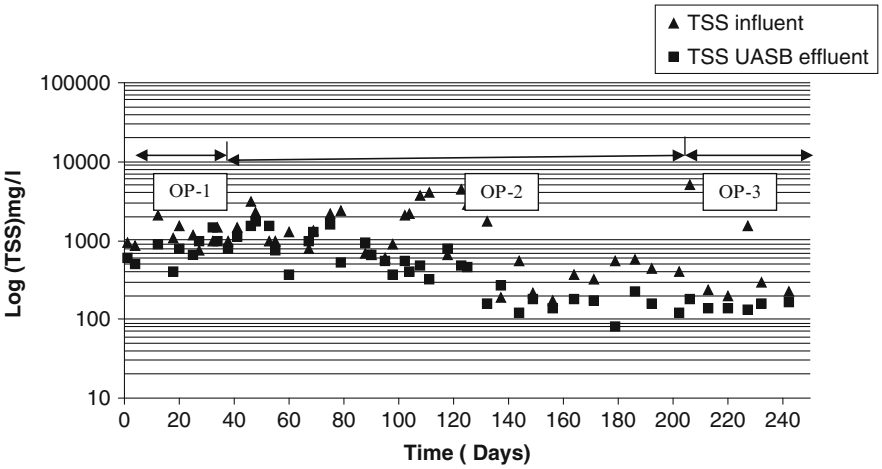

Fig. 18.22. Temporal variation of influent and UASB effluent TSS.

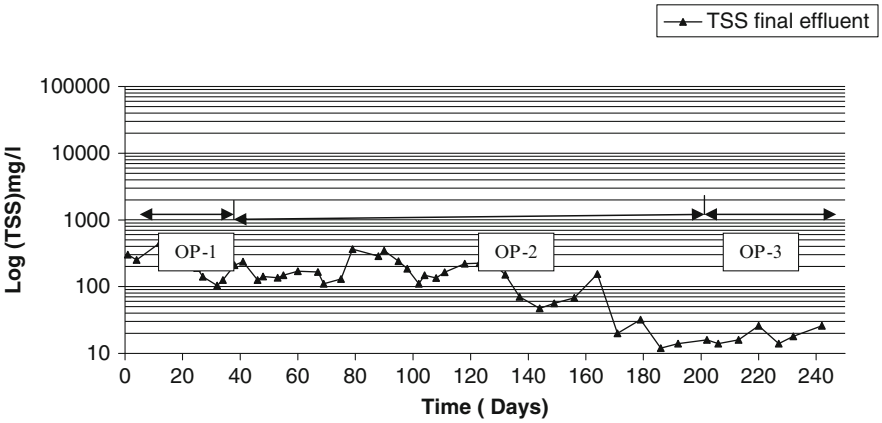

Fig. 18.23. Temporal variation of final effluent TSS.

there was significant difference between them. Scrutiny of the average UASB VSS in the system reveals that during the first 110 days, VSS was increasing in the reactor to as high as 30,330 mg/L and, therefore, it is plausible that the influent VSS were not biodegraded but rather entrapped physically in the sludge blanket. After reducing SRT from 100 days to 65 days on day 150, VSS concentration in the UASB decreased to stabilize at 13,000–14,500 mg/L. After 130 days of operation, the actual gas production closely matched the theoretical one. The methane content of gas varied from 78 to 82%.

As shown in Figs. 18.22 and 18.23, due to differences in wastewater batches, there were significant variations in the TSS concentration fed to the UASB reactor. However, effluent TSS concentrations after aerobic treatment were very low in the range of 200 mg/L, and after

200 days of operation and upon incorporation of the anoxic bioreactor, TSS in the final effluent was reduced to less than 20 mg/L.

4.3.4. Impacts of Process Parameters

Table 18.17 shows the effect of OLR and upflow velocity on COD removal and biomass activity in the UASB reactor. As shown in Table 18.17, the substantial increase in COD removal and biomass activity corroborates that indeed OLR is the predominant contributing factor during the treatment of readily biodegradable food processing wastes.

Higher recycle rates can be utilized to improve VFA/alkalinity ratio and to reduce the alkalinity requirements. By adopting a recirculation ratio of seven times the feed flow, VFA to alkalinity ratio decreased from 0.2 to 0.1, in addition to the decrease in bicarbonate consumption, from 2 g/L of Na_2CO_3 to 0.5 g/L.

4.3.5. Inert Accumulation

For the tomato processing waste, influent suspended solids constituted 11% of COD ($R^2 = 0.73$, not shown). Habets (46) stated that for high rate UASB processes the influent suspended solids should constitute no more than 10% of total COD. Above this level, pretreatment may be required.

Figure 18.24 depicts the temporal variation of volatile and nonvolatile suspended solids (NVSS) or "fixed suspended solids" at the bottom of the UASB reactor. The UASB reactor was seeded with digested sludge from an anaerobic digester treating alcohol industry wastewater. Initially in the UASB reactor, the NVSS were around 15% of the TSS. After 100 days of operation, the NVSS accumulated to around 40% of TSS, which increased to 60% of TSS after 160 days of continuous UASB reactor operation. Analysis of sludges from various ports of the UASB reactor revealed an NVSS accumulation of 60% of TSS in the bottom part, and 40% of TSS in the top part.

Sludge wastage is often used to control NVSS accumulation and hence SRT was reduced from 100 days to 65 days after 150 days of operation. The effect of reducing SRT, as reflected in Fig. 18.24, was to maintain NVSS concentration at 60% of TSS in the UASB reactor. The performance of UASB reactor in terms of COD removal and biogas generation was steady at an NVSS fraction of 60% of TSS in the reactor. This clearly indicates that for this particular application, operation at an SRT of 65 days, corresponding to a sludge wastage rate of 3.2 g TSS/day and an observed yield of 0.042 g TSS/g COD removed, is feasible despite the very high NVSS accumulation.

4.3.6. Post-UASB Treatment

The A/O system for treating effluent from the UASB reactor was an activated sludge system. The operating conditions and treatment performance of the (A/O) system are summarized in the above-mentioned Tables 18.14–18.16 for the three operating conditions. Although COD removal efficiency across the aerobic system was low at 5–65%, the overall system removal efficiency was very high in the range of 95–99%. The relatively low organic removal efficiency observed in the aerobic system is attributed to the excellent COD removal achieved by the UASB. $SBOD_5$ results, as verified by a certified environmental laboratory, during OP-3 were well below 15 mg/L. The COD to BOD_5 ratios in the final effluent from the aerobic system were 8–10:1.

Table 18.17
Effect of OLR on COD removal rate and biomass activity

Days		HRT days	Avg. OLR, kg COD/m^3-day	Upflow velocity, m/h	COD, % removed	Biomass activity, g COD/g VSS-day	Biogas yield, L of gas/g COD removed	Methane yield, L of gas/g COD removed
130–160	Phase I	1.3	2.7 ± 0.4	0.09 ± 0.01	92.2 ± 3.3	0.21 ± 0.04	0.41 ± 0.12	0.32 ± 0.09
160–180	Phase II	1.3	2.9 ± 0.3	0.12 ± 0.00	93.55 ± 1.4	0.19 ± 0.03	0.39 ± 0.05	0.31 ± 0.04
200– 250	Phase III	0.7	7.75 ± 3.4	0.22 ± 0.01	95.6 ± 1.5	0.52 ± 0.1	0.43 ± 0.2	0.35 ± 0.17

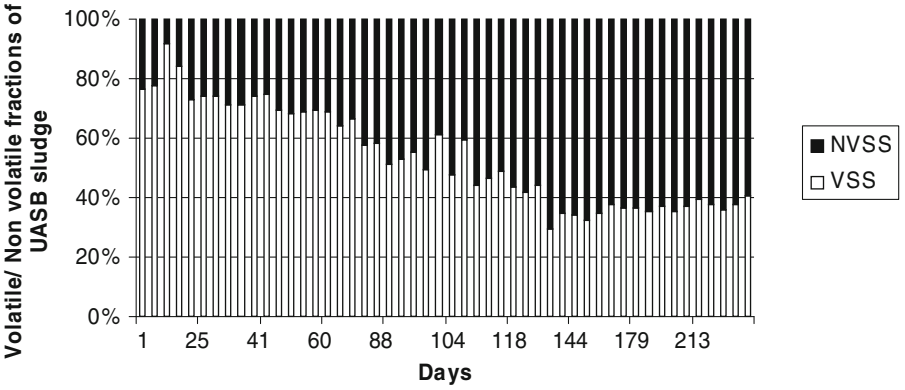

Fig. 18.24. Temporal variations of inert solids in UASB bottom sludge.

Overall results show excellent removal of carbonaceous organic matter. The effluent BOD_5 ranged from 6 to 55 mg/L during OP-2 and averaged 24 mg/L with BOD_5 removal efficiency of 98.5%. Similarly, final effluent $SBOD_5$ ranged from 6 to 45 mg/L during OP-2, with an average of 17.5 mg/L, which decreased to an average of 8.25 ± 1.9 mg/L during OP-3. These data clearly indicate that the system can reliably achieve <15 mg/L BOD_5 (100% during OP-3 and 64% during the entire period).

The variation of the final effluent TSS concentrations is depicted in Fig. 18.23. The sludge volume index (SVI) of the aerobic mixed liquor varied from 10 to 82 mL/g throughout this study and ranged from 28 to 62 ml/g from day 75 to day 156. Following the implementation of the anoxic tank, final effluent TSS concentration continued to decline to as low as 10 mg/L. During the period from day 180 to day 250, effluent TSS concentrations, based on 12 samples averaged 19.2 ± 6mg/L.

Figure 18.25 illustrates the temporal variation of ammonia and nitrates in the final effluent. Nitrification was stable despite the wide variability in influent NH_4-N concentration from 85 mg/L to 200 mg/L with the effluent NH_4-N concentration of <3 mg/L. Nitrification efficiency was 99.5% with effluent ammonia from the aerobic reactor steady at 0.38 ± 0.4 mgNH_4-N/L.

It must be asserted that throughout the study, when nitrification efficiency was stable, i.e., after day 100, nitrites in the final effluent remained extremely low at 0–0.2 mg/L. As evident from Fig. 18.25, the effluent NO_3-N concentration decreased from 120 mg/L to 60 mg/L after introducing an anoxic step in UASB-aerobic configuration, i.e., after 190 days of operation.

5. WASTEWATER CHARACTERIZATION AND MODELING

5.1. Characterization of Tomato-Processing Wastewater

5.1.1. Introduction

Activated Sludge Model No.1 of the IAWPRC Task Group (47) is frequently adopted to predict the performance of biological wastewater treatments plants. This simulation model

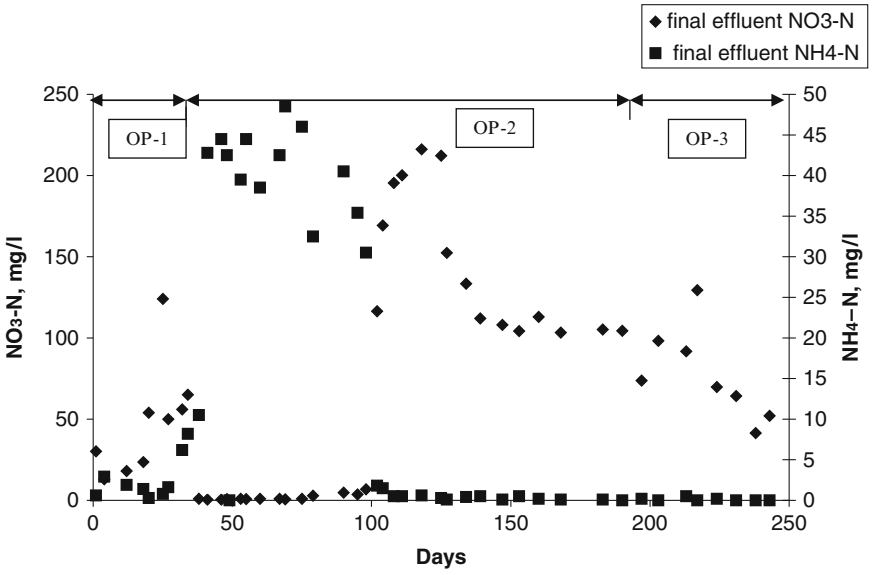

Fig. 18.25. Temporal variations of final effluent ammonia and nitrates.

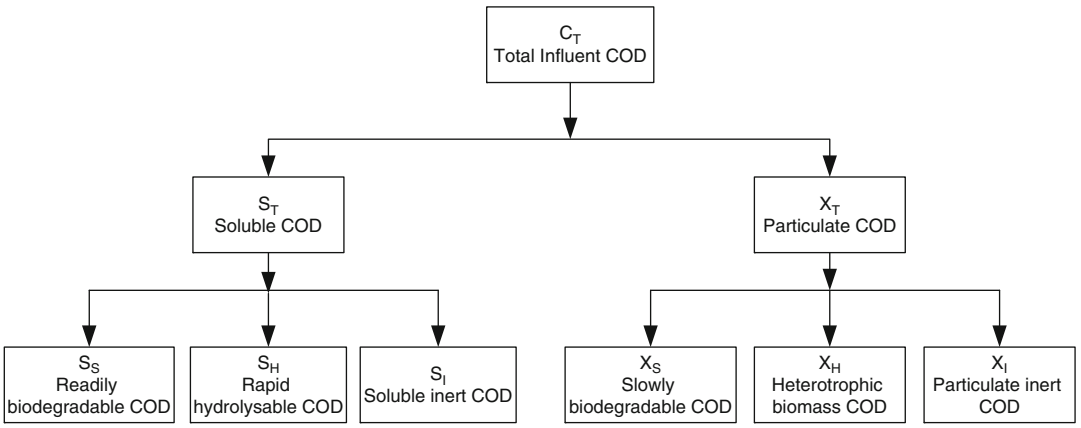

Fig. 18.26. COD fractions in wastewater.

delineates wastewater fractions such as readily biodegradable substrate, slowly biodegradable substrate, soluble and inert organic matter. Among all these major fractions, as shown in Fig. 18.26, S_I and X_I are nonbiodegradable fractions, and others are biodegradable fractions. Kinetic coefficients such as maximum heterotrophic growth rate μ_{max}, endogenous decay coefficient b_H, half saturation coefficient K_S, hydrolysis rate constant K_h, and yield coefficient Y_H are pertinent for the accurate prediction and simulation of the biological wastewater treatment system.

5.1.2. Experimental System Setup

Oxygen uptake rate (OUR) was measured via respirometric methods to determine the various COD fractions and kinetic coefficients of the tomato-processing wastewater. A respirometer was adopted to measure the OUR and facilitate wastewater characterization and biokinetics determination. Filtered wastewater samples via 47 mm diameter and 0.45 μm pore size membrane filter were mixed with preaerated and acclimatized sludge at initial substrate to biomass ratio (S_0/X_0) of 4 mg COD/mg VSS. A stock solution of allylthiourea (ATU) was added to the mixture in 250 mL or 500 mL reaction bottles to inhibit nitrification at an initial concentration of 20 mg/L. Respirometric studies were conducted on the raw wastewater as well as NH_4^+-N augmented raw wastewater. A stock solution of NH_4^+-N was also added to the bottles at an initial concentration of 150 mg/L and a BOD:NH_4^+-N ratio of 100:5 to overcome the potential nitrogen limit in the wastewater samples. KOH tubes were inserted into each vessel to absorb carbon dioxide. Wastewater samples and sludge in the reaction bottles were mixed by magnetic stirrers and all the bottles were put in a 25°C water bath maintained by temperature control module.

5.1.3. Determination of Wastewater Fractions and Biokinetic Coefficients

To evaluate the biological treatability of tomato-processing wastewaters, batch respirometer tests were carried out to determine wastewater fractions, i.e., readily biodegradable substrates S_S, rapidly hydrolysable substrates S_H, inert soluble organic materials S_I, slowly biodegradable substrates X_S, heterotrophic organisms X_H, and particulate inert organic materials X_I using the following methods. Kinetic coefficients studied included yield coefficient Y_H, maximum heterotrophic growth rate μ_{max}, endogenous decay coefficient b_H, saturation coefficient K_S, and hydrolysis rate constant K_h.

5.1.3.1. Determination of Readily Biodegradable Substrate S_S and Yield Coefficient Y_H

Respirometric tests with filtered wastewater and activated sludge at S_0/X_0 of 4 mg COD/mg VSS were conducted to determine S_S and Y_H. A control blank with deionized water and same amount of activated sludge as the above test samples, was run simultaneously in the experiment. Temporal variations of total and soluble COD as well as TSS and VSS were monitored. OUR decreased rapidly and dropped to a lower level when S_S was depleted. S_S can be calculated from the equivalent oxygen consumption in the test sample after subtracting the oxygen consumption of the blank in accordance with the following Eq. (2).

$$S_S = \frac{\Delta O_2}{1 - Y_H}. \tag{2}$$

Yield coefficient Y_H can be calculated using the following equation by plotting net oxygen consumption vs. SCOD reduction (49, 50).

$$Y_H = 1 - \frac{\Delta O_2}{\Delta SCOD}. \tag{3}$$

5.1.3.2. DETERMINATION OF μ_{max} AND b_H

The maximum heterotrophic growth rate μ_{max} is again determined by means of respirometer test on the basis of initial OUR value at S_0/X_0 ratio of 4 mg COD/mg VSS. The method developed by Kappeler and Gujer (51) was used. For the observed net OUR profile (after subtracting the blank), the following linear expressing can be derived, with a slope of $\mu_{max} - b_H$.

$$\ln \frac{\text{OUR}}{\text{OUR}_{\text{initial}}} = (\mu_{max} - b_H)t. \tag{4}$$

The method to calculate b_H involves plotting the change of OUR with time in a respirometer test with only seed sludge and completely devoid of substrate, on the basis of the following Eq. (5).

$$\ln \text{OUR} = [\ln(1 - f_e)b_H X_{H0}] - b_H t, \tag{5}$$

where X_{H0} was measured as particulate COD (PCOD) and the coefficient for the production of inert COD from endogenous respiration (f_e) was set at a determined value of 0.2 g COD/g COD (52).

5.1.3.3. DETERMINATION OF X_H

A respirometer run on raw wastewater only was conducted to determine the concentration of heterotrophic biomass in wastewater X_{H0}. If b_H, Y_H, and f_e are known, X_{H0} can be calculated according to the following Eq. (6).

$$\text{OUR}_{\text{initial}} = \frac{1 - Y_H}{Y_H}\mu_{max} X_{H0} + (1 - f_e)b_H X_{H0}. \tag{6}$$

5.1.3.4. DETERMINATION OF K_S, K_h AND X_S

K_S, K_h, and X_S affect oxygen respiration if growth is limited by substrate. The substrate half-saturation coefficient K_S, hydrolysis constant K_h, and slowly biodegradable substrate X_S can be determined by graphical comparison of the measured respiration with the simulated one using iterative curve fitting (51). Modeling software GPS-X (Hydromantis Inc., Hamilton, ON) was adopted for the curve fitting to get the values of K_S, K_h and X_S.

5.1.3.5. DETERMINATION OF S_I AND X_I

Orhon et al. (53) introduced this method to determine S_I and X_I as a modification of previous method proposed by Germirli et al. (54). The method involved three batch runs, two with the wastewater to be studied and the third with glucose. The first wastewater reactor was started with nonfiltered wastewater sample, total COD C_{T0}, and the second with filtered wastewater sample, soluble COD S_{T0}, whereas the initial COD in the third glucose reactor was adjusted to S_{T0}. An S_0/X_0 ratio of 4 mg SCOD/mg VSS was applied and same amount of activated sludge was adopted in all three reactors. Nutrients, i.e., nitrogen and phosphorus were added to three reactors according to the ratio of BOD:NH_4^+-N:PO_4^{3-}-P of 100:5:1 for the biomass growth. In all the above three reactors, profiles of SCOD vs. time were monitored until all the degradable COD was entirely depleted. As shown in Fig. 18.27, the residual soluble substrate concentration in the glucose reactor S_{RG} reached a level characterizing residual

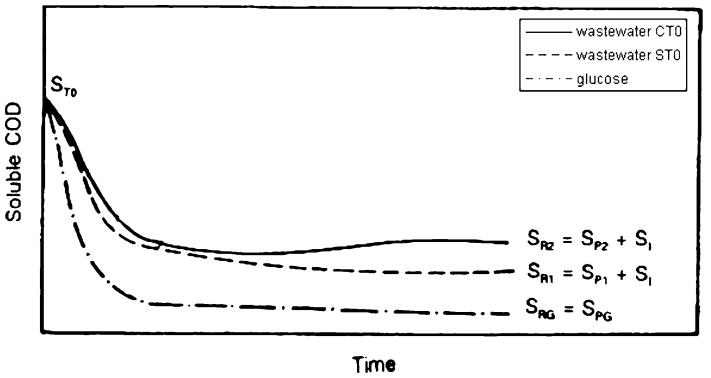

Fig. 18.27. Mathematical simulation for the estimation of S_I and X_I.

soluble microbial products concentration S_{PG} alone. In the wastewater run started with S_{T0}, the COD would stabilize at a minimum level S_{R1}, the sum of S_I and S_{P1}. As a result, the SI fraction could be calculated as the following equation with the assumption $(S_P)_{wastewater} = (S_P)_{glucose}$, i.e., $S_{P1} = S_{PG}$ since the two reactors were started with the same S_{T0}.

$$S_I = S_{R1} - S_{PG}. \tag{7}$$

The first reactor started with nonfiltered wastewater C_{T0} ($C_{T0} > S_{T0}$), and same soluble fractions S_{T0} would demonstrate a higher residual soluble COD S_{R2} because of a higher S_{P2}. S_{P2} was calculated when S_I was known.

$$S_{P2} = S_{R2} - S_I. \tag{8}$$

In an experimental study long enough for $\theta_C = \infty$ and $S_S = 0$, X_I was finally calculated in Eq. (9) based on the assumption that residual soluble microbial products concentration, S_P, was directly proportional to the total input biodegradable substrate concentration, C_{S0}, as shown in Eq. (10).

$$X_I = C_{T0} - C_{S0} - S_I, \tag{9}$$

$$C_{S0} = \frac{S_{P2}}{S_{P1}} S_{S0}, \tag{10}$$

where

$$S_{S0} = S_{T0} - S_I. \tag{11}$$

5.1.3.6. Determination of Remaining Fractions

Rapidly hydrolysable substrates S_H could be calculated based on soluble COD balance. X_S could also be calculated based on particulate COD balance instead of the iterative curve fitting method mentioned above in sect. 5.1.4 if all other fractions were known.

$$S_H = S_T - S_S - S_I, \tag{12}$$

$$X_S = C_T - S_T - X_I - X_H. \tag{13}$$

5.1.4. Characterization Results

Respirometric studies were carried out on three raw wastewater samples after primary clarification and three anaerobic effluent samples corresponding to the three raw wastewater samples collected from the field at the beginning, in the middle, and at the end of the canning season. Respirometer samples were collected every 2 h in the first 10 h and then every day for the remaining period during the 2–4 day run. TCOD, SCOD, TSS, and VSS were measured for the samples collected routinely. To assess potential nutrient deficiency, raw wastewater was tested with and without ammonia–nitrogen, respectively.

It can be found from the COD fractions as shown in Table 18.18 that the S_S fraction of different batches was quite stable and ranged from 37.5 to 42.6% of raw wastewater SCOD although the SCOD of wastewater fluctuated from 3,140 to 5,200 mg/L. Since the biodegradability of tomato wastewater was low confirmed by the low readily biodegradable fraction S_S% and low maximum heterotrophic growth rate max of μ_{max} of 37.5–42.6% and 0.95–2.26/day compared with the typical value of 40–60% (55) and 6/day (47), an anaerobic prefermentation tank was adopted in the first stage of a pilot-scale anaerobic/aerobic wastewater treatment system to enhance the biodegradability of tomato-processing wastewater. For the runs of

Table 18.18
COD fractions in the tomato-processing wastewater

Raw wastewater	Sample 1 (without ammonia)	Sample 1 (with ammonia)	Sample 2 without ammonia)	Sample 2 (with ammonia)	Sample 3 (without ammonia)	Sample 3 (with ammonia)
S_S	446	1,992	1,195	1,950	246	1,336
S_H	4,594	3,048	3,895	3,140	2,844	1,754
S_I	110		110		50	
X_S^a	1,179		1,419		466	
X_H	670		400		275	
X_I	111		111		39	
S_S/S_T, %	8.7	38.7	23.0	37.5	7.8	42.6
S_T	5,150		5,200		3,140	
C_T	7,110		7,130		3,920	
Anaerobic effluent[b]	Sample 1		Sample 2		Sample 3	
S_S	1,354		426		655	
S_H	1,656		1,246		66	
S_I	120		120		63	
X_I	58		58		62	
S_S/S_T, %	43.3		23.8		83.5	
S_T	3,130		1,792		784	
C_T	3,630		2,050		1,190	

[a] Numbers in parentheses were simulated from GPS-X modeling software and numbers not in parentheses were calculated from COD balance.

[b] Anaerobic effluent sample was settled for 30 min and supernatant was used for the respirometric studies to exclude the COD fractions from MLVSS.

Table 18.19
Kinetic coefficients for the tomato-processing wastewater treatment

		Influent (without ammonia addition)	Influent (with ammonia addition)	Anaerobic effluent (without ammonia addition)
Sample 1	Y_H, g COD/g COD	0.76	0.71	0.77
	μ_{max}, day^{-1}	2.26	2.05	2.05
	b_H, day^{-1}	0.22	0.27	0.24
	K_S, mg COD/L	–	175	75
	K_h, day^{-1}	–		–
Sample 2	Y_H, g COD/g COD	0.74	0.73	0.74
	μ_{max}, day^{-1}	0.95	1.25	2.69
	b_H, day^{-1}	0.28	0.27	0.15
	K_S, mg COD/L	50	50	80
	K_h, day^{-1}	0.65		–
Sample 3	Y_H, g COD/g COD	0.75	0.68	0.75
	μ_{max}, day^{-1}	2.19	1.20	5.64
	b_H, day^{-1}	0.26	0.27	0.24
	K_S, mg COD/L	175	150	100
	K_h, day^{-1}	0.6		–

influent wastewater without ammonia augmentation, S_S was underestimated because of nitrogen limitation, and hence rapidly hydrolysable substrate S_H was overestimated according to the COD balance. S_I measured from batch tests was 50–110 mg/L, which was close to clarifier effluent COD, indicating the measurement of S_I was reasonable and reliable. As shown in Table 18.19, measured yield coefficient Y_H ranged 0.68 g COD/g COD–0.76 g COD/g COD for raw wastewater and 0.74 g COD/g COD–0.77 g COD/g COD for anaerobic effluent which demonstrated that the measured Y_H was reproducible. K_S, half velocity concentration of Monod, ranged from 50 to 175 mg COD/L in the influent and 75–100 mg COD/L in the anaerobic effluent, which is higher than the typical municipal wastewater of 20 mg COD/L (47), demonstrating that the tomato-processing wastewater contains some fraction of highly suspended organics and thus has a slow response of oxygen uptake. K_h ranged 0.60–0.65/day, well below the 3.0/day for municipal wastewater (47). K_S and K_h were solved by comparison of measure OUR and simulated OUR via iterative simulation using GPS-X as shown in Figs. 18.28 and 18.29.

5.2. Modeling of Tomato-Processing Wastewater Treatment System

5.2.1. Introduction

5.2.1.1. Development of Models

Development and publication of International Association of Water Quality (formerly IAWPRC, then IAWQ, now IAW) Activated Sludge Model No. 1 (47) has provided a consistent frame work for modeling suspended growth biological wastewater treatment systems. Systems

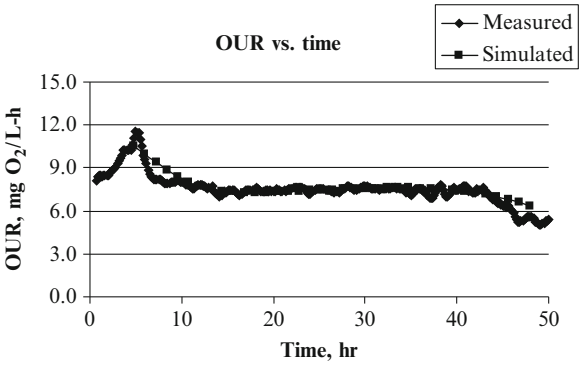

Fig. 18.28. Simulation of influent sample 3 (without ammonia augmentation), $K_S = 175\,\text{mg COD/L}$.

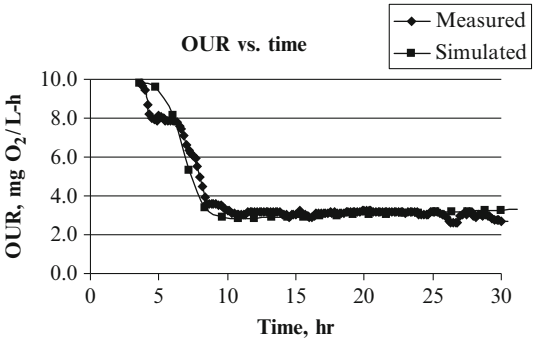

Fig. 18.29. Simulation of influent sample 3 (non-filtered wastewater only), $X_S = 1,000\,\text{mg COD/L}$, $k_h = 0.6/\text{day}$.

modeled include those for carbon removal, combined carbon oxidation and nitrification, and single sludge nitrification and denitrification (56). Many basic concepts of ASM1 were adapted from an earlier model called the University of Cape Town (UCT) model. Model development and refinement is an ongoing activity and new models are continuously being developed or modified by researchers as fundamental knowledge improves or applications are better understood. The Activated Sludge Model No. 2 (57) was introduced as a further development of ASM1. ASM2 introduced phosphorus accumulating organisms (PAOs) and allowed to simulate the behavior of biological nutrient removal activated sludge systems. For example, the ASM2 model, which includes biological phosphorus removal, has 19 processes and 20 state variables. The Activated Sludge Model No. 2d (58) was based on the ASM2 and was expanded to include the denitrifying activity of the PAOs. This extension of ASM2 allows for improved modeling of the processed, especially with respect to the dynamics of nitrate and phosphate. Similarly, the Activated Sludge Model No. 3 (59) is a further refinement of ASM1, wherein substrate degradation is modeled as a two-step process involving substrate storage and then mineralization.

5.2.1.2. System Modeling Softwares

In order to determine the most suitable design, optimize the control, and predict the behavior of the full-scale plants under varying operating conditions, computer simulation programs are increasingly used today in the wastewater treatment industry. GPS-X (Hydromantis Inc., Hamilton, ON), EFOR (DHI Software, Hørsholm, Denmark), AQUASIM (Swiss Federal Institute for Environmental Science and Technology (EAWAG), Dübendorf, Switzerland), STOAT (DHI Software, Hørsholm, Denmark), SSSP (Bidstrup and Grady, Clemson University), SASSPro (Science Traveller International, Inc., Seattle, Washington), WEST (Hemmis N.V., Kortrijk, Belgium), and ASIM (Holinger Ltd., Liestal, Switzerland) are among the WWTP modeling software packages available in the market.

5.2.1.3. System Modeling Practices

In recent years, mathematical modeling of wastewater treatment processes has become an accepted tool in engineering practice and is extensively used by consulting engineering firms and regulatory agencies. Applications of mathematical models range from research to treatment plant design, operation, control, and troubleshooting (60). Orhon (61) reviewed the basic steps in the design of activated sludge systems on the basis of process modeling concepts. However, the application of the models is limited at most treatment plants because of lack of advanced input of parameter values required by the model (48). Daigger and Nolasco (56) evaluated the design of a full-scale WWTP using Single Sludge Simulation Program (SSSP), developed by Bidstrup and Grady (62) and the General Purpose Simulation (GPS-X), which is the state-of-the-art computer-based dynamic wastewater treatment modeling program Hydromantis Inc. (Hamilton, ON). Harper and Jenkins (63) investigated the suitability of anaerobic and aerobic (AnA) process for treating phosphorus-deficient wastewaters with highly variable influent COD loading in AnA and completely aerobic (CA) sequencing-batch reactors (SBRs) and model predictions suggested that the AnA process was stable when treating loading pattern (LP) 1 simulating daily-load variations but eventually failed when treating LP 2 simulating weekend shutdowns. Meijer et al. (64) simulated the start-up of a full-scale biological phosphorus and nitrogen removing (BPNR) WWTP using ASM2d and showed high model sensitivity to operational data especially temperature. Nuhoglu et al. (65) simulated a full-scale WWTP of Erzincan city, located in Eastern Turkey and serving 124,000 population equivalents. Simulations were performed using precompiled model and layout implemented in GPS-X simulation software and gave a reasonable match for the investigated variables.

However, most of the modeling practices were carried out on municipal WWTPs. Although some attemps were made on municipal wastewater with industrial effluents (66), very few of them simulated industrial wastewater treatment processes. In this study, the ASM model for this specific tomato-processing wastewater was calibrated and subsequently used to simulate the performance of pilot-scale and bench-scale anaerobic/aerobic systems. ASM1 as adopted in the simulation and the commercial package GPS-X was used for the process model.

5.2.2. Model Calibration

The most important step in any modeling work is model calibration, which ensures that the developed process model satisfactorily represents the actual system. The calibration process for the model includes the following steps as shown in Fig. 18.30:

- Definition of the process flow diagram including all physical dimensions of the system.
- Entering all pertinent influent characterization data. It must be asserted that the fractionation of organic matter in this model is indeed very complex, requiring laborious and expensive experimental methods in this project. Major fractions, such as readily biodegradable COD, inert soluble COD, inert particulate COD, particulate substrate, and heterotrophic biomass, were defined using respirometric results as shown in the Influent Advisor, a model based influent wastewater characterization tool. Minor fractions such as autotrophic biomass were defined using a default value of 0 in the model.
- Adoption and/or modification of the kinetic parameters. The kinetics of different processes which are incorporated in ASM1 are shown in Appendix III. Kinetic coefficients, i.e., μ_{max}, b_H, K_S, K_h, determined by the respirometric studies were adopted and modified.

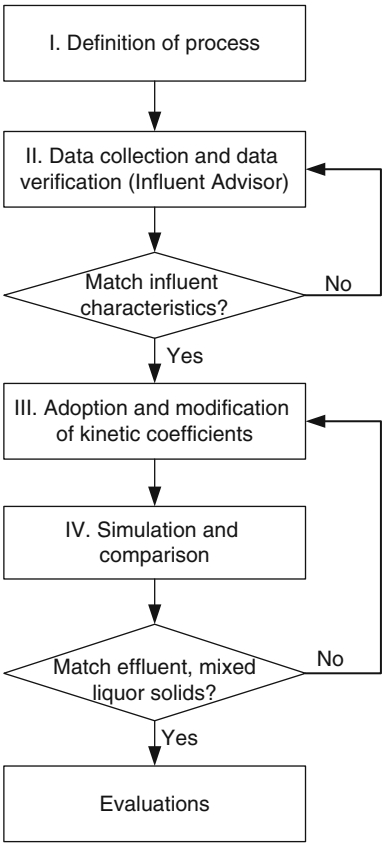

Fig. 18.30. Model calibration protocol of GPS-X.

- Simulation and comparison of model predictions with the measured data for the final effluent and the aeration tank biomass.

It must be emphasized that the process identified in steps 2–4 above is an iterative process since an initial fractionation will rarely result in proper calibration.

5.2.3. Model Scenario

GPS-X was adopted to simulate the following scenario based on the steady-state performance of pilot-scale system. The operating conditions of the pilot-scale system include anaerobic HRT = 0.25 day, aerobic HRT = 1 day, SRT = 5 days, and temperature = 25°C.

5.2.4. Modeling Results

The experimental results were compared with simulated results using GPS-X for the pilot-scale and bench-scale anaerobic/aerobic systems in terms of the following parameters, i.e., total and soluble COD (TCOD and SCOD), total and soluble BOD (TBOD and SBOD), mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solids (MLVSS), total and soluble TKN (TKN and STKN), NH_3-N, and NO_3^--N. These parameters were compared in influent, anaerobic reactor effluent, aerobic reactor effluent, and clarifier effluent. All the influent characterization data were input into Influent Advisor of GPS-X. As an important step of the calibration procedure, model predicted influent parameters were calibrated to fit the corresponding experimental parameters by adjusting the nitrogen compounds and nutrient fractions. In addition to the influent characterization, the model requires input of the sludge settling characteristics, i.e., SVI, as well as kinetic coefficients. The comparisons in pilot-scale and bench-scale systems are shown in Figs. 18.31 and 18.32. Generally, the model predicted the trends reasonably well although some discrepancies between predicted and observed values existed. Since ASM1 does not incorporate biological phosphorus removal, P was not predicted in the simulation using GPS-X based on ASM1 model.

In general, the model predicted soluble species in all process effluents well. The model predicted the final effluent parameters that are close to the experimental data except for TSS and VSS which confirmed that the model is well calibrated to the specific industrial application. The measured values of TSS and VSS are higher than the model prediction due to the short circuiting in the clarifier and lack of sludge collection mechanism. MLTSS/MLVVS data confirm that the model-predicted biomass concentrations agreed fairly well with most steady-state VSS data within the standard deviations of experimental measurements.

The calibrated process model was then used to simulate the full-scale system performance based on the following reactor sizes: anoxic tank = 925 m^3, aeration tank = 1,850 m^3, secondary clarifier = 782 m^3, secondary clarifier area = 154 m^2, and Q = 2,300 m^3/day. Two scenarios were primarily evaluated: the average concentration and the 95% concentration. Tables 18.20 and 18.21 include the results of modeling based on the average influent concentration and the 95% influent concentration. It is evident that the modeling results corroborate the achievability of the required dry-ditch criteria for the full-scale system without any requirements for nutrient addition.

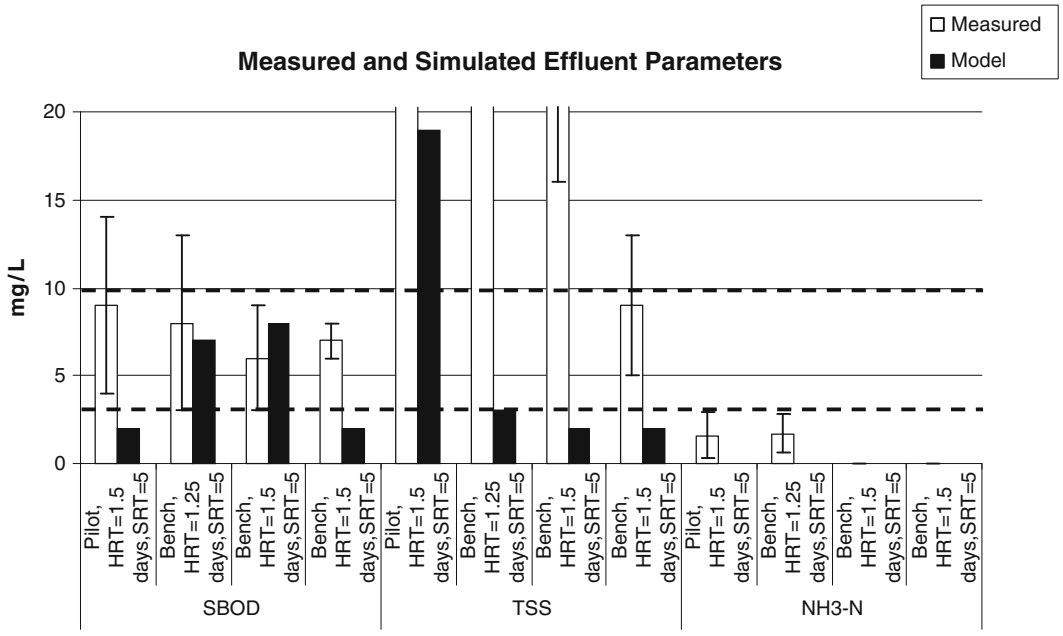

Fig. 18.31. Comparison of measured and simulated effluent parameters.

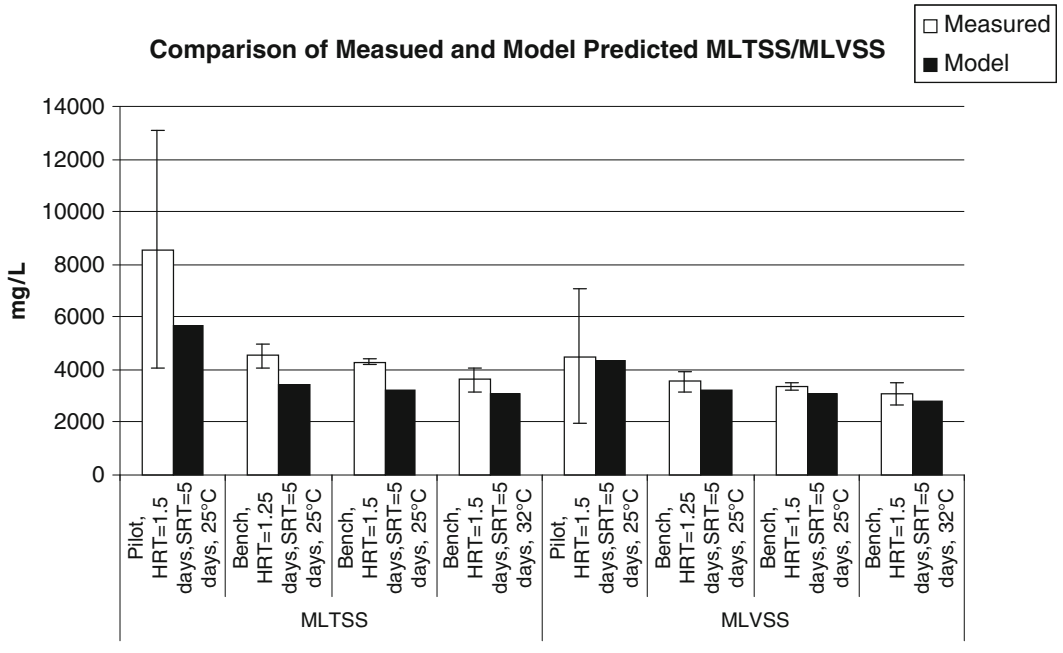

Fig. 18.32. Comparison of measured and simulated mixed liquor concentration.

Table 18.20
Model prediction of system performance (Unit, mg/L)

	Influent (50%, 95%)	50% Effluent	95% Effluent
TCOD	4,620 (9,100)	178	180
SCOD	3,910 (6,180)	104	112
TBOD	2,000 (4,400)	11	13
SBOD	1,650 (3,000)	1	2
NH_3-N	29 (112)	0	0
NO_3^--N	1	0	0
TSS	520 (2,870)	47	48
VSS	420 (2,280)	42	39
TKN	115 (244)	6.6	6.2
STKN	65 (132)	0.9	0.6

Table 18.21
Model prediction of system operating conditions

	50% influent basis	95% influent basis
MLSS, mg/L	3,983	7,089
MLVSS, mg/L	3,636	5,840
OUR, kg/day	5,166	8,827
F/M, anaerobic		
kg COD/kg VSS-day	2.5	3.1
kg BOD/kg VSS-day	1.1	1.5
Clarifier solids loading, kg/m^2-day	132	215
SRT, day	5	3
Sludge production		
kg VSS/day	2,949	7,796
kg TSS/day	3,180	9,143

6. DESIGN EXAMPLE

The optimized design criteria for the two systems, i.e., anaerobic/aerobic and the UASB are illustrated in Table 18.22. This criteria was used to design a $2,300\,m^3$/day full-scale system with the influent characteristics as shown in Table 18.1. Tables 18.23 and 18.24 list the full-scale process unit sizes required respectively for the A/O and UASB-A/O systems based on the optimized operating conditions delineated during the study.

Table 18.22
Optimized design criteria for anaerobic/oxic and UASB-A/O systems

UASB-A/O system		Anaerobic/Oxic system	
UASB reactor		*Anaerobic tank*	
Design loading	10 kg COD/m^3-day	HRT	10.4 h
Anoxic tank		Mixing intensity	8.8 W/m^3
Hydraulic retention time (HRT)	4 h	*Aeration tank*	
Mixing intensity	9.7 W/m^3	HRT	24 h
Aeration tank		Design MLSS	4 kg SS/m^3
HRT	12 h	Internal recirculation	2Q
Design MLSS	4 kg SS/m^3	*Clarifier*	
Clarifier		HRT	8.2 h
HRT	4 h	Return activated sludge	0.5Q
Return activated sludge	4Q		

Table 18.23
UASB-A/O system

1	UASB reactor	
	Volumetric flow rate (Q)	2,300 m^3/day
	Reactor size	40′(H) × 30′(W) × 40′(L)
	Total volume	1,350 m^3
	Total liquid reactor volume	1,150 m^3
	Design loading	10 kg COD/m^3-day
2	Anoxic–oxic system	
2.1	Anoxic	
	Hydraulic retention time (HRT)	4 h
	Volume	385 m^3
	Mixer	5 hp
2.2	Aeration tank	
	Volume	1,200 m^3 – with 5.0 m SWD
	HRT	12 h
	Average oxygen demand (AOD)	1,060 kg/day
	Peak oxygen demand (POD)	2,500 kg/day
	E_{OTE}	10%
	Air required	$70\,m^3/\,min = 2,500\,ft^3/\,min$
	Design MLSS	4 kg SS/m^3
2.3	Clarifier tank	
	SS loading	120 kg/m^2day
	Area	77 m^2
	Diameter	10 m with 5.0 m SWD
	SS_{peak}	240 kg/m^2day
	HRT	4 h at $Q_{average}$

Table 18.24
Anaerobic/oxic system

	Volumetric flow rate (Q)	2,300 m^3/day or 600,000 US gal/day
1.	Anaerobic tank	
	Volume	930 m^3 with 5.0 m SWD
		26.5 m(L) × 7 m(W) × 5 m (D)
	Hydraulic retention time (HRT)	10.4 h
	2 mixers ea.	11.0 hp, 580 rpm, 0.58 m propeller diameter
2.	Aeration tank	
	Volume	2,300 m^3
		27 m(L) × 17 m(W) × 5 m(D)
	HRT	24 h
	3 blowers ea.	55 hp (1 duty and 2 standby)
	Diffused air system	Numbers 500, 10-cfm coarse bubble
3.	Clarifier	
	Volume	782 m^3
	Height	5.08 m with 5.0 m SWD
	Diameter	14 m
4.	Internal recirculation system	
	2 pumps ea.	
5.	Return activated sludge (RAS) flow	3,450 m^3/day

7. ECONOMIC EVALUATION OF TREATMENT ALTERNATIVES

Table 18.25 presents a comparison of the preliminary full-scale system costs predicated on the results and findings of the study. For the economic analysis, the incremental differences in operation between the two treatment alternatives are: the added value of methane, the differences in sludge production, and the added cost of additional carbon required to build up biomass prior to the canning season, necessitated by the almost order of magnitude change in organic loading.

It should be noted that notwithstanding the economic advantages the system may have over the other, two issues have to be considered in technology selection. The UASB system can not sustain influent TSS concentrations over 500 mg/L, thus necessitating significant optimization of primary clarification. Due to the large variations in loadings, between the canning season and the off-canning-season spaning close to one order of magnitude, the "turn-down" and "turn-up" ratios for the UASB system are extremely important, particularly during the transition prior to the canning season. This may entail build-up of anaerobic granular sludge activity over a period of few months by addition of readily biodegradable organics such as acetate.

Table 18.25
Comparison of the preliminary full-scale system costs

		Cost of A/O system, in CAN $	Cost of UASB-A/O system, CAN $	Capital cost differentials, CAN $* 1,000
Capital costs[a]				
A	Clarifier			
	Concrete	160,000	120,000	−40
	Equipment	150,000	120,000	−30
	Savings in cost by UASB-A/O system			70
B	Anoxic tank (AxT)	210,000	120,000	−90
	Mixers	50,000	25,000	−25
	Savings in cost by UASB-A/O system			115
C	Aeration tank (AT)	500,000	300,000	−200
	Aeration equipment	350,000	280,000	−70
	External recirculation (ASR)	Same cost involved		
	Internal recirculation (AT to AxT)	Same cost involved		
	Savings in cost by UASB-A/O system			270
D	UASB		1,500,000	1,500
	UASB recycle pumping system	–	40,000	40
	UASB concrete foundation	–	140,000	140
	Total of D			1,680
E	Gas storage	–	500,000	500
Operating costs[b]				
A	Pumping cost for feed	Same cost involved		
	UASB recirculation pumping cost	–	20 KW × 24 × 360 × 0.1	−17.3
B	Additional labor cost		17,600	−17.6
C	Sludge production	During canning season sludge production = 3,450 kg/day During off-canning season sludge production = 345 kg/day	During canning season sludge production = 780 kg/day During off-canning season sludge production = 78 kg/day	

(*Continued*)

Table 18.25
(Continued)

		Cost of A/O system, in CAN $	Cost of UASB-A/O system, CAN $	Capital cost differentials, CAN $* 1,000
	Sludge production yearly	= (3,450 × 60) + (345 × 300) = 310,500 kg/year	= (780 × 60) + (78 × 300) = 70,200 kg/year	
	Volumetric sludge (at 30% solids)	= 310.5/0.3 = 1,03 m^3/year	= 70.2/0.3 = 234 m^3/year	
	Cost incurred in sludge processing (considering 150$ for 27.3 T sludge processing)	= 5,686	= 1,285	4.4
D	UASB chemicals			
	Methanol to build loading for initial two months period (start up)		= 5,000 kg COD/day × 0.3 × 60 day	−90
E	Power savings in mixers and mechanism		= 14 hp × 0.746 × 24 × 360 × 0.1	9.03
	Power saving in aeration energy		= 100 hp × 0.746 × 24 h/day × 365day/year × 0.1/KWH	65.35
F	Cost of methane (considering $10.44/GJ)	–	Methane during canning season = 125.3 × 60 GJ = 7,518 GJ Methane during off-canning season = 12.53×240 = 3,007.2 GJ Methane during build-up = 125.3 × 0.5 × 60 = 3,759 GJ	
			Total energy from methane = 14,284.2 GJ	149.2

[a]Total cost involved in UASB-A/O system construction = (Total of D − Total savings in cost) + Gas storage cost.
Total savings in cost = sum of A + B + C = CAN $ (70 + 115 + 270) K = CAN $ 455K.
So, total cost involved in UASB-A/O system construction = (Total of D − Total savings in cost) + Total E = CAN $ [(1,680 − 455) + 500] K = CAN $ 1,725K.
[b]So, total savings in operating cost = A + B + C + D + E + F = CAN $(−17.3–17.6 + 4.4–90 + 9.03 + 65.35 + 149.2) K = CAN $ 102.7K.

The assumptions used to calculate the capital and annual operation and maintenance costs for the full-scale systems, based on the sizes specified are:

- Cost of power is $ 0.1/KWH
- Cost of natural gas is $ 10.44/GJ
- Cost of UASB system as quoted by Biothane® is 1.25 million US $ with an exchange rate of 0.83 CAN $ to 1 US $
- Methanol addition is required for a period of 2 months per year to "turn-up" the loadings from the off-canning, so that the system can meet the criteria from the beginning of the canning season. Cost of methanol is $ 0.3/L

It is evident from the capital and annual O&M costs presented in Table 18.25 that the payback period, assuming a 7% rate of return, is about 28 years. Furthermore, the effluent from the UASB-A/O system is much richer in nitrates than the A/O system and accordingly should future regulations target nitrates or total nitrogen, the return on investment might be even lower.

8. SUMMARY

The successful execution of this study culminated in the development of a process design that can be used effectively to overcome nutrient limitations and enhance biodegradability for many particulate-laden food processing wastewaters, which will be implemented at full-scale. Although the other system developed comprising the UASB-anoxic–oxic system is not economical because of seasonality, its technical viability has been proven.

Kinetic coefficients derived from respirometric studies were applied in the modeling of anaerobic/aerobic systems. The entire comparison between model predicted parameters and measured parameters in the pilot-scale and bench-scale anaerobic/aerobic systems demonstrated that the model was well calibrated and that the performance of the systems can be reasonably predicted using the fractionation and kinetic coefficients established from respirometry.

The calibrated process model was then used to simulate the full-scale system, and the modeling results corroborated the achievability of the required dry-ditch criteria for the full-scale system without any requirements for nutrient addition.

