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Biological Treatment of Industrial Effluents

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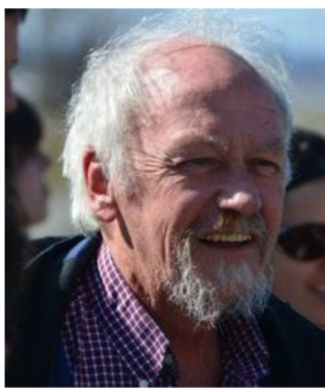

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Preface

This book is a part of the comprehensive series *Current Developments in Biotechnology and Bioengineering* (Editor-in-Chief: Ashok Pandey), comprising nine volumes. To this series, the present volume brings an extensive and thorough treatment of various waste and wastewater treatment processes by internationally recognized experts. Up-to-date coverage of recent advances is given for each specific subject area, and the remaining challenges over the wide range of processes are highlighted. As will be evident, enormous quantities of municipal, industrial, and agricultural wastewaters are generated globally each year.

With increasing global urbanization, municipal wastewater treatment has taken on worldwide importance. This is no small problem, with domestic wastewater production estimated to be 330 km^3/year. This is enough to provide, if all were treated, sufficient water and nutrients for millions of acres of food crop production and enough energy, through anaerobic digestion, for millions of households. Of course, we are far from achieving total recovery of the valuable resources present. This waste stream is particularly well characterized and of rather similar composition everywhere. However, even here there are treatment challenges related to climatic variations or nutrient removal requirements. These subjects are treated in part by several of the chapters in the present volume, including one on wastewater treatment in cold climates and another on the recently developed anammox process for effective tertiary treatment through the removal of fixed nitrogen.

The quantity of annual agricultural wastewater produced globally is enormous because of the large water usage requirement: on average 1000 L/kg for plant crops and at least three times this amount for meat production. Agricultural wastewater, largely runoff, is difficult to treat because it is a non-point-source emission, but has substantial pollution potential due to its content of fertilizers, leading to eutrophication of natural water bodies, and to its content of herbicides and pesticides. Agricultural runoff is causing enormous problems worldwide, with more than 70% of rivers and 50% of lakes in the United States being affected. Some types of agricultural wastewaters can be effectively treated using anaerobic digestion, a topic that is discussed in several chapters in the present volume: microbiology and biochemistry of anaerobic treatment; anaerobic bioreactors/digesters, design and development; and by-products of anaerobic treatments—methane and manure.

Industrial wastewater effluents have a significant potential for pollution of local water resources with important consequent impacts on human health and ecosystems, problems that have been encountered in actual fact over the past century with the direct discharge of enormous amounts of wastes into lakes, rivers, and oceans. In addition, many industrial processes in current use have very high water demands. Coupled with increasing water scarcity in many parts of the world, this means that effective wastewater treatment will become imperative to return useable water to local environments for reuse.

Some treatment technologies have already been brought into practice and are currently deployed in at least some developed countries, but their widespread adoption by developing countries remains to be implemented. With increased industrialization of developing countries, effective treatment of industrial wastes will become a challenge of ever greater importance in the future. In addition, the efficiency of many present treatment technologies,

both in terms of energy usage and in terms of treatment efficacy, has room for significant improvement. This, together with the large scope of treatment required, presents ample challenges for dedicated research and development efforts, as detailed by a number of authors in this volume.

Unlike municipal and agricultural wastewaters, which are fairly uniform in composition throughout the world, industrial wastewaters are highly variable in both the quantity of polluting materials present and their composition. Each type of industry produces a waste stream with a distinct chemical composition that is a direct reflection of the particular chemical/biochemical processes involved. Hence, treatment processes need to be specifically tailored for each type of wastewater that is to be discharged into the environment. This volume contains chapters specifically discussing treatment of low-strength and high-strength wastewaters. As well, a large number of examples of treatment processes for many important industrial sectors are presented, including effluents from the food and beverage industries, the textile industry, aquaculture, the pharmaceutical and personal care products industries, the petroleum industry, the pulp and paper industry, mining, and the electronic and electrochemical industries. Additional chapters discuss some specific treatment aspects relevant to industrial wastewaters, including dechlorination processes, treatment of recalcitrant wastes, and removal of toxic components of wastewaters.

Finally, two chapters discuss topics of general interest in wastewater treatment, the advantages and disadvantages of anaerobic treatments versus aerobic treatments and the application of molecular biological tools to monitor process efficiency. Taken together, we believe this volume presents an authoritative and comprehensive review of selected topics in wastewater treatment that should be of use to practitioners, researchers, and teachers and students.

We would like to acknowledge the reviewers for their valuable comments to improve the final quality of the chapters included in this volume. In addition, we would also like to thank Dr. Kostas Marinakis, Book Acquisition Editor; Ms. Anneka Hess; and the entire production team at Elsevier for their help and support in bringing out this volume. Without their commitment, efficiency, and dedicated work, this volume could not have ever been accomplished.

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1

Aerobic Treatment of Effluents From Textile Industry

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1.1 Introduction

Rapid industrialization of the developing world has contributed to unsustainable pollution levels [1,2]. In the past few decades, an increase in the demand for textile products has led to a steep rise in water pollution [3,4]. Textile effluent is tagged as the most polluting as it consumes a large quantity of water and chemicals for the processing of fabrics throughout the world and in turn these industries generate pollution by discarding the wastes [5–7]. Similarly, increasing financial constraints pave the way for discharge of untreated effluents into the environment [8]. Globally, 280,000 tons of dye is discharged into textile industry wastewater every year [9,10].

Although the use of textile dyes is important, it causes serious environmental problems. Textile wastewater contains a mixture of inorganic and organic compounds, which are complex in nature [11]. According to a recent report from China, each year about 70 billion tons of wastewater from the textile and dyeing industry is generated and needs adequate treatment before ultimate discharge into the environment. Surprisingly, about 10–15% of the dyes used in the dyeing process do not fix with the textile fibers and, therefore, they are carried by the wastewater in their original forms and concentrations [12]. The major pollutants present in textile wastewater are recalcitrant organics, residues of reactive dyes, aerosols, leveling agents, acids, alkalis, amines, heavy metals, chlorophenol, chlorine, halogen carriers, formaldehyde, biocides, and softeners [13–16]. Table 1.1 presents the major pollutants and chemical types present in textile wastewater and their main processes of origin. Accordingly, the various unit processes, such as sizing, desizing, bleaching, mercerizing, dyeing, and printing, generate high levels of biochemical

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Table 1.1 Major Pollutants Present in Textile Wastewater

Pollutant	Major Chemical Types	Main Processes of Origin
Organic load	Starches, enzymes, fats, grease, waxes, surfactants, acetic acid	Desizing, bleaching, dyeing
Color	Dyes, scoured wool impurities	Dyeing
Nutrients	Ammonium salts, buffers, sequestrants	Dyeing
pH and salt effects	NaOH, mineral/organic acids, NaCl, silicate, sulfate, carbonate	Scouring, desizing, bleaching, mercerizing, dyeing
Sulfur	Sulfate, sulfide, hydrosulfide salts, sulfuric acid	Dyeing
Toxicants	Heavy metals, reducing agents, oxidizing agents, biocides, quaternary ammonium salts	Desizing, bleaching, dyeing, finishing
Refractory organics	Surfactant dyes, resins, chlorinated organic compounds, carrier organic solvents	Bleaching, desizing, dyeing, finishing

Adapted from H. Patel, R.T. Vashi, Characterization and Treatment of Textile Wastewater, Butterworth Heinemann, Elsevier, USA, 2015 [18].

oxygen demand (BOD), chemical oxygen demand (COD), suspended solids, dissolved solids, alkalinity, pH, and strong odorous conditions in the wastewater stream [17].

The low biodegradability of textile wastewater is generally attributable to the existence of recalcitrant organics including dyes, sizing agents, and dyeing aids. These pollutants, directly or indirectly, are known to cause several chronic diseases to living beings. Also, their dark color disrupts the penetration of sunlight, resulting in the prevention of photosynthesis of the ecosystem, leading to eutrophication of water bodies [19]. In addition, odorous compounds such as hydrogen sulfide may be produced under anaerobic conditions. The biological activity of the receiving water body will be affected by the presence of dissolved sulfide species. Again, in the textile industry, volatile aromatic hydrocarbons are also emitted owing to the use of fossil fuels and other industrial chemicals [20]. Many dyes and their breakdown products have evidenced harmful effects on humans and in mammalian assays. In addition, they can be mutagenic or carcinogenic in nature and they tend to bioaccumulate in the food chain [21–24]. The release of textile wastewater into the environment not only affects the water resources, but also alters the soil productivity, marine life, and ecosystem.

The textile industries are constantly identifying, tracking, and addressing their pollution-related vulnerabilities to satisfy the increasing demands from regulatory boards and policy makers [25]. Globally about 7×10^5 tons of dye is produced per year. Of this, 10–15% ends up in the effluent during the dyeing process as mentioned earlier [26,27]. Dyes like triphenyl methane and azo dyes are extensively used in textile industries for dyeing of nylon, wool, silk, and cotton [28]. The dyes employed in textile industries are composed mainly of aromatic organic compounds [29]. The aromatic benzene structures are usually complex in nature and highly resistant to light, biological activity, ozone, and other environmental conditions. Because of these limitations, the application of conventional wastewater treatment processes still remains a major challenge for the textile industry.

1.2 Dye Composition and Classification

Dye is a mixture of two main components, namely, a chromophore and an auxochrome. The colored compound that contains a certain unsaturated group is called the chromophore. The usual chromophores seen in ancient dyes are nitro, azo, keto, thioketo, *ortho*-, and *para*-quinoid ring chromophores. A compound with a chromophore is described as a chromogen. The basic meaning of the word auxochrome is color enhancer. When a chromogen contains groups other than those mentioned earlier, it is called an auxochrome. The auxochrome may be either acidic or basic, like −OH or $-NH_2$; however, other auxochromes include $-SO_3H$, $-NR_2$, and −NHR. In general, dyes can be classified into natural dyes and synthetic dyes based on their origin (Fig. 1.1). The use of natural dyes was practiced during the bronze age. Natural dyes are a group of dyes that are extracted from biological material like plant and animal residues. Even though they are extracted from biological material, such dyes are considered mordant dyes because they need addition of one or more of copper, aluminum, chromium, iron, and other metallic salts. Such mordant components are required for ensuring the fastness of the dyes when exposed to light and during the washing process. On the other hand, synthetic dyes were commercialized only in the middle 1860s. Synthetic dye production rapidly replaced the use of conventional natural dyes as they impart a variety of colors to fabrics [30]. Based on the chemical composition and mode of application in the dyeing process, synthetic dyes can be classified as follows.

1.2.1 Basic Dyes

These are also called cationic dyes and usually they are water soluble. Basic dyes along with a mordant are primarily employed to dye acrylic fibers like polyesters, and nylon, among others. These basic dyes are not appropriate for other fibers than acrylic. Hence, they are normally used for subsequent treatment of fabric that was previously dyed with acid dyes.

1.2.2 Direct Dyes

Direct dyes or substantive dyes are used to color cellulose-based fibers without the help of a mordant. These dyes are used to color wool, silk, and rayon, among others. These dyes are dull and have meager fastness to washing.

1.2.3 Mordant Dyes

Mordant dyes are acidic in nature. They are also called as chrome dyes. Sodium or potassium salts are used to treat fabrics during or after the completion of the process of dyeing for strong binding of the chrome. They are mainly used for wool. They are less effective for dyeing cotton, linen, silk, rayon, and nylon.

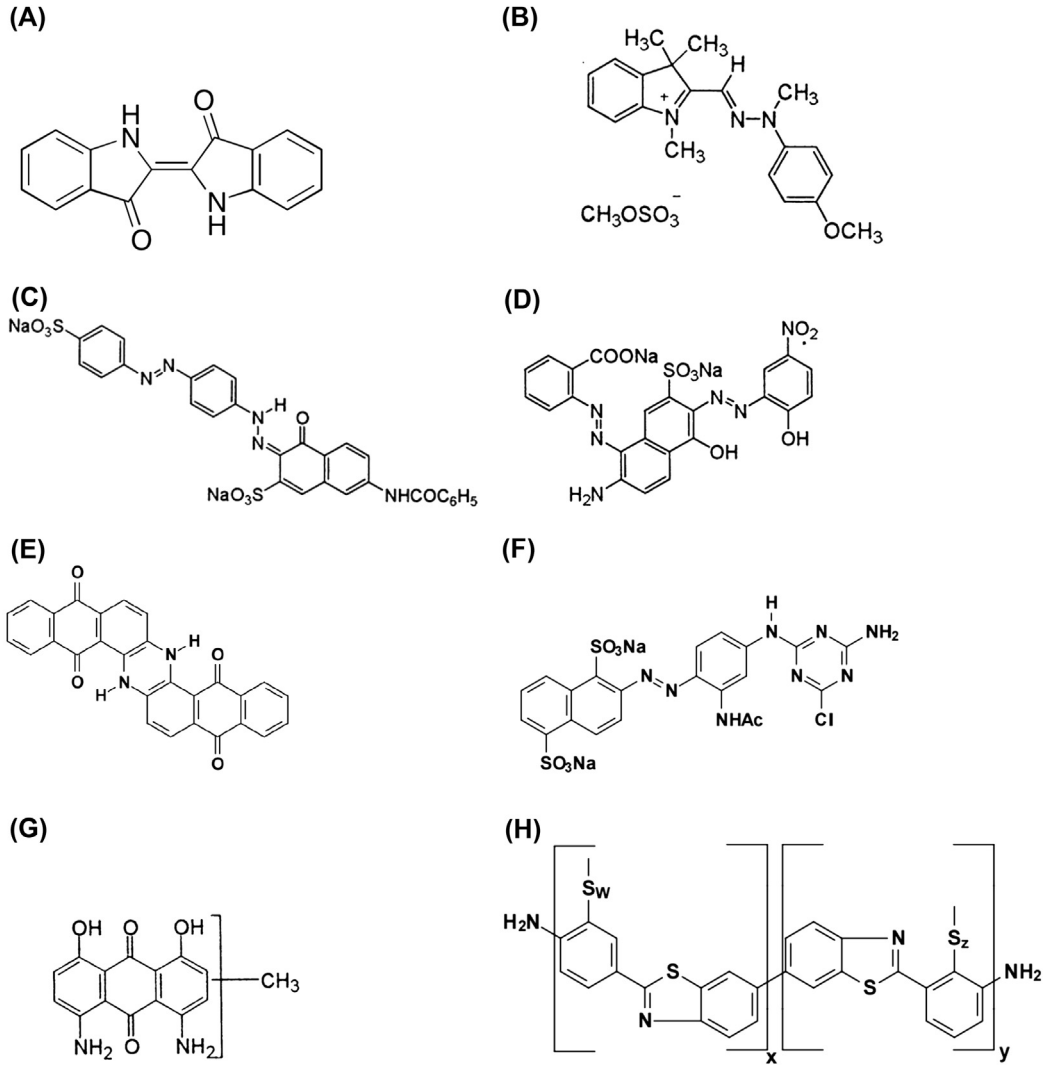

FIGURE 1.1 Representative chemical structures of various dyes used in the textile industry. (A) Natural, (B) basic, (C) direct, (D) mordant, (E) vat, (F) reactive, (G) disperse, and (H) sulfur.

1.2.4 Vat Dyes

Vat dyes are naturally water insoluble. To make them soluble and to fix with fabrics, they are reduced with alkali salts. Further oxidation tends to restore their insoluble nature. Without the addition of mordant, these dyes are used to color cotton, linen, and rayon, whereas with the addition of mordant, they are used to color wool, nylon, polyesters, and acrylics.

1.2.5 Reactive Dyes

Reactive dyes were initially used for cellulose-based fibers. Occasionally, heat treatment is required for these reactive dyes for generating various shades. Once the dyeing process is completed, the fabrics are washed using soap to take away every unfixed dye. Applications include dyeing wool materials, silk fibers, and nylon blends.

1.2.6 Disperse Dyes

Disperse dyes are insoluble in water and marketed in the form of powder or paste. Traditionally, disperse dyes were prepared to dye cellulose materials. These days, they are employed to dye acrylic and nylon fibers.

1.2.7 Sulfur Dyes

The insoluble nature of sulfur dyes is made soluble by treating them with soda and sodium salts. Usually, treatment at high temperature and the addition of salts are required to impart color to the fabrics. Once the dyeing process is completed, the unfixed salts and dyes are removed. These dyes are mainly employed to impart color to cotton and linen materials.

1.3 Main Processes Involved in the Textile Industry

The four main stages of textile processing involve yarn production, fabric production, wet processing, and garment manufacturing [31]. Yarn fabrication is the process in which the conversion of raw fiber into yarn or thread occurs. The second step, fabric production, involves weaving, knitting, and tufting. The next step is wet processing, which includes pretreatment, dyeing, printing, and finishing. In this process, the sizing elements are detached from the gray fabric by treating them with acids or enzymes. The scouring process is done to remove impurities such as oils, waxy materials, and fatty acids, among others, seen in the fabric, under alkaline conditions, high pressure, and high temperature. Bleaching is performed to whiten the fabrics and yarns using bleaching agents like hypochlorite and hydrogen peroxide. During the mercerizing process, the fabrics or yarns are treated with a sodium hydroxide (cold) solution, which increases the tensile potency, gleam, and dye uptake by making the fabric swell. The dyeing process is carried out to give a finished texture to the fabric by diffusion.

1.4 Aerobic Processes to Treat Textile Industry Effluents

Aerobic treatment systems are high-rate oxidizers of soluble organic and nitrogenous compounds. Commercially available aerobic treatment reactors promote the removal of color, suspended solids, and pathogens and the reduction of COD, BOD, and other

toxins from textile wastewater. According to Khelifi et al. [32], aerobic processes for the treatment of textile wastewater are efficient and cost-effective. Most of the aerobic reactors operate as constant volume reactors with complete mixing and intermittent flow, as the influent is not continuous in small-scale textile industries. Complete mixing of contents is ensured in the aeration chamber to promote and maximize the contact between dissolved oxygen, microbes, and wastewater. The effluent from the aeration chamber moves into a clarifier. The rate of discharge of the effluent is a direct response to the influent flow rate. Typical bioprocesses that will be discussed in this chapter for the treatment of wastewater from textile industries include the extended aeration process and suspended and attached growth processes.

1.4.1 Extended Aeration

In practice, most aerobic reactors operate as extended aeration units. The term extended is associated with the duration of aeration. It is characterized by long-term aeration, long retention times, low food-to-microorganism (F/M) ratio, and low biomass accumulation. To ensure the endogenous growth phase of microorganisms, excess dissolved oxygen and minimal soluble organic matter are provided, and microbes will readily deplete the bioavailable organic carbon, including biomass. The focus is to establish the right balance between the amount of new cells produced and the amount of cells endogenously degraded per day [33].

1.4.2 Suspended-Growth Bioreactors

The suspended-growth aerobic units are termed as scaled-down activated sludge plants (Fig. 1.2). The nature of activated sludge is a heterogeneous microbial culture comprising bacteria, protozoa, rotifers, and fungi. Assimilation of organic matter is achieved by the presence of the bacteria [34]. The segregation of dispersed bacteria from the bio-aerated unit treated effluent is gained by the existence of protozoa and rotifers, which serve as the predators. The biomass is thoroughly blended with the biodegradable organic fraction and individual organisms agglomerate together (flocculate) to form a

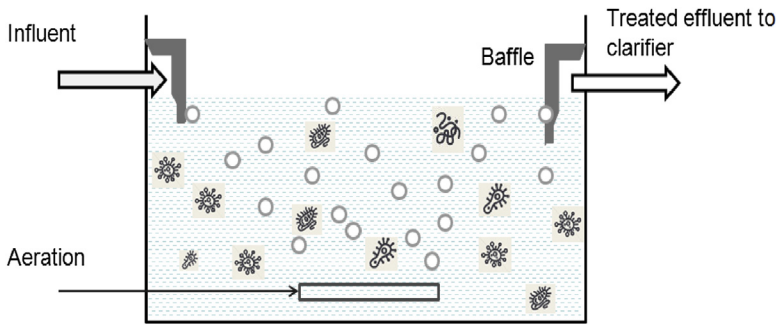

FIGURE 1.2 Schematic of an aerobic suspended-growth process.

progressive mass of microbes, also referred to commonly as biological flocs. This slurry of biological flocs and wastewater is named the mixed liquor.

1.4.3 Attached-Growth Bioreactors

Attached-growth systems are also referred in the literature as fixed-film reactors (Fig. 1.3). For the attachment of microbes, an inert medium is provided as the carrier [34]. The cross flow of the wastewater on the medium facilitates the absorption of the fine, suspended, colloidal, and dissolved organic solids by the biological film. The contact of wastewater and dissolved oxygen with the attached microorganisms is achieved by: (1) pumping the liquid over the medium or (2) moving the medium through the liquid. The configuration and the design of the attached-growth biotreatment unit provide attached growth in the same basin as suspended-growth and this is therefore named "coupled-contact aeration." It enhances the attainment and quantity of aeration units. This dual system has the following advantages: (1) a higher grade of microbial population strength and (2) lesser amounts of residual suspended solids and BOD in the treated effluent.

For turbulent flow conditions, channels with a larger diameter are provided and attached-growth areas are submerged to flow over the surfaces. The suspended-growth microbes will flourish in the large channels. Aeration is provided by (1) direct air injection and/or (2) circulation of water into the air-liquid interface. Excessive attached growth will come off and settle down, which should be removed periodically as part of maintenance.

1.4.4 Factors Influencing the Biodegradation of Textile Effluent

Microorganisms and their growth are the driving force for the degradation process [35]. To achieve high rates of degradation of textile wastewater, a suitable environment should be provided for the microorganisms to perform and thrive [36]. The selection, biochemical oxidation rate, survival, and maintenance of microorganisms are influenced by the following factors.

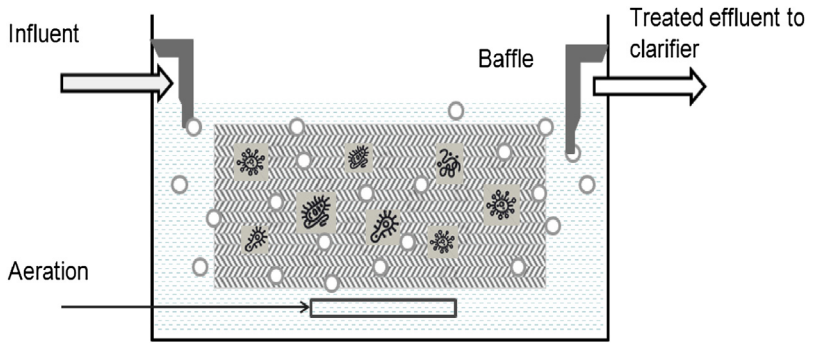

FIGURE 1.3 Schematic of an aerobic attached-growth process.

1. Temperature
2. pH
3. Food-to-microorganism (F/M) ratio
4. Hydraulic retention time (HRT)
5. Nutrient availability
6. Aeration/oxygen transfer rate
7. Hydraulic and organic loading rates

Temperature: The rate of biological oxidation is a function of temperature. Various microbial species have optimal temperatures for survival and cell synthesis: psychrophilic (12−18°C), mesophilic (25−40°C), and thermophilic (55−65°C).

pH: The influent pH has a significant effect on textile industry wastewater treatment [37]. By adding a suitable acid/base, it is possible to treat textile wastewaters over a wide pH range. However, the optimum pH for microbial growth is between 6.0 and 7.5. It is commonly viewed that, at a slightly alkaline pH, the bacterial growth is prime. Similarly, it has also been shown that the algal and fungal growth is stimulated in slightly acidic wastewater. The response to pH is largely due to changes in the acidic environment caused by enzymatic activity.

F/M ratio: The F/M ratio indicates the correspondence between the amount of substrate, bioavailable organic compounds, charged into the aeration chamber each day and the amount of microorganisms accommodated within the aeration chamber. The physicochemical properties of the substrate (food) as well as the type of biocatalyst, whether pure or mixed, strongly determine the overall efficiency of the treatment process. The F/M ratio is explained in terms of mass of BOD per mass of microbes in the treatment unit per day of operation. This life-sustaining parameter has a significant influence on the microbial population and its activity. A sudden disturbance in the organic loading rate (OLR) affects the microbial population.

HRT: The average time spent by the soluble compound present in wastewater within the bioreactor is known as the HRT. It is a ratio between the volume of the aeration tank and the influent flow rate. Textile wastewaters have been successfully treated using an activated sludge process at HRTs varying between 18 and 36 h [38,39].

Nutrient availability: Nutrients, such as N and P, enhance the growth of microorganisms in the aerobic reactor, which increases the treatment efficiency. Nutrient type depends on the nature of the microorganism adopted, namely, bacteria, fungi, yeasts, etc. The optimum nutrient requirement for the maximum decolorization of textile effluent is the major concern. The effects of the presence and absence of nutrients on textile industry wastewater treatment using *Trametes versicolor* were reported by Mullai and Vishali [40].

Aeration/oxygen transfer rate: Aeration is the process by which air is mixed with or circulated through the textile wastewater. The theoretical requirement of oxygen for the aeration process depends upon the total microorganisms present for oxidizing the waste. It can be evaluated based on BOD content, ammonia-nitrogen oxidized, oxidized

nitrogen denitrified, and the dissolved oxygen (DO) required for microbial growth. Large quantities of oxygen must be provided to maintain aerobic conditions [33]. In a conventional wastewater treatment plant, an aerobic treatment system is designed to maximize the contact interface (surface area) between the gas and the liquid phases, to escalate the opportunity for oxygen transfer.

Hydraulic and organic loading rates: Both hydraulic and organic loading rates form the basis for an aerobic bioreactor specification [33]. The OLR indicates the availability of the food (incoming colloidal and soluble BOD) compared to the availability of the microbial population to assimilate the food (F/M ratio). The quality of the treated textile effluent will be high if there are more microbes than food, and the reverse is also possible.

1.5 Mechanism of Aerobic Treatment of Textile Effluent

Biological degradation or bioremediation is the key area of interest for treatment of various pollutants and effluent wastes. Various microbial strains are selected and acclimatized to grow and metabolize in the presence of toxic effluents such that they can transform the pollutants to less harmful by-products. Apart from being environmentally friendly and cost-effective, they do not consume large volumes of water compared with various physicochemical methods involved in treating these effluents. Major mechanisms by which textile wastewater can be treated using microorganism can be classified into biosorption and enzymatic degradation. A wide variety of microorganisms, both fungi and bacteria, have been isolated with the ability to degrade different classes of dyes commonly used in the textile industry. Some of these include *Aspergillus*, *Bacillus*, *Enterococcus*, *Pichia*, *Pseudomonas*, *Shewanella*, and *Staphylococcus* [41–45].

1.5.1 Biosorption

Microorganisms are known to effectively remove soluble toxic organic and inorganic substances by a passive process, commonly known as biosorption. Biosorption takes place because of the presence of various functional groups on the microbial surface. Biosorption leads to sequestration of dyes or metals from solution by (1) complexation, (2) chelation, (3) precipitation, and (4) ionic interactions. Azo dyes, which are major contributors to the color of the textile effluent, are removed by biosorption particularly by fungi and yeasts. The cell wall of a microbe is considered to be the primary site of biosorption. In the case of biosorption by yeasts, peptidoglycans or proteins present on the cell wall or active groups such as polysaccharides, lipids, and amino acids [46–48] play a vital role. Biosorption depends upon the solution pH, temperature, initial dye concentration, and dosage. *Saccharomyces cerevisiae* showed maximum sorption at pH 6.0 [49]. A dead biomass of *Aspergillus niger* has been effectively utilized as a biosorbent for which the optimum pH was found to be 5.0 [50]. Agricultural residues have also been used as biosorbents for treating wastewater. Wheat straw [51], corn cob, and barley husk

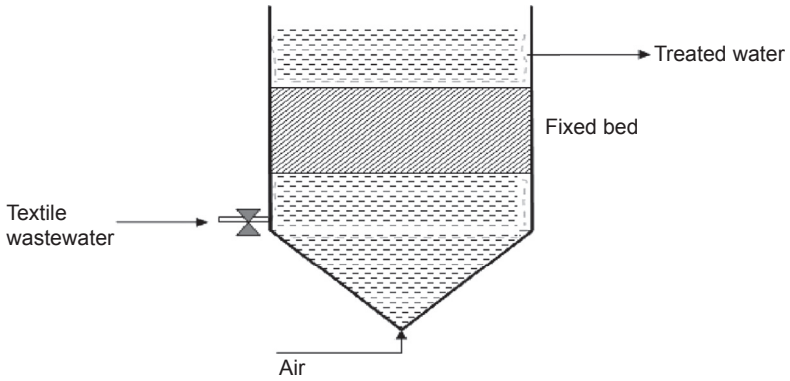

FIGURE 1.4 An up-flow fixed-bed reactor for textile wastewater treatment, specifically for the removal of dyes from wastewater.

[52] have shown very efficient removal of color, with corn cob and barley showing 75% removal. The most commonly used reactor configuration for decolorization of textile wastewater is an up-flow fixed-bed bioreactor (Fig. 1.4). Decolorization of various dyes by biosorption using various biomass types is presented in Table 1.2.

1.5.2 Enzymatic Degradation

Azo dyes in comparison to natural dyes are stable, inexpensive, and easy to synthesize and provide a wide variety of colors to be used by the textile industry [58]. Three major enzymes in the microbial system that contribute to azo dye degradation are azoreductases, laccases, and peroxidases.

Table 1.2 Dye Removal by Biosorption Using Various Biomass Types

		Decolorization Conditions				
Biomass	**Dyes Tested**	**pH**	**Temperature (°C)**	**Time (min)**	**Biosorption Capacity (mg/g) or Decolorization (%)**	**References**
Aspergillus parasiticus	Reactive red 198	2	50	50	1.03×10^{-4} mol/g	[53]
Aspergillus fumigates	Methylene blue	Alkaline	30	120	93%	[54]
Trichoderma sp.	Orange G	2	NA	NA	0.45 mg/g	[55]
Orange peels	Golden yellow Black B Red 6BL	7	Room	120	60–70%	[30]
Peanut hull	Reactive Black 5	NA	60	NA	55.55 mg/g	[56]
Kluyveromyces marxianus IMB3	Remazol Black B Cibacron orange	NA	NA	720	37 mg/g 8.5 mg/g	[57]

NA, not available.

1.5.2.1 Azoreductases

Azoreductases are flavoproteins that either extracellular in nature or may be localized in the cytoplasm of bacteria belonging to the species *Clostridium*, *Bacillus*, and *Pseudomonas* [59,60]. Complete decomposition of azo dyes by microbial cells occurs in two steps. In the first step, under anaerobic conditions, colorless metabolites like aromatic amines are generated by the reductive cleavage of the azo bond. During the second step, under aerobic conditions, the intermediates are decomposed into stable end products. The proposed mechanism through which these enzymes reduce the dyes involves electron transfer from the enzyme redox center to the mediators (NADH, NADPH, FMN, etc.) and subsequent transfer to the dyes [61–63]. The proposed mechanism of the action of azoreductases is illustrated in Fig. 1.5.

1.5.2.2 Laccases

Lignin and various aromatic compounds are acted upon and degraded by laccases. These are Cu-dependent enzymes and require the presence of oxygen for their function. Laccases are produced by both fungi and bacteria; however, the laccases obtained from white rot fungi in particular are considered to be very efficient in the degradation of dyes. Aromatic amines, which are the intermediates of dye degradation by azoreductases, are not produced using laccases but, in turn, transform these intermediates into simpler nontoxic forms. Laccases are involved in the oxidation of azo dyes by accepting an

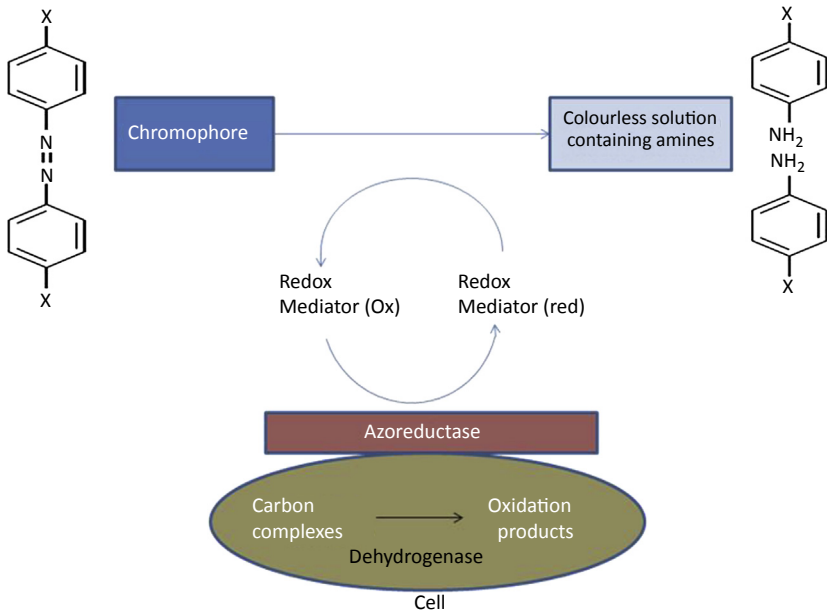

FIGURE 1.5 Mechanism of azo dye degradation by azoreductases in bacteria.

electron from the dye and transferring it to O_2 (via mediators), and subsequently the dye breaks down. Laccases can act upon a wide range of substrates and, therefore, can be used for the treatment of wastewaters that contain various azo dyes [64–66].

1.5.2.3 Peroxidases

Lignin peroxidases are heme-containing oxidoreductases and are known for the degradation of various aromatic compounds like phenyls and synthetic dyes [59]. Chivukula and Renganathan [67] proposed a mechanism of azo dye degradation by lignin peroxidases, in which a radical is produced at the carbon involved in the azo linkage (−N═N−) owing to the oxidation of the phenol group by lignin peroxidase. This is followed by the attack of a water molecule on the phenol group, releasing phenyldiazine, which is subsequently oxidized to generate N_2 [68]. Lignin peroxidases from the white rot fungus *Phanerochaete chrysosporium* are reported to degrade a variety of azo dyes [69].

1.6 Biocatalysts for Textile Wastewater Treatment

1.6.1 Algae

Algae have three different mechanisms for decolorization or assimilation of the colored compounds. The chromophores are utilized (1) for the production of algal biomass, carbon dioxide, and water; (2) for transformation of the colored compounds to uncolored ones; and (3) for adsorption of the dye on the algal biomass. *Chlorella* and *Oscillatoria* have been reported to degrade azo dyes to aromatic amines to simple compounds and subsequently to CO_2 [70]. They have been shown to degrade over 30 different dyes [71]. Several researchers have reported the potential of various algal strains to treat textile wastewater owing to their ability to degrade the azo bonds [72,73]. Algae can be grown symbiotically with aerobic microbes such that the algae would provide O_2 to these aerobes, which can utilize the aromatic amines released by the degradation of the chromophores or dyes [74]. *Scenedesmus bijugatus* showed 68% decolorization of azo dyes after 6 days of incubation [75]. Khataee et al. [76] reported 83.5% decolorization of Basic Red 46 by a green macroalga belonging to *Enteromorpha* sp. within 5 h of batch incubation at 25°C.

1.6.2 Bacteria

Bacteria have been preferred over fungi for the treatment of textile wastewaters as the rates of decolorization and mineralization of the dyes present in the effluents are higher. In addition to this major advantage, the use of bacteria for treatment leads to less sludge generation and the process is cost-effective as well. Species belonging to the genera *Pseudomonas*, *Bacillus*, *Aeromonas*, and *Proteus* are some of the extensively studied bacteria for the degradation of dyes and other toxic effluents [77,78]. *Pseudomonas aeruginosa* has been reported to decolorize a commercial textile dye, Navitan Fast Blue

SSR, under aerobic conditions [79]. Kolekar et al. [80] reported a decrease in COD and color of a mixture of dyes by using *Shewanella* strain KMK6. Reductions in COD and color by 66.7% and 96.9%, respectively, were observed when *Bacillus* MK-8 strain was used in the form of granules [81]. Table 1.3 presents the bacterial degradation of various dyes under different process conditions.

Bacterial decolorization by pure cultures is rapid, but leads to the formation of toxic intermediates like aromatic amines [82]. Mixed cultures have been shown to be more efficient in the degradation of wastewaters and dyes owing to the synergistic metabolism of the microbes present that can utilize toxic intermediates to form nontoxic by-products [83]. A microbial consortium of *Bacillus*, *Sphingobacterium*, and *Pseudomonas* was reported to decolorize textile wastewater rapidly compared to the pure cultures [84].

1.6.3 Fungi

Fungi have been found to be very effective in the decolorization and degradation of textile wastewaters because of the presence of various nonselective enzyme systems, which can act upon a wide range of substrates, enabling them to survive under harsh conditions [85]. The secretion of laccase, lignin peroxidases, and manganese peroxidase helps them in degrading the recalcitrant components of the wastewater [86]. White rot fungi, in particular, have been found very effective in the degradation of various dyes and other xenobiotics [87]. Amaral et al. [88] and Assadi et al. [89] reported 92% and 98%, respectively, decolorization of a raw textile effluent by *T. versicolor*. The application of white rot fungi in the effluent treatment is limited by the long growth cycle, which in turn requires prolonged hydraulic retention times. The need for nitrogen-limiting conditions and preserving the dominance of fungal cultures in the reactor system is the major challenge that prevents the use of fungi for full-scale applications [58,90]. A mixed fungal culture of *Pleurotus ostreatus* and *Coriolus versicolor* reduced the COD and BOD of wastewater along with achieving its decolorization [91]. Table 1.4 lists the various fungal strains that have been applied for treatment of textile wastewater and degradation of various dyes.

1.6.4 Yeasts

Interest in textile wastewater treatment using yeasts is due to the ability of the yeast biomass to absorb and accumulate toxic chromophores as well as to degrade them into simpler compounds. Dead biomass of yeast has been utilized as a biosorbent for the biosorption of dyes as discussed in a previous section. Yeasts also possess an enzyme system that can degrade dyes present in the textile wastewater. Peroxidases, reductases, and laccases are some of the enzyme systems present in yeast that take part in the dye degradation process. *Candida krusei*, *Trichosporon beigelii*, *Galactomyces geotrichum*, *S. cerevisiae*, etc., have been well reported for the biodegradation of dyes [92–94].

Table 1.3 Bacteria-Based Textile Dye Decolorization Under Various Process Conditions

		Decolorization Conditions					
Bacterium	**Dyes Tested**	**Initial Dye Concentration (mg/L)**	**pH**	**Temperature (°C)**	**Mode of Operation**	**Time (h)**	**Decolorization (%)**
Micrococcus glutamicus NCIM 2168	Reactive Green 19 A	50	6.8	37	Static	42	100
Bacillus sp. VUS	Navy Blue 2 GL	50	7.0	40	Static	18	94
Acinetobacter calcoaceticus NCIM-2890	Direct Brown MR	50	7.0	30	Static	48	91.3
Enterococcus gallinarum	Direct Black 38	100	NR	NR	Static	20 days	100
Pseudomonas sp. SU-EBT	Congo red	1000	8.0	40	Static	12	97
Rhizobium radiobacter MTCC 8161	Reactive Red 141	50	7.0	30	Static	48	90
Comamonas sp. UVS	Direct Red 5B	1100	6.5	40	Static	13	100
Exiguobacterium sp. RD3	Navy Blue HE2R	50	7.0	30	Static	48	91
Proteus mirabilis	Red RBN	1000	6.5–7.5	30–35	Static	20	95
Aeromonas hydrophila	Red RBN	3000	5.5–10.0	20–35	NA	8	90
Citrobacter sp.	Azo and triphenylmethane dyes	5 μM	7–9	35–40	Static	1	100
Paenibacillus azoreducens sp. nov.	Remazol Black B	100	NR	37	Static	24	98
Bacteroides fragilis	Amaranth, Orange II and tartrazine	0.1 mM	8	35	Static	NA	95
Bacillus fusiformis KMK5	Disperse Blue 79 and Acid Orange 10	1500	9	37	Anoxic	48	100

NR, not reported.

Table 1.4 Fungus-Based Textile Dye Decolorization Under Various Process Conditions

		Decolorization Conditions			
Fungus	**Dye Tested**	**pH**	**Temperature (°C)**	**Time (days)**	**Decolorization (%)**
Aspergillus ochraceus	Reactive Blue-25	NA	30	7	100
Ganoderma australe	Poly R-478	6.7	25	18	93.4
Pleurotus ostreatus sp. 3					66.25
Polyporus sp. 2					86.53
P. ostreatus	Disperse Orange 3	5	30	5	57
	Disperse Yellow 3				57
Trametes versicolor	Remazol Black B	4	30	12	88.4
Cunninghamella elegans	Orange II	5.8	28	4	88
Lentinula edodes	Poly R-478	6.5	30	11	72 mm decolorized zone
Ganoderma sp. WR-1	Amaranth	NA	28	8 h	96
Pycnoporus sanguineus MUCL 41582	Acid blue 62	NA	25	11	99

NA, not available.

1.7 Bioreactor Configurations for Textile Wastewater Treatment

1.7.1 Rotating Biological Contactor

Suspended-growth and attached-growth bioprocesses are merged in the design of rotating biological contactors (RBC) (Fig. 1.6). On a common shaft, closely placed circular disks are attached/fixed and are slowly rotated. The RBC is designed in such a way that ~35–45% of the disk surface is submerged under water and the shaft is located just above the liquid surface. This arrangement is provided to ensure the exposure of both air and textile wastewater (periodically) to the disk surface when it is rotating. An inert material such as polystyrene or polyvinyl chloride is used as the disk material. The biomass grows on the disk surface and during every cycle of submergence, the microorganisms are exposed to the substrate present in the textile wastewater. To achieve a high removal of organic compounds and nitrification, the set of disks is arranged in series and sometimes in stages [95]. Thus, in each stage, the OLR decreases along the length of the reactor. Oxygen is absorbed when the reactor exposes the fixed film to the ambient air during rotation. If the absorbed oxygen is in excess, it is commixed with the bulk liquid as the contactor surface moves back through the wastewater. When the attached biomass thickness increases on the disk, a portion of the excess biomass is sloughed off by its own weight or it can be trimmed off during maintenance.

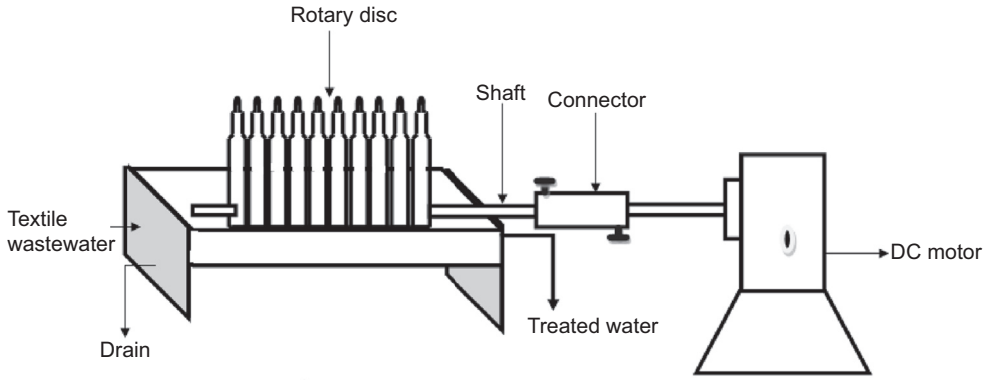

FIGURE 1.6 Schematic of a rotating biological contactor reactor for textile wastewater treatment.

The material of construction of RBC tanks is reinforced concrete or steel. To prevent the discharge of nuisance odors and to offer suitable environmental conditions the tanks are usually closed. RBCs can be scaled down for domestic use or scaled up for municipal wastewater treatment plants as a secondary treatment [96]. RBCs have proven to be efficient for the treatment of highly complex textile and dye wastewaters [95,97,98]. The main advantage of RBCs is the high interfacial area formed by the rotating disk, giving good interaction between microbial species and the pollutants in the system. For textile wastewater treatment, biological systems involving bacteria are ideal because of the quicker degradation rate, but a mixed bacterial community would be essential for its complete biodegradation [99].

1.7.2 Sequencing Batch Reactor Systems or Periodic Processes

The sequencing batch reactor (SBR) is a suspended-growth-activated sludge batch treatment system [100]. In SBRs, time-sequenced processes of flow equalization, aeration, clarification, and biomass wasting are conducted in the same tank (Fig. 1.7). SBRs present some advantages for the biodegradation of dyes and xenobiotic compounds. The periodic operation of the reactor executes selective pressures that can select a defined microbial population that is able to degrade problematic (target) compounds. Thus, the use of this type of reactor could be interesting to treat difficult to degrade wastewaters such as textile dye wastewaters [101].

A single cycle of SBR operation has five basic modes of operation: fill, react, settle, decant, and idle [102].

1. *Fill*: During this step, ~25% of the total cycle time may be used. The reactor is filled with textile wastewater from the primary treatment unit. At this stage, the supply of oxygen may or may not be there to offer alternating cycles of high or low DO.

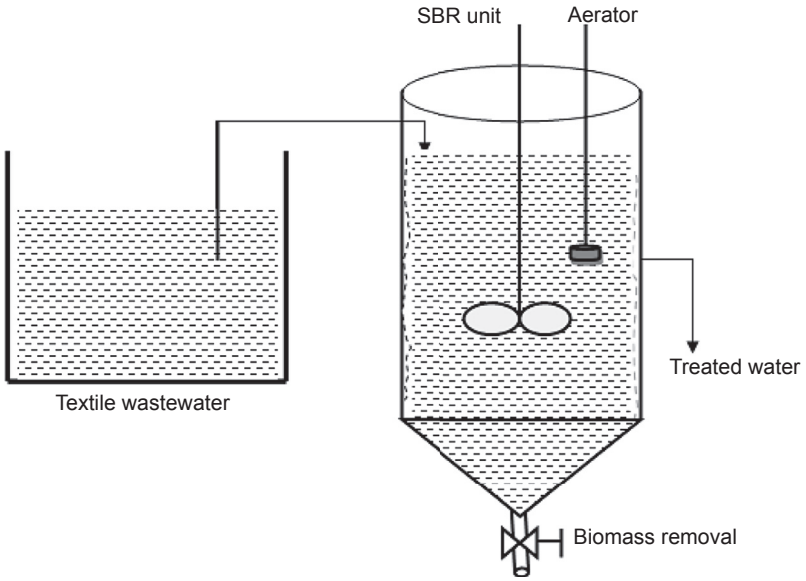

FIGURE 1.7 Schematic of a sequencing batch reactor (SBR) unit for textile wastewater treatment.

2. *React*: Of the total cycle time, ~35% may be needed to fulfill this mode. To obtain rapid biodegradation of organic and nitrogenous compounds, aeration is ensured.
3. *Settle*: Settling is nothing but clarification, in which liquid–solid separation takes place, and this step will require ~20% of the overall cycle time. The supply of air is expelled to allow the wastewater to become anoxic (for denitrification) and to allow for dormant conditions that facilitate effective liquid–solid separation in the form of supernatant and sludge, respectively.
4. *Draw*: The treated effluent, which is in the form of clarified supernatant, is removed using adjustable weirs, floating weirs, and submersible pumps. The settled biological solids in the bottom are removed systematically. The decanting step generally takes ~15% of the total cycle time.
5. *Idle*: Total cycle time is allotted for the first reactor to complete its full cycle and then to shift the flow into the second reactor for parallel operation.

To maintain the longevity of SBR operation, the important parameter is that a tank is never completely emptied; rather a portion of the settled solids is left in the reactor. The settled biological solids are then used as the inoculum for the next cycle and this periodic, time-sequenced operation has been viewed as an advantage for high-strength wastewater treatment. Several studies have been performed using aerobic SBRs to investigate the decolorization and degradation of textile wastewater, including azo dye-containing wastewater [103], azo dye Acid Red 151 [101], and reactive, sulfonated, monoazo and diazo dyes [104].

1.7.3 Membrane Bioreactors

The conglomeration of conventional activated sludge treatment and filtration processes, achieved with the help of microfiltration (0.4 μm) and ultrafiltration (10 nm) membranes, is the basis for membrane bioreactor (MBR) technology. An MBR allows easy sludge separation because the membrane surface acts as a barrier. All the particles, colloids, bacteria, and viruses are retained on the membrane while the disinfected, treated wastewater passes through the membrane. This greatly reduces the reactor volume and sludge production and allows operation at higher sludge concentrations up to 12,000 mg/L. Two main process configurations of biomass rejection MBR are submerged or immersed (iMBR) and sidestream.

The schematic of an iMBR is shown in Fig. 1.8. It consists of a tank coupled to membrane modules equipped with air diffusers and a sludge drain. The reactor is usually seeded with activated sludge. Permeate can be withdrawn using a pump. A pressure sensor monitors the transmembrane pressure generated by the membrane module, often a result of vacuum generation.

Flat-sheet membrane module (FS) and hollow-fiber membrane module (HF) are the two basic types of commercial membrane modules used in iMBRs. In comparison to FS, the HF consists of a thinner space between the membranes, due to which a higher packing density is achieved. However, it leads to more vulnerable membrane clogging complications, and it can also make cleaning tougher [105]. Many researchers have reviewed the current advances, mechanisms, and factors influencing membrane fouling in MBRs. Generally, these factors have been classified into four distinct groups:

1. the nature of the sludge,
2. the operating parameters,
3. the characteristics of the membrane/module, and
4. the composition of the feed wastewater

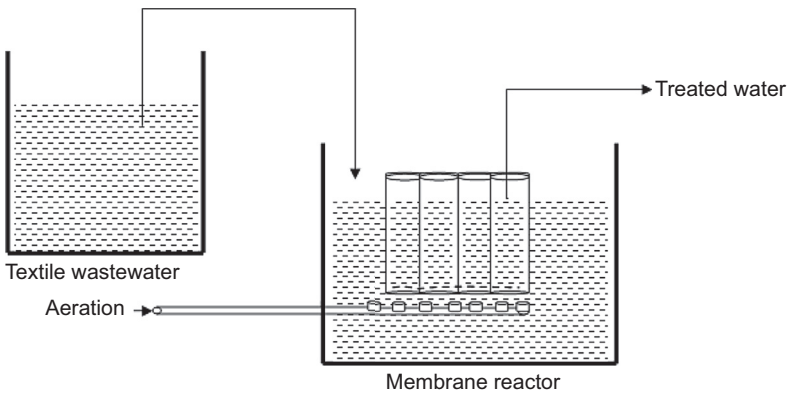

FIGURE 1.8 Schematic of a membrane bioreactor for textile wastewater treatment.

Although membrane fouling is an important factor in MBR operation, for full-scale applications the following aspects need to be addressed cautiously [106]:

1. Pretreatment and screening of feed wastewater
2. Membrane and aerator clogging
3. Loss of membrane integrity
4. Biosolids formation
5. Hydraulic overloading or system design

1.7.3.1 Advantages and Challenges of Membrane Bioreactors

The advantages and challenges of using an MBR in wastewater treatment can be summarized as follows;

1. High solid–liquid separation efficiency, low energy consumption, easy operation, and no pollution [13].
2. Complete solid removal, good physical disinfection capability, high efficiency for carbon, nitrogen, and color removal [107].
3. Decoupling of the sludge retention time (SRT) from the HRT, as a result of complete biomass retention in the aerobic reactor.
4. The volume of the reactor is reduced and/or the OLR is increased by letting the biomass concentration proliferate in the reaction basin.
5. Feed wastewater needs to be screened (1–3 mm) to remove large solids to avoid membrane damage. By doing this pretreatment step, the MBR replaces three individual processes of the conventional wastewater treatment scheme and becomes a more compact reactor than a conventional activated sludge process (Fig. 1.9).

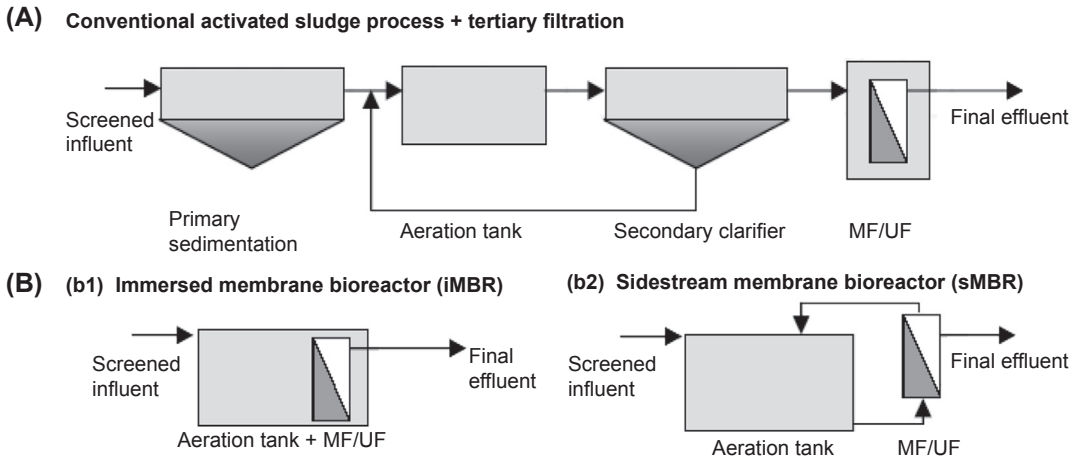

FIGURE 1.9 (A) Conventional activated sludge process and (B) membrane bioreactors in both configurations: (b1) immersed and (b2) sidestream. *MF*, Microfiltration; *UF*, Ultrafiltration.

1.7.3.2 Design and Operational Considerations

The iMBR type represents the most widely used configuration in full-scale applications. This section provides some design and operational considerations to address the following aspects:

1. Pretreatment
2. Design flux
3. Membrane fouling control and cleaning
4. SRT and biomass concentration
5. Membrane life

Pretreatment: The raw wastewater should be pretreated before passing through the membrane, because membranes are very sensitive to damage. Coarse solids like plastics, wood, rags, papers, and leaves and fine particles like hair should be removed from wastewater. To protect the membrane and prolong its operational life fine screening is always required [106].

Design flux: Several differences are usually noticed in the design and operational process flux of larger MBR plants. For both types of membrane systems, the net flux during operation is over 18 L/h m^2. It has been shown that the averaged trends in the designed maximum net flux and operation mean flux have been moderately boosted by only 3 L/h m^2 since 2009 [108]. On close comparison between the hybrid system and the MBR designed to manage maximum flow conditions it can be ascertained that ~57% higher average energy is required for the full-flow MBR [109]. This is presumably due to the high requirements of membrane aeration and lack of efficiency using the available membrane area.

Membrane fouling control and cleaning: An understanding of the reasons for membrane fouling and the optimal operation conditions of an MBR is important for successful textile dye wastewater treatment [5,110]. To minimize the negative effects of fouling, conventional operating strategies include: (1) air sparging, (2) physical cleaning techniques (i.e., back flushing and relaxation), and (3) chemical cleaning. According to literature reports, by monitoring the permeability, a control system for back washing can be automatically initiated. This control system back flushes the membrane as a function of the magnitude of membrane fouling, and by doing so, a reduction of up to 40% in the back-flushing water required can be achieved [111,112].

SRT and biomass concentration: The SRT contributes to system economics through a distinct treatment performance and membrane filtration. Specifically, these parameters are influenced by biomass concentration (measured as mixed liquor suspended solids; MLSS), formation of soluble microbial products (SMP), and oxygen transfer efficiency [113]. An increase in the SRT increases the concentration of sludge solids, in other words, a decrease in the volume of bioreactor needed. A better treatment performance along with low sludge generation can be achieved by the low growth rates of some microorganisms (specifically nitrifying bacteria). In addition, it has been predicted that high

values of SRT can increase membrane permeability by reducing SMP production. In contrast, a high solids concentration ends in a higher viscosity of the microbial suspension. As a consequence, it decreases the air sparging efficiency and oxygen transfer rate to the microbes, resulting in a higher energy demand as well as raising membrane fouling and the risk of membrane clogging. For economical reasons, most full-scale facilities are designed for MLSS ranging between 8000 and 12,000 mg/L and SRT varying from 10 to 20 days.

Membrane life: Membrane is a relatively new technology; as a result, limited information on the life of membranes is available. For some wastewater treatment applications, after 7 years of operation, a loss in permeability correlation was reported, which indicates the reach of the nonoperative nature of a membrane. Proper functioning during membrane cartridge life, determined by the welding strength at its perimeter, seems to be related to the total volume of water permeated and the total mass of oxidant (NaOCl) used during chemical cleanings [114]. According to Crawford et al. [115], from a practical viewpoint, an adequate membrane life guarantee from the membrane manufacturer should be secured using appropriate membrane procurement strategies/negotiations.

1.7.4 Fluidized-Bed Reactors

Large volumes of textile wastewater can be processed using fluidized-bed reactors. Originally, these reactors were developed for catalytic cracking of petroleum naphtha during the refining process. When the small solid particles are suspended in the upward direction, fluidization is achieved. If the liquid velocity is steadily increased, the pressure drop and the drag on individual particles increase, and the particles start to travel and become suspended in the fluid and the suspension behaves like a dense fluid. In this reactor type, the microorganisms are suspended in the liquid phase and a porous supporting disk placed at the bottom facilitates the uniformity of wastewater flow. To maintain adequate aerobic conditions, air is passed below the distributor plate at a low flow rate. Once the bed is fluidized, the pressure drop across the bed stays constant, but the bed height continues to increase with the increasing flow. The bed can be operated at quite high velocities with very little or no loss of the suspended microorganisms. Fig. 1.10 shows the various fluidization conditions achieved in a fluidized-bed reactor. As shown in Table 1.5, fluidized-bed reactors have been used for the treatment of textile effluents containing various azo and reactive dyes.

1.7.4.1 Advantages

The advantages of fluidized-bed reactors include: (1) the solid is vigorously agitated by the fluid passing through the bed, and the mixing of solids ensures that there are practically no temperature gradients in the bed; (2) violent motion of the solids provides maximum heat transfer rates; and (3) separation of solids is easily done.

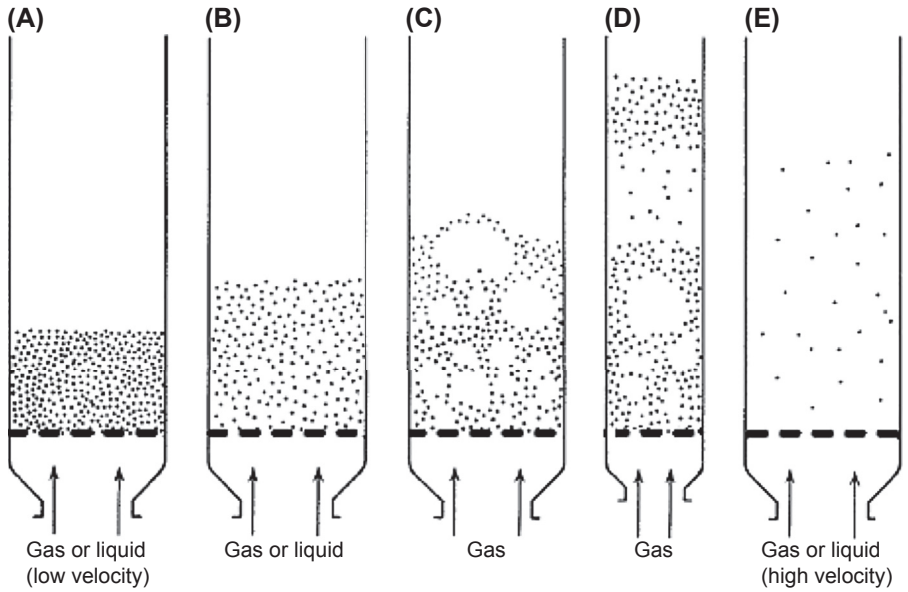

FIGURE 1.10 Various types of solid–fluid contact patterns in a fluidized-bed reactor. (A) fixed bed, (B) incipient fluidization, (C) aggregative fluidization, (D) slugging fluidization, and (E) turbulent fluidization. *Adapted from D. Geldart, Types of gas fluidization, Powder Technology 7 (1973) 285–292.*

Table 1.5 Literature Reports on the Performance of Various Bioreactor Configurations for Textile Wastewater Treatment

Wastewater	Reactor Type	Removal Efficiency (%)	Operating Conditions	References
Reactive azo dyes	Fixed-film reactor	Color: 99.5 COD: 97.5	Initial conc.: 300 mg/L COD: 7200 mg/L HRT: 24 h	[117]
Synthetic reactive azo dyes	Continuous-biofilm reactor	Color: 70–80	Initial conc.: 30 mg/L COD: 500–900 mg/L HRT: 12 h	[118]
Textile	Continuous-biofilm reactor	Color: 89	Initial conc.: 200–320 dilutions COD: 750–1175 mg/L HRT: 12 h	
Textile	Submerged-membrane bioreactor	Color: 20–70 COD: ~90	COD: 2450 mg/L Permeate flux: 4 L/m^2 h TMP: 50 mbar MLSS: 12 g/L pH: 8.2 to 10.5 Temperature: 18 ± 2°C HRT: 40–80 h	[119]
Textile	Air-pulsed bioreactor using *Trametes versicolor*	Color: >90	Initial conc: 150 mg/L HRT: 48 h	[120]

Table 1.5 Literature Reports on the Performance of Various Bioreactor Configurations for Textile Wastewater Treatment—cont'd

Wastewater	Reactor Type	Removal Efficiency (%)	Operating Conditions	References
Polyester finishing mill	Membrane bioreactor	Color: 87 COD: 60–95	VLRs ranging between 0.35 and 3.6 g/(L day)	[121]
Procion Blue 2G	Biochemical oxidation using *Pseudomonas aeruginosa*	Color: 100 COD: 90	COD: 2579 mg/L	[122]
Azo dye Acid Orange 7	Membrane bioreactor	Color: ~100 COD: 60–80	Initial conc.: 50–400 mg/L COD: 95–550 mg/L HRT: 4–24 h	[123]
Textile wastewater	Packed-bed column	Color: ~93–100 COD: ~50	Packing: *Candida tropicalis* within sodium alginate matrix Initial pH: 3.0–6.0 Bed height: 5–15 cm Flow rate: 0.5–1 mL/min	[92]
Anionic dye Orange II	Packed-bed column	Color: 74–94	Packing: Bimetallic chitosan Initial pH: 3.0–6.0 Bed height: 3–7 cm Concentration: 50–200 mg/L Flow rate: 4–10 mL/min	[124]
Cotton dyeing	Packed-bed column	Color: ~97 TOC: ~74 COD: 66 BOD: 72	Packing: Fe/activated carbon Initial pH: 3.0–6.0 Bed height: 3–7 cm Concentration: 50–200 mg/L Flow rate: 4–10 mL/min Temperature: 50°C	[125]
Synthetic textile effluent	Fluidized-bed reactor	Color: 76 COD: 60 Sulfate: 50 Chromium: 93	Cr(VI): 5–45 mg/L Initial COD: 2000 mg/L	[126]
Reactive Black 5, Reactive Orange 16, and Reactive Blue 2	Fluidized-bed reactor	Color: 82–100 COD: 57–91	HRT: 100 min	[127]
Reactive Blue 13	Fluidized-bed reactor	Color: 83 COD: 91	pH: 7.0 Residence time: 70 h Glucose conc.: 2 g/L HRT: 70 h	[128]
Azo dye (Red RBN)	Fluidized-bed reactor	Color: 90	Polyvinyl alcohol-immobilized cell beads Initial dye conc.: <2200 mg/L HRT: 8 h	[129]

BOD, biochemical oxygen demand; *COD*, chemical oxygen demand; *HRT*, hydraulic retention time; *MLSS*, mixed liquor suspended solids; *TMP*, transmembrane pressure; *TOC*, total organic carbon; *VLR*, volumetric loading rate.

1.7.4.2 Disadvantages

The disadvantages of fluidized-bed reactors include: (1) incomplete mixing and poor contact between the liquid and the solid phases, (2) erosion of vessel internals, and (3) attrition of solids [130].

1.7.5 Packed-Bed Reactors

In this reactor type, wastewater (adsorbate) is introduced at the top of a clean bed of packing (an adsorbent). Packed-bed reactors have been tested widely for color removal from textile wastewater using pure and mixed cultures of bacteria and/or fungi. In addition to achieving high color removal, these reactors have also proved to be successful in COD and BOD reduction (Table 1.5). Initial solute removal largely occurs in a narrow band at the top of the column. This band, known as the adsorption zone, begins to extend downward until its lower edge reaches the bottom of the column. At this point, termed the breakthrough point, the effluent concentration rises rapidly. When the effluent concentration (C) approaches 90% of C_0 (initial adsorbate concentration), the adsorbent is considered to be exhausted [131]. The breakthrough time and the shape of the breakthrough curve are very important determinants of the dynamic response of the adsorption column. The *breakthrough time* is defined as the time of adsorption when the outlet concentration is ~5−10% of the inlet concentration. Depth of the exchange zone, time required for the exchange zone to move its own height, adsorption rate, and adsorption capacity are some of the main parameters to be considered when designing an adsorption column [132]. Other design parameters for a packed-bed reactor include the following: the adsorption capacity of pollutants at breakthrough and exhaustion times, the breakthrough time, the time equivalent to the total capacity of the column, the exhaustion time, the total or stoichiometric amount of solute adsorbed, the total amount of solute sent to the column, the volume of effluent treated, and the empty bed residence time.

The sorption process in a packed-bed reactor can be described by the following steps:

1. As a first step, the adsorbate molecules move from the bulk of the liquid to the external surface of the adsorbent (by film diffusion).
2. In the next step, these molecules move from the surface of adsorbent into the interior of the adsorbent (by particle diffusion).
3. Finally, the molecules get adsorbed on the interior of the porous adsorbent.

Any one of the above-mentioned steps or a combination of the three steps may be the rate-controlling step. In full-scale wastewater treatment plants, transport within the solution may be a rate-determining factor. Based on literature reports, many experimental sorption systems have been designed to eliminate the effect of transport in the solution by the process of rapid mixing so that it does not become rate limiting. From a modeling perspective, to ascertain the rate-limiting steps of the overall adsorption process, models proposed by Weber and Morris, Boyd, Urano-Tachkawamodel, and Mathews-Weber can be used [133].

1.8 Conclusions

For practical applications, the use of bacterial cultures is preferred over other biocatalysts for the treatment of dye-containing textile effluent because of the high decolorization and mineralization efficiencies. Several bioreactor configurations involving attached growth, such as a fixed-bed reactor, and suspended growth, such as an sequencing batch reactor, have been successfully tested for the removal of textile dyes in effluent. The high interfacial area generated in the case of an RBC facilitates good contact between the microbial species and the pollutants. New bioreactor configurations involving membranes have been developed specifically for dye wastewater treatment. Among these, the iMBRs are optimized and configured to achieve high treatment efficiencies. A combination of physicochemical and biological or advanced oxidation techniques will yield more promising results for treating complex high-strength textile wastewaters.

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2

Aerobic Treatment of Effluents From the Aquaculture Industry

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2.1 Introduction

2.1.1 Aquaculture Industry

Aquaculture is the practice of nurturing various types of aquatic (freshwater or marine) organisms to supplement the food supply. It has been exercised as small-scale fish farms in Asian countries for approximately 4000 years [26]. However, in the past few decades, aquaculture has become the fastest growing animal food-producing sector. The global level of cultured fish production rose by a magnitude of more than 20 times from 1970 to 2008 [14]. In the first decade of the 21st century alone, the production rate of aquaculture increased from 32.42 million to 52.55 million tonnes [14]. The Asian region contributed to nearly 90% of global production, of which China accounted for 62.3% (Fig. 2.1). It is expected that under the pressure of limits on captured numbers and of the increased demand for fish consumption, the aquaculture industry will experience further upsurges in growth. The World Bank [65] predicted that aquaculture would make up 50% of the total fish supply with 93.6 million tonnes from 2015 to 2030.

Aquaculture practices vary from region to region. Whereas small-scale fishing farms are still predominant in Asian and African countries, more intensive and industrial-scale farms are found in Europe and North and South America.

2.1.2 Characteristics of Effluent From the Aquaculture Industry

The fast-growing rate of aquaculture in the past few decades has triggered concerns about its impact on the water environment. Some countries have developed regulations for effluent discharges from aquaculture activities but the majority of developing countries, which account for a larger portion of productivity, have not. Indeed, pollutant profiles of aquaculture effluents differ greatly from one farm to another. This variability reflects the differences in production scale, types of culture systems, cultured species,

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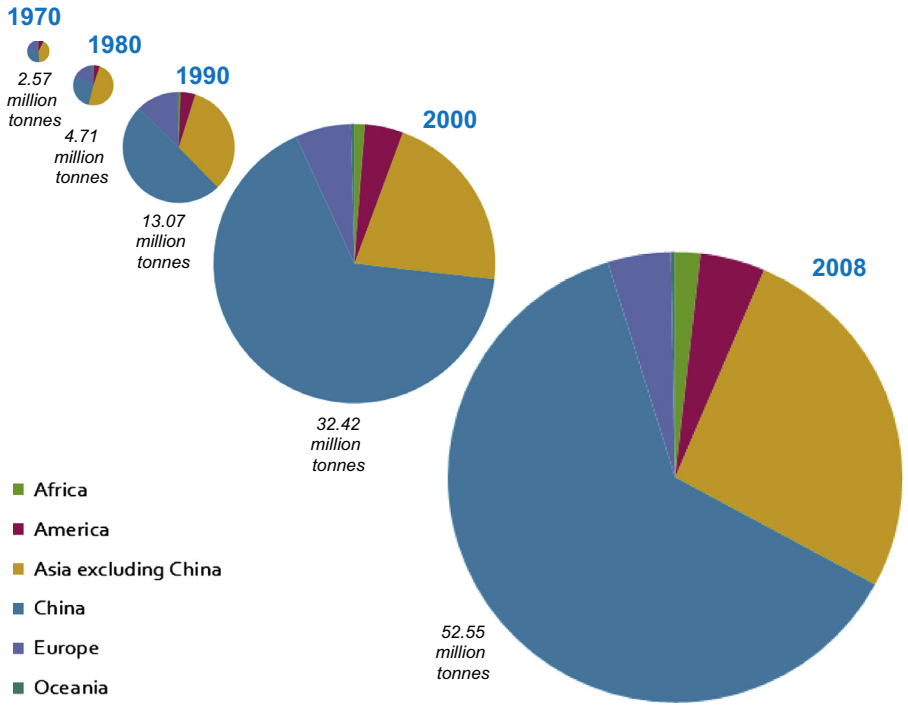


FIGURE 2.1 Aquaculture productivity by region. Sizes of the pie charts represent the relative proportion of a year.

feeding patterns, inline treatment processes, and management procedures [54]. This section is devoted to exploring discharge patterns, pollutants of concern, and environmental impacts of various aquaculture systems.

2.1.2.1 Discharge Patterns

The effluent discharge of an aquaculture farm is strongly dependent on its employed culture system. Therefore, this section will explore various types of culture systems with regard to their discharge behaviors. There are five basic types of aquaculture systems, including: (1) flow-through systems, (2) pond systems, (3) recirculating systems, (4) net pens and cages, and (5) floating and bottom culture systems [59]. Other types of aquaculture such as alligator farming could be encountered elsewhere; however, these practices are insignificant in comparison to the aforementioned systems.

2.1.2.1.1 FLOW-THROUGH SYSTEMS

Flow-through systems are mostly used for cultivating salmonid species. They often take upstream water from a watercourse of sufficient quantity and quality (rivers, wells, springs, etc.) to create an internally steady flow for cultivating aquatic animals (Fig. 2.2). In fact, water is not directly consumed; instead it passes through the system to supply oxygen and clean water and then flushes wastes out of the system. The hydraulic retention time is normally less than 1 h. Owing to this distinctive characteristic, a flow-through system produces a continuous and large-volume discharge. The flow rate of

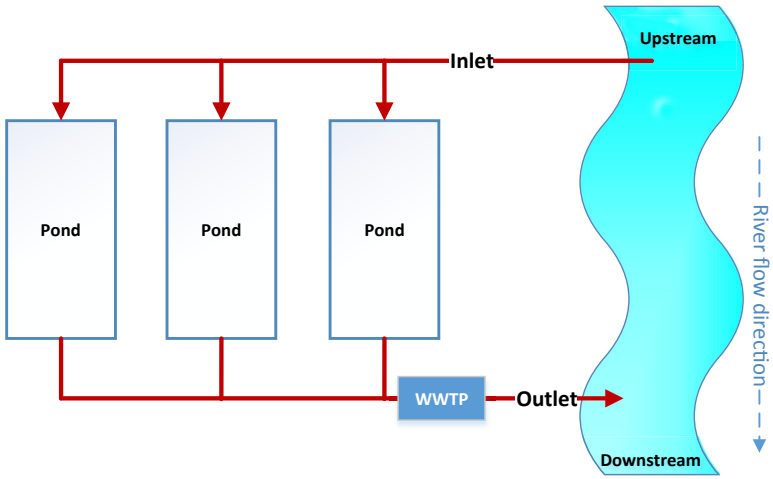


FIGURE 2.2 Diagram of flow-through systems. *WWTP*, wastewater treatment plant.

water discharge could be approximately 300−420 m^3/kg of product for a single-raceway system or 66 m^3/kg for a series of raceways [59]. Flow-through systems usually produce a high-volume but much diluted waste stream.

2.1.2.1.2 POND SYSTEMS

Ponds can be classified as levee ponds, watershed ponds, or depression ponds, according to their water supply. A levee pond consists of a surrounding berm structure to prevent any unwanted water coming into the pond as pumps or piping systems closely control its water level. In contrast, watershed ponds collect runoff water with an acceptable quality from its basin, while depression ponds receive mainly groundwater infiltration and sometimes rainfall. Water volume in watershed and depression ponds receives little intervention, except in cases when water is filled or withdrawn to ensure their proper functions.

Pond sizes fluctuate in a wide range of values from 100 to 100,000 m^2, depending on their production scale, site-specific conditions, and species types. Table 2.1 presents the sizes of common commercialized ponds. In most cases ponds have an average depth of 1.2−1.5 m. Ponds may incorporate aerators to deliver adequate oxygen demand for organisms (Fig. 2.3).

The drainage frequency of pond systems is subject to the cultivated species. For example, it can be 1 year for striped bass, shrimp, and crawfish or up to 6 years for catfish [59]. Nonetheless, a majority of ponds do not discharge more than 30 days per year.

2.1.2.1.3 RECIRCULATING SYSTEMS

Nowadays, aquaculture farmers frequently make use of recirculating systems to reduce their discharge. A typical recirculating system consists of a solid separation device, a biofilter, and an oxygenation recharger (optional), which actively treats and reuses water

Table 2.1 Characteristics of Aquaculture Systems

No.	System	Characteristics	Configuration	Species	Water Use (m^3/kg Production)	Discharge Frequency	Water Quality Maintenance Inside Systems
1	Flow-Through Systems						
	Cold-water species	Consist of single- or multiple-pass units with constantly flowing culture water, using raceways and circular/rectangular tanks.	Some common sizes: • Trout: 24 m long × 2.5 m wide × 0.8 m deep • Trout and catfish: 30.5 m long × 3 m wide × 1 m deep • Series of cells: 9 m long × 3–6 m wide × 3 m deep	Mostly salmonid (rainbow trout) and other types such as freshwater shrimp	54.16–528.26	Continuous	Aeration, water exchange
	Warm-water species	Usually require artificial oxygenation.		Catfish, sunfish, tilapia	274.56	Continuous	Aeration, water exchange
2	Ponds						
	Levee ponds	Suitable for flat land. Soil taken from excavation is reused to build levees. It can be a single unit or a series of ponds.	Size: Varies from less than 4000 to 100,000 m^2. For commercial ponds, an area of 80,000 m^2 is preferable for balance between construction cost and ease of operation. Average depth: 1.2–1.5 m	Baitfish, catfish, crawfish, hybrid striped bass, ornamentals, perch, shrimp, and sports fish	1.79	Infrequent	Aeration, water exchange, natural physical, chemical, and biological processes
	Watershed ponds	Could be built in hilly areas. Size and shape of watershed ponds depend on the local territory.	A pond area of less than 80,000 m^2 is easier to operate.	Baitfish, hybrid striped fish, ornamental fish, sports fish, sunfish, and yellow perch			

3	**Recirculating Systems**						
	Cold-water species Warm-water species	Intensively engineered culture systems, which circulate and reuse water many times through active treatment before discharging	Tanks or raceways, often accompanied by solid removal facilities, biological filtration/ aeration to maintain the influent water quality	Almost all species, but predominantly hybrid striped bass and tilapia	3.29 0.13	Varies from infrequent to continuous	Clarifiers, biological filters, aerators
4	**Net Pens and Cages**	Suspended systems onshore or offshore, which hold farmed species inside Depending on natural conditions such as tidal and water current regimes	Sizes and shapes of the systems vary.	Salmonid species, steelhead trout, cobia, and redfish	—	—	Natural water movement
5	**Floating and Bottom Culture Systems**	Farming bivalve species on bottom beds	Take advantage of water current to supply food for shellfish	Various types of shellfish: clams, mussels, oysters, scallops, etc.	—	—	Natural water movement

Modified from FAO, World Aquaculture 2010. Aquaculture Service, Fisheries and Aquaculture Resources Use and Conservation Division, FAO Fisheries and Aquaculture Department, Rome, 2011 and U.S. EPA, Technical Development Document for the Final Effluent Limitations Guidelines and New Source Performance Standards for the Concentrated Aquatic Animal Production Point Source Category (Revised August 2004). Office of Water, Washington, DC, United States, 2004.

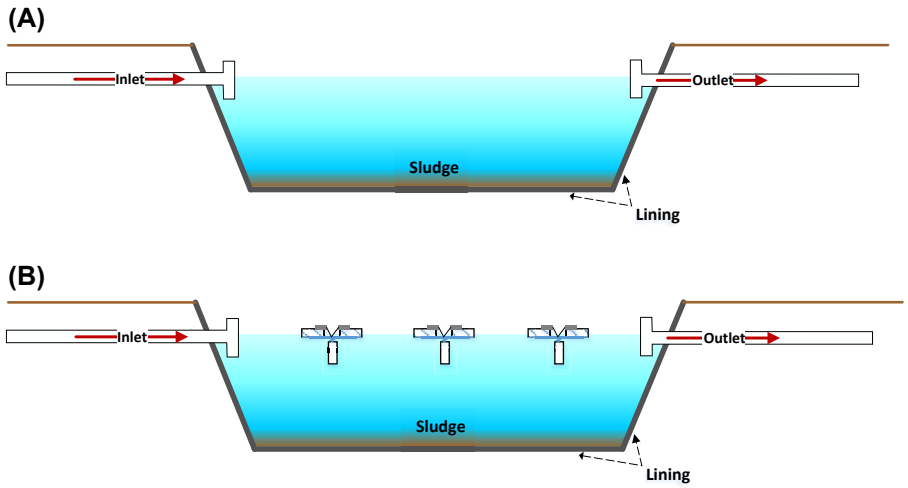


FIGURE 2.3 Diagram of aquaculture ponds. (A) Without surface aerators and (B) with surface aerators.

several times before final discharging (Fig. 2.4). Owing to the recirculation, these systems consume very small amount of water (only 5–10% per day for makeup water to compensate for the loss caused by evaporation, solids removal, etc.). Its small but concentrated effluent can be released continuously or infrequently after storage.

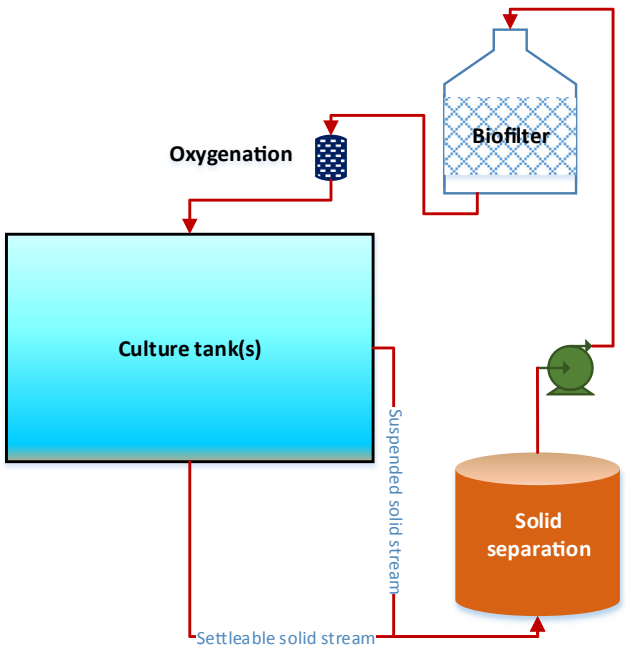


FIGURE 2.4 Diagram of recirculating aquaculture systems.

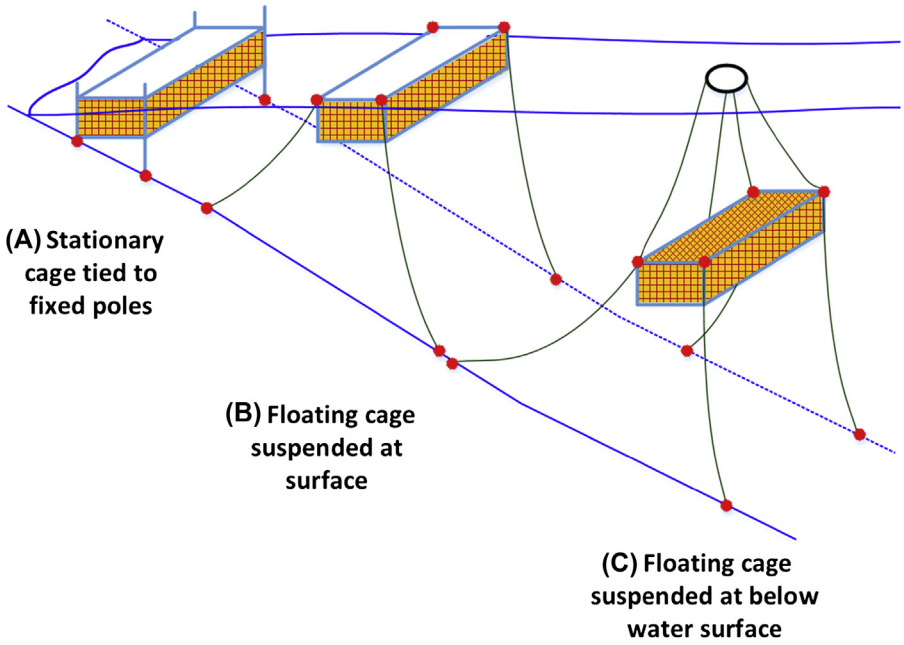


FIGURE 2.5 Diagram of net pens and cages.

2.1.2.1.4 NET PENS AND CAGES

Net pens and cages are suspended or floating holding systems in which some cultured species are grown (Fig. 2.5). These systems are completely governed by the natural watercourse's features, from water quality [temperature, pH, and dissolved oxygen (DO)] to self-biodegradation to food supply. It is impossible to control the water pollution of these systems. Rather, managerial methods must be applied such as restrictions on siting, number of systems per site, capacity of each system, and so on.

2.1.2.1.5 FLOATING AND BOTTOM CULTURE SYSTEMS

Floating and bottom culture systems are employed to grow mollusk shellfish (mussels, scallops, and clams). The principles of floating and bottom culture systems are similar to those of net pens and cages. They also rely on the water movement to supply essential nutrients for the development of cultured bivalves. Three common types of bottom culturing are illustrated in Fig. 2.6.

2.1.2.1.6 SUMMARY

The characteristics of these aquaculture systems are summarized in Table 2.1. Ponds and recirculating systems produce less flow rate but more concentrated effluents, whereas flow-through systems discharge a much higher flow rate but at a low pollutant concentration. Net pens, cages, and floating and bottom culture systems will not be addressed further, as their pollution control and management are less relevant to treatment technologies, which are the focus of this chapter.

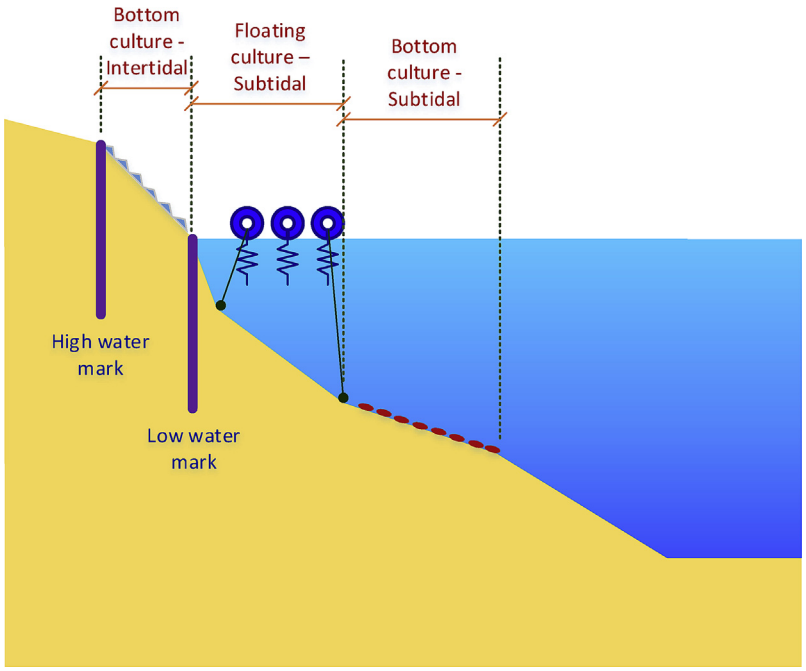


FIGURE 2.6 Floating and bottom culture systems.

2.1.2.2 Solids, Organic, and Nutrient Pollution

2.1.2.2.1 SOURCES

Despite the fluctuations in effluent concentrations between systems, discharges from aquaculture activities are commonly rich in solids, organic matter, and nutrients such as nitrogenous and phosphorous compounds (Table 2.2). These components originate from residual feed, feces, or dead bodies of aquatic species. The rate of pollutants released into the environment is basically a function of the amount of food consumed and the digestibility of the food [54]. One study [2] concluded that 1 kg of product fish discharged approximately 150−600 g of solids. Likewise, Jegatheesan et al. [26], in their analysis of the aquaculture industry in southeast Asia showed that:

- In a day, 1 ton of cultured fish emitted 0.8 kg of nitrogen and 0.1 kg of phosphorus through its uneaten food and excreta.
- Through its lifetime, an average kilogram of farmed fish produced 577 g of biological oxygen demand (BOD), 90.4 g of nitrogen, and 10.5 g of phosphorus.

Indeed, feeding is the primary source of solids, organic, and nutrient pollution [63]. A majority of feed is discarded as waste. This process is characterized by the ratio between waste feed and total feeding load. This ratio depends on cultured species, facility sizes, feed types, and feeding patterns. For example, it is approximately 60% in a concentrated catfish farm or up to 70% for salmonid species [26]. Even an insignificant amount of feeding residues could considerably increase the effluent pollutant concentrations [23].

Table 2.2 Characteristics of Aquaculture Effluents

Culture System	Types of Aquatic Species	Area	Production Yield (tonnes/year)	Pollutants: pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	Nitrogen: NH_4 (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	Total N (mg/L)	Phosphorus: PO_4 (mg/L)	Total (mg/L)	Coliform (MPN/100 ml)	Notes	References
Flow-through	Trout	–	–	6.9–7.8	1.2–3.8	0.5–1.9	0.8–62	0.02–0.6	–	–	–	–	–	–	Surveyed three trout farms in Virginia, USA (1998)	[59]
	Various	–	9–91	–	–	1.4–4.7	3.6–11.5	0.10–0.36	–	–	–	–	–	–		[63]
	Salmonid smolt	–	3–15	–	–	3–200	0–1000	–	–	–	0.2–20	–	0.02–5	–	Surveyed in Norway in 1985	[2]
	Salmonid smolt	–	40–250	–	–	3–10	2–10	–	–	–	0.5–2	–	0.05–0.3	–	Surveyed in Norway in 2000	[2]
Pond	*Chanos chanos*	0.2 ha	–	–	–	–	–	0.16–3.31	0.03–0.647	0.26–2.26	0.45–4.48	2.39–10.45	–	–		[31]
	Oncorhynchus mykiss	–	0.49–1.4	6.5–8.1	–	–	–	0.006–0.65	0.004–0.72		0.58–4.8	0.003–0.075	0.022–0.95	–	Surveyed semiintensive ponds from four farms	[34]
	Hybrid striped bass	–	–	–	–	1.4–64.4 (11.5)	0–370 (49)	0.02–7.29 (0.95)	0–2.94 (0.07)	0–4.61 (0.36)	–	–	0–1.9 (0.31)	–	Surveyed 20 commercial hybrid bass ponds in South Carolina, USA (1998). Numbers in parentheses represent mean values.	[59]
	Shrimp	–	–	–	–	50	1000	–	–	–	–	–	–	–	Water exchange rate of the pond was 2% per day	[59]
Recirculating	Shrimp	–	–	–	1215–1427	–	–	85–95	82–114	160–186	–	–	–	–		[15]
	O. mykiss	–	–	–	–	1030–1220	1346–1990	3.4–4.8	–	–	65.5–81.3	–	42.8–71.6	$1.4 \times 10^7 \pm 5.2 \times 10^6$		[47]
	O. mykiss	–	35 million	–	–	560–756	1889–2581	1.9–2.3	0.34–0.48	3.5–6.3	76–96	–	42–58	–		[48]
	Litopenaeus vannamei	96 m^2	–	7.4–8.2	–	1.9–4.3	3.7–22.1	0.04–0.54	0–0.24	2.08–9.68	–	0.03–2.09	–	–		[32]
	Various	–	–	–	1043	–	752	2.96	5.35	109.0	–	5.98	28.6	–		[59]

Continued

Table 2.2 Characteristics of Aquaculture Effluents—cont'd

Culture System	Types of Aquatic Species	Area	Production Yield (tonnes/year)	Pollutants											Notes	References
				pH	COD (mg/L)	BOD_5 (mg/L)	TSS (mg/L)	Nitrogen				Phosphorus		Coliform (MPN/100 ml)		
								NH_4 (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	Total N (mg/L)	PO_4 (mg/L)	Total (mg/L)			
US EPA [59]		Standard		6.0–9.0	–	30	30	–	–	–	–	–	–	–	Apply for: • Recirculation systems: Discharge water at least 30 days a year • Flow-through and net pen systems: Produce at least 45,359 kg/year	[59]
Global Aquaculture Alliance		Standard		6.0–9.0	–	30	50	3	–	–	–	–	0.3	–		[3]
Standard of International Finance Corporation		Standard		6.0–9.0	–	50	50	–	–	–	–	–	–	400		[3]

BOD_5, biochemical oxygen demand in 5 days; *COD*, chemical oxygen demand; *TSS*, total suspended solids; *MPN*, most probable number.

In many cases, fertilizers are employed as a supplementary feed in fishing farms. Fertilization helps to promote the development of phytoplankton and aquatic invertebrates, which are an important source of food for cultured animals. They could be animal wastes (manures from chicken, pigs, cows, and even human) or organic fertilizers as well as inorganic fertilizers (ammonium phosphate and urea) [16]. The application of animal manure in aquaculture is very favored in low-income areas because of its economic advantages and abundant availability. In contrast, this type of feeding is not common in higher-income countries as it can create a negative profile for the industry.

2.1.2.2.1.1 FLOW-THROUGH SYSTEMS As mentioned before, the most distinctive feature of flow-through system effluent is high flow rate with low concentration. According to the literature review, Wang et al. [63] observed the increase in concentration between influent and effluent in flow-through systems to be as follows: total suspended solids (TSS) ranging from 0 to 100 mg/L, 5-day BOD (BOD_5) from 0 to 15.6 mg/L, total ammonium nitrogen (TAN) from 0.01 to 1.52 mg/L, and total phosphorus (TP) from 0 to 0.591 mg/L. In their own study on six West Virginia flow-through aquaculture facilities for trout (rainbow trout, brown trout, and brook trout), effluent TSS, BOD_5, and TAN increased from 1.9 to 9.0, 0 to 3.3, and 0.03 to 0.33 mg/L, respectively, in comparison to those of influent. Discharge from flow-through systems generally falls below the regulated limits during the normal operation regime (Table 2.2), whereas substantial changes between influent and effluent are often seen during feeding, harvesting, or cleaning periods.

2.1.2.2.1.2 POND SYSTEMS Discharges from water exchange and drainage as well as overflow during heavy rains frequently have components similar to those of the pond water. Unlike the other practices, pond systems have a unique waste-assimilation capacity [3]. When water is stored over an ample period of time, physical, chemical, and biological processes can reduce concentrations of organic matter, nitrogen, and phosphorus inside the pond (Table 2.2). A part of the organic constituents is consumed by microbial decomposition, whereas phosphorus is mainly lost by precipitating and settling [59]. The loss of total nitrogen can be assigned to bacterial decomposition (bacteria, fungi, *Nitrosomonas*, and *Nitrobacter*) of nitrogen particulates, denitrification, and vaporization (Fig. 2.7). The complex nitrogen assimilation cycle in a fish pond is well documented in the literature. Notwithstanding this, ponds still have higher pollutant concentrations than their receiving water bodies.

2.1.2.2.1.3 RECIRCULATING SYSTEMS Recirculating systems produce a small volume of wastewater as a result of an integrated treatment system. Regardless of this small volume, its discharge is rather intense in terms of TSS, chemical oxygen demand (COD), BOD_5, total coliform, and nitrogenous and phosphorous compounds (Table 2.2). Conversely, the main component of total nitrogen produced in flow-through systems was ammonia, whereas that of recirculating systems (after biofilters) was nitrate [8].

In commercial farms, COD can range from 1043 to 1427 mg/L, BOD_5 from 560 to 1220 mg/L, and TSS from under 1000 to about 3000 mg/L [15,47,48,59]. Total nitrogen concentrations are also very high in all wastewater samples, in which they can reach approximately 200 mg/L. Total phosphorus concentrations have a trend similar to that of nitrogen, but to a lesser extent (28.6–71.6 mg/L). All of these values exceed the regulated requirements of the US EPA National Pollution Discharge Elimination System, the Global Aquaculture Alliance, and the International Finance Corporation (Table 2.2).

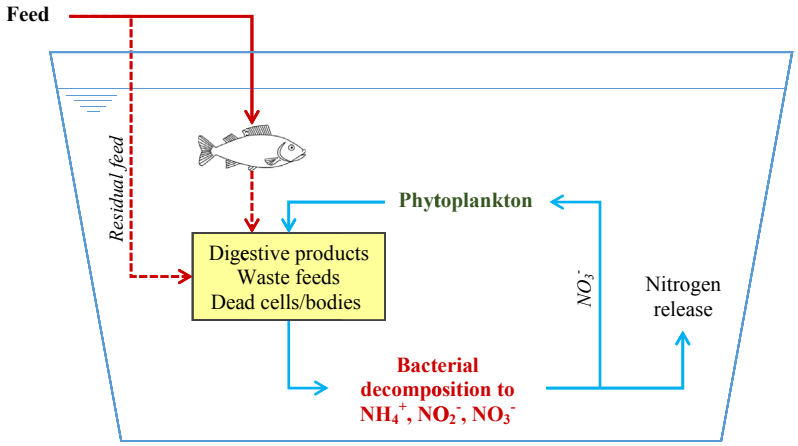


FIGURE 2.7 Schematic of nitrogen assimilation in a fish pond.

2.1.2.2.2 IMPACTS

Aquaculture effluents with a high concentration of solids, organic matter, and nutrients lead to the deterioration of water quality in receiving water bodies. This is particularly problematic in Asian and African countries with the dominance of small-scale farming systems, as they regularly discharge raw water directly into rivers or the ocean.

First, suspended solids (uneaten pellets, feces) reduce the light penetration through the water, which inhibits the photosynthesis of phytoplankton and seagrass, causing an increased fatality of these organisms. Subsequently, bacterial degradation of dead plants will consume oxygen in the water and adversely affect the aquatic culture. In extreme circumstances, aquatic creature profiles may shift into sediment-tolerant species, which affects the aquatic food chain right at the root. In addition, sediments can settle to the bottom where their organic content can biologically degrade and consequently turn the bottom over to anaerobic conditions. This alteration causes a significant change in the composition of the benthic organism community. Some products of anaerobic decomposition processes such as sulfide and ammonia are toxic to aquatic organisms.

Second, various studies have been done on the changes in seagrass (an important element in marine food chains) in response to discharges of nutrients and organic pollutants from fish farms. The immediate effect of fish farm discharge on seagrass could be observed for areas right underneath fish cages. The seagrass in these areas rapidly disappeared. Meanwhile, seagrass in surrounding regions was severely damaged, with weakened roots and increased deaths as well as reduced biodiversity and density [21]. The same phenomenon was also observed in three flow-through trout farms in Virginia, USA [59], where the benthic aquatic life was adversely affected.

In addition, many problems have been documented from the use of human and animal waste in aquaculture. Of significant concern is the possibility of transmission of diseases from these types of waste to humans. These types of waste could deteriorate the physical characteristics of aquaculture water quality, such as odor, color, turbidity, and solids.

The greatest concern related to aquaculture discharge, however, is a waterborne nutrient-enriched phenomenon called eutrophication [41]. As Herbeck et al. [21]

reviewed, the existence of nutrients from fish farms could be detected several kilometers away. The eutrophication effect is discussed in great detail in Ref. [6]. The impact of this scenario is that it will eventually affect human health, either directly or indirectly. The excessive growth of algae in watercourses demands higher chlorination, in case those sources of water are used for domestic purposes. This, in turn, leads to heightened concern because chlorination by-products can result in cancer risks. Finally, some types of microbes (i.e., *Pfiesteria piscicida*) and algae are toxic to human health.

2.1.2.3 Antibiotic and Chemical Pollution

2.1.2.3.1 SOURCES

In addition to solids, biological wastes, and nutrients, discharges from the aquaculture industry may contain various types of chemicals as a result of the application of algicides, disinfectants, and antibiotics. Algicides and disinfectants are commonly used in shrimp farming to prevent the development of unfavorable phytoplankton, fish, bivalves, and algae.

Aquatic animals are usually raised in very crowded densities that never occur in the natural environment. Two immediate outcomes derive from this scenario. First, it increases friction and psychological stress among farmed animals. Fighting or colliding with one another easily wounds cultured fish. Second, the high-population density is a favorable environment for spreading diseases. As a result, pathogens are often found in aquaculture effluents. These effluents may affect both the fish and the humans in contact.

To control this risk and to ensure aquaculture productivity, antibiotics have been widely applied. Chloramphenicol, oxytetracycline, and oxolinic acid are the most popular of 26 antibiotics often used in the aquaculture industry [26]. Surprisingly, animal wastes, which are used as a food source in some farms, can release their ingested antibiotics into the water environment, even though only in small amounts. The rate of applied antibiotics ranges from 1 g/ton of fish production in Norway to as high as 700 g/ton in Vietnam [43]. While some countries (for example, United States and Norway) have strict conditions for using antibiotics, other countries, especially those with predominantly small-scale farming systems, use inadequate antibiotics controls.

2.1.2.3.2 IMPACTS

A large amount of antibiotics is not retained in the bodies of farmed animals and will be discarded into the environment through metabolic wastes [13]. For example, oxytetracycline has a low adsorption rate in fish; therefore, it must be applied in a high dose of 100−150 mg/kg fish per day for 10−15 days continuously [43]. More than 70% of this amount is released into the water via fish metabolites.

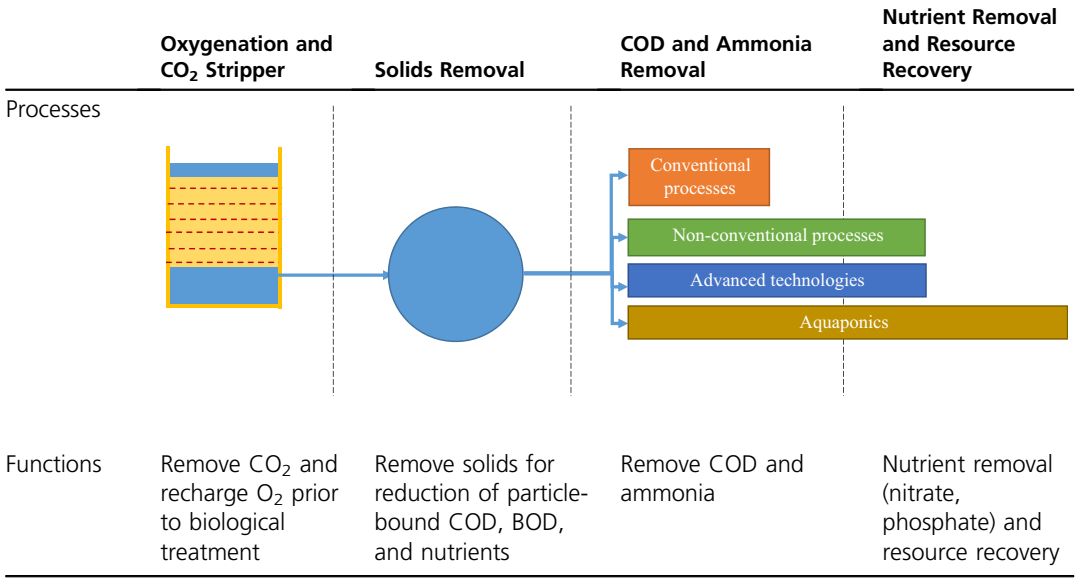
The excessive use of antibiotics leads to bacterial antibiotic resistance not only inside the farming boundaries but also in the surrounding water environment. Romero et al. [43] reported a phenomenon of antibiotic resistance in various types of organisms such as *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Edwardsiella tarda*, *Edwardsiella ictaluri*, *Vibrio anguillarum*, *Vibrio salmonicida*, *Pasteurella piscicida*, and *Yersinia ruckeri*. The antibiotic-resistance genes of bacteria can be transferred to human pathogens whenever the conditions are favorable [43]. A survey carried out by Duran and

Marshall [9] on ready-to-eat shrimps found that at least 140 in 162 bacteria species were antibiotic resistant. Among them appeared four common types of human pathogen, including *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio* spp. Bacterial antibiotic resistance can accumulate in humans through the food chain.

2.2 Treatment of Effluents From the Aquaculture Industry

When wastewater contains biodegradable organic substances and nutrients, there are two types of biological treatment processes that could be employed—aerobic treatment and anaerobic treatment. The advantages and disadvantages of these two processes have been well studied. For aquaculture effluents, because of its low-strength organic content (less than 1000 mg/L) and high nutrient availability, aerobic treatment technology is the more favorable option [26]. Other advantages of aerobic alternatives include: (1) reduction of unpleasant odors, (2) production of stable and reliable treatment outputs, (3) suitability for space-restricted areas, (4) reduction of the additional demands for oxygen before discharging, and (5) elimination of pathogens. However, aerobic treatments are not effective at removing NO_3^- and PO_4^{3-}; thus further steps (denitrification or phosphorus removal) must be implemented.

In aerobic methods, the wastewater influents are often treated preliminarily with oxygenation and solid separation (Section 2.2.1). Although solid removal can partially reduce the magnitude of pollution, a majority of dissolved substances such as TAN, nitrite, phosphorus, BOD_5 and coliform bacteria still existed and required further treatment. Sections 2.2.2–2.2.4 will present three different approaches—conventional, nonconventional, and advanced technologies.



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	Oxygenation and CO_2 Stripper	**Solids Removal**	**COD and Ammonia Removal**	**Nutrient Removal and Resource Recovery**
Technologies	• Stripping towers • Surface aerators • Diffused aerators	• <10 μm: Diatomaceous earth filter, cartridge filter, ozonation, foam fractionator • 10–50 μm: Sand filter, cyclone • 50–100 μm: Cyclone, bead filter, microscreen filter • >100 μm: Bead filter, microscreen filter, settling tank	*Conventional processes*: Fluidized-bed sand filters, moving-bed biofilm reactors, rotating biological contactors, trickling filters, etc. *Nonconventional treatment*: Wetland *Advanced technology*: Membrane bioreactors, membrane biofilm reactors	*Aquaponics*: "Green water" systems

2.2.1 Pretreatment Methods

2.2.2.1 Oxygenation and CO_2 Strippers

Oxygenation and CO_2 stripping can be done at the same time to supply sufficient oxygen demand for the following treatments as well as to remove CO_2. CO_2 is among the important parameters to control in the process of aquaculture practice as it reduces the capacity of hemoglobin to transport oxygen and increases fish dysfunctions. The safe concentrations of CO_2 for tilapia, striped bass, and trout are 60, 60, and 9–30 mg/L, respectively [55]. O_2 regeneration and CO_2 stripping options are presented in Table 2.3.

2.2.2.2 Solids Removal

As a large portion of organic compounds is accompanied by solids, removal of solids can significantly reduce the concentration of BOD and total phosphorus. As Davidson et al. [8] reviewed, the percentages of total phosphorus present in solid phase in a recirculating rainbow trout system and in flow-through effluents were 85% and 30–84%, respectively. The percentage of nitrogen in particulate form was about 7–32% of total nitrogen [25]. Solids removal should be done as soon as possible to prevent the destruction of large particles into smaller pieces, which increases the dissolved concentration of pollutants (BOD and nutrients). This is particularly important for aquaculture wastewater, as most particles are uneaten feed and discarded feces, which are loosely bonded. In addition, the partial removal of solids can decrease the organic concentration, which in turn reduces the competence of heterotrophic carbonaceous microorganisms over autotrophic nitrifying bacteria in biofilters (Section 2.2.2).