NOMENCLATURE

A/O = Anoxic–oxic
b_H = Endogenous decay coefficient, day^{-1}
BOD = Biochemical oxygen demand, mg/L
C_{S0} = Total input biodegradable substrate, mg/L
C_{T0} = Total input substrate, mg/L
C_T = Total substrate, mg/L
COD = Chemical oxygen demand, mg/L
DO = Dissolved oxygen, mg/L
DSVI = Diluted sludge volume index, mL/g

F/M = Food to microorganism ratio, mg COD/mg VSS
HRT = Hydraulic retention time, day
K_S = Saturation coefficient, mg COD/L
K_h = Hydrolysis rate constant, day^{-1}
MLVSS = Mixed liquor volatile suspended solids, mg/L
ORP = Oxidation-reduction potential, mV
OUR = Oxygen uptake rate, mg/L-day
RBCOD = Readily biodegradable chemical oxygen demand, mg COD/L
S_0/X_0 = Initial substrate to biomass ratio, mg COD/mg VSS
S_H = Rapidly hydrolysable substrates, mg/L
S_I = Inert soluble substrate, mg/L
S_P = Residual soluble microbial products, mg/L
S_R = Residual soluble substrate, mg/L
S_{S0} = Input biodegradable substrate, mg/L
S_S = Readily biodegradable substrate, mg/L
S_T = Total soluble substrate, mg/L
SBOD = Soluble biochemical oxygen demand, mg/L
SCOD = Soluble chemical oxygen demand, mg/L
SBR = Sequencing batch reactor
SP = Soluble phosphorus, mg/L
SRT = Solids residence time, day
STKN = Soluble total Kjeldahl nitrogen, mg/L
SVI = Sludge volume index, mL/g
TBOD = Total biochemical oxygen demand, mg/L
TCOD = Total chemical oxygen demand, mg/L
TMP = Transmembrane pressure, Pa
TP = Total phosphorus, mg/L
TSS = Total suspended solids, mg/L
TKN = Total Kjeldahl nitrogen, mg/L
UASB = Upflow anaerobic sludge blanket
VFA = Volatile fatty acids, mg/L
VSS = Volatile suspended solids, mg/L
WAS = Waste activated sludge
X_T = Particulate substrate, mg/L
X_S = Slowly biodegradable substrate, mg/L
X_H = Heterotrophic biomass, mg/L
X_I = Inert particulate organic matter, mg/L
Y_H = Yield coefficient, mg COD/mg COD
μ_{max} = Maximum heterotrophic growth rate, day^{-1}

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Animal Glue Production from Skin Wastes

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CONTENTS

Abstract Animal glue is the most important protein adhesive obtained from animal hides, skins, and bones through hydrolysis of the collagen. Animal glue production has long been a lucrative business in various parts of the world. This chapter discusses pretreatment and conditioning techniques including acidic, alkali, and enzymic proteolysis, which are involved during animal glue production. The extraction methods, including denaturation and thermal treatment, are also discussed. The possible improvement of pot life and moisture resistance of animal glue using chemical modification technique is presented. The application of micro-bubble technique for glue production from cow skin is also introduced.

1. INTRODUCTION

Animal wastes primarily consist of solid manure and liquid effluent generated from farms and feedlot. Large quantities of animal wastes containing high levels of organic pollutants are also produced by slaughterhouses and poultry processing centers. Waste streams from meat processing such as blood, hides or skin, bones and, offals are potential contributors both in terms of solid waste, also called animal biomass, and liquid effluent. The application of animal wastes as useful products is widely practiced worldwide as part of the pollution prevention and waste minimization strategy adopted in many countries. In some parts of the world, glue

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_19,

production from animal bones and hides has been a lucrative business. Proper handling and disposal of animal waste are important in protecting human health (1).

This chapter focuses on the utilization of animal skins or hides for the production of glue. Usually, the hides are processed into various products for the leather industry, but a broader perspective of converting the wastes into aqueous gel or gelatin also deserves examination.

1.1. Animal Skin Generation Rates

Knowledge of the quantities of animal skin or hides generated and collected for further processing is of fundamental importance to the management of animal wastes and byproducts. The quality and available quantities of skin or hides also determine the method to be used for economic reasons. The world's production of animal skin or hides is estimated to be 10.8 million metric tons per annum in 2003. Literature review reveals the annual production of animal skin or hides from sheep, goat, cattle, and buffalo. Cattle hides account for more than 68% of the total animal skin production (1). In global terms, Asia leads the world by producing more than 2.47 million tons of animal skins in 2003. In contrast, Europe and the United States produced about 1.30 million tons and 1.04 million tons of cattle hides, respectively, in 2003 (2).

1.2. Hide Removal from Cattle and Sheep

In animal skin or hide processing, slaughterers normally remove hides from cattle and sheep to reduce the potential contamination of carcasses by hair, dirt, and manure. Usually, hides are removed from cattle and sheep mechanically after the removal of the head, tail, and hooves. The process of hide removal begins with some initial separation from the carcass manually, using either conventional or air-driven knives, to enable attachment of mechanical pullers. The pullers then remove the hide by either pulling up from the neck to the tail or pulling in the reverse direction, which is less common. Typically, pulling of the hide is carried out manually with good results, but it requires experienced slaughterhouse personnel to do it efficiently (3).

The hide must be preserved to prevent degradation due to rapid microbial attack. On-site hide processing can consist of salting for preservation before shipment to leather tanning operations, or it can involve washing, defleshing, and salting before shipment. However, on-site hide processing options may also include curing before shipment for off-site tanning or complete processing followed by the marketing of tanned hides (4).

2. ANIMAL GLUE

Glue production from animal bones and hides has long been a lucrative business in various parts of the world. Animal glue historically refers to "hide or bone glue" adhesives, which need to be heated and melted before usage. Animal glue is the most important protein adhesive obtained from cattle and other animal hides and bones by hydrolysis of the collagen. Hide glue is higher in molecular weight than bone glue and therefore stronger (5).

The popularity of this product is due to its unique combination of properties, which cannot be achieved by other synthetic resins, such as the ability to deposit a viscous, tacky film from a hot aqueous solution that forms a firm gel while cooling. Other properties include ease of

preparation, high tack, fast set, simple application, good matching properties, and high bond strength. Animal glue, however, is soluble in water, and this property has limited use in dry application only.

Most research studies nowadays concentrate on the effort to increase the yield of extracted glue and gelatin, using new methods for the pretreatment of the raw material to liberate the noncollageneous material from the stock without affecting the collagen-containing material or the specifications of the final product such as gelling strength and viscosity. These pretreatment methods are developed based on conventional methods in the pretreatment but with different conditions or by using new enzymes in the pretreatment of raw material stocks. On the other hand, a new process has also been developed to increase the yield of glue and gelatin within shorter times, fewer extraction stages, and high conversion of raw materials into glue or gelatin. All these considerations are based on economic factors.

2.1. General

The origin of animal glue in antiquity and its wide range of adhesive applications have contributed to the persistent use of the term "glue" to refer to any adhesive. Animal glue refers to the material extracted from protein collagen, which is found in specific animal tissues such as skin, hides, bones, sinews, and tendons. Contrary to popular assumptions, animal glue cannot be made from horns, hooves, or hair because these are primarily composed of keratin protein, which is not readily hydrolyzed, and by the nature of its structure, it will not lend itself to produce the helical configuration consistent with the characteristics of animal glue and gelatin.

Animal glue and gelatin are almost identical as they are produced by the same methods and from the same raw materials. In some instances, animal glue is referred to as technical gelatin; however, the two differ in purity and quality. Gelatin requires clean and edible raw materials, more purification, and more stringent processing conditions and control. Animal glue, however, can be processed from raw materials that otherwise would be wasted. The color and purity of animal glue, unlike that of gelatin, do not necessarily indicate its effectiveness as an adhesive.

2.2. Type

Commercial animal glue usually is named according to the raw materials from which the product is made; thus, there are bone and hide glues. Bone glue has light yellow/dark brown color and is shaped like pearls with a diameter of 3–4 mm.

Hide glue and technical gelatin are a mixture of glutin and a smaller quantity of its fission products. It is produced by means of extracting non-tanned hides and hide-cuttings in warm water. The obtained water extracts are thickened, then cooled and dried. Hide glue is light yellow to dark brown in color. It is delivered in irregular-sized grains: in ground form (grain size 1.5–2.5 mm) and unground form (grain size 3.5–4.5 mm). The technical gelatin has a light yellow color and has a similar consistency as hide glue in ground form.

Further delineation of glue is also possible. For example, green bone glue refers to glue produced from bones fresh from packing or boning houses; extracted bone glue is made from bones that have been precooked to remove fat prior to glue extraction; chrome glue is extracted

from scraps and shavings of chrome-tanned leather; and coney glue is made from rabbit skin residues from the production of felt. Fish glue, which is made from the non-edible parts of fish, is generally liquid at ambient temperature and is clearer and lighter than bone and hide glues.

2.3. *Properties and Chemical Composition*

Animal glue is marketed as a dry solid, and its color ranges from light yellow to tan, brown, and sometimes almost black. In an unground state, it is almost hornlike and can be obtained as non-uniform particles, plates, or squares or as fine beads called pearls. Commercial glue is usually sold ground to a size of 2–2.4 mm (8–10 mesh). Special grinds are also available in 20, 100, and 325 mesh sizes (4). Animal glues are described as hydrolyzed collagen with the following formula:

$$C_{102}H_{149}O_{38}N_{31} + H_2O \rightarrow C_{102}H_{151}O_{39}N_{31}$$

The factors affecting this hydrolysis are temperature, pressure, time and amount of solvent used (6). The molecular weight of hide glue has a wide range from 20,000 to 250,000. The higher the gel strength, the higher would be the molecular weight (6). The approximate chemical compositions are 51–52% carbon, 6–7% hydrogen, 24–25% oxygen, and 18–19% nitrogen (7).

Animal glue is composed of α amino acids joined in polypeptide linkages to form long chain polymers. In an aqueous solution of animal glue, polypeptide chains assume random configurations of essentially linear form. It has been indicated that most glue molecules consist of single chains terminated at one end by amino group and at the other by carboxyl group. The molecules may also have side chains, contain cyclic structures, and may in part conform to the oriented chain in the original collagen. Polypeptide chains vary in length and molecular weight (5).

Amino acid studies corroborated by various analyses indicate that there are 18 different amino acids present in collagen and animal glue. Table 19.1 shows the level of amino acids in collagen and animal glue (4). The acidic and basic functional groups of amino acid side and terminal groups confer poly-electrolyte characteristics on protein chains. The chains

Table 19.1
Comparison of yield percentage between new process and conventional extraction process (26)

Temperature (°C)	Yield (%)				
	New extraction				Conventional extraction
	4,000 rpm	4,500 rpm	5,000 rpm	5,500 rpm	
75	16.27	16.86	17.24	18.18	12.12
85	41.38	49.97	46.43	45.47	16.94
95	17.59	22.51	19.24	19.49	7.88
Total	75.24	89.34	82.91	83.14	36.94

contain both amine and carboxylic groups, which are reactive and ionizable. These electrically charged sites affect the interactions among protein molecules and between protein molecules and water. These polar and ionizable groups are believed to be largely responsible for the formation of gelatin and the characteristic rheological properties of animal glue. Cross-linkage between protein molecules is possible through hydrogen, ionic, and covalent bonds.

Animal glue is amphoteric because the amine and carboxyl groups contained in the polypeptide protein chain are reactive and ionizable. In a strong acid solution, protein is positively charged and acts as a cation. In a strong alkaline solution, it is negatively charged and acts as an anion. The intermediate point, where the net charge on protein is zero, is known as the isoelectric point (IEP) and is designated in pH units. The IEP varies, depending on whether the pretreatment of collagen is acidic or alkaline. During processing, acidic or alkaline treatments are used to hydrolyze the amide groups in collagen to a greater or lesser extent, liberating the acid functions. Acid-processed glue (little amide group modification) has an IEP near 9.0, and alkaline-processed glue (low residual amide groups) has an IEP close to 4.8 (5).

Another important characteristic in gelatin production is the gelation of animal glue upon cooling. Gelation involves both intra- and intermolecular reorientation upon cooling of the solution. It is caused by the formation of random primary and secondary bonds. Intermolecular network formation is primarily the result of a cross-linking mechanism between molecular chains by a hydrogen bond.

2.4. Manufacturing

The ultimate objective of glue production is the conversion of material containing collagen of different degrees of insolubility into a maximum quantity of soluble and highly purified glue with good physical–chemical properties such as gel strength, viscosity, low ash content, and clarity. The fundamental production process is based on three stages (8):

1. Preparation of raw material, i.e., the elimination of non-collageneous components from the stock material with or without the reduction of cross-linkage between collagen components
2. Conversion of purified collagen into glue or gelatin
3. Refinement and recovery of glue or gelatin in dried form

Preparation of animal glue is essentially a treatment of a collagen source with heat and water in order to hydrolyze it to a soluble product as rapidly and as efficiently as possible. The resulting solution is filtered, centrifuged to remove fat, concentrated to a suitable concentration in an evaporator, chilled to gel the concentrate, extruded or cut into particles, dried, ground, and analyzed. The quality and cleanliness of the raw materials, their cleaning and preprocessing, and the speed of extraction, concentration, and drying determine the quality of the product. The quantity of glue obtained is affected by the quantity of hides or bones used initially, the nature of the raw material, the extraction temperature, the time of extraction, the amount of water used and the number of extractions carried out (8).

All the raw materials are washed with large volumes of cold water to remove all contaminants such as blood, manure, salt, and dirt. The duration of washing will depend on the cleanliness of the raw material at the start. The wash is complete when the water runs clear

or, in the case of salted hides, when it is salt-free. Dry and salted stock requires long washes. Gelatin production can differ in the pretreatments that are necessary to extract the collagen using hydrolysis. Normally, higher collagen concentration found in the raw material provides a more efficient hydrolysis step. Thus, the pretreatment of hides will be different from that of bones and depending on the method used, some pretreatment can change the characteristics of the glue (8).

3. PRETREATMENT AND CONDITIONING

The first stage in glue or gelatin production is the pretreatment and curing process. Pretreatment is the process by which tissue is soaked in either acid or alkali in order to increase the availability of collagen to the hydrolytic environment. The pretreatment procedure used depends upon the facilities and the nature of the stock. In its natural state, collagen is water-insoluble and must be conditioned to solubilize the protein. Collagen molecules are triple helices of amino acid sequence and contain both non-polar and charged acidic and basic side chains. The conversion of collagen to soluble protein of animal glue involves the breaking of the intra- and intermolecular polypeptide bonds through the use of acid or alkali pretreatment before the extraction takes place.

3.1. Acidic Pretreatment

In this type of pretreatment, collagen is soaked in a dilute acid, such as hydrochloric or sulfuric acid, and then extracted at a pH of about 4. Many noncollageneous proteins of the tissues are isoelectric at about this pH and are therefore less soluble and more readily coagulated under the extraction conditions. The gelatin that is obtained after this pretreatment can be low in impurities or heavily contaminated.

The type of acid used appeared to have a considerable effect on the yield of the product and properties of the acid process. Poppe et al. (9) showed that 5% sulfuric acid was more suitable than 5% hydrochloric acid but had a longer soaking time. Furthermore, Reich (10) studied the relationship between the animal age at slaughter and acidic treatment, which suggested that suitable skin age of cattle should be in the order of 2–3 years old.

3.2. Alkali (Lime) Pretreatment

Lime suspension pretreatment for the manufacture of glue and gelatin was probably the first method for the production of high quality glue and gelatin. In terms of yield and properties and purity of products, it is a very effective method for preparing glue and gelatin from mature cattle tissues.

Babloyan (11) showed that sodium thiosulfate was as effective as sodium sulfate in the pretreatment of animal hides or skin and that sodium chloride and sodium carbonate gave reduced yield. Poppe et al. (9) claimed that alkali pretreatment also caused chemical alteration (hydrolytic reactions) in collagen without appreciable solubilization. It required only the breaking of weak physical forces that maintained the fibrous collagen structure.

Fadilah et al. (12) studied the effect of size and treatment methods on the conversion of dried cattle hide into glue. A pretreatment of alkaline using calcium oxide (CaO) was carried

out at two different temperatures of 5 and 10°C for soaking periods of 5, 10, 15, 20, 25, and 30 days with vigorous stirring every 70–72 h. Yield improvement was obtained, with a yield of 34 g per l00 g of sample at soaking conditions of 5°C for 30 days, while at 10°C the yield was about 29 g per l00 g for the same period.

3.3. Enzymic Proteolysis

Typically, the soaking process in alkaline or acidic suspension for an extended period involves large quantities of materials. Any process that might reduce the conditioning time of hide is worthy of investigation. One such process is that of enzymic proteolysis. The hide is treated in buffer solution at pH 12.5–10.0 at temperature 24–28°C for 2 days, followed by washing and acid treatment in a tumble. The acidification step denatures the enzyme and prevents further proteolysis of extracted gelatin (13).

4. EXTRACTION

The extraction process consists of transferring the pretreated washed material into an extraction vessel containing hot water (60–65°C), heating with agitation for a suitable time, and draining the liquor from the bottom. More water is added and the step is repeated at a slightly higher temperature for shorter periods. Several extractions are made in this fashion until the liquor that is removed contains only 1% solids. At this point, the economics of the extraction prevents further processing. Residue (grout) in the extraction vessel is removed and may be dumped, but it is usually dry-rendered to recover fat. As the extraction proceeds, the volume of material shrinks and, thus less water is required. In order to alleviate poor extraction, hot water and agitation are used to prevent packing in the extraction vessel, which causes poor extraction.

Rapid operations are essential for the formation of glue material. Glue liquor usually hydrolyzes readily at a concentration of 2–9%. Deterioration of quality in dilute glue at the formation stage is also common due to favorable conditions for bacteria and fungi to form a natural breeding medium. In practice, the liquor is then filtered as rapidly as possible to remove any suspended fines and transferred to evaporators. These usually are multiple-effect vacuum machines that can concentrate liquors to 20% solids if the quality of glue is not too high. Further concentration of liquor can be accomplished with wiped-film-type evaporators or plate evaporators for maximum concentration. The concentrated liquors or heavy liquors (as they are sometimes called) are ready for a variety of drying processes. The liquor may be chilled for gelling by passing over a chill roll and then through a slicer that forms long strips for the drying oven. The liquor may also be chilled by pumping it to a refrigerated rotator that chills and extrudes it under pressure onto a belt where the noodles are carried to a dryer.

Cole and McGill (13) reported that the extractability of the hide may be greatly decreased as animal age increases. The parallel increase in insoluble residue with animal age is to be expected, but the same relationship of gelatin yield to animal age would not necessarily follow, because the yield depends upon the variable salt, hair, and fat contents. If one assumes that calves average 6 months, young animals 20 months and aged animals 60 months, then for the conditions given, the first extraction extractability decreases by some 0.5% for each month

of animal age. The more readily extractable the gelatin, the better are the properties, with viscosity a good indicator of this trend (12). Cole and Roberts (14) also reported that collagen from 10-month-old animal hide exhibited very high extractability after a very moderate liming.

4.1. Denaturation

It has been shown that the simplest route from collagen to glue or gelatin is through the denaturation of soluble collagen (15). In this process, the triple helical structure is destroyed to produce one, two, or three random gelatin molecule chains. This change occurs in mild conditions by heating the pretreated collagen containing material at 40°C. Steven and Tristram (16) used the same process as of Flory and Weaver (15) with an additional hydrogen bond breaker at room temperature or lower to convert collagen-containing material into glue or gelatin. It involved breaking only the hydrogen bonds and hydrophobic bonds that helped to stabilize the collagen helix. Under these mild conditions, no covalent linkages were destroyed in the time required.

Von Hippel and Wong (17) defined the transition from collagen to glue or gelatin by a denaturation temperature (TD). This was determined by heating the liquor of soluble collagen at a series of fixed temperatures for 30 min each. The liquor was then cooled to a fixed temperature (20°C), and the new viscosity or optical rotation was measured. By plotting the viscosity or optical rotation against the temperature of the 30-min heating, a sharp fall was demonstrated in the denaturation region. The temperature at which 50% of this change occurred is defined as the denaturation temperature.

4.2. Thermal Treatment

Insoluble mature mammalian collagen contains material that can be converted to glue or gelatin by prolonged hot water extraction alone, although the glue (gelatin) produced by this means is of little commercial value.

Nasrallah and Ghossi (18) feed the initial extraction water to an extraction vessel at a temperature of 52°C or below. The vessel content was then gradually externally heated to 40°C by passing a portion of the aqueous liquid from the vessel through a heat exchanger and back to the vessel. Alternatively, heat could be added to the extractor content to gradually raise the temperature of the content to the desired temperature. Operating in this manner, the extractable gelatin was never in contact with the aqueous liquid that had a temperature above 52°C before being subjected to low temperature extraction; thus, even localized gelatin modification was avoided. After the initial low temperature extraction, the collagen-containing material reached an elevated temperature, typically at about 40°C, and subsequent extractions could begin by feeding water which was at or only slightly above the desired extraction temperature.

Nasrallah and Ghossi (18) also found that gentle and uniform agitation of the vessel content during extraction was extremely effective in improving the yield of the resulting gelatin extraction process. Agitation should be sufficient to remove the bulk of the gelatin from the surface without emulsifying or dispersing the oil present in the collagen-containing material. They found that an improved yield of 8–14% and increased gelatin quality (bloom strength) of up to 6% in the finished product could be obtained by making at least two extractions below

46°C, for a period of 3.5 h or less at a water to collagen-containing material ratio of about 1.5–2.5:1 to remove the increased amount of gelatin at relatively low temperature. Several more extractions were possible with the extraction temperature maintained below 52°C, and the time of contact of hot water with the collagen-containing material being maintained at 3 h or less. Finally, they found that water added in the low-temperature extractions permitted a more efficient extraction and improved the recovery of gelatin in a relatively shorter period.

Fadilah et al. (12) used the conventional extraction method in the production of animal glue at different starting temperatures. Samples of collagen-containing material were fed to a glass beaker containing 60°C distilled water for 1 h. The fat emulsion skimmed up on the water surface was easily separated. The samples were extracted in three stages at increasing temperatures of 60, 70, and 80°C for 8 h each. The extracted glue was then filtered, and the clear glue liquid obtained was dried in a temperature controlled water bath at 60°C for 10–14 h.

Lakoche et al. (19) reported an improved process for the manufacture of gelatin from collagen-containing material by utilizing a caustic solution. Collagen-containing material was demineralized to produce Ossein, which was homogenized or ground. The Ossein was added to a water solution of sodium hydroxide or potassium hydroxide at a concentration of at least 4% by weight and a swelling restraining salt (sodium sulfate) at a concentration of at least 3% by weight for 10–120 h to form a reacted slurry. The slurry was heated at a temperature of at least 45°C for 30 min to produce a gelatin-containing solution that was then clarified by raising the pH of the solution to greater than 9.8. Sulfate salt was added to the gelatin solution to reduce the pH ranging between 7 and 8. Phosphoric acid was added to the solution to reduce the pH ranging between 5 and 6. Following extraction and clarification, the gelatin solution was filtered, oxidized or de-ionized to achieve the desired level of micro-constituents, prior to concentration and drying.

5. CHEMICAL MODIFICATION

Animal glue can be produced using a chemical modification process. The modification of the chemical constituent of animal glue involves reactions either with the polypeptide backbone of the molecule or more commonly with the side-chain groups along the polypeptide backbone, which are the amino group and the carboxyl group.

Gupta et al. (20) developed a process to convert animal glue into a hot set adhesive in which both the pot life and the moisture resistance of animal glue were improved. They indicated that animal glue can be applied to plywood along with paddy husk gel as an extender with a small percentage of potassium dichromate and paraformaldehyde to give a composition, which when passed at about 100°C (±5°C) met the requirement for tea chest plywood. They also suggested that the hot set composition of gluing, based on animal glue, had an advantage over the urea-formaldehyde based composition of reducing the cost of gluing to about 25%.

Stotts (21) invented a new thermal insulating foam from clear animal glue. This foam replaced highly flammable polyurethane foams, and flame-retardant types, which had a tendency to produce significant quantities of toxic smoke during a fire. The new foam produced was also better than cellulose insulation materials, including either flame retardants which

leached out over time, or organic flame retardants which produced large quantities of toxic smoke. The new invention also replaced other thermal insulation materials, such as fiberglass and mineral wool, since the latter were often backed by readily combustible paper. The new foam overcomes the drying phenomenon of collagen foams at ambient temperature without collapsing before being applied the surface.

Kormanek (22) reported that under certain conditions, animal glue exhibited improved adhesive properties when the inventive hardener composition was added. The animal glue hardener consists of:

1. The product of the reaction between urea and formaldehyde that formed a hydroxy-methyl compound of urea, such as dimethylol urea (DMU)
2. A stabilizer consisting of a latex copolymer such as vinyl-acetate-dibutyl-meleate, or polysaccharide, including dextrin, corn starch and sugar, or ammonia. The stabilized hardener was added to animal glue to form a strengthened glue gel composition

Kormanek (22) showed that the DMU reacted well as a cross-linker with hydrophilic groups contained in the macromolecular chain of the animal glue protein. When added to DMU, a latex or polyhydroxyllic stabilizer controlled the reaction of the DMU with the protein chain of the glue to prevent an over-reaction or over cross-linking of the chain.

Bishop and Lasser (23) cross-linked and hardened animal protein gel with a new composition so that the final product could be used for a variety of industrial and medical applications. The methods consisted of adding a transglutaminase to a composition of a temperature-sensitive gel-forming protein; and incubating the composition and transglutaminase to convert them into a cross-linked gel.

6. APPLICATION

Hide and bone glues are used in paper, textile, wood-working, and polygraphic and chemical industries. Technical gelatin and technical protein colloid sometimes designate animal glue for applications other than the adhesive field. Jelly glue, flexible glue, non-warp glue, and composition glue refer to formulations that contain glue but are compounded so as to give specific changes in properties such as greater flexibility, non-curling, and so on. Other glue-formulated products are designated by their applications, e.g., freezer glue, sizing glue, and plating glue.

7. CASE STUDY: PRODUCTION OF GLUE

Animal glue research has seen few published works in the last 15 years. It is well known that tanneries have dominated the direct reuse or utilization of animal hides or skins. Other uses of hides such as for gelatin and glue production will be competing with established leather related industries. However, the demand for gelatin in the medical and food sectors and specifically animal glue, will continue to rise and therefore attract a great deal of interest in improved methods for glue production.

This section describes the research carried out at the University of Putra Malaysia, on glue production from cow skin using an innovative micro-bubbles technique. Brandon documented

the background of using micro-bubbles as solid bodies in the extraction of glue from collagen-containing material to reduce the extraction time and the number of extraction stages (24). He proposed that bubbles in fluid having a critical size (0.5 mm) behave like solid spheres. By implementing micro-bubbles in the extraction of glue, the micro-bubbles would blow inside the extraction vessel at a certain speed by the action of disperse-mixing process and strike the collagen-containing material from all sides to soften it and to form pores through the collagen-containing material. McDonough (25) proposed applying a wide 6-bladed high shear mixer to shear air bubbles into micro-bubbles and to disperse them into the extraction vessel.

Mohammed Issam (26) achieved greater improvement in glue yield using dispersed micro-bubble technique within a shorter time period. He carried out the extraction of animal glue at different speeds of disperser (4,000–5,500 rpm) over a series of different temperatures (75–95°C). As Table 19.1 shows, there was a great improvement (50–60%) in glue yield using the new extraction process compared with that obtained using conventional extraction method.

This extraction technique did not affect the physical properties of glue, whereby the viscosity and the gelling strength of samples (Table 19.2) fell within the same range as required by National Association of Glue Manufacturers (5, 26)).

The plywood industry is one of the industries in which such adhesives can be potentially of use and where urea formaldehyde (UF) resin is the main binder. With an increased environmental consciousness regarding formaldehyde emission (a toxic gas released by UF resin at high temperature and humidity), this high moisture-resistant animal glue may offer a better alternative. Mohammed Issam (26) conducted research to increase the moisture resistance of animal glue with incorporation of melamine without significant increase in cost.

He claimed that blending the animal glue with melamine urea formaldehyde (MUF) and fortifying with melamine formaldehyde cross-linker (MF_{cl}) gave a superior animal glue suitable for bonding plywood. The optimum condition of MF_{cl} to be blended with MUF resin was 1.25 g water/g glue swelling capacity, pH 8.2 and viscosity of 310 cP (37°C).

Table 19.2
Viscosity and gelling strength of produced animal glue (26)

Mixer speed (rpm)	Temperature (°C)	Gelling strength (g)	Viscosity (millipoise)
4,000	75	421.7 ± 2.38	174 ± 5.40
	85	343.8 ± 2.30	156 ± 6.18
	95	289.7 ± 2.67	118 ± 5.93
4,500	75	425.2 ± 3.48	183 ± 6.42
	85	345.1 ± 2.37	157 ± 3.96
	95	287.1 ± 1.06	123 ± 5.01
5,000	75	423.9 ± 1.67	172 ± 3.43
	85	339.2 ± 1.34	161 ± 3.19
	95	281.0 ± 2.78	126 ± 3.78
5,500	75	417.7 ± 2.05	182 ± 3.03
	85	339.5 ± 2.10	159 ± 3.80
	95	287.5 ± 2.14	120 ± 3.91

Furthermore, Mohammed Issam studied the bacterial resistance of the cured adhesives against bacteria and fungus. Bacterial attack was observed in the animal glue sample after 3 days. For the cross-linked animal glue with MF_{cl}, the bacterial growth was observed after 1 week. No bacterial growth was observed on the cross-linked animal glue blended with MF_{cl} and MUF, cross-linked animal glue blended with MUF after a 2-week experimental period (26).

Mohammed Issam documented that the blending of animal glue with melamine-based resins had greatly improved the pot life of blended adhesives compared to the pot life of MUF resin used alone. For instance, the pot life obtained for the animal glue blended with MUF was 45 min, and for MUF alone, it was was 15 min, showing 66.6% improvement. The pot life obtained from blended animal glue and MF_{cl} and MUF was 30 min, showing 50% improvement over MUF (26). Additional glue factory waste management information can be found from the literature (27).

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An Integrated Biotechnological Process for Fungal Biomass Protein Production and Wastewater Reclamation

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CONTENTS

Abstract An integrated biotechprocess has been developed for fungal biomass protein production and wastewater reclamation from starch processing wastewater. The process resulted in producing 9.0 g/L fungal biomass, and removing total suspended solids, 95% BOD and 75% nitrogen. The biomass products contained 45% protein and appreciable quantities of amino acids, and they would be nutritive and edible for animal consumption. The reclaimed wastewater could be used for farm irrigation. This technology appeared to be technically feasible and economically beneficial for food and agricultural industries.

1. INTRODUCTION

Each year, billions of tons of organic substances are processed through the food and other processing industries. A significant portion of the organic substances become organic pollutants through wastewater streams. This is a major environmental concern in most parts of the world. Yet, much of these organic substances can potentially be utilised as valuable resources. Sustainability requires not only the treatment of the wastewater streams but also the recovery and utilization of resources, both the water itself and the substances in it. Bioconversion of wastes is the natural way to recover the useful resources. In industries, biotechnology can be

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_20

used to facilitate the natural recycling processes. Biotechnological treatment of wastewater streams can produce valuable end-products, such as microbial biomass protein (MBP), utilizing the organic substances in wastewater as substrates.

In order to achieve the dual objectives of water reclamation and MBP production, it is necessary to have (a) suitable organism species for any given wastewater streams, which can utilise the organic pollutants in wastewater as the substrates with no or minimum pre-treatment and nutrient supplementation, and (b) suitable bioreactor system that can be operated under non-aseptic conditions and facilitate both the MBP production and water reclamation. The cultivation of suitable fungal species with starch processing wastewater (SPW) in external air lift bioreactors (EALB) can achieve these objectives. Pilot plant studies have demonstrated that the system is technically feasible and economically beneficial for the simultaneous production of fungal biomass proteins (FBP) and wastewater reclamation (1, 2). These types of processes can potentially be extended to the treatment and utilization of organic wastewater streams from other industries.

2. FUNGAL BIOMASS PROTEIN PRODUCTION

2.1. Fungal Biomass Protein

Microfungi play an important role in food industries. They have a number of properties, which make them important both scientifically and industrially. They have a wide range of enzymes and are capable of metabolizing complex mixtures of organic compounds occurring in most wastes (3–5). The production of biomass proteins from microfungi is particularly attractive for a number of reasons. These include the following: (a) the cells of most species of microfungi contain reasonably high levels of proteins, (b) microfungi contain low levels of nucleic acids when compared to yeasts and bacteria, (c) FBP products are relatively stable and can be easily separated from the cultivation media and (d) food produced from fungi is traditionally eaten in many parts of the world (6). In addition, fresh fungal biomass has a pleasant odour, which further facilitates the utilization of the biomass. For example, fresh dewatered fungal biomass products could be directly supplied to an animal farm as stock feeds (7). In this case, the processing costs of drying are avoided.

Another distinctive advantage of microfungus cultivation is the easiest way of separating fungal biomass from the culture media. The cost of separating the biomass from the spent cultivated broth is a significant part of the capital and operating costs for biomass protein production. In the case of microfungus cultivation, the filamentous or pellet form of the microfungi leads to an easy and cheap harvesting of mycelial biomass.

The nutrient contents of the FBP produced by *Aspergillus oryzae* DAR 3,699 and *Rhizopus arrhizus* DAR 2,062 from SPW have been analysed in detail (7–9). The biomass of both *Aspergillus* and *Rhizopus* contain more than 45% (w/w) crude proteins. The analytical results for crude proteins, metabolizable energy, fat, fibre and other nutrient quality parameters are listed in Table 20.1; and the amino acid compositions of the FBP are listed in Table 20.2. Tables 20.1 and 20.2 indicate that the nutrient quality of the FBP is relatively high. For example, Table 20.2 shows that the fungal biomass contained appreciable quantities of essential amino acids that are clearly superior to those of the FAO reference protein of UN WHO (10),

Table 20.1
Nutrient analysis of *Aspergillus* and *Rhizopus* biomass

Nutrient parameter	Unit	*Aspergillus*	*Rhizopus*
Crude protein (N × 6.25)	%	45.7	49.7
Fat (N/C)	%	1.9	1.1
Total diet fibre	%	16.2	14.6
Available detergent fibre (N/C)	%	5.5	5.7
Metabolizable energy (poultry)	MJ/kg	16.1	16.3
Metabolizable energy Rum (dry)	MJ/kg	19.3	19.5
Ash	%	6.4	5.8
Moisture (N/C)	%	8.4	8.1
Available CHO	%	2.4	6
Total CHO	%	24.3	21.5
In vitro digestibility	%	82.54	84.35
Calcium	%	0.18	0.16
Phosphorus	%	1.59	2.05
Sodium	%	0.42	0.17
Zinc	ppm	50	59
Potassium	%	1.4	0.46

except for amino acids tryptophan and tyrosine, which appear to have slightly lower contents than the FAO references.

2.2. Fungal Biomass Protein Production

The production of FBP from raw materials is a field with the largest volume capacity in modern industrial biotechnology. It is one of the most investigated topics in biotechnology. Microfungi have been used extensively in the fermentation industries as a traditional beverage and for fermented foods in the Orient for more than 2,000 years (3, 11, 12).

Because of the properties of easy harvesting, low nucleic acid content and acceptability as traditional food, filamentous fungi have become more and more attractive in MBP production and biotechnological waste treatment processes. A considerable amount of research is being devoted to the growth of cellulose fungi such as *Trichoderma sp*. on cheap cellulosic materials or waste products. Two microfungi, *Trichoderma viride* and *Geotrichum candidum*, are grown in a submerged culture for 60 h, giving rise to a product containing 20% crude protein and 23% fibre with an in vitro digestibility of about 65% (13, 14). Attempts have also been made to grow filamentous fungi on plant cell biomass. Fungi, including *Botritis cinerea* and *Trichoderma viride*, grow well on waste plant cell biomass as the sole nutrient source (15). *Botritis cinerea*, a plant pathogen, which has a recognised ability to degrade plant cells rapidly, is a particularly suitable fungus for MBP production when grown on waste plant cells. The starch using fungi, such as *Aspergillus niger* or *R. arrhizus*, is hydrolysed to glucose and the protein content increases as the fungus grows (16, 17).

In the Pekilo process, mycelia of the filamentous fungus *Paecilomyces variothi* are continuously cultivated in a medium, which contains dissolved carbohydrates. The yield of

Table 20.2
Amino acid composition (g of amino acid per 16 g nitrogen) of the fungal biomass

Amino acid	*A. oryzae*	*R. arrhizus*	FAO standard
Essential			
Phenylalanine	11.08	9.45	2.8
Tyrosine	2.06	2.28	2.8
Isoleucine	5.45	6.42	4.2
Leucine	8.76	7.85	4.8
Lysine	15.54	17.85	4.2
Methionine	2.45	3.06	2.0
Cysteic acid	2.34	2.09	2.2
Threonine	4.56	5.68	2.8
Valine	4.57	6.24	4.2
Tryptophan	0.67	0.87	1.4
Total essential	57.48	61.79	31.4
Non-essential			
Aspartic acid	5.87	4.82	
Serine	6.25	5.89	
Glutamic acid	9.57	6.78	
Glycine	8.04	7.64	
Alanine	3.57	5.21	
Total non-essential	33.4	30.34	

biomass approached 55% of the reducing substrate consumed a value exceeding that originally anticipated. The dried Pekilo protein is sold to feed compounding mills and has a crude protein content of 52–57% (18). Several fungal processes in a submerged culture for the treatment of starch wastes have been described. Balagopal and Maini (19) grew fungi in a suspension culture containing 25 g/L of cassava starch wastes and found *Aspergillus sp*. NRRL 330 and *Rhizopus sp*. to be superior in terms of mycelial weight and protein production. *Trichoderma harzianum* can be grown in suspensions of cassava meal (4%), and an enriched product with 38% protein from the original cassava containing 2.4% protein can be obtained. *Aspergillus niger* mutants have been applied for increasing the protein content of cassava starch wastes up to 20%. *Penicillium notatum* and *P. digitatum* also grew well on potato processing wastes, resulting in a biomass of 9–24 g/L. Solid-state cultures are of minor importance today, although a number of enzymes are still produced from *Aspergillus*, *Mucor,* or *Rhizopus* species. Fungi can be grown with almost any waste products that contain carbohydrates, such as confectionery and distillery waste, vegetable waste, and wood processing effluents (4, 20–22).

2.3. Fungal Biomass Protein Production from Starch Processing Wastewater

The manufacturing of starch products from wheat, corn and potato uses large quantities of water. The high level of water usage results in the generation of vast quantities of SPW.

Table 20.3
Composition and characteristics of starch processing wastewater

Parameter	Unit	Value range	Typical
Suspended solids	mg/L	3,200–4,650	4,200
Volatile suspended solids	mg/L	1,860–2,300	2,200
Total solids	%	1.86–2.96	2.64
Total insoluble solids	%	1.32–2.15	1.84
Soluble protein	%	0.16–0.39	0.28
Starch	mg/L	2,150–3,140	2,950
Insoluble carbohydrates	mg/L	2,860–3,950	3,560
Total organic carbon (TOC)	mg/L	4,520–7,560	6,840
BOD	mg/L	12,600–16,800	15,400
Soluble COD	mg/L	6,860–11,470	8,960
Total COD	mg/L	16,870–22,800	20,670
Sugars	%	1.24–1.88	1.63
Phosphate	mg/L	92.0–106.0	96.0
Sulphate	mg/L	68.0–83.0	76.0
Total Kjeldahl-N	mg/L	440.0–620.0	605
pH		5.22–5.88	5.75
Temperature	°C	35–41	38

The wastewater streams contain high levels of chemical oxygen demand (COD) and other pollutants, as shown in Table 20.3 for a typical SPW stream (1, 2), which contained an organic loading of 16–22 g/L COD and 2.1–3.1 g/L starch. Therefore, they are highly polluting and can impose heavy loads on the environment or could be expensive in terms of sewer disposal. On the other hand, SPW represents an important energy-rich resource, with a relatively high percentage of carbohydrates, cellulose, protein and plant nutrients (Table 20.3). This resource has been shown to be a suitable substrate for biological conversion to FBP (1, 2, 20).

In addition, starch waste materials also offer the advantages of availability and consistent quality, being a readily convertible material at competitively low costs from which a wide variety of products can be produced. The pH range of SPW (5.2–6.0) and the temperature (around 38°C) of SPW are also highly suitable for fungal cultivation. The low pH range of the SPW is particularly important as this inhibits the growth of contaminating bacteria that may be present in a non-aseptic culture environment.

A system of FBP production and SPW reclamation has been extensively studied (1, 2, 7–9, 15) and is used as a model system here. In this system, SPW with characteristics as listed in Table 20.3 were used as the culture media, and two enzyme producing fungal species of *A. oryzae* DAR 3,699 and *R. arrhizus* DAR 2,062 were used for FBP production. The two fungal species were selected from a group of potential fungal species in a comprehensive screening and selection study and were found to be particularly suitable for FBP production and wastewater reclamation (1). Laboratory testing indicated that they have fast growth kinetics and the biomass produced is readily separated from the liquid phase. The optimal temperature for the cultivation was also determined to be around 35°C.

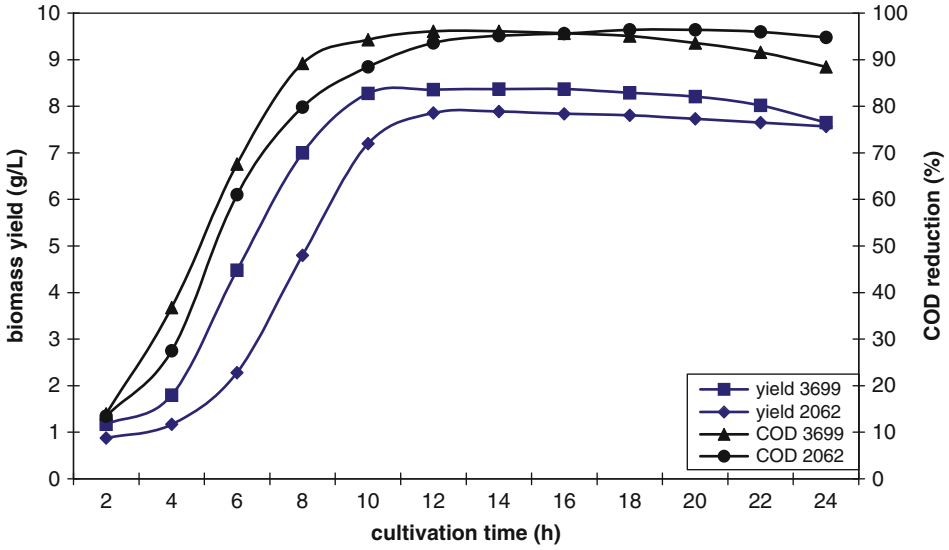


Fig. 20.1. Experimental profiles for fungal biomass yield and COD reduction during the cultivation of *A. oryzae* 3,699 and *R. arrhizus* 2,062 on SPW medium over a period of 24 h. Means of the tested cultures, two analytical replications: fungal biomass yield $1.6\% < \mathrm{SE} < 2.4\%$; COD reduction $1.8\% < \mathrm{SE} < 3.6\%$.

The seed cultures used in this system were prepared in a three-stage process as spore, suspensions and pre-cultures. The strains of the fungal species were maintained on potato dextrose agar (PDA) slants at 4°C and recultured bimonthly. Phialospore suspensions were prepared from PDA slants on Petri dishes. The slants were incubated at 28°C for 4 days. Spores were harvested from the surface of each slant into 10 mL of sterile water. This suspension containing 1×10^7–1×10^8 spores per mL, determined by haemocytometer counts, was used as inoculum.

The fungal biomass growth kinetics of the system is illustrated in Fig. 20.1, in which the amount of biomass produced (biomass yield) are plotted against the batch cultivation time (7, 8), together with corresponding COD reaction during the cultivation process. The biomass growth follows the typical characteristics of microbial growth kinetics. The kinetics profile contains four growth phases. The first phase is the lag phase. In this phase of the first 2 h of cultivation, there is little fungal growth, as the fungal cells grow under an incubation process in the culture medium. After this particular incubation period, the growth rate of the fungal cells increases dramatically, as they enter the exponential growth phase. In Fig. 20.1, the exponential growth phase occurs between 6 and 10 h, and 8 and 12 h for *A. oryzae* 3,699 and *R. arrhizus* 2,062, respectively.

During the exponential growth, the growth kinetics of the biomass can be described by the Monad kinetics as follows:

$$\mu = \frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{\mu_{\max} C_\mathrm{s}}{1 + K_\mathrm{m} C_\mathrm{s}}, \tag{1}$$

where μ is specific growth rate of the biomass, X is the biomass concentration, t is time, C_s is the concentration of the limiting substrate, and μ_{max} and K_m are constants. The specific growth rates of the system in Fig. 20.1 can be estimated to be $0.18\,h^{-1}$ for *A. oryzae* 3,699 and $0.14\,h^{-1}$ for *R. arrhizus* 2,062.

At the end of the exponential growth phase, the maximum fungal biomass concentration is reached. Beyond that, the biomass growth moves into a stationary phase. During the stationary phase, the microfungi are stable in the growth medium for more than 10 h (Fig. 20.1). The last phase is the decline phase or death phase. In this phase, a number of viable fungus cells and autolysis of cells occur. Usually, contamination with other species also occurs, especially due to an increasing pH (higher than 6.5) during the incubation period, which is conducive to bacterium growth competing for the limited nutrients. For the cultivation of the fungal species, as shown in Fig. 20.1, the decline phase starts at around 22 h.

The COD reduction profiles in Fig. 20.1 show that COD reductions of around 90–95% are achieved after the exponential growth phase and maintained during the stationary phase. The COD reductions start to deteriorate at the start of the decline phase, corresponding to the autolysis of the fungal cells, which releases COD into the water phase. It is also noted that, although biomass yield and the COD reduction remained relatively stable, and the variations are relatively small, during the stationary phase, the maximum biomass yields and the maximum COD reduction may not occur at the same time. Therefore, optimal operating conditions for maximum biomass production and those for maximum COD reduction may be slightly different.

Table 20.4 shows the typical water quality parameters of the reclaimed wastewater for the fungal cultivation with SPW (2, 23). Table 20.4 demonstrates a high efficiency of biodegradation and the removal of starch materials with high bioconversion rates. Associated with valuable FBP production, removal of more than 95% organic loading and insoluble solids, and approximately 75% N and P from the SPW are achieved. The reclaimed wastewater contains low organic compounds and very low minerals, and may be used for a number of applications such as farm irrigation.

Table 20.4
Removal efficient and water quality of reclaimed wastewater

Parameter	Removal (%)	Residual
BOD_5	91.8–96.4	550–880 mg/L
COD	88.6–97.5	750–2,100 mg/L
TOC	80.6–85.2	980–1,40 mg/L
Suspended solids	96.8–98.6	70–85 mg/L
Total solids	86.1–93.4	0.18–0.34%
Insoluble solids	92.4–97.5	0.05–0.12%
Total K-N	71.2–76.4	150–180 mg/L
Phosphate	76.8–81.2	25–34 mg/L
pH		6.6–7.8
Total dissolved solids		210–240 mg/L

Another key parameter for microbial cultivation is the conversion yield. For the fungal cultivation with SPW, the COD can be used as the measure of substrate concentration and the conversion yield from COD to biomass can be defined as

$$Y_{\mathrm{COD}/X} - \frac{X}{\Delta \mathrm{COD}} \tag{2}$$

Example 1
From the growth kinetics in Fig. 20.1, estimate (a) the specific growth rate of *A. oryzae* and (b) the COD to biomass yield.

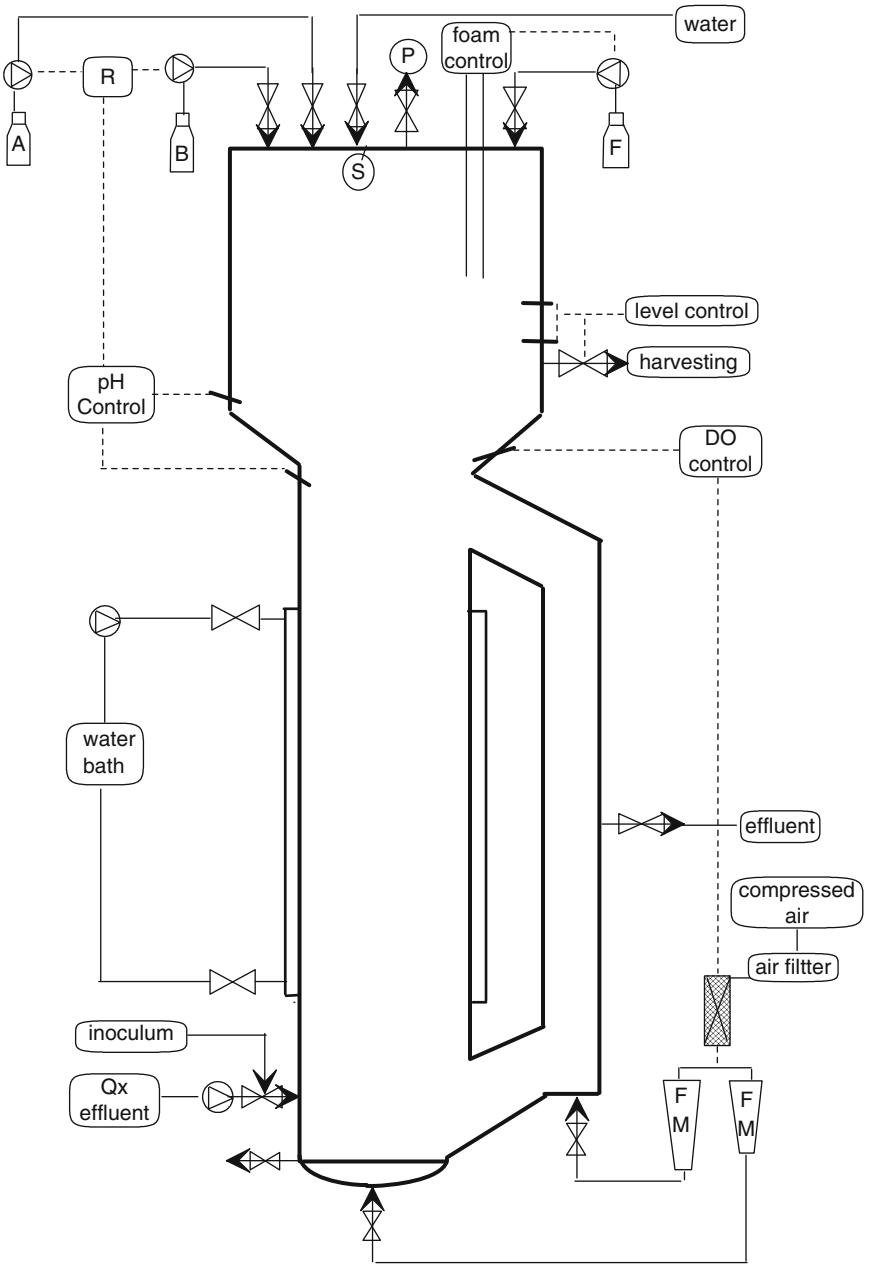
Answer
(a) From Fig. 20.1, at the cultivation time of 8 h, the biomass growth rate can be estimated from the slope of the growth phase to be about 1.25 g/L/h and the biomass concentration is about 7.0 g/L. Therefore, the specific growth rate is 1.25/7.0 = 0.18 L/h. (b) From Fig. 20.1, the maximum biomass concentration is about 8.3 g/L and the corresponding COD reduction is about 96%. The initial COD value of the SPW is 20,670 mg/L = 20.67 g/L. Therefore, the change in COD is $-0.96 \times 20.67 = -19.8$ g/L, and the conversion yield is $-8.3/(-19.8) = 0.42$.

3. REACTOR CONFIGURATION AND PROCESS FLOW DIAGRAM

3.1. Reactor Configuration

The central processing unit in an FBP production plant is the external air-lifted bioreactor (EALB), which is schematically illustrated in Fig. 20.2. The bioreactor has three major sections: a riser (main column in the diagram), a downcomer (side column) and a gas separator (top column), as well as the controlling and monitoring accessaries. At the bottom of the riser, an air sparger is fitted to provide for aeration. The liquid flows upwards in the riser and downwards in the downcomer. The gas flows as bubbles together with the liquid. The gas flow is actually responsible for inducing the circulation flows, which is necessary to provide the mixing and to facilitate the oxygen transfer processes. To further facilitate the oxygen transfer, a second air sparger may also be fitted at the bottom of the downcomer. In this case, the air flow in the downcomer is also upwards, counter-current to the liquid flow. It has been shown that the use of a second air diffuser can significantly increase the oxygen transfer efficiencies (24, 25). The riser provides the main reaction volume and may also be fitted with a water jacket to control the reaction temperature. The gas separator at the top of the reactor is needed to provide the space for gas liquid separation and the mechanism for the removal of products. A micro screen may also be fitted in the gas separator for the separation and recycling of the fungal biomass during continuous cultivations.

In designing the dimensions of an EALB, the ratio between the cross section areas of the downcomer and the riser is a key parameter, which is in the range of 0.3–0.6. Another important ratio is that of length to diameter of the riser and the downcomer (the lengths of the two are about the same), which can be within the range of 6–12. A higher ratio increases the retention times of the air bubbles and hence the oxygen transfer rate, while a lower ratio leads to better mixing characteristics and a lower pressure drops. The diameter of the gas separated



A: acid liquid; B: alkali liquid; F: antifoam liquid;
R: relay; FM: air flow meter; S: spray ball;
Qx: SPW streams P: pressure indicator with gas outlet.

Fig. 20.2. Schematic diagram of external air-lift bioreactor system.

is usually 1.5–2 times of that of the riser to ensure effective gas liquid separation. The height of the gas separator can be in the range of 1–1.5 times of the diameter to provide sufficient space for gas liquid separation and product removal, as well as the accommodation of accessaries.

Example 2
Design the key dimensions of an EALB with a reaction volume of 1,000 l (1.0 m^3), if the area ratio and the length (L) to diameter ratio (d) are to be 0.48 and 8, respectively.