Table 2.3 Oxygenation and CO_2 Stripping Options

		Design Parameters		
Option	**Principle**	**Parameter**	**Low Range**	**High Range**
Stripping towers	Wastewater flows downward uniformly by distribution systems while the air is supplied from the bottom	Water distribution	Drip late	Spray nozzles
		Hydraulic loading	10 kg/m^2 s	30 kg/m^2 s
		Water breakup	Splash screens	Random packing
		Tower height	0.5—2 m	
		Volumetric gas-to-liquid ratio	1—20	
Surface aerators	Use a motor with a propeller-type blade floating on the water surface	Standard aeration efficiency	1.0 kg/kWh	3.0 kg/kWh
		Motor size	0.5—2 HP	
		Spray pattern	Boil	Fountain
		Spray height	0.5 m	2 m
		Spray diameter	1 m	4 m
Diffused aerators	Use porous diffusers to deliver air bubbles through the water column	Standard aeration efficiency	0.5 kg/kWh	2.0 kg/kWh
		Motor size	0.25 HP	3 HP
		Diffuser depth	0.25 m	2 m
		Bubbles	Fine	Coarse

Modified from S. Summerfelt, et al., Carbon Dioxide Stripping — Fundamentals of Computer Design Model (Recirculating Aquaculture Systems Short Course). College of Agriculture and Life Sciences, The University of Arizona, USA, 2012.

Particle sizes and concentrations are important for designing subsequent facilities. Conventional solids separation methods such as settling tanks and moving screens can remove roughly 50% of the total solids [8]. Removal techniques for a particle are dependent on its size (Fig. 2.8), as follows:

- Large particles, >100 μm: settleable, thus easily settled out
- Medium particles, >50 μm: filterable with a screen
- Small particles, <10 μm: difficult to filter.

From a review of the available literature on the size distribution of solids in recirculating agricultural systems, for culturing mature fish, more than 90% of solids had diameters of less than 30 μm. The situation is different for nursery systems in which more large-size particles are observed [25].

2.2.2.2.1 SETTLING TANKS

Settling tanks are preferable for removing solids in wastewater, as they are cheap and simple. Moreover, head loss of this type of equipment is often negligible. Settling tanks used in aquaculture wastewater treatment include settling basins, tube settlers, plate settlers, swirl separators, and similar systems. These facilities target particles larger than 100 μm.

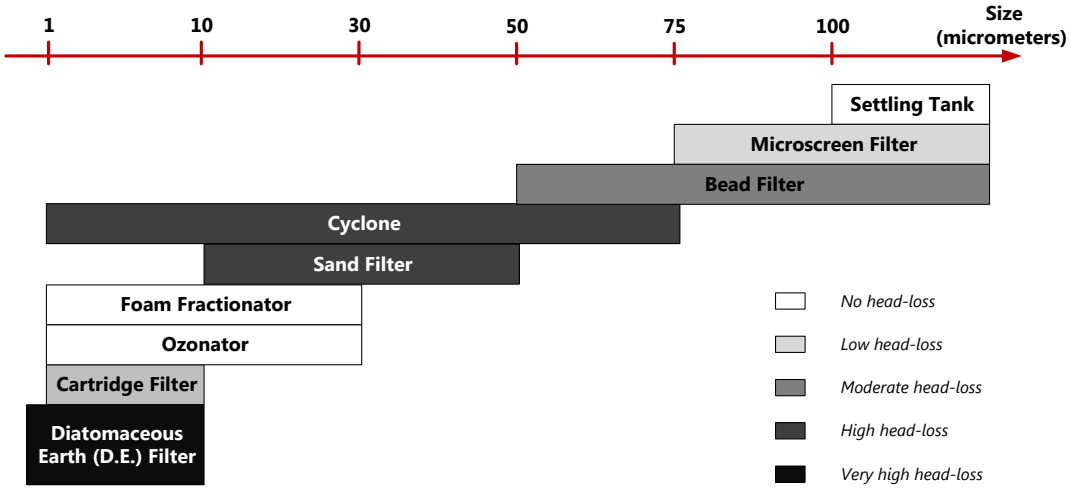


FIGURE 2.8 Typical solids removal technologies in aquaculture wastewater treatment. *Modified from R. Strange, Recirculation Aquaculture Systems. University of Tennessee, USA, 2004.*

2.2.2.2.2 MICROSCREEN FILTERS

Utilizing the moving motion and a continuous back-flushing regime, this type of filter can remove a wide range of particles without periodic shutdown. It is effective at removing solids larger than 75 μm.

2.2.2.2.3 FILTERS

Common filters are sand filters and bead filters. They can remove a wide range of particles of different sizes. For example, sand filters are claimed to deal with particles of sizes as small as 10 μm. The removal efficiency and head loss of reactors depend greatly on the size of the media and size of the particles. In some cases, bead filters can serve as a mechanical filter as well as a biological filter.

2.2.2.2.4 FRACTIONATORS

This type of solid separator focuses on removing fine particles (less than 30 μm) and colloids by attaching them to the surface of air bubbles (supplied by an air blower at the bottom of the reaction tank). The air bubbles and attached substances float to the water surface and are retained there as foams, which are easily removed by a skimmer. The fractionators often work better in saline water (with higher buoyance than freshwater). Nevertheless, fractionators cannot completely replace other types of solid removals.

2.2.2 Conventional Treatment

Activated sludge processes and biofiltration are the two most common conventional aerobic treatment methods in wastewater treatment. Whereas activated sludge systems are very popular for municipal wastewater, their application in aquaculture is rather limited. The main reason for this problem is their unsatisfactory performance of

nitrification (which is one of the main targets of aquaculture wastewater treatment) [66]. Hence, this section will mainly discuss biofiltration.

Indeed, biofilters have been applied extensively for aquaculture wastewaters because of their ability to remove organics and ammonia. They are evidently appropriate for recirculating aquaculture systems in which biofilters aim to covert ammonia and nitrite (two toxic compounds to fish, even in small amounts) into nitrate. The nitrification in biofilters for aquaculture wastewater is an ammonia-limiting process, rather than an oxygen-limiting process, for municipal or industrial wastewater treatment [22].

Typical aerobic biofilters used in aquaculture wastewater treatment are fluidized-bed sand filters, moving-bed biofilm reactors, rotating biological contactors, and trickling filters. In the case of submerged-bed filters, classical designs are often disadvantageous in aquaculture as they have low specific surface area, high potential of biofouling, and high construction cost as well as large and heavy structures. Improvements in medium materials and water flow distribution systems make submerged-bed filters more suitable for aquaculture application; however, their nitrification rate is rather low [8].

The following sections discuss in detail the applications of these biofilters in aquaculture via a pragmatic approach. The design processes of these biofilters, which are referenced in classical textbooks, will not be repeated here.

2.2.2.1 Fluidized-Bed Sand Filters

Fluidized-bed sand filters (FBSFs) utilize the upflow velocity of water to keep biofilm-coated sands suspended in reactors. Heterotrophic carbonaceous microorganisms and autotrophic nitrifying bacteria grown on the surface of the sand grains will help to digest the organic content, ammonia, and nitrite of the wastewater. Biofilms developed on the surface of sand grains will gradually increase the grain size while decreasing the grain density [8], which makes the sands move toward the water surface. The advantage of this process is that it expands the bed medium volume, which allows higher contact between water and biofilm. Collisions between sand grains, shear stress of water flow, and turbulence inside reactors act as a self-cleaning mechanism for FBSFs.

The design and management of FBSFs in aquaculture have been presented in great detail in Ref. [56]. The most common design parameters for FBSF are: (1) sand size, (2) head loss, (3) water distribution, (4) bed volume expansion, (5) flow rate, (6) TAN removal efficiency, and (7) removal methods of aging sands.

- **Sand selection:** Hard, whole grain, and finely graded crystalline silica sand with specific gravity of approximately 2.65 is recommended [56]. D_{10}, the effective size that allows less than 10% of particles to go through, is used as a parameter to select sand size. The common range of sand diameters is 0.1−1.0 mm. To investigate the effects of sand size on biofilm characteristics, Nam et al. [39] studied two sand sizes, D_{10} of 0.23 and 0.60 mm. In this research, smaller particles had more surface area for hosting microorganisms, which leads to thick, porous, and rough biofilms with an average thickness of 16.28 μm for 0.23-mm particles. In contrast,

larger-size particles are subject to higher shear forces; therefore, their biofilms are thinner and smoother, with an average thickness of 5.42 μm for 0.60-mm particles. Nonetheless, the ratio between biofilm surface area and biofilm volume was consistently constant.

- **Head loss:** Head loss through an expanded bed is calculated as follows [56]:

$$\frac{H_{\text{bed}}}{L} = \frac{\rho_{\text{p}} - \rho}{\rho}(1-\varepsilon) = (SG_{\text{p}} - SG_{\text{w}})(1-\varepsilon),$$

in which L is the depth of the static bed (cm), SG_{p} is the specific gravity of the particle, SG_{w} is the specific gravity of water; ε is the static bed porosity (0.42−0.47), ρ_{p} is the density of sand (2.65 g/cm^3) and ρ is the density of water (1 g/cm^3).
In general, 1.0 m of an initially static sand depth requires 0.87−0.98 m of water head.

- **Uniform water flow distribution:** This is the most critical factor for ensuring FBSF performance reliability. A short circuit in the water flow, if it were to occur, would significantly compromise treatment performance. Various kinds of flow distributor are presented in Table 2.4.
- **Bed volume expansion:** Commonly designed static sand height is 1−1.25 m. Bed volume expansion rates are designed from 25% to 100% [28] or 2−5 m [56]. Yet, bed volume expansion will change over time as a result of biofilm development on the surface of grains.
- **Flow rate:** The FBSF flow rate should be at least 190 L/min to ensure the suspension of bed volume [18].
- **TAN removal efficiency:** TAN concentration discharged from a culture tank with an integrated FBSF is calculated via the formula presented in Ref. [56]:

$$\text{TAN}_{\text{out}} = \left\{\frac{1}{1-(R\times(1-f_{\text{rem}}))}\right\} \times \left\{\frac{r_{\text{TAN}}}{Q_{\text{biof}}} \times \frac{10^6\ (\text{mg})}{1\ (\text{g})} \times \frac{1\ (\text{day})}{1440\ (\text{min})}\right\},$$

in which TAN_{out} is the TAN concentration discharged from a culture tank (mg/L), f_{rem} is the TAN removal efficiency of the biofilter, r_{TAN} is the average daily rate at which TAN is produced (kg/day); R is the fraction of water recirculated through the biofilter, and Q_{biof} is the biofilter flow rate (L/min).

- **Removal methods of aging biomass-coated sands:** Although FBSF has a high capacity for self-cleaning; siphoning or shearing the aging grains is necessary to control the development of microorganisms inside the reactor. The siphoning method reduces energy use as it uses only static hydraulic difference to remove excessive biomass. However, it could lead to a more labor-intensive sand-cleaning process. In addition, biomass loss is hard to control and, consequently, reduces the treatment efficiency [8]. In contrast, shearing can reduce the amount of sand removed and the labor force while increasing the treatment efficiency. The problem with the shearing method is its higher energy demand.

Table 2.4 Characteristics of Typical Water Flow Distribution Types

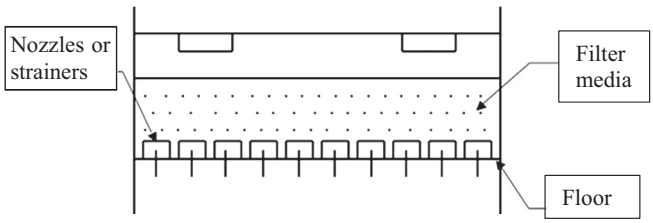
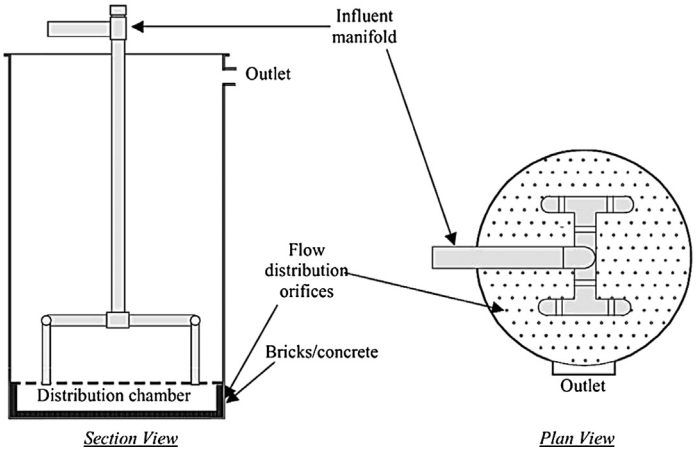
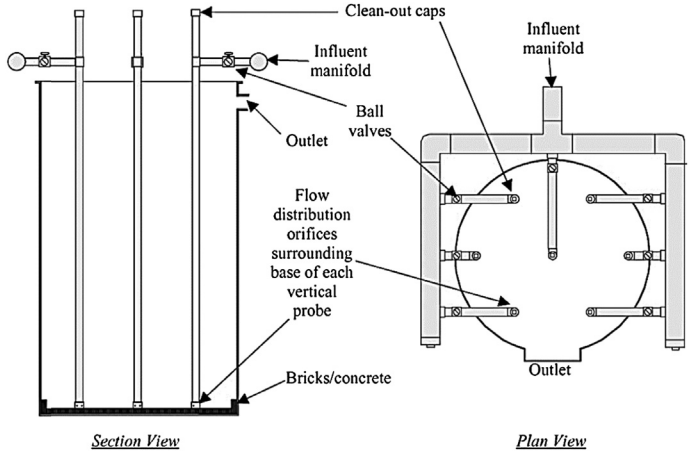
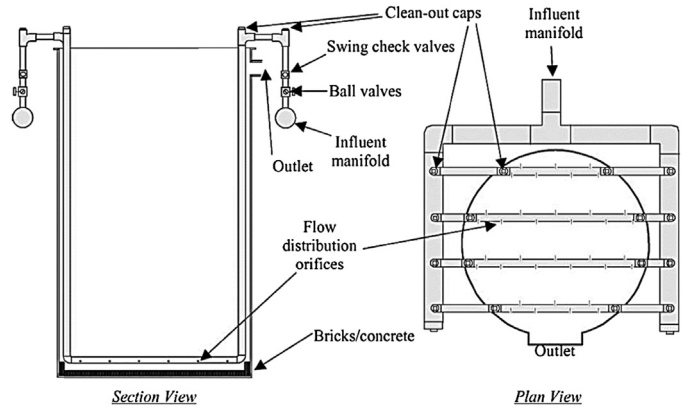
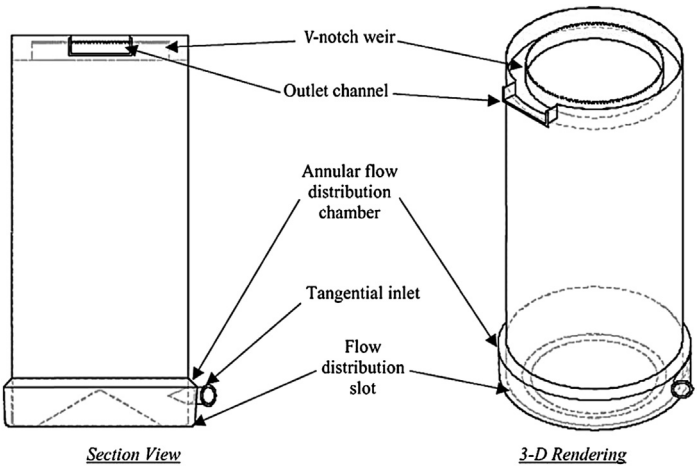
Type of Water Flow Distribution	Principle and Illustration	Applications, Advantages, and Disadvantages
Gravel-covered horizontal pipe	One to four layers (about 7.6 cm per layer) of gravel (with an ascending order of gravel size from the top to the bottom) over the top of distribution or pipeline.	Applied for small-scale systems (−) High water flow rate may dislocate gravels. (−) Susceptible to clogging from solid trap and biofilm development
Nozzle-type flow distributors with false-floor manifold		Limited application (−) Subject to plugging or fouling (−) High cost and proprietary nature restrict its applicability
False-floor orifice distribution plate	The pipe system conducts water from the top to the bottom distribution chamber lying under a false-floor support. 	Commercialized and currently applied for recirculating aquaculture systems

Table 2.4 Characteristics of Typical Water Flow Distribution Types—cont'd

Type of Water Flow Distribution	Principle and Illustration	Applications, Advantages, and Disadvantages
Vertical pipe manifold	The system of vertical pipes is used to conduct the water from the influent manifold to the reactor bottom through flow distribution orifices.  	Successfully applied in the Conservation Fund Freshwater Institute (USA) and recirculating aquaculture systems (–) Sand size must be small to retain an acceptable performance
Horizontal pipe manifold	Water is distributed through the evenly spaced orifices from a set of vertical pipes and horizontal laterals. • Distance between orifices and between pipe laterals is 7.5—30 cm. • Total area of orifices:cross-sectional area of bed is 0.0015—0.005 • Cross-sectional area of pipe-lateral:total area of orifices served is 2—4 • Cross-sectional area of manifold:total area of pipe-laterals is 1.5—3  To eliminate abrasion from sands, a concrete or brick layer is built at the floor (10—15 cm from the distribution pipes and 10—20 cm from the sides)	Widely applied in recirculating aquaculture systems

Continued

Table 2.4 Characteristics of Typical Water Flow Distribution Types—cont'd

Type of Water Flow Distribution	Principle and Illustration	Applications, Advantages, and Disadvantages
CycloBio with slotted inlet manifold	Developed by Neil Helwig of Marine Biotech, Inc., the CycloBio acts as a cyclone, using continuous water flow as a driving force for rotation. A bottom-inverted cone at the bottom is used to accelerate water upflow velocity. 	Applied in recirculating aquaculture systems (+) Low head loss (about 10% of horizontal pipe manifold) (+) More uniform expansion (+) Simple operation and maintenance

Modified from S.T. Summerfelt, Design and management of conventional fluidized-sand biofilters. Aquacultural Engineering, 34 (3) (2006) 275–302.

FBSF has been widely applied in large-scale plants, especially in cold weather. Summerfelt [56] reviewed the performance of TAN removal of FBSF. In general, it can remove 50–90% of TAN for each pass and maintain the effluent ammonia and nitrite concentrations at 0.1–0.5 and <0.1–0.3 mg/L, respectively. Davidson et al. [8] reported on the performance of TAN, BOD_5, and total coliform removal for 0.11-mm-sand FBSF with the biofilm shearing method as being 86–88%, 66–82%, and 1–2 $\log_{10}$, respectively.

2.2.2.2 Moving-Bed Biofilm Reactors

Developed in the late 1980s in Norway, moving-bed biofilm reactors (MBBRs) utilize carriers (plastics or otherwise) to cultivate and grow biomass for nitrifying and removing organic compounds. These carriers can move freely inside the reactor thanks to turbulence caused by air diffusers located at the bottom. As a result, the whole volume of the reactor becomes a pool for microorganisms to develop.

Important design parameters for an MBBR are:

- **Carriers:** These are commonly high-density polyethylene materials (e.g., Kaldnes material) with various active surface areas (300–500 m^2/m^3) and density.
- **Filling fraction:** The filling fraction of carriers can be easily adjusted, but the fraction below 70% (typically 50–70%) was recommended by [44]. This value ensures a proper mixing efficiency.
- **Empty bed hydraulic retention time (HRT):** 2–5 min.
- **Air supply:** Turbulence caused by the aeration–mixing process is crucial to maintaining biofilm thickness for maximizing the nitrification rate. The ideal biofilm thickness is less than 100 μm.
- **Retention sieves:** These aim to retain media inside a reactor. They could be in different forms such as vertically rectangular mesh or cylindrical bar sieves.

The main aim of MBBRs used in aquaculture is nitrification. Rusten et al. [44] summarized the influence of various factors on the TAN removal process, such as organic loading, DO concentration, influent TAN (Fig. 2.9), temperature, pH, and alkalinity. The nitrifying bacteria are very sensitive to the inhibitor concentrations such as nitrite; therefore, pH and alkalinity should be carefully controlled.

The TAN removal rates of MBBRs for a freshwater Atlantic salmon (*Salmo salar*) smolt plant and a "brown trout and arctic char juveniles" plant were 0.4–0.5 and 0.3 g NH_4/m^2 day, respectively [44]. In saline water systems, the removal rate was decreased significantly. Nitrification rate constants of saline systems were reduced by

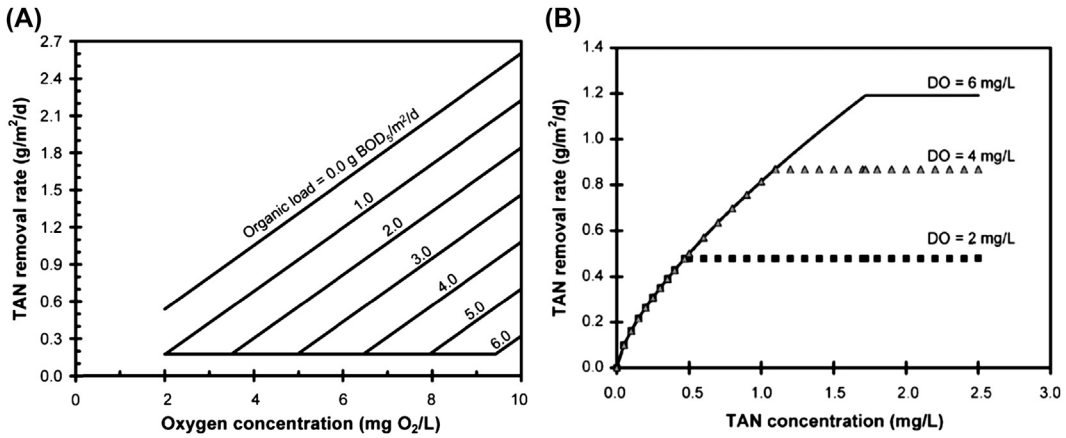


FIGURE 2.9 Influence of various factors on total ammonium nitrogen (*TAN*) removal in a Kaldnes moving-bed biofilm reactor at 15°C. (*Left*) Organic load and reactor dissolved oxygen (*DO*) concentration at TAN_{in} excess (≥2.5 mg N/L), and (*right*) TAN and DO concentrations at 0.4 g BOD_5/m^2 day organic load. *Adapted from B. Rusten, et al., Design and operations of the Kaldnes moving bed biofilm reactors. Aquacultural Engineering, 34 (3) (2006) 322–331.*

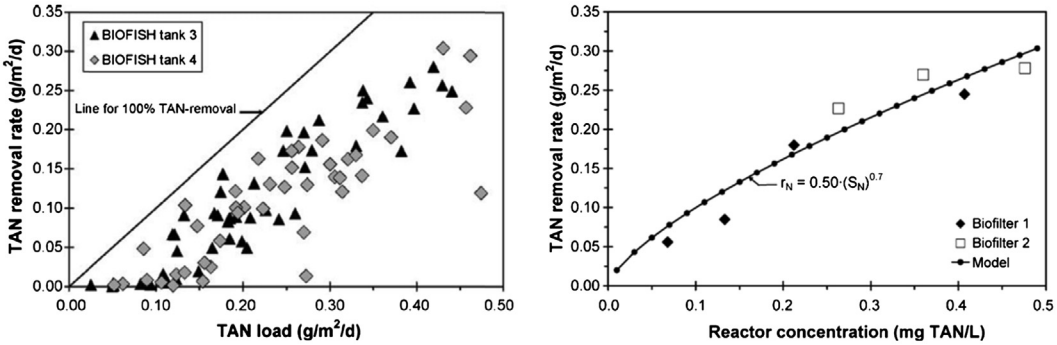


FIGURE 2.10 Total ammonium nitrogen (*TAN*) removal rate by moving-bed biofilm reactors in full-scale plants in a freshwater culture system and a saline system. *Adapted from B. Rusten, et al., Design and operations of the Kaldnes moving bed biofilm reactors. Aquacultural Engineering, 34 (3) (2006) 322–331.*

40% in comparison to freshwater systems [44] (Fig. 2.10). However, Malone and Pfeiffer [35] were doubtful of this effect.

2.2.2.3 Rotating Biological Contactors

Rotating biological contactors (RBCs) are often composed of circular biofilm-fixed plates or disks, which rotate around a central horizontal axle at a slow speed. The disk is always semisubmerged (35–40%) in wastewater [22]. As a result, in one half of the rotating cycle, the biofilm receives substances from wastewater; and in the other half, it will be supplied with natural oxygen from the air. Current technological advances in medium materials and rotating mechanisms help to overcome common issues of RBCs such as large land size and mechanical breakdown.

Design parameters of RBCs are as follows:

- **Medium material:** In the past, flat or corrugated fiberglass and plastic were normally used in RBCs. However, these traditional materials had low specific surface areas (less than 100 m^2/m^3). Fortunately, new materials have been developed to increase the specific surface area up to 300 m^2/m^3.
- **Rotational speed**: A rotational speed of 1–5 rpm and peripheral velocity of 0.18–0.40 m/s are suggested for RBC operation [4,22]. Within these velocity ranges, increasing the rotating velocity incrementally will generally increase the removal performance until a threshold value is reached, at which point the performance remains constant [4]. An overranged speed can accelerate the shearing force of the biofilm, which reduces the attachment of microorganisms on the disk surface. In contrast, an underranged speed will reduce the oxygenation for bacteria submerged in water and dry the biofilm exposed to the air.
- **Disk diameter**: Disks used in aquaculture often have a diameter of 3 m or less [22].
- **Space between disks:** The distance between two disks should not be less than 13 mm to prevent clogging between disks [28].

- **Mass and hydraulic loading**: The suggested value for maximum hydraulic loading for RBC application in aquaculture is 300 m^3/m^2 day [22]. In commercialized applications, this value can be achieved at 407 m^3/m^2 day [4]. Nevertheless, it was compensated for by a lower nitrification rate (0.43 ± 0.16 g/m^2 day).
- **Organic loading**: An increase in organic loading will reduce nitrification activities, as heterogenic carbonaceous bacteria will outcompete nitrifying bacteria. When the ratio between organic compounds and ammonia (TAN) was higher than 3.5, the nitrification rate fell by 55% [4].
- **Staging**: Staging is performed in RBCs to increase the treatment efficiency through a series of three to five contiguous RBCs. The improved performance is contributed to by separating carbon removal and nitrification. As heterogenic carbonaceous bacteria grow five times faster than nitrifying bacteria, the carbon removal often occurs in the first RBC and nitrification happens in the following reactors [22]. Consequently, at the same total medium and hydraulic volume, a series of small RBCs will perform better than a single large RBC [4].

Typical influent concentrations of ammonia originating from aquaculture practices are 3–5 mg/L [4]. The performance of commercial-scale RBCs in treating aquaculture (0.43 ± 0.16 g/m^2 day) is comparable to those of trickling filters (0.24–0.55 g/m^2/day) and microbead filters (0.45–0.6 g/m^2 day). Although CO_2 removal is not the main objective of RBCs, RBCs can remove approximately 39% of CO_2 [4].

2.2.2.4 Trickling Filters

A trickling filter is composed of two main sections—a top water distribution system and a base located at the bottom of the filter. The base contains a layer of medium, a supporting floor for the medium, and a water collecting structure. Water is evenly supplied from the top distribution system, adsorbs oxygen from the air, and trickles on the biofilm on the medium's surface. In this way the biofilm will receive both nutrients and oxygen. If properly designed, a trickling filter is very sustainable as it does not require an external oxygen supply, is stable over a long period of time, and rarely clogs. Trickling filters are often employed in warm-water aquaculture systems but not in cold-water systems as nitrification efficiency is reduced significantly [10].

Important design parameters for a trickling filter include:

- **Water distribution system:** Uniformity of water distribution is very important in a trickling filter design. Poor water distribution leads to a reduced efficiency that results in uneven contact between microorganisms and water. There are three types of water distribution systems: a moving arm, a perforated screen, and a nozzle. A moving arm with a rotating beam is often applied for random media. Meanwhile, a perforated screen is used only for small-scale plants, whereas a pressure spray system (nozzle) is usually applied for larger plants [11].
- **Type of medium:** Polymeric materials are preferred in aquaculture applications as they are lighter and have higher specific surface area (100–300 m^2/m^3) [10].

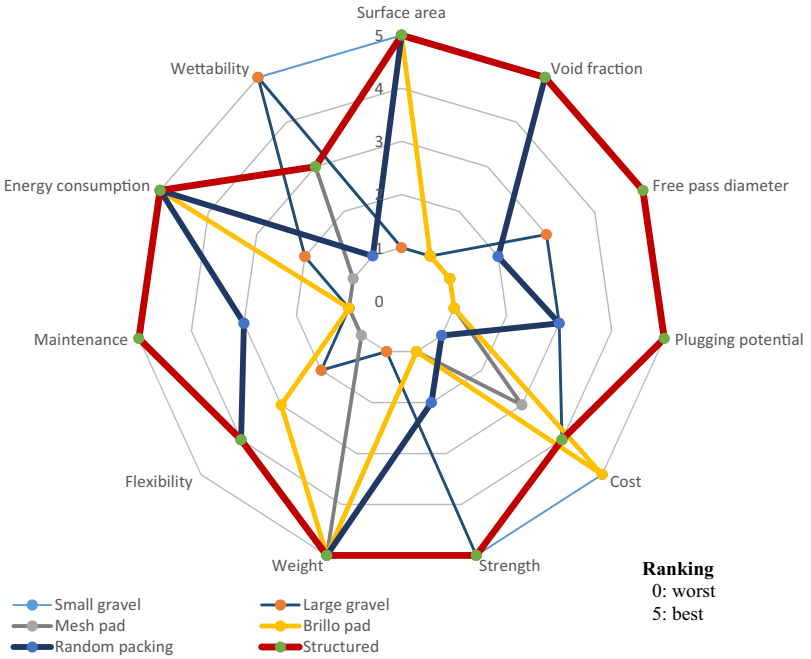


FIGURE 2.11 Comparison of types of media applied in aquaculture wastewater treatment. *Data adapted from M. Smith, Biological Filters for Aquaculture. L S Enterprises, USA, 2013b.*

Materials with high void space (>90%) will increase the possibility of contact between water and biofilm on the surface of the material. Smith [52] assessed six types of media for fixed-film biofilters against 11 aspects such as surface area, void fraction, free pass diameters, plugging potential, cost, mechanical strength, weight, flexibility, difficulties in maintenance, total energy consumption, and wettability (Fig. 2.11). Structured media are often preferred to random packing because of their diverse benefits such as flexibility (with module or block) and simplicity in usage. Structured media can provide cost savings as they do not need a supporting frame like that required for random media.

Information on specific surface area and void fraction of common types of media for trickling filters is presented in Table 2.5.

- **Hydraulic loading rate (HLR)**: Selected HLR will depend on the choice of material. Eding et al. [11] assessed minimum and maximum values of various materials (Table 2.6). Hochheimer and Wheaton [22] recommended that HLR for a trickling filter should be in the range of 50–300 m^3/m^2 day to both ensure wetting of surface and prevent the scouring of biofilm. Specifically, Ebeling [10] narrowed this range down to 100–250 m^3/m^2 day.
- **Reactor depth:** Smith [51] presented a good discussion on the depth of trickling filters in aquaculture applications. Accordingly, the optimal depth of a

Table 2.5 Specific Types of Media Researched for Trickling Filters in Aquaculture

Type of Medium	Specific Surface Area (m^2/m^3)	Void Fraction
Finturf artificial grass[a]	284	—
Kaldnes rings[a]	500	—
Norton rings[a]	220	—
Leca (light-weight clay aggregate)[a]	500—1000	—
Random-flow medium Filterpak-CR50[b]	200	0.93
Vertical-flow medium Bionet[b]	200	0.95
Cross-flow medium FKP319[b]	150	0.92
Structural packings AccuPac CF-3000[c]	105	95
Norpak[d]	164	

[a]Ref. [29].[b]Ref. [11].[c]Ref. [10].[d]Ref. [17].

Table 2.6 Hydraulic Loading Rates for Various Types of Material

Type of Medium	Min HLR (m^3/m^2 day)	Max HLR (m^3/m^2 day)	References
Random flow medium Filterpak-CR50	100	200	Bovendeur et al [66], quoted in Ref. [11]
Randomly packed plastic pall rings	32—55	72—188	Roberts [67], quoted in Ref. [11]
Randomly packed Norton Actifil medium	29		Grady and Lim [68], quoted in Ref. [11]
Dow Surfpac	—	234—350	Kamstra et al [69], quoted in Ref. [11]

trickling filter should be about 1.2—3 m to strike a balance between energy demand and land area.

In a lab-scale experiment, ammonia removal efficiency was 28—68% or 0.11—1.29 g/m^2 day for the influent TAN concentration of 0.5—3.5 mg/L and flow rate of 3—10.5 L/min for nylon pot scrubber media [38]. This performance was rather poor compared to the results obtained by Greiner and Timmons [17] of 0.94—3.92 g/m^2 day for influent TAN concentrations between 0.81 and 4.63 mg/L.

2.2.2.5 Conclusion

Typical criteria for selecting a good biofilter are: (1) small footprint, (2) inert materials of construction, (3) low capital cost, (4) good mechanical strength, (5) low energy consumption, (6) low maintenance requirements, (7) portability, (8) reliability, (9) monitorability, (10) controllability, (11) turndown ratio, (12) safety, (13) utility, and (14) scalability [51]. Inevitably, one single filter type cannot satisfy all criteria. Each of them has its own advantages and disadvantages (Table 2.7).

Table 2.7 Comparison of Common Aerobic Biofilters Used in Aquaculture Wastewater Treatment

No.	Filter	Oxygen Transfer Mechanism	Biofilm Management	Specific Surface Area	Advantages	Disadvantages
1	Fluidized sand beds	Flow transport	Continual abrasion	Very high	• Reasonable efficiency • Cost-effectiveness for construction • Low maintenance requirements • As the typical shape of FBSF is a tall column, it consumes less land area than any other method. • It is the most efficient reactor in terms of active surface area per unit of reactor volume as active surface for biofilm growth is expanded as a result of upflow water velocity.	• Changes in bed expansion volume over time are troublesome for adjusting water flow velocity to prevent the washout of biomass. The flow variation should be less than 30% to ensure proper bed expansion [56]. • Difficult to control the uniform distribution of water flow. • FBSF itself does not integrate any oxygen supply equipment; rather, it requires a high concentration of DO in the wastewater influent (up to 90% of saturated DO) [56]. Therefore, it needs to be located immediately after an oxygenation and CO_2 stripping tower. • Operational cost is higher than RBC and trickling filters. • Difficult after restart
2	Moving bed biofilm reactors	Direct aeration	Continual abrasion	Moderate	• Very low head loss • No sludge recycle demand • Insignificant rate of wear and tear for biofilm carriers (even after 15 years of operation as proven in Ref. [44]). • Capable of dealing with a wide variety of loadings.	• Energy demand for keeping media suspended

3	Rotating biological contactors	Cascading	Sloughing	Low	• Self-oxygenation • Small hydraulic head • Low operating cost • Could be integrated in the raceway • Effective (more than 60% removal efficiency) in a wide range of loadings • Passive CO_2 degassing • Easy to observe biofilm development and problems	• Low specific surface area • High capital cost • High mechanical failure. Air-lift and water-jet RBC may overcome this weakness
4	Trickling filters	Cascading	Sloughing	Low	• Self-aeration • Robust and stable operation • Simple design, construction, operation, and maintenance • Able to treat a wide range of nutrient concentrations • Acts as an air stripper (CO_2, H_2S, and other gases)	• Low specific surface area • Requires some pumping head • Not volume-effective • Clogging of media if not properly designed

DO, dissolved oxygen; *FBSF*, fluidized-bed sand filter; *RBC*, rotating biological contactor.

Modified from J.N. Hochheimer, F. Wheaton, Biological filters: trickling and RBC design, in: Proceeding 2nd International Conference Recirculating Aquaculture, 1998; R.F. Malone, T.J. Pfeiffer, Rating fixed film nitrifying biofilters used in recirculating aquaculture systems. Aquacultural Engineering, 34 (3) (2006) 389–402M. Smith, A Review of Biofiltration Packings, L S Enterprises, USA, 2013a.

2.2.3 Nonconventional Treatment

Whereas conventional aerobic treatment methods normally demand high energy and technical skills, nonconventional methods are often less sophisticated but land-extensive. A frequently applied nonconventional treatment method is constructed wetlands, either free-water surface (FWS) or subsurface flow (SSF). These wetlands mimic ecological processes between macrophytes, solids, detritus, microorganisms, and aquatic fauna [33].

The design of a wetland was based on the fundamental equation developed by Kadlec and Wallace [27]:

$$\frac{dC}{dx} = -\frac{k}{q_w}(C - C^*),$$

in which C is the concentration of the pollutant (mg/L), x is the fraction of the area through the wetland along the flow path, k is the areal rate constant (m/day), q_w is the HLR of the wetland (m/day), and C^* is the background concentration (mg/L).

The equation was then modified by Tilley et al. [57] for aquaculture ponds:

$$A_w = -\ln\left[\frac{C_o - C^*}{C_i - C^*}\right] \times \frac{q_p A_p}{k_z},$$

in which A_w is the wetland treatment area (m^2), C_o is the targeted concentration of the pollutant (mg/L), C_i is the initial concentration of the pollutant (mg/L), C^* is the background concentration (mg/L), q_p is the HLR from the aquaculture pond (m/day), A_p is the aquaculture pond area (m^2), and k_z is the areal rate constant for constituent z (m/day), which is calculated by the following equation [27]:

$$k_z = \ln\left[\frac{C_o - C^*}{C_i - C^*}\right] \times \frac{q_w}{x}.$$

For recirculating aquaculture tanks, Lin et al. [32] proposed the following equation to calculate the surface area of wetland with the assumption that background concentration C^* was negligible:

$$\frac{A_w}{A_t} = \frac{r \times h_t \times (\ln C_i - \ln C_o)}{k \times \varepsilon \times h_w}$$

in which A_w is the surface area of the wetland (m^2); A_t is the surface area of the culture tank (m^2); r is the recirculating ratio = daily flow of recirculating water)/(total water in the culture tank (day^{-1}); h_t is the water depth of the culture tank (m); k is the first-order removal rate constant (day^{-1}); C_i is the initial concentration of the pollutant (mg/L); C_o is the targeted concentration of the pollutant (mg/L); ε is the porosity of wetland (assumedly, 0.85 for FWS and 0.45 for SSF); and h_w is the water depth of the wetland (m).

Treatment performance is strictly reliant on HRT. Common designed HRT values for wetlands ranged from 1 to 12.8 days (most often 2–5 days). Tilley et al. [57] claimed that wetlands were still effective at removing total phosphorus and inorganic suspended solids from shrimp pond effluents at an HRT of less than 1 day. Longer HRT generally

resulted in better treatment performance and a larger land requirement. Nonetheless, HRT exceeding 15–20 days may increase salinity and anaerobic conditions [57]. Therefore, there is always a trade-off between HRT, treatment efficiency, and land area required in the wetland design process.

Applications of constructed wetlands are rather versatile from freshwater to saline environments. The salinity of water is a critical factor for selection of plant types, along with adaptability to local climatic conditions, easy and rapid growth, and high removal performance [33]. Nevertheless, the selection of plants does not depend only on technical knowledge. Natural selection processes under real conditions will finally determine suitable plant types. For example, among 10 saline plant species selected for wetlands of 3–8 ppt salinity (*Avicennia germinans*, *Borrichia frutescens*, *Chara* spp., *Hydrochloa caroliniensis*, *Juncus effusus*, *Nymphaea odorata*, *Pithophora* spp., *Ruppia maritima*, *Sesbania drummondii*, and *Typha latifolia*), after only 1 year of operation, *T. latifolia* became predominant, whereas the other types gradually vanished [57]. To ensure ecological diversity as well as sustainability in constructed wetlands, more studies need to be conducted to simulate distribution and conditions for various types of plants under natural conditions.

Various mechanisms such as plant uptake, photooxidation, and assimilation occurring in wetlands are advantageous for removing pollutants. As a result, they are not only ecologically beneficial and low cost, but also effective at removing pollutants. Suspended solids (SS), BOD_5, TAN, NO_2^-, and NO_3^- were successfully removed from catfish, shrimp, and milkfish pond effluents in the range of HLR 0.018–1.95 m/day by 55–71%, 24–54%, 57–66%, 83–94%, and 68%, respectively [26]. These results were comparable to the wetlands used for treating a 6.9-ha catfish pond in Alabama, USA (76–87%, 37–67%, 1–81%, 43–98% for SS, BOD_5, TAN, and NO_2^-, respectively) [46]. Furthermore, a subsurface wetland system was used to treat a trout raceway at an HLR of 10.6 m/day (35%, 37%, and 87% removal for SS, BOD_5 and TAN) [50]. However, the magnitudes of phosphorus removal were significantly different in each case, between 59% and 84% for [46] and 0–5.4% [26,32,50]. The differences might be a result of different HLRs, HRTs, and plant types. Table 2.8 presents results of studies on constructed wetlands.

Not only are wetlands able to remove pollutants, but surprisingly, they also have a buffer capacity in controlling salinity. For instance, Tilley et al. [57] observed a constant value of total dissolved solids (TDS) in the wetland effluent (about 3.1 ppt) at all times after 2 years of operation.

However, the biggest problem with wetlands is that they are very land-extensive. They can consume 70–270% of pond area owing to low HLR and long HRT [32]. For example, to treat 13,600 m^3 of wastewater from an 8.1-ha shrimp pond in the Loma Alta Shrimp Aquaculture Facility, a 7.7-ha wetland was required. This area was equivalent to 95% of the total pond area [57]. This is economically unviable unless cultivated plants such as *Distichlis spicata* are used for livestock feeding purposes [34].

Another point to consider is the depletion of DO at the effluent of the wetland. It has created some problems in the reuse of water for aquaculture systems [32,57]. Clogging of aggregate materials was also observed after 1.5-years of prolonged use [50].

Table 2.8 Studies on Performance of Constructed Wetlands in Aquaculture Wastewater Treatment

Type of Wetland	Type of Wastewater Treated	Type of Plants	Scale	Capacity	HRT (h)	Depth (m)	Performance	References
FWS	Mesohaline shrimp pond effluent	Cattail (*Typha latifolia*)	Full scale	13,600 m^3/day, HLR = 0.177 m/day	24	0.15–0.45	TAN $\leq$ 1.8 mg/L (65%) NO_3^- $\leq$ 0.42 mg/L (76%) BOD_5 $\leq$ 9 mg/L (31%)	[57]
FWS	Shrimp (*Litopenaeus vannamei*) recirculating tank	Cattail (*T. latifolia*) and reed (*Phragmites australis*)	Full scale	HLR = 1.57 –1.95 m/day	42.7 $\pm$ 5.7 m^3/day (3 months) 53.9 $\pm$ 18.4 m^3/day (5 months)	0.40	SS = 55–66% BOD = 37–54% TAN = 64–66% NO_2^- = 83–94%	[32]
SSF	Trout (rainbow trout, *Oncorhynchus mykiss*; brown trout, *Salmo trutta*; and brook trout, *Salvelinus fontinalis*)	35% *Phragmites communis* 35% *Phalaris arundinacea* 30% swamp and land plants	Full scale	HRT = 10.4 –28.9 m/day	0.014 day	1.00	SS = 35% BOD = 37% TAN = 87%	[50]
SSF	Rainbow trout (*O. mykiss*)	*Distichlis spicata*	Pilot	HLR = 4.8 (L/m^2/day)	-	0.15	TN = 58–88% NH_3 = 58–97% TP = 85–95%	[34]

BOD, biological oxygen demand; BOD_5, biochemical oxygen demand in 5 days; *FWS*, free-water surface; *HLR*, hydraulic loading rate; *HRT*, hydraulic retention time; *SS*, suspended solids; *SSF*, subsurface flow; *TAN*, total ammonium nitrogen; *TP*, total phosphorus; *TN*, total nitrogen.

2.2.4 Advanced Technology

2.2.4.1 Membrane Bioreactors

Membrane bioreactors (MBRs) have been extensively studied and developed since the 1970s for treating municipal and industrial wastewaters. By incorporating activated sludge and membrane filtration, MBRs can retain microorganism communities inside a reactor (mixed liquor volatile suspended solids in the range of 15,000−30,000 mg/L) and thus, allow longer sludge retention time (SRT of 6.2 to more than 100 days), while permitting a more versatile HRT control [47]. With membrane pore sizes of 0.01−10 μm, MBRs can hold not only microorganisms but also a wide range of pollutants such as SS, particulate-bound nitrogen, and phosphorus. In general, MBRs can produce a high-quality effluent suitable for reclamation. There are two main types of MBRs being applied in agriculture—conventional activated sludge MBR (AS-MBR) and hybrid biofilm MBR (BF-MBR). BF-MBRs include a submerged membrane filtration unit integrated inside an MBBR. In cases in which an aerobic MBR is used together with an anoxic MBR for nitrogen removal, the DO concentration should be kept at around 2 mg/L [47].

Sharrer et al. [47] studied a pilot-scale AS-MBR for treating 22 m^3 of concentrated backwash flow from a 35-mtonne rainbow trout (*Oncorhynchus mykiss*) production under various saline conditions (salinity of 0, 8, 16, and 32 ppt). With the SRT of 64 ± 8 days and F/M ratio kept at 0.05−0.15 per day, TSS and total volatile solids removal efficiencies remained high (more than 99%) with outlet concentrations of 0.3−2.5 and 0.1−0.4 mg/L, total heterotroph bacteria of 2−5.6 $\log_{10}$ removal (2−121 cfu/mL), total coliform bacteria of 3.2−7.0 $\log_{10}$ removal (0−80 cfu/mL), $cBOD_5$ achieved 99.8% ($cBOD_5 = 0.6$−1.3 mg/L) at all salinity levels [47]. These results were also confirmed by Pulefou et al. [41] and Sharrer et al. [48]. Conversely, TAN removal was somewhat affected by salt concentrations. Different studies revealed conflicting results about the effects of salinity on nitrification processes. Sharrer et al. [47] confirmed that nitrification was not affected at salinity levels of up to 8 ppt. Beyond that, higher salinity levels (i.e., 16 ppt) required an acclimation period of at least 110 days before the nitrification achieved a steady state. Whereas Hamoda and Al-Attar [20] and Dahl et al. [7] claimed that salt concentrations up to 30 g/L would not affect the biological processes, Sánchez et al. [45] offered a different explanation. They found that the nitrification rate declined proportionally when salinity increased. This was explained as being due to the impacts of salinity on the *Nitrosomonas* spp. population.

The most apparent advantage of MBRs is their compact areal footprint (under 10% of the pond, in contrast with 70−150% for wetlands) [47]. The MBR's effluent can be reused in fish culture tanks to preserve heat, alkalinity, salts, and water under a proper control manner. In a comparative study, Holan et al. [24] observed the capacity of the BF-MBR to halt bacteria blooms in the recirculating aquaculture system (RAS) system. By filtering all the recirculating water in RAS tanks twice a day, the BF-MBR can also reduce the mortality of cod larvae (*Gadus morhua*) by 3.5% and improve their growth rate by 13%

[24]. This was explained by the reductions in colloidal particles and harmful bacteria by 44% and 80%, respectively, in comparison with conventional biofilters.

In contrast, its main concern is the high probability of membrane fouling. Membrane foulants can be particulate/colloidal, organic, bio-originated, or inorganic [19], and dissolved and colloidal particles play an important role. Indeed, feed types can significantly affect the transmembrane pressure by the generation of submicrometer particles that were believed to be the major component contributing to membrane fouling. Holan et al. [25] studied the influence of three types of feed for cod larvae, including (1) rotifers and algae paste, (2) live *Artemia* nauplii, and (3) Gemma Micro Diamond 300 dry feed, on particle size distributions and membrane fouling, at a pilot scale. They found that the first two types of feed produced more colloids at smaller sizes [i.e., <1 μm (+4.5−7.7%) and <50 nm (+6.0−15.1%)], in comparison with live *Artemia*. Another disadvantage of the MBR is its high energy consumption. In addition, MBRs must have a regular maintenance schedule to ensure proper function (Table 2.9).

2.2.4.2 *Innovative Membrane Biofilm Reactors for Denitrification*

Although nitrate is much less toxic than ammonia and nitrite to the development of fish, it can be a contributor to eutrophication of water bodies. Some public aquariums require the nitrate level to be under 20 mg/L [49]. Nitrate concentrations in aquaculture wastewater, especially in marine environments, were too low to be effectively removed by physical or electro(chemical) techniques [49]. Traditional biological denitrification methods regularly required more space or equipment for an additional loop of treatment because optimal conditions for microorganisms between nitrification and denitrification differed greatly. Therefore, several teams have tried innovative denitrification technologies that could be incorporated with available aerobic biofilters. Among these new innovations are ion-exchange MBRs (IE-MBRs) [37], hydrogen-permeable hollow fiber membranes [36], and ethanol-packed membrane biofilm reactors [49].

Table 2.9 Maintenance Frequency of Membrane Bioreactors

Frequency	Items	Parameters
Daily	Functions of crucial components (pumps, mixers, blower units)	Transmembrane pressure; dissolved oxygen
Weekly	Retain appropriate concentration of microorganisms by withdrawing solids	Mixed liquor suspended solids
Biannually	Chemical membrane cleaning to reduce biofouling and $CaCO_3$ precipitation	Transmembrane pressure

Data adapted from M.J. Sharrer, et al., Membrane biological reactor treatment of a saline backwash flow from a recirculating aquaculture system. Aquacultural Engineering 36 (2) (2007) 159−176..

2.2.4.2.1 ION-EXCHANGE MEMBRANE BIOREACTORS

Matos et al. [37] researched the use of anion-exchange membranes to transform nitrate ion into nitrogen gas in a biocompartment under three flux rates: 0.9, 1.5, and 7.7 L/m^2 day, equivalent to HRTs of 28, 17, and 3 h, respectively (Fig. 2.12). The hydraulic regime of the biocompartment was separated from the water stream to control its HRT. In this study, the chosen biocompartment's HRT was 5 days to reduce its effluent wastage. Movement of nitrate ion through the membrane was regulated by adjusting the counterion concentration (i.e., Cl^-). The experiment mimicked the real conditions of oceanic aquarium water in Lisbon.

The removal of nitrate in the IE-MBR was calculated from the model developed by Velizarov et al. [62]:

$$J_{NO_3^-} = \frac{\frac{C_{NO_3^-,1}}{C_{Cl^-,1}} - \frac{C_{NO_3^-,2}}{C_{Cl^-,2}}}{\left[\frac{L}{P_{NO_3^-} \times Q}\right] + \left[\frac{\delta_1}{D_{NO_3^-,w} \times C_{Cl^-,1}}\right] + \left[\frac{\delta_2}{D_{NO_3^-,w} \times C_{Cl^-,2}}\right]},$$

in which $C_{NO_3^-,1}$ is the concentration of nitrate in the water (mg/L), $C_{NO_3^-,2}$ is the concentration of nitrate in the biocompartment (mg/L), $C_{Cl^-,1}$ is the concentration of chloride in the water (mg/L), $C_{Cl^-,2}$ is the concentration of chloride in the biocompartment (mg/L), δ_1, δ_2 is the thickness of the boundary layers adjacent to membrane surfaces at the water and biocompartment sides ($\delta_1 = \delta_2 = 38$ μm), L is the membrane thickness (μm), $P_{NO_3^-}$ is the membrane permeability to NO_3^-, Q is the ion-exchange capacity of the membrane (mol/L), and $D_{NO_3^-,w}$ is the diffusion coefficient of the counterion NO_3^- in water (1.9×10^{-5} cm^2/s).

This system can reduce the initial nitrate concentration from 251 mg/L to less than 27 mg/L at fluxes of 0.9 and 1.5 L/m^2 h, but not at the flux of 7.7 L/m^2 h. The compositions of feed water and the biocompartment's water are alike, except for the absence of nitrate in the biocompartment's water. Consequently, the treated water can be reused

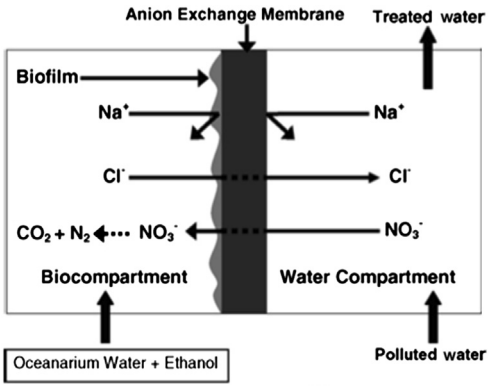


FIGURE 2.12 Schematic diagram of nitrate transport and bioreduction in the ion-exchange membrane bioreactor. *Adapted from C.T. Matos, et al., Nitrate removal in a closed marine system through the ion exchange membrane bioreactor. Journal of Hazardous Materials 166 (1) (2009) 428–434.*

directly in the oceanic aquarium after the nitrate ions are removed. However, this method is very difficult to manage and requires a high level of expertise to operate.

2.2.4.2.1 HYDROGEN-PERMEABLE HOLLOW FIBER MEMBRANE AND ETHANOL-PACKED MEMBRANE BIOFILM REACTORS

Hydrogen-permeable hollow fiber membrane and ethanol-packed membrane biofilm reactors generally have two main components—an existing aerobic biofilter and a series of submerged denitrifying biofilm-supporting media with an integrated substrate supply mechanism. Basically, denitrifying biofilm is attached on the surface of a hydrogen-permeable hollow fiber membrane, polyethylene or poly(ethylene-vinyl acetate) film. The substrate will be gradually released through these materials to the biofilm. In addition, DO cannot reach the inner layer of the biofilm, which ensures suitable conditions for denitrification, even under high DO concentration. The design parameters (i.e., membrane design, module design, membrane packing, and reactor configuration) and influence factors (i.e., biofilm management strategy and pH) of membrane biofilm reactors are well discussed in Ref. [36].

The denitrification rate of these reactors depends greatly on (1) initial concentration, (2) type of substrate, and (3) rate of substrate release. An example of an ethanol-packed membrane biofilm reactor treating wastewater from *Chaetodon miliaris*, *Scyllarides haanii*, and *Panulirus brunneiflagellum* production is illustrated in Table 2.10. In the lab-scale experiment, the denitrification degree was found to increase in the first order with the substrate supply rate. Under different initial nitrate concentrations, 10.8 g COD (in lab scale), 13.8 g COD (full scale, low initial nitrate concentration), and 5.3−7.5 g COD (full scale, high initial nitrate concentration) were required for each gram of nitrogen removed [49]. These figures were much higher than typical biological treatment methods that incorporate deoxygenation equipment. However, as mentioned earlier, the biggest advantage of this denitrification technology is its simple installment, versatile incorporation in existing aerobic tanks, and economic viability.

Table 2.10 Types of Film and Denitrification Rates

Type of Film	Initial Nitrate Concentration (mg/L)	Ethanol Permeate Rate at 25°C (g COD/m^2 day)	Denitrification Rate (g N/m^2 day)
Lab scale			
0.3-mm-thick PE	50	2.5	0.1 ± 0.1
0.1-mm-thick PE	50	7.6	0.8 ± 0.3
0.05-mm-thick PE	50	15.2	1.4 ± 0.3
0.1-mm-thick EVA	50	20.7	1.9 ± 0.3
Full scale			
0.07-mm-thick PE	4−9	-	0.68
	20−40	-	1.1

EVA, poly(ethylene-vinyl acetate); *PE*, polyethylene.

Data adapted from T. Shoji, et al., Demonstration of a novel ethanol-packed membrane biofilm reactor for denitrification at the Tokyo Sea Life Park. Aquacultural Engineering 63 (2014) 45−53.

2.3 Future Perspectives

The future trend in aquaculture aims to reduce waste generation while making the best use of resources (feed, water, land, and energy) to achieve sustainability. It aims to create aquaculture ecology where both resources and wastes are recycled within the system boundary. One strategy is to marry recirculating aquaculture systems and hydroponics in a symbiotic environment, which is termed "aquaponics." Aquaponics is not a substitute for traditional biofiltration methods; rather it is used as a supplement to make the best use of feed resources and as a sink for nitrate and phosphorus elimination. In this system, flora not only acts as a biofilter, but also provides a valuable source of food. Actually, this type of practice is not something new. It was employed by ancient Asian and Aztec civilizations dating back 3000 years ago. However, with the emergence of intensive aquaculture farming, its presence gradually vanished. Modern aquaponics is targeted more at large-scale, intensive systems. It can be termed as "integrated multitrophic aquaculture" or "integrated marine recirculating aquaculture system," depending on the targeted object [58].

The design of an aquaponic system greatly depends on local conditions (light intensity, air and water temperature, soil types, etc.), types of aquaculture production, feed types, and selection of cospecies and plants as well as their relative ratio. A good knowledge of the interaction between nutrient inputs, its transformation, and outputs will determine the correct sizing of the system [5]. An insufficient number of plants means low nutrient removal, whereas too many plants will result in suboptimal conditions for their development. The recommended ratios between fish feed rate and plant growing area vary greatly. For tilapia production, this ratio is between 50 and 100 g feed per square meter of growing area [1,42]; whereas for catfish production, it is 15−42 g/m^2 [12].

Because the greatest concern with aquaponic systems is the harvesting and use of plants, the commercial value of plants must be taken into account. Some combinations have been suggested, such as fish−phytoplankton−shellfish or fish−seaweed−macroalgivore, for more profitable coproducts.

One type of aquaponics incorporates algae in fish pond or tank systems to serve two purposes: (1) making use of excessive feed and discarded nutrients from fish for algae development and (2) harvesting algae for commercial purposes or clean energy production. This system goes by the name "green water," to differentiate itself from "clean water" systems [51]. Wang et al. [64] applied the macroalgae *Ulva pertusa* for cleaning water from a recirculating tank of sea cucumber juveniles and distributing treated water back to the tank. *Ulva pertusa* reduced 68% of TAN with the rate of 0.459 g N/m^2 day and 26% of orthophosphate from wastewater, whereas the survival rate of sea cucumber juveniles remained high (87%). This treatment also helped to preserve the loss of heat and necessary minerals for juvenile growth, especially in the winter. In another attempt, Nasir et al. [40] cocultivated *Chlorella* sp. and the sewage fungus *Aspergillus niger* to treat catfish *Clarias gariepinus* wastewater at lab scale. Whereas *Chlorella* sp. proved to be effective at removing 97% of nutrients (TAN and phosphorus) at the optimal inoculation dosage of

30% (v/v) after 10 days, *A. niger* (with the optimal inoculation dosage of 30 mg/L) was used to control the population of *Chlorella* sp. and gave the water a clearer appearance.

Van Den Hende et al. [61] studied microalgal bacterial flocs (MaB-flocs) in sequencing batch reactors at three scales: lab scale, pilot scale, and full scale. Despite good results being obtained from lab reactors, the upscaling to full scale produced various unforeseeable challenges. Although settling characteristics of MaB-flocs was significantly improved, their removal efficiency was reduced by a magnitude of 1−3 and their volumetric biomass fell by 10−13 times. Subsequently, the effluents cannot meet the discharge standard for nitrite and nitrate. In addition, pH values of the system increased dramatically (pH over 9.5), which then required surging of flue gas to control pH. This could be explained by the change in microorganism structure inside the reactors. Van Den Hende et al. [61] observed the reduction of filamentous cyanobacteria (*Phormidium* sp.) and increase of filamentous microalgae (*Ulothrix* sp. and *Klebsormidium* sp.) from small-scale to large-scale reactors.

Lettuce (*Latuca sativa*) and nasturtium (*Tropaeolum majus*) were tested to remove TAN, NO_3^-, and PO_4^{3-} from wastewater generated in rainbow trout (*O. mykiss*) raceways [5]. The TAN removal efficiency of nasturtium (about 80%) was much faster and higher than that of lettuce (48%). However, with the low influent concentration (0.56 mg inorganic nitrogen per liter), the results were at best ambiguous. Other studies with higher initial concentrations revealed higher nutrient removal rates [30,42].

Several salt-tolerant flora types with high-use values were also suggested by Turcios and Papenbrock [58], such as *Salicornia* spp. and mangrove. The coculture of *Salicornia* spp. in aquaponics was more advantageous than in land-based planting owing to the following factors: easy control, mass production, and hygiene [58]. It has: (1) a great sorption capacity of nitrate and phosphate; (2) prominent nutritious value for human consumption with a substantial content of minerals, vitamins, proteins, and polyunsaturated fatty acids; and (3) high potential for biogas production. Likewise, the ecological value of mangrove forests has been well recognized. They are outstanding in terms of tidal attenuation, tsunami prevention, and coastal protection. UNEP-WCMC [60] valued each hectare of mangrove as equivalent to US$200,000−900,000. Above all, mangrove forests provide an excellent habitat for various types of fish, shrimps, and other species. The application of fish culturing under the mangrove canopy originally began in southeast Asian countries (i.e., Philippines, Vietnam, and Thailand).

Owing to the diverse nature of aquaponics, operation of such systems requires a wide range of specific knowledge and expertise. More research needs to be conducted to establish a substantial literature on this treatment method.

2.4 Conclusion

The rapid development of aquaculture that is diverse in character brings more challenges for water resource management. Five major culturing methods have been

discussed with regard to their discharge behavior. Nevertheless, discharges from all aquaculture systems can be characterized by high concentration of nutrients. This raises concerns not only about their toxicity, but also about eutrophication and ecological degradation.

Various aerobic treatment technologies have been presented in this chapter to tackle the above-mentioned problem. The treatment technologies have been assessed based on five aspects—basic principles, design parameters, treatment efficiency, advantages, and disadvantages. While traditional biofiltration methods are still applied widely, more alternatives are being considered. First, nonconventional methods such as wetlands offer a low-cost and ecologically friendly opportunity. Second, because of the high demand for reusing water and compositions within the aquaculture industry, MBRs have been developed in this field. Third and last, aquaponic practices have emerged to create a more sustainable aquaculture industry. Their ultimate goals are to: (1) reduce the discharge of pollutants into the environment, (2) utilize waste and uneaten feed as a source of nutrients for other species (i.e., microalgae, macroalgae, plants, etc.), (3) bring economic values from plants, and (4) ensure ecological balance.

Nevertheless, aerobic treatment is mostly effective in removing TAN or, in other words, nitrification. They are not very efficient in removing nitrate and phosphorus, with the exception of some wetlands (Section 2.2.3), innovative membrane biofilm reactors for denitrification (Section 2.2.4.2), and aquaponic systems. Therefore, they need to be incorporated into a denitrification process.

List of Abbreviations

AS-MBR	Activated sludge membrane bioreactor
BF-MBR	Biofilm membrane bioreactor
BOD	Biological oxygen demand
BOD_5	Biochemical oxygen demand in 5 days
COD	Chemical oxygen demand
D_{10}	Effective size that allows less than 10% of particles to go through
DO	Dissolved oxygen
EVA	Poly(ethylene-vinyl acetate)
FAO	Food and Agriculture Organization of the United Nations
FBSF	Fluidized-bed sand filter
FWS	Free-water surface
HLR	Hydraulic loading rate
HRT	Hydraulic retention time
IE-MBR	Ion-exchange membrane bioreactor
IMRAS	Integrated marine recirculating aquaculture system
IMTA	Integrated multitrophic aquaculture
MaB-flocs	Microalgal bacterial flocs
MBBR	Moving bed biofilm reactor
MBR	Membrane bioreactor
MLSS	Mixed liquor suspended solids

Continued

MLVSS	Mixed liquor volatile suspended solids
PE	Polyethylene
pH	Potential of hydrogen
RAS	Recirculating aquaculture system
RBC	Rotating biological contactor
SRT	Sludge retention time
SSF	Subsurface flow
TAN	Total ammonium nitrogen
TMP	Transmembrane pressure
TP	Total phosphorus
TSS	Total suspended solids
TVS	Total volatile solids
US EPA	US Environmental Protection Agency
WB	World Bank

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3

Aerobic Treatment of Petroleum Industry Effluents

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3.1 Introduction

The oil and petrochemical industry is among the human activities known to generate annually large amounts of contaminants, which are released into the atmosphere, soil, and natural water bodies. Total petroleum hydrocarbons (specifically aliphatic hydrocarbons), jointly with other aliphatic, aromatic, and polyaromatic compounds, are among the most common pollutants found in effluents, resulting from various stages involved in the petrochemical process and other incidences related to oil industry operations such as accidental spills, leakages from storage, wash-down operations, or vessel cleanouts [1].

The activities of the oil industry have several impacts on the environment due to the large amounts of oily wastes that are generated. Oily sludge is a semisolid material composed of a mixture of clay, silica, and iron oxides contaminated with oil, produced water, and the chemicals used in the production of oil. Therefore, the treatment and management of oily sludge is essential to promote the sustainable management of the profitable extraction of natural resources, with a preference for the reduction, reutilization, and recycling of these oily wastes. Biological, physical, and chemical processes can be used serially and/or in parallel to decrease environmental contamination by petroleum hydrocarbons and other contaminants to levels permitted by environmental legislation [2]. The management of oily wastes involves the characterization of oily sludge (physical, physicochemical, and chemical properties) and the technologies to treat it. The classification of oily sludge consists of identifying each process that generates it, determining the physicochemical characteristics of the waste, and comparing it to those of wastes and substances with known impacts on health and the environment as described by international guidelines and directives [3,4].

The International Petroleum Industry Environmental Conservation Association has identified the primary sources of the petroleum industry's oily wastes as (1) oily sludge

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with detergents or washing liquids and carrying rust and reaction residues, (2) oily sludge with nonmineral skimmed foam and grease, (3) light oily sediments containing mineral material, and (4) heavy oily sediments containing mineral material [4–6].

Many physical, physicochemical, and biological processes are available to treat oily sludge, such as landfilling, incineration, coprocessing in clinkering furnaces, microwave liquefaction, centrifugation, destructive distillation, low-temperature conversion, thermal plasma, incorporation into ceramic materials, development of nonpermeable materials, bio-piles, and bioreactors [2]. Also, a wide variety of conventional and nonconventional technologies have been reported for the treatment of petrochemical-related wastewater effluents [7,8], including phase-change technologies and chemical, physical, physicochemical, and some nonconventional emerging technologies. Just to mention some examples, several authors have reported the application of a coagulation–flocculation (CF) process to wastewater effluents contaminated with surfactants and/or oil derivatives using a wide variety of synthetic coagulants and flocculants such as ferric chloride [8–10], ionic polyelectrolytes [11], aluminum sulfate [12], alum polychloride, and lime [8]. In more recent work, natural coagulants have been identified as providing interesting possibilities with potential application in high-load chemical industry effluents, performing comparably to alum sulfate, ferric chloride, and other commercial coagulants [8]. Natural coagulants such as *Moringa oleifera* seeds [13], guar gum and its derivatives [14], tara gum, locust bean gum, and *Prosopis laevigata* seed gum [15] have been tested in the past for the treatment of wastewater through the CF process for the improvement of water quality.

Among all of these, biological treatments (suspended and/or immobilized cell processes) have been identified as suitable, cost-effective treatment methods for generated wastewater [8]. Aerobic submerged biofilters, for example, have been reported for the treatment of wastewater contaminated with high concentrations of various pollutants such as pesticides [16,17], phenols, and chlorophenols [9], as well as domestic wastewater and the wastewaters generated by the surfactant-enhanced soil washing process using a single bacteria culture [16,17]. However, several other authors have found that symbiotic associations among different bacteria genera yield higher treatment efficiencies compared with single bacterial systems [19,20].

These conventional processes when used alone, however, often present many different operational problems such as inhibition due to relatively high concentrations of toxic chemicals, long retention times and/or start-up periods, and the generation of large amounts of sludge [21]. One possibility to avoid many of the previously described inconveniences is the use of coupled treatment systems capable of enhancing microbial growth, improving nutrient removal efficiency, allowing continuous process operation, and increasing the system's capability for handling toxic pollutants [9,22,23].

Because the challenge of treating petrochemical wastewater effluents will continue for the next few decades, searching for novel approaches and applications for biological degradative systems is a hot spot of great interest among the scientific community. This chapter aims to review the latest trends and ultimate developments related to aerobic treatment as well as identifying challenges and proposing alternatives for biological treatment of effluents from the petroleum industry.

3.2 Petroleum Biodegradation

Petroleum and its products are formed mainly by hydrocarbons. Every chemical in the mixture has a different boiling point, carbon number, chemical family, and structural isomers. Hydrocarbons present in petroleum and its products can be classified as an aliphatic, aromatic, asphaltene (phenols, fatty acids, ketones, esters, or porphyrins), or resin (pyridines, quinolines, carbazoles, sulfoxides, and amides) [24]. Some relevant compounds found in crude oils are the relatively water-soluble light aromatics benzene, toluene, ethylbenzene, and various xylenes. If water is contaminated with gasoline, it is also possible to find gasoline additives such as methyl *tert*-butyl ether in the mixture.

A wide variety of bacteria, molds, yeast, cyanobacteria, and green algae have been confirmed to be able to oxidize hydrocarbons aerobically (Table 3.1). There is evidence that hydrocarbons can also be degraded by bacteria in the absence of oxygen. The majority of the organisms that have been found to be able to biodegrade hydrocarbons aerobically are bacteria. Most of the hydrocarbon-biodegrading bacteria belong to the groups Firmicutes and α-, β-, γ-, and δ-Proteobacteria [25]. Yakimov et al. reported the identification of obligate hydrocarbon bacteria (hydrocarbonoclastic bacteria) found in marine samples that belong mainly to the genera *Alcanivorax*, *Marinobacter*, *Thallassolituus*, *Cycloclasticus*, and *Oleispira* [26].

Petroleum hydrocarbons can be biodegraded when the right microorganisms are present and the required nutrients (N and P) and oxygen are supplied. Biodegradation of petroleum hydrocarbons can be a complex process depending on the nature and concentration of the hydrocarbons present, as well as on the physical and chemical characteristics of the effluent. The rate of aerobic biodegradation depends on the complexity of the molecule. A rather complex molecular structure makes it challenging for microorganisms to find a location for an initial enzymatic attack. It has been observed that large hydrocarbons with much branching or containing many aromatic rings are more difficult to degrade. Thus, in general, contaminants with a relatively high molecular weight will be harder to biodegrade in comparison to compounds with a lower molecular weight. Another aspect that hinders biodegradation is low water solubility. In accordance, the preference of bacteria for the degradation of hydrocarbons present in petroleum and its products can be ranked as follows: linear alkanes, branched alkanes, small aromatics, and cyclic alkanes [4,5]. Some compounds, such as the high-molecular-weight polycyclic aromatic hydrocarbons (PAHs), are very difficult to degrade.

Because all known hydrocarbon-oxidizing enzymes are cell bound, microorganisms must incorporate these insoluble substrates by direct contact or by emulsifying the hydrocarbons in water. Therefore, many hydrocarbon-degrading organisms produce extracellular emulsifiers or biosurfactants, as further explained in the next section of this chapter. By reducing the size of the oil droplets, the rates of both direct contact and solubility increase significantly. Nevertheless, microbial hydrocarbon solubility must be greater than or equal to the biodegradation rates. It was observed by Tellez et al. [30] that microorganisms metabolize water-insoluble substrates using three strategies: (1) dissolving the substrate in

Table 3.1 Microorganisms Identified as Capable of Degrading Petroleum Hydrocarbons

Name	Domain	Environment	References
Cycloclasticus sp.TUi26, *Marinobacter hydrocarbonoclasticus* ATCC 49840, *Geobacillus thermodenitrificans* NG80-2	Bacteria (hydrocarbonoclastic)	Freshwater	[35]
Alcanivorax sp. DG881, *Marinobacter algicola* DG893, *M. hydrocarbonoclasticus* VT8, *Oceanicaulis alexandrii* HTCC2633, *Oleispira antarctica* RB-8, *Alcanivorax borkumensis* SK2, *Cycloclasticus pugetii* PS-1	Bacteria (hydrocarbonoclastic)	Marine	[35]
Rhodococcus opacus B4 PD630	Bacteria (hydrocarbonoclastic)	Soil	[35]
Micrococcus sp., *Corynebacterium* sp., *Flavobacterium* sp., *Bacillus* sp., *Pseudomonas* sp.	Bacteria	Crude oil-contaminated soil samples	[36]
Pseudomonas putida, Pseudomonas maltophilia, Burkholderia cepacia, Acinetobacter sp., *Nocardiodes* sp. strain CF8, *Rhodococcus* mutant, *Alcanivorax* sp.	Bacteria	Pure cultures	[37]
Phormidium and *Oscillatoria* cyanobacteria	Bacteria	Cyanobacterial mats inhabiting a heavily polluted site in a coastal stream	[38]
Marinobacter spp.	Bacteria	Petroleum-contaminated brine soil	[39]
Alcanivorax, *Marinobacter, Pseudomonas, Acinetobacter,* Rhodobacteraceae	Bacteria	Deepwater Horizon oil spill in the Gulf of Mexico	[40]
Acremonium, Aspergillus, Aureobasidium, Beauveria bassiana, *Cunninghamella* spp., *Fusarium, Gliocladium, Graphium, Hansenula, Mortieriella* spp., *Paecilomyces, Phoma* spp., *Scolecobasidium obovatum, Sphaeropsidales*, *Tolypocladium inflatum, Trichoderma, Verticillium* spp.	Fungi	Marine/soil	[28]
Prototheca zopfii	Algae	Creek sediment	[41]
Candida, Cladosporium resinae, Rhodosporidium, Rhodotorula, Saccharomyces, Sporobolomyces, Trichosporon	Yeast	Not mentioned	[28]

water, (2) metabolizing the compound after excreting a bio-surfactant, or (3) using a mechanism involving physical contact with the insoluble phase of the substrate [30].

Fuel hydrocarbons are transformed through primary metabolism, whereby the compounds are used as growth substrates and their degradation yields energy for the

organisms [31]. Once the microorganisms have established contact with the hydrocarbons, the first step in hydrocarbon degradation by bacteria is the introduction of oxygen into the molecule using an enzyme called oxygenase. The hydrocarbon is then oxidized by two electrons for every hydroxyl (−OH) group added. Thermodynamically, oxygenation reactions are costly, because the available electrons that might better be used to reduce NAD^+ to NADH are used to reduce O_2 to H_2O, a reaction that does not capture the electrons or produce energy for the cells. Because of this energy demand the yield for the organism per electron equivalent of hydrocarbon oxidized is lower than for other organic compounds [6,8]. Monod kinetic parameters for the aerobic degradation of hydrocarbons have been reported in the literature. The cell yield coefficient ranges from 0.01 to 1.56 g cells/g carbon [9−11,32,33]. The gain for the microorganism is that the products of the oxygenation reactions are more available and easier to attack [29].

Bioremediation can take place naturally (intrinsic) or with the aid of engineered treatment systems. Intrinsic bioremediation refers to degradation on-site by indigenous bacteria. This process can take hundreds of years even if the right conditions for the bioremediation of the contaminants exist. The common engineered systems used to treat hydrocarbon-contaminated water can be classified as in situ or ex situ systems. The systems classified as in situ are treatments that transform the contaminants at the source (i.e., groundwater, river, produced water), whereas ex situ systems are treatments requiring the transport of the polluted fluid to a treatment facility [29]. In situ treatments are further classified as amended and/or bio-augmented systems. Amendment, also referred to as bio-stimulation, is defined here as the measures taken to provide the right conditions for bacteria to ensure the biotransformation of pollutants and/or to increase the biotransformation rate. These measures usually involve the addition of nutrients (inorganic, organic), micronutrients, and/or electron donors/acceptors to increase the biotransformation rate of indigenous bacteria present at the site [42−46]. On the other hand, bio-augmentation refers to the addition of specially selected microorganisms capable of degrading the compound(s) of interest [29,48].

Bio-stimulating indigenous microorganisms have been observed to increase the biotransformation rate of hydrocarbons by approaching a C/N/P ratio of 100:10:1 [18−21]. The addition of oxygen can be achieved via air sparging or the construction of oxygen-releasing barriers [4,22,23,47]. Bio-augmentation has been observed to have less of a positive effect on increasing biodegradation rates compared to bio-stimulation [4,14,24]. Ex situ systems consist of conventional water treatment systems such as activated sludge [49], trickling filters [26,27], fluidized bed reactors [50], aerated lagoons [51], and constructed wetlands [52].

The factors that have been identified that influence the efficiency and even the success of aerobic biodegradation of petroleum hydrocarbons are divided into two categories: abiotic and biotic [28]. The source water characteristics (in addition to those previously mentioned) that have been reported to have a negative effect on bioremediation are low temperature (optimal temperature for biodegradation is in the range

20−30°C), extreme acidic or basic pH values, salinity (greater than 3%), pressure (greater than 10 atm), and the presence of recalcitrant compounds [5,6,13,46].

As for the biotic factors, these include having microorganisms capable of degrading the petroleum hydrocarbons in the water source under the prevailing environmental conditions, competition for nutrients or oxygen with indigenous bacteria, and predation due to the presence of protozoa [53]. For bio-augmentation, it has been observed that acclimatization of the inoculum to the contaminants and environmental conditions in the water to be treated has a beneficial effect [28]. This is achieved in batch reactors by exposing the bacteria at standard conditions to lower concentrations of the contaminants and relatively higher concentrations of nutrients, until degradation of the contaminants of interests is achieved. Later, the concentration of nutrients is reduced to the values found in the water source. Other parameters such as temperature, pH, and/or salinity are also changed gradually [16,24]. Given the complex mixture of compounds present in oil or its derivatives, mixed cultures have been used as inocula, because no single species has been observed to be able to degrade most of the compounds present in petroleum products or intermediate bioremediation products [5,12].

3.3 Petroleum Bioavailability

The ideal petroleum biodegradation technology needs to be implemented in nonsterile natural environment(s) where the degradative microorganisms encounter a variety of biotic and abiotic factors [54−56]. Many of these factors have adverse effects on the efficiency of the degradation process by different mechanisms, giving advantages to the bioavailability of the pollutants. With this idea, an optimum petroleum biodegradation can be apparently performed in a controlled environment, as happens with ex situ bioremediation, in which various procedures are used to enhance the process.

For ex situ bioremediation, different conditions have been tested at different scales. For example, phenol degradation has been tested at the laboratory scale using a polymer bead bioreactor [50:50 poly(butylene terephthalate):polyether glycol] [57]. The use of a two-phase bioreactor system for degradation of PAHs or spent mushroom compost as bulking agent for low-molecular-weight PAHs has also been reported [58,59]. The circulating electrolyte method has been reported for pentadecane biodegradation [54], or use of bark chips as a soil-bulking agent for petroleum oil bioremediation at field-scale study [60]. Poultry litter, coir pith, and rhamnolipid bio-surfactant have also been used for gasoline biodegradation [36]. The disadvantage is the need to transport the soil or water to where the conditions are controlled, meaning extra cost. The most common restoration method is in situ bioremediation [61], because it does not need sample transportation, being, therefore, less expensive and avoiding the release of volatile chemicals in unpolluted places.

In general, the main goal for in situ petroleum bioremediation is to accelerate the pollutant degradation rate either by introduction of efficient petroleum-degrading strains (bio-augmentation) [62,63] or by stimulating the natural attenuation process by adding carbon, nitrogen, phosphorus, potassium, or electron acceptors/donors as

acetate, nitrate, sulfate, glutamate, and gaseous formulations to the contaminated environment (bio-stimulation) [64,65], or both.

As stated above, it is important to consider various environmental factors during petroleum bioremediation, such as the physicochemical nature of the contaminated environment; nutrient availability; presence, chemical nature, and concentration of cocontaminants; extent of contamination; community structure of the indigenous microbial communities; pH; inorganic carbon; humidity; and aeration, among others [66–68]. All these factors have been found to significantly influence the bioremediation process in a case-by-case manner; knowing the effect of every factor can be very useful for planning the parameters for petroleum bioremediation and estimating the success of the process.

During the petroleum bioremediation process, keeping the microbial population constant by using the proper conditions for the strains or finding strains with flexible adaptation capabilities are key issues. Among the adaptation capabilities of the biodegrading strains are the ability to build biofilms and to produce bio-surfactants. Biofilms are formed by exopolymers, a complex mix of lipopolysaccharides, glycolipids, lipids, proteins or peptides, and nucleic acids [69,70]. Environmental conditions usually dictate the key properties of biofilms such as porosity, density, water content, charge, sorption and ion-exchange properties, hydrophobicity, and mechanical stability [69]. The physiological characterization of the biofilm's structure has demonstrated that over 95% of the biofilm matrix is constituted by water or some non-aqueous-phase liquid [71–73]. The biofilm, therefore, forms a static yet mobile microbial environment, which also helps to move the microbes over long distances (on the microscopic scale) from the point of biofilm formation, resulting in improved pollutant bioavailability [74]. Also, the biofilm helps the bacteria generate aggregates using the produced exopolymers, which helps to produce different enzymes to degrade the pollutants and, at the same time, protect the cell community from the toxic concentrations of the various petroleum contents, permitting a gradient formation through the biofilm and the use of hydrocarbons as nutrients.

The hydrophobic nature of petroleum compounds makes them scarcely or not at all available for enzyme cleavage or being assimilated by bacterial cells. To improve the bioavailability of petroleum, some strains have the ability to synthesize bio-surfactants, helping to generate emulsification and better hydrophobic component assimilation, as reported for *Pseudomonas aeruginosa* for hexadecane degradation [75], or improvement of petroleum biodegradation by various bio-surfactant-producing *Bacillus* strains [76].

3.4 Synthetic Surfactants and Bio-surfactants

3.4.1 Bio-surfactants

As stated earlier, bio-augmentation and bio-stimulation are the best ways to improve petroleum bioremediation; however, for full success in the restoration process it is important to consider three major factors that are involved: (1) the bioavailability of the

toxic substrate to be degraded, (2) the diffusivity and availability of the toxic substrate for the degrading microorganisms, and (3) the capability of the degrading microorganisms to survive in the polluted environment [77]. One way to improve bioavailability is by enhancing the solubility of petroleum and its derivatives; the best way to dissolve hydrophobic pollutants is by the use of surfactants. Surfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between the fluid phases of different polarities, such as oil and water. These properties render surfactants capable of reducing surface and interfacial tension and lead to the formation of microdrops (micelles) in which hydrocarbons can be solubilized in water. Micelles can surround and sequester hydrocarbons and other hydrophobic compounds, increasing their solubility in water. However, a prerequisite for surfactant-enhanced biodegradation is that the degradative microorganisms not be adversely affected by the surfactant. Because of their amphipathic nature, many surfactants can also dissolve bacterial cell membranes and act as effective disinfectants [78], although particular bacterial strains can employ a variety of mechanisms (e.g., cell impermeability, efflux pumps, and surfactant degradation) to counter these negative effects [79]. Thus, the net effect of surfactants on biodegradation is variable [77,80]. To eliminate that problem Plante et al. isolated different bacterial strains resistant to surfactants in environments polluted with hydrocarbons; some of the genera isolated were *Vibrio*, *Spongiobacter*, and some related to *Bacillus* [81]. As well, other genera, including *Pseudomonas*, *Flavobacterium*, and *Streptomyces* [82a], have been reported to be resistant to surfactants; their resistance is due to the modification of the components of their outer membrane, such as outer membrane proteins, reducing the lipopolysaccharide and making the external envelope more hydrophobic, as reported for *P. aeruginosa* [82b].

One concern for the use of synthetic surfactants is related to their biodegradability. A common approach to avoid this problem is to use bio-surfactants able to drive hydrocarbon dissolution, improving the pollutant's bioavailability and enhancing its biodegradation. A list of bio-surfactants and the strains that produce them is given in Table 3.2. Certainly, it is important to consider the potential toxic effects of these bio-surfactants because many of them have an antibiotic effect and can eliminate the autochthonous microbial community or, even worse, the biodegrading strains used for bio-augmentation.

3.4.2 Synthetic Surfactants

Synthetic surfactants have gained a lot of attention in recent years as they have opened the way for the dissolution of various organic compounds in micelles. Owing to their unique structure and properties, synthetic surfactants show higher efficiency and effectiveness in applications such as lowering surface tension and lowering the critical micelle concentration, among others. Surfactant molecules accumulate at the liquid/liquid interfaces and lower the surface as well as interfacial tensions. Surfactant micelles

Table 3.2 List of Bio-surfactants and Producing Microorganism Strains

Bio-surfactant	Microorganism(s)	References
Cellobiose lipids	*Ustilago maydis*	[83]
Serrawettin	*Serratia marcescens*	[84,85]
Polyol lipids	*Rhodotorula glutinis, Rhodotorula graminis*	[86]
Trehalose lipids	*Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia erythropolis, Corynebacterium* sp., *Mycobacterium* sp.	[87]
Ornithine lipids	*Pseudomonas* sp., *Thiobacillus thiooxidans, Agrobacterium* sp.	[88]
Viscosin	*Pseudomonas fluorescens, Leuconostoc mesenteriodes*	[89–91]
Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas chlororaphis, Serratia rubidaea, Bacillus subtilis*	[92–94]
Carbohydrate–lipid	*P. fluorescens, Debaryomyces polmorphus*	[95]
Protein PA	*P. aeruginosa*	[96]
Diglycosyl diglycerides	*Lactobacillus fermentum*	[97]
Whole cell	*Cyanobacteria*	[98]
Fatty acids/neutral lipids	*Clavibacter michiganensis* subsp. *insidiosus*	[99]
Sophorolipids	*Candida bombicola, Candida antartica, Torulopsis petrophilum, Candida botistae, Candida apicola, Candida riodocensis, Candida stellata, Candida bogoriensis*	[100]
Liposan	*Candida. tropicalis*	[101]
Monnosylerythritol lipids	*C. antartica, Kurtzmanomyces* sp., *Pseudozyma siamensis*	[102]
Surfactin/iturin	*B. subtilis, Bacillus amyloliquefaciens*	[103–106]
Subtilisin	*B. subtilis*	
Amino acid lipids	*Bacillus* sp.	[107]
Lichenysin	*Bacillus licheniformis, B. subtilis*	[108]
Peptide lipids	*B. licheniformis*	[109]
Phospholipids	*Acinetobacter* sp.	[110]
Vesicles and fimbriae	*Acinetobacter calcoaceticus, Pseudomonas marginilis, Pseudomonas maltophilia*	[88]
Emulsan	*A. calcoaceticus*	[111]
Alasan	*Acinetobacter radioresistens*	[112]
Massetolide A	*Pseudomonas* SS101	[113]
Putisolvin	*Pseudomonas putida* PCL1445	[114]
Amphisin	*Pseudomonas* sp. DSS73	[115]
Syringomycin	*Pseudomonas syringae* pv. B728a, B301D, B3A	[116,117]

solubilize the water-insoluble compounds such as oil and its derivatives. Depending upon their polarity, oil molecules are solubilized at the palisade layer or into the core of the micelles. A large body of literature exists on the equilibrium partitioning of various environmentally significant oils and derivative solutes, including PAHs, in the micellar solutions [118–121].

Solubility of oil and oil derivatives may be strongly influenced by the micellar characteristics of the surfactants employed. The efficiency of surfactant-enhanced remediation at the oil-contaminated sites or effluents depends upon the capability of surfactant micelles for the solubility of different oil components. The surfactant

solution may enhance solubility but, at the same time, accumulate in the soil or water. Tatsumi et al. [122] have reported the use of cationic surfactants with two quaternary ammonium groups in the polar head and two hydrolyzable amide groups in the lipophilic portion. The hydrophilic nature of the spacer contributes to a higher water solubility that helps in hydrolysis and degradation processes [123–126]. Cationic surfactants have been successfully employed to interact with the negatively charged surfaces or biomolecules such as prokaryotic/eukaryotic cells, antigenic proteins, and lipids. Quaternary ammonium compounds (QACs) are usually used to obtain cationic liposomes. Generally, these have the ability to interact with various microbial species and cultured biological cells [127,128]. Unfortunately, the major problem related to the use of QACs, and synthetic surfactants in general, is their high degree of toxicity. Table 3.3 gives some relatively new examples of synthetic surfactants reported as being capable of enhancing oil and/or oil derivative solubility to increase their bioavailability.

Table 3.3 Various Synthetic Surfactants Used to Enhance Solubility and Bioavailability

Surfactant	Oil Derivative Tested	Main Results	References
Dodecyltrimethylammonium chloride, Brij 58, SDBS, and mixtures	Anthracene, pyrene	Solubility enhancement was observed for both solutes. To determine the toxicity and to quantify its interaction with erythrocytes, hemolytic assessment was performed. Biodegradability test showed that surfactant mixture is less toxic and readily biodegradable.	[126]
Alkyl polyglucoside–sorbitan ester	Oil spills	Laboratory flood experiment with alkyl polyglucoside–sorbitan ester formulation recovered 94% of initial oil in place	[125]
SDS, SDBS, Texapon N40, Sulfopon 30, and Surfacpol A14104, Tween 80, Tween 20, Span 80, Brij 35, Emulgin W600, Polafix CAPB, Polafix LO	TPHs, BTEX, polycyclic aromatic hydrocarbons	Oil derivatives solubility was significantly enhanced as well as their biodegradation.	[129]
Surfacpol 203, Surfacpol G, Surfacpol A1404, Emulgin 600, Tween 20, Brij 35, Tween 80, SDS, Polafix, SDBS, Texapon 40, Polafix CAPB	TPHs	Use of synthetic surfactants enhanced TPH biodegradation as high as 37%	[18]

BTEX, benzene, toluene, ethylbenzene and different xylenes; *SDBS*, sodium dodecylbenzene sulfonate; *SDS*, sodium dodecyl sulfate; *TPHs,* total petroleum hydrocarbons.

3.5 Genetically Modified Organisms for Petroleum Biodegradation

The proper combination of key genes from different biodegrading microorganisms in one recipient can produce genetically modified microorganisms (GMOs), which, in theory, can achieve enhanced bioremediation capabilities [77,130]. Several new genome sequences of biodegrading bacteria and the genetic regulation of gene products involved in biochemical pathways have been used for the successful development of GMOs with improved degradation skills [131–133].

GMOs are usually produced with one or more of the following objectives [134]:

1. To improve or alter the degradation capacity of a given strain
2. To provide the bacteria with resistance to environmental conditions
3. To monitor the presence of added bacteria
4. To measure the bioavailability of contaminants

The first reported GMO capable of degrading multiple compounds present in hydrocarbons was engineered in 1976 by D.A. Friello, J.R. Mylroie, and A.M. Chakrabarty. The organism was a multiplasmid-containing *Pseudomonas* strain capable of oxidizing aliphatic, aromatic, terpenic, and polyaromatic hydrocarbons [28]. The first patent for living organisms was registered in 1981 by Ananda M. Chakrabarty for *Pseudomonas aeruginosa* NRRL B-5472 with camphor, octane, salicylate, and naphthalene degradative pathways in the form of plasmids and *Pseudomonas putida* NRRL B-5473 with camphor, salicylate, and naphthalene degradative pathways and drug resistance factor RP-1, all in the form of plasmids [135].

The only reported field release of a GMO for bioremediation purposes was conducted by the University of Tennessee in collaboration with Oak Ridge National Laboratory in 1996 on a site contaminated with naphthalene, anthracene, and phenanthrene. The GMO used was *Pseudomonas fluorescens* HK44. The *P. fluorescens* HK44 strain had a naphthalene plasmid pUTK21 and a bioluminescence-producing lux gene fused within a promoter for the naphthalene catabolic genes. Thus, exposure of strain HK44 to naphthalene or the intermediate metabolite salicylate resulted on a bioluminescent response. Strain HK44 served as a reporter for naphthalene bioavailability and biodegradation [31,34,136].

Fu-Min et al. [137] reported a list of GMOs that were designed to possess higher degradative capacity than wild-type strains and bioremediation process monitoring, strain monitoring, stress response, end-point analysis, and toxicity assessment. Examples of organisms that were designed for biodegradation of hydrocarbons are presented in Table 3.4.

Another strategy to create highly effective hydrocarbon-degrading microorganisms is by inducing random mutations into hydrocarbon-degrading enzymes using UV radiation. Malkawai et al. [138] observed that, after UV irradiation, three bacterial isolates

Table 3.4 GMOs Designed for Bioremediation of Hydrocarbons (Cited from Ref. [137])

Microorganism	Modification
Pseudomonas putida KT2442	Pathway
Escherichia coli JM109 (pSHF1003)	Substrate specificity
Pseudomonas pseudoalcaligenes KF707-D2	Substrate specificity
E. coli FM5/pKY287	Regulation
P. putida TVA8	Process monitoring
Pseudomonas fluorescens 10586s/pUCD607	Stress response
P. fluorescens 10586s/pUCD607	Toxicity assessment

showed an increase in their hydrocarbon degradation rate compared to the wild types. The three microorganisms modified with this technique were *P. aeruginosa* TDJ2, *P. putida* TDJ6, and *Pseudomonas mallei* TDJ4. Borah and Yadav [139] found that after UV irradiation, *Bacillus* spp. strains were able to degrade and withstand higher concentrations of hydrocarbons.

While significant advances have been made in the development of GMOs, their application for in situ bioremediation has been restricted because of the unpredicted risks associated with their release into the environment. One of the most commonly anticipated concerns is the "horizontal gene transfer" to the natural microbial population [140]. As well, the GMOs released may multiply within the new environment and affect negatively the autochthonous equilibrium of the microbial community diversity. Consequently, microbiologists have recommended the incorporation of genetic routes into GMOs that may restrict their proliferation to only the environment(s) in which they can achieve the targeted degradation [132,140].

One method used to restrict the proliferation of GMOs is "bacterial control systems" that work on the basis of distinctive bacterial phenotypes in the environment [141] by selectively killing the GMOs when they attempt to grow outside the set environment [142]. GMOs designed with a "killing-based" bacterial control system are often referred to as "S-GMOs" and use a "killer gene" to induce cell death in response to environmental changes. Some of these genes have been used for the construction of efficient S-GMOs used for environmental purposes, eg, in situ bioremediation. However, these kinds of new GMOs need to be tested, because, in the same way that horizontal transfer from GMOs to the natural microbial community can happen, these also can be inverted, and the bacterial control system can be blocked, permitting continued growth of the GMOs, affecting the equilibrium of the natural environment.

GMOs can be targeted to ex situ bioremediation systems, however, where the pollutant can be contained, and the presence or absence of the GMOs in the system may be reviewed at the end of the process and, if necessary, disinfection processes may be used to eliminate residual GMOs.

3.6 Sequentially Coupled Physical and Chemical Petroleum Treatment

Biological petroleum wastewater treatment may be complicated mainly because of a low biodegradability rate. The 5-day biological oxygen demand/chemical oxygen demand (BOD_5/COD) ratio in petroleum, petrochemical, and/or oilfield effluents is usually quite low, making aerobic treatment difficult and not very cost-effective [143]. Biological degradation implies the elimination of a pollutant through the metabolic activity of microorganisms, usually bacteria and fungi, present in the water or soil [144]. As a result, conventional biological processes alone may not be able to generate satisfactory results, particularly for application to industrial wastewaters with high toxicity and contaminant loads in which many of the substances included are refractory to biological treatment [145]. For these cases, a very attractive alternative for the removal of biologically persistent contaminants or to increase wastewater biodegradability is the combination of biological treatment with the use of advanced treatment technologies. The use of sequentially coupled physical–chemical processes jointly with biological treatment has led to cost-effective treatment options demonstrating the convenience of these methodologies for application for petroleum wastewater.

Relatively few methodologies have been tested to enhance oil industry wastewater biodegradability and applied sequentially coupled to aerobic biodegradation. Table 3.5 depicts the relationship of reports on the use of sequentially coupled technologies for the improvement of treatment efficiency in the oil industry.

As shown in Table 3.5, Malakahmad et al. [146] evaluated the performance of a lab-scale sequential batch reactor (SBR) to treat a synthetic petrochemical wastewater containing Hg and Cd (9.04 ± 0.02 and 15.52 ± 0.02 mg/L, respectively). The removal

Table 3.5 Reports on Applications of Sequentially Coupled Technologies for Oil Industry Wastewater Treatment

Sequentially Coupled Process	Petroleum Wastewater Treated	Removal Efficiency Achieved	References
SBR	Petrochemical wastewater containing Hg^{2+} and Cd^{2+}	76–90% Hg; 96–98% Cd removal	[146]
Membrane SBR	Petroleum refinery wastewater	97% hydrocarbons removal; high dependence on hydraulic retention time	[147]
Natural coagulants–aerobic biofilter	Petrochemical wastewater	>90% TPHs removal; >60% COD removal; toxicity removal	[148]
Coagulation–flocculation biofilter	Surfactant-enhanced soil washing wastewater	73% hydrocarbons removal	[18]
Electrocoagulation–fixed-film aerobic bioreactor	Oil refinery wastewater	98% TPHs removal; 95% COD removal	[149]

SBR, sequential batch reactor; *TPHs*, total petroleum hydrocarbons.

ranges obtained were 76−90% for Hg and 96−98% for Cd. They found that COD removal was affected by the concentration of mercury and cadmium in the wastewater and the same trend was found for the concentration of microorganisms, probably resulting from the toxicity of the metals [146]. Finally, these authors analyzed the consortium of microorganisms in the SBR and found *Rhodospirillum*-like and sulfate-reducing-like bacteria as well as *Gomphonema*-like algae among the main species.

In the same way, Shariati et al. [147] tested the effect of hydraulic retention time (HRT) on the performance and fouling characteristics of a membrane-sequencing SBR. They found that hydrocarbon removal efficiencies higher than 97% were possible using a coupled process with HRT values of 8, 16, and 24 h. Accordingly, they also found that the rate of membrane fouling increased with the decrease in HRT values [147]. The effects of the use of natural and synthetic coagulant agents in the CF process coupled with aerobic biodegradation of hydrocarbons in a petrochemical effluent and in the wastewater effluent from the surfactant-enhanced soil washing process have been reported by our research group [18,148]. We found that the use of natural coagulants may generate the same removal efficiencies in the CF process but with the main advantage of increasing the biodegradability of the produced effluent as well as the sludge generated. The use of, for example, locust bean gum (a natural coagulant) generated a total petroleum hydrocarbon (TPH) removal efficiency on the same order as some synthetic coagulants (i.e., Polafix CAPB, Texapon 40, sodium dodecylbenzene sulfonate). Nevertheless, when the biodegradation of the CF effluent was carried out, the highest efficiency for the hydrocarbon removal was achieved for the wastewater containing the natural coagulant [19].

In the case of petrochemical effluents, a high efficiency in total suspended solids and turbidity removal was achieved using *Opuntia* spp. powder as natural coagulant (77% removal), quite close to the results for the use of alum (88% removal) but without the drastic change in the water pH produced by the latter. *Opuntia* spp. was found to be the most efficient for COD removal from the water through the CF process (36% COD removal). When the effluent from the CF using the natural coagulant was used for the biodegradation process using the aerobic biofilter, 95% of TPHs remaining in the treated wastewater were removed after 30 days of biological treatment and a similar trend was found for COD biodegradation. The petrochemical effluent was characterized as nontoxic in a test performed using *Lactuca sativa* L. var. *capitata* after the sequentially coupled process compared with the classification as very toxic of the influent to the treatment system.

Finally, in a 2015 work, Perez et al. tested the sequential coupling of electrocoagulation with a fixed-film biological process for the treatment of oil refinery wastewater. These authors report the assessment of electrocoagulation using different current intensities, electrode number, and electrolyte concentration for the removal of contaminants in the water and increase of the effluent biodegradability. They found high TPH removal (>80%) and a significant increase in the biodegradability (from BOD/COD = 0.15 to 0.5) after the electrocoagulation process [149]. The further application of the biodegradation process removed 98% of the TPHs and up to 95% of the COD in the oil refinery wastewater.

3.7 Future Perspectives

Bioremediation of petroleum or its derivatives using in situ or ex situ technologies can be improved by bio-augmentation or bio-stimulation or both, depending on the pollutant type and concentration. The use of different and new GMOs constructed to improve the rate of biodegradation is ideal, but considering the risk of these strains to negatively modify the natural environment, their current application may not be the best option. According to the ideas presented in this chapter, a new way to improve the elimination of petroleum-polluted effluent or sites is to help the natural system to dissolve, predigest, or reduce the toxicity of the complex mix. One way to achieve these goals is the use of purified enzymes. Nevertheless, many of them are expensive to isolate and to purify owing to their relatively low stability. One advantage is that they can be engineered to be resistant to different pH values or temperatures or to have an increased degradability half-time. These new traits can help to reduce the cost of treatment, isolation, and purification by enhancing the overall process. Another possibility is to mix the enzymes with bio-surfactants or synthetic surfactants with low toxicity to increase the availability of the petroleum to interact with the active sites of the enzymes. In a similar fashion, it might be possible to take advantage of the biofilm matrix produced by various petroleum biodegradative strains and use it as a scaffold to attach bio-surfactants and a mix of enzymes, producing a perfect microenvironment for a petroleum biodegradation cell-free system [150–153].

Another new approach is the introduction of novel nanomaterials with catalytic properties able to use the dissolved oxygen and favoring the production of reactive oxygen species that initiate the oxidation of all hydrocarbons and producing new metabolizing by-products or to use the nanomaterials as a support for enzymes and bio-surfactants to enhance the bio-elimination of petroleum and its derivatives where, owing to their concentration, there is no support for any living things.

3.8 Conclusions

Microbial remediation of hydrocarbons has been observed to be feasible under diverse conditions, aerobic/anaerobic, mesophilic/psychrophilic, nonhalophytic/halotolerant, etc., as well as in different engineered systems: in situ or ex situ. Many microorganisms belonging to diverse taxa have been identified as able to use hydrocarbons as a carbon source or even as obligate hydrocarbon degraders. Owing to the complex mixture of contaminants that exist in hydrocarbons, it has been observed that consortia work better compared to pure cultures. The aerobic and anaerobic degradation pathways and the enzymes involved in the process have been studied and advances in this area continue to be made.

The amendment of microorganisms (bio-stimulation) to start or to enhance the biodegradation of hydrocarbons in a polluted site or from a water pumpout has also been observed to positively influence the bioremediation of hydrocarbons. Several

studies have contributed to elucidating the environmental factors that inhibit bioremediation as well as the nutritional requirements of hydrocarbon-degrading bacteria.

Even though genetically engineered microorganisms have been developed and proven to work in the field, their use in hydrocarbon bioremediation has been limited to laboratory applications and blocked in field applications because of current regulations and the rejection of the public. Thus, there is only one study in which a GMO was used to degrade hydrocarbons in a polluted site [53].

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4

Aerobic Treatment of Effluents From Pulp and Paper Industries

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4.1 Introduction

The pulp and paper industry is one of the most pollution-riddled industries that the world has known [1]. The production process includes digesting wood chips to form pulp, washing and bleaching the material to achieve whiteness, and generating steam as a means to dry the paper. Pulping is the primary stage of such a process and is considered the main source of pollutants of this industry. During pulping, wood chips are treated to remove lignin and improve fibers for making paper. Washing and bleaching are the last steps of the process and they employ a significant amount of energy and water [2]. Water consumption varies depending on the process and it can be as high as 60 m^3/ton of paper produced, regardless of the most modern and best available technologies used [1]. Market research published by Frost and Sullivan [3] showed that more than 85% of the water is consumed by the pulp and paper industry for processing, which generates large volumes of contaminated wastewater.

The characteristics of the wastewater generated depend on the type of process, type of wood, process technology, management practices, internal recirculation, and amount of water used. Usually, wastewater generated from production processes contains high concentrations of chemicals such as sodium hydroxide, sodium carbonate, sodium sulfide, bisulfites, elemental chlorine or chlorine dioxide, calcium oxide, hydrochloric acid, chlorinated lignosulfonic acids, chlorinated resin acids, chlorinated phenols, and chlorinated hydrocarbons [2,4]. Therefore, the effluent of a kraft mill is extremely contaminated and has high organic content (20–110 kg chemical oxygen demand (COD)/air dried ton paper), dark brown coloration, adsorbable organic halide (AOX), and toxic contaminants [5]. Also, the alkaline extraction stage of bleach plant effluent is the major source of color and is mainly due to lignin and its various derivatives.

Lignin-containing effluent is discharged from the pulping, bleaching, and chemical recovery section of a production line. Lignin is a heterogeneous, three-dimensional polymer, composed of oxyphenylpropane units [6]. The high chlorine content of bleached plant reacts with lignin and its derivatives and forms highly toxic and recalcitrant

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compounds that are responsible for high biological oxygen demand (BOD) and COD. Furthermore, highly toxic and recalcitrant compounds such as dibenzo-*p*-dioxin and dibenzofuran are also formed in the effluent of pulp and paper mills. It has been estimated that the total effluent discharged annually from these mills is about 40,000 million m^3, assuming an average of 200 m^3 of effluent per ton of pulp and paper [7].

If untreated effluent from pulp and paper mills is discharged into surrounding water bodies, the water quality is severely impaired. The effluent is characterized by the dark brown color visible over a very large distance and contains high BOD and COD and lignin compounds and their derivatives. Such a dark brown color is due to the formation of lignin degradation products during the processing of lignocellulosics from paper and pulp production [8]. These untreated effluents are toxic to aquatic organisms and demonstrate a strong mutagenic effect [7]. Furthermore some compounds in the effluents are resistant to biodegradation and can bioaccumulate in the aquatic food chain [9].

Owing to the large amount of water used in these processes, recycling is mandatory and recirculation of the process waters is now commonly practiced. However, water reuse may lead to organic and inorganic concentration and this can affect the production process. Unfortunately, environmental concerns are not restricted to the high consumption of water and energy and production of a highly contaminated effluent. Generation of solid wastes including sludge from wastewater treatment plants and air emissions are other problems that need immediate attention and effective disposal; effective treatment approaches are therefore essential. Main solid wastes include, but are not limited to, lime mud, lime slaker grits, green liquor dregs, boiler and furnace ash, scrubber sludges, and wood processing residuals. Inappropriate disposal of these solid wastes may cause significant environmental problems because of high organic content, partitioning of chlorinated organics, pathogens, ash, and trace amounts of heavy metal content [4]. The major air emissions that originate in mills are nitrogen oxides (NO_x), sulfur oxides (SO_x), sulfur gases, and volatile organic compounds (VOCs) including ketones, alcohols, and solvents such as carbon disulfide methanol, acetone, and chloroform [10].

It is of the highest importance to take into account the amount, type, and characteristics of these wastes to provide the best treatment and waste management technology. Various physicochemical and biological as well as combined treatment methods and approaches are used extensively for pulp and paper mills. Physicochemical methods such as sedimentation/flotation, coagulation and precipitation, adsorption (color removal in particular), membrane filtration (to remove AOX, COD, and color), chemical oxidation, and hybrid methods are widely discussed in the scientific literature [2]. Biological treatments including fungal or bacterial, aerobic and anaerobic, as well as a combination of both technologies, are preferred for treatment of pulp and paper mills because wastewater composition consists of high organic compounds and economical aspects. In numerous countries, tertiary treatment is applied in combination with secondary treatment options to obtain the discharge limits of regulations [1]. Furthermore, hybrid systems that combine biological and physicochemical methods are gaining the attention of pulp and paper mill operators worldwide [2].