Answer
As the cross sectional area is proportional to the square of the diameter, the diameter ratio between the downcomer and the riser is $0.48^{0.5} = 0.69$. If the diameter of the riser is d, then the total working volume of the reactor is

$$\pi/4 \times (1 + 0.48)d^2 \times 8d = 1.0\,\mathrm{m}^3$$

$$d = 0.48\,\mathrm{m}$$

$$L = 8 \times 0.48 = 3.8\,\mathrm{m}$$

3.2. Process Flow Diagram

A process flow diagram of a pilot plant for the FBP production and wastewater reclamation is illustrated in Fig. 20.3 (7). The pilot plant consists of the cultivation, separation and drying stages. Drum rotary filter with 200 μm stainless steel mesh is used for separating the fungal biomass from cultivated broth. The separated wet biomass is dewatered by belt-pressure filter, and then followed by a flash air drying process to dry the final products. The filtered effluent is collected as the reclaimed water.

In practical operations, there may be two processing options. After a simple filtration, the wet FBP products could be directly supplied to an animal farm due to their appetizing flavours. In this case, the drying process is unnecessary. Another option is to transfer the dewatered product for stockfeed production, which not only reduces the costs of capital and operation but may also produce a new stockfeed with high protein content as well.

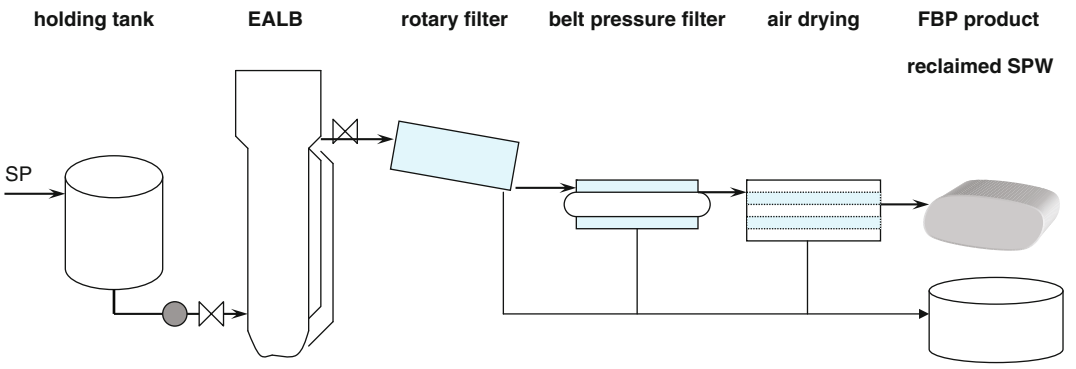


Fig. 20.3. Process scheme for FBP production and wastewater reclamation from starch processing wastewater.

4. OXYGEN TRANSFER AND HYDRODYNAMICS

4.1. Oxygen Transfer

The cultivation of microfungi in EALB is an aerobic process and sufficient oxygen supply in the liquid phase is needed to ensure that the cultivation process is viable. Oxygen supply is usually provided through aeration with air. Therefore, the rate of oxygen transfer from the gas phase to the liquid phase is critical. In fungal cultivation processes, it is usually required that the dissolved oxygen (DO) level in the liquid phase to be at least 50% of the saturation value (24, 25).

The oxygen transfer rate is calculated by the following equation,

$$N_{O_2} = K_L a(\mathrm{DO}^*\text{–DO}) \tag{3}$$

where N_{O_2} is the oxygen transfer rate per unit reactor volume, $K_L a$ is the mass transfer coefficient for oxygen transfer, DO is the dissolved oxygen concentration in the liquid phase and DO* is the saturation oxygen concentration in the liquid phase. The value of DO^* can be obtained from Henry's law and the partial pressure of oxygen in the gas phase.

Example 3
Calculate the unit volume oxygen transfer rate if the oxygen transfer coefficient is 600 L/h. Assume that the DO level in the liquid phase is 0.1 mmol/L and the saturation value is 0.25 mmol/L.

Answer

$$N_{O_2} = 600 \times (0.25 - 0.1) = 90\,\mathrm{mmol/h}.$$

From Eq. (3), the oxygen transfer rate can be increased by the value of (DO^*–DO) (driving force). As a minimum DO level must be kept in the liquid phase in order to maintain a viable cultivation process, the driving force for oxygen transfer may be increased by increasing the partial pressure of the oxygen in the gas phase. For example, instead of atmospheric air supply, pressurized air or pure oxygen can be used as the gas phase.

Example 4
Calculate the oxygen transfer rate if the air pressure is doubled in the previous example. Assume that the oxygen transfer coefficient, the DO level in the liquid phase, remains the same.

Answer
The new oxygen transfer rate is $600 \times (0.25 \times 2 - 0.1) = 240\,\mathrm{mmol/h}$.

In practice, the improvement in oxygen transfer rate by increasing the air pressure will be much less that in the previous example, in the range of 20–40%, as the DO level in the liquid phase will also be higher.

The oxygen mass transfer coefficient is affected by a range of parameters, including the diffusion coefficient of oxygen in the liquid phase, the quantity and size distribution of the air bubbles, which in turn are determined by the rheological properties of the liquid phase, aerator design and the hydrodynamic characteristics (mixing, velocity and gas hold-up) of

Table 20.5
Properties of cultivation broth

Biomass concentration (g/L)	Density (kg/m^3)	Viscosity (Pa s)	Surface tension (N/m)
2.0	1,180	6,340	66,860
4.0	1,470	12,400	46,400
8.0	1,680	18,600	38,200

the reactor system. For a given system, when the reactor design is completed, these effects will be determined by the operational parameters, principally the aeration rate. Therefore, the determination of a proper aeration rate is critical to the operation of an EALB system.

The oxygen transfer coefficient is usually determined through experimentation and correlation, in which the following equation can be used for the cultivation of filamentous fungi (25).

$$K_L a = \frac{G(y_1 - y_2)}{V\left(\frac{P_T y_1}{H} - \mathrm{DO}\right)} \tag{4}$$

where G is molar air flow rate (mol/h), y_1 and y_2 are the oxygen content of inlet and exit air (mol %), V is the liquid phase volume in reactor (l), P_T is the total pressure in system (atm), DO is the dissolved oxygen level in liquid phase (mol/L) measured at top of the riser, and H is Henry's constant (8.345×10^2 l atm/mol).

4.2. Rheological Properties and DO levels

The fluids of mycelial culture contain suspended mycelial particles and exhibits non-Newtonian flow behaviours. This is further complicated by the changes in the concentration of fungal cells during the course of the cultivation. A set of typical properties of the fluids at an operating temperature of 25°C for the model fungal cultivation system are given in Table 20.5, and the profiles of mycelial biomass concentration (broth density), viscosity and DO level in the culture broth during a batch cultivation are shown in Fig. 20.4. The variation of mycelial biomass concentration follows a typical logarithmic growth phase. The rheological characteristics of the cultivated broth become increasingly viscous and non-Newtonian as the biomass concentration increased. It was observed that turbulence in the riser subsides considerably and the bubble size distribution also changes. Even at a relatively low biomass concentration of 2 g/L, large spherical-capped bubbles (4 mm in diameter) become predominant in the riser. As the broth became highly viscous, large bubbles are present in the riser along with some very small bubbles. The large bubbles rise rapidly through the riser and disengage at the top gas separator, while the smaller bubbles remain trapped inside the reactor (26).

In Fig. 20.4, the variations in DO concentrations within the broth have four phases during mycelial biomass growth: a high lag phase, a decrease phase, and increase phase and a low lag phase. During the high lag phase within the first few hours of cultivation, the DO remains at a relatively constantly high level, approaching the saturation, due to no or little oxygen consumption. A rapid decrease phase in DO level is followed during the exponential growth

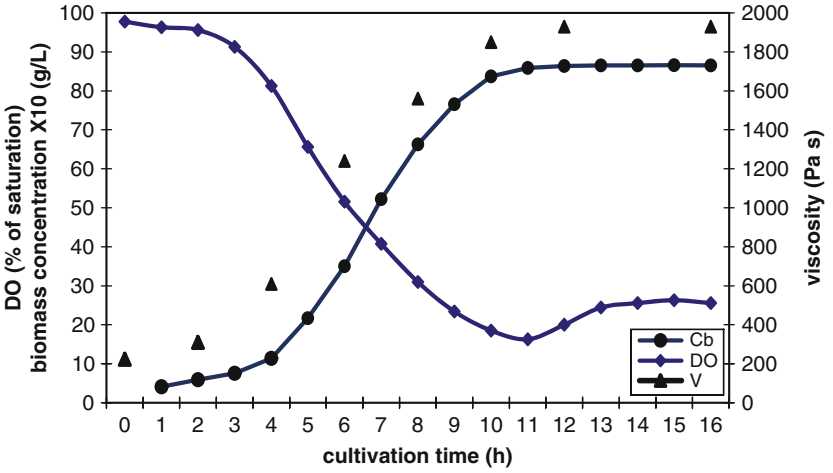


Fig. 20.4. Profiles of mycelial biomass concentration (C_b), viscosity (V) and DO level of culture broth as a function of the cultivation time.

phase of mycelial biomass, as the O_2 uptake by the mycelium is higher than the O_2 transfer into the medium. The DO level increases slowly as the biomass growth shifts to the biomass stationary phase. This DO increase phase, however, extends over a relatively short time. After that, it remains at a constant level. This behaviour reflects the dependence of mycelial biomass growth on sufficient oxygen supply, as mycelium growth limited the O_2 transfer into the cultivated broth.

4.3. Hydrodynamic Characteristics and Oxygen Transfer Coefficient

The gas velocity and the gas hold up are important parameters which affect the oxygen mass transfer coefficient. The measurement of the gas hold-up can be expressed as an average or overall hold-up, and the volume expansion method can be used. It is estimated as the percentage increase in volume of the liquid phase compared with that when there is no aeration in the liquid medium. Figure 20.5 shows the profiles of gas superficial velocity, gas hold-up, and the corresponding oxygen transfer coefficient in a cultivation process of the model system. The gas hold-up decreases significantly with the increase of broth viscosity, but slightly during the saturation phase. This phenomenon has been observed in other non-Newtonian solutions (27, 28). The variations of superficial gas velocity and oxygen transfer coefficient have a similar trend during the cultivation of mycelial biomass. Both decrease with biomass growth, and then remain at a constant value when mycelial biomass is at a stationary growth phase.

4.4. Aeration Rate and Oxygen Transfer Coefficient

As mentioned earlier, the aeration rate is a critical parameter for the hydrodynamic characteristics and oxygen mass transfer coefficient. Therefore, fundamental relationships between the air flow rate and superficial velocity, gas hold-up, oxygen transfer coefficient and DO level in the reactor are needed, and these are usually determined experimentally under different

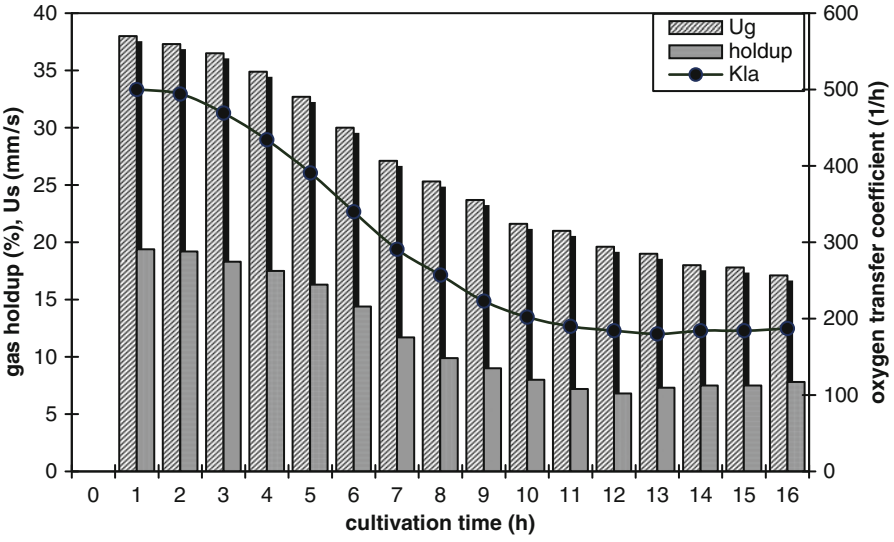


Fig. 20.5. Hydrodynamic profiles of gas hold-up, superficial gas velocity and oxygen transfer coefficient as a function of the cultivation time.

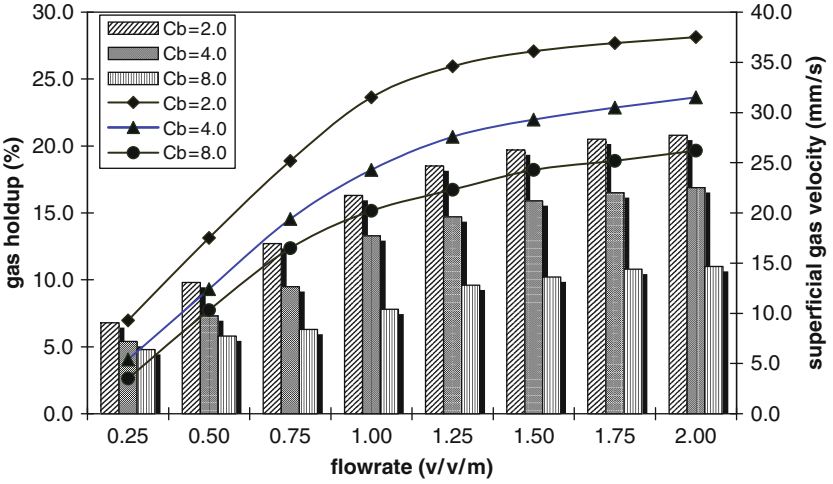


Fig. 20.6. Effect of air flow rate and mycelial biomass concentration (C_b) on gas hold up (*column*) and superficial gas velocity (*line*).

levels of biomass concentration. A set of typical relationships are shown in Figs. 20.6 and 20.7 for the model system. Here, the aeration rate is measured as the relative volume of air to the volume of the liquid phase per unit time.

From the relationships in Fig. 20.6, it can be seen that the superficial gas velocity increases linearly with the air flow rate in the range of 0–1.00 v/v/m, while a further increase in the air

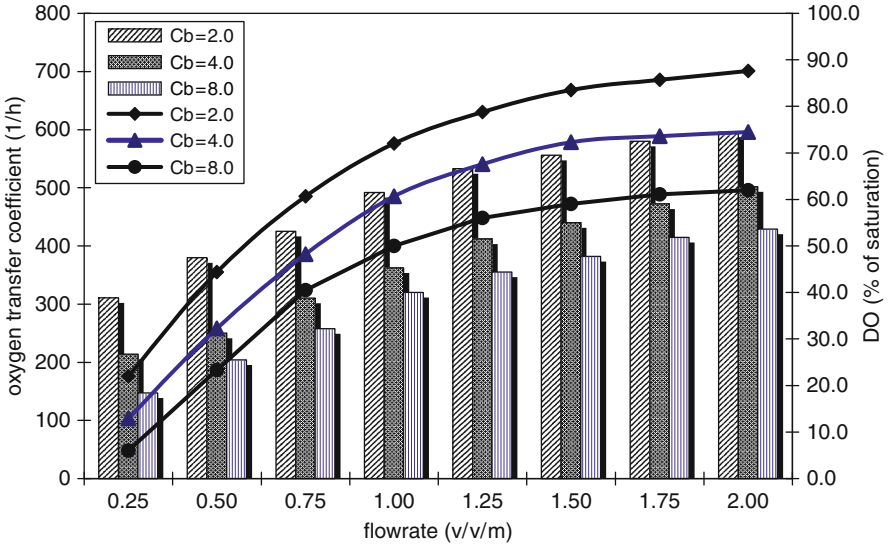


Fig. 20.7. Effect of air flow rate and mycelial biomass concentration (C_b) on oxygen transfer coefficient (*column*) and DO level (*line*).

flow rate leads to a slower increase in gas velocity. The riser superficial gas velocity is usually represented by the aeration flow rate. However, Fig. 20.5 here indicates that the relationship between gas velocity and air flow rate varies with the mycelial biomass density.

From Figs. 20.6 and 20.7, the gas hold-up and the oxygen transfer rate also increases with air flow rate. Similar to the case of gas velocity, in the lower range of air flow rate, the relationships is close to linear, and then the influence of the flow rate becomes less pronounced in an increasing range of 1.25–2.00 v/v/m, when the mycelial biomass is highly concentrated in the bioreactor.

It can also be seen that the DO level can be improved dramatically by increasing the air flow rate, but the increasing rate of DO level decreases as the air flow rate exceeds a critical value (1.250 v/v/m) in the EALB (Fig. 20.7). At this level, the DO saturation rate is approximately 50%. Moreover, the enhancement of the DO level by increasing air flow rate in the cultivated broth with high biomass concentration is limited because of the highly viscous culture broth. For example, in Fig. 20.7, an increase in air flow rate from 1.25 to 2.00 v/v/m increases the DO by approximately 12% of saturation at biomass concentration of 2.0 g/L, by only approximately 8% increase in DO at C_b 4.0 g/L, and a negligible increase in DO at C_b 8.0 g/L. Clearly, an increase in air flow rate at a high concentration of biomass would not achieve a desired DO level to meet sufficient oxygen consumption for mycelial biomass growth.

The fundamental relationships, as shown in Figs. 20.6 and 20.7, can be used to design the aeration requirements for an EALB system. For most systems, the aeration rate can be set at a value at or higher than the critical value for the systems.

Example 5
Design the aeration requirements for a 1,000 L EALB from the fundamental relationships in Figs. 20.6 and 20.7.

Answer
From Figs. 20.6 and 20.7, the critical value of aeration rate is about 1.25 v/v/m. Therefore, the design aeration rate can be set at slight higher at 1.5 v/v/m, and the aeration requirement is determined to be $1.5 \times 1{,}000 = 1{,}500$ L/m.

Example 6
Estimate the oxygen transfer coefficient and the oxygen transfer rate at an air flow rate of 1.5 v/v/m. Assume that the saturation DO is 0.25 mmol/L.

Answer
From Fig. 20.6, the oxygen transfer coefficient ranges from 360 to 540 L/h at various biomass concentrations, and the corresponding saturation DO ranges from 45 to 70%. Therefore, the oxygen transfer rate ranges are as follows:

$$360 \times (0.25 - 0.45 \times 0.25) = 49.5\,\text{mmol/h/L}$$

$$540 \times (0.25 - 0.70 \times 0.25) = 40.5\,\text{mmol/h/L}$$

5. PROCESS DESIGN AND OPERATION

5.1. Batch Process

The EALB can be operated in batch, semi-continuous, and continuous modes of operations. In the batch process, the SPW production medium is inoculated with a small amount of pre-culture, usually in the range of 5–8% (v/v), and the reactor system is operated without influent and effluent water streams. The operating temperature is usually controlled at the optimal cultivation temperature (e.g. 35°C), and the pH of the cultivation medium can be adjusted into the optimal pH range of the culture species (e.g. 5.5–7.0). As the oxygen transfer rates usually decreases during the course of batch operations, the aeration rate is also regulated, typically starting with a lower aeration rate (e.g. 0.6 v/v/m) to a higher aeration rate (e.g. 1.2 v/v/m) to maintain a DO level above 50% of saturation.

In practice, batch cultivation finishes after the stationary phase is reached, either when the maximum biomass production or the maximum COD reduction is achieved. The reactor can then be prepared for the next batch process. Therefore, the operating time of a batch operation consists of both the cultivation time and the preparation time needed for the next batch of cultivation, both of which should be considered in the design of the reactors. The total volume (V_t) of the bioreactors are designed as the product of the volumetric processing rate (F) and the total operating time (t):

$$V_t = Ft \tag{5}$$

Example 7
Design the bioreactor volume required to batch process 1 l/d of SPW for the model system kinetics as given in Fig. 20.1.

Answer
From Fig. 20.1, the stationary phases for the two microfungi are reached around 10–12 h. If the operation time is set as 12 h, the reactor volume required will be

$$V_t = 1 \times 12 = 12 l$$

5.2. Semi-continuous Process

The semi-continuous mode of operation is also known as repeated fed batch process. It is conducted in a similar fashion as in the batch mode of operation. In addition, at the end of the initial cultivation cycle, when the maximum growth of the biomass has been reached, the cultivated broth is withdrawn from the bioreactor at a fixed V_{out}/V_t value (ratio of the volume drawn out to the total culture volume). Afterwards, SPW medium is added into the bioreactor and the cultivated broth remained in the bioreactor serves as the inoculum for the next cycle. A set of typical results obtained from the semi-continuous cultivation of *A. oryzae* 3,699 and *R. arrhizus* 2,062 in SPW are shown in Figs. 20.8–20.10 for three different values of V_{out}/V_t (8).

In the semi-continuous process, the value of the V_{out}/V_t affects the productivity of the bioreactor. A lower V_{out}/V_t value leaves more inoculum in the bioreactor and reduces the

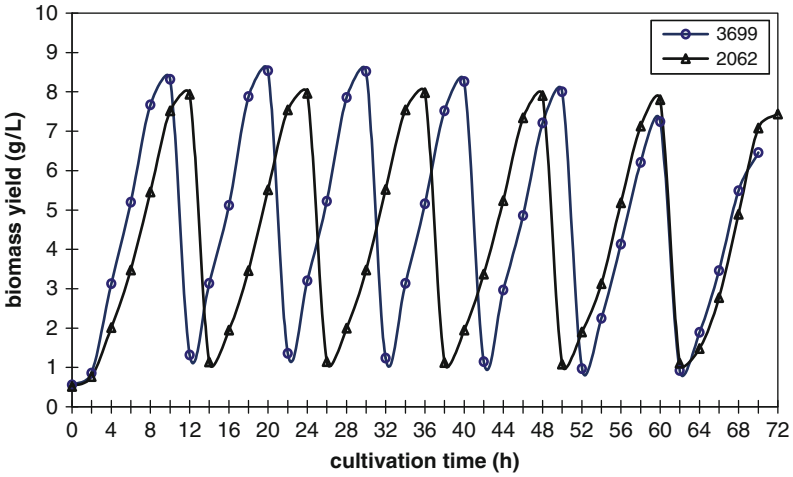


Fig. 20.8. Growth characteristic of the fungal biomass of *A. oryzae* 3,699 and *R. arrhizus* 2,062 during the semi-continuous cultivation at V_{out}/V_t ratio 0.90. Means of the tested cultures, two analytical replications: fungal biomass yield 1.6% < SE < 3.2%.

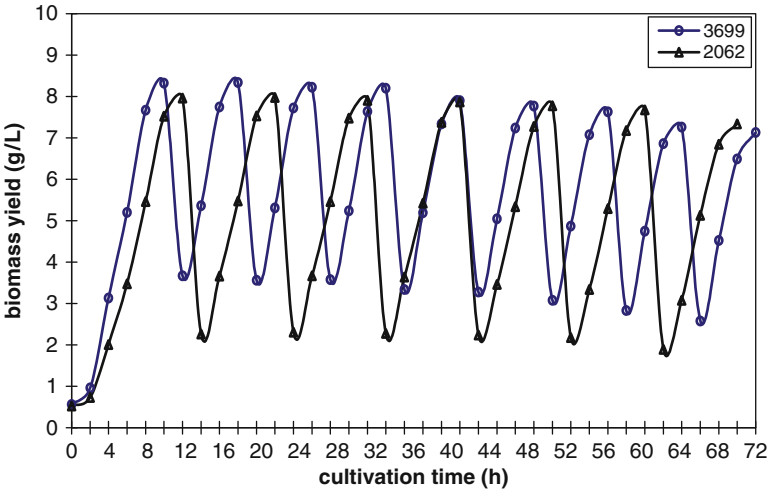


Fig. 20.9. Growth characteristic of the fungal biomass of *A. oryzae* 3,699 and *R. arrhizus* 2,062 during the semi-continuous cultivation at V_{out}/V_t ratio 0.70. Means of the tested cultures, two analytical replications: fungal biomass yield $2.0\% < \text{SE} < 2.8\%$.

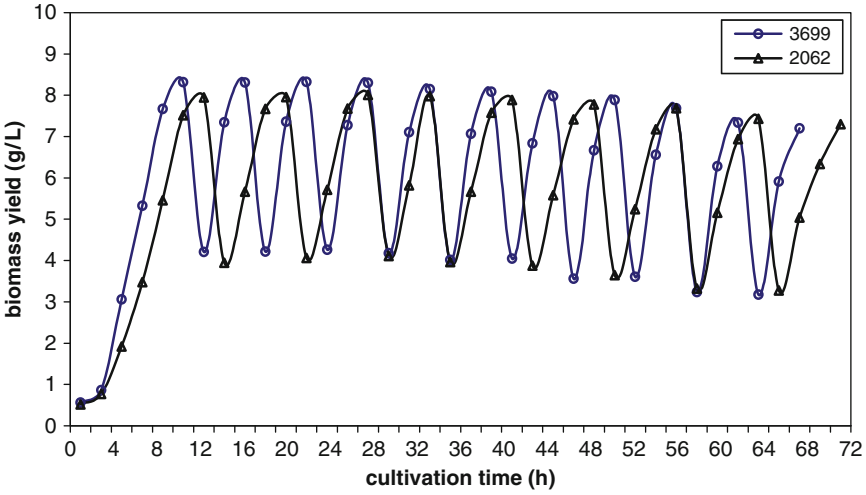


Fig. 20.10. Growth characteristic of the fungal biomass of *A. oryzae* 36,993 and *R. arrhizus* 2,062 during the semi-continuous cultivation at V_{out}/V_t ratio 0.50. Means of the tested cultures, two analytical replications: fungal biomass yield $1.8\% < \text{SE} < 3.4\%$.

shock to the microorganisms. The bioreactor shortens the lag phase needed, which in turn shortens the operation time needed and increases the overall productivity of the bioreactor. On the other hand, a higher V_{out}/V_t value produces more products per cycle and reduces the preparation times needed between the cycles. Typically, a value of 0.5–0.9 is used for EALB.

The design equation for semi-continuous mode is similar to that of the batch operation, except a correction factor of V_{out}/V_t is needed.

$$V_t = Ft/(V_{out}/V_t) \tag{6}$$

where t is the operation time needed for one cycle of cultivation.

Example 8
Design the bioreactor volume required to process 1 l/d of SPW for the system in Fig. 20.9 with Microfungus *A. oryzae* and a V_{out}/V_t value of 0.7.

Answer
From Fig. 20.9, the operation time needed for one cycle of cultivation is 8 h. Therefore,

$$V_t = 1 \times 8/0.7 = 11\,l$$

5.3. Continuous Process

For a large scale process, the continuous operation mode is usually more efficient. However, this mode of cultivation is much more difficult to operate. For example, the biomass may accumulate on the walls and probes inside the culture vessel. Air supply also becomes a problem because the mycelium clogs up the air sparger, which leads to a low level of oxygenation and eventually unstable operations.

A continuous operation is initiated towards the completion of a batch cultivation, starting at the beginning of a stationary phase and then run at fixed liquid and air flow rates. The SPW medium is continuously fed into the bioreactor and the cultivated broth is continuously withdrawn. A recycle stream from the effluent can also be used to increase the stability of the continuous operation. When a steady state is established, the following design equation can be obtained:

$$FX = r_X V_t \tag{7}$$

where X is the biomass concentration and r_X is the biomass growth rate. Equation (7) can be rewritten as

$$F/V_t = r_X/X \tag{8}$$

or

$$D = \mu \tag{9}$$

where D is the dilution rate.

The productivity and the COD reduction rate of the bioreactor is strongly affected by the dilution rate. This can be illustrated as in Figs. 20.11 and 20.12 for the continuous cultivation of *A. oryzae* 3,699 and *R. arrhizus* 2,602 on SPW at dilution rates ranging from 0.06 to 0.20 L/h (7). The results clearly demonstrate that an optimal dilution rate based on either the biomass productivity or COD reduction rate can be obtained. For example, a maximum biomass productivity of 0.92 g/L/h for *A. oryzae* 3,699 and 0.87 g/L/h for *R. arrhizus* 2,062

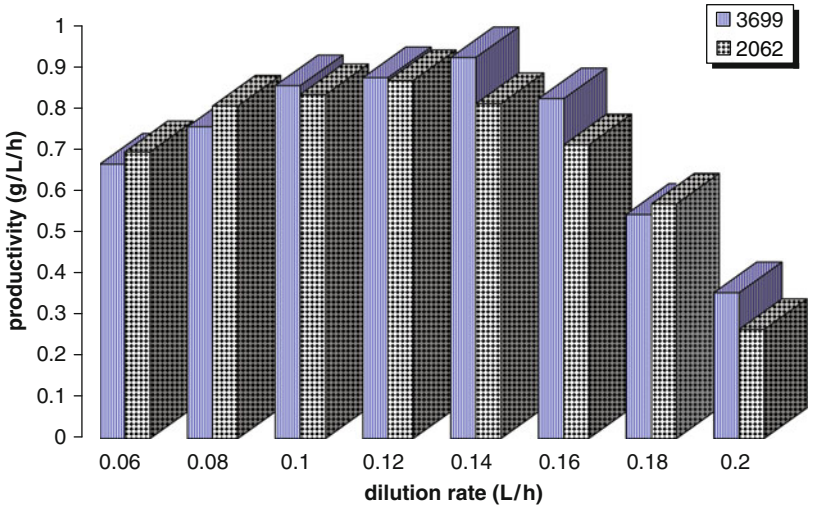


Fig. 20.11. Fungal biomass productivity in the continuous cultivation of *A. oryzae* 3,699 and *R. arrhizus* 2,062 as a function of the dilution rate in the EALB. Means of the two tested cultures, two analytical replications, fungal biomass productivity: $1.8\% < \text{SE} < 3.6\%$.

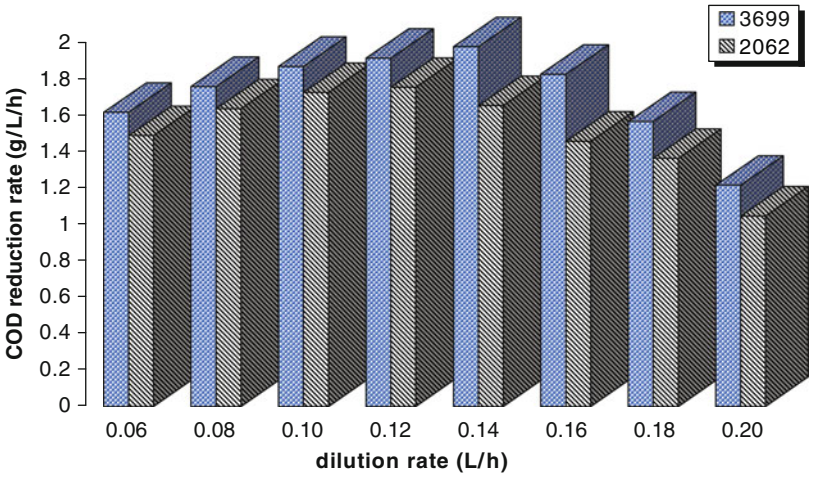


Fig. 20.12. COD reduction rate in the continuous cultivation of *A. oryzae* 3,699 and *R. arrhizus* 2,062 as a function of the dilution rate in the EALB. Means of the two tested cultures, two analytical replications, COD reduction rate: $2.1\% < \text{SE} < 3.8\%$.

are obtained at a dilution rate of 0.14 and 0.12 L/h, respectively. Similarly, from Fig. 20.12, the highest COD reduction rate of 1.91 g/L/h for *A. oryzae* 3,863 is obtained at a dilution rate of 0.14 L/h with 96.5% COD reduction and for *R. arrhizus* 2,062, a maximum COD reduction rate of 1.75 g/L/h with 95.8% COD reduction at a dilution rate of 0.12 L/h.

Example 9
Design the bioreactor volume required to continuously process 1 L/d of SPW for the system in Figs. 20.11 and 20.12 with microfungus *A. oryzae* for maximum biomass production and COD reduction.

Answer
From Figs. 20.11 and 20.12, the optimal dilution rate for both biomass productivity and COD reduction for *R. arrhizus* 2,062 is 0.14 L/h. Therefore, from Eq. (8),

$$F/V_t = 0.14,$$

$$V_t = F/0.14 = 1/0.14 = 7.1\,\text{L}.$$

6. SUMMARY AND CONCLUSIONS

With the development of an EALB system, simultaneous production of fungal biomass protein and wastewater reclamation from starch processing wastewater can be effectively achieved. In particular, microfungi *A. oryzae* and *R. arrhizus* can be successfully cultivated using raw SPW, while treating the SPW. The processes have a number of advantages including (1) a high bioconversion efficiency of starch materials and a short cultivation time, (2) high protein contents of the biomass produced and high nutrient qualities of amino acids that may be safe for human and animal consumption and (3) high COD reduction rates from SPW. The temperature and pH conditions of the SPW are also close to the optimal operating conditions of the microfungal species, and thus the operational costs can be reduced.

In the cultivation of the microfungal species, the oxygen transfer rate and the resulting DO level is critically important. Sufficient aeration rate is needed to maintain the level of DO above 50% of the saturation value. A higher oxygen pressure can be used to increase the oxygen transfer rates in the bioreactor.

The EALB can be operated in batch, semi-continuous and continuous processes. While the continuous process has higher efficiency of operation, a smaller bioreactor can be used and the batch mode of processing offers flexibility and ease of operation. The performance of the semi-continuous process is between the other two modes of operation. The bioreactor volumes can be designed from the fungal growth kinetics and optimal operating conditions obtained from pilot studies, as illustrated by design examples. The fundamentals of fungal biomass can be found from the literature (29–31).

NOMENCLATURE

C_s = Substrate concentration
D = Dilution rate
DO = Dissolved oxygen concentration
DO* = Equilibrium dissolved oxygen concentration
F = Volumetric flow rate
G = Molar air flow rate
H = Henry's constant

y_1 = Inlet oxygen mole fraction
y_2 = Exit oxygen mole fraction
N_{O_2} = Oxygen transfer rate
$K_L a$ = Oxygen mass transfer coefficient
K_m = Monad kinetic constant
P_t = Total pressure
r_X = Biomass growth rate
t = Time
V = Liquid phase volume in reactor
V_{out}/V_t = Ratio of volume withdrawn to total culture volume
V_t = Total reactor volume
X = Biomass concentration
$Y_{COD/X}$ = COD to biomass yield
μ = Biomass specific growth rate
μ_{max} = Maximum biomass specific growth rate

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21
Algae Harvest Energy Conversion

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CONTENTS

Abstract Algae harvest energy conversion to biofuel technology is a promising alternative to fossil fuel that has inherent pollution attachment. With present resources available for the microalgae mass production and hence, high oil yield, microalgal can sufficiently be a new source of renewable energy to replace the fossil fuels. In this chapter, algae description, composition, cultivation, its conversion to biofuel, and commercial prospects and problems are presented.

1. INTRODUCTION

1.1. Algae Description

Algae are microscopic, single-celled plant, growing in an aqueous environment – both fresh water and marine (1). The term microalgae are now being used to cover various oleaginous species. Thus, microalgae are organisms made up of simple cellular structure and a large surface to volume body ratio (2). These comprise of a vast group of photosynthesis and heterotrophic organisms which make use of sunlight as energy source and simple inorganic nutrients like CO_2, soluble nitrogen components, and phosphorus for growth (1).

There are edible as well as poisonous algae. Edible algae found in different locations world wide include *Spirulina*, *Dulse chlorella, Purple laver* (Porphyra), *Chondorus cripus* (Irish moss), *Ulva lactuca*, *Alaria esculenta* and are as such used as food, food supplements, and agents to improve desired quality of some foods and drinks (3–5). Algae are equally excellent

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_21,

sources of inorganic nutrients like potassium; they also serve as fertilizers. Certain microalgae produce hydrogen and oxygen through the process of biophotolysis while others naturally manufacture hydrocarbons (6). Other valuable substances from microalgae include vitamins, color pigment, essential fatty acids and amino acids, pharmaceutically active substance, and other chemicals (7).

Microalgae can be cultivated under aqueous conditions of both freshwater and saltwater. They thrive in moist, black earth in the desert, and in all the contents in-between (6). In general, low rainfall, high temperature, and sunshine hours substantially favor algae growth (2).

1.2. Composition of Algae

Generally, algae biomass contains three main components, which are carbohydrates, protein, and natural oils (8). The percentage composition of algal dry biomass is presented in Table 21.1. Also, Table 21.2 presents the lipid contents of algae.

1.3. Classification of Microalgae

Microalgae are often classified, by the biologists, according to their pigmentation, life cycle, and basic cellular structure. The classes are presented in Table 21.3.

Table 21.1
Percentage composition of algal dry biomass

Carbon	46%
Nitrogen	10%
Phosphates	1%
Others	43%

Source: Ref. (2).

Table 21.2
Lipid content of different algae

Strain	% Liquid (on dry basis)
Scenedesmus sp.	12–40
Chlamydomanas sp.	21
Chlorella sp.	14–22
Spirogyra sp.	11–21
Dunaliella sp.	6–8
Euglena sp.	14–20
Prymnesium sp.	22–38
Porphyridium sp.	9–14
Synechoccus sp.	11

Source: Ref. (6).

Table 21.3
Classification and properties of microalgae

No	Name	Habitat	Occurrence	Stored compound
1	Diatomes (Bacillariophyceae)	Fresh brackish water	Approximately 100,000 species exist	Silica in cell walls. Carbon in form of natural oils or polymer of carbohydrates known as Chyrsolaminarin
2.	Green algae (Chlorophyceae)	Fresh water, e.g., swimming pool	Single cells or colonies	Starch in oil (derivable under certain conditions)
3	Blue-green algae (Cyanophyceae)	Variety of habitat	Approximately 2,000 known species exist	Similar to nitrobacter, helps in fixing nitrogen from the atmosphere
4.	Golden algae (Chrysophyceae)	Fresh water system	Approximately 1,000 species exist	Complex pigment system in varying colors. Store oil and carbohydrates

Source: Ref. (8).

2. CULTIVATION

Advancement in research and development as well as energy issues in terms of security alternative and sustainable sources have been the driving force behind mass production of microalgae. Presence of other components that serve as raw materials for food and chemical industries also encourages its production. Oswelld and Golueke in 1960 (9) first proposed microalgae culture as a source of renewable fuel (10). They described a large-scale systems of algae culture, harvesting of the biomass, and the anaerobic digestion of the algal sludge to produce nutrients that serve as food for the growth of algae and production of biogas that could be used to generate electricity and the flue gas (CO_2) (11). In 1978, Benemann et al. (12) reported a more detailed design and engineering analysis of the algae cultivation, which showed that production of biogas, from algal culture, is favored economically above fossil fuel.

Since early 1980, other research institutes and centers like the US DOE (13) intensified research and development on acceptable commercial production of biogas and use of the algal culture, particularly for fuel and fixation of CO_2, a greenhouse gas (GHG) (14–19). Today in the algae industry, alga culture is growing fast with over 10,000 dry tonnes annually (2).

2.1. Factors Affecting Cultivation

Several factors are considered when cultivating algae, because different algae have different requirements. These factors range from algal strains to weather and culture techniques. Generally, under optimal conditions of sunlight and temperature, average algal biomass products are projected to be as high as $30\,g/m^2/d$ (20).

2.1.1. Algal Strain

Microalgae, like enzymes, are very specific in nature. Specific strains survive in specific conditions. The conditions that favor selection of microalgae culture are importantly equal to those that can withstand the invasion by "weed" algal strain or grazing by zooplankton (2). Current techniques require the development of inoculum production in case the ponds are contaminated. Microalgae, called extremphiles, e.g., Spirulina and Dunaliella, which can survive and thrive well in extreme environment are now being incorporated in current commercial microalgae technology.

2.1.2. CO_2 Enrichment

One of the most essential nutrients needed for survival by microalgae is CO_2, which is being converted to biomass. Algal culture, therefore, need stable supply of concentrated CO_2 that can be sourced from power plant flue gases and other sources. In recent time, microalgae CO_2 bio fixation is given attention not only to achieve biomass production but also to abate GHG. Some power plants in Hawaii are located close to microalgae pond to supply the CO_2 required by the pond through the flue gas generated (10).

2.1.3. Microalgae Physiology

The physiological make up of the algal cells influence the nutritional uptake and the cultivation environment. Naturally, microalgae grow to cover surface of pond or the culture environment, but if the physiology is favorable, it facilitates the production of biomass with high contents of starches or oils (10). However, certain physiological responses are unfavorable to microalgae survival in algae ponds. High concentrations of accumulated O_2 in culture ponds do inhibit the growth of algae (10). Moreover, light saturation is an associated problem with algae physiology. The large amount of chlorophyll and other pigments in algal cell result in cells near the surface of the ponds capturing more photons than their photosynthetic components need, therefore rendering the excess wasted and unavailable to algal cells down the ponds. Thus, recent research and development approaches through physiological and genetic has been to reduce the light harvesting pigment (21).

2.1.4. Sunlight

Microalgae comprise of a vast group of photosynthetic organisms, which require abundant sunlight for photosynthesis. They grow in sheet form over ponds, lakes, or other provided habitat in order to have access to sunlight using the large amount of chlorophyll and other pigment capable of undergoing photosynthetic processes (8).

2.1.5. Habitat

Microalgae can survive in aquatic environment, fresh or mangrove water. However, land is hardly a limitation to algal culture. Earth's vast hydrosphere can be used without competing for the small arable land. Moreover, a commercial practice for economic gains is driving algal culture landwards. The general design of the culture pond commonly used is about 20–40 cm deep earthen ponds mixed with paddle wheels (10). The productivity per area is about five higher as compared with traditional agricultural crops and fast growing "energy crops" (1). Equally, lower quality water like wastewater and energy effluent of biological

waste treatment facilities can be used for growing algae. Thus, the land use demands for microalgae compliment, rather than compete with other biomass-based fuel technologies (8).

2.2. Cultivation System

Algal culture is going commercially worldwide, in order to meet up with the demands of products derivable from microalgae; some of the cultivation systems have passed research and development (R and D) while others are undergoing R and D in order to meet commercial purposes. Though different algae have different requirements, all cultivation systems must attend to the temperature, nutrients, and light demands of the microalgae. Some of these systems are listed below

2.2.1. Open Pond System

The open pond system of microalgae culture is widely applied and has been the birth of algal culture. Here, algae are grown in ponds, lakes, lagoons, and other water entrapment habitats. Commercially, the open pond systems, designed in a "raceway" form are shallow ponds, (Fig. 21.1), which facilitate effective intake of CO_2 dissolved in water (8).

The shallow depth of the ponds keeps the algae exposed to sunlight and at a position that the sunlight can penetrate. Most of the ponds are fitted with paddle wheels, which provide the flow to circulate the algae, water, and nutrients around a racetrack. Paddle wheel-mixing provides a controllable and flexible mixing medium than pumps and also facilitates effective management of the ponds to promote algal cells that tend to flocculate and settle (11).

The pond systems maximize the advantages of nature, particularly, sunshine and warmth, and as such are operated continuously. Nutrients and water are constantly fed into the pond, and algae-containing water is withdrawn simultaneously. *Spirulina* is a common algae grown in this system, particularly, in large raceway type open pond of about 0.4 ha, mixed by paddle wheels. Nutrients, mostly CO_2, purchased from commercial sources are added to the ponds and the mature algae are harvested by fine mesh screens for further processing (20). In a typical

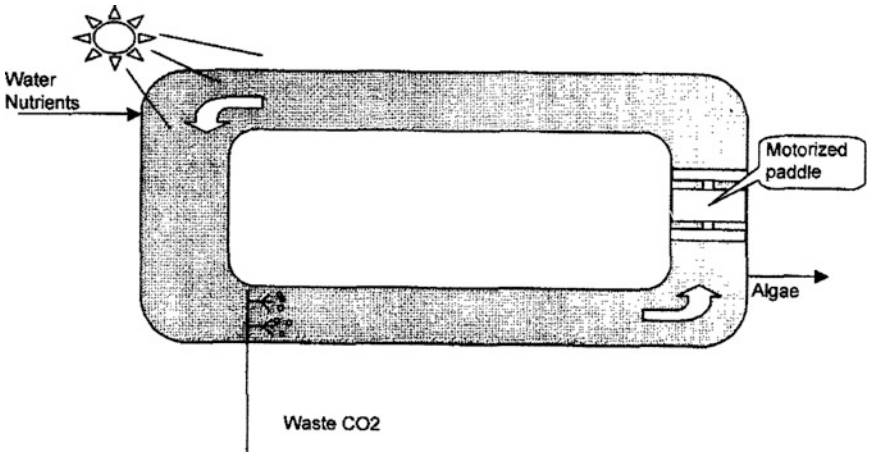

Fig. 21.1. Racepond with paddle. Source: Ref. (8).

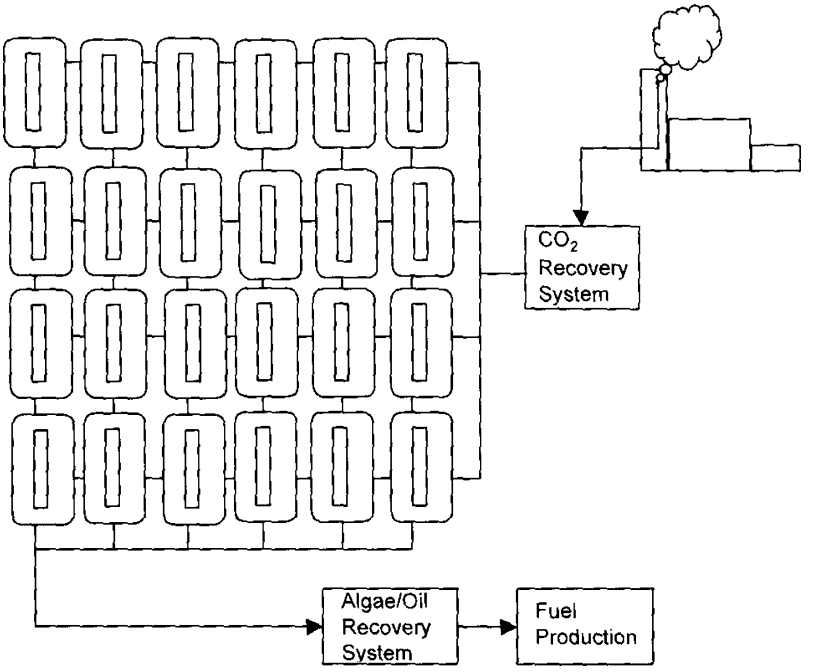

Fig. 21.2. Algae farm. Source: Ref. (8).

Spirulina production facility in Hawaii, a small power plant installed to generate electricity, produces stack gas at about 480°C at 20 scm/min equivalents to 188 kg/h of CO_2, which is transferred to the bottom of a CO_2 absorption tower of 2.4-m diameter and with 6.4-m high packing material (20). Today, for commercial purposes, the open pond systems combined many of such ponds as "algae farm" (Fig. 21.2).

The productivity of these farms is measured in terms of biomass produced per day per unit of available surface area and not volume, since surface area is essential to the algal to capture sunlight. Furthermore, the open system is now being used as GHG abatement (10, 21).

2.2.1.1. Advantages Of Open Pond System

(a) Relatively cheaper to construct, operate, and maintain.
(b) Viable for algae that require extreme conditions to survive, e.g., algae like *Spirulina sp.* and *Dunaliela selina* grow well in water with high concentration of sodium bicarbonate and extremely salty water, respectively.
(c) Open pond system facilitates easy inoculation of new pond with a desired concentration and strain of algae through an outflow pipe. The algae, mostly diatoms, are collected in a "pillow case" of fine mesh cloth through the outflow pipe and are sent into new ponds or used as feed for shrimp larvae.
(d) Open pond system maximizes the use of natural sunlight for light and photosynthesis.

2.2.1.2. Disadvantages Of Open Pond System

(a) Open pond system exhibits higher algae cell densities, which make algae removal or harvesting difficult in some ways.

(b) This system requires relatively large land space as well as water.
(c) This system is vulnerable to contamination or attack by invasive algae species, bacterial, and others.
(d) Water, temperature as well as light conditions of open pond system are very difficult to control.

These largely depend on nature.

(e) CO_2 and other nutrients like N and P must be provided to such quantity required by a designed pond. CO_2 requires careful control of pH and other conditions before it can be introduced.
(f) Accumulation of high concentration of oxygen in culture ponds inhibits the growth of algae that are susceptible to such concentration of oxygen.

2.2.2. Closed System

Close system, as opposed to an open pond system, is a type of microalgal culture system where carbon dioxide, sunlight, and nutrient-rich water are made available and introduced for the survival and growth of typical algae in an enclosed phenomenon. It is commercially referred to as photobioreactor (PBR) which means that source of light is incorporated into the system. An open pond covered with a greenhouse is sometime considered as PBR. At required operating conditions of typical PBR, the excess culture overflows and is harvested. Maximum productivity of a PBR is achieved when the time to exchange one volume is equal to the doubling time of the algae.

Research and development carried out in different research institutes, worldwide, has rolled out improved PBR to overcome one associated short coming or the other. A current commercial application of closed PBRs is in the cultivation of *Haematococcus pluvialis*, unicellular alga, which is a source of expensive pigment astaxanthin used in salmon aquaculture as well as antioxidant in food supplements (20). PBRs vary from simple, externally illuminated glass jars to highly engineered fermenters (6). They include the following:

2.2.2.1. Airlift Bioreactor

This is a type of pneumatic contacting device in which fluid circulation takes place in a defined cycle pattern through channels built specifically for this purpose (Fig. 21.3) (1). The bioreactor of a riser tube, a gas separator and a down comer tube, appear in triangular form. Liquid circulation is achieved by the force developed due to apparent fluid densities between the riser and down comers. This model, developed by Greenfuel Technologies Corporation, a Massachusetts-based research company, employs photomodulation process that rotates the algae in and out of the sunlight. And because of its low level and homogenous distribution of hydrodynamic shear, the reactors have great potential for industry bioprocesses (1).

2.2.2.2. Bubble Column Bioreactor

This is a type of PBR mainly used for inoculum production of special or desired algal strain (22). The bubble column bioreactor system (Fig. 21.4) consists of a cascade-type open cultivation system with a number of basins placed in series. In principle, the system allows large-scale selective cultivation of broad range of algal species. Result of this system used for mono-algal cultivation of *Mondus subterraneous* and *Chlorella fusca* shows a promising industrial application (22). Low maintenance, stable long-term uni-alga production (over

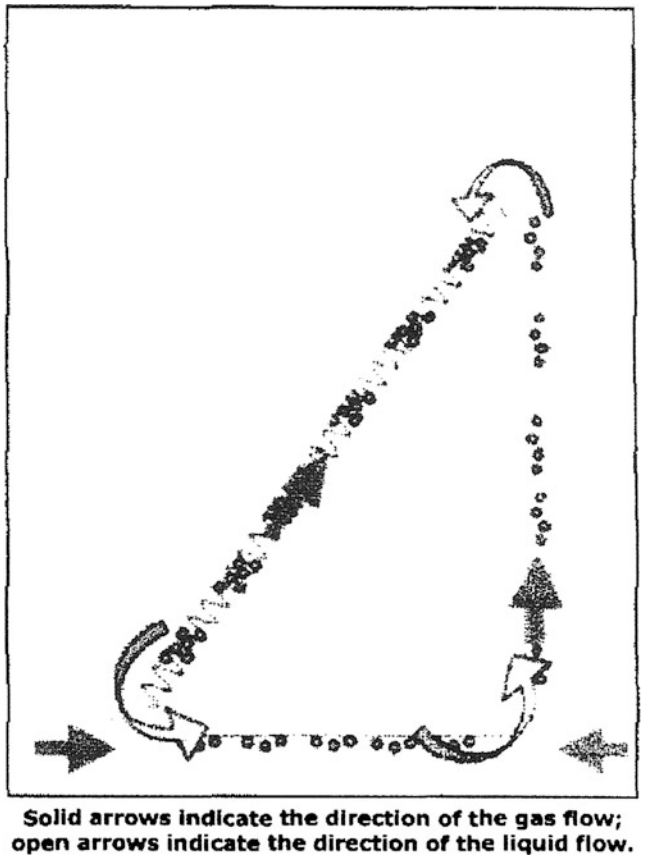

Fig. 21.3. Schematic Diagram of an airlift bioreactor. Source: Ref. (8).

1 year) and yield, which is independent of algae concentrations, are advantages of this model (22).

2.2.2.3. Advantages Of Closed System

(a) The close system PBR produces algae that are generally of higher nutrient than old senescent algae.
(b) This system favors unialgal production in large number over a long time.
(c) Strands under cultivation are protected from invasive strands and contamination by organisms carried in the wind.
(d) Close culture system is independent of all variation in the climatic conditions, such as irregular intensity of sunlight, etc.
(e) The system can be used to culture parent algae or inoculums of open pond systems

2.2.2.4. Disadvantage Of Closed System

(a) Major problem of the PBRs is the distribution of light in the algae culture. The algae located at the surface of the reactor absorbed the light, thereby shielding those few millimeters below in darkness, leading to inhibition in the total algae growth.

Fig. 21.4. Bubble column bioreactor. Source: www.personal.psu.edu/faculty/w/r/wrc2/RESEARCH/bioRxN.

(b) Close system is expensive to maintain and requires some specialties for operations.
(c) This system can only be operated on small-scale units, because they are difficult to maintain in mass cultures.

2.2.3. Semiclosed Systems

This type of algal culture systems combines both the outdoor and indoor (i.e., open and close) factors and variables for the survival and growth of algae. Its design varies, depending on factors set to improve the system, such factors take prominence in the design of the system. They include the following:

2.2.3.1. FLAT-PLATE AIRLIFT REACTOR

This is a few centimeter thick glass-reactor that employs the principle of airlift reactor (ALR). It consists of flat bubble columns with a constant flow of bubbles of air and carbon dioxide. Static mixers located in the stream channel produce flocculation, thereby sending

each alga to the surface of the reactor in order to be exposed to the light for fractions of a second. This "Flashing light effect" is sufficient to produce algae cultures of great density (7).

2.2.3.2. Penthouse-Roof′ Tubular Reactor

This is a closed tubular PBR, often called "penthouse-roof," which consists of solar concentrators mounted in a climate-controlled greenhouse on top of the laboratory complex combining features of indoor and outdoor cultivation units. The dual-purpose system was designed for algae biomass production in temperate climate zones under well-controlled cultivation conditions and for heating service water with surplus solar energy. This technology is developed at Academic and University Centre in Nove Hrady (1).

2.2.3.3. Optical Fiber Reactor

The optical fiber reactor, similar to ALR, is made up of 60 by 120-cm membrane of woven fibers resembling window screens interspersed between glow plates, on which the algae is grown. Fiber optic cables channel sunlight into the glow plates and about 140 cm^3 of CO_2-rich hot flue gas is injected via ducts while water moves by capillary effect to the algae. In this system, the algae use the available carbon dioxide and water, and give off pure oxygen and water vapor. Having grown to maturity, the algae fall to the base of the bioreactor where they are harvested and further processed for onward use, particularly, as feed stock and fertilizers (1).

2.2.3.4. Advantages Of Semiclosed System

(a) The semiclosed bioreactors have good selectivity and process control.
(b) It yields high biomass density and high area productivity. Its photochemical efficiency ranges between 10 and 15% (22).

2.2.3.5. Disadvantages Of Semiclosed System

(a) Like other close system reactors, the semiclose bioreactor is of high cost both in operation and maintenance.
(b) Also the energy demand is relatively high (22).

2.3. Harvesting

Algae harvesting is the process employed for effective removal of algal biomass from both open and closed systems for range of applications that include food, industrial, and agro chemicals, as well as energy and others. The basic principle behind algae harvesting is the use of filter medium, particularly microscreens, to screen out the algae from the culture media. A range of technologies from flotation, concentration to flocculation has been tested.

2.3.1. Flotation

Flotation followed by mechanical dewatering and final sand filtration or membrane filtration shows satisfactory performance with respect to cost and energy use (22). In froth flotation, the water and algae are aerated in froth and the algae are then removed from the water (23).

2.3.2. Flocculation

This involves the use of flocculants on mature algae-laden water, which results in overflow of improved clarity and underflow of higher concentration of sediments. Flocculation

efficiency depends on the time required for flocculation and flow-setting velocities. Chemical flocculants like alum and ferric chloride are used to harvest but it is often too expensive for large operations. Severing or interrupting the supply of CO_2 to an algae culture system causes the algae in the system to flocculate on its own. This process is known as autoflocculation. Spontaneous flocculation of algae cells removed from the mixed pond, followed by settling of the flocs, is observed in some algae culture and is called bioflocculation or microstraining (24). It often results in biomass slurry containing about 5% solids. This harvesting technique is of low cost but has not been tested on a large scale (2).

2.3.3. Centrifugation

The centrifugation process applicable to algae harvesting is the sedimentation type of centrifuge. It facilitates the concentration of the algae in the water by causing them to migrate through the fluid in radial form toward the axis of rotation. It is based on difference of densities between the algae biomass and the liquid. The technique is equally expensive.

Other harvest techniques, such as ultrasound-based techniques, are still undergoing research and development in various research institutes worldwide (25, 26)

3. BIOFUEL FROM ALGAE

Microalgae cell mass contain lipids and hydrocarbons. The lipids are naturally excreted extracellularly into the colony matrix, though the type and concentration are affected by light, temperature, pH, ion concentration, and other environmental factors.

The concentration of hydrocarbons in some algae like *Botryococcus* is 90% of its dry mass and so is the quantity of lipids (6). Heavy type of algae oil can equally be obtained through liquefaction, a thermochemical process, under high temperature and high pressure. Algae oils can equally be extracted through a variety of methods, which include use of solvents, solvent extraction, enzymatic extraction, expeller press, osmotic shock, supercritical fluid, ultrasonic-assisted extraction, and other methods, particularly those required to extract specific types of oil that would undergo further research and development.

Fuel energy is not only sourced from the oil, but also the algae cell can be used to produce biogas (methane), while its dry biomass matter, after extraction of oil and other fine chemicals, can be burnt as fuel for furnace and other related units (11) to produce heat and electricity (19). The production of biofuels through microalgae has not only attended to the quest for renewable energy source, it also has enormous commercial potential due to the growth rates of microalgae (1). Its acceptability will depend on its price being favorable to the prices of the commonly use mineral oil and other biofuel feed stock (2). Greater potential is to combine algae–fuel production system with co-products and processing (Fig. 21.5).

3.1. Biodiesel

Diesel is virtually the most widely used fuel worldwide to drive light and heavy engines domestically and industrially. Recent discoveries have associated the by-product of diesel from these engines with pollution (27–29). This, as well as scientists' search for renewable energy sources has driven research and development to produce diesel without resulting pollution, common to petroleum diesel (30) from bio-based materials. Biodiesel can be defined

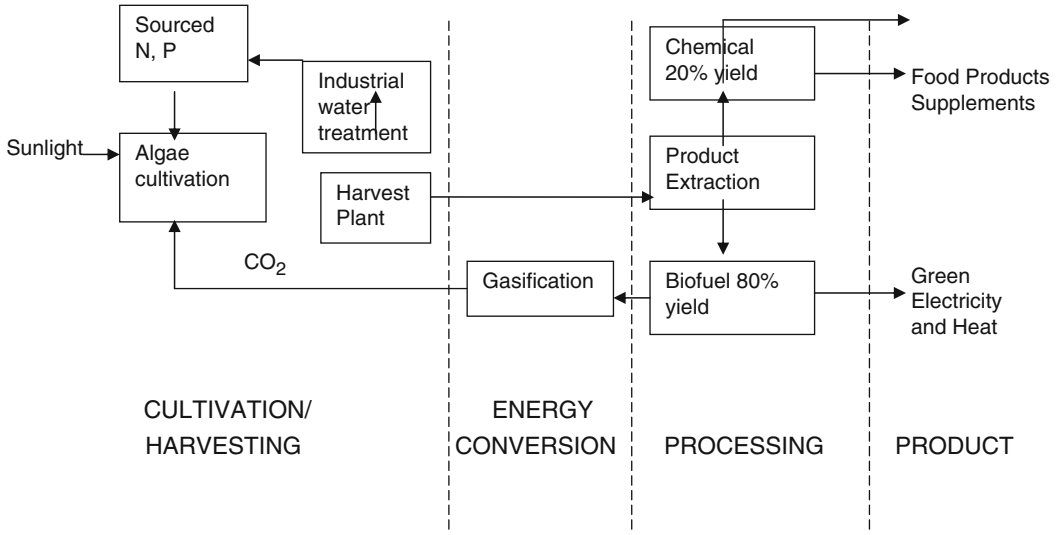

Fig. 21.5. Schematic diagrams for the production of biofuel and fine chemicals from microalgae.

as any biomass-derived diesel fuel substitute. Scientifically, it means specific, chemically modified natural oil, which includes microalgal lipids derived through biological conversion or thermochemical liquefaction of algal biomass (6, 31). Some oilseed crops such as soybeans and rapeseeds, often called "energy crops" have been commercial sources of biodiesel in the United States and Europe. However, alga-based biofuel has emerged as a viable source, which yields about fivefolds higher as compared to these "energy crops" (1). Microalgae systems use far less water than most oilseed crops and land is hardly a limitation to their growth (8).

Triacylglycerols (TAGs) form the bulk of the natural oil found in alga oil and these form about 60% of their body weight (8). TAGs are made up of three long chains of fatty acids attached to the main frame of glycerol. These oils are too viscous for modern diesel engines and must be reduced to ranges of current petroleum diesel (32, 33). This is achieved through "transesterification," a chemical reaction involving TAGs and simple alcohols to produce an alkyl ester commercially known as biodiesel (34), which shares close properties with petroleum diesel fuel.

Biodiesel derived from transesterification process performs as well as petroleum diesel. It reduced emission of gaseous pollutants and particulate matter (27–29) which are toxic and carcinogenic (30). This algae-based biodiesel is biodegradable (35) and as such makes it a promising future fuel (36). Special oil of algae strain, *Botryococcus braunii*, is in focus for biodiesel mass production from microalgae.

Comparative productivity of some crops and microalgal energy worth in Netherlands is given below in Table 21.4.

Unavailability of fast growing algae that are not difficult to culture and harvest and which have high lipid content are the few factors mitigating against mass production from microalgae. Some commercial mass production attempt is going currently in south Africa where about

Table 21.4
Comparative productivity of crop and microalgae energy worth and productivity in The Netherlands

Crop	Productivity ton/ha year	Energy GJth/ha year
Winter wheat (seed + strow)	11	170
Sugar beets (not + leaves)	20	370
Rapeseed	09	180
Willow	10	180
Miscanthus	16	270
Micro algae:	30	600
Current ηPAR5%	60	1,200
Optimixed ηPAR10%		
Theor. Max ηPAR20%	Ca. 120	2,400

Source: Ref. (22).

90 biodiesel reactors with algae as raw materials is expected to produce about 10 million gallons of bio diesel per year (37, 38). Also, "straight vegetable oil" (SVO) is being used in the United State to run modern diesel engines (3).

3.2. Hydrogen Fuel

Hydrogen fuel is the fuel of the future. A fuel cell powered by hydrogen which stores renewable energy is analogous to a personal computer. Hydrogen is stored in the fuel cell and can be converted back to electricity and direct use in transport. The by-product of hydrogen fuel is pure water and heat.

$$2H_2 + O_2 \rightarrow 2H_2O + \text{heat} \tag{1}$$

However, the source of the fuel is equally important to this emerging technology. If sourced from fossil fuel, accompanying pollutants common to fossil fuel and renewability are related problems. Microalgae are photosynthetic organisms and contain some elements like sulfur. However, when microalgae are deprived of sulfur, they switch from production of oxygen, during photosynthesis, to the production of hydrogen with the aid of the enzyme called hydrogenase. (39). A careful fermentation of high starch microalgal biomass can also yield hydrogen (10, 16).

3.3. Biogas

Biogas is a colorless mixture of 60–70% methane (CH_4), 20–30% carbon dioxide (CO_2), and trace amount of hydrogen sulfide (H_2S) and ammonia (NH_4) (40). Biogas is an energy source useful in lighting, heating, and running power generation (11). Biogas can be produced from algal biomass, through anaerobic digestion with the help of some special microorganisms. Algal biomass harvested by a simple flocculation-settling step has been anaerobically digested to produce biogas and CO_2 (11), from concentrated algal sludge.

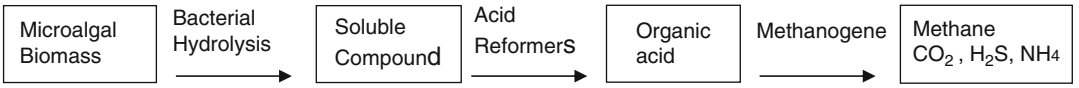

Fig. 21.6. Schematic diagrams of biogas production stages.

Biogas production from biomass consists of three stages: at the first stage, enzymes break the complex polymeric substrates into simpler compounds rendering them soluble. The second stage, anaerobic gasification, involves the digestion of these simpler compounds by a group of acid-forming bacteria to produce organic acids. Methane bacteria, methanogenes, like *methanobacillus, methanococcus, methanospirillum*, and *methosarcina*, finally act on these organic acids to liberate methane and other accompanying gases. Figure 21.6 presents schematic diagram of biogas production stages.

Microalgae biomass contains up to 45% carbon and about 40% of the carbon would be converted to biogas (10). Currently, biogas from algal cells are used to run on-site power generation producing electricity and the flue gas, CO_2, that is recycled to the ponds for microalgae use (24). Equally the nutrients in the digester effluent are used to grow more algae (11).

3.4. Biomass

Harvested microalgae are processed for extraction of oil, fine chemicals, and other nutrients. The residue is fibrous biomass that is often dried and useful in the generation of high-energy biofuel. This can be burnt in the same manner as wood, to produce heat and electricity (22). This has been used in cement industry to fire captive power plants and kilns (1). The combustion products may not be pollutant types associated with combustion of fossil fuel (1). Primary energy in 300 ton of biomass is estimated to be 6600 GJth (22).

3.5. Ethanol

Ethanol is a fuel that can be added, as a blend, in concentrations of 10% without requiring any engine modification. Ethanol, a prominent member of alcohols, is mainly obtained from the fermentation of carbohydrates or carbonaceous materials (1).

4. COMMERCIAL PROSPECTS AND PROBLEMS

4.1. Prospect

Applications of microalgae production in world environmental protection entrench the sustainability of this technology for the future. CO_2, a GHG causing global warming, is one of the essential nutrients for the survival of these microalgae. Consequently, the mass consumption of these gases will help to reduce its menace on world climate. Today, microalgae technology is on the rise (10, 20, 41). Microalgae productions are now adapted to treat municipal and industrial wastewater (42), as well as agricultural drainage water (11). Nutrients for the microalgae are also sourced from sewage (42).

In a new study on the algae biofuels and biomass market, five key strategies emerged as approaches to help producers to reduce costs and accelerate the commercialization of

algae biodiesel, biocrude, and drop in fuels: Fatter, faster, cheaper, easier, and fractionation marketing.

The following summarizes these five key criteria for systems innovations and cost reductions.

4.1.1. Faster

A primary strategy for most algae biofuels producers is to identify algae species that have a high oil content, that will also grow quickly to produce biodiesel, biocrude, and drop-in fuels (43).

It is largely agreed among seasoned practitioners, phycologists, producers, and subject matter experts that algae with high oil content such as *Botryococcus braunii* (Bb) grows slowly and can be harvested only a few times a week, whereas algae with lower oil content such as *Dunaliella* or *Nannochloropsis* (in the 20–40% range) will grow more quickly and can be harvested daily or a few times a day. For this reason, most algae R&D projects and precommercial projects are using algal strains with 20–40% content (43).

4.1.2. Fatter

Algae producers are especially interested in utilizing algal species with a high triglyceride (TAG) oil content for biodiesel and biocrude production. Compared to most algae used today for production with 25% oil content, several scientists and producers are working on identifying species and methods to increase oil content. Most algae systems today can generate from 2,500 gallons up to 5,000 gallons of oil per acre using 30% oil content (43).

If algae producers can utilize fatter algae with 60% oil content, they can help to reduce the size and footprint of algae biofuels production system by as much as half, resulting in significant capital and operating costs for systems twice their size with utilizing algal species with lower oil content. This presents a significant innovation and a welcome improvement for algae producers (43).

4.1.3. Cheaper

Based on the examination of several algae business and economic models, the Algae 2020 study finds the estimated costs to produce algae oils and algae biodiesel today range from $9 to $25 per gallon i ponds, and from $15 to $40 in PBRs today. Reducing these costs are critical for commercial success. An outstanding, significant economic challenge for algae producers is identifying low cost oil extraction and harvesting methods used for algae (43).

A dozen or so companies are now coming up with breakthrough and innovative methods to bring costs down below for extraction and harvesting. Extraction systems can be expensive with estimates up to $15 per gallon of oil produced depending on the extraction method. One company, Missing Link Technology, can extract algae for less than $0.25 per gallon compared to other algae extraction methods, ranging from $2 per gallon up to $12 per gallon (43).

Another example is a harvesting technology from Algae Venture Systems that costs less than $0.30 per gallon of oil produced compared to traditional centrifuge technologies that can cost upwards of $1 or more per gallon of algae oil. Cost reductions in algae production systems are essential for algae producers to establish economically sustainable and profitable enterprises (43).

4.1.4. Easier/Better

The Algae 2020 study has identified that algae producers are now employing far easier and better methods of producing algae for biodiesel, biocrude, and drop-in fuels. Since algae production systems are a complex composite of several subsets of systems (i.e., production, harvesting, extraction, drying, systems), reducing the number of steps and algae biofuels production systems lead to easier, better, and lower-cost systems. For example, OriginOil has developed a technology to combine harvesting and extraction systems into a single process that reduces system complexity and costs for algae producers (43).

Another example is to employ a method that utilizes algae species and cells as mini-processors and refineries in a process referred to as "milking the algae" that will excrete hydrocarbon fuels directly, such as Arizona State's blue-green algae that excrete a kerosene type of jet fuel or Algenol's blue-green algae that consume and excrete ethanol fuel directly. There are also a few species of algae that will naturally excrete oils from the cells (43).

By "milking the algae," these algal microrefineries helps to bypass the harvesting, extraction, and refining systems all together by excreting forms of biofuels directly from the cells. These methods lead to significant cost reductions and help to simplify complex processes for emerging algae producers and customers of new algae biofuels production systems (43).

4.1.5. Coproduct Fraction Marketing Strategies

These are critical to success. Even with algae species with up to 50% oil content, the additional 50% biomass remains. This biomass fraction contains valuable proteins for livestock, poultry and fish feed additives valued from $800 up to $2,500 per ton (43).

Part of the oil fraction, the free fatty acids (FFAs), can produce DHA, Omega 3, and Omega 6 heart-healthy oils, as well as valuable products such as Beta Carotene and other supplements from carotenoids. Other fractions of the algae contain valuable chemicals or molecular compounds that can be used to produce green plastics, green detergents, cleaners, etc that are biodegradable, nontoxic, and can be sold at a premium price over traditional petroleum-based products. The biomass coproduct marketing strategies will be critical to the success for aspiring algae biodiesel and drop-in fuel producers (43).

4.2. Case Study

In Washington State, a study by the University of Washington and the National Oceanic and Atmospheric Administration simulated the impact on coastal counties of Washington State of a yearlong wild algal bloom, which would force the closure of the razor clam season. The study found an impact of $22 million in lost revenue. Algal blooms have caused the loss of 25% of razor clam, and harvesting days have been lost to the blooms, which can spread to cover several square miles of ocean in each event (44).

Companies such as Blue Marble Energy, Aquaflow Bionomic, Bionavitas, among others, have developed large-scale algal harvesting techniques to capture wild algae as a bioremediation step and as feedstock for algal fuel production. For algal build-up in freshwater lakes and ponds, companies such as Parachute Skimmer have developed advanced, manually operated skimmers to collect algae for fuel production (44).

4.3. Problems

Resolution of many workshops on algae harvest energy conversion is that low productivity, high capital intensity in terms of operation and maintenance, respiration, and photoinhibition are few factors militating against viability of microalgae production (20). The factors that most influence costs are biological and not engineering related, thus, projecting costs of biodiesel over the current costs of petroleum-based diesel fuel (8). Field scale testing of algae production systems is still parallel to laboratory tests. Algae species that pass laboratory test have not been promising under conditions encountered in the field (8). Algae harvesting is another technical problem. The most efficient algae harvesting process equipment which is currently available is dissolved air flotation (45).

5. SUMMARY

Algae harvest energy conversion to biofuel technology is a promising alternative to fossil fuel that has inherent pollution attachment. With present resources available for the microalgae mass production and hence, high oil yield, microalgal can, sufficiently, be a new source of renewable energy to replace the fossil fuels. Present stage of microalgae production still requires relatively long-term R&D effort in order to replace fossil fuels in terms of quantity and cost.

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Living Machines

Yung-Tse Hung, Joseph F. Hawumba, and Lawrence K. Wang

CONTENTS

Abstract Ecological studies have revealed that nature has an in-built system to restore itself, thereby sustaining its continuity. In other words, natural ecosystems can act as "Living Machines" in keeping the ecosystems habitable. The biological communities – microbes, plants, and animals – serve as the driving force of several living technological innovations – constructed wetlands, Lake Restores, Eco-Restorers, and Reedbeds. These ecologically based technologies are suitable for environment restoration or mitigation, food production through waste conversions, as well as architecture and landscape design.

1. INTRODUCTION

1.1. Ecological Pollution

Ecosystems comprising estuarine environments, marine shorelines, terrestrial environments, freshwater, groundwater, and wetlands are heavily polluted directly or indirectly by human activities such as mining operations, discharge of industrial wastes, agrochemical usage, and long term applications of urban sewage sludge in agricultural soils, oil spills, vehicles exhausts, and bilge oil as well as anthropogenic organic pollutants. These activities introduce into the various ecosystems a diverse array of pollutants including, heavy

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_22,

metals, volatile organic compounds, nitro-aromatic compounds, phenolic compounds, xenobiotic chemicals (such as polychlorinated biphenyls (PCB) polycyclic aromatic hydrocarbons (PAHs) and pesticides), high nutrient loaded wastewater, and many others (1–7). In the environment, these pollutants pose great health risks to both human and wildlife. The adverse effects of various pollutants depend on their chemical nature and characteristics. For instance, PCBs, PAHs, pesticide residues, owe their toxicity to being recalcitrant, which means they persist in the environment for many years. Organophosphate-based pesticides have been demonstrated to exhibit neurotoxicological properties as well as being associated with the pathology and chromosomal damages associated with bladder cancer (8). Heavy metals, on the other hand, pose the greatest health risk because of the difficulty associated with removal from the environment, which arises from the fact that they cannot be chemically or biologically degraded, making them (heavy metals) ultimately indestructible (2).

When it comes to water, the situation becomes more serious since both the quantity and the quality of fresh water present major problems over much of the world's continents. Fresh water lakes and rivers are polluted by oil spills as well as less satisfactorily treated effluents that come from various processing industries (9). In addition, groundwater pollution is increasingly becoming widespread because of uncontrolled waste deposits, leakages from petrochemical tanks, and continued percolation of untreated sewage, agrochemicals, and other pollutants in the aquifers. Notably, over the last several hundred years, humans have begun living in higher and higher densities, leading to high volumes of sewage output in small geographic areas. This high density of sewage has led to the need to treat the wastewater in order to protect both humans and ecosystem health. Besides, fruits, vegetables, olive oil processing, and fermentation industries also generate solid waste and wastewater, which is nutrient rich. Such wastewater has high biochemical oxygen demand (BOD), (which is a measure of oxygen consumption required by microbial oxidation or readily degradable organic and ammonia), chemical oxygen demand (COD) (9), and is usually acidic (low pH). These wastes often find their way into freshwater bodies (rivers and lakes) where they cause eutrophication (the process of becoming rich in nutrients), which triggers explosive algal blooms. Owing to exhaustion of micronutrients, toxic products, or disease, the algal population eventually crashes. The decomposition of the dead algal biomass by heterotrophic microorganisms exhausts the dissolved oxygen in the water, precipitating extensive fish skills and septic conditions. In some case, eutrophication may not go to this extreme, there are undesirable effects of eutrophication, which may include algal mats, turbid, color water and the shift of fish population from valuable species to less valuable species (10). Besides, it is estimated that between 1.7 and 8.8 million metric tons of oil are released into the world's water every year, of which more than 90% is directly related to human activities including deliberate waste disposal (11, 12). For example, marine oil spills emanating from large scale spill accidents have received great attention because of their catastrophic damage to the environment: (a) the spill of 37,000 metric tons (11 million gallons) of North Sople Crude oil into Prince William Sound, Alaska from the Exxon Valdec in 1989 led to mortality of thousands of seabirds and marine mammals, a significant reduction in population of many inter-tidal and sub-tidal organisms and many long term environmental impacts; (b) minor oil spills and oil contaminations from non-point source discharges (e.g., urban run off and boat

lidge) pollute rivers, lakes and estuaries. As a matter of fact, the US Environmental Protection Agency National Water quality inventory reports non-point source pollution as the Nation's largest source of water quality problem (13, 14), with approximately 40% of surveyed rivers, lakes, and estuaries not clean enough to meet basic uses such as fishing and swimming (12).

1.2. Bioremediation Strategies and Advanced Ecologically Engineered Systems

In order to address these environmental/ecological pollution concerns, several bioremediation (natural or biological remediation approaches) strategies have been devised. For example, to address pollution of the environment by sewage and wastewater, an assortment of technologies, including septic systems in rural areas and sewage treatment plants in urban, has been developed. The purpose of these systems is to remove pathogens, solid waste, and organic carbon from the water. Some also remove nutrients such as nitrogen and phosphorus, which normally cause eutrophication in aquatic systems (42). There are, however, some problems with the current systems for sewage treatment. Septic tanks, in particular, do not effectively remove nutrients, and many larger treatment plants generally rely on chemical treatment to remove some nutrients. Notably, phosphorus removal has largely relied on chemical precipitation. Although nitrogen removal primarily relies on microbiological processes, methanol is often added to stimulate the removal of nitrate. Treatment plants also typically use chemicals such as chlorine or ozone to remove pathogens. Another difficulty of conventional wastewater treatment is the large energy input required. A more fundamental problem with conventional wastewater treatment is its failure to take advantage of the potential resources embodied in wastewater. The nutrients in wastewater are an important resource that is currently going unused. By changing the way wastewater is processed, it is possible to take advantage of these resources (42).

Several biologically-based technological systems, which are currently being developed as alternatives to conventional systems, include the following: (a) the widely studied is the use of natural or constructed wetlands to treat wastes (discussed in Sect. 3.4) and (b) the use of a hybrid between sewage plants and wetlands. The use of a technology based on biological systems: microorganisms and plants (bioremediation/phytoremediation), known as advanced ecologically engineered systems (AEES), is beginning to emerge as promising technology, particularly as a secondary treatment option (12). Specifically, these advanced ecologically engineered systems (AEES) use natural abilities of living organisms to break down macromolecules and metabolize organic nutrients typically found in wastewater and polluted water bodies. The major advantages of using AEES technology are the following: (a) it is less costly, (b) it is less intrusive to the contaminated site, and (c) it is more environmentally benign in terms of its end products (12). However, the choice of any natural bioremediation strategy goes hand in hand with the nature and characteristics of the environment polluted, the nature of the pollutant(s), and the availability of the biological agent(s). It is not the aim of this chapter to exhaust all aspects of application of bioremediation technology. However, this chapter dwells on the application of bioremediation approaches in the remediation of polluted water ecosystems i.e., rivers, lakes, and estuaries.

2. LIVING MACHINES: AS CONCEPT IN BIOREMEDIATION

As already pointed out earlier (Sect. 1), water bodies are on a daily basis being contaminated with waste, and therefore the availability of clean and safe drinking water on earth is continually reducing. Besides, the chemical methods aimed at mitigating the problem, introduce other residual pollutant as a result. On the other hand, bioremediation, which uses biological systems to mitigate the problem, has proven to be a more effective and safe way of restoring the ecosystem to its natural state. Ecological studies have, for long, revealed that nature has an in-built system to restore itself and thereby sustaining its continuity. It is the tilting of the balance in nature that always leads to undesirable consequences. In a typical ecosystem, different populations interact, whereby some of them benefit positively from the interactions while others may be negatively affected by the interactions (10). For example, possible interaction between micro and macro populations can be recognized as negative interactions (competition and amensalism); positive interactions (commensalisms; synergism and mutualism); or interactions that are positive for one but negative for the other population (parasitism and predation).

In simple communities, one or more of the above interactions can be observed. However, in a complex natural biological community, all of these possible interactions will probably occur between different populations concurrently (10). Another important aspect emerging from ecological studies is the observation that positive interactions (cooperation) predominate at low population densities and negative ones (competition) at high population densities. As a result, there is an optimal population density for maximal growth rate (10). In a natural ecosystem a balance always exist whereby different populations interact either positively or negatively until equilibrium is established. In other words, natural population can act as "Living Machines" in keeping the ecosystems habitable by every community member population. Living Machines as concept evolves around the utilization of different biological (microbial, plants and animals) systems to decontaminate the environment of pollutants that are, on a daily basis, released as a result of various human activities. Carefully studied biological systems are selected, and their metabolic and growth requirements are evaluated. Then different community populations that cooperate in their interaction are given particular tasks, after which the product is used by yet another set of cooperative community populations. As the pollutant gets depleted, the populations likewise reduce in sizes. However, the engineered ecosystems (Living Machines) should have systems that reduce the population via the natural food chain. Therefore, instead of population down-sizing through death, prey–predator relations/interactions are introduced. These keep the sizes of the various populations at optimal and thus maintain the performance of the systems. In other words, these systems differ from a typical natural ecosystem in as far combining a variety of natural processes in a structured manner, which artificially accelerate wastewater purification (15). The term Living Machines, describes technologies that employ living organisms of all types and usually housed within a casing or structure made of extremely light-weight materials and powered primarily by sunlight. A typical living Machine comprises a series of tanks or constructed ponds teeming with live plants, trees grasses and algae, koi and gold fish, tiny fresh water shrimps, snails, and a diversity of zooplanktons as well as bacteria (15). In North America, the brothers Eugen

Odum and Howard T Odum laid out the conceptual framework for the practical concepts of ecological designs, and over the last three decades, these concepts have been transformed into part of the Science called "ecological engineering" (16).

Ecological engineering is defined as *the design of sustainable ecosystems that integrate human society with its natural environment for mutual benefit*. It involves creating and restoring sustainable ecosystems that have value to both humans and nature. In so doing, ecological engineering combines basic and applied science for the restoration, design, and construction of aquatic and terrestrial ecosystems. Two major goals are achieved namely (a) the restoration of ecosystems that have been substantially disturbed by human activities such as environmental pollution or land disturbances and (b) the development of new sustainable ecosystems (Living Machines) that have both human and ecological value (17). It is engineering in the sense that it involves the design of the natural environment through quantitative approaches, which rely on basic science, a technology whose primary tool is the self-designing ecosystem, and it is biology and ecology in the sense that the components are all of the biological species of the world (17).

The designing of Living Machines explores the chiefly two of nature's attributes, namely: self-organization and self-designing capacities of ecosystems. Self-design and the related attribute of self-organization are important properties of ecosystems that require clear understanding in the context of creation and restoration of ecosystems. Self-organization, defined as *the property of systems in general to reorganize themselves given an environment that is inherently unstable and non-homogeneous*, is a property that applies very well to ecosystems. This is so because in any ecosystem, species are continually being introduced and deleted, while species interactions, e.g., predation, mutualism, etc., bring about change in dominance, as well as changes in the environment itself. Since ecological engineering often involves the development of new ecosystems as well as the use of pilot-scale models such as mesocosms to test ecosystem behavior, the self-organizing capacity of ecosystems remains an important concept for ecological engineering. Besides, self-organization develops flexible networks with a much higher potential for adaptation to new situations. It is for this reason that it is desirable for solving many of the ecological problems. Therefore, in the construction of Living Machines whereby biological systems are involved, the ability of the ecosystems to change, adapt, and grow according to forcing functions and internal feedbacks is most important (17).

On the other hand, self-design, which is defined as the *application of self-organization in the design of ecosystems*, ensures the continual presence and survival of species in ecosystems after their introduction by nature or humans. As a matter of fact, self-design is an ecosystem's function in which the chance introduction of species ensures continuous sustainability of the system. The ecologically engineered system may be further augmented by multiple seeding of species, which would speed the selection process during the process of self-organization (18). In the context of ecosystem development, self-design means that if an ecosystem is open to allow "seeding" of enough species and their propagation through human or natural means, the system itself will optimize its design by selecting for the assemblage of plants, microbes, and animals that is best adapted for existing conditions. The ecosystem then "designs a mix of man-made and ecological components in a pattern that maximizes performance, owing to

its ability to reinforce the strongest of alternative pathways that are provided by the variety of species and human initiatives" (18).

By applying these biological systems as the driving force, several living technological innovations have been designed (16). Living Machines or AEES are primarily designed either as tank-based systems for treatment of point-source waste or floating systems placed on existing bodies of water that receive non-point source pollution (16). Besides, ecological technologies are also useful in food production through waste conversions, architecture and landscape design, and environmental protection and restoration. It is thus clear that this technology is very advantageous to the conventional pollution management technologies.

2.1. Advantages of Living Machines

Living Machines technology offer a number of advantages over conventional treatment processes:

(a) Living Machines use no chemicals and are thus less costly than conventional treatment plants. For example, in northern climates, some lagoon systems freeze over, making it necessary to find extensive storage space for waste water until the warm discharge season. However, Living Machines can be small enough to be placed in a green house near the source of the pollutant (sewage, waste water from processing industries, Agro waste water etc.) for year – round treatment. Besides, the constant supply of treated effluent water is of such a high standard that it can be used for horticulture and aquaculture production in addition to being recycled to non-potable use such as toilets (15).

(b) Living Machines have sensitive response systems. As such, a sudden influx of toxic pollutants, for example, is quickly obvious when snails move out of the water onto branches of leaves. In a conventional system, it can take days to chemically measure toxicity. The levels of other indicators such as acidity can be determined by the color of the tails of certain species of fish. If these are integral part of the system, it saves both time and money (15).

(c) Owing to their cleanliness and lack of odor, Living Machines may be integrated into buildings, providing an aesthetic dimension while at the sometime reducing energy requirements. Consequently, they are amenable to various designs that not only provide a quality-working environment, but are also an attraction to visitors (15). Such systems serve as direct examples of human processes that are harmonious and symbiotic with natural systems.

(d) They are easy to operate and maintain; that is, the caring for a Living Machine such as a Restorer is less labor intensive since the operator works with living and growing ecologies, other than with bags or tones of chemicals (15, 19).

(e) Living Machines are capable of absorbing or resisting "shock loads" in the waste stream. They owe this capacity to the fact that they are natural and biologically diverse systems, yet they are also mechanically simple. Typical examples are the Lake restorers. Restorer Technology is borrowed from an analogous component in nature called the floating island. Like the floating islands, restorers are an assembly of engineered ecologies incorporated into floating rafts. As the storm blow on the lake, the "Island" or Restorer migrate around with the changing wind. As this is done, the diverse ecologies of plants micro and macro organisms decontaminate the lake thereby restoring the water back to acceptable health standards. In doing all this, any shock load is being resisted (15).

(f) These systems are modular, and can be made in various designs to meet the needs of a growing business or community. This means that the operations and efficiency of the Living Machines can be easily enhanced and improved without excessive costs involved. New Living Machines are

already a third smaller than earlier. As the systems are refined and in some cases miniaturized, it will be possible to integrate them in different ways to support human population without destroying the rest of nature (15).

(g) Since most ecosystems are primarily solar-powered systems, they are self-sustaining. Therefore, once an ecosystem (Living Machine) is constructed, it is able to sustain itself indefinitely through self-design with only a modest amount of intervention.

(h) Living Machines have the ability to self-design. The engineer provides the containment vessels that enclose the Living Machine and then seed them with diverse organisms from specific environments. Within the Living Machine, the organisms self-design the internal ecology in relation to their prescribed tasks and the energy and nutrient streams to which they are exposed (19).

(i) Living Machines have the ability to self-replicate through reproduction by the vast majority of the organisms within the system. This means that, in theory at least, Living Machines can be designed to operate for centuries or even millennia. In Living Machines, the intelligence of nature is reapplied to human ends. They are both garden and machines (19).

2.2. Limitations of Living Machines

In as much as Living Machines offer such versatile advantages, they are not without limitations:

(a) The reliance of Living Machines on solar-power means that a large part of land or water is needed. Therefore, if property purchase (which is, in a way, the purchase of solar energy) is involved in regions where land prices are high, then ecological engineering approaches may not be feasible (18).

(b) Sometimes the species available may not be efficient in degrading very toxic and persistent: recalcitrant wastes. This may result in the persistence of such waste and as a result pollution of such habitats and accompanying health impacts to flora and fauna persists.

(c) Inasmuch as the Natural system is desirable, in some instances, the rate of inflow is so high that it over-shoots the natural rates of removal of the pollutants. This means that a longer residence time may be required to give Nature ample time to do the task. Accordingly, a large piece of land may be required to set up the Living Machine, which may not always be available.

3. COMPONENTS OF THE LIVING MACHINES

3.1. Microbial Communities

The notion that microbial communities are the foundation of Living Machines is obvious. What is less obvious is the diversity in communities of micro-organisms required, if the potential of ecological engineering is to be optimized. On the one hand, bacteria are considered as ubiquitous organisms that organize life on the planet. This is suggested to be through organization, not as distinct species as is conventionally understood in biology, but as unitary society of organisms with no analogous counterparts among other living organisms (20). On the other hand, microbiology maintains that bacteria species have highly specific nutritional and environmental requirements, and the ubiquity principle, which may work over long-term time frames, is inappropriate to the design of Living Technologies (20, 21). In waste or intensive aquaculture, for example, if conditions are not right for nitrifying bacteria, e.g., not enough calcium carbonate as a carbon source, then Nitrosomonas and Nitrobacter will functionally disappear from the system. The only quick way to re-establish

nitrification is through correcting the calcium-carbonate deficiency and re-inoculating the system with culture of appropriate bacteria. For their application in the design of Living technologies, bacterial communities remain a vital component, but unfortunately they largely remain unexplored. Although some 10,000 species have been named and described, and many important reactions characterized, the natural history and ecology of these bacterial species have been little studied, and therefore their distribution and numbers remain obscure (20, 22). Despite this limitation, the use of micro-organisms in designing Living technologies has proceeded in earnest. In their work with the system to degrade coal tar derivatives (PAHs), Margulis and Schwartz (22), inoculated the treatment systems with microbial communities from such diverse locations as salt marshes, sewage plants, and rotting railroad ties; nucleated algae, water molds, slime molds, slime nets, and protozoa. While the bacterial communities provide a diverse array of metabolic pathways for the degradation of the pollutants, the less diverse metabolically than bacteria such as the nucleated algae, water molds, slime molds, slime nets and protozoa are important for the efficiency of the system owing to their exceptionally diverse life histories and nutritional habits. For example, it has been shown that protozoans are important in removing coliform bacteria and pathogens from sewage as well as moribund bacteria thus improving the systems' efficiencies, while fungi are key decomposers in ecological systems (20). Currently, the microbial communities are estimated to comprise about 100,000 species, many capable of excreting powerful enzymes from various metabolic pathways. Such heterogeneous microbial communities are efficient in the removal of organic matter from wastewater (20). Fungi, however, tend to dominate in low pH and terrestrial soils than in aquatic environments. It may, therefore, be important that Living Technologies should incorporate soil-based acid sites linked to the main process cycles into their design.

3.2. Macro-bio Communities (Animal Diversity)

The macro-bio communities comprising various animal species are the regulators, control agents, and internal designers of ecosystems. Unfortunately, they are often little appreciated organisms. It has long been recognized that organisms from every phylogenetic level have a role in the design of Living Technologies and in the reversal of pollution and environmental destruction. For this reason, a search of the vast repository of life forms for species useful to ecological engineers is needed. Odum (23) empathized the need to find control species, meaning those organisms capable of directing living processes toward such useful end points including foods, fuels, waste recovery, and environmental repair. The potential contributions of animals to Living Technologies are therefore remarkable, yet their study has been badly neglected in Biology of Wastewater Treatment. For example, mollusks are not mentioned (24) and in the two volume ecological Aspects of Used Water Treatment, snails are mentioned only once and referred to as nuisance organisms (25, 26). It has now been found that snails play a central to the functioning of Living Technologies. As a matter of fact pulmonate snails, including members of the families; *Physidae*, *Lymnaeidae,* and *Planorbidae*, feed on the slime and sludge communities. Snails also play a dominant role in sludge reduction, tank maintenance, and ecological fluidized bed and marsh cleaning. Ram's horn snails of the family Planorbidae, for example, graze and control filamentous algae mats that would otherwise clog and reduce the effectiveness of the diverse fluidized bed communities. Needless

to say, that some snails digest recalcitrant compounds. The salt marsh periwinkle, *Littorina irrorata*, produces enzymes that attack cellulose, pectin, xylan, bean gum, major polysaccharide classes, algae, fungi, and animal tissues as well as 19 other enzymes interactive with carbohydrates, lipids, and peptides (27). Besides, snails can function as alarms in the Living Machines treating sewage. When a toxic load enters the Providence sewage treatment system, for example, the snails quickly leave the water column and move into the moist lower leaves of the floating plants above the water. Observing this behavior the operator then increases the rate of recycling clean water back upstream into the first cells. Consequently, performance losses are minimized due to the rapid behavioral response of these animals (20).

Virtually all phyla of animals in aquatic environments feed through some filtration mechanism. Bivalves, algivorous fish, zooplankton, protists rotifers, insect larvae, sponges, and others are in this functional category (20). They remove particles of approximately 0.1–50 μm from the water column. Bivalves are significant filterers. For example, Mussels can retain suspended bacteria smaller than 1 μm. Efficiencies may reach 100% for particles larger than 4 μm (28). Individual freshwater clams of the genera Unio and Anodonta filter up to 40 L/day of water, extracting colloidal materials and other suspended organic and inorganic particles. Removal rates 99.5% may be achieved (29). Zooplankton such a micro-crustaceans, on the other hand, can be employed to good effect in applied mesocosms. They feed upon particles 25 μm and smaller and their juvenile stages graze on sub μm sized particles. Since they can exchange the volume of a natural body of water several times per day it is difficult to overstate their importance in ecological engineering (20). In cells within the Living Machines, where fish predators are absent, their numbers are prodigious. Insects play pivotal roles in Living Technologies. Removed from predators in ecologically engineered systems, they proliferate and impact significantly on the water. For instance, chironomid larvae, which feed on sewage, may in turn be feed to fish with water quality improvement was an additional benefit (20).

Vertebrates play key roles in the functioning of Living Technologies. With an estimated 22,000 species, fishes are the most numerous and diverse of the vertebrates. In diet, behavior, habitat, and function, fishes are extraordinarily diverse. Filter and detritus feeding fish are common to all the continents. The filtration rate of algivorous fish may be five orders of magnitude greater than their volume every day (20). In theory, it is possible for the total volume of a fishpond to pass through algae-filtering fish on a daily basis. There are edible fish species like the Central American Characin, *Brycon guatemalensis*, which are capable of shredding and ingesting tough and woody materials. Members of the South American armored catfish family *Plecostomidae* may be used to control sludge build up in waste treatment, and as well as food in culture Living Technologies. Tilapia, *Oreochromis* spp., may be used to harvest small plants like duckweed and aquatic ferns. In several Living Machines minnows, including the golden shiner, *Notemigonus crysoleucas*, and fathead minnow, *Pimephales promelas* feed on organic debris and rotting aquatic vegetation. They breed among rafted higher plants grown on the surface of the water. Excess minnows may be sold as bait fish. Therefore research into the aquarium and ichthyologic literature will be valuable to ecological engineers (20).

3.3. Photosynthetic Communities

Ecological engineering was founded on recognition of the role of sunlight and photosynthesis. By way of contrast, algae and higher plants are seen in civil engineering as nuisance organisms to be eliminated physically and chemically from the treatment process. Contemporary intensive aquaculture takes a similar view. The ecosystem-based solar aquaculture developed at the New Alchemy Institute in the 1970s and its successors constitute an exception to this trend (20). Algae-based waste treatment systems were pioneered by Oswald (1988), Lincoln, and Earle (1990) in the US, Fallowfield; Garrett (1985) in the UK; Shelef et al. (1980) in Israel, and a host of scientists in China and India (Ghosh 1991). In these systems floating higher aquatic plants are used in a variety of waste treatment approaches. For instance, the use of emergent marsh plants and engineered marsh-based systems for waste treatment has gained prominence and technical sophistication over the last few decades. Notably, employing plant diversity can produce Living Technologies that require less energy, aeration, and chemical management. Root zones are superb micro-sites for bacterial communities. There have been, for instance, observed enhanced nitrification in treatment cells covered with pennywort, *Hydrocotyle umbellata*, and water hyacinth, *Eichhornia crassipes*, as compared with comparable cells devoid of higher plants. Some plants sequester heavy metals. One such species of mustard, *Brassica juncea*, has been found to remove metals from flowing waste streams, and accumulating up to 60% of its dry weight as lead. Metals can subsequently be recovered from harvested, dried, and burned plants. Apart from metal sequestering, certain species of higher plants such as *Mentha aquatica* produce anti-microbial compounds or antibiotics that may kill certain human pathogens. Such plants are vital as components of the Living technology design. Besides pollution reductions or mitigations, there is economic potential of plants from Living Machines. Flowers, medical herbs, and trees used in rhizofiltration in a waste treatment facility may subsequently be sold as byproducts. For example, the Frederick, Maryland Living Machine sewage treatment facility produces horticultural crops for the water gardening industry (20).

3.4. Nutrient and Micro-nutrient Reservoirs

Carbon/Nitrogen/Phosphorus ratios need to be regulated and maintained. A full complement of macro and trace elements needs to be in the system so that complex food matrices can be established and allowed to "explore" a variety of successive strategies over time. This will support biological diversity. In designing Living Machines, mineral diversity should include igneous, sedimentary, and metamorphic rocks. With a rich mineral base they should support a wide variety of biological combinations and give the systems greater capacity to self-design and optimize. While mineral diversity provides the long-term foundation for nutrient diversity, in the near term microorganisms and plants require nutrients in an available form. If carbon is recalcitrant, or phosphorus in n insoluble state, or the NPK ratios are out of balance, or trace elements are missing, the ecosystems can become impoverished. There should, therefore, be a system to replenish the Living Machine of its vital nutrients. As a general rule, it is preferable that use is made of organic and rock-based amendments to correct imbalances and kelp meal for trace minerals and potassium (20).

4. TYPES OF LIVING MACHINES OR RESTORERS

4.1. Constructed Wetlands

Natural wetland systems have often been described as the "earth's kidneys" because they filter pollutants from water that flows through on its way to receiving lakes, streams, and oceans. For the reason that these systems can improve water quality, engineers and scientists construct systems that replicate the functions of natural wetlands. Constructed wetlands are accordingly defined as treatment systems or Living Machines that use natural processes involving wetland vegetation, soils, and their associated microbial assemblages to improve water quality (30). The concept of using constructed wetlands for the treatment of wastewater has evolved from years of observing the high water quality inherent to natural wetlands, despite contaminated effluent. This natural process has been simulated in constructed wetlands, which are designed to take advantage of many of the same processes that occur in natural wetlands, but accomplish them within a more controlled environment. Some of these systems have been designed and operated with the sole purpose of treating wastewater, while others have been implemented with multiple-use objectives in mind, such as using treated wastewater effluent as a water source for the creation and restoration of wetland habitat for wildlife use and environmental enhancement. Moreover, constructed wetlands also control pollutants in surface runoff, create wildlife habitat, and add aesthetic value (30, 32).

In general, these systems should be engineered and constructed in uplands and outside floodplains in order to avoid damage to natural wetlands and other aquatic resources, unless the source water can be used to restore a degraded or former wetland. The degree of wildlife habitat provided by constructed treatment wetlands, or sections of these wetlands, varies broadly across a spectrum. At one end of the spectrum are those systems that are intended only to provide treatment for an effluent or other water source, in order to meet the requirements of the Clean Water Act (CWA), and these provide little or no wildlife habitat. At the other end are those systems that are intended to provide water reuse, wildlife habitat, and public use, while also providing a final polishing function for a pretreated effluent or other water source. By harnessing and encouraging the complex ecologies present in these natural treatment systems, constructed wetlands can provide basic or advanced treatment for organic nutrient loads (30, 31). There are many advantages of using constructed wetlands in treatment of water pollution. (a) Constructed wetlands provide simple, low energy, low-maintenance alternatives to conventional treatment methods. Accordingly, constructed wetlands can be integrated into a complete system including pretreatment, disinfection, and re-use. Options for re-use include subsurface irrigation, wash-down water, toilet flushing, and industrial use. Besides, constructed wetlands can stand-alone or function as an upgrade to conventional systems, (b) constructed wetlands reduce residual wastewater sludges that typically require disposal, they are passive, exhibit reliable performance with minimal maintenance and operational costs, (c) they are simple to operate, simple to construct, (d) they can be operated year-round except in the coldest climates, and (e) can provide wildlife habitat, sites for wildlife observation, and environmental education.

There are two types of Constructed Wetlands (32): Subsurface Flow system (SFS) and Free Water Surface (FWS). Subsurface flow systems are designed to create subsurface flow

through a permeable medium, keeping the water being treated below the surface, thereby helping to avoid the development of odors and other nuisance problems. Such systems have also been referred to as "root-zone systems," "rock-reed-filters," and "vegetated submerged bed systems." The media used (typically soil, sand, gravel, or crushed rock) greatly affect the hydraulics of the system. Free Water Surface Systems, on the other hand, are designed to simulate natural wetlands, with the water flowing over the soil surface at shallow depths. Both types of wetlands treatment systems typically are constructed in basins or channels with a natural or constructed subsurface barrier to limit seepage. Constructed wetlands treatment systems have diverse applications and are found across the US and around the world. While they can be designed to accomplish a variety of treatment objectives, for the most part, Subsurface Flow Systems are designed and operated in a manner that provides limited opportunity for benefits other than water quality improvement. On the other hand, Free Water Surface Systems are frequently designed to maximize wetland habitat values and reuse opportunities, while providing water quality improvement (32).

The operations of constructed wetlands follow the same principle as other Living Technologies. Treatment of dissolved biodegradable material in wastewater is achieved through the synergistic work involving decomposing microorganisms, which are living on the exposed surfaces of the aquatic plants and soils, plants species, as well as various animal species. Decomposers such as bacteria, fungi, and actinomycetes are active in any wetland, breaking down dissolved and particulate organic material to carbon dioxide and water. This active decomposition in the wetland produces final effluents with a characteristic low dissolved oxygen level with low pH (32). The effluent from a constructed wetland usually has a low BOD as a result of this high level of decomposition. Aquatic plants, on the other hand, play an important part in supporting these removal processes through such mechanisms as pumping atmospheric oxygen into their submerged stems, roots, and tubers. The oxygen is then utilized by the microbial decomposers attached to the aquatic plants below the level of the water. Plants also play an active role in taking up nitrogen, phosphorus, and other compounds from the wastewater. This active incorporation of nitrogen and phosphorus can be one mechanism for nutrient removal in a wetland. Some of the nitrogen and phosphorus is released back into the water as the plants die and decompose. In the case of nitrogen, much of the nitrate nitrogen can be converted to nitrogen gas through denitrification processes in the wetland (32). While the use of wetlands is a promising idea, there are several potential obstacles. To be effective these wetlands require a large land area. In addition, wastewater added to wetlands must be pretreated to remove solids, reducing the energetic saving. Another problem is that in temperate climates these marshes exhibit reduced functionality for much of the year (32).

4.2. Lake Restorers

Restorers are an assembly of engineered ecologies incorporated into floating rafts. Restorer Technology is borrowed from an analogous component in nature known as the floating island, which is formed as dense mats of vegetation. Typically, they are made up of cattails, bulrush, sedge, and reeds, which normally extend outward from shoreline wetlands. As the water gets deeper and the roots no longer reach the bottom, this vegetation uses the oxygen in their root mass for buoyancy, while the surrounding vegetation provides support that is crucial

for retaining their top-side-up orientation. Moreover, the area beneath these floating mats is exceptionally rich in aquatic biota. Eventually, storm events may tear whole sections free from the shore. These resultant floating islands migrate around a lake with changing winds, occasionally reattaching to a new area of the shoreline, or breaking up in heavy weather (33).

Unlike the natural floating islands, lake restorers are construction involves making rafts or wire cages that can float of water. They are then planted with different species of plants, which later provide habitats to various micro and macro organisms. Efficient airlift pumps and fine-bubble air diffusion systems incorporated in the design of restorers add oxygen to the water as well as circulate water and nutrients over the Restorer's biological surfaces to stimulate the natural healing process. It is the complete body of water that treats itself. The resultant rafted floating ecologies, can treat wastewater, assist in the upgrade of outdated and overloaded facultative lagoons, suppress algal growth or help maintain the health of ponds and lakes. These diverse "floating islands" are installed in new or existing lagoons and ponds to provide a simple, robust, and beautiful method of treating waste and cleaning up polluted waters. The robustness of Restorers lies on the utilization the widely recognized benefits of fixed bio-films to accelerate the natural processes found in a river, lake, pond, or constructed lagoon by the following: (a) Introducing oxygen and circulation to the stressed environment that often lacks sufficient oxygen-rich surface areas necessary to maintain a balanced ecology; (b) Utilizing native higher plants and artificial media as bio-film substrate to support rich microbial, algae, and animal communities; (c) Acting as a chemostat and incubator by producing great volumes of beneficial microorganisms that flow into the surrounding water and feed on excess nutrients and organic pollutants; and (d) Providing opportunities for benthic communities to establish themselves in the bottom areas that were once oxygen poor (33).

4.3. Eco-Restorers

Eco-Restorers, unlike Lake Restorers, are more expensive to construct yet less energy efficient to operate. These systems, many of which were originally built under the name "Living Machines," are ideal for situations where there is either very little land available or where a significant element of visitor interest and interpretation is required (33). In 1995, Jonathan Porritt opened Europe's first Eco-Restorer System, a Living Machine, at the Findhorn Foundation. This ecologically engineered plant is designed to treat sewage from the population of up to 300 people living at the Findhorn Foundation and provides a research and educational facility to promote this technology throughout Europe. Diverse communities of bacteria, algae, micro-organisms, numerous species of plants and trees, snails, fish, and other living creatures interact as whole ecologies in tanks and bio-filters. In this Living Machine system, anaerobically treated sewage flows into a greenhouse containing a series of tanks. These tanks contain species which breakdown the sewage naturally as it moves through. In many systems there are by-products of fish and plants being produced that can then be sold. Living Machines mirror processes that occur in the natural world, but more intensively. At the end of the series of tanks, the resulting water is pure enough to be recycled. The technology is not only capable of meeting tough new sewage outflow standards, but uses no chemicals, and has a relatively inexpensive capital cost attached.