Solid waste disposal strategies for the pulp and paper industry vary depending on the geographical location and regulations. After sorting and handling and dewatering, thermal applications such as combustion and aerobic as well as anaerobic digestion and deposition in landfills are generally accepted approaches. However, solid wastes from the pulp and paper industry must be monitored after landfilling because of the toxicity of compounds present in the waste stream [4]. Moreover, gaseous contaminants are other environmental hazards generated by this industry. To minimize contamination and its adverse effects on the environment and people, various physicochemical methods and techniques such as adsorption, thermal and catalytic oxidation, and condensation are utilized [11].

In this chapter, waste generation, its characterization, and its management by the pulp and paper industry along with aerobic treatment of effluents using various conventional and novel approaches are discussed in detail.

4.2 Waste Generation

In addition to wastewater, various types of solid wastes and sludge are generated in the pulp and paper industry during the production processes [1,7,10]. Solid waste generated at pulp mills consists of [4]:

1. Rejects: The rejects consist of sand, bark, and wood residue from wood handling. They have a low moisture content, have significant heating value, can be easily dewatered, and are generally burned in the mill's boiler for energy recovery.
2. Green liquor sludge, dregs, and lime mud: These are inorganic sludges separated from the chemical recovery cycle. They are usually landfilled after dewatering and drying.
3. Wastewater treatment sludge: This originates in primary sludge and biological sludge generated in the second clarifier. These sludges can be blended together with added polymers to dewater to obtain 25–40% dry solid content.
4. Chemical flocculation sludge: This derives from water treatment and is taken to the landfill because of the high content of inorganic materials and water.

On the other hand, solid waste generated at paper mills consists of [4]:

1. Rejects: Rejects from recovered paper are various impurities and lumps of fibers, staples and metals from ring binders, sand, glass, and plastics. Rejects have a very low moisture content and significant heating value and can be easily dewatered just like rejects from pulp mills. They are incinerated for energy recovery or placed in landfills.
2. Deinking sludge: This contains short fibers, coatings, fillers and ink particles that contain heavy metals, extractive substances, and deinking additives. These can be reused in the cement and ceramics industries or incinerated despite having a rather low heating value.

3. Primary sludge: This type of sludge is generated in the clarification of process water by "kidney treatment," e.g., dissolved air flotation. The sludge usually consists of fines and fillers and can be easily dewatered. Generally, mills landfill the primary sludge or mix with deinking or secondary sludge.
4. Secondary or biological sludge: This is generated in the clarifier of the biological units of wastewater treatment and is either recycled into a new product (cardboard industry) or thickened, dewatered, and then incinerated for energy recovery or disposed of in a landfill. Secondary sludge volumes are smaller in comparison to primary sludge, because most of the heavy, fibrous, and inorganic solids are removed in the primary clarifier. Unfortunately, secondary sludges are very difficult to handle because of a high microbial protein content and they need to be mixed with primary sludge to achieve adequate dewatering.

It is important to point out that treatment of wastewater generated at pulp and paper mills is the main source of wastewater treatment sludge and deinking sludge. For instance, Balwaik and Raut [12] reported that more than 300 kg of sludge is produced for each ton of recycled paper. However, the amount of waste generated varies greatly in different regions owing to varying recycling rates.

The amount and composition of waste generated from a mill using secondary fiber are different from those of a mill that uses virgin material. Significantly larger amounts of rejects are produced when processing recycled fiber, because of the unrecyclable filler proportion in the raw material. This may pose a significant problem in mills producing recycled paper from office waste, using highly filled grades as the raw material. Also, deinking mill sludge generally has a higher ash content, whereas the kraft pulp mill sludge has high sulfur content [13]. According Elliott and Mahmood [14], about 40−50 kg of sludge (dry) is generated in the production of 1 ton of paper at a paper mill in North America. Of that approximately 70% is primary sludge and 30% is secondary sludge. The primary sludge can be dewatered relatively easily in comparison to the secondary sludge because the secondary sludge consists mostly of excess biomass produced during the biological process [15].

Disposal of solid wet wastes is expensive, whereas thermal destruction is both an expensive and a very energy-demanding process, which is generally deemed economically unfeasible. Currently, the scientific and industrial communities are concentrating efforts on minimizing the production of sludge by using advanced biological treatment processes coupled with various hybrid approaches. For instance, the dominating method used to decrease sludge formation is the use of a prolonged sludge retention time in the activated-sludge processes, which will be discussed in detail later in this chapter. Briefly, during the activated sludge process, most of the organic material is metabolized by aerobic microorganisms and converted into carbon dioxide and water. The method has been shown to successfully reduce sludge production, but unfortunately at the expense of an increased use of electrical energy for aeration.

4.3 Waste Characterization

The pulp and paper industry is the fifth largest energy user, and approximately 4% of total energy is used globally every year. Three different raw materials are used in the pulp and paper industry, nonwood fibers and soft and hard wood materials. Also, more than 100 million kg of toxic pollutants are released every year from this industry [16].

4.3.1 Process Description

The first step of the production is known as the pulping process. The main stages here are debarking, wood chipping, chip washing, chip digestion, pulp screening, thickening, and washing [17].

- Debarking converts the plant fiber into smaller pieces called chips and removes the bark. In this step raw materials such as hard wood, soft wood, and agricultural residues are used, typically resulting in the transfer of tannins, resin acids, etc., present in the bark to process waters.
- Pulping turns the chips into pulp. This process removes the majority of lignin and hemicellulose content from the raw material, resulting in a cellulose-rich "pulp." Pulping can be carried out by several different methods, such as mechanical, chemical, kraft, sulfite pulping, etc.
- Bleaching is employed on the brown pulp obtained after pulping to meet color requirements. Several bleaching agents, including chlorine, chlorine dioxide, hydrogen peroxide, oxygen, ozone, etc., may be used. This is the stage when lignin, phenols, and resin acids get chlorinated and then transformed into highly toxic xenobiotics.
- Washing removes the bleaching agents from the pulp. Generally caustic soda serves to extract color and bleaching agents from the pulp.

According to Sumathi and Hung [18], mechanical and chemical operation processes are used in most pulp and paper mills worldwide. Mechanical processes include but are not limited to mechanical pressure, disk refiners, heating, and light chemical processes to increase pulping yield. Chemical processes involve cooking of wood chips in pulping liquors at high temperature and under pressure. Also, mechanical and chemical processes may be combined for some specific operations. Despite the yield of mechanical processes being higher (90–95%) compared to chemical processes (40–50%), the overall quality of the pulp obtained from mechanical processes is poorer in addition to color and fibers [2]. Thus, based on these properties, chemical pulping in alkaline or acidic media is highly preferred. In alkaline media, generally referred as the kraft process, the wood chips are cooked in liquor including sodium hydroxide (NaOH) and sodium sulfide (NaS_2). A mixture of sulfurous acid (H_2SO_3) and bisulfide ions (HSO_3^-) is used in the acidic process and this is referred to as the "sulfide" process.

During the pulp processing, approximately 5–10% of the lignin that comes from the raw materials cannot be removed and is responsible for the product's dark color. The production of white paper (pulp bleaching) includes five or six treatment steps with elemental chlorine, alkali, optional hypochlorite, chlorine dioxide, and chlorine dioxide.

4.3.2 Wastewater

All the processes during the pulping phase are water and energy intensive. As previously mentioned, approximately 200 m^3 water is used per ton of produced pulp and most of this wastewater is highly contaminated, especially when generated from the chemical pulping process. Wood preparation, pulping, pulp washing, screening, washing, bleaching, paper machine, and coating operations are the most important pollution sources during the various process stages. Wastewater generated from the pulping stage mostly contains wood debris, soluble wood materials, and various chemicals from chemical pulping. However, the bleaching process produces wastewater of a very different quality. Despite having lower strength than pulping wastewater, these wastewaters contain a large variety of toxic compounds. The kraft process is used worldwide and approximately 60% of all pulp production includes both mechanical and chemical pulping [19].

Thus, wastewater generated from the pulping process contains large amounts of wood compounds such as lignin, carbohydrates, and extractives and the treatment of these wastewaters utilizing biological methods is extremely difficult if not economically unfeasible. The wastewater also contains toxic compounds such as resin acids, unsaturated fatty acids, diterpene alcohols, and chlorinated resin acids [2]. One of the most important steps in the bleaching process is the oxidation of chlorine, which results in the formation of toxic chlorinated organic compounds or AOX [18].

4.3.3 Gas Emissions

Air pollutants and gas emissions are additional concerns from the pulp and paper industry. The most important gas emission is water vapor. Furthermore, solid particulates, nitrogen oxides, VOCs, sulfur oxides, and total reduced sulfur compounds are also observed and they need to be taken care of.

4.4 Waste Management

As discussed previously, various types of waste are produced from different pulp and paper production stages and all these wastes pose significant environmental problems. In order to solve them: (1) waste minimization can be achieved using new and best available technologies and (2) end-of-pipe treatment technologies should be used before the discharge and/or disposal.

4.4.1 Waste Minimization

Currently, waste minimization is achieved through chemical recovery and/or recycling and through application of best available techniques (BATs). Chemical recovery and/or recycling (e.g., in the chemical pulping process) significantly reduces contaminants and presents additional economic benefits through the recovery of resources [20]. Chemical recovery is used because of economic viability in the kraft process. On the other hand, BATs reduce cost, liability, and the regulatory burdens of hazardous waste management. Subsequently, hazardous waste generation can be reduced by several waste management approaches, including:

1. Production, planning, and sequencing
2. Process adjustment and/or modification
3. Raw material replacement
4. Housekeeping waste segregation and separation
5. Recycling

Some examples of new BATs are presented below:

1. Organic solvent pulping: This process is economically feasible for small- and medium-scale plants for significant recovery and reuse of chemicals. During this process, organic solvents like ethanol and methanol are used. Unfortunately, this process is more energy intensive compared to other conventional approaches [18].
2. Acid pulping: Acetic acid is used under high pressure to treat raw wood chips. The disadvantage of this process is a significant loss of acid; however, there are already reports documenting significant recovery and reuse [18].
3. Bio-pulping: Microorganisms or microbial enzymes such as xylanases, pectinases, cellulases, hemicellulases, and ligninases and their combination are used in the pulping process to improve the properties of pulp [21]. Bio-pulping is preferred because of the reduction of chemicals and energy consumption, reduction in subsequent pollution, and increase in yield and strength of pulp.
4. Elemental chlorine-free and total chlorine-free bleaching: These are used to reduce the resulting chlorinated organic wastes [18].
5. Bio-bleaching: Fungal cells and/or their enzymes are used for pretreatment of pulp. A number of studies showed that application of fungi reduces the chemical dosage of bleaching and enhances the brightness of paper [8,9,22–27].
6. Extended delignification: Lignin removal before the bleaching step can be achieved using ozone and various catalysts [28–34]. These types of treatment positively affect other bleach effluent quality parameters such as COD, BOD, color, and AOX.

4.4.2 Solid Waste Handling

Wastewater treatment is a process in which waterborne contaminants are removed from the larger wastewater stream and concentrated into a smaller side stream. Usually, the

side stream is too large to be disposed of directly; therefore further concentration processes are necessary. These processes are called "solid waste handling" operations.

4.4.2.1 Stabilization/Digestion

Sludge stabilization is a treatment technique applied to biological sludge to reduce odor or toxicity. As a desirable side effect, such a treatment often reduces the amount of solids through volume and mass consolidation. Anaerobic and aerobic digestion, lime treatment, chlorine oxidation, heat treatment, and composting fall into this category.

Anaerobic digestion: The biochemical reactions that take place during various stages are as follows:

$$\text{Organics} + \text{acid-forming organisms} \rightarrow \text{volatile acids}$$

$$\text{Volatile acids} + \text{methane formers} \rightarrow \text{methane} + \text{carbon dioxide}$$

Sludge volume decreases owing to the conversion of biomass to methane and carbon dioxide. Subsequently, methane can be recovered for its heating value.

Aerobic digestion: Aerobic digestion is the aeration of sludge in an open tank. Oxidation of biodegradable matter, including cell mass, occurs in an air-rich environment. Similar to anaerobic digestion, there is a decrease in sludge solids observed and the sludge is well stabilized with no odors present. Capital costs are less than those of anaerobic digestion, but operating costs are higher and there is no by-product methane production for energy recovery.

Lime treatment: Stabilization by lime treatment does not result in the reduction of organic matter. Lime is added to maintain the pH of the sludge above 11.0 for 1−14 days. It is assumed that after approximately 2 weeks most bacteria are destroyed.

Composting: A natural digestion process, composting usually incorporates sludge material that consequently will be spread onto the soil. Sludge is combined with a bulking material, such as other solid wastes or wood chips, and piled in specially designed pits. Aeration is provided by periodic turning of the sludge mass or by mechanical mixers/aerators. The energy produced by the decomposition reaction can raise the waste temperature to 140−160°F, destroying pathogenic bacteria. At the end of the composting period, the bulking material is separated, and the stabilized sludge is applied to land or disposed of at a landfill.

4.4.2.2 Sludge Conditioning

Sludge from a final liquid−solids separation unit may contain from 1% to 5% total suspended solids. It is economically feasible to remove water from sludge and thus handle smaller amounts of material. Unfortunately, it is impossible to remove water from sludge using only mechanical dewatering. Dewatering processes or equipment are designed to remove water in a much shorter time compared to natural gravity. To speed up the process, an energy gradient is used to promote rapid drainage. However, energy application requires frequent conditioning of the sludge prior to the dewatering step. Thus, conditioning is required because of the nature of the sludge particles. Both inorganic and

organic sludge consists of colloidal (less than 1 μm), intermediate, and large particles (greater than 200 μm). The large particles, or flocs, are usually compressible. Under an energy gradient, these large flocs compress and prevent water from escaping. Consequently, the pressure drops throughout the sludge cake because of the decrease in porosity and pore sizing that exceeds available energy and dewatering stops. The main purpose of sludge conditioning is to induce or maintain significant porosity and pore size sufficient for the water to drain. Typically, biological sludges are conditioned with $FeCl_3$, lime, and synthetic cationic polymers, either separately or combined. Heat conditioning and low-pressure oxidation are also used for biological sludges. Inorganic sludges are conditioned with $FeCl_3$, lime, and either cationic or anionic polymers.

Thus, two sludge management practices are currently available in the pulp and paper industry: first, mechanical dewatering followed by composting to produce material applicable for land amendment or covering material for landfills, and second, mechanical dewatering followed by incineration with placement of formed ash into landfill. There are no regulations that force mills to treat sludge on-site, and therefore many mills outsource the composting or drying of sludge to a contractor, which in turn increases transportation costs. This cost is directly proportional to the mass of solid waste and may reach a significant amount for larger mills. Unfortunately, most of the problems that sludge management faces are linked to dewatering properties. These properties vary depending on the type of sludge, with an acceptable dewaterability for primary sludge and very poor dewaterability for biological and chemical—flocculation sludge. The mechanical dewatering of pulp and paper mill sludge is usually performed using a series of process units, such as a gravity table or rotary thickener followed by a belt press or a screw press, as each process unit operates in different ranges of the total solids content. Pure secondary sludge and digestate from anaerobic digestion typically require a centrifuge for the mechanical dewatering process. Filtrate from the mechanical dewatering units often has a high content of organic substances and requires renewed wastewater treatment. Composting is the biological decomposition of biodegradable organic matter under aerated conditions, and it is carried out in either windrows or reactors. Wastes with a high moisture and low fiber content need considerable amounts of moisture sorbing material and structural support to compost well [35].

4.4.2.3 Dewatering

There are numerous ways to dewater the sludge [36—41]. These are explained in more detail below.

Belt filter press: Belt filter presses have been used in Europe since the early 1960s and in the United States since the early 1970s. In these belt filter presses sludge is sandwiched between two tensioned porous belts and passed over and under rollers of various diameters. At a constant belt tension, rollers of decreasing diameters exert increasing pressure on the sludge, thus squeezing out water. Although many different designs for belt filter presses are available, they all incorporate a polymer conditioning unit, a gravity drainage zone, a compression (low-pressure) zone, and a shear (high-pressure) zone.

Polymer conditioning unit: Polymer conditioning can be done in a small tank, in a rotating drum attached to the top of the press, or in the sludge line. Usually, the press manufacturer supplies a polymer conditioning unit with the belt filter press.

Gravity drainage zone: The gravity drainage zone is a flat or slightly inclined belt, which is unique to each press model. In this section, sludge is dewatered by the gravity drainage of free water. The gravity drainage zone should increase the solids concentration of the sludge by 5−10%. If the sludge does not drain well in this zone, the sludge can squeeze out from between the belts or the belt mesh can become blinded. The effectiveness of the gravity drainage zone depends on sludge type, quality, and conditioning, along with the screen mesh and design of the drainage zone.

Screw press: Screw presses are most effective for primary sludge, producing cake solids of 50−55%, but are also appropriate for primary and secondary blended sludge. Sludge is conditioned and thickened prior to dewatering. A slowly rotating screw, analogous to a solid bowl centrifuge, conveys and compresses the solids. The screw has the same outer diameter and pitch for the entire length of the press. In some models, the diameter of the screw shaft increases toward the discharge end of the screw press to enhance dewatering. The compression ratio (the ratio of the free space at the inlet to the space at the discharge end of the screw) is selected according to the nature of the material to be dewatered and the dewatering requirement. Dewatered cake is discharged as it is pressed against the spring or hydraulically loaded cone mounted at the end of the screw press. Filtrate is collected in the collecting pan located under the screw press, and the cake is transported to the next stage.

Vacuum filters: Vacuum filtration utilizes various porous materials as filter media, including cloth, steel mesh, and tightly wound coil springs. Under an applied vacuum, the porous medium retains the solids, but allows water to pass through. The relative importance of cake dryness, filtrate quality, and filter cake yield can vary from one system to another. A lower drum speed allows more time for drying of the sludge to increase cake dryness. However, this also decreases the filter cake yield. Polymers can assist in the production of a drier cake without the problem of a smaller filter cake yield. Synthetic polymers improve cake dryness by agglomerating sludge particles that may hinder the removal of water. This agglomeration also increases the solids capture across the unit, which results in a higher-quality filtrate.

Centrifuges: Centrifugal force, 3500−6000 times the force of gravity, is used to increase the sedimentation rate of solid sludge particles. The two principal elements of a continuous solid bowl centrifuge are the rotating bowl and inner screw conveyor. The bowl acts as a settling vessel and the solids settle because of the centrifugal force from its rotating motion. Typically, operation of centrifugal dewatering equipment is a compromise between quality, cake dryness, and sludge throughput.

Plate and frame press: A plate and frame filter press is a batch operation consisting of vertical plates held in a frame. A filter cloth is mounted on both sides of each plate. Sludge pumped into the unit is subjected to pressures of up to 25 psig as the plates are

pressed together. As the sludge fills the chamber between individual plates, the filtrate flow ceases, and the dewatering cycle is completed.

Sludge drying beds: Sludge drying beds consist of a layer of sand over a gravel bed. Underdrains spaced throughout the system collect the filtrate, which usually is returned to the wastewater plant. Water is drained from the sludge cake by gravity through the sand and gravel bed. This process is complete within the first 2 days. All additional drying occurs by evaporation, which takes from 2 to 6 weeks.

4.4.2.4 Sludge Disposal

Disposal of the sludge depends on current environmental regulations, for example, the Resource Conservation and Recovery Act (RCRA) in the United States; geographical location; and sludge characteristics.

Reclamation: Because of the high costs associated with disposal of sludge, this waste stream should be evaluated for its reclamation potential. Therefore, potential energy value, mineral content, raw material makeup, and by-product markets for each type of sludge must be carefully assessed.

Incineration: Biological sludge can be disposed of by incineration; the carbon, nitrogen, and sulfur are removed as gaseous by-products, and the inorganic portion is removed as ash.

Land application: Sludge produced from biological oxidation of industrial wastes can be used for land application as a fertilizer or soil amendment. However, a detailed analysis of the sludge needs to be carried out to evaluate toxic compound and heavy metal content, leachate quality, and nitrogen concentration in sludge ready for land application.

Landfill: Landfill is the most common method for disposing of various types of wastewater sludge. However, landfilling must be assessed to avoid contamination of groundwater. Many US states require impermeable liners, defined as having a permeability of 10^{-7} cm/s in landfill disposal sites. This requirement limits liners to a few natural clays and commercial plastic liners.

4.5 Environmental Regulations

In the United States, many governmental regulations have been established in recent years for the protection of the environment. The Clean Water Act (CWA) and the RCRA are among the most significant. The CWA of 1972 established regulations for wastewater discharge, provided funding for publicly owned treatment works (municipal waste treatment plants), and authorized the National Pollutant Discharge Elimination Systems to regulate and establish wastewater discharge permits for industrial and municipal plants. The RCRA of 1976 provided regulations for management of hazardous solid wastes, cleanup of hazardous waste sites, waste minimization, underground storage, and groundwater monitoring.

4.6 Aerobic Treatment of Effluent

Aerobic systems are the following, and examples include but are not limited to activated sludge (liquid waste) and composting (solid waste):

$$\text{Organic matter} + \text{oxygen (energy)} \rightarrow \text{carbon dioxide} + \text{new cells}$$

Furthermore aerobic systems require air (100 kWh of energy expenditure), typically receive influent of 100 kg of COD, and produce effluent of 2–10 kg of COD and sludge of 30–60 kg with significant heat losses. When choosing a BAT for aerobic treatment, it is extremely important to choose it based on the following sustainability criteria:

- The BAT must have high removal efficiencies for COD/BOD, suspended solids, nitrogen, phosphorus, etc.
- It must be a stable technology with adequate response to energy cuts, toxicity, overloads, etc.
- It should be simple, especially its maintenance, operation, and control.
- It should have only a few stages of treatment.
- It should not pose disposal problems.
- It should not generate odors.
- It should be unrestricted by the size of operation.
- It should provide by-product recovery options.

Typical aerobic process conditions include:

- hydraulic retention time ranging from 4 to 8 h for conventional systems;
- sludge age from 5 to 25 days;
- dissolved oxygen concentration from 1.5 to 2.0 ppm;
- BOD/N/P ratios of 100:0.8–3.5:0.3–0.6; and
- temperature from 35 to 37°C.

Conventional aerobic treatment approaches include activated sludge treatment and aerated lagoons. These biological processes are the most commonly used in the pulp and paper industry. During these processes, dissolved organic matter is converted into carbon dioxide, water, and new cells by microbial growth sustained by aerobic respiration.

4.6.1 Activated Sludge Process

One of the biggest advantages of an activated sludge process (ASP) is the production of a high-quality effluent with very reasonable operating and maintenance costs [42,43]. ASP uses microorganisms to feed on organic contaminants present in wastewater that consequently produce high-quality effluent. Basic principles include [44–47] the growth of microorganisms and their attachment to one another, forming flocs that are allowed to settle to the bottom of the tank, leaving a rather clear liquid free of organic materials and suspended solids. Furthermore, screened wastewater is mixed with a predetermined amount of recycled liquid with microorganisms from a secondary clarifying tank, which

then becomes a so-called mixed liquor. This mixture is stirred in the presence of air to provide oxygen and maintain solids in suspension. After some time, the mixed liquor is directed to a clarifier where it settles. A large portion of the microorganisms is removed as they settle and the partially treated water is taken for further treatment. The resulting settled solids—activated sludge—are returned to the first tank to repeat the process.

Such a conventional treatment is used to remove BOD, COD, suspended solids (SS), AOX, and other specific compounds such as chlorinated phenols, guaiacols, catechols, vanillins, 1,1-dichlorodimethyl sulfone, and chlorinated acetic acid. For instance, more than 70% of filtered COD and almost all BOD_5, resins, and fatty acids can be removed with this process [48–51]. The main operational problems at pulp and paper mills are the nitrogen and phosphorus limitation in the system, growth of the filamentous microorganisms, and bulking problems. Bulking is caused by the lack of oxygen, low organic loading rates, and low amounts of nitrogen as well as phosphorus. However, these drawbacks can be overcome by adding chlorine, ferrous salts, or lime.

4.6.1.1 Aeration

Aeration is a critical stage in the ASP. Various approaches to aeration are used:

1. High-rate aeration. It operates in the log-growth phase. The biomass population is fed with excess food provided by recirculation. Thus the effluent contains high levels of BOD because the oxidation process is not complete. However, the settling characteristics of the produced biomass are rather poor. Therefore, increased sludge return rates are needed to offset poor settling and to maintain a high and healthy biomass population. Poor settling also increases the SS content of the effluent. Therefore, poor effluent is produced that highly limits the use of this approach. However, the advantage of high-rate aeration is low capital investment due to much smaller tanks and basins required because of the short oxidation time.
2. Conventional aeration. This is the most widely used approach by municipalities and industries that operate in the endogenous phase to produce effluent with desired BOD and total suspended solids (TSS) levels. Conventional aeration is called the “middle of the road” approach because investment and operating costs are higher in comparison to the high-rate process but lower than those of the extended aeration plants.
3. Extended aeration. These plants operate in the endogenous phase but also use longer oxidation periods to reduce effluent BOD levels. Thus, it requires higher investment and operating costs because of the larger tanks and basins as well as more air. Also, extended aeration produces a relatively high-SS-containing effluent when optimum natural settling ranges are exceeded.
4. Step/tapered aeration. In the plug flow basin the head receives the most concentrated waste. Consequently, metabolism and oxygen demand are the greatest at that point. As the waste flows through the basin the rate of oxygen

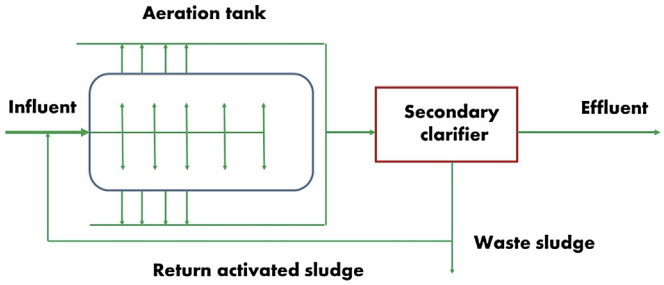
uptake decreases, which reflects the advanced stage of oxidation. Importantly, step/tapered aeration overcomes this disadvantage. Tapered aeration delivers more oxygen at the head and slowly reduces its supply to match the demand as the waste flows through the basin/tank. Step aeration also alters the supply of the influent. The basin is typically divided into several stages and the raw influent is introduced to each stage subsequently. All return microorganisms are provided at the head of the basin. Such design reduces aeration time to 3–5 h, while BOD removal efficiency is maintained. The shorter aeration time significantly reduces installation capital because a smaller basin can be used. However, operating costs are very similar to those of a conventional plant.

Overall the advantages of ASP include: (1) effective removal of BOD, COD, and nutrients; (2) a flexible process that can be tailored to meet specific requirements; and (3) being the most widely documented and accepted form of secondary wastewater treatment. The disadvantages include: (1) high investment and operating as well as maintenance costs; (2) constant energy supply requirement; (3) requirement of highly trained operators who can monitor the system and react to changes immediately; and (4) availability of spare parts and chemicals.

4.6.1.2 Types of Activated Sludge Process

Common ASP types include the following:

- Conventional–complete mix: If properly installed, the concentration of microorganisms and the BOD (e.g., oxygen demand) are uniform throughout the aeration tank. This type is great at handling slug and toxic loads but is prone to filamentous sludge bulking (i.e., exhibits poor settling in secondary clarifier) (Fig. 4.1).
- Conventional–plug flow: Its length is bigger than its width and therefore there is little or no longitudinal mixing. It is more efficient than a complete-mix ASP. Variable oxygen demand along the tank with high demand (thus high aeration requirement) at the front of a tank is present. Unfortunately it is not suitable for handling slug or highly toxic loads (Fig. 4.2).
- Extended aeration ASP: This can be designed as complete mix or plug flow and operates at very high hydraulic retention time (>20 h) and high sludge retention time (SRT) (>20 days). Sludge production here is relatively low and it can produce highly treated effluent with low BOD. It can also suffer from poor-settling pin flocs and unfortunately requires a relatively large aeration tank with high aeration requirements.
- High-purity oxygen (e.g., UNOX, OASES): For this type, oxygen is introduced into covered staged tanks. It is highly efficient, with high volumetric BOD loading, and this aeration tank is relatively compact. Unfortunately it is more complex to install, operate, and maintain (Fig. 4.3).
- Sequencing batch reactor: The same tank can be used in batch mode for aeration and settling. A preceding storage basin or additional sequencing batch reactor



Aeration tank is constantly mixed
Influent: flow rate, substrate (BOD_5) concentration and microorganism concentration
Aeration tank: substrate (BOD_5) concentration, microorganism concentration and volume
Return activated sludge: flow rate, substrate (BOD_5) concentration and microorganism concentration
Waste sludge: flow rate, substrate (BOD_5) concentration and microorganism concentration
Effluent: flow rate, substrate (BOD_5) concentration and microorganism concentration

FIGURE 4.1 Conventional—complete mix activated sludge process.

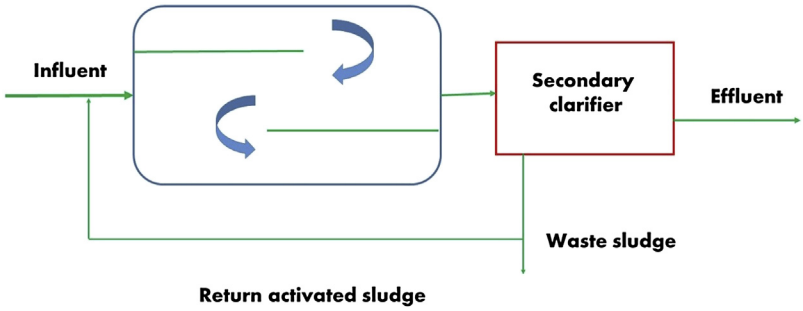


FIGURE 4.2 Conventional—plug flow activated sludge process.

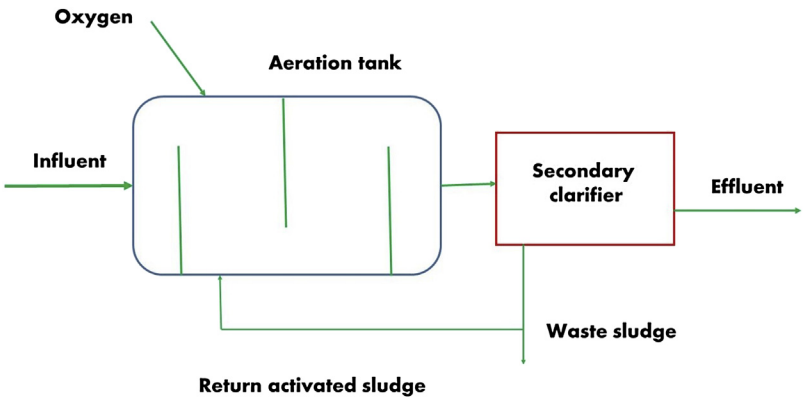


FIGURE 4.3 High-purity-oxygen activated sludge process.

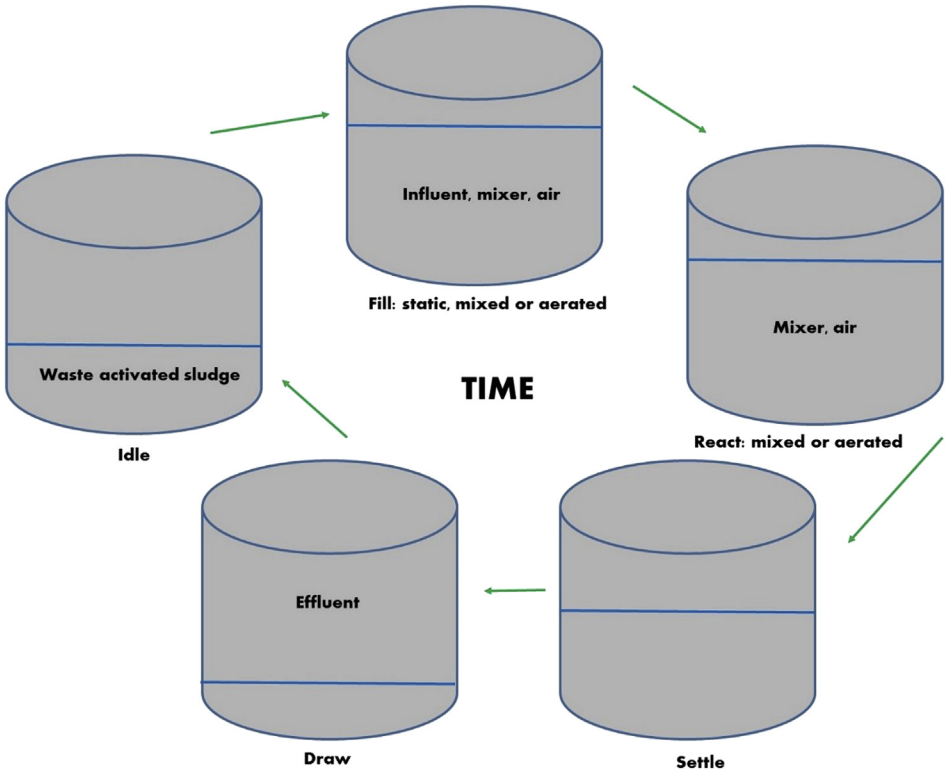


FIGURE 4.4 Sequencing batch reactor activated sludge process.

(SBR) is necessary with continuous wastewater flow. It requires less space and has relatively low capital costs. It is relatively easy to automate but requires highly skilled maintenance operators (Fig. 4.4).

ASP control methods include:

- constant SRT;
- constant food to microorganisms ratio;
- constant mixed liquor suspended solids or mixed liquor volatile suspended solids concentration; and
- return activated sludge percentage or ratio.

These choices are based on the following: (1) ASP performance, (2) variability of BOD load, (3) ease of implementation, and (4) operator preference.

4.6.2 Aerated Lagoons

Typically, aerated lagoons (ALs) are characterized by a large volume, a long retention time for the water, and no continuous removal of bio-sludge. These lagoons shelter

various complex microbial communities, which are selected according to the physicochemical parameters of the wastewater, the design and operation of the lagoon, and the ambient environmental conditions. Microbial communities in ALs are responsible for degradation of contaminants and their transformation to desired carbon dioxide and water. Activity and stability of these microorganisms are essential for constant contaminant degradation. Unfortunately, these microorganisms are influenced by changing environmental conditions such as variations of influent pH, temperature, organic loading rates, and toxic compound levels, as well as seasonal climate changes. These changes highly affect the microbial community's composition and overall performance. Furthermore, the AL process does not involve recirculation of biomass, which is the primary difference between an AL and the ASP. The settled sludge is removed once every 1−10 years. Unfortunately, there is a lack of scientific data on the structure and dynamics of microbial communities in ALs that would aid a better understanding of the microbial ecology processes within ALs and assist in better design and use of ALs in wastewater treatment.

Despite the fact that lagoons are simple and economical, they are not as popular as ASPs because of the requirement for much land and basin volumes as well as much higher energy needs and low energy efficiency in terms of aeration and mixing. Furthermore, they may have problems associated with effluent foaming and extensive smell. The removal and appropriate disposal of settled sludge can also be problematic. Treatment efficiency highly depends on the type of effluent, design of the treatment system, and operating conditions. It has been reported that COD can be removed up to 60% and AOX up to 70% in ALs [52,53].

4.6.2.1 Facultative Lagoons

Facultative lagoons are very cheap because they do not require electrical input and rely solely on wind to mix the oxygen into the water and induce decomposition. Despite their affordability, these lagoons have several drawbacks such as BOD accumulation, sludge buildup, and odors. For instance, insoluble BOD accumulates on the bottom for anaerobic decomposition of solids, whereas the soluble BOD stays in the water column.

According to the experience of many municipalities throughout the United States, facultative lagoons demonstrate a faster rate of sludge generation, typically in the range from $^1/_4$ to $^1/_2$ inch a year. The amount of sludge buildup relies heavily on the depth of the lagoon and the amount of wind it receives. For instance, if the lagoon is less than 4 feet deep and under windy conditions, sludge builds up at a very slow rate. Conversely, if the lagoon is surrounded by trees (BOD), sludge builds up at a faster rate. In northern parts of the United States, Canada, and Europe, harsh winters contribute to a faster sludge buildup due to cold weather stopping the activity in the lagoon. Another drawback of this type of lagoon is the problematic removal of ammonia. Furthermore, odor is also a problem. A so-called spring turnover (surface water is at about 39.5°F and the densest) significantly contributes to the odor problem.

4.6.2.2 Problems Associated With Aerated Lagoons

ALs may have problems in the following areas:

1. Effluent TSS. TSS indicates that there is too much algae in the effluent. Algae will increase the pH of the lagoon and will block the sunlight, so that sunlight will not be able to destroy the contaminants and thus the lagoon will be deemed to be ineffective.
2. BOD. If TSS increases, BOD also increases as the lagoon is flooded with food.
3. Ammonia. Nitrifiers usually exhibit a very slow growth rate and in lagoons with water temperature of at least 60°F (15.5°C) their growth could last up to a week.
4. Sludge buildup. Sludge will inevitably build up in the corners of the lagoon and around the aerator. Thus, naturally occurring bacteria must be stimulated to reduce the amount of sludge and increase the lagoon's efficiency.
5. Effluent dissolved oxygen (DO) and pH. There are very tight environmental regulations for controlling the minimum limit of DO and the maximum limit of pH.
6. Phosphorus. It is necessary to control phosphorus in the discharge with various polymers and ferric chloride.
7. Foaming and odor. Especially in cold climate zones, when the ice melts, lagoons can exhibit increased odors and elevated concentrations of ammonia.

4.7 Novel Approaches to Treating Effluents Aerobically

It is obvious that there is no single best available wastewater treatment process based on a sole biological, chemical, or physical approach. Furthermore, one method is usually not effective enough to meet the increasingly stringent discharge requirements of paper and pulp industries [1]. Most of the time, it is necessary to include a tertiary step to either reach the desired efficiency or meet stringent regulations in terms of effluent quality. Typically, the tertiary step is some kind of enhanced biological treatment, chemical precipitation, chemical oxidation, or a combination of all or several treatment types. For instance, chemical precipitation is a common approach where metal ions, mostly iron(III) or alumina(III), are used to break the repulsive forces—form flocs/entities—between the negatively charged organic ions that are present in the wastewater. Then polymers are used to form even larger flocs, which can be removed in sedimentation or flotation units based on their density. Unfortunately, energy requirements for flotation are as high as those for aeration in biological wastewater treatment. Consequently this method should be avoided if possible.

4.7.1 Flocculation–Coagulation

Several reactors are known to be used worldwide to treat wastewater from pulp and paper mills. The SBR is a fill-and-draw activated sludge system applicable for many types of wastewater [46,54–57]. In SBRs, wastewater is added to a single "batch" reactor,

subjected to various treatments to remove contaminants, and then discarded [55]. The newly developed fed-batch reactor (FBR) involves the slow addition of highly concentrated wastewater into an aeration tank with no removal of the effluent until the tank is full [58]. Combined biological (FBR) and coagulation treatment followed by immediate sand filtration has been effectively utilized and resulted in a total COD and BOD reduction of 93% and 96.5%, respectively [59]. Toxicity tests showed that untreated effluent was toxic, whereas treated effluent did not exhibit any toxicity to fish that were exposed to this wastewater for more than 72 h.

Flocculation−coagulation is the addition of metal salts to form larger flocs from smaller particles [60]. Thus, aluminum chloride as coagulant and starch-g-PAM-g-PDMC, a natural polymer, as a flocculant have been added to the pulp and paper effluent. Optimal doses of 871 and 22.3 mg/L of coagulant and flocculant, respectively, at pH 8.35 resulted in 95.7% turbidity and 83.4% lignin removal [61].

Flocculation can also be enhanced by adding polyelectrolytes such as polydiallydimethylammonium chloride (polyDADMAC) coupled with polyacrylamide (PAM) to enhance the treatment effectiveness [62]. Tests were performed with polyDADMAC and PAM dosages of 0.4−2.0 and 0.4−8.0 mg/L, respectively, with mixing speed of 200 rpm for 2 min followed by 30 rpm for 10 min and settling time of 5 min. It was found that 98% and 96% of COD and TSS were removed, respectively. Furthermore, increasing the dosages of polyDADMAC increased the destabilization of particles and produced small flocs, whereas the addition of PAM increased the size of flocs for a better synergistic flocculation process.

The use of high-molecular weight (HMW) polyDADMAC resulted in a much higher treatment efficiency than lower-molecular-weight flocculants, with more than 90% reduction in COD [63]. On the other hand, chitosan has proven to be a superior flocculant that reduced more than 85% of COD, 85% of turbidity in comparison to conventional polyaluminum chloride (up to 45% and 60%), respectively [64]. Chemical precipitation using 5 g/L CaO, together with Fenton and Fenton-like processes, resulted in more than 90% COD removal. Doses of Fe(III) and hydrogen peroxide, pH, and contact time were 1 g/L, 3 g/L, 6.9, and 1 h, respectively [65].

The combination of coagulation with electricity also resulted in very promising results for treating pulp and paper mill effluents [66−70]. These methods may be technically and economically feasible for larger scale operations [71]. For instance, aluminum and iron electrodes can significantly reduce COD, lignin, phenol, and BOD as well as color [66−68].

4.7.2 Advanced Oxidation Processes

Despite the effectiveness of biological treatment in enhancing the quality of pulp and paper effluent, it cannot remove nonbiodegradable recalcitrant organic matter (ROM), in particular HMW (MW 41000 Da) oxidized lignin compounds from bleaching operations [28]. Thus, advanced oxidation processes (AOPs) are among the most promising and

understood technologies that have a bright future in wastewater treatment [29,30,72–74]. Typically, AOPs employ a strong oxidant (ozone or hydrogen peroxide) with UV radiation, ultrasound, or hydroxide ion to produce hydroxyl radicals (*OH)—an extremely powerful oxidizing agent able to nonselectively destroy various compounds [72,74]. The ability of hydroxyl radicals to destroy the molecular structure of chemical compounds is advantageous for partial oxidation of nonbiodegradable HMW organics or dechlorination of ROM [28].

Unfortunately, complete oxidation and mineralization of target contaminants are not economically effective because of the large energy expenditure and chemicals that are necessary to carry out these reactions. Therefore, AOPs may not be the best available technology alone for pulp and paper mill effluents and must be combined with other remediation approaches. For instance, AOPs coupled with biological treatment provide a viable alternative and could help remove ROM from the effluent economically and effectively. For instance, effluent with toxic and inhibitory compounds can be pretreated by AOPs to produce biodegradable intermediates, which are then readily treated biologically [28]. For instance, ozonation (doses of 0.7–0.8 mg O_3/mL) performed in a semibatch bubble column reactor at pH 5 and 11 demonstrated 30% higher total organic carbon (TOC) demineralization in comparison to sole ozonation or biotreatment. Furthermore, ozone addition enhanced the biodegradability of the effluent (21% COD reduction and 13% BOD5 enhancement), making the removal of more pollutants possible [28]. The conversion of HMW to low-molecular-weight compounds was an important factor in the overall biodegradability enhancement of the alkaline effluent. In another study, 45 min of ozone treatment yielded almost colorless effluent with over 90% decolorization efficiency and with a corresponding ozone capacity rate of 20.0 mg O_3/L. Unfortunately, the ozonation capacity used did not mineralize the compounds and thus the TOC did not change [32]. The BOD/COD ratio increased from 0.10 to 0.32 with ozone flow rate of 4.0 L/min.

Treatment of high-residue (lignin) effluent content (COD higher than 70,000 mg/L) can also be enhanced by a simultaneous use of ozonation and chemical precipitation with concentrated sulfuric acid (97.1%) at pH 1 and 3 [75]. It was discovered that 77% of COD and 96.1% of color had been reduced. The biodegradability of the effluent treated with ozonation increased by up to 0.29. Furthermore, when ozonation was used together with a catalyst (TOCCATAs process), up to 76% of COD was removed [30]. On the other hand, ozonation enhanced with carbon adsorption resulted in almost 98% of COD and 98% of color removal. When ozonation was enhanced with the addition of hydrogen peroxide, only color had been removed better [31]. Furthermore, ozonation with photocatalysis and biological treatment (membrane bioreactor, MBR) has indicated promising results [33]. For instance, the consumption of 2.4 g O_3/L of ozone in combination with a photocatalysis (TiO_2) in an MBR resulted in more than 60% COD reduction in kraft pulp mill effluent. In a case in which photocatalysis was used alone, only 20–30% of COD was removed.

Various other AOPs like UV, UV enhanced with hydrogen peroxide, UV-assisted TiO_2, and UV in the presence of hydrogen peroxide and TiO_2 are used to degrade contaminants in pulp and paper mill effluents. Several studies found that TiO_2-assisted photocatalysis (solar/TiO_2 and UV/TiO_2) resulted in the highest TOC and toxicity removals under alkaline conditions compared with the other AOPs tested. Approximately 79.6% TOC and 94% toxicity removals were obtained by the TiO_2-assisted photocatalysis (Pt/TiO_2 and UV/TiO_2) with a titanium dioxide concentration up to 0.75 g/L at pH 11 within 30–60 min irradiation [29,72,76,77]. Furthermore, during solar/TiO_2 treatment, at an optimum dose of 0.75 g/L TiO_2 and pH of 6.5, 75% COD removal of wastewater was achieved within 180 min solar irradiation time. A reduction of 80% of TSS was also obtained using the same operating conditions [78].

Furthermore, when a photocatalytic system (TiO_2 and ZnO) on aluminum foil and Luffa cylindrical supports was used with a biological system (fungus, *Trametes pubescens*, immobilized on polyurethane foam), TOC, 2-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol decreased by more than 96%, 97%, 90%, and 99%, respectively [79].

4.7.3 Adsorption

There is a huge and available variety of adsorbents such as activated carbon, silica, nanomaterials, coal ash, ion-exchange resins, etc., to remove contaminants from effluents [60,80]. For instance, ion-exchange resins in combination with activated carbon resulted in 72% and 76% reduction in dissolved organic carbon (DOC), respectively [81]. Delayed petroleum coke was used to produce activated carbon that served to remove color and chlorinated contaminants from pulp mill wastewater using a fixed-bed reactor [82]. More than 90% of COD, DOC, and AOX was removed with an adsorbent dose of 15,000 mg/L.

Furthermore, polyaluminum chloride (PAC) as a coagulant and bagasse fly ash (BFA), which was generated in sugar mills, as an adsorbent were used to remove COD and color from pulp and paper mill effluents. Under optimal conditions of pH 3 and initial PAC dosage of 3 g/L, more than 80% COD and 90% color removal were achieved. The optimal conditions for the adsorptive removal of 55% COD and color with BFA were pH 4 and BFA dosage of 2 g/L [83]. Two-stage treatment with 3 g/L PAC and 2 g/L BFA resulted in 87% COD and 95% color removal without pH adjustment.

4.7.4 Biological Techniques

Biological techniques use various microorganisms such as fungi, algae, bacteria, and enzymes, sometimes alone or usually as in combination with several physicochemical treatment methods. These techniques are considered to be cost-effective, sustainable, and “green” and are applicable to significantly reduce the BOD and COD in pulp and paper mill effluents. Unfortunately they typically fail to remove color or degrade recalcitrant compounds [60].

4.7.4.1 Fungi

Fungi can be used to treat pulp and paper effluents because they produce extracellular enzymes and can withstand higher toxic effluent loads in comparison to bacteria [8,9,24]. White rot fungi are microbes with the ability to degrade lignin and phenolic and other recalcitrant compounds in the effluent by producing enzymes, e.g., lignin peroxidases and laccases [23,25,84–90]. For instance, with the use of *T. pubescens*, *Phanerochaete chrysosporium*, *Merulius aureus*, or *Fusarium sambucinum* in the presence of bagasse, nearly 80% color, 79% lignin, and 90% COD can be reduced in the first 4 days [24], as well as 82–93% of AOX in 4–12 days [91]. Conversely, *Pleurotus sajor caju* and *Rhizopus oryzae* reduced the relative absorbance of effluent by up to 46% at 250 nm and up to 75% at 465 nm, and destroyed 81% of COD in less than 2 weeks [22]. *Emericella nidulans* has been reported to reduce color by almost 70% and lignin by 40% after optimizing the conditions using the Taguchi approach. The optimum conditions were temperature 30–35°C, rpm 125, dextrose 0.25%, tryptone 0.1%, inoculum size 7.5%, pH 5, and duration 24 h [9]. It has been reported that the main mechanism involved in lignin remediation process using fungi entirely follows the metabolism pathway. In contrast, color and chlorinated compounds removal follows metabolism and transformation pathways [60,91].

On the other hand a combination of biological treatment with AOPs provide additional benefits to the overall effectiveness of the treatment. For instance, *T. pubescens* in combination with TiO_2/UV successfully removed chlorophenols from pulp and paper effluent with moderate costs and an easy to implement system [92]. Unfortunately, if high oxygen content and high pH as well as low glucose content are present in the system, fungal treatment may be rather limited and then require additional enhancements [84,91,93]. Thus, it is very important to take into account all the reaction conditions and effluent parameters and characteristics prior to using fungi for treatment.

4.7.4.2 Bacteria

Bacteria can also be used to degrade lignin and recalcitrant contaminants in pulp and paper effluent owing to their tolerance to pH fluctuation, biochemical versatility, and adaptability [84,94]. For instance, *Streptomyces viridosporus* and *Streptomyces coelicolor, Rhodococcus* sp., *Rhodococcus jostii*, *Rhodococcus erythropolis*, *Nocardia autotrophica*, *Sphingobium* sp., *Pseudomonas putida*, and *Acinetobacter* sp. have been reported to almost completely degrade lignin [95]. *Bacillus cereus* GN1 was able to degrade 2,4-dichlorophenol up to 78% in 2 days [96]. Furthermore, *Pseudomonas aeruginosa* (DSMZ 03504 and DSMZ 03505) and *Bacillus megaterium* (MTCC 6544) successfully reduced 76% of COD within 10 h, almost 70% BOD in 24 h, and 7% total dissolved solids (TDS) as well as AOX and color in 1 day [94].

4.8 Conclusions

This chapter discussed waste generation and characterization as well as sole and enhanced aerobic treatment of effluent emanating from the pulp and paper industry. It

provided information on waste management and potential strategies to select the best available technology to treat the effluent. The pulp and paper industry is having to adjust to the changing regulatory environment and reform its production processes to be competitive and maintain profits.

To overcome regulatory and process drawbacks, various novel and conventional biological and physicochemical treatment methods must be developed and employed. Over the years, such methods were used to remove solid materials from wastewater using various coagulants and flocculants. Numerous laboratory-scale studies and real-scale applications proved that these methods are effective in reducing COD, BOD, turbidity, and lignin concentration in the effluent. Novel pretreatment methods such as electrocoagulation in combination with biological methods significantly improved COD removal, prevented color formation, and reduced odor during the treatment. Furthermore, adsorption and advanced oxidation methods in combination with biological methods were even more successful to reduce color, concentration of recalcitrant compounds, COD, and BOD, among other parameters. Adsorption is able to remove both soluble and insoluble compounds of higher molecular weight fractions. Despite their effectiveness, most of the advanced oxidation methods are relatively expensive and need well-trained personnel to operate them. Fungi and bacteria can be effectively utilized to remove lignin and color from pulp and paper wastewater. Unfortunately, extreme environmental conditions such as pH and oxygen content as well as potential lack of glucose may severely limit their use. ALs have always been a primary choice for pulp and paper mills owing to their ability to reduce BOD. However, unfortunately, they lack the capacity to remove color and recalcitrant compounds. ASPs in combinations with physicochemical methods have attracted more interest from municipalities and industries since 2005 because of their attractive costs in terms of return on investment and process effectiveness.

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5

Aerobic Treatment of Effluents From the Mining Industry

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5.1 Introduction

Water is an integral part of mining operations and is used in all aspects including exploration, mining, mineral processing, and closure. Mines obtain their water from a variety of sources including allocation from the bulk water infrastructure (third-party water), groundwater, and surface water (rainfall and runoff), but the mines in arid regions rely heavily on groundwater of variable quality. Some mining operations can also use lower quality or alternative sources of water [1,2]. Water is used in mining for various purposes including mine workings, dust suppression (in underground mines, roadways, etc.), coal washing, mineral separation, etc. Water is returned to the environment (output) after contact with mining or processing activities, supplied to other users such as to towns, lost through evaporation from storage dams, or reused for mine operations after treatment. In certain cases, release to creeks or rivers is highly restricted or controlled. The interactions of water streams in mining operations with inputs and outputs are shown in Fig. 5.1. An improved water balance [3,4] will provide an account of the various types of water required and the amounts of water used for mine operations or stored within and exiting the mine site. This will help in developing better water management practices.