A typical design of an Eco-Restorer, using the Findhorn example, has five major components, which are housed in a single-span greenhouse, approximately 10 m wide by 30 m long. They comprise the anaerobic septic tanks, closed aerobic reactor, open aerobic reactors, the clarifiers, and the ecological fluidized beds (EFBs). This Living Machine at Findhorn receives about 60 m^3 wastewater per day from. The raw wastewater is received in the first component of the system; the anaerobic septic tanks. Typically, three anaerobic bioreactors are buried outside the greenhouse and their function is to reduce significantly the organic material and inorganic solids in the wastewater. The absence of oxygen in the wastewater promotes the growth of anaerobic and facultative bacterial populations. After the anaerobic digestion, the effluent from the anaerobic tanks flows into a closed aerobic tank in the greenhouse. Air is introduced through fine bubble diffusers to convert the wastewater from an anaerobic to an aerobic state. Gases from the closed aerobic tank pass through an air filter system to eliminate odors. After this treatment, the effluent moves the open aerobic reactors. The Living Machine at Findhorn has four aerobic tanks containing diaphragm aerators and each is planted with plant species with large root masses on floating plant racks. The BOD and TSS are reduced at this stage and ammonia nitrified. The primary function of the plants is to provide favorable environments for enhanced microbial activity. Bacteria and other micro-organisms attach themselves to the large surface area of submerged plant roots. These attached biofilms contribute significantly to the treatment process. The secondary plant functions include nutrient removal, metal sequestering, pathogen destruction, and some control of gas exchanges. The main objective is to have a healthy and diverse sequence of ecosystems present. The wide variety of plant species filling ecological niches in the system is a key to the robust nature of natural treatment systems. The ecological network of species creates internal biological redundancies compared with a purely microbial system, or a monoculture duckweed system. This gives the potential for improved efficiency and greater resilience. Despite the efficiency of both micro-organisms and plants, the effluent from the open aerobic tanks still contains some un-degraded suspended solids. The solids kept in suspension in the aerobic tanks are removed in the Clarifier. The clarifier is a settling tank with cone-shaped bottom. The suspended solids settle at the bottom of the tank and are returned to the anaerobic primary tanks. In the Clarifier tanks you may see tiny water creatures such as Cyclops living in the water. They perform an important part in both treatment and in creating a complex food chain. The clarified effluent now is set to enter the final phase of treatment by the ecological fluidized beds (EFBs).

The Ecological Fluidized Beds in each train are filled with light rock media. For aerobic operation, airlift pumps raise the water from the bottom of the fluidized bed to the surface, where the water flows down through the bed. Recycle rates can be varied up to 100 times the flow rate through the component. The aerobic operation provides reductions in BOD and TSS and nitrification. For the anaerobic operation of the fluidized beds for denitrification, mechanical pumps circulate water up through the bed. The fluidized beds are planted and benthic animals graze the surface. The first fluidized bed is usually run aerobically to nitrify any remaining ammonia in the waste stream. The second fluidized bed can be run anaerobically to denitrify. The third and final fluidized bed is run for final denitrification and polishing. The underlying concept behind the design involves rapid flows of water by recycling through the media filled zones. The key attributes of an Ecological Fluidized Bed are: stable high

surface area micro-environment sites for bacteria, ultra rapid exchanges across biological surfaces, direct NH_4/NO_3 uptake, nitrification and denitrification cycles, the support of higher plant life and root systems within the media and in the aquatic environments, and Self-cleaning. The biology is managed as a balanced ecosystem. The levels of dissolved oxygen, and carbon to nitrogen ratios, as well as recycle rates and bioaugmentation, are adjusted with the overall objective of reducing levels of BOD, ammonia (NH_3), total nitrogen (TN), faucal coliform, and solids. Information on the efficiency of the Restorer system/Living Machine at Findhorn, showed that the system treats sewage to advanced wastewater treatment (tertiary) standard. Specifically, biological oxygen demand (BOD), total suspended solids (TSS), total nitrogen in water (TKN), ammonium (NH_4), nitrate (NO_3^-), and total phosphorous (TP), which were 250, 160, 40, 50, 10, and 7 mg/L, respectively before treatment, were less than 10 mg/L after treatment for BOD, TSS, and (TKN); 2 mg/L for NH_4 and 5 mg/L for NO_3, and TP, respectively (33).

4.4. Reedbeds

Reedbeds are natural systems, which are ideal for treatments on small-scale or where there are no land restrictions. They are cost effective to install and simple and inexpensive to run. They do however take up larger areas of land than Restorers. Currently, there are several different alternative designs: (a) horizontal flow reedbeds (HFR); in this design the wastewater is fed in and flows slowly through the bed in a horizontal path below the surface until it reaches the outlet zone. Here it is collected before leaving via the level control arrangement at the outlet. As it flows, the wastewater comes into contact with a network of aerobic, anoxic, and anaerobic zones. The Reed rhizomes open up the bed to provide new hydraulic pathways, (b) vertical flow reedbeds (VFR); these systems are often used to reduce on-site sludge production. The sludge is added to the reedbed and is degraded in the oxygen rich environment by the plant roots, (c) pond and reedbed systems (PRS); the pond and reedbed systems are individually-designed, robust, and self-maintaining, and can treat domestic, municipal, agricultural and industrial waste water to very high standards. They consist of a series of shallow outdoor ponds, fringed with various species of emergent plants, and are linked by areas of aggregate-filled constructed wetland. These systems can be built for as few as 5 and as many as 3,000 people. Land requirements are approximately 10 m^2 per person equivalent, depending on conditions (33).

5. PRINCIPLE UNDERLYING THE CONSTRUCTION OF LIVING MACHINES

As has been pointed out earlier (Sect. 2), Living Machines construction relies on the principles of ecology, and the resultant technological innovations, defined broadly as advanced ecologically engineered systems (AEES), are being considered for application to number of problem areas. Potential applications include: (a) The replacement of or provision of designs of ecological systems (ecotechnology) as alternatives to man-made/energy-intensive systems to meet various human needs (for example, constructed wetlands for wastewater treatment). (b) The restoration of damaged ecosystems and the mitigation of development activities. (c) The management, utilization, and conservation of natural resources. (d) The integration of

society and ecosystems in built environments (for example, in landscape architecture, urban planning, and urban horticulture applications). These potential applications govern or offer a basis for the underlying principles for the construction of Living technologies. Bergen and co-workers summarize these principles into five general principles to guide those practicing ecological engineering in any context or ecosystem (34). There are specifically five principles governing the construction of Living Machines which are here-below briefly explored.

5.1. Living Machine Design to be Consistent with Ecological Principles

This principle emphasizes the importance of understanding the characteristics and behaviors of the natural systems. The designs accordingly produced with regard for, and taking advantage of, the characteristic behavior of natural systems, shall be most successful. Also notable is the fact that when natural structures and processes are included and mimicked, then nature is treated as a partner in design, and not as an obstacle to be overcome and dominated. This is because the capacity of ecosystems to self-organize is recognized and put into use. Mitsch and Jørgensen state that it is this "capability of ecosystems that allows nature to do some of the 'engineering' and that the ecological engineers participate as choice generators and as facilitators of matching environments with ecosystems, but nature does the rest." The key attributes of an ecosystem that allow for self-organization are complexity and diversity. Ecosystems can be complex structurally and in the temporal and spatial scales of processes. Significant ecological changes are often episodic, and critical processes, which occur at rates spread over several orders of magnitude, but clustered around a few dominant. Ecosystems are also heterogeneous, displaying patchy and discontinuous textures at all scales and do not function around a single stable equilibrium. They are rather defined by the functionally different states, which are created from the "destabilizing forces far from equilibria, multiple equilibria, and/or absence of equilibria define, and movement between states. These maintain structure and diversity of the ecosystems." The structure and diversity produced by the large *functional space* occupied by ecosystems is what allows them to remain healthy, or to persist. The large functional space required for sustainable ecosystems is directly at odds with traditional engineering design practices that create systems that operate close to a single, chosen equilibrium point. Another important characteristic of ecosystems is that the outputs of one process serve as the inputs to others. No waste is generated and nutrients are cycled from one trophic level to the next. In constructing Living Machines, this concept should be well understood. A final characteristic of natural systems is that they tend to function near the edge of chaos or instability. Designing systems to include ecological characteristics would, therefore, depart from common engineering practice. Designing for ecological rather than engineering resilience would mean encouraging diversity and complexity, while allowing systems to self-organize, mature, and evolve. How to design systems to perform like ecosystems and still function as desired is explored in the remaining principles (34).

5.2. Living Machine Design to Deal with Site-Specific Situation

The complexity and diversity of natural systems cause a high degree of spatial variability. While the ecological characteristics discussed earlier are generally applicable, every system and location is different. The second principle suggests that one has to gain as much

information as possible about the environment in which a design solution ought to function. Furthermore, the spatial variability rules out standardized designs, which means that the solutions should be site-specific and small-scale. Standardized designs imposed on the landscape without consideration for the ecology of a place will take more energy to sustain. In addition, knowledge of the place also allows for more holistic designs. Such design take into account both the up-stream and downstream affects of design decisions. For upstream issues such as what resources must be imported and appropriated to create and maintain a solution are considered while for downstream the site-specific and off-site impacts of the design on the environment are considered. In addition to the physical context of a design, knowledge of the cultural context is important. Designs are more likely to succeed and to be accepted by the local community when the people who live in a place are included in the design process. They bring knowledge of the particularities of a place and are empowered through direct participation in shaping their environment. Attention to group dynamics and conflict mediation is important for successful stakeholder participation (34).

5.3. Living Machine Design to Maintain the Independence of Its Functional Requirements

Ecological complexity adds high and often irreducible levels of uncertainty to the design process. Even under conditions of certainty, the amount of relevant information in possession may be overwhelming and often unmanageable, yet it is desirous that the solutions are kept simple and workable. Under these circumstances a strategy for dealing with such uncertainty would be to set the *tolerances* on the design functional requirements as wide as possible. The third principle describes that the functional requirements (FRs), which are the specific functions that a design solution is required to provide, are satisfied, individually, by the design parameters (DPs). This means that the design parameters are the physical elements of the solution chosen to satisfy FRs. Therefore, best designs are those that have independent (not coupled) FRs and one and only one DP to satisfy each FR. Consequently, when modifying one DP affects more than one FR, then a design is described as being coupled. In these circumstances, wide tolerances on FRs can make the design essentially uncoupled. This is so because wide design tolerances allow a larger functional range for a system while the outputs remain within acceptable ranges. However, when interacting with ecological systems, the concept of functional independence becomes a lot less clear. This is so because ecosystems are complex with many levels of interconnection between components, which means that many elements of the system may be involved in more than one process. Since ecosystems can function and provide benefits to society without human intervention, the design FRs are incorporated or considered in any undertaking of Living Technology designs to satisfy unmet human needs. Therefore, the FRs for design follow from the statement of these needs, while the ecosystem processes that are in existence and their preservation needed while designing for unmet needs, act as constraints on design. Although the independence principle predicts that successful designs may be obtained when the FRs are kept uncoupled in the solution, in reality, however, it would be foolish not to take advantage of the multiple, coupled services an ecosystem can provide (34).

5.4. Living Machine Design to Enhance Efficiency in Energy and Information

The fourth principle follows from taking advantage of the self-organizing property of ecosystems. To let nature do some of the engineering means that the free flow of energy into the system from natural sources, primarily the Sun should be put to maximum use. At the same time the energy expended to create and maintain the system directed, by design, from off-site sources, such as fossil fuels, large-scale hydroelectric sources, etc should be minimized. While utilizing free flowing energy, however, it is important to follow where the energy would go without intervention, to make sure that it is not more critically needed downstream and that there is minimal adverse impact. This could be achieved by keeping the information content of the design to the minimum or simply stated making designs simple yet successful. For example, the energy input needed to restrict a stream channel to a confined space tends to be high and ultimately fails when a large flood occurs. A better design would recognize the expected variability in stream flows and design the system to withstand large variations in flow (wide tolerance) yet still maintain its ecological and engineering functions i.e., minimizing information content. In this way the extra information required would be balanced by utilizing self-organization and wide tolerances. In other words, this can be considered as an upfront capital investment in diversity that would gain overall efficiency later through reduced energy requirements and a reduced risk of failure. Therefore, diversity provides insurance against uncertainty in addition to contributing to ecological resilience. In the case of an engineered wetland, for example, a wide range of species may be included in the initial construction, but natural processes are allowed to select those best suited for the imposed environment. Similarly, the first and second principles advocate an up-front investment in knowledge of the design context to minimize uncertainty and to allow less information to be transferred during design implementation (34).

5.5. Living Machines Design to Acknowledge and Retain it Values and Purposes

The major goal of Living technologies is the provision of ecologically oriented designs that would benefit both society and the natural environment. Moreover, most engineering codes of ethics state at least that engineers have a responsibility to serve and protect society. From an ecological engineering perspective, this code has been explicitly broadened to include the responsibility of sustaining the natural systems that support life. Regardless of specific ideology, however, design practices that acknowledge the motivating values and purposes would be more successful. Recall that the third principle recommends using wide tolerances under conditions of uncertainty. Consequently, it follows a precautionary approach for ecological engineering, and itought to be adopted at all time. A precautionary approach should act as a form of insurance against unpleasant surprises in the future. In Living technologies innovations, classical engineering should be applied sparingly, and complex solutions avoided where possible. Furthermore, design solutions that are both fail-safe and safe-fail should be pursued to avoid catastrophic failures. As opposed to traditional fail-safe approaches, safe-fail solutions acknowledge that our original functional requirements for a design may not be met or that there may be unexpected results. Failure in this case is not catastrophic. Therefore, in selecting the design, alternatives that have the best worst-case outcome should be advocated for (34).

6. OPERATIONALIZATION OF LIVING MACHINE TECHNOLOGY

The operationalization of the Living Machine technology relies on the incorporation of plants and animals in many of the same basic processes (e.g., sedimentation, filtration, clarification, adsorption, nitrification and denitrification, volatilization, and anaerobic and aerobic decomposition) that are used in conventional biological treatment systems. A typical Living Machine comprises six principle treatment components: (a) an anaerobic reactor, (b) an anoxic tank, (c) a closed aerobic reactor, (d) aerobic reactors, (e) a clarifier, and (f) "ecological fluidized beds" (EFBs) (Fig. 22.1a). While the open aerobic reactors and EFBs are found in almost all Living Machines, the other components are not always utilized in the treatment process. The specific components used are selected by the designers depending upon the characteristics of the wastewater to be treated and the treatment objectives. Sometimes additional process components may be added if considered necessary by the designers (43).

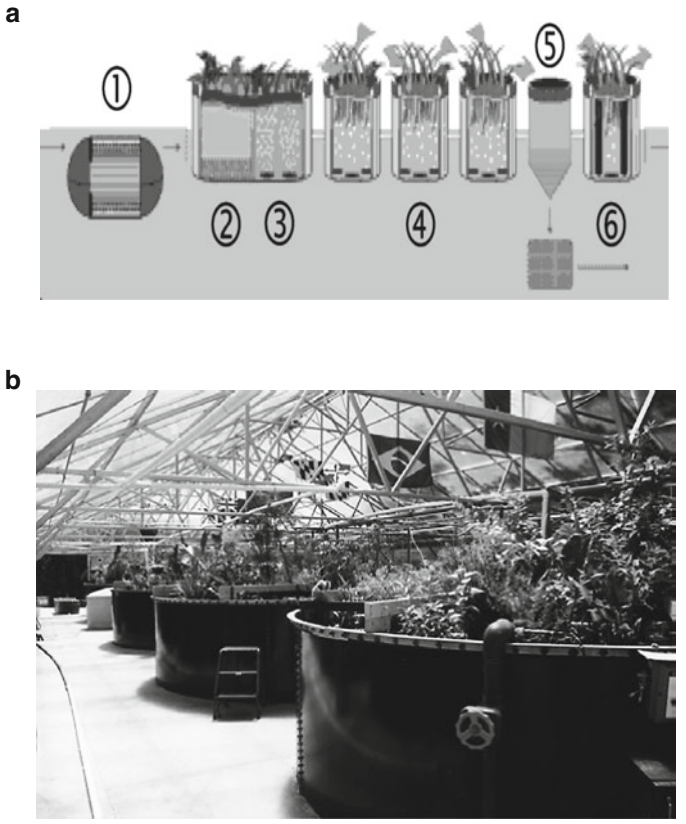

Fig. 22.1. (**a**) Illustrates the operational set-up and components of the Living Machine®: (1) anaerobic reactor, (2) anoxic reactor, (3) closed aerobic reactor, (4) open aerobic reactors, (5) clarifier, and (6) "ecological fluid bed." (**b**) Illustrates the operational set up of the open aerobic tanks of the Living Machine in south Burlington, VT. A series of tanks in a greenhouse are shown (Adapted from US Environment Protection Agency (43)).

6.1. Anaerobic Reactor (Step 1)

In case it is incorporated into the treatment process, the anaerobic reactor serves as the initial step of the process. The reactor, which is similar in appearance and operation to a septic tank, the anaerobic reactor reduces the concentrations of BOD5 and solids in the wastewater prior to treatment by the other components of the process. Raw influent enters the reactor, which acts as a primary sedimentation basin. Some of the anaerobic reactors used have an initial sludge blanket zone, followed by a second zone for clarification. Additionally, strips of plastic mesh netting are sometimes used in the clarification zone to assist with the trapping and settling of solids, and to provide surface area for the colonization of anaerobic bacteria, which help to digest the solids. Sludge is typically removed periodically via perforated pipes on the bottom of the reactor, and wasted to a reed bed or other biosolids treatment processes. Gases produced are passed through an activated carbon filter or biofilter for odor control (43).

6.2. Anoxic Reactor (Step 2)

The primary purpose of the anoxic reactor is to promote growth of floc-forming microorganisms, which will remove a significant portion of the incoming BOD5. The anoxic reactor is mixed and has controlled aeration to prevent anaerobic conditions, and to encourage floc-forming and denitrifying microorganisms. Mixing is accomplished through aeration by a coarse bubble diffuser. These diffusers are typically operated so that dissolved oxygen is maintained below 0.4 mg/L. The space over the reactor is vented through an odor control device, which is usually a planted biofilter. In addition, an attached growth medium may be placed in the compartment to facilitate growth of bacteria and other microorganisms. Settled biosolids from the clarifier (Step 5), and nitrified process water from the final open aerobic reactor (Step 4) are recycled back into this reactor. The purpose of these recycles is to provide sufficient carbon sources to the anoxic reactor to support denitrification without using supplemental chemicals, such as methanol (43).

6.3. Closed Aerobic Reactor (Step 3)

The purpose of the closed aerobic reactor is to reduce the dissolved wastewater BOD5 to low levels, to remove further odorous gases, and to stimulate nitrification. Aeration and mixing in this reactor are provided by fine bubble diffusers. Odor control is again achieved by using a planted biofilter. This biofilter typically sits directly over the reactor and is planted with vegetation intended to control moisture levels in the filter material.

6.4. Open Aerobic Reactors (Step 4)

Next in the process train are the open aerobic reactors, or aerated tanks. They are similar to the closed aerobic reactor in design and mechanics (i.e., aeration is provided by fine bubble diffusers); however, instead of being covered with a biofilter, the surfaces of these reactors are covered with vegetation supported by racks. These plants serve to provide surface area for microbial growth, perform nutrient uptake, and can serve as a habitat for beneficial insects and microorganisms. With the variety of vegetation present in these reactors, these units (along with the Ecological Fluidized Beds – Step 6) set the Living Machine apart from other

treatment systems in terms of their unique appearance and aesthetic appeal (Fig. 22.1b). The aerobic reactors are designed to reduce BOD5 to better than secondary levels and to complete the process of nitrification. The size and number of these reactors used in a Living Machine design are determined by influent characteristics, effluent requirements, flow conditions, and the design water and air temperatures (43).

6.5. Clarifier (Step 5)

The clarifier is basically a settling tank that allows remaining solids to separate from the treated wastewater. The settled solids are pumped back to the closed aerobic reactor (Step 3), or they are transferred to a holding tank, and then removed for disposal. The surface of the clarifier is often covered with duckweed, which prevents algae from growing in the reactor.

6.6. Ecological Fluidized Beds (Step 6)

The final step in the typical Living Machine process are the "ecological fluidized beds" (EFBs). These are polishing filters that perform final treatment of the wastewater, and one to three are used in series to reduce BOD_5, TSS and nutrients meet final effluent requirements. An EFB consists of both an inner and outer tank. The inner tank contains an attached growth medium, such as crushed rock, lava rock, or shaped plastic pieces. The wastewater flows into the EFB in the annular space between the inner and outer tanks and is raised by air lift pipes to the top of the inner ring that contains the media. The bottom of the inner tank is not sealed, so the wastewater percolates through the gravel media and returns to the outer annular space, from where it is again moved back to the top of the gravel bed. The air lifts also serve to aerate the water and maintain aerobic conditions. The unit serves as a fixed bed, downflow, granular media filter and separates particulate matter from the water. Additionally, the microorganisms that occupy the granular media surfaces provide any final nitrification reactions. As sludge collects on the EFB, it reduces its ability to filter. This would eventually clog the bed completely. Therefore, additional aeration diffusers beneath the gravel bed are periodically turned on to create an upflow airlift, reversing the flow direction. This aeration is intended to "fluidize" the bed and release the trapped sludge (hence the name of this unit). This sludge is washed over and accumulated at the bottom of the outer annular space where it can be collected manually, and wasted along with the biosolids from the anaerobic reactor. Consequently, the name "ecological fluidized bed" is somewhat misleading for this unit since, in its treatment mode; it acts like a typical, conventional, down-flow coarse media contact filter unit. Only during backwash cleaning does the bed become partially fluidized. After this last step, the wastewater should be suitable for discharge to surface waters or a subsurface disposal system, or reused for landscape irrigation, toilet flushing, vehicle washing, etc. (43).

7. CASE STUDIES OF CONSTRUCTED LIVING MACHINE

7.1. Sewage Treatment in Cold Climates: South Burlington, Vermont AEES, USA

Ocean Arks International, which is a not-for-profit organization dedicated to the development of ecological design and its implementation into society, in 1995 constructed a tank-based "advanced ecologically engineered system" (AEES) or Living Machine in south

Burlington, Vermont, to determine if the technology is capable of treating sewage to high standards in a northern New England climate, particularly during the cold and short day-length seasons (16). The AEES facility, housed within a 725 m^2 (7,800 ft^2) greenhouse, contained two parallel treatment systems designed to treat 300 m^3/day (80,000 gallons/day) of sewage from the city of south Burlington to advanced tertiary wastewater standards for 5-day carbonaceous biochemical oxygen demand ($CBOD_5$), total suspended solids (TSS), total Kjeldahl nitrogen (TKN), ammonia (NH_3), nitrate (NO^-_3), and total nitrogen (TN). The performance target for removal of fecal coliforms in the system was 2,000 cfu/100 mL without disinfection. The Vermont Living Machine was biologically diverse. Over 200 species of vascular and woody plants were evaluated for their effectiveness and suitability for waste treatment between 1995 and 2000. Plants were evaluated for: (a) their ability to tolerate sewage, (b) the extent of the root zones, (c) disease and pest resistance, (d) ease of management, and (e) secondary economic value. The plants were physically supported on the surface of the water by rigid plant racks designed to provide gentle flow over the roots in a highly aerated and turbulent surrounding environment. The system was designed to utilize microbial communities attached to plant roots, as well as flocculating bacteria in the open water to affect treatment. Invertebrates including micro-crustaceans and freshwater clams provided biological filtration, while snails and fish were incorporated into the design to digest residual bio-solids. The flow was split between two 150 m^3/day (40,000 gallons/day) treatment trains with a hydraulic retention time (HRT) of 2.9 days. The facility was started in December 1995, operated at its design flow capacity by May 1996, and was maintained at this steady state until the end of 1999. Each treatment train comprised nine tanks connected in series and each tank was 4.6 m wide × 4.6 m deep (15 ft × 15 ft). Raw effluent entered and was mixed in an anoxic reactor. To control odors normally associated with raw sewage, an ecological gas scrubber, employing higher plants, and a soil/bark/compost media, was mounted over the anoxic reactor tank. The wastewater flowed from the anoxic reactor into four aerobic reactors. Dense plantings were maintained on surface racks. The waste then flowed to a clarifier covered with floating aquatic plants. Bio-solids from the clarifier were recycled to the anoxic reactor or wasted (16).

Downstream of the clarifier were three tanks containing ecological fluidized beds (EFBs) in series. The EFB is in essence serves as a submerged trickling filter capable of supporting plants mounted over an outer ring of open water. The media that comprises the inner part of the EFB physically supports benthic organisms, including mollusks. Depending upon water quality and their position in the series, the EFB's could be operated anoxically to aid denitrification, or aerobically for polishing and final filtration. The facility met and exceeded its design parameters, for CBOD5, TSS, TKN, NH_3, NO^-_3, and TN as well as fecal coliform bacteria. A high level of performance was maintained even during the coldest months. In addition, phosphorus design standards were also met, but the AEES technology has yet to demonstrate phosphorus removal beyond what would be expected in a nitrifying activated sludge process.

One of the goals of the project was to grow organisms that not only provided treatment but also had potential economic benefits. Botanicals with economic value included young trees such as *Taxodium distichum* L. (bald cypress), *Zantadeschia aethiopica* L. (Calla Lily), and plants used for environmental remediation or wetland mitigation. Fish grown and harvested

from the system included *Notemigonus crysoleucas* M. (golden shiners) and other bait fish, *Pimephales promelas* R. (fathead minnows), and ornamental fish including *Carassius auratus* L. (goldfish) and Japanese koi. All of the fish species fed upon organic material and plankton produced internally within the facility. One of the most striking aspects of the Vermont facility was its beauty. It remains a frequently visited educational facility and is currently operated as a test facility for the treatment of different types of high strength organic wastes including brewery wastes. It is also a site where new economic by-products from both liquid and solid waste conversion processes are being developed (16).

7.2. Environmental Restoration: Flax Pond, Harwich, Massachusetts, USA

The Flax Pond, which is a 15-acre (6 ha) pond in Harwich, Massachusetts, has for decades been heavily impacted by leachates from an adjacent landfill and unlined septage holding lagoons. By 1989, the pond was closed to recreation and fishing because of contamination caused by the daily intrusion of 295 m^3 (78,000 gallons) of leachate from the landfill (16, 35). The pond had low oxygen levels, high coliform counts, excessive sediment build up, and organic pollutants in the water column including volatile organic compounds (VOCs). Macro-benthic organisms were absent from many of the bottom sampling stations. Flax Pond had unusually high sediment concentrations of total phosphorus (300 times greater) and iron (80 times greater) compared with other Cape Cod ponds (16, 36). Ammonia levels in the sediments were found to be as high as 8,000 mg/kg. The pond is delineated into an eastern zone and a western zone; the cloudier eastern zone being the predominant zone of impact from the landfill. The pond also had a maximum depth of 6 m and stratifies in its western end. In the autumn of 1992 construction of the first floating Pond Restorer was completed and anchored at the eastern end. It employed a windmill and solar panels for electrical generation and was capable of circulating through its nine cells up to 380 m^3/day (100,000 gallons/day) of water drawn from the bottom of the pond. The first three cells were filled with semi-buoyant pumice rock that supported diverse benthic life including freshwater clams of the genera *Unio* and *Onodonta*. Since phosphorus was limiting in the pond's water column, a slow release form of a clay-based soft phosphate was added to the EFB cells in the Restorer. Moreover, bacterial augmentation and mineral enrichment in the first three cells was routinely done. The final six cells supported over two-dozen species of terrestrial plants on racks. The Restorer was not operated during the winter months to allow the pond to freeze completely.

The first noticeable effect of the Restorer on the pond was the return of a positive oxygen regime to the bottom. By 1995, the sediment depth throughout the pond had been reduced by an average of 64 cm representing a total of 38,000 m^3 of digested sediments. Between the years 1999 and 2001, dramatic changes in the sediments took place, including large reductions (exceeding 50%) in total phosphorus, ammonia, and TKN. However, total iron increased in the western end and decreased slightly in the eastern end of the pond. Alkalinity followed a similar pattern. The investigators could not establish which internal mechanisms were involved in the changes in sediment phosphorus, although TKN reduction was with certainty be associated with nitrification and denitrification in the sediments (i.e., nitrates were below detectable limits in all sediment samples in both 1999 and 2001). Water clarity and the overall health of the pond have improved over the past decade, and biodiversity has increased (16).

7.3. Organic Industrial Wastewater Treatment from a Poultry Processing Waste in Coastal Maryland: Using Floating AEES Restorer

In the late 1990s, the design of the Pond Restorer used in Flax Pond evolved into a linear AEES Restorer design for use on new and existing wastewater treatment lagoons. This technology combines the benefits of the small footprint AEES tank-based technology (Sect. 6.1) with the simplicity and efficiency of constructed wetlands. The first large-scale wastewater application of the floating AEES Restorer technology was installed in June 2001 on a wastewater treatment lagoon that treats 3,785 m^3 (1 million gallons/day) of high strength poultry processing waste in coastal Maryland. The Restorers were installed in a 34,100 m^3 (9 million gallon) storage lagoon downstream of a lagoon that had been run as a Sequencing Batch Reactor (SBR) for over 15 years (16).

Twelve Restorers run 43 m (140 ft) each across the lagoon and are secured from the banks in multiple cells, creating a serpentine flow pattern with floating baffles. Twenty-five species of native plants (25,000 individuals) were installed in plant racks on the outside edges of the Restorers. The plants are a critical element in the technology. Their root system provides surface areas and nutrient support for microbial communities, some nutrient uptake and they shade/inhibit suspended algae in the lagoons. Water is treated in the open areas on each side of the Restorers with fine bubble linear aerators installed at the bottom of the lagoon. The center zones of the Restorers, with suspended fabric media, provide surface area for attachment and growth of microbial communities and as such are submerged, aerobic, fixed film reactors. The transition between the old SBR system and the new Restorer lagoon took place in October 2001. Although definitive quantitative data is not yet available, qualitative successes of the project in these early stages are worth noting.

Since start-up of the Restorer system, effluent standards have not exceeded state permit levels. The electrical energy use in the lagoons has been reduced by approximately 74% compared to the former sequence batch reactor (SBR) system (40, 41). Energy reduction is the result of higher biological reaction rates in the Restorer lagoon and the efficiency of the new aeration design. Sludge has been trucked for 20 years from the poultry processing plant for land application at nearby farms. The sludge comes from a variety of locations within the wastewater system, including the lagoons. Since installation of the Restorers the average truckloads of sludge leaving the processing facility have decreased significantly. This overall sludge reduction is the direct result of reduced sludge coming from the Restorer lagoon. Operation of the former SBR system required wasting of sludge for 8 h every day from the lagoons. Following installation of the new Restorer system, sludge is wasted for approximately 1 h every few weeks. In addition, 45 sludge judge samples have been taken monthly within the Restorer lagoon. Since August 2001 total sludge levels have decreased by approximately 10 cm (4 in.). This decrease indicates that sludge degradation is faster than sludge accumulation, even as the lagoon treats waste (16, 33).

7.4. Architectural Integration: Oberlin College, Ohio, USA

In recent decades architecture has begun to include ecologically designed systems within structures for air purification, humidity control, water re-use, waste treatment, and food

production. The bio-shelters developed by the Todds are being integrated in ecologically designed systems for living and life support (37). A number of new buildings employ ecologically engineered technologies for waste treatment, water reuse, and education including the Ontario, Canada, Boyne River School, and the Kitchener/Waterloo YMCA rural campus. The most recent of these is the Lewis Environmental Studies Center at Oberlin College in Ohio. The building includes renewable energy, natural day-lighting, and non-toxic and recyclable materials. Within the structure is an AEES system or Living Machine for sewage treatment and biological research. This system, similar to the Vermont AEES, includes tanks connected in series and a constructed wetland within the building. The tanks support a diverse community of tropical and temperate plants. The purified wastewater is sterilized with UV before reuse in the toilets in the building. There is a growing interest in redefining the functioning of buildings in ecological terms. This drives some architects toward conceptualizing buildings as "organisms." New light transmitting designs and self-regulating technologies optimize internal climates and support a diversity of ecological elements within the buildings. Nature is increasingly being brought indoors for practical and aesthetic reasons (16).

7.5. Tyson Foods at Berlin, Maryland, USA

The poultry processing facility acquired by Tyson Foods at Berlin, Maryland, came with a wastewater treatment system that was known to be the worst in the state. The major problem with this system was that it discharged its contents to Chincoteague Bay, which is a protected bay used for fishing and harvesting crabs abs scallops. Owing to its inability to comply with the State of Maryland discharge standards, the downstream aquatic ecosystems could not be protected. This 1-million gallons/day poultry-processing waste treatment system required a wastewater treatment upgrade to meet effluent treatment standards and to reduce energy costs and the use of chemical treatment (33). Ocean Arks International (OAI) installed such restorers. Adding Restorers to existing waste treatment lagoons provided a robust and flexible treatment option. In the modified treatment system an existing aerated lagoon is maintained with subsurface aeration only. At the beginning of this lagoon is an anoxic denitrifying cell. Wastewater is polished in a 9 million-gallon lagoon using 12 linear Restorers. The nitrified effluent can be recycled back to the anoxic zone. This treatment method has reduced energy input by 70–80%. Twelve floating Restorers (2,100 ft^2 each) were installed in the lagoon and secured from the banks in four separate cells, created with suspended fabric baffles. Water flows through the Restorer lagoon in a serpentine path to maximize treatment, gently aerated and circulated by subsurface, fine-bubble aeration. The wastewater is treated both beneath the Restorers and in the open channels between them. The plant roots and the curtains of suspended fabric media act as submerged, aerobic, fixed film reactors.

The biological design of the Restorers and their placement within the lagoon provides diverse habitat (in the water column, sediments, and the Restorers) for a variety of microbial communities, each of which performs an important function in the treatment process. Approximately 25,000 plants of 25 species were planted on the Restorers, only a handful of the 500 species that Ocean Arks has researched for use in wastewater treatment. Aquatic and water-loving species native to the region were chosen for their treatment properties, their ease of maintenance, and root mass area. The operation and maintenance of the Restorers

is simple and low in cost. Walkways provide access to the plants. In addition to the newly planted diversity, several local plants as well as turtles have migrated into the system, creating a unique self-organizing ecosystem.

8. FUTURE PROSPECTS OF LIVING MACHINES

8.1. Integration of Industrial and Agricultural Sectors: Proposed Eco-Park in Burlington, Vermont, USA

Ecological design concepts are starting to be applied to the development of integrated economic systems in an industrial context. A good example of one such system is the development or construction of Eco-industrial Parks. An Eco-Industrial Park has been defined as, "a community of businesses that cooperate with each other, and with the local community, to efficiently share resources (information, materials, water, energy, infrastructure and natural habitat) leading to economic gains, improved environmental quality, and equitable enhancement of human resources for business and local community" (38). This idea is clearly illustrated by the work pioneered by the city of Burlington and the Intervale Foundation established the Intervale Community Enterprise Center (ICEC). The ICEC undertook to develop a year round, agriculturally based Eco-Park in a 280 ha flood plain within Burlington's city limits. The Eco-Park would derive most of its energy from the utilization of waste heat from the 53 MW McNeil power station. The project has brought together a number of allied businesses including a brewery, several food processors, a restaurant, and a host of Intervale growers and suppliers to the Eco-Park. The University of Vermont's ecological design studio would also be housed in the complex (16). The structure that will support the project combines greenhouses with a conventional light manufacturing facility in a $3,800\,m^2$ ($40,900\,ft^2$) structure. The food culture team at Ocean Arks International (OAI) has been developing some of the agricultural components for the Eco-Park. Their approach has been to start with readily available organic wastes and through ecological processes convert the wastes to high value products. The main goal is ecological and economic amplification of organic materials in an integrated manner similar to that developed by Yan and Ma (39). On a pilot scale the materials being used include spent grain from a local brewery, straw, and bedding from an organic poultry operation. There are several stages in the conversion of materials.

Stage 1: The organic materials are blended, pasteurized, and inoculated with oyster mushroom spawn (*Pleurotus ostreatus* (Jacq:Fr.)). The substrate is placed in plastic bags punched with holes and placed in a mushroom incubator room. When the bags are fully colonized by the mushroom mycelium they are transferred to a grow room for fruiting and harvest. Biological efficiency of conversion, the ratio of wet weight of harvested mushrooms to the dry weight of the substrate, has exceeded 60%. After harvest the remaining substrate has the potential to be used as a high quality animal feed for livestock. In the process of mushroom production, the vegetative forms of fungi colonize the straw and spent grains and produce essential amino acids such as lysine. Tests with cattle and the fish tilapia have demonstrated a ready acceptance of the material.

Stage 2: The spent mushroom substrate is placed in earthworm or vermiculture chambers. The earthworms rapidly convert the materials to enriched compost. The earthworms, a product of the process, are then blended with aquatic plants, *Azolla* sp. (water fern) and *Lemna* spp. (duckweeds), to produce protein-rich fish feeds.

Stage 3: The mushroom/earthworm-based compost is then utilized in the growing of tropical plants in pots and the culture of salad greens. No additional fertilization to the compost is required for the production of greens. After several harvests of salad greens the medium is then utilized as a soil amendment or as a potting soil.

8.2. Aquaculture

Another key component in the design of integrated food systems for urban settings is aquaculture. The food team at OAI has designed re-circulating systems based upon four tank modules for the culture of aquatic animals. To date, OAI has successfully cultured *Oreochromis* sp. (tilapia) and *Perca flavescens* M. (yellow perch) in these systems. The system is designed to produce feeds for the fish internally, including attached algae turfs and their associated communities, floating aquatic plants including Lemna and *Azolla*, zooplankton, and snails. External feeds to the system include earthworms and commercial feeds. These ecosystem based fish culture systems have proven to be efficient. The multiplicity of pathways for nutrients and materials to flow in the production of a diversity of crops is an integral part of ecological design. If such an approach proves to be economically viable in an urban setting, the larger issue of food security can be addressed through the application of applied ecological concepts (16, 40, 41).

NOMENCLATURE

PCB = Polychlorinated biphenyls
PAH = Polycyclic aromatic hydrocarbons
EPA = Environmental protection agency
BOD = Biochemical oxygen demand
COD = Chemical oxygen demand
NAS = National Academy of Sciences
AEES = Advanced ecologically engineered systems
SFS = Surface flow systems
FWS = Free water surface
m = Meter
m^3 = Cubic meters
EFB = Ecological fluidized beds
TSS = Total suspended solids
NH_4 = Ammonium
NH_3 = Ammonia
HFR = Horizontal flow reedbed
VFR = Vertical flow reedbed
PRS = Pond and reedbed system

CBOD = Carbonaceous biochemical oxygen demand
TKN = Total Kjeldahl nitrogen
NO_3 = Nitrate
TN = Total nitrogen
HRT = Hydraulic retention time
VOC = Volatile organic compounds
SBR = Sequencing batch reactor
UV = Ultra violet
ft = Feet
TP = Total phosphorous

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Global Perspective of Anaerobic Treatment of Industrial Wastewater

Kuan Yeow Show, Joo Hwa Tay, and Yung-Tse Hung

CONTENTS

Abstract While anaerobic process had been widely used for stabilizing concentrated solids, the process long suffered a poor reputation because of lack of understanding regarding its fundamentals. Nearly a century later, anaerobic treatment is now arguably the most promising and favorable wastewater treatment system for meeting the desired criteria for future technology in environmentally sustainable development. The development of anaerobic processes, anaerobic biochemistry and microbiology, global applications, and applications of anaerobic processes for industrial wastewaters are discussed.

1. GLOBAL PERSPECTIVE OF ANAEROBIC TREATMENT

The anaerobic treatment of wastewaters and sludges has been in practice for more than a century. While it had been widely used for stabilizing concentrated solids, the process long suffered a poor reputation because of lack of understanding regarding its microbiology and biochemical components. The anaerobic treatment was perceived as a sensitive process that was easily upset and difficult to control. It was also known for producing obnoxious odors, as well as requiring long initial start-up periods and high temperatures (35°C) for effective

From: *Handbook of Environmental Engineering, Volume 11: Environmental Bioengineering*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-031-1_23

waste stabilization. Another possible reason that the anaerobic process had not found general acceptance was that the practical feasibility of direct treatment processes had yet to be proven on specific industrial effluents (1).

Nearly a century later, anaerobic treatment is now arguably the most promising and favorable wastewater treatment system for meeting the desired criteria for future technology in environmentally sustainable development.

Since 1973, the European Union's environmental legislation has developed within a framework set by different Environmental Action Programs, which show how the EU proposes to develop its environmental policy and legislation. The fifth program, titled "*Towards Sustainability: A European Community Program of Policy and Action in relation to the Environment and Sustainable Development*," has already been finished (1993–2000), and a new program, the sixth ("*Environment 2010: Our Future, Our Choice*"), has been approved by the European Commission for the next decade (2001–2010). The general approach and strategy of the fifth program differs from that of the previous programs. As its title "*Towards Sustainability*" implies, the program sets longer term objectives and focuses on a more global approach. The Sixth Environmental Action Program has the same overall perspective, focusing on areas where more action is needed.

Similarly, a recent U.S. National Research Council committee (2) stated, "We are convinced that socially compatible and environmentally sound economic development is possible only by charting a course that makes full use of environmentally advantageous technologies. By this, we mean technologies that utilize resources as efficiently as possible and minimize environmental harm while increasing industrial productivity and improving quality of life." Again, the main term of this program is "sustainable development."

Achieving an integrated prevention and control of pollution requires an integrated control of emissions to air, water, and land, as well as the efficient use of energy and raw materials. The anaerobic treatment process would appear to meet these criteria well. The first reason is the fact that anaerobic treatment is a natural process in which a variety of different species from two entirely different biological kingdoms, the Bacteria and the Archaea, work together to convert organic wastes through a variety of intermediates into methane gas, an excellent source of energy. Methane gas can be used to heat the waste stream to give a higher rate of stabilization or to supplement in-plant power requirements. Pathogenic microorganisms are reduced, and objectionable organic matter is eliminated. The net result is the production of biosolids that are also useful as soil conditioner and are widely used as such. Additionally, with industrial wastewater treatment, the amount of biosolids produced is far less than with aerobic treatment, and the biosolids are already stabilized for land application. Nutrient requirements for anaerobic treatment are smaller in amount than with aerobic treatment.

A unique characteristic of anaerobic treatment by methane fermentation is that no electron acceptor such as oxygen or nitrate needs to be present or added for the process to work. Organic matter itself or the carbon dioxide resulting from its destruction serves this need. As a result, organic loadings to anaerobic reactors can be much higher than to aerobic reactors because oxygen mass transfer limitations are not involved, and energy requirements for mixing are greatly reduced. Therefore, reactors can be much smaller. The absence of a need for an external electron acceptor is also of great advantage when groundwater is contaminated

with biodegradable organics, since sufficient external electron acceptors are often absent. Anaerobic processes leading to methane formation make possible the intrinsic bioremediation of organic groundwater contaminants.

Not only is anaerobic treatment of value for wastewater treatment, but it is also the process used in sanitary landfills that results in the stabilization of organic wastes, converting them to methane gas, which is becoming increasingly valued as an energy source. Some opponents would cap landfills to prevent water from entering and the natural bioconversion to methane to occur, but this practice needs to be questioned. Methane is a powerful greenhouse gas, but if captured for use, it acts instead as a good renewable energy source.

As an added advantage, an unexpected scientific finding, over the past several decades, is that the same anaerobic process is capable of destroying most chlorinated hazardous compounds, including pesticides and chlorinated solvents, and converting polychlorobiphenyls (PCBs) to less harmful forms. Aerobic processes that are so widely used do not have this capability.

Of even greater surprise was the finding that some anaerobic organisms obtain energy for growth from the dehalogenation process (3). Anaerobic processes can also destroy some inorganic pollutants, such as nitrates and perchlorates.

In summary, anaerobic treatment results in net energy production, produces biosolids that are good soil conditioners, requires less reactor volume, and destroys troublesome hazardous chemicals. By itself, the process is capable of meeting the criteria for sustainable development.

2. DEVELOPMENT OF THE ANAEROBIC PROCESSES

2.1. History of Anaerobic Treatment

The first recorded research of anaerobic treatment was accidentally made while evaluating the fertilizer value of digested and undigested manure. In 1808, Davy collected gas containing 30% methane from the digested manure. At the same time, however, Volta is credited with having recognized first that anaerobic biological processes result in the conversion of organic matter to methane (4). In 1776, he showed that "combustible air" was formed from sediments in lakes, ponds, and streams, and concluded that it was derived from the plant material in the sediment.

In 1856, Reiset found methane being liberated from decomposing manure piles and proposed that this process be studied to help explain the decomposition of organic material in general (5).

The first full-scale application of anaerobic treatment was also for domestic wastewater but in a configuration more like a septic tank. This air-tight chamber was described in the French journal *Cosmos* (6) by Mouras, and was called "Mouras' Automatic Scavenger" in which suspended organic material was "liquefied." The article indicated that the invention had been in use for 20 years, which would place the beginning of its application in the 1860s. Targe indicated in the article that this was "the most simple, the most beautiful, and perhaps, the grandest of modern inventions," and "a complete solution of the problem which for centuries had been an insolent menace hurled in the face of all humanity."

Perhaps, the first hybrid anaerobic system was that described in Metcalf and Eddy's historical text on American sewerage practice (7). In about 1890 or 1891, Moncrieff constructed a tank with an empty space below and a bed of stones above. Thus, this was a hybrid of a tank digester and an anaerobic filter. The wastewater of 10 people entered the tank first and then passed up through the anaerobic filter. After 7 years of operation, the sludge remaining in the bottom was removed and readily disposed. Other studies on this system by Houston in 1892 and 1893 confirmed that there was a great reduction in sludge volume to be handled by this system.

One of the first anaerobic filters was a bed of sand at the Massachusetts experimental station (8) to which wastewater was applied with a pore space detention time of about 8 days. After 14 years of operation, 89% of the organic impurities applied to it were claimed to have been removed through biological activity.

Another experiment described was with a filter containing broken stone 0.5–2 in. in diameter. Domestic wastewater was applied with a surface loading rate of about 2 m/day, and 85% organic removal was indicated. A thin film of bacteria covered the stone, indicating that the removal was by bacterial action. The Massachusetts State Board of Health also indicated the advantages of holding wastewater solids for a period of time to achieve hydrolytic or bacteriolytic action on waste solids, resulting in the conversion of a portion of the organic matter into inoffensive gases or soluble compounds that pass out with the wastewater (7).

A "septic tank," which appears to be modeled after the Automatic Scavenger, was constructed in Exeter, England, in 1895 by Cameron to treat about 230 m^3/day of waste water, for which Cameron was awarded a patent (7). Because of its success, the City of Exeter, in 1897, approved the treatment of the entire city's wastewater by this means.

A similar system was designed by Talbot for Urbana, Illinois, in 1894, and for Champaign, Illinois, in 1897. The Talbot design had vertical baffles reaching 0.6–1 m below the surface of the wastewater in the tank. Thus, a sort of baffled reactor is indicated. Cameron recognized the value of the methane gas produced in the septic tanks, and at Exeter, the gas was collected and used for heating and lighting at the disposal works.

In 1897, waste disposal tanks at a leper colony in Matunga, Bombay, were reported to also have been equipped with gas collectors, and the gas was used to drive gas engines (5). While septic tanks began to be used widely, the effluents were often black and offensive and contained indigestible material that clogged contact beds often used for subsequent treatment.

Clark suggested in 1899 that this problem could be reduced if the sludge was fermented by itself in a separate tank at Lawrence, Massachusetts (9). This is perhaps the first indication of a move towards separate sludge digestion.

In 1904, Travis put into operation a new two-stage process in which the suspended solids settled into a separate chamber for digestion (7, 9). Travis believed it was desirable to pass some wastewater through the "hydrolyzing" chamber, as the sludge digestion chamber was called, but this created problems with suspended solids and septic conditions in the effluent. A Travis tank began operation in Emscher, Germany, in 1905, but was modified by Imhoff to prevent wastewater from flowing through the "hydrolyzing" chamber. The sludge was allowed to stay in this chamber from a few weeks to several months, after which it was inoffensive and could be withdrawn and disposed without nuisance.

The Imhoff tank greatly reduced the cost of sludge disposal and rapidly came into favor. By the end of 1914, about 75 cities and many institutions in the United States had received licenses to use the Imhoff tank (7).

The anaerobic process was then beginning to move away from treatment of wastewaters to the treatment of settled sludge. The Imhoff tank was obviously not the complete solution to wastewater treatment and had problems that needed addressing. The tanks were tall, and the digestion chamber had to be connected intimately with the sedimentation tank. Efforts then began with separate digestion of sludges (10). This was not practically successful until 1927 when the Ruhrverband at Essen-Rellinghausen installed the first sludge-heating apparatus in a separate digestion tank (11). The efficiency of treatment greatly exceeded that available with Imhoff tanks, and separate digestion grew rapidly in popularity, particularly in larger cities.

The value of methane gas produced by digestion became more generally recognized. In addition to its use for heating digesters, it can be used for other purposes such as a medium for digester liquid mixing through biogas recirculation. In 1923, methane gas was collected on a large scale by the Emschergenossenschaft and delivered to the municipal gas system at the Essen-Rellinghausen plant (11).

In 1927, the Ruhrverband utilized the sludge gas in Iserlohn and then in Essen-Rellinghausen to generate power for a biological treatment plant, and they used the cooling water from the motors for heating he digestion tanks. Such use of digester gas is now common practice at wastewater treatment plants throughout the world.

By the 1930s, many German cities added compressing plants to store the gas in steel cylinders for use as a motor fuel (11). This practice also has been used on and off in modern times.

Along with these applications, there were many studies during the 1920s and 1930s of the separate anaerobic sludge treatment process, such that by the end of the 1930s, a sufficient understanding had developed to allow wide-scale practical applications.

During this period, the use of anaerobic process to treat wastewaters evolved together with further development to treat sludges. The following section, however, will focus solely on the history and development of industrial wastewater treatment.

2.2. Industrial Wastewater Treatment

Initial strong interest in applying the anaerobic process for industrial wastewater treatment can perhaps best be attributed to Buswell. Beginning in the 1920s, he and his colleagues conducted extensive research on the nature of the process and its potential application for treatment of industrial wastewaters and agricultural residues (5, 9, 12–16).

These important studies were hampered in application as the single tank anaerobic digester was generally used, which offered no provision for separating microorganisms from the wastewater for long residence time in the reactor. Nevertheless, Buswell's contributions to anaerobic treatment development are significant.

Recognition of the importance of solids residence time for reducing reactor size and detention time began in the 1950s, drawing from experiences with aerobic treatment in activated sludge plants and trickling filters.

One of the leaders of this new movement was Stander (17, 18). By separating the anaerobic bacteria from the effluent stream and keeping them in the reactor, he demonstrated that the detention time for efficient treatment of several different wastewaters from the fermentation industry could be lowered to 2 days, compared with the two weeks or more with conventional digesters.

Stander (19) later demonstrated the validity of these concepts in full-scale treatment of winery wastewater in an anaerobic "clarigester," which employed a settling tank over a tank reactor. This differed from the Imhoff tank in that wastewater was introduced directly into the reactor for treatment and then moved upward into the settling tank. Bacteria and other solids settled in the settling tank and were returned by gravity to the reactor, creating a long biological solids retention time.

Another approach was to use a reactor followed by a settling tank with organism recycle, similar to an activated sludge plant (20). Here, dilute packinghouse waste was treated, and organism recycle allowed reduction of the detention time to less than 1 day.

These applications and those by Stander indicated that efficient treatment of dilute industrial wastewaters was possible by anaerobic processes when solids retention time concepts were applied. These studies then led to various modifications of anaerobic reactors to achieve efficient treatment of wastewaters in general.

Since taking advantage of the principles of aerobic-activated sludge treatment (a dispersed growth reactor) appeared to work well for anaerobic treatment, many researchers in the 1960s proposed applying a biofilm reactor, which also retained microorganisms, and was also widely used for aerobic treatment. As noted with early developments, the anaerobic filter for treatment of dilute municipal wastewaters was one of the first applications of the anaerobic process. It appeared worthwhile to return to this early concept for modern evaluation.

The first large-scale application of the anaerobic filter was reported in 1972 for the treatment of wheat starch wastewater (21). The process has seen many applications since then.

Another biofilm concept was that of the expanded-bed reactor (22), which McCarty had earlier applied successfully for denitrification (23). This system is particularly suitable for very dilute wastewaters because of the large retention of microorganisms, short detention time potential, and freedom from bioclogging.

The most successful new reactor design in its broad application to a variety of industrial and municipal wastewaters, however, is the upflow anaerobic sludge blanket reactor (UASB) process conceived by Lettinga. The developments and applications of anaerobic treatment stemming from Lettinga's work have been considerable.

3. ANAEROBIC BIOCHEMISTRY AND MICROBIOLOGY

Several techniques have been developed and adapted to isolate and study anaerobic bacteria (24). The anaerobic ecosystem is the result of complex interactions among organisms of different species. Generally, there are four major stages in the production of methane and carbon dioxide from organic matter. The first stage involves hydrolysis of large organic compounds into smaller sizes. In the second stage, the smaller-sized organic compounds undergo fermentation through extracellular enzymes produced by fermentative bacteria. Acidogenesis occurs

with the formation of hydrogen, carbon dioxide, acetate, organic acids, and other organic intermediates. The third stage involves acetogenesis, in which the organic acids produced in acidogenesis are converted to acetate and hydrogen. In addition, a proportion of the available hydrogen and carbon dioxide is converted to acetate by homoacetogenic bacteria. In the final stage, methanogenic bacteria reduce the carbon dioxide and the decarboxylate acetate to form methane.

Other organisms may play an important role in the initial fermentative stages. These are termed "passenger organisms" as they do not become established in the reactor but are continuously added with the feed. The constant addition of these facultative bacteria does not significantly change the established hydrolytic anaerobic flora.

3.1. Hydrolysis

Hydrolysis and liquefaction converts complex insoluble organic compounds into smaller, simpler molecules that may be utilized as an energy source. The biopolymers protein, carbohydrate, and lipid are hydrolyzed to amino acids, simple sugars, and fatty acids, respectively, by extracellular enzymes.

Starch and cellulose are quantitatively the most important of these polymers. The genera of bacteria associated with cellulose degradation are *Bacteroides, Ruminococcus, Clostridium, Cellobacterium,* and *Butyrivibrio. Clostridium*, obligate bacteria that are strict anaerobes sensitive to oxygen, is the major group. It produces spores to survive in aerobic conditions.

Flavobacteriem, Alcaligenes, Achromobacter, and various enteric bacteria are common facultative microorganisms that have been identified in wastewater treatment systems. Cellulolytic bacteria require ammonia as a nitrogen source, cysteine and sulfides as sources of sulphur, vitamin B, hemin, menadione, and mineral salts, especially sodium.

The hydrolysis of polysaccharides, such as hemicellulose and pectin, yields hexose and pentose sugars. Starch is degraded more readily in anaerobic reactors than cellulose. Lipids are broken down by hydrolysis, 4–5% being incorporated as lipids in the bacteria. The neutral fats are hydrolyzed to long-chain fatty acids and glycerol. Long chain fatty acids are then degraded via the betaoxidation cycle.

The extracellular hydrolysis of proteins to polypeptides and amino acids is catalyzed by proteases. This usually is accompanied by the formation of ammonia, carbon dioxide, and volatile fatty acids. Deamination is done by fermentative bacteria, *Bacteriodes ruminicolai, peptococcus*, and other bacteroides species.

3.2. Acidogenesis

The end products from the first stage are converted into short-chain volatile acids such as acetic acids, propionic acids, and to a lesser extent, butyric, valeric, and caproic acids (25). Acetate is considered the most important intermediate formed from the fermentation of proteins and fats.

Hydrogen and carbon dioxide are formed as well. The final products of the acidogenic bacterial metabolism depend on initial substrate and environmental conditions, especially hydrogen partial pressure. Low hydrogen partial pressure favors the formation of acetate,

carbon dioxide, and hydrogen. High hydrogen gas partial pressure favors the formation of propionate and other higher organic acids, lactate, and ethanol (26).

Maintenance of low hydrogen partial pressures, below 0.1 kPa, has been demonstrated in cocultures of fermentative hydrogen-producing organisms and methanogenic hydrogen-utilizing organisms (27).

3.3. Acetogenesis

The third stage, acetogenesis, consists of two groups of bacteria:

1. Hydrogen-producing acetogens that catabolize organic acids, alcohols, and certain aromatic compounds into acetate and carbon dioxide.
2. Homoacetogens (or hydrogen-consuming acetogens) that use hydrogen and carbon dioxide to form acetate. Although homoacetogens are thought to synthesize only 1–2% of the total acetate at 40°C (28), their exact role is unclear.

Carbon dioxide may be reduced by hydrogen to produce acetate and subsequently utilized in methane production. Short-chain fatty acids are also produced from hydrogen and carbon dioxide. Homoacetogenic bacteria are chemolithotrophic hydrogen and carbon dioxide utilizers with high thermodynamic efficiencies.

Balch et al. (29) isolated and identified two such homoacetogenic bacteria, *Clostridium aceticum* and *Acetobacterium woodii*. Other organisms responsible for acetate synthesis from carbon dioxide include *Clostridium formicoaceticum* and *C. aceticum*. *Eubacterium limosum* is able to synthesize butyrate and acetate from hydrogen and carbon dioxide (28). Though homoacetogenic metabolism may contribute to the maintenance of low hydrogen partial pressures, hydrogen utilizing methanogens have a lower substrate constant value, K_s, for hydrogen. Theoretically, they should outcompete the homoacetogens for hydrogen at the concentrations prevalent in a stable reactor.

3.4. Methanogenesis

Methanogenic bacteria belong to the group *archaebacteria*, a phylogenetically distinct group (27). A limited number of substrates are used by the 47 known species of methanogenic bacteria.

Two major groups of methanogenic bacteria have been identified:

1. Group 1 consists of 33 species belonging to the families of *Methnobacteriaceae, Methanothermaceae, Methanococcaceae, Methanomicrobiaceae*, and *Methanoplanaceae*. These species reduce carbon dioxide and hydrogen and/or utilize formate in the formation of methane.
2. Group 2 consists of 14 species belonging to the family of Methanosarcinaceae. These species utilize acetate, methylamines, and or methanol. *M. barkeri* and *M. vacuolata* are the most versatile as they use all known methanogenic substrates except for formate.

All methanogens obtain energy for growth from the formation of methane. Most methanogenic bacteria (as shown in Group 1) can utilize hydrogen and carbon dioxide as their sole energy source (29), but few are known to split acetate (acetotrophic methanogens), for example, like those in Group 2.

The slower catabolism and growth rate of acetotrophic methanogens can limit the overall rate of reaction (30, 31), leading to toxic level accumulation of acetic acid. The degradation of acetate to methane is thought to be the rate-limiting step in the overall conversion of substrate to methane (32–34). Complex polymers and fats are the exception; here, hydrolysis is the rate-limiting step (35).

Coenzymes are specific nonprotein units required for activity of a particular protein. Coenzyme F_{420} (36) and coenzyme M (37) are unique to methanogens; both have potential for use in identification and numeration of methanogens.

4. COMPARISON BETWEEN AEROBIC AND ANAEROBIC PROCESSES

In recent years, tighter restrictions on sludge disposal site location, air pollution, hazardous waste disposal, odor control, in addition to other factors, have had a substantial impact on the applicability of aerobic treatment of industrial wastewaters. In order to manage a successful scheme for the treatment of wastewaters, the development of processes combining both a high efficiency, low construction and maintenance costs has become a major priority.

In this context, anaerobic wastewater treatment is becoming increasingly popular worldwide. In comparison with conventional aerobic treatment (38), the main advantages of anaerobic wastewater treatment are as follows:

(a) Lower treatment costs
(b) High flexibility, since it can be applied to very different types of effluents
(c) High loading rate operation, which implies smaller space requirements
(d) Smaller volume of excess sludges
(e) Anaerobic organisms can be preserved unfed for long periods of time

Several aspects in the comparison are addressed below:

1. *Reduced generation of waste biomass*

A very important issue of anaerobic technology is the significantly reduced production of excess sludges (5–20%), when compared with aerobic-based processes.

Presently, landfills for organic wastes are near the point of closure, with more limitations for agricultural applications, etc. Technologies producing smaller amounts of waste sludges will put landfills in a better situation.

2. *Higher loading rates at higher concentrations*

Anaerobic systems are also characterized by the possibility of applying higher loading rates, commonly varying from 5 to 20 kg chemical oxygen demand (COD)/m^3/day, whereas the usual loads to aerobic systems are around $0.5-3\,kg/m^3/day$. This implies a substantial reduction of the reactor volume and the available space required and, therefore, lower installation costs.

On the other hand, modern industries are achieving a high degree of reuse and recycling of process water, which results in reduced flows of the final liquid effluent to be discharged, but with higher pollutant concentrations (39). Therefore, processes based on anaerobic technologies are especially indicated for the treatment of smaller flows of highly polluted wastewaters.

The comparison between different technologies can be made in terms of the organic matter removed per unit of area required.

Conventional aerobic systems (rotating biological contactors, trickling filters, activated sludges) have typical values between 1 and 10 kg biological oxygen demand removed ($BOD_{removed}$)/m^3/day (40). Full-scale anaerobic systems, however, are included in a higher range, from 20 to 40 kg biological oxygen demand (BOD)/m^3/day.

3. *Stability and fewer operational problems*

An in-depth knowledge of anaerobic principles and applied research will make feasible the replacement of many aerobic processes, which continue to present ongoing operational (bulking, biomass washout) and disposal problems. On the other hand, most anaerobic technologies are based on immobilized biomass (by granulation or adhesion), which minimizes washout problems.

4. *Treatment of seasonal wastewaters*

An important advantage of anaerobic technology is its ability to treat wastewaters generated seasonally, such as those resulting from sugar manufacturing or the fish canning industry, which normally produce effluents only 2–6 months per year (41).

Sludges can be maintained active for long periods of time, with a small decrease in viability. The re-start-up can be performed in a short period of time. Also, the possibility of maintaining the reactor unfed during holidays or even weekends is feasible.

5. *Biotransformation and biodegradation of xenobiotics*

Recent research indicates that certain organic compounds, non-biodegradable under aerobic conditions, can be anaerobically biotransformed.

For instance, anaerobic processes can successfully dehalogenate highly chlorinated organic compounds from the pulp and paper industry (42). Other research shows that wastewaters with high levels of formaldehyde can be effectively detoxified in an anaerobic reactor, through its conversion to methanol then into methane, provided that the proper hydraulic residence time in the anaerobic reactor is used (43).

In the near future, this detoxifying capability may become a major reason for many industries to select anaerobic biotechnology. Moreover, anaerobic wastewater treatment has successfully achieved the complete mineralization of different anthropogenic compounds. Aromatic compounds such as phenols and methylated phenols (cresols) are commonly encountered pollutants in complex effluents, such as those generated by petrochemical industries, which have been reported to be degraded anaerobically (44, 45).

A review of the recent developments and latest applications of anaerobic technologies to complex effluents containing terephthalic acid, synthetic resins, carboxymethylcellulose, maleic acid, etc., has been reported by Macarie (46). In many cases, these effluents are poor in nutrients. The small quantity of nutrients required for the anaerobic biomass makes the economic impact of nutrients addition negligible.

6. *Emission of volatile hazardous compounds*

Increasingly restrictive controls are now being placed on air emissions of volatile organic contaminants from industrial production, including fugitive emissions from aerobic treatment reactors.

Many organic contaminants are volatile and tend to be air-stripped from the wastewater during aerobic treatment before they are biodegraded, thus contributing to air pollution (47). This is one of the main advantages of anaerobic technology when an overall environmental analysis is conducted.

7. *Energy efficiency*

Anaerobic treatment produces energy in the form of biogas (1.27×10^7 J/kg $COD_{converted}$), while aerobic treatment usually requires between 0.5 and 2 kWh/kg O_2, depending on the technology applied (47).

This characteristic makes energy conservation and the consequent ecological and economic benefits possible. Part of the energy may be used to heat the digester, while excess energy can be converted into electricity (1.05×10^7 J required per 1 kWh produced).

8. *A flexible technology*

The anaerobic process can be used to treat high, medium, and low strength, hot and cold, and complex and simple wastewaters. The capacity for adaptation shown by anaerobic biomass has been frequently reported as a key factor in the development of systems to treat new effluents.

The experiments carried out on the wastewaters generated by seafood canning factories showed the potential of these technologies even for the treatment of highly saline effluents (41).

Moreover, anaerobic systems range from the extremely simple, with only a control system to maintain temperature, to quite complex ones, such as the expended granular sludge bed (EGSB) systems, fluidized beds, etc., which require accurate mechanisms to control the hydraulics and the stability of the process (48).

However, there are some drawbacks in the anaerobic process. A major limitation has been the low yield and long doubling times of the methane-producing bacteria. Hence, it is generally a slower process than the aerobic process, requiring a longer start-up period. Other problems that need addressing are process reliability, as it is more sensitive to environmental factors (pH, temperatures, changing organic loadings etc), toxicity causes and effects, and a better understanding of refractory organic degradation. However, because of the increased environmental awareness since the 1970s, anaerobic treatment has gained more attention in terms of research and technology development. This resulted in a better understanding of the complex microbial processes and in a number of improvements of the technology. The successful cultivation and retainment of biomass in the reactor via immobilization or granulation appeared to be the key to developing a better system. Research towards an even broader application is clearly of importance and appears to be headed in a promising direction to overcome the limitations associated with the anaerobic treatment process.

5. GLOBAL APPLICATIONS OF ANAEROBIC TREATMENT

Anaerobic technology is now being employed in more than 65 countries, and a total of approximately 1,400 plants were built by the 16 leading vendors of such systems. These plants account for approximately 65% of the total number of anaerobic treatment plants for industrial applications, which is estimated to be around 2,000. From a database analysis of 1,215 plants, it appears that the UASB technology, as originally developed in the Netherlands, is the most predominant process. The higher capacity EGSB type systems are also gradually replacing at least some of the UASB applications.

The following section presents a historical overview of the various processes, their application areas, and geographic distribution.

5.1. The Number of Anaerobic Treatment Plants Installed Worldwide

Information on the implementation of anaerobic technologies used for the treatment of municipal and industrial wastes and wastewaters was collected (49). A detailed overview of the anaerobic systems currently used for the worldwide treatment of industrial wastewaters was reported (50).

Developing countries such as India and Brazil have installed extremely impressive numbers of anaerobic treatment plants. The potential for anaerobic treatment has not yet been fully exploited yet in China, the largest country in Asia.

Netherlands, being the homeland of Lettinga, and one of the greatest contributors in the advancement of anaerobic treatment process, shows its support in building a large number of full-scale anaerobic plants.

The data below shows that the anaerobic technology is accepted in both industrialized and less developed countries.

A database on cumulated number of anaerobic treatment plants installed by the vendors (ADI, Biothane, Degremont, Grontmij, Kurita, Paques, Proserpol, Purac and VA TECH) was compiled. The total number was 1,215 installed in 65 different countries in 2000. The data shows that anaerobic treatment plants were not at all favorable before 1970. However, with increasing understanding and acceptance towards this process, a growing interest and belief in the anaerobic application to treat industrial discharges has resulted in an exponential growth rate in the anaerobic plants installed over the last three decades.

The total number of plants built was ± 100 per year from l992 to 1997, which is a phenomenal rate. It appears that the number of plants built has stabilized in Europe to a level of 20–30 per year, while in North America the number seems to be decreasing since 1997, from its peak of 13 per year in 1988 to 6 per year in 2000. Given the size of the North American market and its industrial activity, it may be concluded that the potential for anaerobic treatment has not yet been fully exploited in this region. The number of anaerobic plants built in South America has increased to 84%. This is a significant increase relative to the percentage of 76% over the period from 1990 to 1996.

5.2. Types of Anaerobic Treatment Plants Installed Worldwide

The key to the successful application of anaerobic treatment is to un-couple the hydraulic retention time (of wastewater) and the solids retention time (of active biomass) in the reactor system.

In order to achieve high system loading rates, short hydraulic retention times should be applied, while at the same time maintaining positive net solids (biomass) retention. Thus, various reactor designs were developed over the past two decades that are based on various ways of retaining biomass within the reactor system.

5.2.1. Anaerobic Contact Process

The anaerobic contact process utilizes a clarifier to settle out biomass solids external to the actual bioreactor. These solids are subsequently recycled back to the reactor, hence increasing biomass retention time. This system involves bulk volume tanks and is generally considered not competitive except in those cases of treatment of high solids or high fat/protein content wastewater. Purac from Sweden and Biothane from the Netherlands are active promoters of this process for specific applications.

5.2.2. Upflow Anaerobic Sludge Blanket

The UASB design as originally proposed by Lettinga was one of the earliest systems to rely on the establishment of a granular biomass (51). Due to the excellent settling characteristics of this granular biomass, good sludge retention is assured also by virtue of specially designed three phase (biogas, water and biomass) separators. This technology originates from the Netherlands and is promoted by companies such as Biothane (from The Netherlands), Biotim (from Belgium), Grontmij (from The Netherlands), Haskoning (from The Netherlands), Kurita (from Japan), and Paques (from USA).

5.2.3. Fixed Film or Anaerobic Filter

Immobilizing biomass on a fixed carrier is an alternative method of retaining biomass. One common system in use is the fixed film system (sometimes referred to as fixed bed) in either upflow or downflow mode. The apparent disadvantage of this system is the cost associated with (bulky) carrier material and its relatively low loading potential. The system is successful in operation, e.g., on chemical wastewater (52). Proserpol from France actively promotes this process.

5.2.4. Fluidized Bed System

The Fluidized Bed (FB) system, developed in the early 1980s, uses the phenomena of immobilization of biomass on a fluidized carrier material (like sand, basalt, pumice and the like). The problems encountered in this system were based on excessive growth on the carrier under mild shear conditions (top part of reactor) and no growth on the carrier under high shear conditions (low part of reactor) necessarily required to fluidize the carrier. The last FB biomass on carrier system build was by Degremont in 1996. Nowadays, the biomass on carrier system seems to be disappearing from the marketplace.

5.2.5. Hybrid

The hybrid system combines both features of the fixed bed system (in the top of the reactor) as well as the UASB system (use of granular biomass). ADI is actively promoting this process, e.g., for chemical applications.

5.2.6. Expanded Granular Sludge Bed and Internal Circulation Systems

The latest generation of anaerobic treatment system is the EGSB process (53) and Internal Circulation (IC) process (54). To cope with the aforementioned problems with the FB system, Biothane turned the FB system, initially developed by Gist brocades (55), into an EGSB system (56), making use of a granular biomass rather than fixing to a carrier. This system uses a granular biomass, which is expanded by gas and hydraulic forces. Biothane actively promotes it as the Biobed process.

5.3. Scope of Industrial Applications

The anaerobic treatment process has been successfully applied in various types of industrial wastewaters ranging from food wastewaters to chemical wastewaters. Food, breweries, and beverages industries have the largest share of anaerobic treatment plants built to treat their wastewaters (50). The data presented also affirms that anaerobic treatment is an established technology for a wide variety of industrial applications.

The fact that chemical wastewater can be treated via the anaerobic process is quite significant. Recent advances in chemical technology have led to the production of many and new potentially dangerous compounds called xenobiotics in wastewater streams. Each of these new substances, representing a wide array of compounds ranging from phenols to pesticides, presents its own individual problems when dealing with its ultimate disposal. New and innovative techniques have been introduced for the treatment of these wastes, many of which employ anaerobic processes.

Brewery, beverages, distillations, and fermentation wastewaters present problems similar to those of sugar processing, namely generation of high organic strength wastewater. These wastewaters are composed of the residues of the fermented material that contributes to a high BOD as high as 25,000 mg/L after the alcohol has been distilled off. Anaerobic processes have been used successfully to treat these wastewaters, thus explaining the large percentage (54%) of anaerobic applications in this area.

Food processing industries cover a diverse scope from fish processing, pear, and pineapple wastes to bean-blanching wastes. The anaerobic treatment process has proven to have encouraging results. Again, 32% of the anaerobic process plants, almost one-third, are dedicated to treat food wastewaters.

5.4. The Development of UASB and EGSB

The granular sludge-based processes UASB and EGSB comprise a large portion of applications. Although UASB still is the predominant technology in use, at present, ESGB type processes are gaining more popularity driven by economics.

The database (50) indicates that the design load for EGSB systems is approximately double that of the UASB process, which results in a competitive advantage over lower loaded systems.

It should, however, be noted that the data presented represents approximately 50–60% of total anaerobic systems installed, and contribution of EGSB and IC systems may be relatively high in the current database relative to total number of systems installed. It follows that the average (design) loading rate for the 198 EGSB plants in the database is somewhat over 20 kg COD/m^3/day. This is two times higher than the average loading rate for 682 UASB, which is 10 kg COD/m^3/day.

The higher design loading rates determine lower cost for reactors, which contributes to the overall cost competitiveness of the process.

6. APPLICATIONS OF ANAEROBIC PROCESSES FOR INDUSTRIAL WASTEWATER

6.1. Anaerobic Fluidized Bed Reactor

Very few industrial wastewater plants have been built using the fluidized bed technology. However, the advantages of this technology must be highlighted here: high concentrations of biomass on dense supports, easily retained within the reactor vessel, are achieved; a large area of mass transfer is generated through small size fluidized support particles; and high velocities of fluid flow allow the treatment of very diluted wastewater or high recycle ratio, which create adequate alkalinity conditions and resistance to sporadic organic overload.

Anaerobic fluidized bed reactor (AFBR) was developed from the extension of the fixed film concept (22). With this development, clogging of the anaerobic filter was prevented by attaching the biomass to the mobile particulate surfaces to form a biofilm. Sand is the most common biomass support particle used, but gravel, plastic, and even granulated carbon have been considered.

A high surface area to volume ratio is created by the small particles; this enables the reactor to maintain a large active mass of attached microorganisms at high liquid flow rate. Wastewater passes upwards through the bed of attached microbial sludge, along with recycled effluent, at a rate sufficient to cause fluidized and biomass settlement.

However, the main disadvantages of the AFBR are power requirements for fluidization, and the close monitoring and control required for such a highly engineered process.

The most recent development in the study of AFBR is in the design and its startup (57). An investigation was conducted to find the optimum conditions for a 10-L ABFR to work. Beach sand was used as the solid support for the biomass. The system attained a COD removal of over 85% for a recommended organic loading rate (OLR) of 3.4 kg COD/m^3/day. A removal efficiency of 92% was obtained at an OLR of 1.04 kg COD/m^3/day, with a hydraulic retention time (HRT) of 12 h. This is a very good COD removal efficiency (between 82 and 92%), and this compares very well to the maximum biodegradability of the inflow, which is 95%. The best gas production of 1.8 m^3_{biogas}/m^3/day is equivalent to a production of 0.16 m^3/kg COD removed. Using ethanol as the carbon source enabled the best start-up results.

Low concentration synthetic and municipal wastewaters could be treated in an anaerobic inverse fluidized bed (58). Both bioreactors showed gas hold up due to the liquid down flow pattern of the prototype. The bioreactor had a removal efficiency of 83%, specific activity of 4.5 kg $COD_{removed}$/kg Immobilized Volatile Solids (IVS). The reactor treating

municipal wastewater had a removal efficiency of 44% while the specific activity was 4.2 kg $COD_{removed}$/kg IVS. The biomass concentration was 13.8 and 1.1 kg IVS/m^3 for synthetic and municipal wastewaters, and the scanning electron microphotographs (SEM) showed a bacterial diversity for the first run and only cocci cells for the second run. The system does not remove suspended solids, so a polishing posttreatment to improve water quality must be implemented.

While the AFBR is still in its infant stages of development, the great potential of the system can be explored further.

6.2. Upflow Anaerobic Sludge Blanket Reactor

The sludge blanket concept was first used in the Reversed Flow Dorr Oliver clarigester, which is a modified version of the contact process. Unlike the contact process, it is unmixed, and feed flows upward through a dense bed of flocculated bacteria. Flocs collect in the settling compartment and return to the reactor by gravity. The Dorr Oliver clarigester improvements due to the biomass loss in effluent led to the Upflow Sludge Blanket process and the UASB process. The UASB has an integrated gas solids separator to help retain sludge, and mechanical agitation is minimized or omitted.

UASB reactors have been applied to a wide range of industrial wastewater, including those containing toxic or inhibitory compounds. The process is also feasible for treating domestic wastewater with temperatures as low as 14–16°C or lower.

Since the early 1980s, considerable research and development has been undertaken with respect to anaerobic wastewater treatment systems and, specifically, UASB. Reductions in BOD of 75–90% have been noted in tropical conditions. The UASB technology is feasible in an urban, developing world context because of its high organic removal efficiency, simplicity, low-cost, low capital and maintenance costs, and low land requirements. Anaerobic treatment processes are suitable in tropical conditions because anaerobic treatment functions well in temperatures exceeding 20°C.

Anaerobic wastewater treatment systems are characterized by low sludge production and low energy needs. The UASB is typically constructed with entrance pipes delivering influent to the bottom of the unit and a gas solids separator at the top of the reactor to separate the biogas from the liquid phase (water and sludge) and of sludge from the water phase; overall, this prevents sludge washout.

The UASB process was first described in an international journal with studies on the treatment of methanol with laboratory UASB reactors (1), and its potential for dilute wastewaters in general was described (59, 60). Subsequently, many reports by Lettinga and his colleagues emerged on the application of the UASB process for treatment of a variety of wastewaters including those from sugar beets (61, 62), piggery (63), alcohols (1), fatty acids (64–67), slaughterhouse (68, 69), potato starch (70), milk fat (71, 72), and pulp and paper wastes (73–75).

Of growing interest is the application of the UASB process for the treatment of domestic wastewaters, which was clearly demonstrated as feasible (76–80). The following section highlights the most recent full-scale and pilot-scale findings in the anaerobic treatment of industrial wastewaters.

A control system was developed to manage the start-up of an UASB reactor, using a reduced number of process variables (81). Two different start-up strategies were applied: fed-batch and continuous operation. In the fed-batch mode, results show that starting from an OLR of lower than 0.5 kg COD/m^3/day, a load of higher than 8 kg COD/m^3/day was achieved in only 33 days, and the COD removal efficiency was over 90%. In the continuous system, results show 24 h of an excellent value, and also, starting from an OLR of lower than 0.5 kg COD/m^3/day, a load of 9–12 kg COD/m^3/day was achieved in 40 days, and the COD removal efficiency was over 95%. Comparing the standard deviation of the process parameters, the fed-batch mode had a better process efficiency. However, the continuous mode has a better capacity to treat the organic load by enabling the system to operate at a more stable influent ORL. This is especially useful during the first 2 weeks of the start-up phase.

The microbial mechanism of thermophilic granulation and sludge retainment during start-up was studied (82). Development of well-settleable granular sludge was the key factor for successful operations of the UASB process. Inoculum was taken from thermophilic digested sewage sludge. At an operating temperature of 55°C, the feed solution was composed of sucrose, yeast, and volatile fatty acids, which are acetate and propionate. Results showed that the granule's sludge volume index (SVI) finally settled at about 13 mL/g volatile suspended solid (VSS) upon maturation of the thermophilic granules. As a result of establishment of the whole granulated sludge bed, the reactor allowed a maximum volumetric COD loading of 45 kg COD/m^3/day with a COD removal efficiency of 90%. The maximum sludge loading achieved was 3.7 gCOD/gVSS d, which is 2–3 times that of sludge grown under mesophilic conditions. Both acetate and hydrogen utilizing methanogenic activities exhibited their optima at 65°C, while that of propionate fed methanogenic activity was at 50°C. Methanogenic activities of the retained sludge increased finally up to 110 times for acetate, 25 times for propionate, and 3.6 times for hydrogen, when compared with those of the seeded sludge. This relatively low value for propionate implies that the propionate degradation is most likely to be a rate-limiting step in the thermophilic anaerobic process.

Another way to encourage a fast start-up is to adjust the Microbial Load Index (MLI) values (83). Findings show that under high MLIs of 0.8 and 0.6, granulation developed well in 3–4 months of operation, providing for a fast start-up. However, with low MLIs of 0.3 and 0.2, there was still no granulation after 6 months. Three phases were observed during the process of granulation, namely acclimation, granulation, and maturation. A stepwise and gradual increment in the sludge loading rate (SLR) must be followed to avoid overloading or starving at different stages.

In its early development in the 1980s, UASB had been used to treat food industry wastewaters such as beet sugar, corn, and potato starch processing. Recent studies showed that UASB can be applied in treating wastewaters containing concentrated proteins (84) and aromatic compounds such as phenol (85). Changing the rate of effluent recirculation is widely used to prevent toxic impact to the working microorganisms. Recirculation, together with biogas production, results in higher superficial upflow velocity that causes the washing out of biomass. Low hydraulic loading rate on the treatment of wastewater containing high concentration of phenol using a Recirculated UASB (RUASB), operating under mesophilic condition (35°C) has been encouraged (86). As the hydraulic loading rates decreased from 2.5 to 1.6 m/h, the relative bacterial activity also decreased from 80% to 50%.

Granulation is generally a slow process, but it is a prerequisite of the optimum performance of UASB-like reactors. Use of polymers enhanced the anaerobic granulation process (87). Chitosan, natural polymer, outperformed Percol 763, a synthetic polymer in terms of granules formation rate. Chitosan yielded a granulation rate as high as 56 m/day, compared to 35 m/day with Percol 763 in acidic pH. Under alkaline conditions, chitosan is progressively neutralized, thus resulting in a less effective flocculation of suspended sludge. The high granulation yield of chitosan was most probably attributed to its polysaccharides structure, acting similarly to the extracellular polymeric substances (EPS) in aggregating anaerobic sludge.

Competition exists between methanogenesis and sulfidogenesis in anaerobic wastewater treatment (88). High concentrations of sulfate in wastewater can adversely affect the methane production in anaerobic treatment processes. Sulfidogens degrade substrates into bicarbonates and intermediates in the process of reducing sulfate to sulfide. Sulfidogens and methanogens coexist in many anaerobic ecosystems as they have similar physiologies. They are strictly anaerobes and in favor of similar optimum temperature and pH. Results showed that after acclimation, a benzoate removal efficiency of 99.5% was consistent regardless of the sulfate concentrations. Sulfidogenesis slowly outcompeted methanogenesis during the acclimation phase. This was indicated by the increased sulfate reducing efficiency of from 48% up to 99% while it was accompanied by the decrease in methane production from 1.02 to 0.39 L methane/L.d.

Supplement glucose improves the anaerobic degradation of phenol (89). Phenol is present in the wastewater of some industries, like coal gasification, coke production, pharmaceutical, pesticide, fertilizer, dye manufacturing, synthetic chemical, and pulp and paper. The maximum concentration of phenol could go as high as 6,000 mg/L, which is toxic to living aquatic organisms. Glucose is used as a co-substrate to achieve effective and constant anaerobic biodegradation of phenol. Phenol can be degraded to methane and carbon dioxide through phenol metabolizers and hydrogen-utilizing and acetotrophic methanogens. The phenol removal efficiency was also the best at 98%, compared with 88% without the supplement glucose. Moreover, it also exhibited greater resistance to those adverse conditions and the system recovered faster than the other system without the glucose supplement.

The results of the pilot study, together with the results from the intensive laboratory studies, suggest the feasibility of thermophilic anaerobic treatment for food industry wastewaters (90). The reactor was operated at 55°C and placed on the premises of a factory manufacturing deep-frozen goods from vegetables. The hot (greater than 80–90°C) and concentrated (COD 14–79 g/L) wastewater streams, deriving from steam peeling and blanching of carrot and potato were used. More than 80% COD removal was achieved.

Removal of chlorinated phenols (CP) is possible in UASB reactors (85). Halogenated organic pollutants are labeled as toxic and recalcitrant in the environment. Effluents containing CPs and related compounds are especially problematic to treat due to their persistence and their high solubility in fat. Once introduced into water ecosystems, accumulation within river sediments and bioaccumulation within the tissues of organism have been observed. CP compounds were able to be metabolized to mineral end products to a large extent at loading rates where the reactor's performance was not hindered. There was no accumulation of phenol in any of the reactors in the experimental conditions.

Treatment of polyethylene terephthalate (PET) wastewater with UASB is proven feasible in full-scale application (91). PET is generated by the direct esterification of terephthalic acid (TPA) with ethylene glycol. The raw material of highly purified TPA is readily available in the market. Wastewater from the esterification process is composed of mainly unreacted raw material, largely ethylene glycol, and products of the secondary or degradation reactions, such as terephthalic acid esters, methanol, acetaldehyde, and crotonaldehyde being the major part. There is also another wastewater stream, called the second stream, from the melt spinning process where a bath of chemicals is showered to improve the physical characteristics of the fiber. Anaerobic biodegradability was 90% and 75% for esterification wastewater and second stream wastewater, respectively.

Anaerobic treatment of wastewater from a fish-canning factory has also been proven feasible in a full-scale UASB reactor (92). The wastewater comes from 2 main streams, mussel cooking, which is seasonal, and tuna cooking. Most of the organic load from mussel cooking wastewater is composed of carbohydrates (74.5%), while that of tuna cooking wastewater has a significant percentage of fat (23.5%) and protein (73.0%). Thus, the high fluctuation in wastewater characteristics causes a high variance in the reactor's efficiency. However, performance is improved when a mixture of both streams is treated due to the high degradable carbohydrate content of the mussel cooking wastewater. Through alkalinity control, it is possible to operate the system properly with a COD removal between 70 and 90% for influent ranges from 2 to 8 kg $COD/m^3/day$.

UASB technology can also be used to treat crab-processing wastewaters (93). Crab cooker wastewater contains high concentrations of COD, total suspended solids (TSS), and total Kjeldahl nitrogen (TKN). Using the UASB process, the BOD_5 and COD removal efficiency was over 90%. Acidification of the feed wastewater improves treatment as it reduces the concentrations of the wastewater feed suspended solids.

It is feasible to treat tapioca starch industry wastewater effectively (94). After removal of suspended solids by simple gravity settling, starch wastewater was used as a feed. COD conversion efficiencies that are greater than 95% and gas productivity of 5–8 $m^3_{biogas}/m^3/day$ were obtained. Removal of starch solids from wastewater by simple gravity settling was sufficient to obtain satisfactory performance using the UASB process.

6.3. Upflow Anaerobic Filter

In the upflow anaerobic filter (UAF), wastewater passes through a packed bed with retained biomass. A high proportion of the biomass (50%) is held in the interstitial spaces of the film rather than being firmly attached to the media surface. Soluble organic compounds in the influent wastewater are consumed by microorganisms in the biomass and converted to methane and carbon dioxide. UAF is used especially for treatment of high strength industrial wastewater. UAF has reasonable tolerance to organic load shocks and temperature variance. This is because the biomass attached to the support hinders biomass washout.

While UAF is effective in treating high organic loadings, its disadvantages should not be overlooked: reduced reactor volume of 10–40% due to support material, presence of dead zones, and channeling due to the accumulation of solids especially in the lower part of the reactor, and clogging especially at high OLR of substrates, such as carbohydrates.

Moreover, the stratification of trophic groups, which makes the hydrolytic, acetogenic, and methanogenic activities concentrated at certain area, decreases the operation efficiency, minimizing substrate–biomass contact. Wastewater with a high solids concentration has a greater tendency to cause filter blockage.

Hybrid anaerobic filters were invented to improve the performance of the UAF. One hybrid filter consists of a lower zone that is an upflow anaerobic sludge blanket and an upper zone that is the UAF. Another hybrid uses 2-stage modular anaerobic filters.

Conventionally, a UAF reactor is single-fed at the lower part. A multifed reactor (MFR) system is more efficient than single-fed reactor (SFR) system in terms of COD removal and stability to resist hydraulic and organic overloads (95). Due to thorough mixing in the liquid phase (hydrodynamic behavior of multifed influent entry), a more homogeneous bacterium consortium was developed throughout the MFR. That means the specific methanogenic and nonmethanogenic activities were similar from the bottom to the top, and the worry of stratification had been minimized. The biomass developed was much smaller but much more active in the MFR. The advantages of MFR over SFR include: no recycling flow is needed to increase mixing in the MFR; and a working volume of more than 85% in the MFR, compared with only 65% in the SFR. However, two rooted drawbacks are still associated with the use of UAF; preferential paths and the clogging of the bed, and the relative lower efficiency at the upper part of the reactor.

A nutrient removal plant configuration was successfully designed and tested in pilot-scale for the treatment of piggery wastewater (96). The core of the process is represented by a hybrid upflow anaerobic filter in which both anaerobic digestion and denitrification takes place. The anaerobic reactor is slightly overloaded in order to provide volatile fatty acids (VFA) for denitrification. The effluent of the anaerobic reactor is fed to the following phosphorus-removal stage, which is composed of a sludge predenitrification step, an anaerobic phosphorus-release step, an aerobic nitrification tank, and a final settler. The overall plant removal efficiency was around 96% for COD, 92% for nitrogen, and 92% for phosphorus. The anaerobic digester contributed 80% to the overall denitrification capacity.

Tuna processing wastewater was treated with an anaerobic filter (AF) and downflow stationary fixed film (DSFF) reactor (97). The AF removed up to 75% of the influent COD concentrations, whereas the DSFF reactor removed 70%. Thus, AF shows a much better performance, allowing higher organic loadings and COD removal efficiencies than the DSFF reactor.

The anaerobic treatment of cheese whey is also applicable (98) with a system consisting of a continuous stirred tank reactor as the acidogenic reactor and an upflow anaerobic filter as the methanogenic reactor. The acidification rate increased up to a maximum of 50%. A 90% soluble effluent COD removal efficiency was obtained with an outmost biogas yield of $0.55\,m^3/kg\,COD_{removed}$.

UAF has also been successfully applied to treating dairy wastewater (99). Microscopic examination showed that the number of autofluorescent methanogens varied from 15 to 28% of the number of total bacteria along the upflow anaerobic filter, while the number of viable methanogens was 10–70 times less than that of autofluorescent methanogens. The most dominant species was found to be *Methanococcus* followed by short rods, medium rods, long

rods, filaments, and *Methanosarcina*. The quantity of attached biomass was the highest at the bottom and lowest at the top. An average of 50% reduction in compressive strength of the sintered glass media was measured after 8 months of operation. An average of 80% COD removal efficiency was achieved for most of the operating period.

6.4. Anaerobic Fixed Bed Reactor

The effluent water quality is improved remarkably by packing of the filter media (100). Packing of the filter media promotes the degradation of insoluble matter as well as soluble matter of the influent. Insoluble matter (cellulose) in the influent does not accumulate in the interstitial space of the filter media and on the surface of the filter media, but it acclimates in the lower part of the reactor. Packing of the filter media promotes the accumulation of lipolytic bacteria, acetate-consuming methanogenic bacteria, and hydrogen-consuming methanogenic bacteria in the space where filter media were packed. Hydrolysis reaction of cellulose is promoted by packing of the filter media.

The relationship between the filter media and the behavior of anaerobic bacteria was also studied using anaerobic fixed-bed reactors (101). The number of suspended acidogenic bacteria was higher than those attached to the filter media. On the other hand, the number of attached methanogenic bacteria was more than ten times as higher than that of suspended ones. Decreasing the HRT of the reactor promoted the accumulation of attached bacteria. The number of acidogenic bacteria in the reactor packed sparsely with the filter media was higher than that in the closely packed reactor. The number of methanogenic bacteria in the sparsely packed reactor was lower than that in the closely packed reactor.

Kozariszczuk and coworkers (102) recommended interdisciplinary cooperation between microbiologists and chemical engineers in the development of small anaerobic wastewater treatment plants. Microbiological parameters should be added to the conventional parameters of process engineering because microorganisms play a major part in the success of treatment process. With modern methods from molecular biology, such as fluorescence in situ hybridization (FISH), quantification of uncultured microorganisms can also be achieved. Moreover, the assessment results using modern microbiological methods are fast and usually available within hours. The results of these measurements lead to a better understanding of the correlation between changing process parameters and the state of the microbial population. The incorporation of microbiology enhances the maintenance of a stable anaerobic treatment process.

An anaerobic fixed-bed reactor immobilized with *Clostridium bifermentans* DPH-1, a strict anaerobe and perchloroethylene (PCE) dechlorinating bacterium, can effectively remove tetrachloroethylene (103). Ceramic beads provided a large surface area for the development of a cell mass in the column. The volumetric degradation rate was relatively higher than those of other reactors. In order to maintain the efficiency of PCE dechlorination, 20 h or more HRT in the reactor system was required.

6.5. Anaerobic Baffled Reactor

The most common bioreactor type used for anaerobic digestion is the Continuously Stirred Tank Reactor (CSTR). The main problem of this reactor type, i.e., the fact that the active biomass is continuously removed from the system leading to long retention times, has been

overcome in a number of systems based on immobilization of the active biomass. Two representative types are the UASB and the anaerobic baffled reactor (ABR). The success of these reactor systems rests on the highly flocculated, well settling, compact methanogenic sludge granules that develop in these reactors.

A novel reactor type named Periodic Anaerobic Baffled Reactor (PABR) has been designed (104) and offers the following major advantage: it may be operated as an ABR, a UASBR, or at an intermediate mode. The PABR hydraulic behavior has been characterized using residence time distribution experiments at different retention times. Simulating the PABR behavior, the dependence of the reactor performance on the switching frequency is determined as a function of the retention time. In particular, it is found that for high retention times, the ABR mode is superior, whereas for low retention times, the UASBR mode is preferred. In order to establish the accuracy of the predictions of the simulation study, the PABR behavior was experimentally verified using three different stable periodic states.

The coupled anaerobic/aerobic process can be used to treat high-sulfate wastewater with sulfate reduction and biological sulfide oxidation (105). Pharmaceutical wastewater with a COD concentration of 40,000 mg/L and a sulfate concentration of 5,000 mg/L was effectively treated with an anaerobic baffled reactor. COD removal efficiencies were greater than 50% and the conversion of influent sulfate was greater than 95% with effluent sulfide concentrations of less than 20 mg/L. The major product observed from degradation of isopropyl acetate was acetic acid. Coupled anaerobic/aerobic removed sulfur from the wastewater stream and helped to stabilize the pH in the reactor system.

Palm oil mill effluent can also be treated by anaerobic baffled reactors (106). A recycle of more than 15 times is needed to maintain the system pH higher than 6.8 without alkalinity supplementation, and the imposition of recycle is an effective means to reduce alkalinity requirements. Moreover, the kinetic model for substrate utilization and methane was able to show the domination of certain culture in anaerobic processes. The model was also found to predict the experimental data of the present study with good accuracy.

Wool wax in the wool scouring wastewater can be degraded using an anaerobic baffled reactor (107) in a full-scale plant. COD efficiencies ranged between 72% and 47%. No inhibition by long-chain fatty acids was observed. Considering the results of grease content determination and Thin Layer Chromatographic (TLC) analysis in both reactors, it could be assumed that wool wax is hydrolyzed forming sterols and free fatty acids, and that free fatty acids are degraded while sterols are accumulated in the sludge.

As the latest development, split feed anaerobic baffled reactor (SFABR) shortens the start-up period and gives a higher process performance (108), when using improved seed material, even for the treatment of particularly problematic wastewater, i.e., ice-cream wastewater. SEM revealed that the granulation process occurs relatively rapidly in the SFABR compared with other reactor configurations, and that the reactor contained a highly mixed population of methanogens in all compartments. The use of polymer-conditioned anaerobic sludge and granular sludge as seed proved advantageous over the use of suspended growth anaerobic sludge, and the "improved" SFABR consequently performed more efficiently and showed greater stability than the conventional ABR.

6.6. Expanded Granular Sludge Bed Reactor

The start-up of the anaerobic sludge bed (and in particular the EGSB) reactor systems is rapid, within a few days with granular seed sludges, and may be applied across a wide range of conditions and strengths of wastewater (109). EGSB systems are particularly suited to low temperatures (10°C) and very low strengths (very much smaller than 1,000 mg/L) and for the treatment of recalcitrant or toxic substrates.

New insights into the anaerobic degradation of very different categories of compounds, and into process and reactor technology will lead to promising new generations of anaerobic treatment systems (110). These concepts will provide a higher efficiency at higher loading rates, and will be applicable for extreme environmental conditions (e.g., low and high temperatures) and to inhibitory compounds. Moreover, by integrating the anaerobic process with other biological methods (sulfate reduction, microaerophilic organisms) and with physical–chemical methods, a complete treatment of the wastewater can be accomplished at very low costs, while at the same time valuable components can be recovered for reuse.

The anaerobic treatment of chemical and brewery waste water with a new type of anaerobic reactor; the biobed® EGSB reactor, has been very effective (111). The new ultra high loaded type of anaerobic reactor is in full-scale implementation to treat the wastewaters from the chemical industry and the brewery. The chemical factory involved is Caldic Europoort in the Netherlands, which produces formaldehyde from methanol. The wastewater is characterized by high concentrations of these compounds (formaldehyde till 5 g/L and methanol till 10 g/L). Due to the special configuration of the employed anaerobic reactor, it is possible to acquire removal efficiency for both compounds of more than 99%. At the brewery involved, the Biobed® reactor is installed before an existing aerobic treatment. Here, the reactor serves as "COD remover," which results in a decreased COD load to the aerobic posttreatment, causing lesser sludge production and lesser energy consumption. It is possible to treat wastewater containing toxic but degradable chemical compounds.

The Biobed® EGSB system addresses the shortcomings of the upflow anaerobic sludge blanket reactor in the chemical industry (53). The most striking feature is the growth of biomass in a granular form, similar to the UASB granules: no carrier material is used. The process is especially suitable for treating wastewater that contains compounds that are toxic in high concentrations and that can be degraded only in low concentrations (chemical industry). It is also possible to operate the reactor as an ultra high loaded anaerobic reactor (to 30 kg COD/m^3/day) for applications in other sectors of industry (e.g., brewery, yeast, sugar, corn ethanol production, etc.,).

Psychrophilic (8°C) anaerobic treatment of partly acidified waste water is also applicable using an EGSB system (112) as the average $COD_{soluble}$, and VFA–COD removal efficiencies were 97% and 90%, respectively. Besides, psychrophilic (2–20°C) anaerobic treatment of low strength synthetic and malting wastewater is also possible (113). The COD removal efficiencies found in the experiments exceeded 90% in the single module reactor. When a two-module EGSB system was used at the temperature range 10–15°C, soluble COD removal and volatile fatty acids removal of 67–78% and 90–96%, respectively, were achieved. The mineralization of anthracitic acid as the only carbon and energy source is possible at low influent concentrations (114).

Mesophilic conditions (35°C) appear to be a feasible option for treating slaughterhouse wastewater in an EGSB system (96). The average COD removal efficiency was 67%. Total suspended solid (TSS) was 90% removed. Fats were 85% removed and no accumulation of fats was observed on the sludge. The specific methanogenic activity of the sludge was about 3 times higher than that of the sludge inoculated into the reactor. Sludge activity did not change significantly after 1 year of operations.

Thermophilic sulfite and sulfate reduction offer good prospects as part of an alternative technology to conventional off-gas desulfurization technologies (115). Methanol can be efficiently used as an electron and carbon source to obtain high sulfite and sulfate elimination rates in thermophilic bioreactors.

In Germany, there are currently 125 full-scale anaerobic treatment plants treating industrial wastewater (beet sugar, starch, pectin brewery, distillery, vegetable, potato processing). The first EGSB reactor at a German potato processing factory as well as the first municipal wastewater treatment plant combined with a separate anaerobic stage to successfully treat a wastewater mixture from several small factories (116).

The behaviors of EGSB and UASB reactors in dilute (e.g., ethanol, diluted beer) and concentrated (e.g., coffee) Wastewater Treatment has been compared. There were no big differences in the removal rates during the operation with coffee wastewater. Probably, in this effluent, the process is limited by the reaction kinetics instead of by mass transfer, because of the complex nature of the waste. With diluted beer, EGSB reactor yields a better performance than the UASB (117).

6.7. Hybrid Anaerobic Reactors

A model has been developed for the simultaneous removal of trichlorfon, with glucose added as carbon source for degradation requirement of trichlorfon, in a hybrid bioreactor (118). The hybrid bioreactor has both suspended and magnetically immobilized biomass. The respective roles of these two types of biomass were evaluated with a mathematical model, which also verified well with experimental results. It was found that the suspended biomass plays a key role in removing both substances in the system. This is due to the coexistence of both trichlorfon-degrading and glucose-removing bacteria in each type of the biomass. Such a system would be applicable to the treatment of complex industrial wastewaters that contain easily biodegradable organics as well as refractory pollutants.

The use of an upflow anaerobic hybrid blanket (UAHB) reactor has been proven to provide better stability in treating fermentation process wastewater consisting of high sulfate and ammonia, when water-absorbing polymer particles (WAP) are added (119). The granules could be developed in an UAHB process, in which a filter is installed in the upper part of reactor and WAP are also added into inoculum, for treating sulfide- and ammonia-rich wastewater.

An industrial pilot scale treating wastewater from a fiberboard-processing factory with an anaerobic hybrid UASB–UAF bioreactor was monitored with an advanced data acquisition and control system, called Programmable Logic Controller (PLC), which manages the on-line data acquisition, monitoring, and supervision (92). Monitoring of CO concentration did not permit the prediction of destabilization of the bioreactor. However, H_2 concentration is quite a sensitive variable, which must be analyzed together with other parameters such as methane

composition or gas flow-rate. Besides, alkalinity is easy to measure and provides immediate information about the state of the plant, as was shown through the off-line measurements.

Anaerobic thermophilic (55°C) treatment of thermomechanical pulping whitewater (TMP) in reactors based on biomass attachment and entrapment was studied (120) using three different reactor configurations. In all reactors, up to 70% COD removals were achieved. The anaerobic hybrid reactor, composed of an UASB and a filter, gave degradation rates up to 10 kg COD/m^3/day. The anaerobic multistage reactor, consisting of three compartments, each packed with granular sludge and carrier elements, gave degradation rates up to 9 kg COD/m^3/day. Clogging and short-circuiting eventually became a problem in the multistage reactor, probably caused by excessive packing of the carriers. The anaerobic moving bed biofilm reactor performed similar to the other reactors at loading rates below 1.4 kg COD/m^3/day, which was the highest loading rate applied. The use of carriers in the anaerobic reactors allowed short HRT with good treatment efficiencies for TMP whitewater.

A full-scale, two-stage anaerobic treatment plant treating the wastewaters from a purified terephthalic acid (PTA) production facility in South Korea for over 4 years was studied (121). The system provided stable operation with COD removals consistently averaging over 90%. The removal of specific phthalic acid isomers and related chemicals has been essentially 100% except for terephthalic acid, which has averaged about 90%, and paratoluic acid, which had only about 30% removal. About 80% of the removal occurred in the first stage of the two-stage hybrid process. A companion single-stage anaerobic contact process removed only 80–85% of the COD and only 35–67% of the phthalic acids.

A three-stage anaerobic treatment process for highly concentrated pig wastewater for small producers was proven to be economical (122). The system provided a series of mixing, homogenization, biological reaction, and final stabilization of concentrated pig waste (total solid content of 8–10%). The process provided stable operational performance, simple operational procedure and well-stabilized sludge effluent. It was also found that the system is economically feasible in Hawaii. Compared to other treatment processes for highly concentrated pig waste, this process is considered an appropriate alternative for the application of the small producers in land limited and tropical conditions. Also, the present treatment system can be easily developed into a prefabricated package plant, which can minimize on-site labor and building costs.

Previous research on the anaerobic treatment of olive oil mill effluent (OME) has shown that the presence of lipids, especially unsaturated long-chain fatty acids, inhibits methane production. However, a two-reactor anaerobic system with partial phase separation is useful for treating any lipid-containing wastewater, such as the treatment of OME (123). It consists of an "acidogenic" reactor and a "methanogenic"' reactor. An almost quantitative biotransformation of unsaturated long-chain fatty acids to palmatic acid is obtained in the first reactor; hence, this drastically lowered the lipid inhibition on methanogenesis in the second reactor. In the second reactor, it had the main percentage of COD conversion, which was over 70%.

The anaerobic treatment of cane-molasses alcohol stillage was studied (124) in a thermophilic two-phase system comprising two bioreactors for the acidogenic and methanogenic phases, in comparison with the single-phase process. The treatment efficiency was essentially maintained at BOD_5 and COD removal of over 85% and 65%, respectively, in the two-phase

process even with higher substrate loading. The acidogenic phase provided a satisfactory conversion of initial COD to VFA averaging 15.6% in the degree of acidification. The methanogenic culture pH of both systems was maintained in a range of 7.4–7.8 through self-regulation. The methane content of the biogas generated from the two-phase process was significantly higher by about 17% than that from the single-phase process, both decreasing with increasing substrate loading and shorter HRT.

Mining effluents are often acidic, containing high concentrations of sulfate and metals. Usually, the treatment is done by lime addition. Sulfate can also be biologically removed as sulfide or sulphur, provided that there is a suitable carbon and energy source. Two completely mixed reactors configured in such a way that an anaerobic reactor followed by a clarifier improves the efficiency of metal removal from acid mine water and the sludge characteristics (125). The sulfate in the acid mine water was removed from 3,000 to less than 200 mg/L (as SO_4) and the formed sulfides to less than 200 mg/L.

Pentachlorophenol (PCP) is remarkably and efficiently degraded in a hybrid reactor supplied with a mixture of fatty acids (propionic, butyric, acetic, and lactic) and methanol (126). The reduction of COD was around 97%, and methane was found to be 86% in the biogas production. The efficiency of volatile fatty acids breakdown was 93%, 64%, and 74%, respectively, for butyric, propionic, and acetic. PCP total removal of more than 99% was reached by granular sludge activities formed during 21 months of reactor operation. Methanogenic microorganisms predominance was noticed with 10^5–10^6 cells/mL during enumeration on methanol or lactate added to sulfate culture media. The removal rate was 1.07 mg PCP/g Volatile solid (VS)/day during the highest PCP concentration addition.

7. THE FUTURE OF ANAEROBIC TREATMENT

Anaerobic treatment is well over a century old. Its initial development was for the treatment of domestic wastewaters, using anaerobic filters and hybrid processes that are still of interest today. It then progressed in application to separate sludge digestion, then to treatment of dilute industrial wastewaters. Several processes have been developed that accomplish efficient treatment of wastewaters in short detention times.

The anaerobic treatment process has long been known for its unique ability to convert highly objectionable wastes into useful products. With global concerns over energy shortages and greenhouse gas formation through combustion of fossil fuels, more efforts towards renewable energy supplies is clearly needed.