Mining operations in many parts of the world are located in arid or semiarid regions where water is scarce and often there are competing users such as agriculture and towns [5]. In some areas water systems are fully utilized (e.g., in Australia, the Hunter Valley region, Murray–Darling Basin, or parts of the southwest of Western Australia) and it can be difficult to obtain new water entitlements.

Demand for water in the mining industry is likely to increase in the future because of both increasing production and declining ore quality [6,7]. Extreme climate variability [8] creates additional pressures on water resource management and balance between too little water and too much water. For example, severe drought was witnessed during 1993–2008 in most parts of Australia and mine sites began to collect and store water. But

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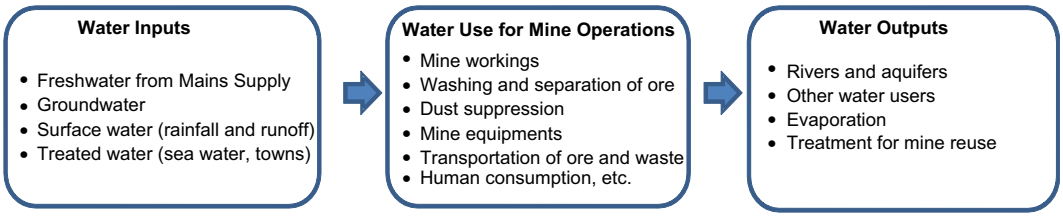

FIGURE 5.1 Water interactions in mining.

Table 5.1 Water Consumption per Tonne of Ore Produced by Various Mineral Industries [13–15]

Mineral Industry	Water Consumption, L/t
Coal	180–220
Aluminum	1588
Zinc	7900
Copper	52,970
Nickel	205,300
Gold	250,000,000

this period was followed by above average rainfall in 2008, 2010, and 2011, resulting in flooding of mines in the Fitzroy Basin.

In Australia, the mining industry consumed 508 GL of water in 2008–09, accounting for an about 23% increase compared to 2004–05 [9]. Water consumption further increased by 21% (to 614 GL) between 2008–09 and 2012–13 [10]. Water requirements can vary depending on the type of ore mined and the metallurgical process adopted. Table 5.1 provides an indication of water consumption for coal and other mineral production. Metal mining requires significantly more water per tonne of metal produced, but coal is by far the largest user of water in the mining sector because of the huge mass of product mined [6]. Whereas the mining industry accounts for 3–4% of total water usage in Australia [11], the total resource export value it brings from metal ores, coal and other mineral fuels, metals, and gold makes up around 60% of the export revenue, and Australia ranks among the top five key mineral producers in the world [12].

5.2 Mine Water Characteristics

The characteristics of mine-impacted water vary from one mine to the other depending on the type of ore being mined and the method used for processing. In Australia, some of the groundwater supplies and inland surface water can have very high salinity levels even before it is used in mining operations [16]. A study on the water quality from some of the coal mines in Australia (New South Wales and Queensland) has shown significant variation in the characteristics of mine water [17]. Similarly, Levay and Schumann [18]

Table 5.2 Water Quality Characteristics of Various Streams in Mining Operations [17,18]

		Values From Ore Processing Mines		
Parameter	**Unit**	**Coal**	**Gold**	**Lead–zinc**
pH		3.5–8.9	2.6–7.6	2.75–8.35
Conductivity	μS/cm	500–21,000	96,000–289,000	1600–13,000
Suspended solids	mg/L	5–50	–	3–579
Calcium	mg/L	20–500	780–4100	65–780
Magnesium	mg/L	25–700	2000–11,000	19–225
Sodium	mg/L	100–6000	13,000–74,000	66–1350
Potassium	mg/L	10–120	100–660	49–52
Aluminum	mg/L	0.01–0.02	25–345	0.31–1700
Iron	mg/L	0–120	2–17	1.1–3400
Manganese	mg/L	0.003–10.0	1.6–3.4	4–260
Chloride	mg/L	200–1300	24,000–110,000	160–1700
Sulfate	mg/L	50–14,000	3800–12,000	265–18,500
Silica	mg/L	0–15	<42	–
Zinc	mg/L	5–250	–	0.17–275

reported the characteristics of various streams from gold-processing and lead and nickel-processing mines. The values given in Table 5.2 are approximate concentration ranges obtained from various streams within the mine sites. The pH of the waters from these mines ranged from acidic to basic in nature. Some streams have high dissolved solids concentration, suspended solids, and characteristics of scale formation.

The values from Table 5.2 also show that each type of ore has distinctive water quality characteristics. In addition to the water quality changes due to different ore processing operations, water quality characteristics are also affected by the practice of blending of various streams and water supplies in some of the mine sites. Also, water quality changes with time. For example, conductivity of the mine water from a lead–zinc ore-processing mine had reduced over 80% over a period of 8 years, although the raw water quality remained unchanged during this period [18].

Microbial activity also influences the water characteristics from coal and metal mining [16,19] and can induce major changes in pH, oxidation–reduction potential, dissolved oxygen, and chemical composition. For example, complex chemical and biological processes occur in the presence of sulfidic minerals in mine waters resulting in acidification of water, increased sulfate concentration, and mobility of dissolved metals. Naturally occurring bacteria can accelerate the production of this acidic mine drainage (AMD) by assisting in the breakdown of sulfide minerals [20,21]. Typical chemical characteristics of AMD from coal and metal mining operations are given in Table 5.3. Dissolved salts, in addition to sulfates, like calcium, magnesium, etc., and heavy metals, are also present in the AMD. The typical concentration range of total dissolved salts is generally 100–30,000 mg/L. Based on its acidity and metal content, AMD is classified as

Table 5.3 Typical Compositions of Acid Mine Drainage From Mining Operations [24–29]

Constituent	Concentration
pH	2.75–5.5
Acidity	100–10,000
Magnesium	80
Calcium	200
Aluminum	50–108
Iron	50–2000
Manganese	20–300
Copper	8–230
Zinc	1–14.5
Sulfate	20–20,800
Arsenic	0.001–340
Cadmium	0.03–1.0
Lead	0.2–11.5

All values except pH are in mg/L.

low, medium, or high strength [22]. Mine waters generally have very little dissolved organic carbon and the main acidity especially in coal mine drainage is proton acidity associated with pH and the mineral acidity arising from the hydrolysis reaction of dissolved iron, aluminum, and manganese. The principal source of alkalinity in mine water is dissolved carbonate (which can exist in bicarbonate or carbonate form) [17,23]. AMD characteristics given in Table 5.3 are generally applicable to both coal and metalliferous mining. However, metals found in mine-impacted water are site specific and can vary significantly depending on the type of exposed minerals. Metals like copper, zinc, cadmium, and lead are of particular concern in metal mining wastewater. Iron, aluminum, and manganese are the major metals of concern in coal mine drainage, although other metals can be present.

Possible impacts from mining on local water sources vary according to local conditions [30,31]. In Australia, mine water discharges into the environment are based on the mine licensing agreements and the regulatory requirements of the respective jurisdiction [32,33]. Guidance in identifying, mitigating, and monitoring key water-related risks is also available [34]. Planned water discharges from mines into the receiving environment are generally controlled and carefully monitored to minimize impact, but some uncontrolled discharges occur owing to extreme weather events and runoffs [35–38]. Ongoing water management initiatives at various mine sites are helping to minimize discharge and maximize water reuse [2,38–40]. There is a paradigm shift toward viewing water as a key business resource with actively engaging communities and not merely managing it as an environmental issue. Part of the water management plan involves developing a suitable water treatment facility. Adopting

efficient treatment technologies on-site would minimize the risk of wet season runoffs and freshwater contamination and allow segregation into different qualities of water to enable greater water recycling.

5.3 Water Treatment Technologies

Mine-affected waters are typically strongly acidic or alkaline and carry high concentrations of salts and trace metals and generally require treatment before discharge into natural waterways. A suitable treatment process for a given site is selected based on water quality and quantity and the treated water quality objectives based on regulatory, economic, and application requirements and availability of space for treatment. Treatment options for mine water typically include physicochemical, biological, or electrochemical processes (Fig. 5.2) and generally require various steps of treatment using more than one technology. Treatment objectives for mine-impacted water generally focus on parameters such as fines and suspended solids, acidity or alkalinity, heavy metals, inorganic salts, and other specific pollutants like ammonia/nitrate, cyanide, organics, radionuclides, etc., and various treatment systems generally applied for the removal of these pollutants present in mining wastewater streams are given in Table 5.4.

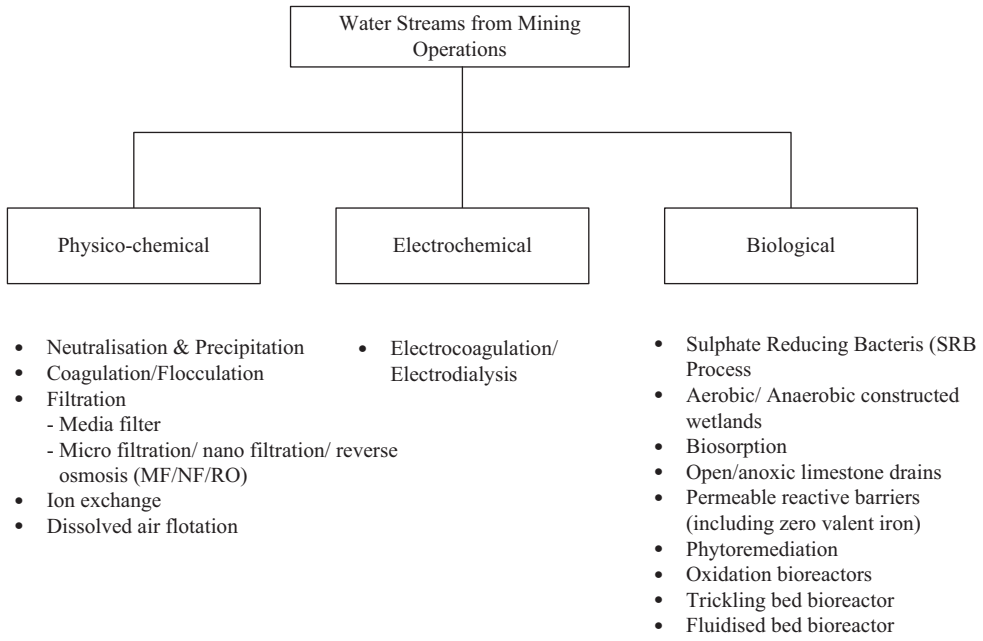

FIGURE 5.2 Various processes adopted for treating mine-impacted water.

Table 5.4 Treatment Systems Adopted for Various Streams From Coal and Metal Mining-Impacted Wastewaters

Acidic/Alkaline Wastewater	Heavy Metals	Fines/Suspended Solids/Colloidal Particles	Inorganic Salts	Specific Pollutants[a]
Neutralization and precipitation, softening	Neutralization and precipitation, biological treatment (passive treatment), adsorption, ion exchange	Coagulation and sedimentation, membrane filtration, dissolved air flotation	Nanofiltration, reverse osmosis, biological treatment (aerobic and anaerobic), evaporation and crystallization	Adsorption, oxidation process (chemical, photo, biological), electrochemical process

[a]Specific pollutants include cyanide, ammonia, nitrate, organic compounds, radionuclides, etc.

5.3.1 Physicochemical Treatment

For the treatment of acidic effluents, the lime neutralization process is generally adopted. Lime (quick lime or hydrated lime) is the simple, low-cost neutralizing agent of choice in most applications for the neutralization process. Lime sludges are heavy, low volume, easy to handle, and easy to clarify [41,42]. Whereas acid is neutralized, metals present in the water are precipitated in the form of metal hydroxides, depending on their solubility. Common heavy metals such as Cu, Zn, Cd, Mn, Pb, and ferrous iron (Fe^{2+}) are effectively precipitated using lime neutralization. Other commonly used chemical reagents are limestone, magnesium hydroxide, soda ash (sodium carbonate), caustic soda (sodium hydroxide), sulfides, and, in some cases, ammonia [43]. In situ treatment of acid mine pit lakes with the addition of magnesium chloride hexahydrate to form a hydrotalcite-based precipitate, has also been carried out [44]. The effluent quality and sludge characteristics are improved with a high-density sludge method through multiple steps of neutralization and sludge recycling [45]. Some of the disadvantages of this process are that the pH of the treated effluent would generally require readjustment to bring it to an acceptable range, it produces large amounts of sludge, and usually requires further treatment or polishing steps [46]. Coagulants (inorganic iron and aluminum salts) and flocculants (polymers) are often used to achieve better solid/liquid separation and better settling of solids. Dissolved air flotation using microbubbles is also adopted in the minerals industry for the removal of oils, heavy metals, and anions [47]. Media filters, like sand filters, or membrane separation, like microfiltration, is used to achieve low-turbidity effluents to meet the required standards [48]. The application of ion-exchange processes in mining waters is for the removal of hardness, alkalinity, ammonia, and metals [49]. Depending on the resin type and the water characteristics, selective metal recovery may be achieved [43]. Some of the main drawbacks using this process when treating mining water include plugging of resins by the mine water, presence of high sulfate, effects of competing ions when selective removal is required, resin regeneration, and disposal of spent resins [49].

Currently, reverse osmosis (RO) is a mature membrane process and one of the most commonly used technologies in mining for salinity reduction [50]. Some mines reuse the RO-treated water for their mine site operations and reduce their reliance on additional freshwater. However, RO membranes can be very sensitive to fouling by various dissolved and undissolved constituents, particulate matter, salt precipitates, and microorganisms, particularly for mine-affected water containing silica. It would require extensive and expensive pretreatment to reduce membrane fouling and to ensure acceptable performance. The short life span of the membrane, membrane scaling, inability to achieve yield to design specifications, and inconsistent output quality water are some of the problems generally encountered in the RO system for the treatment of mine-impacted water [51].

5.3.2 Electrochemical Treatment

Electrocoagulation is the production of a coagulant in water through electrolysis and is achieved by applying a current across the electrode. The metal ions from the electrode are released when an electrical current is applied and reacts with the effluent water to form a precipitate. Gravity sedimentation or filtration is applied as a posttreatment to separate the precipitate. Aluminum or iron is generally used as an anode, and some of the mine water constituents such as arsenic, copper, lead, zinc, cadmium, phosphates, and suspended solids are treated using this method [49,52]. In the mining application, this process can be applied as a pretreatment for RO or for treating tailings water or AMD water.

Electrodialysis is a similar electrochemical process using a series of ion-selective anion- and cation-exchange membranes between an anode and a cathode [53]. When an electrical potential is applied the cations and anions in solution migrate toward their oppositely charged electrode.

The cations pass through the cation-exchange membrane but are retained by the anion-exchange membrane. Similarly, the anions pass through the anion-exchange membrane and are retained by the cation-exchange membrane. Electrolytes are separated into two streams, a concentrated and a dilute stream. This process has been applied to treat AMD water and mine tailings water [54,55].

5.3.3 Biological Treatment

Some of the biological processes applied to treat mining effluents are given in Fig. 5.2. Some of the processes can also be operated in a passive mode (which is designed to have very little operational and maintenance requirement), depending on the contaminant load and flow rate [56,57]. Both aerobic and anaerobic modes of operation are adopted. Some of the process can be further classified as in situ, such as permeable reactive barriers and wetlands, and some as ex situ, such as bioreactors [58]. These processes are applied to treat mine-impacted waters, acid drainage water, and tailings waters for the removal of contaminants like sulfate, thiosalts, heavy metals, cyanide, ammonia, nitrate, cyanide,

selenium, etc. [43]. The performance of bioremediation generally depends on factors such as contaminant concentration, flow rate, pH, acidity or alkalinity, and temperature.

5.3.4 Aerobic Treatment

5.3.4.1 Sulfate Removal and Metal Precipitation

Sulfate removal is achieved under anaerobic/reducing conditions by the sulfate-reducing bacteria, whereby sulfate is converted to sulfide, producing bicarbonate alkalinity, which increases the pH of the acid mine effluent. The dissolved metals form insoluble complexes and precipitate as metal sulfide during the sulfate reduction process [59]. Based on their solubility product the preference of some of the metal sulfide formation follows the order CuS, PbS, ZnS, CdS, NiS, FeS, MnS [59,60]. An aerobic system is used in conjunction with the anaerobic process. Aerobic posttreatment polishes the anaerobic effluent and results in very high overall treatment efficiency [61]. Sulfide formed in the anaerobic process is oxidatively converted to elemental sulfur (THIOPAQ process) [62].

5.3.4.2 Oxidation Bioreactor

The concentrations of metals such as iron, manganese, aluminum, etc., in the mine-impacted water are reduced by oxidation and hydrolysis reactions in an aerobic environment depending on the oxygen availability, pH of the water, microbial activity, and retention time [60]. Biological oxidation of metals like ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}) is an aerobic process using iron-oxidizing microorganisms in a bioreactor [63]. Ferric iron sludges are more stable for settling in the clarifier/thickener [21,64,65]. The pH of mine water is one of the important parameters for metal oxidation and hydrolysis and has an influence on the solubility of metal hydroxide precipitate. Bacterial oxidation of ferrous iron peaks between pH 2 and 3 and reduces at pH >5 [60]. Similarly manganese is oxidized by the catalytic activity of the microorganisms aerobically at pH >6 and the activity ceases below pH 6. Aluminum precipitation is strongly dependent on pH, forming aluminum hydroxide and precipitating at pH 5–8. Cotreatment with municipal wastewater in an activated sludge process was also attempted for the removal of metals like Al, Cu, Fe, Mn, and Zn in mine water [66]. Fluidized-bed aerobic bioreactors with limestone sand or activated carbon medium substrate for microorganism growth are also used [49,67].

Ammonia is found in mining effluent from the hydrolysis of cyanate and dissolution of blasting agent (e.g., ammonium nitrate) residue [43,68,69]. Natural degradation of cyanide forms nontoxic by-products like carbon dioxide and nitrogen. Natural degradation of ammonia involves the transpiration of dissolved ammonia gas. The natural degradation method is generally adopted by the mining industry; however, the removal depends on environmental conditions [43]. Cyanide can be degraded to ammonia by microorganisms under aerobic conditions, which then oxidizes to nitrate (nitrification) [70,71].

Operating conditions such as pH, dissolved oxygen, and temperature are important for the reaction. The optimal pH range for nitrification is 7.5–8.6 [72]. The alkalinity wastewater balances the acid produced during nitrification. The minimum dissolved oxygen in the water must be higher than 1 mg/L [73] and the process significantly slows down below the temperature of 10°C [74]. The most common effluent nitrification reactors are the continuous stirred tank reactor [73], packed-bed trickling bed filter [75], and rotating biological contactor [76]. Simultaneous removal of ammonia, iron, and manganese from mining effluent using the trickling bed filter has also been reported [73,77].

5.3.4.3 Aerobic Wetlands

Aerobic wetlands are built using aquatic plants, crushed rocks and media, and soil. Plants and vegetation take up the metals in the mine water and help the oxidation process to happen. The aerobic microorganisms act as catalysts for the chemical reactions in metal removal. Wetlands can treat acidic, neutral, or alkaline mine waters and metals including iron, manganese, arsenic, nickel, copper, aluminum, zinc, cadmium, and lead [43,49]. Aerobic wetlands are effective for iron removal and also require net alkaline waters. Aerobic wetlands are generally shallow (<1 m) and the water flow can be in the horizontal or vertical direction. Wetlands are more suited to remote locations as they are easy to maintain and require very little monitoring and maintenance. However, a large land area and periodic removal of sediments and precipitate are required for this treatment method and it also generally includes some pre- or post-treatment [78].

5.4 Concluding Remarks

Water is an integral part of mining operations. However, many of these operations are located in arid and semiarid locations and there is an increasing competition for freshwater from other industries. Mining operations can affect the natural water systems (groundwater and surface water) and the discharge of mining-impacted water is strictly monitored and regulated. Many mining companies reuse and recycle water, showing stewardship in adopting water as a key business resource. However, better water management practices are required, including water quality and quantity all through the life cycle of the mine.

Water treatment is part of water management and is site specific. There is a significant variation in the characteristics of mining-impacted water depending on the ore being extracted and processed, site location, various streams within the mine, and whether the mine is in operation or undergoing closure and decommissioning. Mine closure and decommissioning could have long-term water-related issues in dealing with large quantities of saline and acidic mine discharges. Treatment objectives for mine-impacted water generally focus on the removal of suspended and dissolved solids, acidity, heavy metals, cyanide, and ammonia/nitrate. Biological treatment is largely

applied to treat AMD and waters from the tailing stream. Aerobic treatment of mine water is effective for metal removal by the oxidation reaction and ammonia nitrification. Aerobic microorganisms act as catalysts for these processes. Aerobic systems are also used in conjunction with anaerobic processes.

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6

Aerobic Treatment of Effluents From the Electronics Industry

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6.1 Introduction

The electronics industry, especially consumer electronics, is an industry whose products include electronic devices, such as semiconductors, optoelectronics, computers, digital cameras, cell phones, and processing equipment. With products intended for everyday use in communication, entertainment, and offices, it has now become a global industry with annual revenue over US$300 billion. Electronic devices are manufactured and assembled in automatic, advanced processes that are totally different from conventional industrial processes. The relatively low cost of production of microelectronics and communication and display devices has made computers, mobile phones, and other electronic devices inextricable parts of the structure of modern society [1]. Their very fast growth causes effects on the environment: pollution in the manufacturing plants; depletion of raw materials and water, as well as production of electronics waste. There has been profound concern about the management of electronics waste from electronic devices at their end of life [2]. As a result, the industry needs to be ready to take responsibility for resource use efficiency and lean manufacturing, in particular regarding water resource management [3]; the development of eco-design of consumer electronics, which calls for the use of environmentally friendly materials and technology; and legislation of both manufacturing and waste treatment [4]. Even though many studies have been published since 1995 on wastewater treatment for the electronics industry, a comprehensive review of the state-of-the-art treatment technologies is not available. This review aims to introduce the fundamental manufacturing processes of the electronics industry, the characteristics of wastewater from each unit process, and the treatment, disposal, and reuse of electronics industry wastewater. Difficulties and problems encountered in design and operation of wastewater treatment plants are discussed. A future perspective is postulated as well. It is believed that an integrated water management consisting of efficient water use and effective wastewater treatment, reclamation, and reuse will contribute to a sustainable water environment that benefits the electronics industry.

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6.2 Semiconductor Industry

6.2.1 Semiconductor Manufacturing

Electronics manufacture includes many different types of plants. This chapter mainly focuses on electronic wastewater from semiconductor and thin-film transistor–liquid crystal display (TFT–LCD) manufacturing. In the respective manufacturing chains that consist of the various processes, the wafer fabrication process in semiconductor manufacturing and the array process in TFT–LCD manufacturing are the focus because they use vast quantities of ultrapure water (UPW) and produce the largest amount of wastewater that contains complex contaminants. It is noted that these manufacturing plants, unlike other conventional industries, are concentrated in some countries, including the United States, Korea, Taiwan, Japan, and China, some of which are water-stressed countries. It makes water an important issue from the local water supply viewpoint. In fact, water is also critical to ensure a stable global supply chain of consumer electronics.

The manufacturing phase of semiconductors becomes more and more complex as the component size constantly decreases to the nano- and micrometer scale, and a lot of secondary materials are used during manufacturing [1]. Production processes for semiconductors involve many highly complex and delicate unit processes, including silicon growth, photoresist spreading, exposure, lithography, etching, chemical mechanical polishing (CMP), and rinsing (Fig. 6.1). The processes are repeated many times during semiconductor manufacturing and massive amounts of UPW are needed for rinsing. In addition, many organic and inorganic compounds are used. This generates large volumes of wastewater, which must be treated before discharge. Specially designed reclamation systems can help reduce the amounts of effluent and minimize the raw water needed for the production of UPW.

In each unit process of a semiconductor manufacturer, various chemicals are used:

- Lithography: photoresist, tetramethylammonium hydroxide ($N(CH_3)_4OH$; TMAH) as developer, dimethyl sulfoxide ($(CH_3)_2SO$; DMSO) as photoresist stripper.

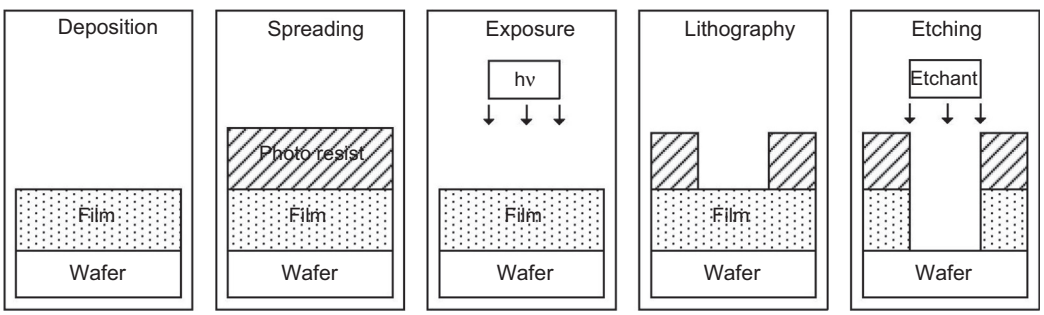

FIGURE 6.1 Unit processes in semiconductor manufacturing.

- Etching: concentrated hydrogen fluoride (HF); HF and ammonium fluoride (NH_4F) (BHF); HF, nitric acid (HNO_3), and acetic acid (CH_3COOH); HNO_3; phosphoric acid (H_3PO_4); sulfuric acid and hydrogen peroxide (H_2SO_4/H_2O_2).
- Rinsing: dilute HF; BHF; HF and HNO_3; hydrochloric acid (HCl) and H_2O_2; H_2SO_4/H_2O_2.
- Others: organic solvents, such as isopropyl alcohol ($(CH_3)_2CHOH$; IPA) for dry cleaning; dispersants and surfactants, such as polyacrylic acid (PAA), ammonium salts, alkyl sulfates, and ethylenediaminetetraacetic acid (EDTA); metal chelates, such as ethanolamine, oxalic acid, and citric acid; and polymers as photoresist.

The results of a survey that covered a total of 167 semiconductor manufacturers in Taiwan are shown in Fig. 6.2 [5]. Although it may not be representative of other countries, it does offer some background information. Depending on the type and scale of the manufacturing process, the wastewater flow rate varies significantly. Typically, it is $<1000\ m^3$/day for wafer probe and packaging plants and $>8000\ m^3$/day for wafer fabrication. It is the wastewater treatment from wafer fabrication that is examined in our study. Throughout more than 2 decades of development, the segregated collection of different streams of wastewater and separate, independent treatments have become common practice among semiconductor manufacturers. For old, existing manufacturers, the drain systems had to go through a series of upgrades and modifications, so that segregated collection could be achieved. However, this is easily realized for new plants. In general, semiconductor wastewater is divided into four streams, namely acidic and basic wastewater, fluoride-containing wastewater, CMP wastewater, and organic wastewater.

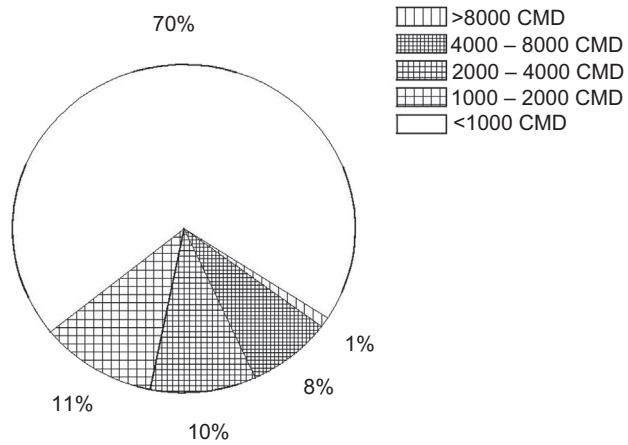

FIGURE 6.2 Distribution of semiconductor wastewater flow rates in Taiwan [5]. *CMD*, cubic meter per day.

6.2.2 Chemical Mechanical Polishing Wastewater

CMP wastewater typically contains spent slurry, contaminants from wafers, and post-CMP cleaning. The majority of the inorganic contents come from slurry abrasive, whereas organic compounds, such as oxidizers, dispersants, surfactants, and chelates, may also be present, because they are used in making the slurry. For independent treatment, coagulation–flocculation by polyaluminum chloride and polymers treats mainly the CMP wastewater in most semiconductor manufacturers in Taiwan [6]. It is common and beneficial to mix CMP wastewater with fluoride-containing wastewater for a combined treatment [7]. Other treatment processes, such as electrocoagulation and microfiltration (MF), have also been examined [8,9]. In full-scale operations, spent CMP slurry is shipped to an off-site reclamation plant, whereas the CMP wastewater is treated by coagulation–flocculation and sedimentation. The effluent can meet the effluent standard of Cu(II) <3 mg/L and total suspended solids <10 mg/L without difficulty. The effluent can also be reused after treatment with activated carbon (AC), ion exchange, and reverse osmosis (RO) (Fig. 6.3). New developments include preconcentration of the spent CMP slurry by ultrafiltration (UF) to 10% of the original volume before shipping out for reclamation, whereas the UF filtrate, c. 90% of the original volume, is treated by RO and directed to UPW systems for further treatment and reused as UPW. Alternatively, CMP wastewater is treated by coagulation–flocculation and sedimentation, followed by MF, and the filtrate is reused as cooling water and cleansing water.

6.2.3 Fluoride-Containing Wastewater

Fluoride-containing wastewater is generated in rinsing and etching units. Whereas the spent strong HF used in etching is shipped to an off-site reclamation plant, fluoride-containing wastewater is divided into dilute (<500 mg/L) and concentrated streams (>500 mg/L). Dilute fluoride-containing wastewater is commonly treated by

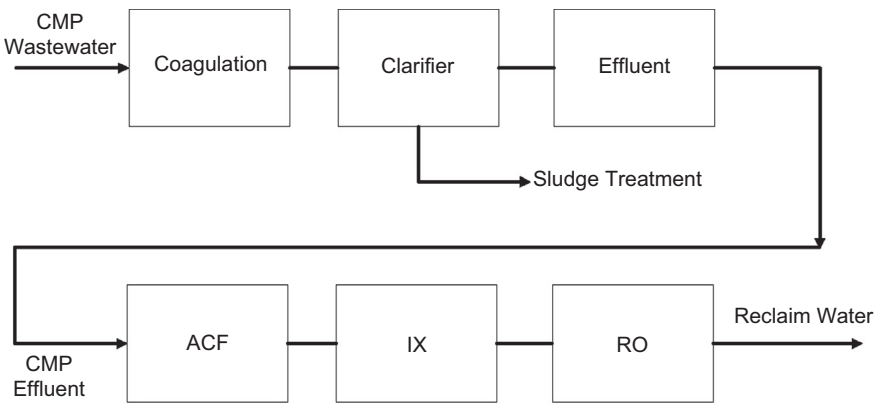

FIGURE 6.3 Chemical mechanical polishing (*CMP*) wastewater treatment and reuse processes. *ACF*, activated carbon; *IX*, ion exchange; *RO*, reverse osmosis.

an ion-exchange process and the effluent is reclaimed and reused. Alternatively, it can be treated by an RO process and the filtrate reclaimed and reused, whereas the concentrate (rejection) is treated in combination with concentrated fluoride-containing wastewater.

The concentrated fluoride-containing wastewater is mainly treated by a chemical precipitation process by using calcium salts, such as calcium chloride ($CaCl_2$) or lime ($Ca(OH)_2$) to generate CaF_2 precipitates [10,11]. In practice, a higher-than-stoichiometric ratio (Ca^{2+}/F^-) is needed, knowing that mixed acids are frequently used in etching, and sulfate and phosphate ions will compete with fluoride ions for calcium. For the flocculation of fine CaF_2 precipitates, anionic polyelectrolytes, such as PAA, with a low molecular weight and high charge density are ideal choices as flocculants [12]. A fluidized-bed crystallization process has been applied for simultaneous wastewater treatment and recovery of CaF_2 sludge as fluorite mineral [13]. There are now several such full-scale plants for semiconductor manufacturers in the science parks of Taiwan. Alternatively, fluoride in the wastewater can be crystallized as cryolite (Na_3AlF_6) mineral [14].

6.2.4 Organic Wastewater

Depending upon total organic carbon (TOC) content, different treatment processes are used for the organic wastewater. For very dilute streams (TOC <20 mg/L), a process consisting of AC and RO units or one with ozone (O_3) and AC is used to treat and reclaim the wastewater. For treatment of medium-strength streams (TOC <50 mg/L), coagulation–flocculation, multimedia filtration, AC, and RO are adopted and the filtrate from RO is connected to a UPW system for further treatment (Fig. 6.4).

Regarding concentrated organic wastewater treatment, it is mostly pretreated before being discharged to the centralized wastewater treatment plant within the science parks, for semiconductor manufacturers located in science parks. For manufacturers located outside of science parks, some contaminants may be difficult to treat by biological processes. Taking TMAH as an example, it is corrosive and highly poisonous, and it has caused several fatal cases of electronic workers' poisoning [15]. Its presence in wastewater is also a problem that needs to be solved. TMAH shows aquatic ecotoxicity, especially through its synergistic action with iodide [16]. Various treatment technologies have been examined [17]. It has been found that pretreatment with ultraviolet/hydrogen peroxide enhanced subsequent biodegradation [18]. In general, aerobic processes are utilized in treating semiconductor wastewater. For some manufacturers, a conventional

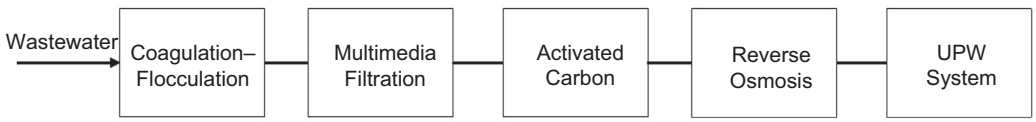

FIGURE 6.4 Treatment process for medium (total organic carbons <50 mg/L) organic wastewater. *UPW*, ultrapure water.

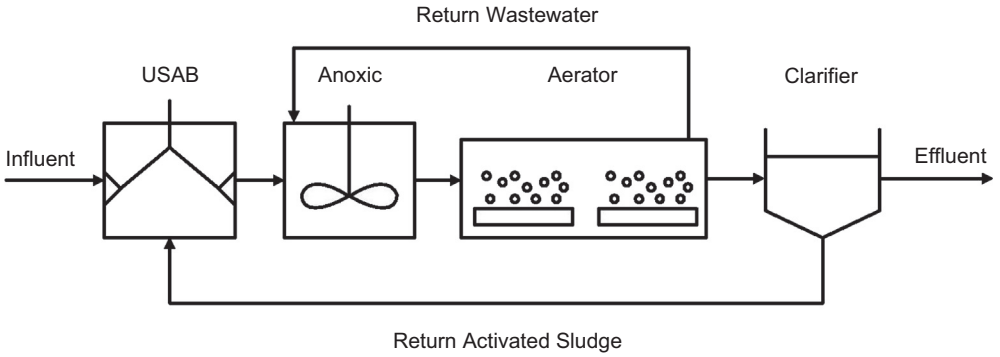

FIGURE 6.5 Anaerobic, anoxic, and aerobic process in treating organic semiconductor wastewater. *UASB*, upflow anaerobic sludge blanket.

activated sludge (CAS) process is used. However, to meet effluent standards for nitrogen (N) and phosphorus (P), some have already changed to an anaerobic, anoxic, and aerobic (A2O) (Fig. 6.5) or anoxic, aerobic, and membrane bioreactor (AO + MBR) (Fig. 6.6) process in place of CAS. A better effluent quality also ensures a higher water reuse rate. A full-scale (550 m^3/day) semiconductor wastewater treatment plant has been retrofitted using a nitritation and anammox process for inorganic wastewater that contains 250–400 mg N/L of ammonia, 2–10 mg N/L of nitrite, and 20–30 mg N/L of nitrate [19]. Combining with denitrification as posttreatment, an effluent of less than 8 mg N/L was obtained. A pilot-scale combination of MBR and RO processes could treat semiconductor wastewater effluent effectively for possible reuse [20]. It is noted that an aerobic bioreactor was used as a pretreatment for the system.

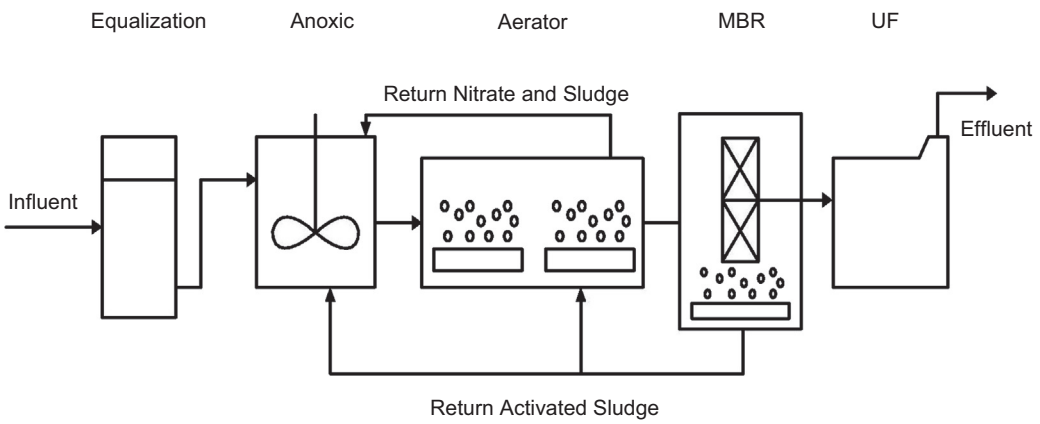

FIGURE 6.6 Anoxic, aerobic, and membrane bioreactor in treating organic semiconductor wastewater. *MBR*, membrane bioreactor; *UF*, ultrafiltration.

6.3 TFT–LCD Industry

6.3.1 TFT–LCD Manufacturing

The manufacturing processes of TFT–LCD consist of array, panel, and module processes and are usually done separately in different plants. It is the array process that produces the most significant amount of wastewater. The array manufacturing processes are similar to those of semiconductors, consisting of deposition, spreading, exposure, photolithography, and etching. The major difference is that the substrate is glass instead of silicate (SiO_2). Some chemicals used in TFT–LCD manufacturing processes are also identical to those used for semiconductors, but may be of different concentration. For example, TMAH is also widely used as a developer in optoelectronic manufacturing at 25%, which is higher than the 2.38% used in semiconductor manufacturing. In addition to TMAH, typical main organic contaminants that are found in TFT–LCD wastewater include photoresist (*n*-butylacetate), stripper (DMSO and monoethanolamine; MEA), dry cleaning solvent (IPA), and chelating agents. Most of these compounds are recognized as slowly biodegradable organic compounds with limited information regarding their biological treatability [21].

Among optoelectronic plants in Taiwan, most are located in science parks. Wastewater from each plant is pretreated first before discharge to the central wastewater treatment plant that treats wastewater from all the plants within the science park. Some manufacturers have their own independent, full-scale wastewater treatment plant. Typical optoelectronic wastewater can be divided into several streams, including stripper (DMSO, MEA), developer (TMAH), and rinsing wastewater [5], and the characteristics are shown in Table 6.1.

Regarding the amount of wastewater, the optoelectronics array process plants tend to have very high flow rates, owing to intensive use of UPW. On the other hand, other plants, such as cell and module processes, produce much lower amounts of wastewater. In a survey of 133 optoelectronics manufacturers in Taiwan [5], there were 11 manufacturers (10%) whose wastewater flow rate was $>5000\ m^3$/day, whereas 74% had a lower wastewater flow rate of $<1000\ m^3$/day (Fig. 6.7).

Table 6.1 Typical Optoelectronic Wastewater Characteristics [5]

	Stripper (DMSO and MEA) Wastewater	Developer (TMAH) Wastewater	Rinsing Wastewater
pH	9–11	10–13	10–11
SS (mg/L)	<10	<10	<10
COD (mg/L)	800–1200	400–600	600–1700
TKN (mg/L)	90–200	100–200	60–90
NH_4^+-N (mg/L)	0–10	2–10	0.1–10

COD, Chemical oxygen demand; *DMSO*, dimethyl sulfoxide; *MEA*, monoethanolamine; *SS*, suspended solids; *TMAH*, tetramethylammonium hydroxide; *TKN*, total Kjeldahl nitrogen.

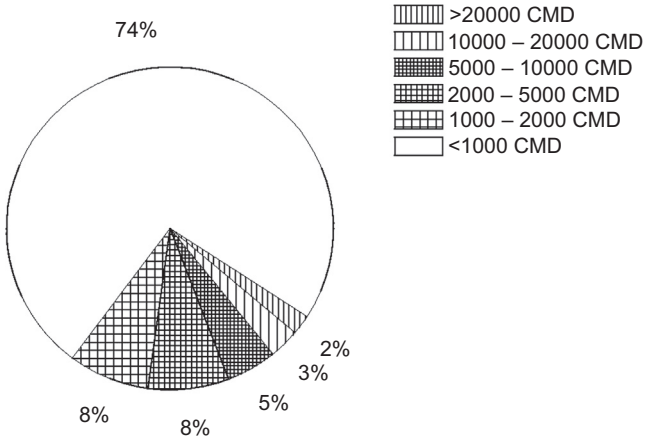

FIGURE 6.7 Distribution of optoelectronics wastewater flow rate in Taiwan [5]. *CMD*, cubic meters per day.

6.3.2 TFT–LCD Wastewater

Because of high N and P contents in optoelectronics wastewater, the CAS process is effective only for COD removal and shows limited removal efficiency for N and P. For some TFT–LCD manufacturers located in science parks, wastewaters without pretreatment may have total nitrogen (TN), NH_4^+-N, and total Kjeldahl nitrogen (TKN) all >800 mg/L, which may cause trouble for the centralized wastewater treatment plant in the science park. Because of the presence of MEA, DMSO, and TMAH, N removal has always been one of the major concerns for optoelectronics wastewater. A full-scale (total volume of 3000 m^3) anoxic, aerobic, and MBR process (Fig. 6.6) was used to treat 5000 m^3/day of MEA/DMSO-containing wastewater with an average influent COD of 800 mg/L [22]. To further improve the nitrification performance, some manufacturers adopted an anoxic, aerobic, anoxic, aerobic, and MBR process, or an aerobic, anoxic, aerobic, and MBR process (Figs. 6.8 and 6.9). The average COD removal was 88.7% and 98.3%, respectively. Examination of the microbial ecology of nitrifying bacteria revealed that *Nitrosomonas oligotropha*-like bacteria were important ammonia-oxidizing bacteria, whereas *Nitrobacter*- and *Nitrospira*-like nitrite-oxidizing bacteria were abundant in the MBR system. Possible inhibitory chemicals and effects of food-to-microorganism ratio and colloidal COD were also investigated [23].

A full-scale methanogenic upflow anaerobic sludge blanket (UASB) followed by a CAS process (Fig. 6.10) with a treatment capacity of 1000 m^3/day and hydraulic retention time (HRT) of 15 h has an advantage over aerobic processes for TMAH treatment because of its superb ability to handle the high strength of TMAH-containing wastewater [21]. Not only was anaerobic sludge under methanogenic conditions favored over the aerobic processes, through molecular analysis, *Methanomethylovorans* and *Methanosarcina* were found to be dominant methanogens involved in the degradation of TMAH [24].

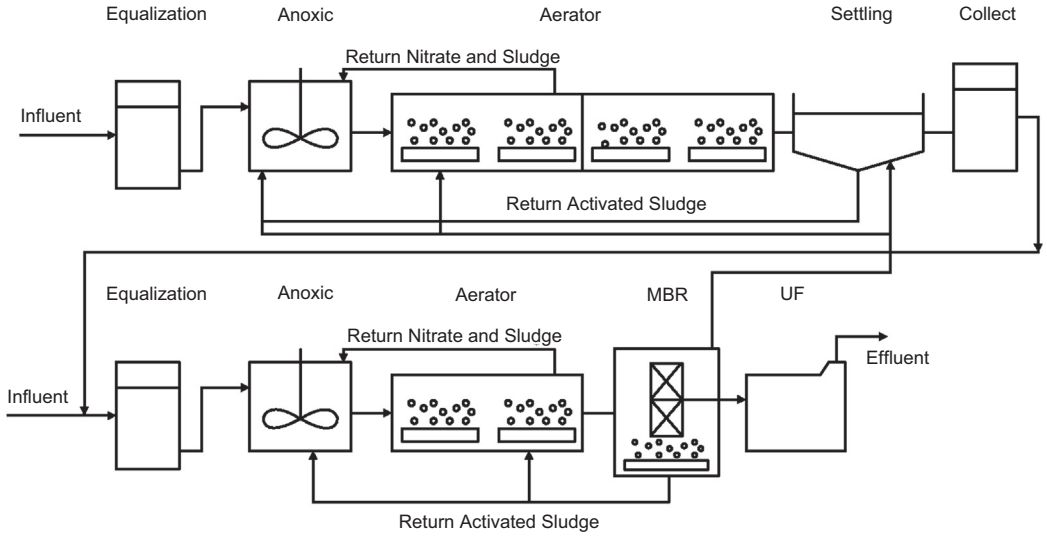

FIGURE 6.8 Anoxic, aerobic, and membrane bioreactor for treating optoelectronics wastewater. *MBR*, membrane bioreactor; *UF*, ultrafiltration.

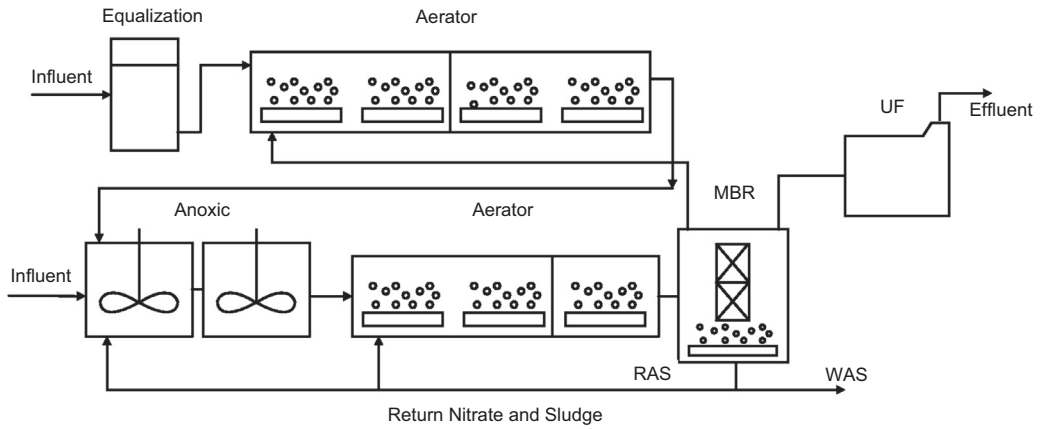

FIGURE 6.9 Aerobic, anoxic, aerobic, and membrane bioreactor for treating optoelectronics wastewater. *MBR*, membrane bioreactor; *UF*, ultrafiltration; *RAS*, return activated sludge; *WAS*, waste activated sludge.

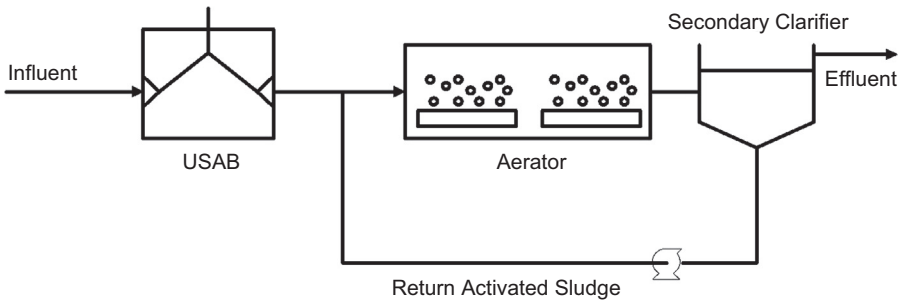

FIGURE 6.10 Upflow anaerobic sludge blanket (*UASB*) and aerobic processes in treating optoelectronic wastewater.

Table 6.2 Optoelectronics Wastewater Treatment Processes and Removal Efficiency [5]

	A2O + MBR	A/O/A/O + MBR	UASB + Posttreatment
COD	98.3	88.7	88.7
TN	98.9	42.1	42.1
TKN	99.2	85.7	85.7
NH_4^+-N	99.8	91.0	91.0
TP	97.5	20.2	20.2
PO_4^{3-}-P	97.3	15.2	15.2

A2O + MBR, Anaerobic, anoxic, and aerobic plus membrane bioreactor process; *A/O/A/O + MBR*, anoxic, aerobic, anoxic, aerobic, and membrane bioreactor; *COD*, chemical oxygen demand; *UASB*, upflow anaerobic sludge blanket; *TN*, total nitrogen; *TKN*, total Kjeldahl nitrogen; *TP*, total phosphorus.

A comparison among these three full-scale processes is presented in Table 6.2 [5]. It seems that A2O + MBR processes could achieve the highest performance.

Some lab-scale research on optoelectronics wastewater treatment has been published. A membrane-coupled methanogenic and facultative bioreactor at bench-scale could treat TFT–LCD wastewater [25]. Laboratory-scale UASB reactors have been shown to be resistant to TMAH at 10,000 mg/L and it can be completely degraded and converted to biogas at a maximum volumetric loading of 7.03 kg TMAH/m^3 day for wastewater from a full-scale TFT–LCD manufacturer [26]. Lei et al. [27] demonstrated that aerobic degradation of TMAH could be achieved in an aerobic and anoxic–oxic sequencing batch reactor (SBR), but aerobic conditions were more effective than anoxic conditions. Two biological activated carbon reactors in series and a biofilter could remove volatile organic compounds, such as IPA and 2-propanone, from semiconductor wastewater. The COD removal rate was higher than 97% at an HRT of 24 h [28].

It is noted that MBR technology (Fig. 6.11) finds wide applications among some representative TFT–LCD manufacturers in Taiwan, particularly at large scale (>5000 m^3/day). The MBR is a very promising technology that boasts a global growth

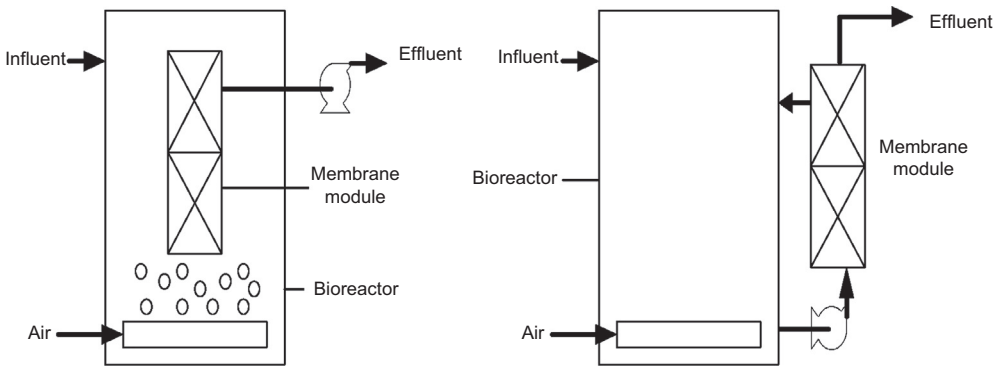

FIGURE 6.11 Configuration of a typical membrane bioreactor process.

rate over 10% annually; nevertheless, large or small, it is mostly used for municipal wastewater treatment [29]. Several problems exist in the operation of electronics wastewater treatment using MBR. First, engineers and technicians are not experienced in this because MBRs were not commonly found previously in Taiwan for municipal wastewater treatment, not to mention the large-scale design and operation for industrial wastewater. In the early stages, operation was not very smooth. Second, some chemicals, such as photoresists and developers, are viscous and tend to foul membranes easily. Third, scaling was troublesome when segregated collection of wastewater was not practiced, because phosphate precipitates, such as calcium phosphate and struvite, formed in pipes and membrane surfaces. Nevertheless, full-scale operation may provide valuable experience in MBR technology as applied in treating industrial wastewater.

In addition, anaerobic MBRs (anMBRs) have been utilized at full scale for treating optoelectronics wastewater in Taiwan. The high biomass retention of anMBRs can ensure high-rate anaerobic treatment (Fig. 6.12). It has a competitive advantage in land-limited countries because of its high volumetric loadings [30]. AnMBRs are effective in the treatment of a wide variety of wastewater types, from municipal wastewater to industrial wastewater; however, the results so far are mostly from bench-scale studies with no description on the industrial scale [31]. Characterized by lower sludge filterability, membrane fouling is more severe than in MBRs. Membrane fouling and other limitations have been discussed for full-scale applications for industrial wastewater treatment [32].