Greater efforts are now needed for broad application of anaerobic treatment for ridding the environment of unwanted organic materials by converting them into methane, a renewable energy source.

The anaerobic process leading towards methane production from wastewaters, solid wastes, and agricultural and forest product residues clearly fits this need. Research towards even broader application is clearly of importance. Problems that need addressing are process reliability, toxicity causes and effects, odor production and control, and better understanding of refractory organic degradation.

From all the numerous and the latest published research on anaerobic processes cited in the earlier section, the anaerobic process is arguably the most promising wastewater treatment system that is able to meet the desired stringent criteria for future technology in an environmentally sustainable development. This process would be able to minimize environmental harm while increasing industrial productivity and improving quality of life.

At the moment, the most popular treatment process is the UASB reactor. However, with the recent development of EGSB and Staged Multi-Phase Anaerobic (SMPA) reactor systems, this may lead to a promising new generation of anaerobic treatment systems (110). The concepts behind the EGSB will provide a higher efficiency at higher loading rates, and are applicable for extreme environmental conditions (e.g., low and high temperatures) and to inhibitory compounds. Moreover, by integrating the anaerobic process with other biological methods (sulfate reduction, microaerophilic organisms) and with physical–chemical methods, a complete treatment of the wastewater can be accomplished at very low costs, while at the same time, valuable components can be recovered for reuse.

It becomes clear that anaerobic treatment is an established technology for a wide variety of industrial applications. The technology is accepted in both industrialized and less developed countries. The granular sludge-based processes UASB and EGSB command a large portion of applications. Although UASB still is the predominant technology in use, ESGB-type processes are gaining more popularity driven by economics. The data shows that the design load for EGSB systems is approximately double that of the UASB process, which results in a competitive advantage over lower loaded systems. It should be noted, however, that the data presented represents approximately 50–60% of total anaerobic systems installed and contribution of EGSB and IC systems may be relatively high in the current database relative to total number of systems installed. It is also foreseen that the higher loaded EGSB type systems are gradually replacing at least some of the UASB applications (50).

In the fields of psychrophilic and thermophilic anaerobic treatments, specific reactor development may serve to further enhance volumetric conversion capacities (127). Due to reduced water usage, both COD and salt concentrations tend to increase for industrial effluents. As a consequence, there is a need for the development of anaerobic reactors retaining flocculant biomass. Membrane bioreactors offer a solution for certain niches in wastewater treatment (128). However, the oxygen transfer economy is poor. There is a need for fundamental research development to obtain a realistic image of this technology.

Recent advances are made possible by adapting the conventional anaerobic high-rate concept to extreme conditions. Staged anaerobic reactor concepts (129) show the advantages under nonoptimal temperature conditions as well as during the treatment of chemical wastewater. In other situations, a staged anaerobic–aerobic approach is required for the biodegradation of specific pollutants, e.g., the removal of dyes from textile processing wastewaters. There are benefits to reactor staging. The staged anaerobic reactor possesses yet more unexploited potential of high-rate anaerobic wastewater treatment.

In the future, not only will treatment technologies experience a global shift towards usage of anaerobic treatment of industrial wastewater but a decentralized approach to management will follow suit to meet the urgent need of integrated environmental protection (EP) and resource conservation (RC) to the available water source (130).

EP and RC-concepts focus on pollution prevention and on a minimum of consumptive use of energy, chemicals, and water in pollution abatement and a maximum of reuse of treated wastewater, by-products, and residues produced in the treatment of waste and wastewater. Consequently, by implementing these concepts, wastewaters like sewage and industrial effluents become an important source of water, fertilizers, soil conditioners, and, frequently, energy instead of a social threat. In addition, a bridge is made between environmental protection and agriculture practice, stimulating urban agriculture in the neighborhood of large cities. Anaerobic treatment is considered the core technology for mineralizing organic compounds in wastewater streams. Additional technologies are required to comply with the reuse criteria.

Sustainable EP makes use of technologies that are able to treat the concentrated wastes at the site. With respect to industrial wastewaters, the on-site approach is nowadays generally applied in Western Europe, forced by political and legislative measures. As a consequence, industrial owners are interested in keeping the wastewater concentrated, minimizing the required volume for the wastewater treatment facility, and thus the investment costs and overall water consumption. A decentralized approach will be observed, as the "polluters pay" principle is the key to proper management of industrial wastewater discharge to maintain a clean and wholesome environment for the enjoyment and benefit of future generations.

Presently, processes based on anaerobic treatment appear to be an excellent option as the core of an integrated process for waste and wastewater treatment (131). Environmental regulations in the European Union, based on the concept of integrated prevention and control of pollution, are oriented towards the sustainability of the production processes, and this leads to better recovery of resources from raw materials, energy saving, etc. This philosophy introduces a new framework to environmental engineers, who have to make efforts concerning waste minimization. During the last few decades, technologies based on the anaerobic treatment of wastewaters and organic wastes have been applied successfully to a wide variety of problems.

8. CONCLUSION

The technology of anaerobic treatment has evolved significantly in recent years. Developments in the 21st century indicate that there is a shift from treatment of sludge to industrial wastewaters. Advancements in the fundamental understanding of biochemistry and microbiology of anaerobic processes have led to successful applications, which show a great deal of promise in overcoming the limitations associated with anaerobic treatment. The technology has been accepted in industrialized countries and appears as an increasingly promising and favorable wastewater treatment process (132). With successful full-scale applications in industries, anaerobic treatment is gaining more attention globally especially in developing countries.

Granular sludge-based anaerobic processes, such as the UASB and EGSB systems, are gradually commanding a large portion of full-scale industrial applications. Although UASB is still the predominant system in use, at present, ESGB-type processes are gaining more popularity driven by economics reasons.

The increasing stringent restrictions on air pollution, sludge disposal on landfills, odor control, and energy consumption have raised questions on the viability of aerobic treatment. There is also a shift in interest from using the aerobic process to using the anaerobic process for biodestruction of organic materials, due to the latter's ability to biotransform pollutants, reduce sludge volumes, and decrease operation cost with energy credit from the methane and hydrogen production (133).

Anaerobic microbial processes have emerged as a focus for scientific and engineering research, involving the participation and contributions of diverse disciplines to further enhance the processes. In the future, the best treatment selection must be the most environment-friendly choice. Anaerobic biotechnology may well become the solution, making energy conservation possible with its concomitant ecological and economic benefits (134–136).

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Appendix: Conversion Factors for Environmental Engineers

Lawrence K. Wang

CONTENTS

Abstract With the current trend toward metrication, the question of using a consistent system of units has been a problem. Wherever possible, the authors of this *Handbook of Environmental Engineering* series have used the British system (fps) along with the metric equivalent (mks, cgs, or SIU) or vice versa. For the convenience of the readers around the world, this book provides a detailed Conversion Factors for Environmental Engineers. In addition, the basic and supplementary units, the derived units and quantities, important physical constants, the properties of water, and the Periodic Table of the elements, are also presented in this document.

Key Words Conversion factors • British units • metric units • physical constants • water properties • periodic table of the elements • environmental engineers • Lenox Institute of Water Technology • mks (meter-kilogram-second) • cgs (centimeter-gram-second) • SIU (Système international d'unités; International System of Units) • fps (foot-pound-second).

1. CONSTANTS AND CONVERSION FACTORS

Multiply	*by*	*to obtain*
abamperes	10	amperes
abamperes	2.99796×10^{10}	statamperes
abampere-turns	12.566	gilberts
abcoulombs	10	coulombs (abs)
abcoulombs	2.99796×10^{10}	statcoulombs
abcoulombs/kg	30,577	statcoulombs/dyne
abfarads	1×10^{9}	farads (abs)
abfarads	8.98776×10^{20}	statfarads
abhenries	1×10^{-9}	henries (abs)
abhenries	1.11263×10^{-21}	stathenries
abohms	1×10^{-9}	ohms (abs)
abohms	1.11263×10^{-21}	statohms
abvolts	3.33560×10^{-11}	statvolts
abvolts	1×10^{-8}	volts (abs)
abvolts/centimeters	2.540005×10^{-8}	volts (abs)/inch
acres	0.4046	ha
acres	43,560	square feet
acres	4047	square meters
acres	1.562×10^{-3}	square miles
acres	4840	square yards
acre-feet	43,560	cubic feet
acre-feet	1233.5	cubic meters
acre-feet	325,850	gallons (U.S.)
amperes (abs)	0.1	abamperes
amperes (abs)	1.036×10^{-5}	faradays/second
amperes (abs)	2.9980×10^{9}	statamperes
ampere-hours (abs)	3600	coulombs (abs)
ampere-hours	0.03731	faradays
amperes/sq cm	6.452	amps/sq in
amperes/sq cm	10^{4}	amps/sq meter
amperes/sq in	0.1550	amps/sq cm
amperes/sq in	1550.0	amps/sq meter
amperes/sq meter	10^{-4}	amps/sq cm
amperes/sq meter	6.452×10^{-4}	amps/sq in
ampere-turns	1.257	gilberts
ampere-turns/cm	2.540	amp-turns/in
ampere-turns/cm	100.0	amp-turns/meter
ampere-turns/cm	1.257	gilberts/cm
ampere-turns/in	0.3937	amp-turns/cm
ampere-turns/in	39.37	amp-turns/meter
ampere-turns/in	0.4950	gilberts/cm

Multiply	*by*	*to obtain*
ampere-turns/meter	0.01	amp-turns/cm
ampere-turns/meter	0.0254	amp-turns/in
ampere-turns/meter	0.01257	gilberts/cm
angstrom units	1×10^{-8}	centimeters
angstrom units	3.937×10^{-9}	inches
angstrom unit	1×10^{-10}	meter
angstrom unit	1×10^{-4}	micron or μm
ares	0.02471	acre (U.S.)
ares	1076	square feet
ares	100	square meters
ares	119.60	sq yards
assay tons	29.17	grams
astronomical unit	1.495×10^{8}	kilometers
atmospheres (atm)	0.007348	tons/sq inch
atmospheres	76.0	cms of mercury
atmospheres	1.01325×10^{6}	dynes/square centimeter
atmospheres	33.90	ft of water (at 4°C)
atmospheres	29.92	inches of mercury (at 0°C)
atmospheres	1.033228	kg/sq cm
atmospheres	10,332	kg/sq meter
atmospheres	760.0	millimeters of mercury
atmospheres	14.696	pounds/square inch
atmospheres	1.058	tons/sq foot
avograms	1.66036×10^{-24}	grams
bags, cement	94	pounds of cement
barleycorns (British)	1/3	inches
barleycorns (British)	8.467×10^{-3}	meters
barrels (British, dry)	5.780	cubic feet
barrels (British, dry)	0.1637	cubic meters
barrels (British, dry)	36	gallons (British)
barrels, cement	170.6	kilograms
barrels, cement	376	pounds of cement
barrels, cranberry	3.371	cubic feet
barrels, cranberry	0.09547	cubic meters
barrels, oil	5.615	cubic feet
barrels, oil	0.1590	cubic meters
barrels, oil	42	gallons (U.S.)
barrels, (U.S., dry)	4.083	cubic feet
barrels (U.S., dry)	7056	cubic inches
barrels (U.S., dry)	0.11562	cubic meters
barrels (U.S., dry)	105.0	quarts (dry)
barrels (U.S., liquid)	4.211	cubic feet
barrels (U.S., liquid)	0.1192	cubic meters
barrels (U.S., liquid)	31.5	gallons (U.S.)

Multiply	*by*	*to obtain*
bars	0.98692	atmospheres
bars	10^6	dynes/sq cm
bars	1.0197×10^4	kg/sq meter
bars	1000	millibar
bars	750.06	mm of Hg (0°C)
bars	2089	pounds/sq ft
bars	14.504	pounds/sq in
barye	1.000	dynes/sq cm
board feet	1/12	cubic feet
board feet	144 sq.in. × 1 in.	cubic inches
boiler horsepower	33,475	BTU (mean)/hour
boiler horsepower	34.5	pounds of water evaporated from and at 212°F (per hour)
bolts (U.S., cloth)	120	linear feet
bolts (U.S., cloth)	36.576	meters
bougie decimales	1	candles (int)
BTU (mean)	251.98	calories, gram (g. cal)
BTU (mean)	0.55556	centigrade heat units (chu)
BTU (mean)	1.0548×10^{10}	ergs
BTU (mean)	777.98	foot-pounds
BTU (mean)	3.931×10^{-4}	horsepower-hrs (hp-hr)
BTU (mean)	1055	joules (abs)
BTU (mean)	0.25198	kilograms, cal (kg cal)
BTU (mean)	107.565	kilogram-meters
BTU (mean)	2.928×10^{-4}	kilowatt-hr (Kwh)
BTU (mean)	10.409	liter-atm
BTU (mean)	6.876×10^{-5}	pounds of carbon to CO_2
BTU (mean)	0.29305	watt-hours
BTU (mean)/cu ft	37.30	joule/liter
BTU/hour	0.2162	foot-pound/sec
BTU/hour	0.0700	gram-cal/sec
BTU/hour	3.929×10^{-4}	horsepower-hours (hp-hr)
BTU/hour	0.2930711	watt (w)
BTU/hour (feet)°F	1.730735	joule/sec (m)°k
BTU/hour (feet^2)	3.15459	joule/m^2-sec
BTU (mean)/hour(feet^2)°F	1.3562×10^{-4}	gram-calorie/second (cm^2)°C
BTU (mean)/hour(feet^2)°F	3.94×10^{-4}	horsepower/(ft^2)°F
BTU (mean)/hour(feet^2)°F	5.678264	joule/sec (m^2)°k
BTU (mean)/hour(feet^2)°F	4.882	kilogram-calorie/hr (m^2)°C
BTU (mean)/hour(feet^2)°F	5.682×10^{-4}	watts/(cm^2)°C
BTU (mean)/hour(feet^2)°F	2.035×10^{-3}	watts/(in^2)°C
BTU (mean)/(hour)(feet^2) (°F/inch)	3.4448×10^{-4}	calories, gram (15°C)/sec (cm^2) (°C/cm)
BTU (mean)/(hour)(feet^2) (°F/in.)	1	chu/(hr)(ft^2)(°C/in)

Multiply	*by*	*to obtain*
BTU (mean)/(hour)(feet^2) (°F/inch)	1.442×10^{-3}	joules (abs)/(sec)(cm^2) (°C/cm)
BTU (mean)/(hour)(feet^2) (°F/inch)	1.442×10^{-3}	watts/(cm^2) (°C/cm)
BTU/min	12.96	ft lb/sec
BTU/min	0.02356	hp
BTU/min	0.01757	kw
BTU/min	17.57	watts
BTU/min/ft^2	0.1221	watts/sq inch
BTU/pound	0.5556	calories-gram(mean)/gram
BTU/pound	0.555	kg-cal/kg
BTU/pound/°F	1	calories, gram/gram/°C
BTU/pound/°F	4186.8	joule/kg/°k
BTU/second	1054.350	watt (W)
buckets (British, dry)	1.818×10^4	cubic cm
buckets (British, dry)	4	gallons (British)
bushels (British)	1.03205	bushels (U.S.)
bushels (British)	1.2843	cubic feet
bushels (British)	0.03637	cubic meters
bushels (U.S.)	1.2444	cubic feet
bushels (U.S.)	2150.4	cubic inch
bushels (U.S.)	0.035239	cubic meters
bushels (U.S.)	35.24	liters (L)
bushels (U.S.)	4	pecks (U.S.)
bushels (U.S.)	64	pints (dry)
bushels (U.S.)	32	quarts (dry)
butts (British)	20.2285	cubic feet
butts (British)	126	gallons (British)
cable lengths	720	feet
cable lengths	219.46	meters
calories (thermochemical)	0.999346	calories (Int. Steam Tables)
calories, gram (g. cal or simply cal.)	3.9685×10^{-3}	BTU (mean)
calories, gram (mean)	0.001459	cubic feet atmospheres
calories, gram (mean)	4.186×10^7	ergs
calories, gram (mean)	3.0874	foot-pounds
calories, gram (mean)	4.186	joules (abs)
calories, gram (mean)	0.001	kg cal (calories, kilogram)
calories, gram (mean)	0.42685	kilograms-meters
calories, gram (mean)	0.0011628	watt-hours
calories, gram (mean)/gram	1.8	BTU (mean)/pound
cal/gram-°C	4186.8	joule/kg-°k
candle power (spherical)	12.566	lumens
candles (int)	0.104	carcel units
candles (int)	1.11	hefner units
candles (int)	1	lumens (int)/steradian
candles (int)/square centimeter	2919	foot-lamberts

Multiply	*by*	*to obtain*
candles (int)/square centimeter	3.1416	lamberts
candles (int)/square foot	3.1416	foot-lamberts
candles (int)/square foot	3.382×10^{-3}	lamberts
candles (int)/square inch	452.4	foot-lamberts
candles (int)/square inch	0.4870	lamberts
candles (int)/square inch	0.155	stilb
carats (metric)	3.0865	grains
carats (metric)	0.2	grams
centals	100	pounds
centares (centiares)	1.0	sq meters
centigrade heat units (chu)	1.8	BTU
centigrade heat units (chu)	453.6	calories, gram (15°C)
centigrade heat units (chu)	1897.8	joules (abs)
centigrams	0.01	grams
centiliters	0.01	liters
centimeters	0.0328083	feet (U.S.)
centimeters	0.3937	inches (U.S.)
centimeters	0.01	meters
centimeters	6.214×10^{-6}	miles
centimeters	10	millimeters
centimeters	393.7	mils
centimeters	0.01094	yards
cm of mercury	0.01316	atm
cm of mercury	0.4461	ft of water
cm of mercury	136.0	kg/square meter
cm of mercury	1333.22	newton/meter^2 (N/m^2)
cm of mercury	27.85	psf
cm of mercury	0.1934	psi
cm of water (4°C)	98.0638	newton/meter^2 (N/m^2)
centimeters-dynes	1.020×10^{-3}	centimeter-grams
centimeter-dynes	1.020×10^{-8}	meter-kilograms
centimeter-dynes	7.376×10^{-8}	pound-feet
centimeter-grams	980.7	centimeter-dynes
centimeter-grams	10^{-5}	meter-kilograms
centimeter-grams	7.233×10^{-5}	pound-feet
centimeters/second	1.969	fpm (ft/min)
centimeters/second	0.0328	fps (ft/sec)
centimeters/second	0.036	kilometers/hour
centimeters/second	0.1943	knots
centimeters/second	0.6	m/min
centimeters/second	0.02237	miles/hour
centimeters/second	3.728×10^{-4}	miles/minute
cms/sec./sec.	0.03281	feet/sec/sec
cms/sec./sec.	0.036	kms/hour/sec

Multiply	*by*	*to obtain*
cms/sec./sec.	0.02237	miles/hour/sec
centipoises	3.60	kilograms/meter hour
centipoises	10^{-3}	kilograms/meter second
centipoises	0.001	newton-sec/m^2
centipoises	2.089×10^{-5}	pound force second/square foot
centipoises	2.42	pounds/foot hour
centipoises	6.72×10^{-4}	pounds/foot second
centistoke	1.0×10^{-6}	$meter^2/sec$
chains (engineers' or Ramden's)	100	feet
chains (engineers' or Ramden's)	30.48	meters
chains (surveyors' or Gunter's)	66	feet
chains (surveyors' or Gunter's)	20.12	meters
chaldrons (British)	32	bushels (British)
chaldrons (U.S.)	36	bushels (U.S.)
cheval-vapours	0.9863	horsepower
cheval-vapours	735.5	watts (abs)
cheval-vapours heures	2.648×10^6	joules (abs)
chu/(hr)(ft^2)(°C/in.)	1	BTU/(hr)(ft^2)(°F/in.)
circular inches	0.7854	square inches
circular millimeters	7.854×10^{-7}	square meters
circular mils	5.067×10^{-6}	square centimeters
circular mils	7.854×10^{-7}	square inches
circular mils	0.7854	square mils
circumferences	360	degrees
circumferences	400	grades
circumferences	6.283	radians
cloves	8	pounds
coombs (British)	4	bushels (British)
cords	8	cord feet
cords	$8' \times 4' \times 4'$	cubic feet
cords	128	cubic feet
cords	3.625	cubic meters
cord-feet	$4' \times 4' \times 1'$	cubic feet
coulombs (abs)	0.1	abcoulombs
coulombs (abs)	6.281×10^{18}	electronic charges
coulombs (abs)	2.998×10^9	statcoulombs
coulombs (abs)	1.036×10^{-5}	faradays
coulombs/sq cm	64.52	coulombs/sq in
coulombs/sq cm	10^4	coulombs/sq meter
coulombs/sq in	0.1550	coulombs/sq cm
coulombs/sq in	1550	coulombs/sq meter
coulombs/sq meter	10^{-4}	coulombs/sq cm
coulombs/sq meter	6.452×10^{-4}	coulombs/sq in
cubic centimeters	3.531445×10^{-5}	cubic feet (U.S.)

Multiply	*by*	*to obtain*
cubic centimeters	6.102×10^{-2}	cubic inches
cubic centimeters	10^{-6}	cubic meters
cubic centimeters	1.308×10^{-6}	cubic yards
cubic centimeters	2.6417×10^{-4}	gallons (U.S.)
cubic centimeters	0.001	liters
cubic centimeters	0.033814	ounces (U.S., fluid)
cubic centimeters	2.113×10^{-3}	pints (liq.)
cubic centimeters	1.057×10^{-3}	quarts (liq.)
cubic feet (British)	0.9999916	cubic feet (U.S.)
cubic feet (U.S.)	0.8036	bushels (dry)
cubic feet (U.S.)	28317.016	cubic centimeters
cubic feet (U.S.)	1728	cubic inches
cubic feet (U.S.)	0.02832	cubic meters
cubic feet (U.S.)	0.0370	cubic yard
cubic feet (U.S.)	7.48052	gallons (U.S.)
cubic feet (U.S.)	28.31625	liters
cubic feet (U.S.)	59.84	pints (liq.)
cubic feet (U.S.)	29.92	quarts (liq.)
cubic feet of common brick	120	pounds
cubic feet of water (60°F)	62.37	pounds
cubic foot-atmospheres	2.7203	BTU (mean)
cubic foot-atmospheres	680.74	calories, gram (mean)
cubic foot-atmospheres	2116	foot-pounds
cubic foot-atmospheres	2869	joules (abs)
cubic foot-atmospheres	292.6	kilogram-meters
cubic foot-atmospheres	7.968×10^{-4}	kilowatt-hours
cubic feet/hr	0.02832	m^3/hr
cubic feet/minute	472.0	cubic cm/sec
cubic feet/minute	1.6992	cu m/hr
cubic feet/minute	0.0283	cu m/min
cubic feet/minute	0.1247	gallons/sec
cubic feet/minute	0.472	liter/sec
cubic feet/minute	62.4	lbs of water/min
cubic feet/min/1000 cu ft	0.01667	liter/sec/cu m
cubic feet/second	1.9834	acre-feet/day
cubic feet/second	1.7	cu m/min
cubic feet/second	0.02832	m^3/sec
cubic feet/second	448.83	gallons/minute
cubic feet/second	1699	liter/min
cubic feet/second	28.32	liters/sec
cubic feet/second (cfs)	0.64632	million gallons/day (MGD)
cfs/acre	0.07	m^3/sec-ha
cfs/acre	4.2	cu m/min/ha
cfs/sq mile	0.657	cu m/min/sq km

Multiply	*by*	*to obtain*
cubic inches (U.S.)	16.387162	cubic centimeters
cubic inches (U.S.)	5.787×10^{-4}	cubic feet
cubic inches (U.S.)	1.0000084	cubic inches (British)
cubic inches (U.S.)	1.639×10^{-5}	cubic meters
cubic inches (U.S.)	2.143×10^{-5}	cubic yards
cubic inches (U.S.)	4.329×10^{-3}	gallons (U.S.)
cubic inches (U.S.)	1.639×10^{-2}	liters
cubic inches (U.S.)	16.39	mL
cubic inches (U.S.)	0.55411	ounces (U.S., fluid)
cubic inches (U.S.)	0.03463	pints (liq.)
cubic inches (U.S.)	0.01732	quarts (liq.)
cubic meters	8.1074×10^{-4}	acre-feet
cubic meters	8.387	barrels (U.S., liquid)
cubic meters	28.38	bushels (dry)
cubic meters	10^6	cubic centimeters
cubic meters	35.314	cubic feet (U.S.)
cubic meters	61,023	cubic inches (U.S.)
cubic meters	1.308	cubic yards (U.S.)
cubic meters	264.17	gallons (U.S.)
cubic meters	1000	liters
cubic meters	2113	pints (liq.)
cubic meters (m^3)	1057	quarts (liq.)
cubic meters/day	0.183	gallons/min
cubic meters/ha	106.9	gallons/acre
cubic meters/hour	0.2272	gallons/minute
cubic meters/meter-day	80.53	gpd/ft
cubic meters/minute	35.314	cubic ft/minute
cubic meters/second	35.314	cubic ft/sec
cubic meters/second	22.82	MGD
cubic meters/sec-ha	14.29	cu ft/sec-acre
cubic meters/$meters^2$-day	24.54	gpd/ft^2
cubic yards (British)	0.9999916	cubic yards (U.S.)
cubic yards (British)	0.76455	cubic meters
cubic yards (U.S.)	7.646×10^5	cubic centimeters
cubic yards (U.S.)	27	cubic feet (U.S.)
cubic yards (U.S.)	46,656	cubic inches
cubic yards (U.S.)	0.76456	cubic meters
cubic yards (U.S.)	202.0	gallons (U.S.)
cubic yards (U.S.)	764.6	liters
cubic yards (U.S.)	1616	pints (liq.)
cubic yards (U.S.)	807.9	quarts (liq.)
cubic yards of sand	2700	pounds
cubic yards/minute	0.45	cubic feet/second
cubic yards/minute	3.367	gallons/second

Multiply	*by*	*to obtain*
cubic yards/minute	12.74	liters/second
cubits	45.720	centimeters
cubits	1.5	feet
dalton	1.65×10^{-24}	gram
days	1440	minutes
days	86,400	seconds
days (sidereal)	86164	seconds (mean solar)
debye units (dipole moment)	10^{18}	electrostatic units
decigrams	0.1	grams
deciliters	0.1	liters
decimeters	0.1	meters
degrees (angle)	60	minutes
degrees (angle)	0.01111	quadrants
degrees (angle)	0.01745	radians
degrees (angle)	3600	seconds
degrees/second	0.01745	radians/seconds
degrees/second	0.1667	revolutions/min
degrees/second	0.002778	revoltuions/sec
degree Celsius	$°F = (°C \times 9/5) + 32$	Fahrenheit
degree Celsius	$°K = °C + 273.15$	Kelvin
degree Fahrenheit	$°C = (°F - 32) \times 5/9$	Celsius
degree Fahrenheit	$°K = (°F + 459.67)/1.8$	Kelvin
degree Rankine	$°K = °R/1.8$	Kelvin
dekagrams	10	grams
dekaliters	10	liters
dekameters	10	meters
drachms (British, fluid)	3.5516×10^{-6}	cubic meters
drachms (British, fluid)	0.125	ounces (British, fluid)
drams (apothecaries' or troy)	0.1371429	ounces (avoirdupois)
drams (apothecaries' or troy)	0.125	ounces (troy)
drams (U.S., fluid or apoth.)	3.6967	cubic cm
drams (avoirdupois)	1.771845	grams
drams (avoirdupois)	27.3437	grains
drams (avoirdupois)	0.0625	ounces
drams (avoirdupois)	0.00390625	pounds (avoirdupois)
drams (troy)	2.1943	drams (avoirdupois)
drams (troy)	60	grains
drams (troy)	3.8879351	grams
drams (troy)	0.125	ounces (troy)
drams (U.S., fluid)	3.6967×10^{-6}	cubic meters
drams (U.S., fluid)	0.125	ounces (fluid)
dynes	0.00101972	grams

Multiply	*by*	*to obtain*
dynes	10^{-7}	joules/cm
dynes	10^{-5}	joules/meter (newtons)
dynes	1.020×10^{-6}	kilograms
dynes	1×10^{-5}	newton (N)
dynes	7.233×10^{-5}	poundals
dynes	2.24809×10^{-6}	pounds
dyne-centimeters (torque)	7.3756×10^{-8}	pound-feet
dynes/centimeter	1	ergs/square centimeter
dynes/centimeter	0.01	ergs/square millimeter
dynes/square centimeter	9.8692×10^{-7}	atmospheres
dynes/square centimeter	10^{-6}	bars
dynes/square centimeter	2.953×10^{-5}	inch of mercury at 0°C
dynes/square centimeter	4.015×10^{-4}	inch of water at 4°C
dynes/square centimeter	0.01020	kilograms/square meter
dynes/square centimeter	0.1	newtons/square meter
dynes/square centimeter	1.450×10^{-5}	pounds/square inch
electromagnetic fps units of magnetic permeability	0.0010764	electromagnetic cgs units of magnetic permeability
electromagnetic fps units of magnetic permeability	1.03382×10^{-18}	electrostatic cgs units of magnetic permeability
electromagnetic cgs units, of magnetic permeability	1.1128×10^{-21}	electrostatic cgs units of magnetic permeability
electromagnetic cgs units of mass resistance	9.9948×10^{-6}	ohms (int)-meter-gram
electronic charges	1.5921×10^{-19}	coulombs (abs)
electron-volts	1.6020×10^{-12}	ergs
electron-volts	1.0737×10^{-9}	mass units
electron-volts	0.07386	rydberg units of energy
electronstatic cgs units of Hall effect	2.6962×10^{31}	electromagnetic cgs units of Hall effect
electrostatic fps units of charge	1.1952×10^{-6}	coulombs (abs)
electrostatic fps units of magnetic permeability	929.03	electrostatic cgs units of magnetic permeability
ells	114.30	centimeters
ells	45	inches
ems, pica (printing)	0.42333	centimeters
ems, pica (printing)	1/6	inches
ergs	9.4805×10^{-11}	BTU (mean)
ergs	2.3889×10^{-8}	calories, gram (mean)
ergs	1	dyne-centimeters
ergs	7.3756×10^{-8}	foot-pounds
ergs	0.2389×10^{-7}	gram-calories
ergs	1.020×10^{-3}	gram-centimeters

Multiply	*by*	*to obtain*
ergs	3.7250×10^{-14}	horsepower-hrs
ergs	10^{-7}	joules (abs)
ergs	2.390×10^{-11}	kilogram-calories (kg cal)
ergs	1.01972×10^{-8}	kilogram-meters
ergs	0.2778×10^{-13}	kilowatt-hrs
ergs	0.2778×10^{-10}	watt-hours
ergs/second	5.692×10^{-9}	BTU/min
ergs/second	4.426×10^{-6}	foot-pounds/min
ergs/second	7.376×10^{-8}	foot-pounds/sec
ergs/second	1.341×10^{-10}	horsepower
ergs/second	1.434×10^{-9}	kg-calories/min
ergs/second	10^{-10}	kilowatts
farad (international of 1948)	0.9995	farad (F)
faradays	26.80	ampere-hours
faradays	96,500	coulombs (abs)
faradays/second	96,500	amperes (abs)
farads (abs)	10^{-9}	abfarads
farads (abs)	10^{6}	microfarads
farads (abs)	8.9877×10^{11}	statfarads
fathoms	6	feet
fathom	1.829	meter
feet (U.S.)	1.0000028	feet (British)
feet (U.S.)	30.4801	centimeters
feet (U.S.)	12	inches
feet (U.S.)	3.048×10^{-4}	kilometers
feet (U.S.)	0.30480	meters
feet (U.S.)	1.645×10^{-4}	miles (naut.)
feet (U.S.)	1.893939×10^{-4}	miles (statute)
feet (U.S.)	304.8	millimeters
feet (U.S.)	1.2×10^{4}	mils
feet (U.S.)	1/3	yards
feet of air (1 atmosphere, 60°F)	5.30×10^{-4}	pounds/square inch
feet of water	0.02950	atm
feet of water	0.8826	inches of mercury
feet of water at 39.2°F	0.030479	kilograms/square centimeter
feet of water at 39.2°F	2988.98	newton/meter^2 (N/m^2)
feet of water at 39.2°F	304.79	kilograms/square meter
feet of water	62.43	pounds/square feet (psf)
feet of water at 39.2°F	0.43352	pounds/square inch (psi)
feet/hour	0.08467	mm/sec
feet/min	0.5080	cms/sec
feet/min	0.01667	feet/sec
feet/min	0.01829	km/hr
feet/min	0.3048	meters/min

Multiply	*by*	*to obtain*
feet/min	0.01136	miles/hr
feet/sec	30.48	cm/sec
feet/sec	1.097	km/hr
feet/sec	0.5921	knots
feet/sec	18.29	meters/min
feet/sec	0.6818	miles/hr
feet/sec	0.01136	miles/min
feet/sec/sec	30.48	cm/sec/sec
feet/sec/sec	1.097	km/hr/sec
feet/sec/sec	0.3048	meters/sec/sec
feet/sec/sec	0.6818	miles/hr/sec
feet/100 feet	1.0	percent grade
firkins (British)	9	gallons (British)
firkins (U.S.)	9	gallons (U.S.)
foot-candle (ft-c)	10.764	lumen/sq m
foot-poundals	3.9951×10^{-5}	BTU (mean)
foot-poundals	0.0421420	joules (abs)
foot-pounds	0.0012854	BTU (mean)
foot-pounds	0.32389	calories, gram (mean)
foot-pounds	1.13558×10^{7}	ergs
foot-pounds	32.174	foot-poundals
foot-pounds	5.050×10^{-7}	hp-hr
foot-pounds	1.35582	joules (abs)
foot-pounds	3.241×10^{-4}	kilogram-calories
foot-pounds	0.138255	kilogram-meters
foot-pounds	3.766×10^{-7}	kwh
foot-pounds	0.013381	liter-atmospheres
foot-pounds	3.7662×10^{-4}	watt-hours (abs)
foot-pounds/minute	1.286×10^{-3}	BTU/minute
foot-pounds/minute	0.01667	foot-pounds/sec
foot-pounds/minute	3.030×10^{-5}	hp
foot-pounds/minute	3.241×10^{-4}	kg-calories/min
foot-pounds/minute	2.260×10^{-5}	kw
foot-pounds/second	4.6275	BTU (mean)/hour
foot-pounds/second	0.07717	BTU/minute
foot-pounds/second	0.0018182	horsepower
foot-pounds/second	0.01945	kg-calories/min
foot-pounds/second	0.001356	kilowatts
foot-pounds/second	1.35582	watts (abs)
furlongs	660.0	feet
furlongs	201.17	meters
furlongs	0.125	miles (U.S.)
furlongs	40.0	rods
gallons (Br.)	3.8125×10^{-2}	barrels (U.S.)

Multiply	*by*	*to obtain*
gallons (Br.)	4516.086	cubic centimeters
gallons (Br.)	0.16053	cu ft
gallons (Br.)	277.4	cu inches
gallons (Br.)	1230	drams (U.S. fluid)
gallons (Br.)	4.54596	liters
gallons (Br.)	7.9620×10^4	minims (Br.)
gallons (Br.)	7.3783×10^4	minims (U.S.)
gallons (Br.)	4545.96	mL
gallons (Br.)	1.20094	gallons (U.S.)
gallons (Br.)	160	ounces (Br., fl.)
gallons (Br.)	153.72	ounces (U.S., fl.)
gallons (Br.)	10	pounds (avoirdupois) of water at 62°F
gallons (U.S.)	3.068×10^{-4}	acre-ft
gallons (U.S.)	0.031746	barrels (U.S.)
gallons (U.S.)	3785.434	cubic centimeters
gallons (U.S.)	0.13368	cubic feet (U.S.)
gallons (U.S.)	231	cubic inches
gallons (U.S.)	3.785×10^{-3}	cubic meters
gallons (U.S.)	4.951×10^{-3}	cubic yards
gallons (U.S.)	1024	drams (U.S., fluid)
gallons (U.S.)	0.83268	gallons (Br.)
gallons (U.S.)	0.83267	imperial gal
gallons (U.S.)	3.78533	liters
gallons (U.S.)	6.3950×10^4	minims (Br.)
gallons (U.S.)	6.1440×10^4	minims (U.S.)
gallons (U.S.)	3785	mL
gallons (U.S.)	133.23	ounces (Br., fluid)
gallons (U.S.)	128	ounces (U.S., fluid)
gallons	8	pints (liq.)
gallons	4	quarts (liq.)
gal water (U.S.)	8.345	lb of water
gallons/acre	0.00935	cu m/ha
gallons/day	4.381×10^{-5}	liters/sec
gpd/acre	0.00935	cu m/day/ha
gpd/acre	9.353	liter/day/ha
gallons/capita/day	3.785	liters/capita/day
gpd/cu yd	5.0	L/day/cu m
gpd/ft	0.01242	cu m/day/m
gpd/sq ft	0.0408	cu m/day/sq m
gpd/sq ft	1.698×10^{-5}	cubic meters/hour/sq meter
gpd/sq ft	0.283	cu meter/minute/ha
gpm (gal/min)	8.0208	cfh (cu ft/hr)
gpm	2.228×10^{-3}	cfs (cu ft/sec)

Multiply	*by*	*to obtain*
gpm	4.4021	cubic meters/hr
gpm	0.00144	MGD
gpm	0.0631	liters/sec
gpm/sq ft	2.445	cu meters/hour/sq meter
gpm/sq ft	40.7	L/min/sq meter
gpm/sq ft	0.679	liter/sec/sq meter
gallons/sq ft	40.743	liters/sq meter
gausses (abs)	3.3358×10^{-4}	electrostatic cgs units of magnetic flux density
gausses (abs)	0.99966	gausses (int)
gausses (abs)	1	lines/square centimeter
gausses (abs)	6.452	lines/sq in
gausses (abs)	1	maxwells (abs)/square centimeters
gausses (abs)	6.4516	maxwells (abs)/square inch
gausses (abs)	10^{-8}	webers/sq cm
gausses (abs)	6.452×10^{-8}	webers/sq in
gausses (abs)	10^{-4}	webers/sq meter
gilberts (abs)	0.07958	abampere turns
gilberts (abs)	0.7958	ampere turns
gilberts (abs)	2.998×10^{10}	electrostatic cgs units of magneto motive force
gilberts/cm	0.7958	amp-turns/cm
gilberts/cm	2.021	amp-turns/in
gilberts/cm	79.58	amp-turns/meter
gills (Br.)	142.07	cubic cm
gills (Br.)	5	ounces (British, fluid)
gills (U.S.)	32	drams (fluid)
gills	0.1183	liters
gills	0.25	pints (liq.)
grade	0.01571	radian
grains	0.036571	drams (avoirdupois)
grains	0.01667	drams (troy)
grains (troy)	1.216	grains (avdp)
grains (troy)	0.06480	grams
grains (troy)	6.480×10^{-5}	kilograms
grains (troy)	64.799	milligrams
grains (troy)	2.286×10^{-3}	ounces (avdp)
grains (troy)	2.0833×10^{-3}	ounces (troy)
grains (troy)	0.04167	pennyweights (troy)
grains	1/7000	pounds (avoirdupois)
grains	1.736×10^{-4}	pounds (troy)
grains	6.377×10^{-8}	tons (long)
grains	7.142×10^{-8}	tons (short)
grains/imp gal	14.254	mg/L

Multiply	*by*	*to obtain*
grains/imp. gal	14.254	parts/million (ppm)
grains/U.S. gal	17.118	mg/L
grains/U.S. gal	17.118	parts/million (ppm)
grains/U.S. gal	142.86	lb/mil gal
grams	0.5611	drams (avdp)
grams	0.25721	drams (troy)
grams	980.7	dynes
grams	15.43	grains
grams	9.807×10^{-5}	joules/cm
grams	9.807×10^{-3}	joules/meter (newtons)
grams	10^{-3}	kilograms
grams	10^{3}	milligrams
grams	0.0353	ounces (avdp)
grams	0.03215	ounces (troy)
grams	0.07093	poundals
grams	2.205×10^{-3}	pounds
grams	2.679×10^{-3}	pounds (troy)
grams	9.842×10^{-7}	tons (long)
grams	1.102×10^{-6}	tons (short)
grams-calories	4.1868×10^{7}	ergs
gram-calories	3.0880	foot-pounds
gram-calories	1.5597×10^{-6}	horsepower-hr
gram-calories	1.1630×10^{-6}	kilowatt-hr
gram-calories	1.1630×10^{-3}	watt-hr
gram-calories	3.968×10^{-3}	British Thermal Units (BTU)
gram-calories/sec	14.286	BTU/hr
gram-centimeters	9.2967×10^{-8}	BTU (mean)
gram-centimeters	2.3427×10^{-5}	calories, gram (mean)
gram-centimeters	980.7	ergs
gram-centimeters	7.2330×10^{-5}	foot-pounds
gram-centimeters	9.8067×10^{-5}	joules (abs)
gram-centimeters	2.344×10^{-8}	kilogram-calories
gram-centimeters	10^{-5}	kilogram-meters
gram-centimeters	2.7241×10^{-8}	watt-hours
grams-centimeters^2 (moment of inertia)	2.37305×10^{-6}	pounds-feet^2
grams-centimeters^2 (moment of inertia)	3.4172×10^{-4}	pounds-inch^2
gram-centimeters/second	1.3151×10^{-7}	hp
gram-centimeters/second	9.8067×10^{-8}	kilowatts
gram-centimeters/second	0.065552	lumens
gram-centimeters/second	9.80665×10^{-5}	watt (abs)
grams/cm	5.600×10^{-3}	pounds/inch
grams/cu cm	62.428	pounds/cubic foot
grams/cu cm	0.03613	pounds/cubic inch

Multiply	*by*	*to obtain*
grams/cu cm	8.3454	pounds/gallon (U.S.)
grams/cu cm	3.405×10^{-7}	pounds/mil-foot
grams/cu ft	35.314	grams/cu meter
grams/cu ft	10^6	micrograms/cu ft
grams/cu ft	35.314×10^6	micrograms/cu meter
grams/cu ft	35.3145×10^3	milligrams/cu meter
grams/cu ft	2.2046	pounds/1000 cu ft
grams/cu m	0.43700	grains/cubic foot
grams/cu m	0.02832	grams/cu ft
grams/cu m	28.317×10^3	micrograms/cu ft
grams/cu m	0.06243	pounds/cu ft
grams/liter	58.417	grains/gallon (U.S.)
grams/liter	9.99973×10^{-4}	grams/cubic centimeter
grams/liter	1000	mg/L
grams/liter	1000	parts per million (ppm)
grams/liter	0.06243	pounds/cubic foot
grams/liter	8.345	lb/1000 gal
grams/sq centimeter	2.0481	pounds/sq ft
grams/sq centimeter	0.0142234	pounds/square inch
grams/sq ft	10.764	grams/sq meter
grams/sq ft	10.764×10^3	kilograms/sq km
grams/sq ft	1.0764	milligrams/sq cm
grams/sq ft	10.764×10^3	milligrams/sq meter
grams/sq ft	96.154	pounds/acre
grams/sq ft	2.204	pounds/1000 sq ft
grams/sq ft	30.73	tons/sq mile
grams/sq meter	0.0929	grams/sq ft
grams/sq meter	1000	kilograms/sq km
grams/sq meter	0.1	milligrams/square cm
grams/sq meter	1000	milligrams/sq meter
grams/sq meter	8.921	pounds/acre
grams/sq meter	0.2048	pounds/1000 sq ft
grams/sq meter	2.855	tons/sq mile
g (gravity)	9.80665	meters/sec^2
g (gravity)	32.174	ft/sec^2
hand	10.16	cm
hands	4	inches
hectare (ha)	2.471	acre
hectares	1.076×10^5	sq feet
hectograms	100	grams
hectoliters	100	liters
hectometers	100	meters
hectowatts	100	watts
hemispheres	0.5	spheres

Multiply	*by*	*to obtain*
hemispheres	4	spherical right angles
hemispheres	6.2832	steradians
henries (abs)	10^9	abhenries
henries	1000.0	millihenries
henries (abs)	1.1126×10^{-12}	stathenries
hogsheads (British)	63	gallons (British)
hogsheads (British)	10.114	cubic feet
hogsheads (U.S.)	8.422	cubic feet
hogsheads (U.S.)	0.2385	cubic meters
hogsheads (U.S.)	63	gallons (U.S.)
horsepower	2545.08	BTU (mean)/hour
horsepower	42.44	BTU/min
horsepower	7.457×10^9	erg/sec
horsepower	33,000	ft lb/min
horsepower	550	foot-pounds/second
horsepower	7.6042×10^6	g cm/sec
horsepower, electrical	1.0004	horsepower
horsepower	10.70	kg.-calories/min
horsepower	0.74570	kilowatts (g = 980.665)
horsepower	498129	lumens
horsepower, continental	736	watts (abs)
horsepower, electrical	746	watts (abs)
horsepower (boiler)	9.803	kw
horsepower (boiler)	33.479	BTU/hr
horsepower-hours	2545	BTU (mean)
horsepower-hours	2.6845×10^{13}	ergs
horsepower-hours	6.3705×10^7	ft poundals
horsepower-hours	1.98×10^6	foot-pounds
horsepower-hours	641,190	gram-calories
horsepower-hours	2.684×10^6	joules
horsepower-hours	641.7	kilogram-calories
horsepower-hours	2.737×10^5	kilogram-meters
horsepower-hours	0.7457	kilowatt-hours (abs)
horsepower-hours	26,494	liter atmospheres (normal)
horsepower-hours	745.7	watt-hours
hours	4.167×10^{-2}	days
hours	60	minutes
hours	3600	seconds
hours	5.952×10^{-3}	weeks
hundredweights (long)	112	pounds
hundredweights (long)	0.05	tons (long)
hundredweights (short)	1600	ounces (avoirdupois)
hundredweights (short)	100	pounds
hundredweights (short)	0.0453592	tons (metric)

Multiply	*by*	*to obtain*
hundredweights (short)	0.0446429	tons (long)
inches (British)	2.540	centimeters
inches (U.S.)	2.54000508	centimeters
inches (British)	0.9999972	inches (U.S.)
inches	2.540×10^{-2}	meters
inches	1.578×10^{-5}	miles
inches	25.40	millimeters
inches	10^{3}	mils
inches	2.778×10^{-2}	yards
inches^2	6.4516×10^{-4}	meter^2
inches^3	1.6387×10^{-5}	meter^3
in. of mercury	0.0334	atm
in. of mercury	1.133	ft of water
in. of mercury (0°C)	13.609	inches of water (60°F)
in. of mercury	0.0345	kgs/square cm
in. of mercury at 32°F	345.31	kilograms/square meter
in. of mercury	33.35	millibars
in. of mercury	25.40	millimeters of mercury
in. of mercury (60°F)	3376.85	newton/meter^2
in. of mercury	70.73	pounds/square ft
in. of mercury at 32°F	0.4912	pounds/square inch
in. of water	0.002458	atmospheres
in. of water	0.0736	in. of mercury
in. of water (at 4°C)	2.540×10^{-3}	kgs/sq cm
in. of water	25.40	kgs/square meter
in. of water (60°F)	1.8663	millimeters of mercury (0°C)
in. of water (60°F)	248.84	newton/meter^2
in. of water	0.5781	ounces/square in
in. of water	5.204	pounds/square ft
in. of water	0.0361	psi
inches/hour	2.54	cm/hr
international ampere	.9998	ampere (absolute)
international volt	1.0003	volts (absolute)
international volt	1.593×10^{-19}	joules (absolute)
international volt	9.654×10^{4}	joules
joules	9.480×10^{-4}	BTU
joules (abs)	10^{7}	ergs
joules	23.730	foot poundals
joules (abs)	0.73756	foot-pounds
joules	3.7251×10^{-7}	horsepower hours
joules	2.389×10^{-4}	kg-calories
joules (abs)	0.101972	kilogram-meters
joules	9.8689×10^{-3}	liter atmospheres (normal)
joules	2.778×10^{-4}	watt-hrs

Multiply	*by*	*to obtain*
joules-sec	1.5258×10^{33}	quanta
joules/cm	1.020×10^{4}	grams
joules/cm	10^{7}	dynes
joules/cm	100.0	joules/meter (newtons)
joules/cm	723.3	poundals
joules/cm	22.48	pounds
joules/liter	0.02681	BTU/cu ft
joules/m^2-sec	0.3167	BTU/ft^2-hr
joules/sec	3.41304	BTU/hr
joules/sec	0.056884	BTU/min
joules/sec	1×10^{7}	erg/sec
joules/sec	44.254	ft lb/min
joules/sec	0.73756	ft lb/sec
joules/sec	1.0197×10^{4}	g cm/sec
joules/sec	1.341×10^{-3}	hp
joules/sec	0.01433	kg cal/min
joules/sec	0.001	kilowatts
joules/sec	668	lumens
joules/sec	1	watts
kilograms	564.38	drams (avdp)
kilograms	257.21	drams (troy)
kilograms	980,665	dynes
kilograms	15,432	grains
kilograms	1000	grams
kilograms	0.09807	joules/cm
kilograms	9.807	joules/meter (newtons)
kilograms	1×10^{6}	milligrams
kilograms	35.274	ounces (avdp)
kilograms	32.151	ounces (troy)
kilograms	70.93	poundals
kilograms	2.20462	pounds (avdp)
kilograms	2.6792	pounds (troy)
kilograms	9.84207×10^{-4}	tons (long)
kilograms	0.001	tons (metric)
kilograms	0.0011023	tons (short)
kilogram-calories	3.968	British Thermal Units (BTU)
kilogram-calories	3086	foot-pounds
kilogram-calories	1.558×10^{-3}	horsepower-hours
kilogram-calories	4186	joules
kilogram-calories	426.6	kilogram-meters
kilogram-calories	4.186	kilojoules
kilogram-calories	1.162×10^{-3}	kilowatt-hours
kg-cal/min	238.11	BTU/hr
kg-cal/min	3.9685	BTU/min

Multiply	*by*	*to obtain*
kg-cal/min	6.9770×10^{8}	erg/sec
kg-cal/min	3087.4	ft-lb/min
kg-cal/min	51.457	ft-lb/sec
kg-cal/min	7.1146×10^{5}	g cm/sec
kg-cal/min	0.0936	hp
kg-cal/min	69.769	joules/sec
kg-cal/min	0.0698	kw
kg-cal/min	46636	lumens
kg-cal/min	69.767	watts
kgs-cms. squared	2.373×10^{-3}	pounds-feet squared
kgs-cms. squared	0.3417	pounds-inches squared
kilogram-force (kgf)	9.80665	newton
kilogram-meters	0.0092967	BTU (mean)
kilogram-meters	2.3427	calories, gram (mean)
kilogram-meters	9.80665×10^{7}	ergs
kilogram-meters	232.71	ft poundals
kilogram-meters	7.2330	foot-pounds
kilogram-meters	3.6529×10^{-6}	horsepower-hours
kilogram-meters	9.80665	joules (abs)
kilogram-meters	2.344×10^{-3}	kilogram-calories
kilogram-meters	2.52407×10^{-6}	kilowatt-hours (abs)
kilogram-meters	2.7241×10^{-6}	kilowatt-hours
kilogram-meters	0.096781	liter atmospheres (normal)
kilogram-meters	6.392×10^{-7}	pounds carbon to CO_2
kilogram-meters	9.579×10^{-6}	pounds water evap. at 212°F
kilograms/cubic meter	10^{-3}	grams/cubic cm
kilograms/cubic meter	0.06243	pounds/cubic foot
kilograms/cubic meter	3.613×10^{-5}	pounds/cubic inch
kilograms/cubic meter	3.405×10^{-10}	pounds/mil. foot
kilograms/m^3-day	0.0624	lb/cu ft-day
kilograms/cu meter-day	62.43	pounds/1000 cu ft-day
kilograms/ha	0.8921	pounds/acre
kilograms/meter	0.6720	pounds/foot
kilograms/sq cm	980,665	dynes
kilograms/sq cm	0.96784	atmosphere
kilograms/sq cm	32.81	feet of water
kilograms/sq cm	28.96	inches of mercury
kilograms/sq cm	735.56	mm of mercury
kilograms/sq cm	2048	pounds/sq ft
kilograms/sq cm	14.22	pounds/square inch
kilograms/sq km	92.9×10^{-6}	grams/sq ft
kilograms/sq km	0.001	grams/sq meter
kilograms/sq km	0.0001	milligrams/sq cm
kilograms/sq km	1.0	milligrams/sq meter

Multiply	*by*	*to obtain*
kilograms/sq km	8.921×10^{-3}	pounds/acre
kilograms/sq km	204.8×10^{-6}	pounds/1000 sq ft
kilograms/sq km	2.855×10^{-3}	tons/sq mile
kilograms/sq meter	9.6784×10^{-5}	atmospheres
kilograms/sq meter	98.07×10^{-6}	bars
kilograms/sq meter	98.0665	dynes/sq centimeters
kilograms/sq meter	3.281×10^{-3}	feet of water at 39.2°F
kilograms/sq meter	0.1	grams/sq centimeters
kilograms/sq meter	2.896×10^{-3}	inches of mercury at 32°F
kilograms/sq meter	0.07356	mm of mercury at 0°C
kilograms/sq meter	0.2048	pounds/square foot
kilograms/sq meter	0.00142234	pounds/square inch
kilograms/sq mm.	10^6	kg/square meter
kilojoule	0.947	BTU
kilojoules/kilogram	0.4295	BTU/pound
kilolines	1000.0	maxwells
kiloliters	10^3	liters
kilometers	10^5	centimeters
kilometers	3281	feet
kilometers	3.937×10^4	inches
kilometers	10^3	meters
kilometers	0.53961	miles (nautical)
kilometers	0.6214	miles (statute)
kilometers	10^6	millimeters
kilometers	1093.6	yards
kilometers/hr	27.78	cm/sec
kilometers/hr	54.68	feet/minute
kilometers/hr	0.9113	ft/sec
kilometers/hr	0.5396	knot
kilometers/hr	16.67	meters/minute
kilometers/hr	0.2778	meters/sec
kilometers/hr	0.6214	miles/hour
kilometers/hour/sec	27.78	cms/sec/sec
kilometers/hour/sec	0.9113	ft/sec/sec
kilometers/hour/sec	0.2778	meters/sec/sec
kilometers/hour/sec	0.6214	miles/hr/sec
kilometers/min	60	kilometers/hour
kilonewtons/sq m	0.145	psi
kilowatts	56.88	BTU/min
kilowatts	4.425×10^4	foot-pounds/min
kilowatts	737.6	ft-lb/sec
kilowatts	1.341	horsepower
kilowatts	14.34	kg-cal/min
kilowatts	10^3	watts

Multiply	*by*	*to obtain*
kilowatt-hrs	3413	BTU (mean)
kilowatt-hrs	3.600×10^{13}	ergs
kilowatt-hrs	2.6552×10^{6}	foot-pounds
kilowatt-hrs	859,850	gram-calories
kilowatt-hrs	1.341	horsepower hours
kilowatt-hrs	3.6×10^{6}	joules
kilowatt-hrs	860.5	kg-calories
kilowatt-hrs	3.6709×10^{5}	kilogram-meters
kilowatt-hrs	3.53	pounds of water evaporated from from and at 212°F
kilowatt-hrs	22.75	pounds of water raised from 62° to 212°F
knots	6080	feet/hr
knots	1.689	feet/sec
knots	1.8532	kilometers/hr
knots	0.5144	meters/sec
knots	1.0	miles (nautical)/hour
knots	1.151	miles (statute)/hour
knots	2,027	yards/hr
lambert	2.054	candle/in^2
lambert	929	footlambert
lambert	0.3183	stilb
langley	1	15° gram-calorie/cm^2
langley	3.6855	BTU/ft^2
langley	0.011624	Int. kw-hr/m^2
langley	4.1855	joules (abs)/cm^2
leagues (nautical)	3	miles (nautical)
leagues (statute)	3	miles (statute)
light years	63,274	astronomical units
light years	9.4599×10^{12}	kilometers
light years	5.8781×10^{12}	miles
lignes (Paris lines)	1/12	ponces (Paris inches)
lines/sq cm	1.0	gausses
lines/sq in	0.1550	gausses
lines/sq in	1.550×10^{-9}	webers/sq cm
lines/sq in	10^{-8}	webers/sq in
lines/sq in	1.550×10^{-5}	webers/sq meter
links (engineer's)	12.0	inches
links (Gunter's)	0.01	chains (Gunter's)
links (Gunter's)	0.66	feet
links (Ramden's)	0.01	chains (Ramden's)
links (Ramden's)	1	feet
links (surveyor's)	7.92	inches
liters	8.387×10^{-3}	barrels (U.S.)

Multiply	*by*	*to obtain*
liters	0.02838	bushels (U.S. dry)
liters	1000.028	cubic centimeters
liters	0.035316	cubic feet
liters	61.025	cu inches
liters	10^{-3}	cubic meters
liters	1.308×10^{-3}	cubic yards
liters	270.5179	drams (U.S. fl)
liters	0.21998	gallons (Br.)
liters	0.26417762	gallons (U.S.)
liters	16,894	minims (Br.)
liters	16,231	minims (U.S.)
liters	35.196	ounces (Br. fl)
liters	33.8147	ounces (U.S. fl)
liters	2.113	pints (liq.)
liters	1.0566828	quarts (U.S. liq.)
liter-atmospheres (normal)	0.096064	BTU (mean)
liter-atmospheres (normal)	24.206	calories, gram (mean)
liter-atmospheres (normal)	1.0133×10^{9}	ergs
liter-atmospheres (normal)	74.735	foot-pounds
liter-atmospheres (normal)	3.7745×10^{-5}	horsepower hours
liter-atmospheres (normal)	101.33	joules (abs)
liter-atmospheres (normal)	10.33	kilogram-meters
liter-atmospheres (normal)	2.4206×10^{-2}	kilogram calories
liter-atmospheres (normal)	2.815×10^{-5}	kilowatt-hours
liter/cu m-sec	60.0	cfm/1000 cu ft
liters/minute	5.885×10^{-4}	cubic feet/sec
liters/minute	4.403×10^{-3}	gallons/sec
liter/person-day	0.264	gpcd
liters/sec	2.119	cu ft /min
liters/sec	3.5316×10^{-2}	cu ft /sec
liters/sec	15.85	gallons/minute
liters/sec	0.02282	MGD
$\log_{10} N$	2.303	$\log_e N$ or ln N
$\log_e N$ or ln N	0.4343	$\log_{10} N$
lumens	0.07958	candle-power (spherical)
lumens	0.00147	watts of maximum visibility radiation
lumens/sq. centimeters	1	lamberts
lumens/sq cm/steradian	3.1416	lamberts
lumens/sq ft	1	foot-candles
lumens/sq ft	10.764	lumens/sq meter
lumens/sq ft/steradian	3.3816	millilamberts
lumens/sq meter	0.09290	foot-candles or lumens/sq
lumens/sq meter	10^{-4}	phots
lux	0.09290	foot-candles

Multiply	*by*	*to obtain*
lux	1	lumens/sq meter
lux	10^{-4}	phots
maxwells	0.001	kilolines
maxwells	10^{-8}	webers
megajoule	0.3725	horsepower-hour
megalines	10^{6}	maxwells
megohms	10^{12}	microhms
megohms	10^{6}	ohms
meters	10^{10}	angstrom units
meters	100	centimeters
meters	0.5467	fathoms
meters	3.280833	feet (U.S.)
meters	39.37	inches
meters	10^{-3}	kilometers
meters	5.396×10^{-4}	miles (naut.)
meters	6.2137×10^{-4}	miles (statute)
meters	10^{3}	millimeters
meters	10^{9}	millimicrons
meters	1.09361	yards (U.S.)
meters	1.179	varas
meter-candles	1	lumens/sq meter
meter-kilograms	9.807×10^{7}	centimeter-dynes
meter-kilograms	10^{5}	centimeter-grams
meter-kilograms	7.233	pound-feet
meters/minute	1.667	centimeters/sec
meters/minute	3.281	feet/minute
meters/minute	0.05468	feet/second
meters/minute	0.06	kilograms/hour
meters/minute	0.03238	knots
meters/minute	0.03728	miles/hour
meters/second	196.8	feet/minute
meters/second	3.281	feet/second
meters/second	3.6	kilometers/hour
meters/second	0.06	kilometers/min
meters/second	1.944	knots
meters/second	2.23693	miles/hour
meters/second	0.03728	miles/minute
meters/sec/sec	100.0	cm/sec/sec
meters/sec/sec	3.281	feet/sec/sec
meters/sec/sec	3.6	km/hour/sec
meters/sec/sec	2.237	miles/hour/sec
microfarad	10^{-6}	farads
micrograms	10^{-6}	grams
micrograms/cu ft	10^{-6}	grams/cu ft

Multiply	*by*	*to obtain*
micrograms/cu ft	35.314×10^{-6}	grams/cu m
micrograms/cu ft	35.314	microgram/cu m
micrograms/cu ft	35.314×10^{-3}	milligrams/cu m
micrograms/cu ft	2.2046×10^{-6}	pounds/1000 cu ft
micrograms/cu m	28.317×10^{-9}	grams/cu ft
micrograms/cu m	10^{-6}	grams/ cu m
micrograms/cu m	0.02832	micrograms/cu ft
micrograms/cu m	0.001	milligrams/cu m
micrograms/cu m	62.43×10^{-9}	pounds/1000 cu ft
micrograms/cu m	$\frac{0.02404}{\text{molecular weight of gas}}$	ppm by volume (20°C)
micrograms/cu m	834.7×10^{-6}	ppm by weight
micrograms/liter	1000.0	micrograms/cu m
micrograms/liter	1.0	milligrams/cu m
micrograms/liter	62.43×10^{-9}	pounds/cu ft
micrograms/liter	$\frac{24.04}{\text{molecular weight of gas}}$	ppm by volume (20°C)
micrograms/liter	0.834.7	ppm by weight
microhms	10^{-12}	megohms
microhms	10^{-6}	ohms
microliters	10^{-6}	liters
microns	10^{4}	angstrom units
microns	1×10^{-4}	centimeters
microns	3.9370×10^{-5}	inches
microns	10^{-6}	meters
miles (naut.)	6080.27	feet
miles (naut.)	1.853	kilometers
miles (naut.)	1.853	meters
miles (naut.)	1.1516	miles (statute)
miles (naut.)	2027	yards
miles (statute)	1.609×10^{5}	centimeters
miles (statute)	5280	feet
miles (statute)	6.336×10^{4}	inches
miles (statute)	1.609	kilometers
miles (statute)	1609	meters
miles (statute)	0.8684	miles (naut.)
miles (statute)	320	rods
miles (statute)	1760	yards
miles/hour	44.7041	centimeter/second
miles/hour	88	feet/min
miles/hour	1.4667	feet/sec
miles/hour	1.6093	kilometers/hour
miles/hour	0.02682	km/min

Multiply	*by*	*to obtain*
miles/hour	0.86839	knots
miles/hour	26.82	meters/min
miles/hour	0.447	meters/sec
miles/hour	0.1667	miles/min
miles/hour/sec	44.70	cms/sec/sec
miles/hour/sec	1.4667	ft/sec/sec
miles/hour/sec	1.6093	km/hour/sec
miles/hour/sec	0.4470	m/sec/sec
miles/min	2682	centimeters/sec
miles/min	88	ft/sec
miles/min	1.609	km/min
miles/min	0.8684	knots/min
miles/min	60	miles/hour
miles-feet	9.425×10^{-6}	cu inches
millibars	0.00987	atmospheres
millibars	0.30	inches of mercury
millibars	0.75	millimeters of mercury
milliers	10^3	kilograms
millimicrons	1×10^{-9}	meters
milligrams	0.01543236	grains
milligrams	10^{-3}	grams
milligrams	10^{-6}	kilograms
milligrams	3.5274×10^{-5}	ounces (avdp)
milligrams	2.2046×10^{-6}	pounds (avdp)
milligrams/assay ton	1	ounces (troy)/ton (short)
milligrams/cu m	283.2×10^{-6}	grams/cu ft
milligrams/cu m	0.001	grams/cu m
milligrams/cu m	1000.0	micrograms/cu m
milligrams/cu m	28.32	micrograms/cu ft
milligrams/cu m	1.0	micrograms/liter
milligrams/cu m	62.43×10^{-6}	pounds/1000 cu ft
milligrams/cu m	$\frac{24.04}{\text{molecular weight of gas}}$	ppm by volume (20°C)
milligrams/cu m	0.8347	ppm by weight
milligrams/joule	5.918	pounds/horsepower-hour
milligrams/liter	0.05841	grains/gallon
milligrams/liter	0.07016	grains/imp. gal
milligrams/liter	0.0584	grains/U.S. gal
milligrams/liter	1.0	parts/million
milligrams/liter	8.345	lb/mil gal
milligrams/sq cm	0.929	grams/sq ft
milligrams/sq cm	10.0	grams/sq meter
milligrams/sq cm	10^4	kilograms/sq km

Multiply	*by*	*to obtain*
milligrams/sq cm	10^4	milligrams/sq meter
milligrams/sq cm	2.048	pounds/1000 sq ft
milligrams/sq cm	89.21	pounds/acre
milligrams/sq cm	28.55	tons/sq mile
milligrams/sq meter	92.9×10^{-6}	grams/sq ft
milligrams/sq meter	0.001	grams/sq meter
milligrams/sq meter	1.0	kilograms/sq km
milligrams/sq meter	0.0001	milligrams/sq cm
milligrams/sq meter	8.921×10^{-3}	pounds/acre
milligrams/sq meter	204.8×10^{-6}	pounds/1000 sq ft
milligrams/sq meter	2.855×10^{-3}	tons/sq mile
millihenries	0.001	henries
milliliters	1	cubic centimeters
milliliters	3.531×10^{-5}	cu ft
milliliters	6.102×10^{-2}	cu in
milliliters	10^{-6}	cu m
milliliters	2.642×10^{-4}	gal (U.S.)
milliliters	10^{-3}	liters
milliliters	0.03381	ounces (U.S. fl)
millimeters	0.1	centimeters
millimeters	3.281×10^{-3}	feet
millimeters	0.03937	inches
millimeters	10^{-6}	kilometers
millimeters	0.001	meters
millimeters	6.214×10^{-7}	miles
millimeters	39.37	mils
millimeters	1.094×10^{-3}	yards
millimeters of mercury	1.316×10^{-3}	atmospheres
millimeters of mercury	0.0394	inches of mercury
millimeters of mercury (0°C)	0.5358	inches of water (60°F)
millimeters of mercury	1.3595×10^{-3}	kg/sq cm
millimeter of mercury (0°C)	133.3224	newton/meter^2
millimeters of mercury	0.01934	pounds/sq in
millimeters/sec	11.81	feet/hour
million gallons	306.89	acre-ft
million gallons	3785.0	cubic meters
million gallons	3.785	mega liters (1×10^6)
million gallons/day (MGD)	1.547	cu ft/sec
MGD	3785	cu m/day
MGD	0.0438	cubic meters/sec
MGD	43.808	liters/sec
MGD/acre	9360	cu m/day/ha
MGD/acre	0.039	cu meters/hour/sq meter

Multiply	*by*	*to obtain*
mils	0.002540	centimeters
mils	8.333×10^{-5}	feet
mils	0.001	inches
mils	2.540×10^{-8}	kilometers
mils	25.40	microns
mils	2.778×10^{-5}	yards
miner's in.	1.5	cu ft/min
miner's inches (Ariz., Calif. Mont., and Ore.)	0.025	cubic feet/second
miner's in. (Colorado)	0.02604	cubic feet/second
miner's inches (Idaho, Kan., Neb., Nev., N. Mex., N. Dak., S. Dak. and Utah)	0.020	cubic feet/second
minims (British)	0.05919	cubic centimeter
minims (U.S.)	0.06161	cubic centimeters
minutes (angles)	0.01667	degrees
minutes (angles)	1.852×10^{-4}	quadrants
minutes (angles)	2.909×10^{-4}	radians
minutes (angle)	60	seconds (angle)
months (mean calendar)	30.4202	days
months (mean calendar)	730.1	hours
months (mean calendar)	43805	minutes
months (mean calendar)	2.6283×10^{6}	seconds
myriagrams	10	kilograms
myriameters	10	kilometers
myriawatts	10	kilowatts
nepers	8.686	decibels
newtons	10^{5}	dynes
newtons	0.10197	kilograms
newtons	0.22481	pounds
newtons/sq meter	1.00	pascals (Pa)
noggins (British)	1/32	gallons (British)
No./cu.cm.	28.316×10^{3}	No./cu ft
No./cu.cm.	10^{6}	No./cu meter
No./cu.cm.	1000.0	No./liter
No./cu.ft.	35.314×10^{-6}	No./cu cm
No./cu.ft.	35.314	No./cu meter
No./cu.ft.	35.314×10^{-3}	No./liter
No./cu. meter	10^{-6}	No./cu cm
No./cu. meter	28.317×10^{-3}	No./cu ft
No./cu. meter	0.001	No./liter
No./liter	0.001	No./cu cm
No./liter	28.316	No./cu ft

Multiply	*by*	*to obtain*
No./liter	1000.0	No./cu meter
oersteds (abs)	1	electromagnetic cgs units of magnetizing force
oersteds (abs)	2.9978×10^{10}	electrostatic cgs units of magnetizing force
ohms	10^{9}	abohms
ohms	1.1126×10^{-12}	statohms
ohms	10^{-6}	megohms
ohms	10^{6}	microhms
ohms (International)	1.0005	ohms (absolute)
ounces (avdp)	16	drams (avoirdupois)
ounces (avdp)	7.2917	drams (troy)
ounces (avdp)	437.5	grains
ounces (avdp)	28.349527	grams
ounces (avdp)	0.028350	kilograms
ounces (avdp)	2.8350×10^{4}	milligrams
ounces (avdp)	0.9114583	ounces (troy)
ounces (avdp)	0.0625	pounds (avoirdupois)
ounces (avdp)	0.075955	pounds (troy)
ounces (avdp)	2.790×10^{-5}	tons (long)
ounces (avdp)	2.835×10^{-5}	tons (metric)
ounces (avdp)	3.125×10^{-5}	tons (short)
ounces (Br. fl)	2.3828×10^{-4}	barrels (U.S.)
ounces (Br. fl)	1.0033×10^{-3}	cubic feet
ounces (Br. fl)	1.73457	cubic inches
ounces (Br. fl)	7.6860	drams (U.S. fl)
ounces (Br. fl)	6.250×10^{-3}	gallons (Br.)
ounces (Br. fl)	0.07506	gallons (U.S.)
ounces (Br. fl)	2.84121×10^{-2}	liters
ounces (Br. fl)	480	minims (Br.)
ounces (Br. fl)	461.160	minims (U.S.)
ounces (Br. fl)	28.4121	mL
ounces (Br. fl)	0.9607	ounces (U.S. fl)
ounces (troy)	17.554	drams (avdp)
ounces (troy)	8	drams (troy)
ounces (troy)	480	grains (troy)
ounces (troy)	31.103481	grams
ounces (troy)	0.03110	kilograms
ounces (troy)	1.09714	ounces (avoirdupois)
ounces (troy)	20	pennyweights (troy)
ounces (troy)	0.068571	pounds (avdp)
ounces (troy)	0.08333	pounds (troy)
ounces (troy)	3.061×10^{-5}	tons (long)
ounces (troy)	3.429×10^{-5}	tons (short)

Multiply	*by*	*to obtain*
ounces (U.S. fl)	2.48×10^{-4}	barrels (U.S.)
ounces (U.S. fl)	29.5737	cubic centimeters
ounces (U.S. fl)	1.0443×10^{-3}	cubic feet
ounces (U.S. fl)	1.80469	cubic inches
ounces (U.S. fl)	8	drams (fluid)
ounces (U.S. fl)	6.5053×10^{-3}	gallons (Br.)
ounces (U.S. fl)	7.8125×10^{-3}	gallons (U.S.)
ounces (U.S. fl)	29.5729	milliliters
ounces (U.S. fl)	499.61	minims (Br.)
ounces (U.S. fl)	480	minims (U.S.)
ounces (U.S. fl)	1.0409	ounces (Br. fl)
ounces/sq inch	4309	dynes/sq cm
ounces/sq. inch	0.0625	pounds/sq inch
paces	30	inches
palms (British)	3	inches
parsecs	3.260	light years
parsecs	3.084×10^{13}	kilometers
parsecs	3.084×10^{16}	meters
parsec	19×10^{12}	miles
parts/billion (ppb)	10^{-3}	mg/L
parts/million (ppm)	0.07016	grains/imp. gal.
parts/million	0.058417	grains/gallon (U.S.)
parts/million	1.0	mg/liter
parts/million	8.345	lbs/million gallons
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{24.04}$	micrograms/liter
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{0.02404}$	micrograms/cu meter
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{24.04}$	milligrams/cu meter
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{28.8}$	ppm by weight
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{385.1 \times 10^{6}}$	pounds/cu ft
ppm by weight	1.198×10^{-3}	micrograms/cu meter
ppm by weight	1.198	micrograms/liter
ppm by weight	1.198	milligrams/cu meter
ppm by weight	$\frac{28.8}{\text{molecular weight of gas}}$	ppm by volume (20°C)
ppm by weight	7.48×10^{-6}	pounds/cu ft
pecks (British)	0.25	bushels (British)
pecks (British)	554.6	cubic inches

Multiply	*by*	*to obtain*
pecks (British)	9.091901	liters
pecks (U.S.)	0.25	bushels (U.S.)
pecks (U.S.)	537.605	cubic inches
pecks (U.S.)	8.809582	liters
pecks (U.S.)	8	quarts (dry)
pennyweights	24	grains
pennyweights	1.555174	grams
pennyweights	0.05	ounces (troy)
pennyweights (troy)	4.1667×10^{-3}	pounds (troy)
perches (masonry)	24.75	cubic feet
phots	929.0	foot-candles
phots	1	lumen incident/sq cm
phots	10^4	lux
picas (printers')	1/6	inches
pieds (French feet)	0.3249	meters
pints (dry)	33.6003	cubic inches
pints (liq.)	473.179	cubic centimeters
pints (liq.)	0.01671	cubic feet
pints (liq.)	4.732×10^{-4}	cubic meters
pints (liq.)	6.189×10^{-4}	cubic yards
pints (liq.)	0.125	gallons
pints (liq.)	0.4732	liters
pints (liq.)	16	ounces (U.S. fluid)
pints (liq.)	0.5	quarts (liq.)
planck's constant	6.6256×10^{-27}	erg-seconds
poise	1.00	gram/cm sec
poise	0.1	newton-second/meter^2
population equivalent (PE)	0.17	pounds BOD
pottles (British)	0.5	gallons (British)
pouces (Paris inches)	0.02707	meters
pouces (Paris inches)	0.08333	pieds (Paris feet)
poundals	13,826	dynes
poundals	14.0981	grams
poundals	1.383×10^{-3}	joules/cm
poundals	0.1383	joules/meter (newton)
poundals	0.01410	kilograms
poundals	0.031081	pounds
pounds (avdp)	256	drams (avdp)
pounds (avdp)	116.67	drams (troy)
pounds (avdp)	444,823	dynes
pounds (avdp)	7000	grains
pounds (avdp)	453.5924	grams
pounds (avdp)	0.04448	joules/cm
pounds (avdp)	4.448	joules/meter (newtons)

Multiply	*by*	*to obtain*
pounds (avdp)	0.454	kilograms
pounds (avdp)	4.5359×10^5	milligrams
pounds (avdp)	16	ounces (avdp)
pounds (avdp)	14.5833	ounces (troy)
pounds (avdp)	32.17	poundals
pounds (avdp)	1.2152778	pounds (troy)
pounds (avdp)	4.464×10^{-4}	tons (long)
pounds (avdp)	0.0005	tons (short)
pounds (troy)	210.65	drams (avdp)
pounds (troy)	96	drams (troy)
pounds (troy)	5760	grains
pounds (troy)	373.2418	grams
pounds (troy)	0.37324	kilograms
pounds (troy)	3.7324×10^5	milligrams
pounds (troy)	13.1657	ounces (avdp)
pounds (troy)	12.0	ounces (troy)
pounds (troy)	240.0	pennyweights (troy)
pounds (troy)	0.8229	pounds (avdp)
pounds (troy)	3.6735×10^{-4}	tons (long)
pounds (troy)	3.7324×10^{-4}	tons (metric)
pounds (troy)	4.1143×10^{-4}	tons (short)
pounds (avdp)-force	4.448	newtons
pounds-force-sec/ft^2	47.88026	newton-sec/meter^2
pounds (avdp)-mass	0.4536	kilograms
pounds-mass/ft^3	16.0185	kilogram/meter^3
pounds-mass/ft-sec	1.4882	mewton-sec/meter^2
pounds of BOD	5.882	population equivalent (PE)
pounds of carbon to CO_2	14,544	BTU (mean)
pounds of water	0.0160	cu ft
pounds of water	27.68	cu in
pounds of water	0.1198	gallons
pounds of water evaporated at 212°F	970.3	BTU
pounds of water per min	2.699×10^{-4}	cubic feet/sec
pound-feet	13,825	centimeter-grams
pound-feet (torque)	1.3558×10^7	dyne-centimeters
pound-feet	0.1383	meter-kilograms
pounds-feet squared	421.3	kg-cm squared
pounds-feet squared	144	pounds-inches squared
pounds-inches squared	2926	kg-cm squared
pounds-inches squared	6.945×10^{-3}	pounds-feet squared
pounds/acre	0.0104	grams/sq ft
pounds/acre	0.1121	grams/sq meter
pounds/acre	1.121	kg/ha
pounds/acre	112.1	kilograms/sq km

Multiply	*by*	*to obtain*
pounds/acre	0.01121	milligrams/sq cm
pounds/acre	112.1	milligrams/sq meter
pounds/acre	0.023	pounds/1000 sq ft
pounds/acre	0.32	tons/sq mile
pounds/acre/day	0.112	g/day/sq m
pounds/cu ft	0.0160	g/mL
pounds/cu ft	16.02	kg/cu m
pounds/cu ft	16.018×10^9	micrograms/cu meter
pounds/cu ft	16.018×10^6	micrograms/liter
pounds/cu ft	16.018×10^6	milligrams/cu meter
pounds/cu ft	$\frac{385.1 \times 10^6}{\text{molecular weight of gas}}$	ppm by volume (20°C)
pounds/cu ft	133.7×10^3	ppm by weight
pounds/cu ft	5.787×10^{-4}	lb/cu in
pounds/cu ft	5.456×10^{-9}	pounds/mil-foot
pounds/1000 cu ft	0.35314	grams/cu ft
pounds/1000 cu ft	16.018	grams/cu m
pounds/1000 cu ft	353.14×10^3	micrograms/cu ft
pounds/1000 cu ft	16.018×10^6	microgram/cu m
pounds/1000 cu ft	16.018×10^3	milligrams/cu m
pounds/cubic inch	27.68	grams/cubic cm
pounds/cubic inch	2.768×10^4	kgs/cubic meter
pounds/cubic inch	1728	pounds/cubic foot
pounds/cubic inch	9.425×10^{-6}	pounds/mil foot
pounds/day/acre-ft	3.68	g/day/cu m
pounds/day/cu ft	16	kg/day/cu m
pounds/day/cu yd	0.6	kg/day/cu m
pounds/day/sq ft	4,880	g/day/sq m
pounds/ft	1.488	kg/m
pounds/gal	454 g/3.7851L = 119.947	g/liter
pounds/1000-gal	120	g/1000-liters
pounds/horsepower-hour	0.169	mg/joule
pounds/in	178.6	g/cm
pounds/mil-foot	2.306×10^6	gms/cu cm
pounds/mil gal	0.12	g/cu m
pounds/sq ft	4.725×10^{-4}	atmospheres
pounds/sq ft	0.01602	ft of water
pounds/sq ft	0.01414	inches of mercury
pounds/sq ft	4.8824×10^{-4}	kgs/sq cm
pounds/sq ft	4.88241	kilograms/square meter
pounds/sq ft	47.9	newtons/sq m
pounds/sq ft	6.944×10^{-3}	pounds/sq inch
pounds/1000 sq ft	0.4536	grams/sq ft

Multiply	*by*	*to obtain*
pounds/1000 sq ft	4.882	grams/sq meter
pounds/1000 sq ft	4882.4	kilograms/sq km
pounds/1000 sq ft	0.4882	milligrams/sq cm
pounds/1000 sq ft	4882.4	milligrams/sq meter
pounds/1000 sq ft	43.56	pounds/acre
pounds/1000 sq ft	13.94	tons/sq mile
pounds/sq in	0.068046	atmospheres
pounds/sq in	2.307	ft of water
pounds/sq in	70.307	grams/square centimeter
pounds/sq in	2.036	in of mercury
pounds/sq in	0.0703	kgs/square cm
pounds/sq in	703.07	kilograms/square meter
pounds/sq in	51.715	millimeters of mercury
pounds/sq in	6894.76	newton/meter^2
pounds/sq in	51.715	millimeters of mercury at 0°C
pounds/sq in	144	pounds/sq foot
pounds/sq in (abs)	1	pound/sq in (gage) + 14.696
proof (U.S.)	0.5	percent alcohol by volume
puncheons (British)	70	gallons (British)
quadrants (angle)	90	degrees
quadrants (angle)	5400	minutes
quadrants (angle)	3.24×10^5	seconds
quadrants (angle)	1.571	radians
quarts (dry)	67.20	cubic inches
quarts (liq.)	946.4	cubic centimeters
quarts (liq.)	0.033420	cubic feet
quarts (liq.)	57.75	cubic inches
quarts (liq.)	9.464×10^{-4}	cubic meters
quarts (liq.)	1.238×10^{-3}	cubic yards
quarts (liq.)	0.25	gallons
quarts (liq.)	0.9463	liters
quarts (liq.)	32	ounces (U.S., fl)
quarts (liq.)	0.832674	quarts (British)
quintals (long)	112	pounds
quintals (metric)	100	kilograms
quintals (short)	100	pounds
quires	24	sheets
radians	57.29578	degrees
radians	3438	minutes
radians	0.637	quadrants
radians	2.063×10^5	seconds
radians/second	57.30	degrees/second
radians/second	9.549	revolutions/min
radians/second	0.1592	revolutions/sec

Multiply	*by*	*to obtain*
radians/sec/sec	573.0	revs/min/min
radians/sec/sec	9.549	revs/min/sec
radians/sec/sec	0.1592	revs/sec/sec
reams	500	sheets
register tons (British)	100	cubic feet
revolutions	360	degrees
revolutions	4	quadrants
revolutions	6.283	radians
revolutions/minute	6	degrees/second
revolutions/minute	0.10472	radians/second
revolutions/minute	0.01667	revolutions/sec
revolutions/minute^2	0.0017453	radians/sec/sec
revs/min/min	0.01667	revs/min/sec
revs/min/min	2.778×10^{-4}	revs/sec/sec
revolutions/second	360	degrees/second
revolutions/second	6.283	radians/second
revolutions/second	60	revs/minute
revs/sec/sec	6.283	rads/sec/sec
revs/sec/sec	3600	revs/min/min
revs/sec/sec	60	revs/min/sec
reyns	6.8948×10^{6}	centipoises
rod	.25	chain (gunters)
rods	16.5	feet
rods	5.0292	meters
rods	3.125×10^{-3}	miles
rods (surveyors' means)	5.5	yards
roods (British)	0.25	acres
scruples	1/3	drams (troy)
scruples	20	grains
sections	1	square miles
seconds (mean solar)	1.1574×10^{-5}	days
seconds (angle)	2.778×10^{-4}	degrees
seconds (mean solar)	2.7778×10^{-4}	hours
seconds (angle)	0.01667	minutes
seconds (angle)	3.087×10^{-6}	quadrants
seconds (angle)	4.848×10^{-6}	radians
slugs	14.59	kilogram
slugs	32.174	pounds
space, entire (solid angle)	12.566	steradians
spans	9	inches
spheres (solid angle)	12.57	steradians
spherical right angles	0.25	hemispheres
spherical right angles	0.125	spheres
spherical right angles	1.571	steradians

Multiply	*by*	*to obtain*
square centimeters	1.973×10^{5}	circular mils
square centimeters	1.07639×10^{-3}	square feet (U.S.)
square centimeters	0.15499969	square inches (U.S.)
square centimeters	10^{-4}	square meters
square centimeters	3.861×10^{-11}	square miles
square centimeters	100	square millimeters
square centimeters	1.196×10^{-4}	square yards
square centimeters-square centimeter (moment of area)	0.024025	square inch-square inch
square chains (gunter's)	0.1	acres
square chains (gunter's)	404.7	square meters
square chains (Ramden's)	0.22956	acres
square chains (Ramden's)	10000	square feet
square feet	2.29×10^{-5}	acres
square feet	1.833×10^{8}	circular mils
square feet	144	square inches
square feet	0.092903	square meters
square feet	929.0341	square centimeters
square feet	3.587×10^{-8}	square miles
square feet	1/9	square yards
square feet/cu ft	3.29	sq m/cu m
square foot-square foot (moment of area)	20,736	square inch-square inch
square inches	1.273×10^{6}	circular mils
square inches	6.4516258	square centimeters
square inches	6.944×10^{-3}	square feet
square inches	645.2	square millimeters
square inches	10^{6}	square mils
square inches	7.71605×10^{-4}	square yards
square inches-inches sqd.	41.62	sq cm-cm sqd
square inches-inches sqd.	4.823×10^{-5}	sq feet-feet sqd
square kilometers	247.1	acres
square kilometers	10^{10}	square centimeters
square kilometers	10.76×10^{6}	square feet
square kilometers	1.550×10^{9}	square inches
square kilometers	10^{6}	square meters
square kilometers	0.3861006	square miles (U.S.)
square kilometers	1.196×10^{6}	square yards
square links (Gunter's)	10^{-5}	acres (U.S.)
square links (Gunter's)	0.04047	square meters
square meters	2.471×10^{-4}	acres (U.S.)
square meters	10^{4}	square centimeters
square meters	10.76387	square feet (U.S.)
square meters	1550	square inches

Multiply	*by*	*to obtain*
square meters	3.8610×10^{-7}	square miles (statute)
square meters	10^{6}	square millimeters
square meters	1.196	square yards (U.S.)
square miles	640	acres
square miles	2.78784×10^{7}	square feet
square miles	2.590	sq km
square miles	2.5900×10^{6}	square meters
square miles	3.098×10^{6}	square yards
square millimeters	1.973×10^{3}	circular mils
square millimeters	0.01	square centimeters
square millimeters	1.076×10^{-5}	square feet
square millimeters	1.550×10^{-3}	square inches
square mils	1.273	circular mils
square mils	6.452×10^{-6}	square centimeters
square mils	10^{-6}	square inches
square rods	272.3	square feet
square yard	2.1×10^{-4}	acres
square yards	8361	square centimeters
square yards	9	square feet
square yards	1296	square inches
square yards	0.8361	square meters
square yards	3.228×10^{-7}	square miles
square yards	8.361×10^{5}	square millimeters
statamperes	3.33560×10^{-10}	amperes (abs)
statcoulombs	3.33560×10^{-10}	coulombs (abs)
statcoulombs/kilogram	1.0197×10^{-6}	statcoulombs/dyne
statfarads	1.11263×10^{-12}	farads (abs)
stathenries	8.98776×10^{11}	henries (abs)
statohms	8.98776×10^{11}	ohms (abs)
statvolts	299.796	volts (abs)
statvolts/inch	118.05	volts (abs)/centimeter
statwebers	2.99796×10^{10}	electromagnetic cgs units of magnetic flux
statwebers	1	electrostatic cgs units of magnetic flux
stilb	2919	footlambert
stilb	1	int. candle cm^{-2}
stilb	3.142	lambert
stoke (kinematic viscosity)	10^{-4}	$meter^2$/second
stones (British)	6.350	kilograms
stones (British)	14	pounds
temp. (degs. C.) + 273	1	abs. temp. (degs. K.)
temps (degs. C.) + 17.8	1.8	temp. (degs. Fahr.)
temps. (degs. F.) + 460	1	abs. temp. (degs. R.)
temps. (degs. F.) − 32	5/9	temp. (degs. Cent.)

Multiply	*by*	*to obtain*
toises (French)	6	paris feet (pieds)
tons (long)	5.734×10^5	drams (avdp)
tons (long)	2.613×10^5	drams (troy)
tons (long)	1.568×10^7	grains
tons (long)	1.016×10^6	grams
tons (long)	1016	kilograms
tons (long)	3.584×10^4	ounces (avdp)
tons (long)	3.267×10^4	ounces (troy)
tons (long)	2240	pounds (avdp)
tons (long)	2722.2	pounds (troy)
tons (long)	1.12	tons (short)
Tons (metric) (T)	1000	kilograms
Tons (metric) (T)	2204.6	pounds
Tons (metric) (T)	1.1025	tons (short)
tons (short)	5.120×10^5	drams (avdp)
tons (short)	2.334×10^5	drams (troy)
tons (short)	1.4×10^7	grains
tons (short)	9.072×10^5	grams
tons (short)	907.2	kilograms
tons (short)	32,000	ounces (avdp)
tons (short)	29,166.66	ounces (troy)
tons (short)	2000	pounds (avdp)
tons (short)	2.430.56	pounds (troy)
tons (short)	0.89287	tons (long)
tons (short)	0.9078	Tons (metric) (T)
tons (short)/sq ft	9765	kg/sq meter
tons (short)/sq ft	13.89	pounds/sq inch
tons (short)/sq in	1.406×10^6	kg/sq meter
tons (short)/sq in	2000	pounds/sq inch
tons/sq mile	3.125	pounds/acre
tons/sq mile	0.07174	pounds/1000 sq ft
tons/sq mile	0.3503	grams/sq meter
tons/sq mile	350.3	kilograms/sq km
tons/sq mile	350.3	milligrams/sq meter
tons/sq mile	0.03503	milligrams/sq cm
tons/sq mile	0.03254	grams/sq ft
tons of water/24 hours	83.333	pounds of water/hr
tons of water/24 hours	0.16643	gallons/min
tons of water/24 hours	1.3349	cu ft/hr
torr (mm Hg, 0°C)	133.322	newton/meter^2
townships (U.S.)	23040	acres
townships (U.S.)	36	square miles
tuns	252	gallons
volts (abs)	10^8	abvolts

Multiply	*by*	*to obtain*
volts (abs)	3.336×10^{-3}	statvolts
volts (internationalof 1948)	1.00033	volts (abs)
volt/inch	.39370	volt/cm
watts (abs)	3.41304	BTU (mean)/hour
watts (abs)	0.0569	BTU (mean)/min
watts (abs)	0.01433	calories, kilogram (mean)/minute
watts (abs)	10^7	ergs/second
watts (abs)	44.26	foot-pounds/minute
watts (abs)	0.7376	foot-pounds/second
watts (abs)	0.0013405	horsepower (electrical)
watts (abs)	1.360×10^{-3}	horsepower (metric)
watts (abs)	1	joules/sec
watts (abs)	0.10197	kilogram-meters/second
watts (abs)	10^{-3}	kilowatts
watt-hours	3.415	British Thermal Units
watt-hours	3.60×10^{10}	ergs
watt-hours	2655	foot-pounds
watt-hours	859.85	gram-calories
watt-hours	1.34×10^{-3}	horsepower-hours
watt-hours	3.6×10^3	joule
watt-hours	0.8605	kilogram-calories
watt-hours	367.1	kilogram-meters
watt-hours	10^{-3}	kilowatt-hours
watt (international)	1.0002	watt (absolute)
watt/(cm^2)(°C/cm)	693.6	BTU/(hr)(ft^2)(°F/in)
wave length of the red line of cadmium	6.43847×10^{-7}	meters
webers	10^3	electromagnetic cgs units
webers	3.336×10^{-3}	electrostatic cgs units
webers	10^5	kilolines
webers	10^8	lines
webers	10^8	maxwells
webers	3.336×10^{-3}	statwebers
webers/sq in	1.550×10^7	gausses
webers/sq in	10^8	lines/sq in
webers/sq in	0.1550	webers/sq cm
webers/sq in	1,550	webers/sq meter
webers/sq meter	10^4	gausses
webers/sq meter	6.452×10^4	lines/sq in
webers/sq meter	10^{-4}	webers/sq cm
webers/sq meter	6.452×10^{-4}	webers/sq in
weeks	168	hours
weeks	10,080	minutes

Multiply	*by*	*to obtain*
weeks	604,800	seconds
yards	91.44	centimeters
yards	3	feet
yards	36	inches
yards	9.144×10^{-4}	kilometers
yards	0.91440	meters
yards	4.934×10^{-4}	miles (naut.)
yards	5.682×10^{-4}	miles (stat.)
yards	914.4	millimeters
years (sidereal)	365.2564	days (mean solar)
years (sidereal)	366.2564	days (sidereal)
years (tropical, mean solar)	365.2422	days (mean solar)
years (common)	8760	hours
years (tropical, mean solar)	8765.8128	hours (mean solar)
years (leap)	366	days
years (leap)	8784	hours
years (tropical, mean solar)	3.155693×10^{7}	seconds (mean solar)
years (tropical, mean solar)	1.00273780	years (sidereal)

2. BASIC AND SUPPLEMENTARY UNITS

A *meter (m)* is 1,650,763.73 wavelengths in vacuo of the radiation corresponding to the transition between the energy levels $2p_{10}$ and $5d_5$ of the krypton 86 atom.

A *kilogram (kg)* is the mass of the international prototype in the custody of the Bureau International des Poids et Mesures at Sevres in France.

A *second (sec)* is the interval occupied by 9,192,631,770 cycles of the radiation corresponding to the transition of the cesium-133 atom when unperturbed by exterior fields.

An *ampere* is the constant current that if maintained in two parallel rectilinear conductors of infinite length of negligible circular cross section and placed at a distance of one meter apart in vacuo would produce between these conductors a force equal to 2×10^{-7} newton per meter length.

A *kelvin* ($^\circ K$) is the degree interval of the thermodynamic scale on which the temperature of the triple point of water is 273.16 degrees.

A *candle* is such that the luminance of a full radiator at the temperature of solidification of platinum is 60 units of luminous intensity per square centimeter.

A *mole (mol)* is the amount of substance which contains as many elementary units as there are atoms in 0.012 kg of carbon-12. The elementary unit must be specified and may be an atom, an ion, an electron, a photon, etc., or a given group of such entities.

A *radian* is the angle subtended at the center of a circle by an arc of the circle equal in length to the radius of the circle.

A *steradian* is the solid angle that, having its vertex at the center of a sphere, cuts off an area of the surface of the sphere equal to that of a square with sides of length equal to the radius of the sphere.

3. DERIVED UNITS AND QUANTITIES

The *liter* was defined in 1901 as the volume of 1 kilogram of pure water at normal atmospheric pressure and maximum density equal therefore to 1.000028 dm^3. This 1901 definition applied for the purpose of the 1963 Weights and Measures Acts.

By a resolution of the 12th Conference General des Poids et Mesures (CGPM) in 1964 the word *liter* is now recognized as a special name for the dm^3, but is not used to express high precision measurements. It is used widely in engineering and the retail business, where the discrepancy of 28 parts in 1 million is of negligible significance.

A *newton (N)* is the force that, when applied to a body of mass of one kilogram, gives it an acceleration of one meter per second per second.

Stress is defined as the resultant internal force per unit area resisting change in the shape or size of a body acted on by external forces, and is therefore measured in *newtons per square meter* (N/m^2).

A *bar* is a pressure equivalent to 100,000 newtons acting on an area of one square metor.

A *joule (J)* is the work done when the point of application of a force of one newton is displaced through a distance of one meter in the direction of the force.

A *watt* is equal to one joule per second.

Dynamic viscosity is the property of a fluid whereby it tends to resist relative motion within itself. It is the shear stress, i.e., the tangential force on unit area, between two infinite horizontal planes at unit distance apart, one of which is fixed while the other moves with unit velocity. In other words, it is the shear stress divided by the velocity gradient, i.e., $(N/m^2) \div (m/sec/m) = N\ sec/m^2$.

Kinematic viscosity is the dynamic viscosity of a fluid divided by its density, i.e., $(N\ sec/m^2)/(kg/m^3) = m^2/sec$.

Density of heat flow rate (or heat flux) is the heat flow rate (W) per unit area, i.e., W/m^2.

Coefficient of heat transfer is the heat flow rate (W) per unit area per unit temperature difference, i.e., $W/m^{2\circ}C$.

Thermal conductivity is the quantity of heat that will be conducted in unit time through unit area of a slab of material of unit thickness with a unit difference of temperature between the faces; in other words, the heat flow rate (W) per unit area per unit temperature gradient, i.e., $W/[m^2(°C/m)] = W/m°C$.

The *heat capacity* of a substance is the quantity of heat gained or lost by the substance per unit temperature change, i.e., J/°C.

Specific heat capacity is the heat capacity per unit mass of the substance, i.e., J/kg°C.

Internal energy is the kinetic energy possessed by the molecules of a substance due to temperature and is measured in joules (J).

Specific internal energy (u) is the internal energy per unit mass of the substance, i.e., J/kg. When a small amount of heat is added at constant volume the increase in specific internal

energy is given by: du $= c_v\, dT$, where c_v is the specific heat capacity at constant volume, and dT is the increase in absolute temperature.

Specific enthalpy (h) is defined by the equation: $h = u + pv$, where p is the pressure and v is the specific volume. Specific enthalpy is measured in J/kg. When a small amount of heat is added to a substance at constant pressure, the increase in specific enthalpy is given by: $-dh = cp\, dT$, where cp is the specific heat capacity at constant pressure.

The *specific latent heat* of a substance is the heat gained per unit mass without an accompanying rise in temperature during a change of state at constant pressure. It is measured in J/kg.

The *entropy* (S) of a substance is such that when a small amount of heat is added, the increase in entropy is equal to the quantity of heat added (dQ) divided by the absolute temperature (T) at which the heat is absorbed; i.e., $dS = dQ/T$, measured in J/°K.

The *specific entropy* (s) of a substance is the entropy per unit mass, i.e., J/kg°K.

A *volt* is the difference of electric potential between two points of a conductor carrying a constant current of one ampere when the power dissipated is one watt.

A *weber* (Wb) is the magnetic flux through a conductor with a resistance of one ohm when reversal of the direction of the magnetic flux causes the transfer of one coulomb in the conductor loop.

Tesla: The magnetic flux density is the normal magnetic flux per unit area and is measured in *teslas*.

A *lumen*, the unit of luminous flux, is the flux emitted within unit solid angle of one steradian by a point source having a uniform intensity of one candle.

A *lux* is an illumination of one lumen per square meter.

Luminance is the luminous intensity per unit area of a source of light or of an illumination. It is measured in candles per square meter.

4. PHYSICAL CONSTANTS

Standard temperature and pressure (S.T.P.)	$= 273.15°\text{K}$ and $1.013 \times 10^5\ \text{N/m}^2$ $= 0°\text{C}$ and $1.013\ \text{bar}$ $= 0°\text{C}$ and $760\ \text{mm Hg}$
Molecular volume of ideal gas at S.T.P.	$= 22.41\ \text{liters/mol}$
Gas constant (R)	$= 8.314\ \text{J/mol}°\text{K}$
$^{RT}(273.15°\text{K})$	$= 2.271 \times 10^3\ \text{J/mol}$
Avogadro constant	$= 6.023 \times 10^{23}/\text{mol}$
Boltzmann constant	$= 1.3805 \times 10^{-23}\ \text{J/K}$
Faraday constant	$= 9.6487 \times 10^4\ °\text{C/mol}\ (= \text{A s/mol})$
Planck constant	$= 6.626 \times 10^{-34}\ \text{J sec}$
Stefan-Boltzman constant	$= 5.6697 \times 10^{-8}\ \text{W/m}^2\ \text{K}^4$
Ice point of water	$= 273.15°\text{K}\ (0°\text{C})$
Triple point of water	$= 273.16°\text{K}\ (0.01°\text{C})$
Speed of light	$= 2.998 \times 10^8\ \text{m/sec}$
Acceleration of gravity (standard) (Greenwich)	$= 9.80665\ \text{m/s}^2$ $= 9.81188\ \text{m/s}^2$ [take g as $9.81\ \text{m/s}^2$]
Universal constant of gravitation	$= 6.670 \times 10^{-11}\ \text{Newton m}^2/\text{kg}^2$
Mass of hydrogen atom	$= 1.6734 \times 10^{-27}\ \text{kg}$

5. PROPERTIES OF WATER

Temperature (°F)	Specific weight, γ (lb/ft^3)	Mass density, ρ (lb-sec^2/ft^4)	Dynamic viscosity, $\mu \times 10^5$ (lb-sec/ft^2)	Kinematic viscosity, $\nu \times 10^5$ (ft^2/sec)	Surface energy, $\sigma \times 10^3$ (lb/ft)	Vapor pressure, ρ (lb/in.^{2})	Bulk modulus, $E \times 10^{-3}$ (lb/in.^{2})
32	62.42	1.940	3.746	1.931	5.18	0.09	290
40	62.43	1.938	3.229	1.664	5.14	0.12	295
50	62.41	1.936	2.735	1.410	5.09	0.18	300
60	62.37	1.934	2.359	1.217	5.04	0.26	312
70	62.30	1.931	2.050	1.059	5.00	0.36	320
80	62.22	1.927	1.799	0.930	4.92	0.51	323
90	62.11	1.923	1.595	0.826	4.86	0.70	326
100	62.00	1.918	1.424	0.739	4.80	0.95	329
110	61.86	1.913	1.284	0.667	4.73	1.24	331
120	61.71	1.908	1.168	0.609	4.65	1.69	333
130	61.55	1.902	1.069	0.558	4.60	2.22	332
140	61.38	1.896	0.981	0.514	4.54	2.89	330
150	61.20	1.890	0.905	0.476	4.47	3.72	328
160	61.00	1.896	0.838	0.442	4.41	4.74	326
170	60.80	1.890	0.780	0.413	4.33	5.99	322
180	60.58	1.883	0.726	0.385	4.26	7.51	318
190	60.36	1.876	0.678	0.362	4.19	9.34	313
200	60.12	1.868	0.637	0.341	4.12	11.52	308
212	59.83	1.860	0.593	0.319	4.04	14.7	300

6. PERIODIC TABLE OF THE ELEMENTS (COMPLIMENTS OF THE LENOX INSTITUTE OF WATER TECHNOLOGY)

Groups ⇨ / Periods & sub-shells ⇩	I IA	2 IIA	3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8	9 VIII	10	11 IB	12 IIB	13 IIIA	14 VIA	15 VA	16 VIA	17 VIIA	18 O
I IS	1 H 1.00794 Hydrogen																1 H 1.00794 Hydrogen	2 He 4.00260 Helium
2 2s2p	3 Li 6.941 Lithium	4 Be 9.01218 Beryllium											5 B 10.811 Boron	6 C 12.011 Boron	7 N 14.0067 Nitrogen	8 O 15.9994 Oxygen	9 F 18.9984 Fluorine	10 Ne 20.179 Neon
3 3s3p	11 Na 22.9897 Sodium	12 Mg 24.305 Magnesium	3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8	9 VIII	10	11 IB	12 IIB	13 AI 26.9815 Aluminum	14 Si 28.0855 Silicon	15 P 30.9738 Phosphorus	16 S 32.066 Sulfur	17 CI 35.4527 Chlorine	18 Ar 39.948 Argon
4 4s3d4p	19 K 39.098 Potassium	20 Ca 40.078 Calcium	21 Sc 44.9559 Scandium	22 Ti 47.88 Titanium	23 V 50.9415 Vanadium	24 Cr 51.996 Chromium	25 Mn 54.938 Manganese	26 Fe 55.847 Iron	27 Co 58.933 Cobalt	28 Ni 58.69 Nickel	29 Cu 63.546 Copper	30 Zn 65.39 Zinc	31 Ga 69,723 Gallium	32 Ge 69.561 Germanium	33 As 74.9216 Arsenic	34 Se 78.96 Selenium	35 Br 79.904 Bromine	36 Kr 83.80 Krypton
5 5s4d5p	37 Rb 85.468 Rubidium	38 Sr 87.62 Strontium	39 Y 88.9059 Yttrium	40 Zr 91.224 Zirconium	41 Nb 92.9064 Niobium	42 Mo 95.94 Molybdenum	43 Tc (98) Technetium	44 Ru 101.07 Ruthenium	45 Rh 102.906 Rhodium	46 Pd 106.42 Palladium	47 Ag 107.868 Silver	48 Cd 112.411 Cadmium	49 In 114.82 Indium	50 Sn 118.710 Tin	51 Sb 121.75 Antimony	52 Te 127.60 Tellurium	53 I 126.90 Iodine	54 Xe 131.29 Xenon
6 6s4f5d6p	55 Cs 132.905 Cesium	56 Ba 137.327 Barium	57 La 138.906 Lanthanum	72 Hf 178.49 Hafnium	73 Ta 180.948 Tantalum	74 W 183.85 Tungsten	75 Re 186.207 Rhenium	76 Os 190.2 Osmium	77 Ir 192.22 Iridium	78 Pt 195.08 Platinum	79 Au 196.97 Gold	80 Hg 200.59 Mercury	81 TI 204.383 Thallium	82 Pb 207.2 Lead	83 Bi 208.98 Bismuth	84 Po (209) Polonium	85 At (210) Astatine	86 Rn (222) Radon
7 7s5f6d	87 Fr (223) Francium	88 Ra (226) Radium	89 Ac (227) Actinium	104 Rf (261) Ruther-fordium	105 Ha (262) Dubnium	106 Sg (263) Seaborgium	107 Ns (262) Bohrium	108 Hs (265) Hassium	109 Mt (266) Meitnerium	110		112						

6 4f	58 Ce 140.116 Cerium	59 Pr 140.91 Praseody-mium	60 Nd 144.24 Neodymium	61 Pm (145) Promethium	62 Sm 150.35 Samarium	63 Eu 107.26 Europium	64 Gd 157.25 Gadolinium	65 Tb 158.925 Terbium	66 Dy 162.50 Dysprosium	67 Ho 104.930 Holmium	68 Er 167.26 Erbium	69 Tm 168.934 Thulium	70 Yb 173.04 Ytterbium	71 Lu 174.967 Lutetium
7 5f	90 Th 232.038 Thorium	91 Pa (231) Protactinium	92 U 238.029 Uranium	93 Np (237) Neptunium	94 Pu (244) Plutonium	95 Am (243) Americium	96 Cm (247) Curium	97 Bk (247) Berkelium	98 Cf (251) Californium	99 Es (252) Einsteinium	100 Fm (257) Fermium	101 Md (258) Mendelevium	102 No (259) Nobelium	103 Lr (262) Lawrencium

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