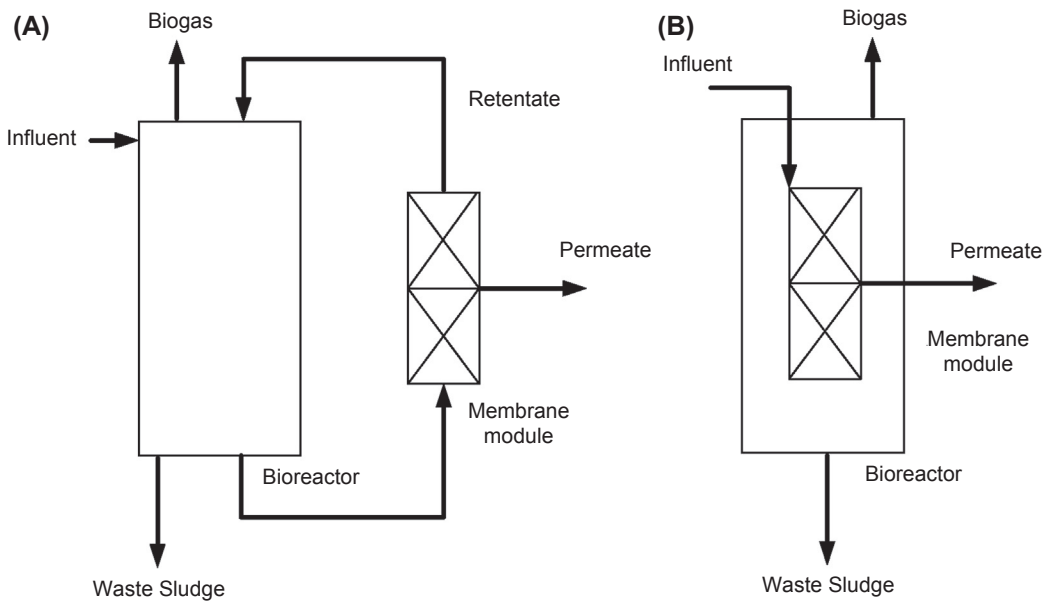

FIGURE 6.12 A schematic of an anaerobic membrane bioreactor (*anMBR*) configuration. (Left) Sidestream anMBR. (Right) Submerged anMBR.

Some lab-scale studies on optoelectronics wastewater treatment have been published. Nitrogen in optoelectronics wastewater that contains 567 mg N/L of ammonia, 7 mg N/L of nitrate, and 572 mg/L of TKN can be removed by the simultaneous partial nitrification, anaerobic ammonium oxidation, and denitrification process in a lab-scale SBR. The system was stable with high TN removal efficiency (93%) and was suitable for high-strength optoelectronics wastewater [33,34]. Satisfactory removal of N and P from electronics wastewater was found in a coupled photo-MBR in which *Scenedesmus* sp. LX1 was cultured [35]. The addition of a carbon source to an anaerobic–aerobic SBR pilot improved the phosphorus removal for semiconductor and optoelectronics wastewater [36].

6.4 Physicochemical Processes

Various physicochemical processes have been used for pretreatment or treatment of electronics wastewater. Phosphate and ammonium in semiconductor fluoride-containing wastewater can be recovered as struvite under appropriate conditions [37]. Phosphate could be selectively removed from semiconductor wastewater using magnesium salts, such as magnesium chloride ($MgCl_2$) or magnesium hydroxide ($Mg(OH)_2$) at the appropriate pH range [38]. A hybrid precipitation–MF process effectively removed phosphate and fluoride from TFT–LCD wastewater [39]. Calcite could also remove phosphate and fluoride from TFT–LCD wastewater [40]. Advanced oxidation processes have been applied to electronics wastewater treatment, mostly for organic contaminants degradation in the wastewater. Ozone in microbubble form enhanced oxidation of DMSO [41]. A fluidized-bed Fenton process could remove 98.9% of the MEA of TFT–LCD wastewater [42]. An electrodialysis process can be used for recycling and concentrating TMAH from optoelectronic wastewater [43]. Graphene oxide has been shown to be very effective for TMAH adsorption, with two times the adsorption capacity of activated carbon [30]. Strong acid-cation exchange resin was more effective than a weak acid-cation resin in removing TMAH from electronics wastewater [44]. Fe- and Al-immobilized catalytic degradation of acetone and IPA in electronics wastewater was examined and a high efficiency was found [45]. The simultaneous reduction of nitrate, hydrogen peroxide, and phosphate could be achieved by a zero-valent iron process [46]. For the effluent from semiconductor manufacturers in science parks, membrane processes, including UF and RO, could polish it for reuse purposes [47].

6.5 Future Perspectives

Waste minimization is an important task for the electronics industry, and there are some successful cases. Waste solvents are mostly reclaimed and reused. Taking IPA as an example, it used to be transported together with spent photoresist and other concentrated organic wastes to cement companies and used as auxiliary fuel in rotary kilns.

However, IPA is now mostly separated from other wastes and reclaimed on-site by distillation or membrane distillation. Ammonia-containing wastewater from a leading semiconductor manufacturer in Taiwan is treated with activated carbon, pH adjustment, and membrane contact, followed by addition of waste H_2SO_4 to make 30% ammonium sulfate ($(NH_4)_2SO_4$) for resource recovery at a daily production rate of 24,500 kg/day. The 30% $(NH_4)_2SO_4$ can be further converted to 21% ammonium hydroxide (NH_4OH) and reused at the production rate of 10 m^3/day. Except for the aforementioned fluorite recovery from fluoride-containing wastewater via crystallization, the CaF_2 sludge is widely reused as raw materials in cement, ceramics, and tile industries in Taiwan [48].

Rainwater harvesting systems (RHSs) are widely established in most semiconductor and optoelectronics manufacturers in science parks. The rooftops of fabricators are ideal for RHSs. The rainwater is treated by simple filtration before use as cooling water makeup, raw water for UPW systems, and irrigation. A representative TFT–LCD manufacturer is equipped with an underground water tank with a capacity of 20,000 m^3.

Vast quantities of UPW are required for both semiconductor and TFT–LCD manufacturing. Cost savings, local or regional water limitations, strategic consideration, and hard lessons in the past have motivated reduction, reclamation, and reuse of water in the electronics industry. For efficient and cost-effective use of water in processing, some successful water conservation techniques have been developed, such as rinse optimization (rinse tank design, idle time, water flow rate, and temperature), segregated collection and treatment of different streams of wastewater, and reclamation and reuse of rejection and regenerants in UPW systems. Water is such a critical issue that recycling and reuse of UPW is a financial necessity for the electronics industry. Some representative companies are keen on the issue. For example, the Taiwan Semiconductor Manufacturing Company claimed that by lowering water consumption and increasing the recycling rate, its water usage per wafer has become a benchmark for global peers and has led Taiwan's semiconductor companies to achieve the lowest average water consumption in the world [49]. Intel Corporation examined its water footprint in semiconductor manufacturing [50]. It is the policy of the Taiwan government to require manufacturing plants in science parks to recycle and reuse >85% of process water and >70% of total water. Most plants meet the requirements, and interestingly, plants with larger water demand performed better in water recovery [3]. Through years of development, water reuse has become a key issue of the electronics industry. Taking rejection from RO units of UPW systems as an example, it is used in water scrubbers for air pollution control, regeneration of the UPW system, cooling water makeup, toilet flushing, and green plant irrigation.

In general, electronics wastewater is treated effectively and efficiently. The water reuse rate is much higher compared with conventional industries, such as petrochemical, pulp and paper, and electroplating. Still, some difficulties exist for treatment of electronics wastewater. The operation of MBRs faces membrane fouling and scaling problems, and the maintenance of a stable operation of the system in a cost-effective way needs experience and continuing research and development. In addition, how to

minimize the water footprint in the electronics industry will still be the biggest challenge as the line-width of semiconductors becomes smaller and even larger amounts of UPW are needed. It highlights the importance of integrated water management for the sustainable development of the electronics industry. It is also an important topic and challenge for water professionals to explore in the near future.

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7

Aerobic Treatment in Cold-Climate Countries

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7.1 Introduction

Treatment of wastewater effluents in cold-climate regions is one of the greatest challenges for environmental and municipal engineers. Significant diurnal, short-term, and longer-term seasonal temperature changes occur frequently in many areas of the world, including northern Europe, Asia, and North America. In the near future, many of these regions will require increasingly efficient treatment systems that are designed to operate even under cold-temperature conditions to meet increasingly stringent effluent discharge guidelines and minimize detrimental effects on receiving environments. These challenges may be largely addressed by the implementation of carefully designed and integrated aerobic biological processes. Although considered robust, the biological treatment processes required for the degradation of organic compounds, nitrification, and denitrification, as well as enhanced phosphorus and pathogen removal, are very sensitive to temperature changes. Microbial activity in a biological wastewater treatment process decreases significantly when the water temperature decreases below 15°C, becomes particularly low at temperatures lower than 10°C, and will exhibit almost no physiological activity at temperatures below 4°C [132].

This chapter aims to demonstrate the effectiveness of aerobic biological treatment of various wastewater effluents under cold climate conditions. The effects of cold temperatures on the performance of both conventional and eco-engineered systems in the removal of biologically treatable constituents are discussed. Finally, aerobic treatment cold-region design considerations and future perspectives for the enhanced performance and widespread implementation of these systems are be recommended.

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7.2 Aerobic Treatment Systems in Cold-Climate Regions

7.2.1 Conventional Systems

Biological treatment of wastewater effluents in conventional systems is typically accomplished by biological processes that can be classified as either suspended- (e.g., activated sludge) or attached-growth (e.g., trickling filter, biofilm) systems. However, traditional suspended-growth processes, such as activated sludge, can exhibit performance limitations when subjected to high hydraulic and organic loads or low temperatures. Two strategies can be applied to increase the performance of activated sludge systems under these conditions: increase in aerobic reactor volume or increase in biomass concentrations inside the aerobic reactor [29].

Scherfig et al. [96] examined the effects of temperature variations on biological unit processes such as nitrification and denitrification. Using US EPA-defined temperature-dependent kinetic coefficients, they estimated that a decrease of 2°C, from 9 to 7°C, would require an increase of 20% and 16% in required reactor volumes, respectively, to maintain nitrification and denitrification treatment performance. In addition to the reduction in biological activity, chemical reaction kinetics and viscosity are also considered in these systems, as they may influence other chemical and physical processes upon which these biological systems are dependent [68,96,118]. Low temperatures also strongly influence adsorption and sedimentation processes, composition of biofilm populations, and oxygen transfer efficiencies in systems requiring aeration [122]. Often, the main difficulty in overcoming these limitations, however, is related to the process capacity, in that expanding clarifier volumes can be logistically difficult or cost-prohibitive. As such, alternate approaches have been developed to overcome treatment limitations associated with temperature fluctuations related to cold-climate operations.

Sundaresan and Philip [102] investigated the performance of three different types of aerobic processes: activated sludge, fluidized bed, and submerged bed. Once the systems had been acclimatized at 35°C and reached steady state, the operating temperature was reduced stepwise from 30 to 5°C. Although the acclimatization time for the submerged bed reactor was longer than for the fluidized bed and conventional activated sludge processes, the submerged bed reactor was generally found to be more robust and efficient. All reactors performed well in terms of constituent removal, as long as the operating temperature was maintained at or above 15°C. At 10°C, the submerged bed reactor exhibited higher removal efficiencies for organic constituents. Once a temperature of 5°C was reached, the treatment efficiencies of the activated sludge and fluidized bed systems were negligible. However, the submerged bed system was still active and was able to regain 90% of its original efficiency once the temperature was raised to 10°C [102], suggesting that submerged bed reactors offer a viable alternative for treating wastewaters in cold-climate regions.

Cold-weather nitrification with a trickling filter/solids contact process has been demonstrated for temperatures as low as 10°C. Moreover, this demonstration was with a medium-density cross for the trickling filter step lower than previously required. Parker et al. [85] investigated the performance of a novel trickling filter/solids contact (TF/SC) treatment scheme in constituent removal under cold climates with average wastewater

temperatures below 10°C. The system benefited from the low concentrations of biodegradable organics in the wastewater and higher density cross-flow media than had previously been applied in the TF/SC process to achieve nitrification [85].

Effective solids reduction, partial nitrification, and biological phosphorus removal have been demonstrated in cost-effective sequencing batch reactors (SBRs) operated using alternating anaerobic/aerobic cycles under cold temperatures [43,47,128–130]. Solids retention time (SRT) control was noted as the most important strategy for cold-temperature operation to achieve nitrification [43,47]. Similarly, the availability of sufficient substrate in the form of volatile fatty acids was noted as the key factor for enhanced biological phosphorus removal [128–130].

Another strategy, referred to as the integrated fixed-film activated sludge (IFAS) process, involves the combination of both types of biomass into one reactor through the introduction of suspended biomass into the aeration/anoxic tank [28,29]. Such systems have been reported to sustain high organic removal rates, as well as nitrification and biological phosphorus removal throughout the winter, without the need to increase suspended biomass concentration and hence, for a corresponding increase in aerobic or clarifier volumes. In the IFAS systems, the attachment media can be either fixed or freely moving inside the reactor [27,29]. One such alternative is to couple the Kaldnes moving-bed biofilm reactor (MBBR) process with a conventional activated sludge process, in which biofilm growth is promoted on small carrier units that are kept in suspension throughout the reactor [26]. Operational advantages of MBBRs compared to fixed-biofilm carrier systems include lower head loss, minimal channeling, and no need for periodic backwashing. MBBRs can also be operated at lower SRTs than conventional activated sludge systems, at low temperature and high organic loading rates, while sustaining nitrification [28].

In other studies, anaerobic pretreatment was demonstrated to be beneficial for biological nitrogen removal. The efficient removal of organic constituents and dissolution of particulate organic matter may enhance nitrification as it may reduce the competition between carbon-utilizing heterotrophic microorganisms and autotrophic nitrifiers [65]. This may be more pronounced at low temperatures as more dissolved organic matter is retained in the anaerobic effluent for denitrifiers. Sequencing batch operation of MBBRs has been examined, along with the application of an MBBR as a posttreatment process following anaerobic wastewater treatment. For nitrogen removal, MBBRs have usually been operated in series with aerobic (nitrifying) and anoxic/anaerobic (denitrifying) units in separate reactors or in a single reactor with alternating aerobic and anoxic phases, through the application of intermittent aeration. The combined two-phase upflow anaerobic sludge blanket (UASB)–septic tank and intermittently aerated MBBR achieved >70% of all constituents, including cold-temperature operation [65].

De Kreuk et al. [24] examined the formation of aerobic granules and their effects on biological conversion processes in an aerobic granular sequencing batch airlift reactor under moderate and low temperatures. They noted that once a reactor was acclimatized at higher temperatures and had reached steady state, it was then possible to operate a stable aerobic granular sludge system at lower temperatures (10°C) [10,102]. The aerobic granules were found to enhance nitrification, although nitrification rates were inhibited

to some extent at low temperatures. Conversely, phosphorus removal efficiency was affected by low temperatures and the carbon/P ratio to a lesser extent [10].

Another cold-climate adaptation strategy has been the introduction of cold-adaptable microorganisms with different carbon sources. These microorganisms have been investigated for systems operated at temperatures as low as 5°C treating synthetic wastewater. A 16S rRNA analysis indicated that *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Collimonas fungivorans* fed with glucose, and *Variovorax paradoxus* and *Acinetobacter* sp. fed with sodium acetate, were dominant, most of which belong to the gram-negative bacteria [81,104].

7.2.2 Naturalized Systems

Another important consideration is that while conventional wastewater treatment technologies are reliable and cost-effective for municipalities serving large populations, they represent a less suitable alternative for small, remote, and rural communities, which are often located in cold-climate regions. In many of these communities, alternative passive, semipassive, or eco-engineered treatment systems, including natural and constructed wetlands, lagoons, wastewater stabilization ponds, bioretention systems, rock filters, and reed beds, have been implemented [1]. They have been proven to be economical alternatives to conventional wastewater treatment practices and are widely used in remote regions by virtue of their ease of operation, minimal energy input, reduced maintenance requirements, and improved sludge thickening [1,13,21,121]. For example, approximately 90% of pond systems in the United States are used in small communities with fewer than 10,000 people and have been shown to be very efficient in treating wastewaters [35]. However, these systems also typically rely on biological processes and, hence, will be influenced by climatic conditions, with greater responses to changes at lower temperatures (<15°C) than for at optimal range of 20–35°C [55]. Although used extensively, concerns associated with the long-term performance and functional reliability of these systems in cold climates compared to conventional, but less sustainable, treatment systems have been raised [50,53,55,94,119,123].

Critical factors limiting the widespread establishment of these eco-engineered treatment technologies have been the lack of a standardized understanding of the effects of temperature on treatment process mechanisms within these systems and a more robust basis of comparison within and between systems as they evolve with time [109]. A number of operational strategies have been proposed to improve treatment efficiencies in cold climates, which generally fall under one of the following categories: improvement of system design and commissioning, optimization of cold-temperature operation, and incorporation of pre- and posttreatment technologies [49,109,111,113].

In the range of available naturalized treatment system approaches, (natural) treatment wetlands are generally considered the least engineered and least energy-intensive alternatives. A number of the biogeochemical processes that govern the removal of organics and nutrients in naturalized systems are affected by temperature, which is

thereby expected to influence overall treatment efficiencies [55]. In the past, they have been used extensively in cold-temperature climates and have been shown to perform very well in polishing primary and secondary wastewater effluents [4,56,66]. These systems, often referred to as tundra wetlands, have also been used extensively in arctic communities [4,41,53,56,126]. Tundra wetland treatment systems are often located in naturally occurring wet depressions on the tundra. Arctic systems, servicing populations of <2500 [53], often treat continuously discharging wastewater from retention lagoons or raw wastewater discharged directly into the wetland, although seasonally decanted systems are also in place. Treatment wetlands inherently have site-specific physiogeographic characteristic that influence plant communities and water retention, which in turn influence the treatment of wastewater discharged into the systems [17,126].

Despite their demonstrated natural ability to act as sinks and transformers in the treatment of wastewaters, the use of natural wetlands to treat wastewater in developed countries has declined. Kadlec and Wallace [56] recommended that this practice be limited to retain their value as part of the landscape and environmental ecosystems, which could be enhanced by reducing extrinsic pollutant loadings. Instead, the use of constructed wetlands, which utilize similar biogeochemical treatment approaches, has been implemented. These systems provide a higher level of control, providing higher treatment efficiencies and more consistent and reliable performance when subjected to colder climates and higher pollutant loadings. However, it should be noted that a number of natural wetlands continue to be employed in temperate and cold-climate regions to polish wastewater from lagoons or conventional treatment facilities [4].

Constructed wetlands include both free-surface, which more closely mimic natural wetland systems, and subsurface flow operational configurations; and these can include both vegetated and nonvegetated systems. Using 4 years of performance data from a free-water surface constructed wetland receiving dairy wastewater in Nova Scotia, Jamieson et al. [48] noted that reaction rate constant values for biochemical oxygen demand in 5 days (BOD_5), total phosphorus, total Kjehldahl nitrogen (TKN), NH_4^+-N, fecal coliform, and total suspended solids (TSS) did not appear to be influenced by temperature or solar radiation, but were positively correlated with the hydraulic loading rate for most parameters, emphasizing the effects of dilution on outlet pollutant concentrations and the importance of wetland hydrology and its influence on overall performance [40]. In studies, the beneficial role of substrate and vegetation in the overall performance of a number of these systems has been reported, despite the short growing season often associated with cold-region systems [2,34,36,41,56,59,60,97–99,105,109,113,126].

Wastewater stabilization pond (WSP) systems, also referred to as maturation or polishing ponds and lagoons, depend on the biogeochemical transformations, as well as physical and hydraulic transport processes, within the pond. For these systems, temperature has been regarded as the most important physical factor influencing performance efficiency [45,56,69,91]. In addition to the physical and biogeochemical treatment processes previously noted, more recently, studies have examined the role of

algae in oxygen, carbon, and nutrient cycling, as well as disinfection control, within these systems [74,114,115]. Algal carbon requirements can be met by a combination of CO_2 released through the oxidation of organic compounds by heterotrophic bacteria and inorganic carbon uptake, whereas photosynthesis provides much of the oxygen needed for BOD removal [91].

Johnson et al. [48] compiled cold-climate performance data for pilot-scale waste stabilization ponds with subsequent naturalized treatment approaches: two tertiary maturation ponds in series, two tertiary maturation ponds in series followed by a reed bed channel, a control rock filter, an aerated rock filter, and a constructed wetland. In general, the maturation ponds and reed bed channel, and the control and aerated rock filters, operated successfully at low temperatures during the UK winter period. It was noted that the maturation ponds and reed bed channel system required a larger land area, whereas the aerated rock filter would require energy input (aeration) [53].

7.2.3 Hybrid Systems

For the context of this chapter, hybrid systems refer to the integration of conventional or mechanical and passive systems, with the aim of enhancing the performance of passive treatment systems. There are tangible benefits that could be associated with the implementation of hybrid systems to improve the winter or cold-temperature performance of naturalized systems. Hybrid aeration pretreatment/constructed wetland systems have been designed and implemented for successful winter/cold-temperature operation in horizontal-flow, vertical-flow, and integrated (combination of horizontal- and vertical-flow treatment cells) constructed wetland treatment systems [53,59,60,66,67,84,98,99,113,120].

Another form of hybrid system adapted for cold-region applications includes an anaerobic pretreatment stage (e.g., septic tank) followed by a vertical-flow aerobic biological filter or vertical-flow constructed wetland [50,75]. In these systems, each treatment cell is typically buried in the ground or landscape, which allows the system to be insulated from extreme climatic conditions or large temperature fluctuations [99]. These types of decentralized hybrid wastewater treatment systems are particularly applicable in remote or cold-region resort communities, where systems may be operating at varying capacities throughout the year [75].

More sophisticated hybrid systems have also been investigated, primarily involving the use of aerated submerged biofilm (ASBF) reactors to enhance the performance of shallow wastewater treatment lagoons or WSPs. The ASBF reactors are designed to encourage the establishment of a nitrifying bacteria biofilm on a submerged surface. The system configuration is designed to maximize contact between rising air bubbles and the submerged biofilm, which is believed to increase the oxygen transfer rate into the biofilm as well as the flux of water, nutrients, and waste products in and out of the biofilm [16]. The rationale for adopting this type of hybrid configuration is that biofilm systems are noted to greatly increase the bacterial mass-to-volume ratio, and hence SRT, in the treatment system, which reduces reactor volume requirements.

7.3 Effects of Cold Climate on Treatment Performance

7.3.1 Organic Matter

Biological treatment processes have been reported to yield poor removal efficiencies of organic constituents in cold regions because microbial activity is typically inhibited at low temperatures. The first-order model has been traditionally employed for predicting removal rates of organic matter in most traditional wastewater treatment processes, with the modified Arrhenius relationship typically used to adjust the removal rate coefficient for temperature [77]. Values of θ range from 1.00 to 1.08, with typical values of 1.04 for activated sludge, 1.08 for aerated lagoons, and 1.035 for trickling filters [77]. However, the responses to temperature fluctuations in conventional and naturalized systems are different as are the approaches to mitigating their effects.

7.3.1.1 Conventional Systems

In conventional wastewater treatment systems, changes in treatment performance have largely been correlated with fluctuations in influent wastewater temperatures, which subsequently affect the biological treatment stage. Sunderesan and Philip [102] examined the effects of temperature (5–35°C) on chemical oxygen demand (COD) removal efficiency for three types of reactors (activated sludge, fluidized bed, and submerged bed) using both synthetic wastewaters and domestic wastewater. They reported that COD removal efficiencies were generally good above 15°C and that most of the systems failed at 5°C. Although the acclimatization time was longer for the submerged-bed reactor, it was generally more robust and efficient compared to the fluidized-bed and activated-sludge processes. At 10°C, the submerged-bed reactor achieved 40% COD removal efficiencies and the fluidized-bed and activated-sludge reactors achieved only 20% removal efficiencies. At 5°C, the submerged-bed reactor exhibited 20% COD removal efficiencies, but was able to regain 90% of its original efficiency once the temperature was raised to 10°C. The COD removal efficiencies of the three reactors were also noted to be higher with synthetic wastewaters than with actual domestic wastewater, which was probably due to the presence of inhibitory substances that would be present in the more complex domestic wastewater [102].

A study of a municipal wastewater treatment plant receiving industrial dyeing effluent containing acid black 1 (AB1) illustrates the potential influence of the presence of inhibitory constituents in wastewater, particularly under cold-temperature conditions [73]. Effluent from a dyeing industry (24–73 mg/L) was combined with domestic sewage and fed to SBRs at 7 and 22°C. COD removal was found to decrease by 50% in dye-bearing wastewater at 7°C and by 20% at 22°C. In addition, the presence of AB1-bearing wastewater led to a general deterioration of the activated sludge process at 7°C, in which excessive foaming and the presence of filamentous bacteria were observed. The recovery period of the activated sludge process was also noted to be longer at the colder operating temperature [73].

The use of MBBRs has been proposed for the treatment of wastewaters with high organic and nutrient loadings. These have been investigated in configurations following anaerobic pretreatment [65], or to enhance an activated sludge process [28,29]. Following the anaerobic pretreatment of dairy wastewater (10°C) and a mixture of black water and kitchen waste (20°C), MBBRs were reported to exhibit 40–70% COD removal efficiencies, whereas continuous- and sequencing-batch operations yielded similar performances. The combination of a UASB as a pretreatment unit, followed by a septic tank with MBBR, provided 92% COD and 99% BOD_7 removals, respectively [65]. When employed in an activated sludge MBBR process combination, the presence of the biofilm (MBBR) would allow the system to operate and maintain system performance at lower temperatures (>9°C) and lower SRTs or higher organic loading rates than the comparable activated sludge process alone [28,29].

ASBF reactors can be used to enhance the performance of activated sludge processes or shallow wastewater treatment lagoons through the addition of specially designed structures that force the direct contact of rising air bubbles against a submerged biofilm [16]. This direct gas-phase contact is believed to increase the oxygen transfer rate into the biofilm and increase the microclimate mixing of water, substrate, nutrients, and waste products into and out of the biofilm. Specifically, the effects of cold temperatures (3.4–6°C) on the removal of COD were investigated for a batch system. In general, heterotrophic bacteria were found to consume the bulk of the COD in the first 8–16 h of the batch test even at lower temperatures, provided sufficient oxygen was not limiting. Wastewater BOD/NH_4-N ratios ranged from 1.9 to 57.5, and wastewaters with a higher BOD/NH_4-N ratio typically sustained larger heterotrophic bacteria populations and smaller autotrophic bacteria populations leading to higher organic removal rates [16]. Xu et al. [122] developed an enhanced physicochemical–biological process to improve the pollutant removal efficiencies under cold-temperature conditions (−30°C ambient outdoor temperature). Micromembrane filtration has been combined with sequential anaerobic–aerobic biofiltration processes. The micromembrane filtration step greatly increased the fraction of dissolved organics and decreased the subsequent biological treatment load, which reduced some of the operational challenges and increased the wastewater treatment efficiency at cold temperatures. Average soluble COD removal efficiencies were increased to 86% when treated using micromembrane filtration and a polyaluminum chloride coagulant dosage of 50 mg/L [122].

Hence, the incorporation of biofilm or submerged-bed configurations with conventional activated sludge processes could be a beneficial adaptation for wastewater treatment facilities endeavoring to meet high effluent discharge standards with biological treatment processes in cold-climate regions. Other considerations would include oxygen limitations. In addition, the study by Martin et al. [73] highlighted the importance of ascertaining inhibition at the lowest operating temperature in the design and testing of biological treatment processes.

7.3.1.2 Naturalized Systems

Conventional unit processes differ considerably from naturalized systems in terms of functional complexity and operating conditions. They are designed to provide intense focus on microbial processes with limited design consideration for other biotic and abiotic components or the spatial heterogeneity of an eco-engineered system [55]. Naturalized treatment technologies for organic constituent removal range from overland flow involving very shallow (a few centimeters depth) water flow over vegetated surfaces, to natural and constructed wetlands involving a variety of vegetated and nonvegetated systems with various surface and subsurface flow configurations and depths of less than 1 m, to WSPs, which typically represent algal systems with typical depths of 1−2 m [55].

Because naturalized systems usually operate in a relatively uncontrolled environment compared with conventional treatment facilities such as activated sludge plants, the efficiency of these systems is expected to change with the climatic conditions. Temperature is often considered the most important physical factor influencing the efficiency of naturalized systems, as it affects the metabolic rate of the microorganisms in the system and thus the rate of degradation of organic matter and subsequent stabilization of inorganic nutrients [69,90]. However, similar (<10% difference) or consistent organic (BOD, COD, total organic carbon (TOC)) removal rates throughout all seasons of operation have been reported in a number of studies investigating these types of systems operated in cold-climate regions, including natural and constructed wetlands [48,55,66,67,84,98,99,105,113], reed beds [34,97], and wastewater stabilization/maturation ponds [44,99,130]. The seemingly limited influence of temperature on overall performance can be attributed to the size and complexity of these naturalized systems, in which effects on known microbial temperature sensitivity are simply dampened by other factors [55]. These masking effects are not always as apparent in temperature-controlled mesocosm or laboratory-scale studies in which the understanding of fundamental processes is targeted, whereas they become more evident in field-scale studies.

Mæhlum and Stålnacke [67] investigated the influence of temperature, flow rate, and influent concentrations over a 3-year period on the treatment efficiency of organics in three integrated horizontal subsurface flow constructed wetlands treating domestic wastewater in Norway, with particular focus on aerobic pretreatment in vertical-flow filters and the treatment efficiency during winter. Aerobic pretreatment followed by constructed wetlands including P sorption media (sand and Filtralite wetlands) removed most organic matter (BOD >80%). Differences in treatment efficiencies between seasons were generally less than 10% and no statistical differences in treatment efficiencies as a result of water temperature could be detected [67]. It was surmised that temperature effects were partially compensated for by the large hydraulic retention time (14 days) and aerobic pretreatment [66,67].

Solano et al. [97] evaluated the treatment performance of a pilot-scale subsurface-flow constructed wetland for the BOD and COD removal from raw municipal wastewater characteristically derived from small villages. They reported high BOD and COD

removal for all treatments studied, including two hydraulic loading rates (150 and 75 mm/day) and two macrophytes [cattail (*Typha* sp.) and reed (*Phragmites* sp.)]. After 2 years of operation a significant correlation was observed between hydraulic loading rate and performance, with the best removal being obtained by those beds receiving the lowest hydraulic loading rate and highest retention time (3 days). High removals were achieved for all the treatment beds without any significant relationship to plant species or to plant biomass (cattail or reed). No seasonal differences were found in BOD and COD removals. According to that finding, removal efficiency could be improved by increasing the retention time [97].

In a study by Ouellet-Plamondon et al. [84], investigating the effects of aeration and vegetation on the treatment performance of wetland mesocosms operated under cold-temperature conditions, average COD removals above 90% were noted for all treatments except for nonvegetated and nonaerated mesocosms, which showed 88% removal. During the summer (25°C), there was a slight increase in COD removal in vegetated compared to nonvegetated mesocosms, but aeration did not enhance removal efficiencies, regardless of whether the system was vegetated or not. In winter (7°C), the expected reduction in COD removal in nonaerated mesocosms was completely compensated for with a notable improvement in corresponding aerated mesocosms, for both the vegetated and the nonvegetated systems. Additional oxygen during cold-temperature operations probably counterbalanced the reduction in biological kinetics resulting from the low temperature and plant dormancy [84].

In a similar study, the suitability of using a subsurface-flow reed bed constructed wetland followed by a duckweed lagoon as a nutrient polishing pond for treating domestic wastewater from small communities in economically underdeveloped cold regions of Iran was investigated by Gholikandi et al. [34]. The reed bed pilot-scale system included four basins, of which two were vegetated reed beds and two were nonvegetated controls. The artificial reed bed constructed wetlands exhibited an average removal of 89% BOD and 78% COD, with further removal of 20% and 10% of BOD and COD, respectively, in the duckweed lagoon.

The presence of plants provides well-documented benefits in treatment wetlands; however, the effects of different species on year-round and seasonal performance are generally not clearly understood. Taylor et al. [105] evaluated the influence of plants on seasonal COD removal in batch-fed (synthetic secondary wastewater) microcosms simulating subsurface-flow treatment wetlands. Nineteen plant species were studied over a 20-month period with temperatures ranging from 4 to 24°C. An average COD removal of 70% was reported for the controls, while removals ranged between 70% and 97% for the individual species. Most plants were noted to enhance COD removal significantly, particularly at temperatures of 4−8°C. It was also found that COD removal decreased at low temperatures in the nonvegetated controls, but exhibited limited seasonal variation in the vegetated microcosms, where removals did not differ for 15 of the species. Two species showed significant negative correlations, better removal at colder temperature, *Carex nebrascensis* and *Carex utriculata*. Species that showed the

highest overall performance were generally in the sedge and rush families (Cyperaceae and Juncaceae), whereas species exhibiting lower treatment performance were largely in the grass family (Poaceae) [105]. The high COD removals throughout the study were strongly associated with high SO_4^{2-} concentrations, particularly at low temperatures, which would suggest that plant performance was probably related to oxidation in the rhizosphere of the plants and their abilities to promote aerobic over anaerobic microbial processes, particularly during colder operational periods.

As previously noted, wastewater stabilization or maturation ponds generally represent aerobic/anaerobic or facultative nonvegetated algal systems with typical operating depths of 1–2 m [55]. In a 1-year study conducted by Rockne and Brezonik [91], carbon C flux through a three-pond wastewater stabilization system operated in a cold region (Minnesota, USA) with an average of 4 months of ice cover was examined. A 90% overall soluble carbonaceous biochemical oxygen demand (sCBOD) removal was reported, with most of the removal occurring in the primary pond. The C balance demonstrated that algal carbon requirements were met by a combination of CO_2 released by bacterial oxidation of organic matter in the wastewater influent, as well as inorganic carbon in the wastewater influent and air–water CO_2 mass transfer. In return, algal photosynthesis provided much of the oxygen needed for heterotrophic sCBOD removal in the primary pond. In effect, soluble organic matter in a pond system can be rapidly oxidized to CO_2 by heterotrophic bacteria, and this inorganic carbon is not factored into the organic carbon measurements until it is assimilated by algae. Hence, it was argued that observed organic carbon removal values do not necessarily reflect true removal efficiencies of influent-derived organic carbon, particularly in the summer, because a large portion of the TOC outflow from the ponds was likely to be algal mass, whereas wastewater influents typically do not contain algae. This conclusion was supported by high TOC concentrations in the secondary pond, even though 90% of the influent sCBOD had been removed in the primary pond.

Mansouri et al. [69] investigated the significance of differences in the seasonal changes in various water quality parameters including COD and BOD_5 in a cold-climate region WSP. The variation of these parameters followed seasonal temperature trends, in which maximum removal efficiencies of COD (76%) and BOD_5 (85%) were noted in the summer, whereas minimum COD and BOD_5 removal efficiencies were observed in the spring (59%) and winter (66%), respectively. However, data analysis revealed that there were significant differences in these water quality parameters between the four seasons in both the influent and the final effluent concentrations [69]. This was attributed to the presence of algae in these systems, particularly during the summer period in which extended photoperiods may prevail in some cold climate regions. *Microcystis aeruginosa*, *Synechococcus*, and *Synechocystis* are typical cyanobacteria species occurring in natural ecosystems and have also been shown to be predominant species in a WSP [80].

Interestingly, Rockne and Brezonik [91] also noted that ~90% of influent TOC was removed during the ice-cover period, which is counterintuitive given that modeled organic carbon removal rate kinetics [44] are expected to decrease considerably at

temperatures expected during ice cover (2−5°C), as previously noted. However, temperature effects were probably dampened by the extended hydraulic retention time (120 days) from the onset of ice cover to its melting, which may allow sufficient time for biodegradation even at reduced rates. Most of the organic matter in the secondary pond consisted of algal biomass and not influent-derived organic material.

Hence, the incorporation of aeration, vegetation, and algal biomass, as well as extended hydraulic retention time, in naturalized treatment system design configurations should be considered viable alternatives for enhancing treatment system performance in cold-climate regions. Extended hydraulic retention times or reduced organic loading rates have been shown to significantly dampen the effects of temperature [55,67,91,97]. It has been reported that factors that enhance electron acceptor availability or enhance root zone oxidation activity can be at least as important as temperature in facilitating organic matter removal [2,3]. Studies have shown that as oxidation decreases, the amount of accumulated residual inert organic matter increases and aggregates in the filtration matrix, which ultimately affects hydraulics through the soil matrix, consequently reducing hydraulic retention time [42,100] and the effectiveness of biological treatment [84]. Increasing oxygen availability with artificial aeration could enhance mineralization and reduce the hydraulic clogging due to increased organic matter accumulation. Thus, for organic matter removal, the incorporation of vegetation as well as artificial aeration in constructed wetlands could be beneficial in winter or cold-climate operation, particularly when vegetation is dormant [84,105].

7.3.2 Nitrogen

The processes leading to nitrogen removal from wastewater primarily involve bacterial transformations. Nitrification refers to the oxidation of ammonium $\left(NH_4^+\right)$ to nitrate $\left(NO_3^-\right)$ by nitrifying bacteria and can occur only under aerobic conditions. Conversely, denitrification is an anaerobic decomposition process in which organic matter is broken down by bacteria using nitrate instead of oxygen as an electron acceptor; nitrate is first reduced to nitrous oxide, which is subsequently further reduced to atmospheric nitrogen (N_2) [112].

Nitrification is the most temperature-sensitive biological process in wastewater treatment, not only with respect to colder temperatures (<10°C), but also in terms of temperature fluctuations [43,46,47,92,129]. Therefore, nutrient removal performance can be difficult to sustain during colder temperature operations. Bacterial nitrifying populations are functionally classified as chemolithotrophic ammonia-oxidizing bacteria (AOB), which oxidize NH_3 to NO_2^-, and nitrite-oxidizing bacteria (NOB), which convert NO_2^- to NO_3^-. The NO_3^- is then converted to nitrogen gas through the anaerobic denitrification process. AOB and NOB share a close symbiotic relationship with one another because of the high toxicity of NO_2^-, forming densely packed microcolonies in wastewater treatment systems [49]. Different populations of AOB and NOB typically coexist in wastewater treatment environments, but changes in temperature can significantly alter the composition of these communities, which ultimately affects nitrification efficiency

[92]. Other factors that can also negatively affect AOBs include pH reduction, low dissolved oxygen (DO) concentrations (<3 mg/L), toxic compounds, solids retention time (SRT) and high organic loads.

7.3.2.1 Conventional Systems

The risk of cold shock is a phenomenon that is particular to conventional wastewater treatment systems operated in cold climate-regions. These can result from rapid snow melt, a common occurrence in the late fall and early spring in cold-temperate climate communities. Sudden, daily temperature fluctuations of 5−7°C are not uncommon [43]. Hwang and Oleszkiewicz [47] characterized the effects of temperature on nitrification, considering both the effects of rapid temperature decreases and those of a gradual temperature decrease. They noted that a sudden temperature decrease affected nitrification much more than predicted using the accepted temperature correction factor (1.072), with a 10°C decrease (20−10°C) yielding a >20% decrease in specific nitrification rate. Conversely, the change in nitrification rate could be predicted fairly accurately for a gradual temperature decrease. It was concluded that in the case of a sudden temperature decrease, the overestimated nitrifier growth rate might cause the washout of the autotrophic organisms; hence, it is essential that measures be in place to avoid nitrifier washout should such conditions arise [47]. A number of studies have been undertaken to develop process strategies to overcome shortfalls in nitrogen removal performance.

Head and Oleszkiewicz [43] introduced the bio-augmentation of nitrifying bacteria for short-SRT nitrification as a relatively low-cost alternative for wastewater treatment facilities operated in cold climates. They suggested seeding a cold SBR with nitrifying bacteria from the anaerobic sludge digestion dewatering liquor or centrate acclimatized at 20°C. The rationale was that the seeded SRTs of the cold SBRs would then be raised above the minimum SRT required for nitrification. Decreases in nitrification rates were observed, however, and complete NH_4^+ removal could be realized as long as seeding to the cold SBRs was sustained [43].

Next, Yuan and Oleszkiewicz [130] developed an SBR process that could achieve partial nitrification and biological phosphorus removal. The study showed that partial nitrification could be achieved at low temperature as long as high DO concentrations (>3 mg/L) were sustained. Controlling SRT was proposed as the operational strategy for successful partial nitrification of an SBR operated under cold-temperature conditions (15°C). From the pilot-scale testing, it was noted that shorter SRT for NOB than for AOB would lead to NOB washout due to the NO_2^- substrate limitation at the beginning of the aeration cycle in the SBR [129,130]. The long-term effects of temperature on partial nitrification were examined by Guo et al. [38]. A larger activation energy (111.5 kJ/mol) was determined for AOB at lower temperatures of 5−20°C than at higher temperatures of 20−35°C (42.0 kJ/mol). As the activation energies of AOB and NOB as well as their sensitivities to temperature changes are distinctly different, this would imply that AOB could outcompete NOB at the higher temperatures and that the rate-limiting step for ammonia oxidation is not identical at different temperature ranges [38].

Another strategy that has been employed to overcome reductions in nitrogen removal at colder temperatures is the use of attached-growth systems, such as trickling filters. Gullicks and Cleasby [37] and Parker et al. [85] examined the nitrification performance of a pilot-scale, separate-stage trickling filter plant and a TF/SC process, respectively. In general, nitrification performance was diminished at low temperatures (10°C), but could be restored effectively during winter operation by introducing continuous dosing and lower hydraulic loading rates. Tower influent aeration, effluent recirculation, and forced-draft ventilation of nitrifying biofilters were also recommended to further improve treatment effectiveness.

As biofilms are generally anoxic at the interface with the attachment medium, there is the potential for denitrification to provide energy inside biofilms in aerated bioreactors should a sufficient carbon source be available, which would be beneficial in the design of bioreactors for nitrogen removal under cold-temperature operation [58]. MBBRs were introduced as part of an innovative activated sludge/attached-growth alternative for operation under cold temperatures and low SRT [28,29,65]. Luostarinen et al. [65] achieved complete nitrification through intermittent aeration, but noted that denitrification was limited by low carbon availability. In essence, Di Trapani et al. [28] introduced suspended carriers, which move freely inside the reactor volume [27], into the aeration/anoxic process to facilitate biofilm attachment and growth in a process referred to as IFAS. This type of process could be used to upgrade conventional activated sludge facilities with the aim of reaching their nitrification objectives under cold temperature operation, without the need of additional volumes. The performance of a pilot-scale, hybrid MBBR process was investigated for operation at relatively low SRT and temperature, with a particular focus on nitrification. Batch tests were conducted to assess nitrification in both the suspended- and the attached-growth systems, to understand the role of each biomass system in the nitrification process. Biofilm nitrification was noted to be higher than that of the suspended sludge, which was not negligible and significantly higher than in a simple activated sludge reactor operated under the same conditions. This was attributed to the seeding effect of nitrifiers from the biofilm to the mixed liquor, which would have contributed to an increase in the apparent nitrification activity of the entire system [28]. In a subsequent study, biofilm nitrification activity was found to increase with decreasing mixed liquor SRT, suggesting that hybrid reactors could be operated with low mixed liquor SRT values, even at low temperatures, and achieve high ammonium removal efficiency [29].

One strategy to compensate for low denitrification rates at low temperatures is to enhance the biomass concentration in the reactor. This can be achieved by introducing more low-temperature-tolerant nitrifying biomass [107,124,125] or by immobilizing the biomass in polymeric matrices [107]. Immobilization of biomass brings additional advantages such as easier separation from the treated wastewater, controlled retention time of the biomass in the system, and protection of the biomass. A psychrotrophic heterotrophic nitrifying–aerobic denitrifying bacterium was isolated by Yoa et al. [124] and identified as *Acinetobacter* sp. It exhibited excellent tolerance to low temperature

from 20 (optimum) to 4°C, facilitating efficient ammonium, nitrite, and nitrate removal at low temperature, under solely aerobic conditions, with little accumulation of intermediates. Next the nitrogen removal performance and metabolic mechanisms of the strain were further investigated under aerobic conditions at 10°C. *Acinetobacter* sp. HA2 was capable of heterotrophic nitrification and aerobic denitrification at low temperature (10°C), efficiently removing ammonium, nitrite, and nitrate [125]. These developments hold promise for future adaptations facilitating nitrification and denitrification for wastewater operations in cold-climate regions.

7.3.2.2 Naturalized Systems

Nitrogen removal in naturalized systems is challenging because of the complexity of the nitrogen cycle, as well as variations of influent nitrogen species. Vegetation, season, temperature, and hydraulic loading most likely influence root zone oxygenation and consequent nitrogen removal, especially for NH_4^+-rich wastewater [2,56]. Nitrogen removal in the form of NH_4^+ or NO_3^- can take place through uptake by algae, heterotrophic organisms, and vegetation. The sequence of mineralization, nitrification, and denitrification is often rate limited by nitrification as the slowest step, whereas denitrification is usually not rate limiting [30,55,112]. Hence, nitrogen removal is often governed by nitrification, which requires aerobic conditions. These aerobic conditions can be facilitated through photosynthetic activity by algae and macrophytes, atmospheric oxygen transfer, and passive and artificial aeration [2,12,55,84,91,98,99,133].

A significant portion of dissolved organic nitrogen can be returned to the water column during breakdown of microorganisms, vegetation, or soil organic matter. Nitrogen release during decomposition is typically higher at warmer temperatures. However, so are nitrogen uptake by algae and vegetation, and nitrification and denitrification processes, which can therefore present confounding effects [55]. NH_4^+ and NO_3^- removal exhibits a strong seasonality in naturalized systems, and nitrate removal has been reported to be much higher in warmer seasons [55,120]. Temperatures approaching 10°C are low enough to partially inhibit nitrate reduction in naturalized systems; however, if the carbon source is sufficient, the nitrate removal rate can be sustained at an appreciable level [55].

In open systems such as wetlands, lagoons, and ponds, seasonal differences in nitrogen removal may be influenced by NH_3 volatilization due to pH increases and uptake resulting from algae growth [41,56,91,114,115]. Significant nitrogen losses resulting from ammonia volatilization can take place particularly when ammonium concentrations are high and the pH rises above 8.0, often due to algal growth, shifting the NH_4^+/NH_3 equilibrium further to the volatile nonionized form. In the absence of algae and related pH increases, the NH_4^+/NH_3 equilibrium shifts further to the nonvolatile ionized form [41,91,114,115].

Allen it al [2]. and Ouellet-Plamondon et al. [84] observed that plant presence and species had a greater effect on total nitrogen and TKN removals, respectively, in wetland

systems than temperature or residence time, achieving approximately twice the nitrogen removal of nonvegetated controls regardless of season and temperature. *Carex* typically outperformed *Typha* and *Schoenoplectus* with less temperature dependency [2]. Artificial aeration was found to improve summer and winter TKN removal in nonvegetated systems, but the additional aeration did not fully compensate for the absence of plants. Ouellet-Plamondon et al. [84] argued that the role of plants extended beyond the addition of oxygen and most likely supported the growth of a more diversified and active microflora in the rhizosphere, confirming the positive effect of aeration (artificial or photosynthetically derived) on nitrifying bacteria.

A number of researchers have argued that the effect of direct nutrient uptake by plants could be significant only when wastewater nutrient and organic loadings are low [2,12,36,84]. In an attempt to address the issues of long-term and cold-climate performance, as well as the importance of plants to overall effectiveness of constructed wetland systems, Gottschall et al. [36] evaluated the contribution of nutrient uptake by emergent macrophytes to total mass nutrient removal in a well-established constructed wetland treating agricultural wastewater in a cold climate. As the wetland was NH_4^+-dominated, there was preferential uptake of NH_4^+ over NO_3^-, and total uptake, as well as biomass, increased with increasing NH_4^+ in the wastewater [36].

Attached-growth biological treatment systems were also shown to be an effective alternative to sustain cold-temperature nitrification as temperatures as low as 4°C. Despite the lack of consistent or prolonged periods of nitrification at low temperatures within suspended-growth treatment systems operated under similar conditions, there is evidence that attached-growth nitrification processes have the potential to achieve ammonia removal at low temperatures for extended periods of time [5,16,25,39].

Biological aerated filters (BAFs) are a unit process that can accommodate both wastewater recirculation and maintenance of high biomass concentrations. BAFs may be added as a treatment unit after aerated lagoons or used directly as a secondary treatment unit. They are generally more compact than other attached-growth processes such as nitrifying trickling filters. In a lagoon system upgrade with a gravel BAF, Ha et al. [39] reported that approximately 95% of NH_3-N could be nitrified with an HRT of 2 h for wastewater entering at an influent temperature of 6.5°C. However, they noted that by recirculating 200% of the effluent back into the BAF for an HRT of 1 h, NH_3-N removal could be improved from 54% to 92% at 6.5°C. Delatolla et al. [25] characterized the effect of cold-temperature exposure time on nitrifying biofilm and nitrifying biomass of BAF implemented as a lagoon system upgrade. They demonstrated that attached-growth AOB and NOB populations were capable of surviving exposure to 4°C and that the AOB population remained fairly consistent throughout the 4-month exposure. The NOB population was noted to decrease significantly with exposure to 4°C, but was maintained for the entire 4-month study [25]. These studies demonstrated that a BAF with recirculation could be employed as an add-on technology to improve nitrification under cold-temperature operations.

The application of psychrophiles in cold-region wetlands has gained interest since 2005. Individual strains (NL01, NL02, NL03) of cold-tolerant bacteria that can sustain nitrification activity and, hence, NH_4^+ removal efficiencies at temperatures below 15°C were investigated by Ying et al. [127]. Moreover, it was also noted that, under the same conditions, a mixture of the three strains was more robust under harsher environmental conditions. Ducey et al. [30] reported on the application of an acclimated lagoon nitrifying sludge (ALNS) capable of high rates of nitrification at temperatures ranging between 5 and 20°C. ALNS was used to inoculate attached- and suspended-growth nitrification processes and consistently exhibited rapid bioreactor start-up and excellent NH_4-N removal performance under cold-temperature conditions. It also formed large flocs that could be settled rapidly, producing a high-quality effluent. Characterization showed that the AOB community was dominated by *Nitrosomonas*, which appeared to form a symbiotic relationship with other cold-tolerant organisms capable of using the accumulated nitrite for nitrogen assimilation and energy production via reduction pathways [30].

Feng et al. (2012) designed a shallow moss constructed wetland (SMCW), constructed with moss (*Bryum muehlenbeckii*) and ornithogenic soil (inoculum) collected from polar regions to enhance nitrogen treatment at cold temperatures (5−20°C). They noted that the highest DO concentrations were detected around moss roots and were significantly higher than in the control wetland. The dominant AOB population characterized in the SMCW included *Nitrosococcus mobilis*, *Nitrosomonas eutropha*, and *Nitrosomonas marina*, which was also different from that in the control wetland. They attributed the SMCW capacity to maintain the nitrification rate at low temperatures to the cold-adapted and tolerant AOB population inoculated from ornithogenic soil and the high oxygen transfer to the moss root zone (Feng et al., 2012).

7.3.3 Phosphorus

7.3.3.1 Conventional Systems

Phosphorus tends to accumulate in aquatic systems because there are no significant gaseous loss pathways; therefore, removal or retention in these treatment systems is regulated by chemical (adsorption, complexation, precipitation), physical (sedimentation, filtration), and biological mechanisms (uptake and release by vegetation, periphyton, and microorganisms). Traditionally, phosphorus removal from wastewater effluents in conventional configurations has largely been through adsorption and chemical precipitation and sedimentation. However, since 1995, a number of studies have focused on elucidating biological phosphorus removal and, more recently, on the effect of temperature on the biological removal pathways and mechanisms.

Biological phosphorus removal (BPR) is essentially a two-stage process, in which microorganisms, known as phosphorus-accumulating organisms (PAOs), pass from an anaerobic stage, in which they store the phosphorus, to an aerobic stage, in which it is released [108]. Ideally, the anaerobic phase is deficient in nitrate $\left(NO_3^-\right)$, nitrite $\left(NO_2^-\right)$,

and DO, and when readily biodegradable carbon substrates such as short-chain volatile fatty acids are available, they are stored as poly-P-hydroxybutyrate (PHB) in the cells. When the system is aerated and aerobic conditions are resumed, the PHB stored in the cell is metabolized and used as an energy source, and thus released back to the aqueous phase [72]. A number of microorganisms, including *Acinetobacter* spp., have been reported to be able to store and release excess phosphorus during anaerobic−aerobic conditions. The efficiency of BPR in conventional treatment systems such as activated sludge processes and SBR is temperature dependent, which can affect its performance and reliability in cold-climate regions [62].

In a 1-year full-scale study, cold-climate BPR was investigated at a wastewater treatment plant equipped with a conventional activated sludge process retrofitted to an SBR, in a small village near the Arctic Circle [72]. Wastewater temperatures typically varied between 3 and 10°C and was below 5°C for approximately 240 days of the year. Effluent soluble phosphorus concentrations with the SBR adaptation were generally <1.0 mg/L for wastewater temperatures above 5°C, but BPR was significantly impeded at temperatures below 4°C [72].

Nitrate has been shown to have a negative effect on anaerobic-phase P release as denitrifiers compete with PAOs for readily biodegradable carbon substrates. It then reasonably follows that denitritation, the second step of denitrification, which also produces NO_2^- and which requires a readily biodegradable carbon substrate, may negatively affect anaerobic-phase P release. Using an SBR system, Yuan and Oleszkiewicz [128] demonstrated that the addition of nitrite alone did not make a significant difference in P-release rate and, in fact, that PAOs were strong competitors for the carbon source with the microorganisms involved in NO_2^- removal. Hence, they concluded that the conversion of NO_3^- to NO_2^- was the key step necessary for the denitrifiers to successfully outcompete PAOs for carbon source. With respect to P uptake, they noted that the aerobic P-uptake rate did decrease with increasing NO_2^- concentration and therefore that aerobic P uptake by PAOs was more sensitive to NO_2^- than anaerobic P release [129]. In addition, BPR and partial nitrification (nitritation) were also achieved at low temperature (15°C), but the availability of short-chain fatty acids was the key to successful P release and subsequent P uptake [129,130].

It has also been shown that the alternating anaerobic and aerobic conditions that promote P uptake and accumulation as intracellular polyphosphate also favor another group of microorganisms known as glycogen-accumulating organisms (GAOs). These microorganisms may also compete with PAOs for the available organic substrate without contributing to P removal [131], and their presence may lead to BPR deterioration [82]. Temperature is one of the key parameters affecting the performance of BPR systems because of its impact on the PAO/GAO competition and community composition [14,64]. Lopez-Vazquez et al. [64] noted that PAOs were the dominant microorganisms at 10°C, because the metabolism of GAOs was inhibited, whereas at 20°C, the growth of PAOs was favored over GAOs only under high pH (>7.5) conditions, and at 30°C, GAOs tended to dominate the competition with higher substrate uptake rates.

Wang et al. [116] demonstrated that BPR from wastewater could be achieved through an aerobic/extended-idle (A/EI) process, suggesting that a strict anaerobic phase, as provided in the anaerobic/oxic (A/O) process, was not critical and that an extended idle period of 210−450 min could achieve similar removals. Chen et al. [15] investigated the effect of temperature (5−30°C) on BPR induced by the A/EI regime. Phosphorus removal induced by the A/EI regime depended strongly on temperature, in that efficiency increased with increasing temperature from 5 to 20°C but decreased when temperatures were increased further to 30°C, and the highest P removal (97.1%) was obtained at 20°C. They demonstrated that the composition of PAOs and GAOs in BPR sludge shifted with the variation of temperature [14,64,132]. The phosphorus removal efficiencies of A/EI SBRs and traditional A/O SBRs were compared at 5 and 20°C by Chen et al. [15]. The results showed the A/EI process yielded higher phosphorus removal efficiencies than the A/O process at both 5 and 20°C, and in both cases, PAOs were more abundant than GAOs, which might be the principal reason for the higher BPR in the A/EI SBRs. Furthermore, successful BPR operation has been observed at temperatures as low as 5°C [14,31]. Studies have demonstrated that the A/EI process could accommodate higher nitrate concentrations [117]. Hence, the higher tolerance for nitrate of the A/EI process might also be a contributing factor in the higher BPR observed in the A/O SBRs [15].

7.3.3.2 Naturalized Systems

Phosphorus in influent wastewater is present in soluble and particulate forms, with both forms containing a certain fraction of inorganic and organic phosphorus constituents, which is dependent on the type of wastewater. For example, municipal wastewater may contain a large fraction (>75%) as inorganic phosphorus in soluble forms. As naturalized systems offer less operational control than their more conventional counterparts, phosphorus behavior is sometimes more challenging to predict. Phosphorus removal in naturalized systems is largely governed by abiotic retention mechanisms including adsorption, complexation, precipitation, sedimentation, entrainment, and entrapment [55,93]. These processes are not as susceptible to temperature conditions as systems that rely on biological processes. A study by Tang et al. [104] examined the effect of cold-resistant bacteria (*Pseudomonas flava* WD-3) seeding of an integrated vertical-flow constructed wetland system on the removal phosphorus during winter operation and found that at high organic substrate loadings, *P. flava* WD-3 dosage and removal rates were positively correlated. However, whereas BPR may still occur under the appropriate environmental conditions, it should be noted that its net contribution to overall phosphorus removal is likely to be overshadowed by other processes.

A number of studies have reported that phosphorus retention is not significantly affected by temperature in cold-climate wetlands [55,67,91]. Kadlec and Reddy [55] suggested that phosphorus removal is likely to be affected by translocation to belowground biomass and slow rate of decomposition of detrital tissue during the cold-temperature season, whereas throughout the warmer seasons, phosphorus removal

is governed by more rapid biological uptake and release during decomposition. Hence, specific removal trends are not always readily apparent owing to the complexity of these systems. The lack of correlation between phosphorus removal and temperature would also suggest that the primary removal mechanism is a physical process [55,67].

The rate of adsorption is largely controlled by the pH and redox conditions of the system, adsorptive surface area (active iron and aluminum or calcium carbonate), and temperature. For instance, under aerobic, neutral to acidic conditions, Fe(III) binds phosphates in stable complexes, whereas under anaerobic conditions, Fe(III) can be reduced to Fe(II), which has a lower adsorption affinity for phosphates and can subsequently lead to their release [112]. Inorganic phosphorus is retained by oxides and hydroxides of iron and aluminum in acid soils and by calcium carbonate in alkaline soils. Similarly, the adsorption of phosphates to calcium occurs only under basic to neutral conditions. However, this can be subject to variability in systems in which the pH fluctuates diurnally as a result of algal photosynthetic activity [41,55]. In addition to the reversible nature of the adsorption process due to these environmental factors, substrates are subject to saturation as each will exhibit a specific adsorption capacity, and when the sites are occupied, no further removal will occur.

Phosphorus uptake by vegetation is expected to be at its highest during the peak growing season, followed by decrease and cessation in the fall and winter. Water column phosphorus can be removed via periphyton uptake. Phosphorus present in detrital plant tissue and algal biomass is rapidly released to the water column during decomposition. However, during long-term periods, a significant fraction of organic phosphorus may remain in the sediment, remain relatively resistant to microbial breakdown, and be considered an important phosphorus sink [55,91]. Both uptake and release are expected to be higher in the spring and summer. The increase in phosphorus flux with temperature suggests that mechanisms of phosphorus release in sediments may be regulated by biological activity including the root zone [66]. For systems that rely on vegetation for P removal (e.g., floating macrophyte mats for *ortho*-phosphate removal) in cold-climate regions, treatment efficiencies have generally been reported to be highly variable throughout the year (0−99.6%), with lower efficiencies during the late fall and higher efficiencies throughout the spring and summer season [109].

7.3.4 Solids

While it is widely accepted that temperature plays a significant role in the performance of wastewater systems, as lower temperatures slow reaction rates and bacterial growth and activity, some studies have also demonstrated that the effectiveness of solids removal can also be affected in both conventional [96] and, particularly, passive treatment systems [120]. As such, various design strategies have been implemented to improve treatment efficiency, including solids removal.

In a study by Xu et al. [122] an enhanced physicochemical−biological wastewater treatment process including micromembrane filtration, anaerobic biofilter, and aerobic

biofilter was developed to improve removal efficiencies under winter operational conditions in northeast China. In particular, micromembrane filtration was found to significantly decrease operating difficulties and increase wastewater treatment efficiency under colder conditions. Full-scale experiments were conducted with outdoor temperatures as low as −30°C. Using micromembrane filtration and a polyaluminum chloride coagulant dosage of 50 mg/L, average suspended solids (SS) removals of 95.8% were achieved.

Mæhlum and Stålnacke [67] examined the influence of temperature, flow rate, and input concentrations on the treatment efficiency of three integrated horizontal subsurface-flow constructed wetlands treating domestic wastewater. The study focused on aerobic pretreatment in vertical-flow filters, filter media with high phosphorus sorption capacity, and treatment performance during winter operations. No statistical differences in the treatment efficiencies due to water temperature were detected, with reported differences generally less than 10%. It was surmised that temperature effects could be partially compensated for by larger hydraulic retention times. The study [67] also reported poor SS removal in the wetland systems with iron-rich ferrohumic podzol sand.

In a 2-year pilot-scale study, Solano et al. [97] assessed the role of macrophytes (*Typha* sp.) and reeds (*Phragmites* sp.) and surface loading rates in a subsurface-flow constructed wetland treating raw municipal wastewater. No significant differences were observed between cattail and reed performance. In addition, no seasonal differences were found in TSS removal, with the exception of winter, during which time removals were significantly lower, although percentage removals never fell below 40%. However, under cold-temperature conditions, higher removals were obtained under lower surface loading rate (150 and 75 mm/day) conditions. A similar study by Gholikandi et al. [34] investigated the feasibility of using subsurface-flow constructed wetland systems followed by a duckweed lagoon as a polishing stage to treat domestic wastewaters in small cold-region communities. The pilot-scale system included two planted reed beds and two nonvegetated controls, followed by the duckweed lagoon. Notably higher TSS removals (92%) were obtained in the reed bed, with a further reduction of 24% in the duckweed lagoon.

A study by Ouellet-Plamondon et al. [84] showed no apparent differences in TSS removals between planted and unplanted horizontal subsurface-flow constructed wetlands during either summer or winter. This was corroborated in studies by Jamieson et al. [49], Mozaheb et al. [80], and Mansouri et al. [69], who actually noted higher removals in the winter (78%) than in the summer (63%). However, Ouellet-Plamondon et al. [84] did observe improvements in TSS removal for both seasons in aerated mesocosms. They achieved significantly higher removals (>95%) than those reported in previous studies (75−85%), suggesting that aeration may have reduced solids accumulation by increasing degradation kinetics and prevented clogging from the initial stages of the pilot-scale process through physical mixing. It should be noted, however, that in many open passive systems there are significant differences in the influent and in the

effluent TSS concentration throughout the different seasons in cold regions and temperate climates. The higher performance observed in the winter may be due to a lower concentration of algal cells [41,80] and heterotrophic organisms in the effluent compared to the summer period, when extended photoperiods are often also experienced in a number of cold-climate regions.

7.3.5 Pathogens

Disinfection is generally the last process in wastewater treatment prior to discharge to a receiving environment or to a distribution network for water consumption or reutilization. It aims to minimize public health risks as a result of exposure to pathogen-contaminated wastewater, as well as enabling water reuse in receiving environments [79]. Removal of a wide spectrum of pathogenic organisms, such as bacterial, viral, protozoan, and helminthic pathogens is often required or expected.

While disinfection methods [ultraviolet (UV) irradiation, chlorination, and ozone] are often applied in conventional and more energy-intensive treatments, they target the removal of only pathogenic bacteria and viruses, not helminth eggs and protozoan (oo) cysts, as these microorganisms behave different from bacteria and viruses and are resistant to these disinfectants [51,52]. Some studies have suggested that passive treatment systems, such as WSPs, may remove up to 6 log units of bacteria and practically all protozoan and helminth eggs, producing a final effluent that meets the WHO guidelines and recent revisions of the use of treated wastewater in agricultural unrestricted irrigation. The reported performance of these systems was generally higher than that observed in conventional treatment processes, such as activated sludge or primary treatments, from which reductions of 1–2 log units for bacteria and 70–99% for protozoan and helminth eggs were noted. Several factors such as sunlight, pH, DO, attachment, sedimentation, retention time, predation, and presence of other organisms were found to contribute to the removal of pathogenic organisms [79,106].

Pond systems have generally been shown to be effective in the removal of pathogens. In previous work by Reinoso et al. [89,90], 100% removal of protozoan pathogens (*Cryptosporidium* and *Giardia*) was reported, as well as 96% and 98% average reductions in *Cryptosporidium* oocysts and *Giardia* cysts. In other studies, up to 6 log units of bacteria, up to 5 log units of viruses, and 99% helminth ova removals were observed in similar pond systems [51,52,89,106]. A number of studies have attempted to explain the factors responsible for pathogen reduction in passive wastewater treatment systems. Jensson et al. [50] noted that a high level of indicator bacteria and <1000 thermotolerant coliforms/100 mL could be achieved in high-performance constructed wetlands consisting of septic tanks, biological filters, and subsurface wetlands in series in Norway under both summer and winter operating conditions.

Typically, an optimally working biochemical treatment process may achieve 90–99% microbial reduction, but in some cases, poor reductions are observed and treated effluents could contain high numbers of fecal microorganisms [90]. Pathogen removal is

considered a complex process involving various mechanisms and factors, such as sunlight, pH, photooxidation, DO, temperature, predation, attachment/sedimentation, and starvation [9,33,54,76,89]. Removal efficiencies of pathogenic microorganisms during wastewater treatment can be inconsistent, and have been shown to vary with treatment process [7,106]. Hence, the roles of these factors, and their relationships, will be discussed further.

7.3.5.1 Sunlight

Sunlight has a lethal impact on coliforms and the die-off rate is proportional to sunlight intensity [18,20,54]. Various wavelength regions of the solar spectrum, such as the UV spectrum (290−400 nm) and photosynthetically active radiation (PAR) (400−700 nm), have been shown to contribute to disinfection in wastewater [13]. A number of studies have investigated the role of sunlight in pathogen inactivation. However, there is debate in the literature regarding the relative contributions of UV-A, UV-B, and visible wavelengths to sunlight disinfection [13,18,20,22].

UV radiation is commonly considered the most potent bactericidal component of sunlight. The UV-B (290−320 nm) range is known to disinfect by directly damaging the DNA, RNA, and other cellular constituents of microorganisms, even those resistant to antibiotics, in processes called direct photoinactivation [13]. However, sunlight is attenuated with depth as it penetrates the water column. In particular, higher light frequencies, such as UV light, are quickly attenuated within the first few centimeters of water and, thus, may not contribute significantly to overall pathogen removal at greater depths [20,54]. Instead, longer wavelengths (PAR spectrum) can penetrate much deeper into the water column, hence their effect can potentially be more important [54]. Kohn and Nelson [61] reported that more than 99% of UV-B light at 290 nm was absorbed in the first 2.5 cm of a WSP, whereas over 99% of visible light at 556 nm was absorbed in the first 8 cm. Sunlight gets attenuated more readily when the water is more turbid owing to higher solids and/or microorganism and/or organic matter concentrations. WSP disinfection can be greatly reduced in algal pond systems with increasing depth, as light can be attenuated by both the water and the algal biomass [110,111]. Therefore, where disinfection is desirable, pond and wetland systems designed for disinfection should be shallow to allow sunlight penetration throughout the depth of the water column.

Disinfection mechanisms vary depending on the wavelength region primarily owing to their differences in energy. In addition to direct photoinactivation induced by exposure to the UV-B spectrum, UV-A (320−400 nm), UV-B, and PAR spectra can also indirectly inactivate microorganisms through photooxidation. Photooxidation is a process in which sensitizers absorb light and transfer this energy to other molecules, leading to the formation of reactive oxygen species (ROS), which react with microorganisms and consequently cause them damage. Photooxidation takes place when ROS are produced by endogenous and exogenous sensitizers as well as by other reactions such as Fenton's reaction [13,19,61]. Endogenous sensitizers, such as flavins and porphyrin derivatives, are found inside the cell of microbes, whereas exogenous sensitizers, such as humic

substances, photosynthetic pigments, and dissolved organic matter, are present in the aquatic environment outside the cell [19,61]. Disinfection as a result of sunlight damage in pond systems has been characterized by three mechanisms: direct damage to DNA by UV-B radiation (280–320 nm), indirect endogenous damage caused by UV-B radiation, and indirect exogenous damage involving the UV-A (320–400 nm), UV-B, and visible spectra up to 550 nm [22,23]. However, it is currently unclear to what extent each of these mechanisms, and the presence of photosensitizers, contributes to disinfection in pond and wetland systems.

7.3.5.2 pH

pH plays an important role in pathogen removal in most wastewater treatment systems. Curtis et al. [20] described high pH as a critical contributor to disinfection because it both increased the rate of photooxidation and made the most penetrating wavelength of light bactericidal. They stated that bacterial cell membranes are the most likely sites of action for exogenously reduced O_2, peroxides, superoxides, and hydroxyl radicals. Observations of similar *Enterococcus* removal efficiencies achieved under both sunlight exposure and dark conditions demonstrate that pH itself is an important mechanism for pathogen removal [8].

In pond and wetland systems, aquatic photosynthesis involving CO_2 uptake and O_2 release often leads to increases in pH and DO concentration [32]. In fact, excessively high pH levels can often be observed in these systems, with values frequently varying diurnally within a range of 7–9.4 [13,18,103]. Neutral to slightly acidic pH conditions (6.5–7.5) have been reported as optimal for fecal bacteria growth [8], whereas pH levels higher than 9 have been found effective for the removal of indicator organisms [6,8,22]. Fluctuations in pH have also been shown to negatively affect the survival of *Escherichia coli* [8].

7.3.5.3 Dissolved Oxygen

Removal of fecal bacteria has been shown to be directly related to DO concentration [18]. As previously noted, oxygen is produced as a by-product of algal photosynthesis, which is a source of oxygen supply in WSPs. Sweeney et al. [103] observed elevated DO concentration of 30 mg/L in pond systems during the summer period. Photooxidation requires the presence of oxygen to form ROS. Therefore, an increase in DO concentration would increase the effect of photooxidation. Light inactivation of *E. coli* and *Enterococcus* increased with increasing levels of DO [19,83]. Endogenous photoinactivation of *E. coli* and enterococci was strongly dependent on DO [23,83].

Being facultative anaerobes, fecal coliforms are able to survive a wide range of DO concentrations. However, they have been found to survive for longer periods under anaerobic conditions than under aerobic conditions. In a study by Marais [71] it was found that aeration enhanced fecal coliform die-off rates. Kaneko [57] also observed that the removal of polioviruses, bacteriophages, and coxsackie virus B3 was enhanced by aeration. Davies-Colley et al. [23] noted that the inactivation of F-DNA virus was

independent of DO concentration, whereas the inactivation of F-RNA virus increased with increases in DO [9]. Conversely, there is very little information available regarding the effect of DO concentration on protozoa and helminth eggs.

7.3.5.4 Temperature

Although optimal rates of bacterial growth are typically restricted to small temperature ranges, bacterial organisms are generally able to survive within broader temperature ranges. A number of studies have reported increased removal efficiencies of fecal coliform with temperature increase [11,63,78,83,86,87,88]. Conversely, Mara et al. [70] concluded that there was no direct relationship between fecal coliform die-off and temperature, as a tertiary pond exhibited higher levels of fecal coliform removal than anaerobic and facultative ponds when operated at the same temperature. In a 2-year pilot-scale constructed wetland study, Solano et al. [97] reported highly variable total coliform, fecal coliform, and fecal streptococci removals, but notably higher reductions in the summer and fall seasons. In both of these studies, factors other than temperature may have contributed to the higher removal efficiency, including vegetation density and type [97,101], hydraulic loading rate [97], and aeration [70], which would support the argument that pathogen removal in pond and wetland systems is likely to be driven by a combination of mechanisms and factors. However, temperature effects on viral, protozoan, and helminthic pathogens have not been investigated extensively.

7.4 Cold-Climate Aerobic Treatment Design Considerations

Aerobic wastewater treatment in cold-climate regions presents unique challenges to ensure that effluents meet required discharge standards. Treatment processes must not only address low-temperature operation efficiency, but also, in some cases, location remoteness, population size, and availability of technical expertise. Processes that have been adapted to overcome these challenges include decreasing the organic and/or nutrient load; increasing the solids and hydraulic retention times; modifying flow configurations, effluent recirculation, or aeration; incorporation of vegetative cover; and dosing with low-temperature tolerant microorganisms.

7.4.1 Flow Configuration

Many environmental factors exhibit seasonal or diurnal cycles that influence whole system performance. Water temperature is one of the important cyclic factors, but flow rates and concentrations into conventional and naturalized systems also affect biogeochemical cycles and can contribute to the observed trends in nutrient and pollutant removal. Various flow configuration strategies can be employed to mitigate the effect of the fluctuations on overall system performance.

A common operational challenge in cold-climate regions is the fact that small-scale domestic wastewater treatment facilities are common. Furthermore, operational stresses

resulting from hydraulic shock loads or fluctuations in constituent loadings are more frequent at small plants than at larger facilities. Hence, they tend to be more vulnerable to the effects of flow fluctuations. As such, there is a need for unit operations or processes to be stable and self-adjusting. For conventional systems using activated sludge treatment, SBR configurations have been beneficial in terms of overcoming the challenges associated with these fluctuations. A batch method offers the advantage of simple and automatic hydraulic time-controlled treatment, which can be configured within a single reactor [72]. This process flexibility allows SBR units to be adaptable for advanced biological nutrient removal, including cost-effective partial nitrification and BPR [47,129] under cold-climate conditions, particularly with the concurrent implementation of SRT control strategies [128,130].

In naturalized treatment systems, external hydrologic inputs such as precipitation, runoff, evaporation, and evapotranspiration can have a large influence on the water budget of the system [48,56]. The less engineered the system, the more important it is to characterize these external hydrologic inputs and their influence on biological rate constants and overall treatment performance [40,41]. Owing to the nature of these systems, it is anticipated that considerations related to site hydrology will be site specific.

7.4.2 Hydraulic Retention Time

In a number of naturalized systems, temperature effects can often be compensated for with large hydraulic retention times (HRTs) [55,66,67,97], which implies lower hydraulic loadings [56], thereby affecting the apparent overall treatment performance. However, decreasing hydraulic loading rates or expanding the operational configuration of a naturalized system to achieve lower effluent concentrations at lower temperatures may not be a cost-effective operational strategy [39,48]. Longer HRTs also tend to lower system reaction rate estimates, especially for heterogeneous wastewater parameters [40,41,48]. Hydraulic loading rates and HRTs should be selected to ensure that the treatment area of a naturalized system is used to its full treatment potential [40,48,56]. This is particularly true in the operational context of conventional systems, in which system expansion is simply not a feasible option. Hence, alternate strategies must be employed to increase residence time within these systems, to enhance the treatment of organics and nutrients, in particular, under cold-climate conditions.

7.4.3 Recirculation

When providing longer retention times in treatment systems to enhance their performance under cold-climate operation may not be practical, as these systems would necessarily need to be more voluminous, one approach to increasing the time that wastewater is in contact with the biomass is to recirculate the wastewater [39]. The benefits of wastewater recirculation for winter operation has been demonstrated in treatment wetlands [56] and vertical-flow constructed wetlands [59,60,98,99,113], as well as in aerated (BAF) lagoon systems [39] and in aerated secondary treatment units

between septic tank and leach fields in decentralized systems [120]. In all cases, improved organic constituent and nitrogen removals (nitrification) were reported.

7.4.4 Enhanced Biomass Systems

The other operation factor that can assist in improving biological treatment at low temperature is to utilize more biomass [39]. Unit processes that allow for the maintenance of high biomass in conventional systems are biological aerated and/or attached-growth and/or fixed-biomass filters. The enhanced cold-temperature performance of a variety of these types of unit process adaptations has been reported and previously discussed, including trickling filters/solids contact processes [85], fluidized beds and submerged-bed reactors [39,98,99,102,113,122], MBBRs [26–29,65,95], and aerobic granules [10,24,102]. Ultimately, these attached-growth systems increase the SRT within the treatment unit, which has been noted to be one of the most effective control strategies for cold-temperature operation to achieve nitrification, BPR, and higher organic matter decomposition [43,47]. They can also be operated at lower SRTs than conventional activated sludge systems, at low temperature and high organic loading rates, while sustaining nitrification [28,102].

Another cold-climate adaptation strategy has been the introduction of cold-adaptable psychrotrophic microorganisms. *Pseudomonas flava* WD-3, *P. fluorescens*, *Pseudomonas denitrificans*, *P. putida*, *C. fungivorans*, *V. paradoxus*, and *Acinetobacter* sp., most of which are gram-negative bacteria, have been investigated for systems operated at temperatures as low as 5°C treating synthetic wastewater [46,81,104,107]. Tang et al. [104] used *P. flava* WD-3 as a cold-temperature bio-augmentation microorganism in an integrated vertical-flow constructed wetland system. Xu et al. [122] and Ying et al. [127] employed mixed psychrotrophs to treat domestic wastewater and demonstrated that cold-resistant strains were more stable and adaptable than single strains at temperatures as low as 5°C. Similarly, other researchers have explored psychrotrophic nitrifying/denitrifying organisms to enhance nitrogen removal from wastewater, where nitrification tends to be most susceptible to temperature [30,46,124,125].

7.4.5 Vegetation

The incorporation of vegetation is particularly relevant for aerobic treatment in naturalized systems, while practically nonexistent in more conventional systems. While the role of vegetation in improving treatment remains a subject of debate, its insulating benefits in cold climates have been noted [41,56,66,67,84,109,120]. Diurnal water temperatures in cold-region climates can vary by approximately 5°C for surface-flow and open-pond systems [55]. Van der Moorten et al. [109] noted that the influence of vegetation on water temperature was not necessarily that higher temperatures could be sustained during colder periods, but that a cool-down effect could be observed at temperatures above 15°C, which sustained microbial activity. A number of studies have investigated the role of vegetation in constructed and treatment wetlands, reed bed

channels, lagoons, and pond systems, but differences in the effects of individual species on annual and seasonal performance are poorly understood.

Emergent macrophytes decrease flow velocity aiding particulate settling and constituent adsorption, transport gases and solutes between aboveground shoots and root zones, take up organic and inorganic compounds, release oxygen and exudates into the rhizosphere, and influence microbial diversity and activity [56,101]. Because vegetation growth, physiology, senescence, and decay vary seasonally, their effects on treatment processes and overall system performance are expected to vary seasonally and be affected by temperature. Taylor et al. [105] investigated the effects of a wide range of plant species on seasonal trends in organic matter removal and root zone oxidation in subsurface constructed wetlands under cold-temperature operations. Species that exhibited higher cold temperature performances were largely in the sedge and rush families (Cyperaceae and Juncaceae), whereas those that performed more poorly were largely in the grass family (Poaceae).

Taylor et al. [105] also demonstrated a capacity to support high organic carbon removal at temperatures as low as 4°C, suggesting that plants, and plant species selection, may be more important in cold-climate regions with low temperatures and extended periods of plant dormancy, than in milder climates. The densities and activities of microorganisms have been reported to be more abundant on root surfaces and within the rhizosphere, contributing to organic matter and nitrogen removal [2,36,84,105,112]. Vegetation contributes to subsurface aeration by transporting oxygen into the rhizosphere, as well as actively and passively releasing exudate composed of a variety of substances that enhance microbial abundance, diversity, and activity around roots [2,105,109]. These factors are believed to contribute significantly to treatment efficacy in systems with high constituent loadings, even at colder operational temperatures.

The use of floating vegetative mats has also been investigated in cold regions. In general, the addition of mats has been shown to improve overall system performance in the removal of organic matter and nutrients, particularly at temperatures of 5 and 15°C. Although the presence of such mats limits oxygen diffusion from the air to the water column, the release of oxygen from the roots was found to be higher than oxygen diffusion from the air and was reported to stimulate oxygen-consuming reactions within the root mat [109].

7.4.6 Aeration

A number of studies have shown that the introduction of aeration, whether vegetation driven, algae driven, passive, or artificial, can greatly improve aerobic treatment of wastewaters in cold-region climates, both in conventional and in naturalized systems. In conventional systems, cold-temperature strategies have included the introduction of aerated attached-growth processes [16,28,29,39,120,122] or sequencing aerobic and anaerobic phases [130]; [43,47,65,72,129]. In naturalized systems, aeration can be achieved through vegetation [2,105,109], passive aeration facilitated by intermittent flow

[59,60,98,99,112], and artificial aeration of the system, often employed as a pretreatment process [50,66,67,84,98,99,113,120]. In most cases, aeration has been reported not only to enhance organic matter removal, but also, more importantly, to promote nitrification and BPR. Hence, this strategy will be important in cold-climate regions, as wastewater effluent discharge guidelines become more stringent and adherence to nutrient removal becomes a priority.

7.5 Conclusion

The performance of aerobic biological treatment processes is significantly influenced by wastewater temperature. Microbiological activity, which affects biological treatment, decreases markedly as water temperature decreases. Biological treatment during winter season or in cold-climate regions, hence, experiences reduced or often limited treatment efficiency. A number of treatment processes and operational strategies have been adopted for conventional systems (e.g., submerged-bed reactors, SBRs), naturalized systems (e.g., constructed wetlands, WSPs), and hybrid systems (e.g., aeration pretreatment/constructed wetlands) to overcome the limitations associated with cold-climate operation to various degrees.

The influence of cold temperature on the performance of both conventional and eco-engineered systems is mainly reflected in the removal of biologically treatable constituents, particularly organic matter, nitrogen, phosphorus, solids, and pathogens. A number of studies have investigated the removal of these constituents in both conventional (Table 7.1) and naturalized systems (Table 7.2) operating in cold temperatures. A combination of aerobic and anaerobic biological processes in conventional systems and aeration, vegetation, algal biomass, and extended HRT in naturalized treatment systems could be beneficial adaptions to improve BOD/COD removal in cold-climate regions. Nitrogen removal, through the processes of nitrification and denitrification, in conventional systems during winter operation, could be sustained through the bioaugmentation of nitrifying bacteria, as well as the use of attached-growth systems, lower hydraulic rates, extended influent aeration, effluent recirculation, and forced-draft ventilation of nitrifying biofilters. In passive systems, in addition to nitrification/denitrification, nitrogen removal can take place through uptake by algae, heterotrophic organisms, and vegetation. The presence of plants or application of a BAF or psychrophiles can all play a role in increasing the removal efficiency of nitrogen. BPR in conventional systems relies on the activity of PAOs as well as anaerobic/aerobic conditions. Temperature is one of the most important factors affecting PAO/GAO competition and dominant species. Phosphorus removal in naturalized systems is mainly achieved through chemical/physical mechanisms, which are less sensitive to temperature fluctuations than systems that are dependent on biological processes. But biological uptake and release of phosphorus can still take place in warmer seasons and contribute to overall phosphorus removal. The effectiveness of solids removal can also be affected by

Table 7.1 Summary of Studies on Conventional Cold Aerobic Wastewater Treatment

Parameters	System Setup	Scale	Wastewater Conditions	References
COD; NH_4-N, NO_2-N, NO_3-N, TKN; SS, VSS	Trickling filter	Pilot scale	Nonsynthetic wastewater; secondary effluent	[37]
BOD; TP; SS, VSS	SBR	Full scale	Nonsynthetic wastewater	[72]
PO_4; MLSS, MLVSS	SBR	Laboratory scale	Inoculated with sludge from anaerobic–anoxic SBR	[14]
BOD; NH_3-N, NO_3-N, TKN; TP; SS	TF/SC; BAF	Pilot scale	Nonsynthetic wastewater; chemical primary effluent	[85]
COD; NH_3-N, NO_2-N, NO_3-N; TSS, VSS	SBR	Laboratory scale	Synthetic wastewater	[43]
BOD, COD; NH_3-N, NO_2-N, NO_3-N, TKN; TSS, VSS	SBR	Laboratory scale	Nonsynthetic wastewater; domestic wastewater	[73]
BOD, COD; NH_4-N, TON, TN; PO_4-P, TP; TSS, VSS	MBBR	Laboratory scale	Nonsynthetic wastewater; anaerobically pretreated wastewater	[65]
NH_4-N; VSS	SBR	Laboratory scale	Synthetic wastewater	[47]
COD; NH_4-N, NO_3-N; TSS, VSS	Hybrid MBBR	Pilot scale	Nonsynthetic wastewater; primary settled wastewater	[26]
BOD, COD; NH_3-N, NO_3-N, TKN	ASP; fluidized-bed reactor; submerged-bed reactor	Laboratory scale	Synthetic and domestic wastewater	[102]
COD; NH_4-N, NO_2-N, NO_3-N; PO_4-P; MLSS	SBAR	Laboratory scale	Synthetic wastewater	[10]
NH_4-N, NO_2-N, NO_3-N; MLSS, MLVSS	SBR	Laboratory scale	Nonsynthetic wastewater	[38]
COD; NO_2-N, NO_3-N; PO_4-P; MLSS, MLVSS	SBR	Laboratory scale	Synthetic wastewater	[130]

BOD, COD; NH_4-N, NO_2-N, NO_3-N, TN; TSS, VSS	Hybrid MBBR	Pilot scale	Nonsynthetic wastewater; primary effluent municipal wastewater	[28]
COD; NO_2-N, NO_3-N	SBR	Laboratory scale	Synthetic wastewater	[107]
COD; NH_4-N; PO_4-N; MLSS, MLVSS	EBPR	Laboratory scale	Non-synthetic; waste activated sludge from WWTP	[128]
COD; NH_3-N, NH_4-N, NO_2-N, NO_3-N; PO_4-P; TSS, VSS	SBR	Pilot scale	Nonsynthetic wastewater; primary effluent	[129]
COD	Activated sludge	Laboratory scale	Synthetic wastewater	[81]
BOD, COD; NH_4-N, NO_2-N, NO_3-N, TN; TSS, VSS	Hybrid MBBR	Pilot scale	Nonsynthetic wastewater; municipal primary effluent	[29]
NH_4-N, NO_2-N, NO_3-N; MLSS	Bacteria consortium in reactors	Laboratory scale	Synthetic wastewater	[124]
NH_4-N, NO_2-N, NO_3-N, TN	Denitrifying bacteria	Laboratory scale	Synthetic wastewater	[125]
COD; NH_4-N, NO_2-N, NO_3-N, TN; SOP; TSS, VSS	A/EI SBRs	Laboratory scale	Synthetic wastewater	[15]
COD; NH_4-N; TP; SS	Micromembrane filtration; biofilters	Full scale	Nonsynthetic wastewater; municipal sewage	[122]

A/EI SBR, aerobic/extended-idle sequencing batch reactor; *ALNS*, acclimated lagoon nitrifying sludge; *ASBF*, aerated submerged biofilm reactors; *ASP*, activated sludge process; *AWL*, aqua treatment technology sand and gravel wetland; *BAF*, biological aerated filter; *BOD*, biochemical oxygen demand; *COD*, chemical oxygen demand; *CW*, constructed wetland; *DOC*, dissolved organic carbon; *EPBR*, enhanced biological phosphorus removal; *FC*, fecal coliform; *FS*, fecal streptococci; *HEV*, culturable human enteric viruses; *HRAP*, high rate algal pond; *ISF*, intermittent dosing sand filters; *MBBR*, moving-bed biofilm reactor; *MLSS*, mixed liquor suspended solids; *MLVSS*, mixed liquor volatile suspended solids; *PVF*, pre-treatment vertical flow filters; *PW*, peat and wood shaving biological trickle filter; *SBAR*, sequencing batch airlift reactor; *SBR*, sequencing batch reactor; *SMCW*, shallow moss constructed wetland; *SOP*, soluble orthophosphate; *SS*, suspended solids; *TAN*, total ammonia nitrogen; *TF/SC*, trickling filter/solids contact; *TKN*, total Kjehldahl nitrogen; *TON*, total oxidised nitrogen; *TP*, total phosphorus; *TSS*, total suspended solids; *VSS*, volatile suspended solids; *WWTP*, wastewater treatment plant.

Table 7.2 Summary of Studies on Hybrid and Naturalized Cold Aerobic Wastewater Treatment

Parameters	Treatment System	Scale	Wastewater Conditions	References
BOD, COD, TOC; NH_4-N, NO_3-N, TN; PO_4-P, TP; SS; bacteria (*Escherichia coli*)	CW	Pilot scale	Nonsynthetic wastewater; domestic wastewater	[66]
Bacteria (*E. coli*)	WSP	Full scale; microcosm	Nonsynthetic wastewater	[78]
BOD, COD, TOC; NH_4-N, NO_3-N, TN; PO_4-P, TP; TSS; bacteria (*E. coli*)	PVF; CW	Full scale	Nonsynthetic wastewater; domestic wastewater	[67]
BOD, COD; TSS	CW	Full scale	Nonsynthetic wastewater	[97]
BOD, COD, TOC; TN; TP; bacteria (thermotolerant coliforms)	Septic tank, aerobic biofilter, CW	Full scale; mesocosm	Nonsynthetic wastewater; domestic wastewater	[50]
COD; NH_4-N, NO_3-N, TKN; TSS	CW	Mesocosm	Reconstituted fish farm effluent	[84]
BOD, TOC; NH_4^+/NH_3-N, TN; TP; TSS	WSP	Full scale	Nonsynthetic wastewater	[91]
NH_4-N, NO_3-N, TKN; DP, TP	CW	Full scale	Non-synthetic wastewater; dairy water	[36]
BOD; NH_4-N, TKN; TP; TSS; bacteria (FC)	CW	Full scale	Nonsynthetic wastewater; dairy water	[48]
BOD, COD; NH_4-N; SS	WSP; CW	Pilot scale	Nonsynthetic, screened wastewater	[53]
COD, SOC, TOC; NH_4-N, NO_2-N, NO_3-N, TKN; soluble P, TP; SS	WSP	Full scale	Non-synthetic wastewater	[103]
BOD, COD; NH_4-N, NO_2-N, NO_3-N, TKN	ASBF	Pilot scale	Nonsynthetic wastewater; TF effluent	[16]
Bacteria (*E. coli*, *Clostridium perfringens*, FS, TC), viruses (coliphages), protozoa (*Cryptosporidium*, *Giardia*), helminths	CW	Full scale	Nonsynthetic wastewater; domestic wastewater	[90]
BOD, COD; TN; TP; TDS, TSS	CW	Pilot scale	Nonsynthetic wastewater; urban wastewater	[34]
NH_4-N, NO_2-N, NO_3-N; MLSS, MLVSS	Suspended biomass reactor with ALNS	Laboratory scale	Synthetic wastewater	[30]
COD; NH_3-N, NO_2-N, NO_3-N, TKN	BAF	Pilot scale	Synthetic wastewater	[39]
Bacteria (*C. perfringens*, enterococci, thermotolerant coliforms), viruses (somatic and male-specific coliphages, HEV)	Aerated lagoons	Full scale	Nonsynthetic wastewater	[63]
BOD; NO_3-N, TKN, TN; TSS	ISF; RTF; suspended-growth aeration tanks	Full scale	Nonsynthetic wastewater; domestic wastewater	[120]

NH_3-N, NH_4-N	CW (psychrophiles)	Laboratory and small scale	Nonsynthetic wastewater; pond effluent	[117]
BOD	WSP	Full scale	Nonsynthetic wastewater	[44]
BOD, COD; TSS	WSP	Full scale	Nonsynthetic wastewater; domestic wastewater	[69]
Bacteria (*E. coli*, FS, TC), viruses (coliphages), protozoa (*Cryptosporidium*, *Giardia*), helminths	WSP	Full scale	Nonsynthetic wastewater; diluted domestic wastewater	[89]
COD	CW	Microcosms	Synthetic wastewater	[105]
Nitrogen (NH_3-N, NO_2-N, NO_2-N)	Nitrifying biofilm	Laboratory scale	Synthetic wastewater	[25]
COD; NH_3-N, NO_2-N, NO_3-N, TKN, TN; TP	PW filter; AWL wetland	Pilot scale	Nonsynthetic; pretreated landfill leachate	[99]
COD; NH_4-N, NO_2-N, NO_3-N; TP	SMCW	Small scale	Nonsynthetic wastewater; primary effluent	[117]
BOD, COD; NH_3-N; TP; TSS; bacteria (*E. coli*, TC)	Natural treatment wetlands	Full scale	Nonsynthetic wastewater	[126]
COD; NH_4-N, NO_2-N, NO_3-N, TN; PO_4	CW	Microcosm	Synthetic wastewater	[2]
COD; NH_4-N, TN; TP	Hybrid CW	Full scale	Nonsynthetic wastewater	[59]
COD; NH_4-N, TN; TP	Hybrid CW	Full scale	Nonsynthetic wastewater	[60]
BOD; NH_3-N, NO_3-N; TP	Tundra wetland	Full scale	Nonsynthetic wastewater; municipal wastewater	[17]
BOD; NH_3-N, TAN, TN; TP; TSS, VSS; bacteria (*E. coli*)	Tundra wetland	Full scale	Nonsynthetic wastewater; municipal wastewater	[41]
BOD; NH_3-N, TAN, TN; TP; bacteria (*E. coli*)	Tundra wetland	Full scale	Nonsynthetic wastewater; municipal wastewater	[40]
COD; NH_4-N; TSS	HRAP	Pilot scale	Nonsynthetic wastewater; urban wastewater	[74]
COD; NH_4-N; TP	CW	Small scale	Nonsynthetic wastewater	[104]
BOD, COD; NH_3-N, NO_2-N, NO_3-N, TKN; hydrolyzable P, *ortho*-phosphate, TP; TDS, TFS, TS, TSS, TVS	PW filter; AWL wetland	Pilot scale	Nonsynthetic, pretreated landfill leachate	[113]

ALNS, Acclimated lagoon nitrifying sludge; *ASBF*, aerated submerged biofilm reactors; *AWL*, aqua treatment technology sand and gravel wetland; *BAF*, biological aerated filter; *BOD*, biochemical oxygen demand; *COD*, chemical oxygen demand; *CW*, constructed wetland; *DP*, dissolved phosphorus; *FC*, fecal coliforms; *FS*, fecal streptococci; *HEV*, culturable human enteric viruses; *HRAP*, high rate algal pond; *ISF*, intermittent dosing sand filters; *MLSS*, mixed liquor suspended solids; *MLVSS*, mixed liquor volatile suspended solids; *PVF*, pre-treatment vertical flow filters; *PW*, peat and wood shaving biological trickle filter; *RTF*, recirculating trickling filter; *SMCW*, shallow moss constructed wetland; *SOC*, soluble organic carbon; *SS*, suspended solids; *TAN*, total ammonia nitrogen; *TC*, total coliform; *TDS*, total dissolved solids; *TFS*, total fixed solids; *TKN*, total Kjehldahl nitrogen; *TN*, total nitrogen; *TP*, total phosphorus; *TS*, total solids; *TSS*, total suspended solids; *TVS*, total volatile solids; *VSS*, volatile suspended solids; *WSP*, wastewater stabilization pond.

lower temperature in both conventional and passive treatment systems, in which higher removal efficiencies at colder temperature are often attributed to lower concentrations of autotrophic and heterotrophic microorganisms. Pathogen removal in conventional systems is generally achieved through disinfection technologies employing UV, chlorination, and ozone, whereas in passive systems, pathogen removal is subject to many environmental factors, such as sunlight, pH, DO, and temperature.

Cold climates pose operational challenges for wastewater treatment plants to meet required and increasingly stringent effluent discharge criteria. To maintain acceptable levels of constituent removals in wastewater treatment facilities operated under cold-climate conditions, operational processes or strategies that have been adopted include modifying flow configurations, increasing HRT, recirculating wastewater, enhancing biomass systems, aeration, and incorporation of vegetation.

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8 Anaerobic Treatment Versus Aerobic Treatment

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8.1 Microbial Metabolism

Microbial metabolism in wastewater treatment involves the use of microorganisms to consume organic matter as substrate. Biodegradable organics are metabolized and converted into carbon dioxide, water, and energy for growth, cell maintenance, and reproduction of the microorganisms. In actual fact, microbial metabolism is the overall biochemical processes that are employed in the destruction of organic compounds, or so-called catabolism, and in the building up of cell protoplasm, termed anabolism. These processes convert chemically bound energy from the organics into energy forms that can be used for microbial life-sustaining processes. Catabolism represents the oxidative, exothermic, enzymatic degradation process that results in the release of free energy from the organic substances. Some of the released energy is available for the construction of new cellular material through anabolism, which is a synthetic process that results in an increase in size and complexity of organic chemical structure [1]. Microbial metabolism can be carried out under both aerobic and anaerobic conditions.

8.1.1 Aerobic Metabolism

Aerobic treatment is an *oxidation* process whereby bacteria degrade organic matter and other pollutants in the presence of oxygen. Aerobic decomposition of organic substances is usually considered to consist of fermentation and respiration (or oxidation), biosynthesis, and endogenous respiration. The end products of the oxidation process consist of carbon dioxide, ammonia, energy, water, and other end products as represented in Eq. [8.1], in which COHNS (carbon, oxygen, hydrogen, nitrogen, and sulfur) represents the waste organic compound in general:

$$\mathrm{COHNS} + \mathrm{O_2} + \text{aerobes} \rightarrow \mathrm{CO_2} + \mathrm{H_2O} + \mathrm{NH_3} + \text{other end products} + \text{energy} \qquad [8.1]$$

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Simultaneously, some of the wastes are converted into new cell tissue using part of the energy released during oxidation through the *synthesis* process (Eq. [8.2]). The mechanisms and kinetics of aerobic decomposition are well established on account of a good understanding of aerobic biochemistry and microbiology:

$$\text{COHNS} + \text{O}_2 + \text{aerobes} + \text{energy} \rightarrow \text{C}_5\text{H}_7\text{NO}_2 \text{ (new cells or sludge)} \quad [8.2]$$

Biodegradable compounds are high-energy forms of organics. The oxidation of such compounds to low-energy forms such as carbon dioxide provides energy for the microorganisms. Understanding how to mix aerobic microorganisms, soluble organic compounds, and dissolved oxygen for high-rate oxidation of organic compounds is part of the fundamental knowledge of wastewater engineers.

Most decomposing microbes prefer aerobic conditions to anaerobic conditions. The aerobic metabolism of organic compounds consumes dissolved oxygen out of the water. If the rate of oxygen supply through aeration is not larger than or equal to the rate of consumption, the dissolved oxygen concentration will eventually diminish below the level needed to sustain a viable aerobic metabolism. In the engineered biochemical oxidation of wastewater, oxygen is supplied to the aerobic microorganisms so that they will consume the substrate (organic carbon) to fuel their metabolism. The result is the conversion of organic pollutants into inorganic compounds and new microbial cells. The net production of cells will form an accumulation of biological material.

8.1.1.1 Fermentation and Respiration

Fermentation is the process in which aerobic and anaerobic heterotrophic microorganisms reduce complex organic compounds to simpler organic forms. As expressed in Eq. [8.3], fermentation is an exothermic and enzymatic breakdown of soluble organic compounds and does not depend on the presence of dissolved oxygen. The fermentation process is often illustrated in two stages: acid fermentation and methane fermentation. End products of the acid fermentation process include volatile fatty acids (VFAs) and alcohols. In the acid fermentation stage, there is little waste stabilization as most of the carbons in the substrate are still in an organic form. In the second stage of methane fermentation, the acid-fermentation end products are converted to methane and carbon dioxide gases. The effect of this conversion is a reduction in the organic waste thereby achieving waste stabilization:

$$\text{COHNS} \rightarrow \text{VFAs} + \text{CO}_2 + \text{CH}_4 + \text{energy} + \text{residuals} \quad [8.3]$$

Aerobic microorganisms can further transform the VFAs (and other degradable organic compounds) from the fermentation process into carbon dioxide, water, and energy. Respiration requires the presence of oxygen (Eq. [8.4]). Oxygen acts as an electron acceptor for the catabolic degradation of the VFAs. Because aerobic microbes can readily convert degradable organic carbon into inorganic carbon, aerobic processes can provide efficient waste degradation:

$$\text{VFAs} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{energy} + \text{residuals} \quad [8.4]$$

8.1.1.2 Biosynthesis

Biosynthesis is considered the most complex and vital energy-requiring activity of all living organisms. The creation of characteristic chemical compounds of cells from simple precursors, and the assembly of these compounds into complex structures such as the membrane systems, contractile elements, mitochondria, nuclei, and ribosomes are accomplished through biosynthesis. Two important components are required for the biosynthesis of cell components. First, the precursors that provide the carbon, hydrogen, nitrogen, and other elements found in cellular structures, and second, adenosine triphosphate (ATP) and other forms of chemical energy needed to assemble the precursors into covalently bonded cellular structures. It should be noted that the cellular components are derived from the wastewater stream and thus, many of the wastewater constituents are being converted into new cells.

8.1.1.3 Endogenous Respiration

Endogenous respiration is a process in which microbes consume other cells at a higher rate than new cells can be produced under substrate-deficient conditions. There is an accumulation of slowly degradable cellular material and other residuals under endogenous respiration. Some aerobic treatment units in the wastewater treatment plants operate in the endogenous respiration phase under extended aeration. This process provides abundant aeration to ensure that once the waste is consumed, the microbes will start feeding on one another. This would reduce the mass of accumulated biomass or sludge that must be treated and disposed of.

8.1.2 Anaerobic Metabolism

The mechanisms of anaerobic processes are much more complicated than those of aerobic processes, because of the many pathways available for an anaerobic community. The anaerobic ecosystem is the result of complex interactions among organisms of various species. The biochemistry and microbiology responsible for the reactions are not fully understood, but during the past 30 years a broad outline of the processes has been reported by various researchers. Several techniques have been developed and adapted to isolate and study anaerobic bacteria [2].

As depicted in Fig. 8.1, the process is performed by two physiologically distinct bacterial populations. In the first stage, organic materials are converted into simple VFAs by a group of facultative and obligate anaerobes commonly termed as “acid formers.” The end products of this first-stage acidogenic conversion comprise predominantly

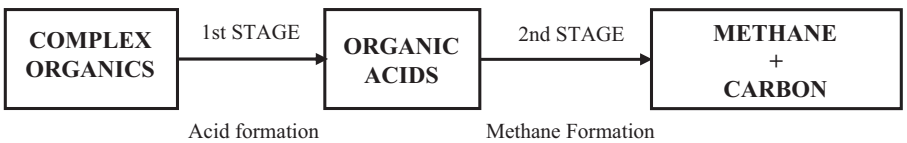

FIGURE 8.1 Two-stage anaerobic digestion.

organic fatty acids and a small portion of biological cells. Although no waste stabilization is brought about during the first stage of treatment, it is normally considered an intermediate reaction to prepare the organic matter in a form amenable for the second stage of treatment. It is in the second stage of treatment that actual waste stabilization occurs. During this stage, 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.

Although anaerobic digestion is commonly described as these two distinct stages, some researchers consider that there are four major stages in the production of methane and carbon dioxide from organic matter. The first stage involves hydrolysis of long-chain complex organic compounds such as carbohydrates, proteins, and fats to simpler molecules. 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. Fig. 8.2 illustrates simplified pathways of

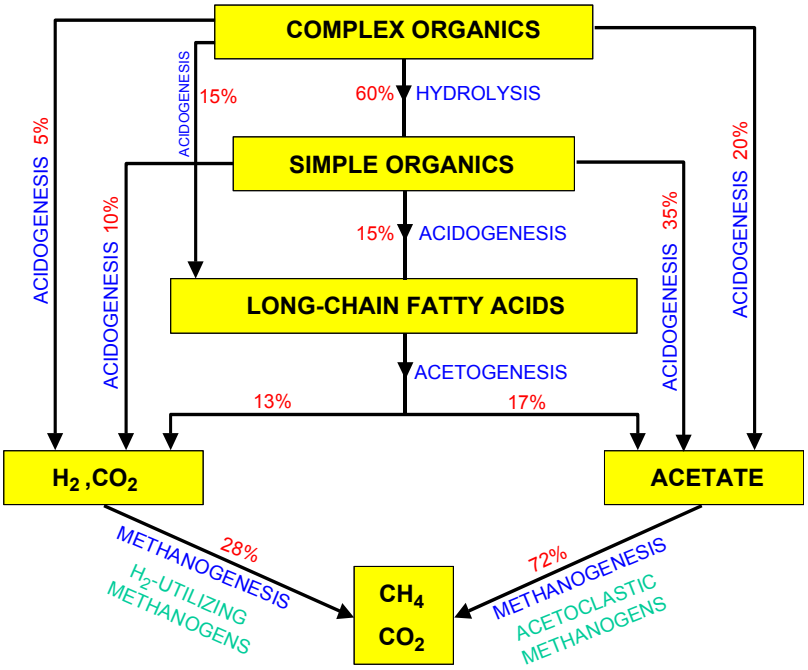

FIGURE 8.2 Pathways of methanogenesis of complex wastes.

methane fermentation of complex wastes. The percentages represent conversion of waste chemical oxygen demand (COD) by various routes.

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.

8.1.2.1 Hydrolysis

Hydrolysis and liquefaction convert complex insoluble organic compounds into smaller simpler molecules that may be utilized as an energy source. The biopolymers proteins, carbohydrates, and lipids 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. They produce spores to survive under aerobic conditions.

Flavobacterium, *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 sulfur, 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 β-oxidation 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 VFAs. Deamination is carried out by fermentative bacteria, *Bacteroides ruminicola*, *Peptococcus*, and other *Bacteroides* species.

8.1.2.2 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 [3]. 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 [4].

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 [5].

8.1.2.3 Acetogenesis

The third stage, acetogenesis, consists of two groups of bacteria, viz., hydrogen-producing acetogens and homoacetogens (or hydrogen-consuming acetogens). Whereas hydrogen-producing acetogens catabolize organic acids, alcohols, and certain aromatic compounds into acetate and carbon dioxide, homoacetogens use hydrogen and carbon dioxide to form acetate. Homoacetogens are thought to synthesize only 1−2% of the total acetate at 40°C [6]; their exact role remains 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. [7] 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 [6]. Though homoacetogenic metabolism may contribute to the maintenance of low hydrogen partial pressures, hydrogen-utilizing methanogens have a lower substrate constant, K_s, value for hydrogen. Theoretically, they should outcompete the homoacetogens for hydrogen at the concentrations prevalent in a stable reactor.

8.1.2.4 Methanogenesis

Methanogenic bacteria belong to the group Archaebacteria, a phylogenetically distinct group [5]. A limited number of substrates are used by the 47 known species of methanogenic bacteria. Two major groups of methanogenic bacteria have been identified. Group 1 consists of 33 species belonging to the families of Methanobacteriaceae, Methanothermaceae, Methanococcaceae, Methanomicrobiaceae, and Methanoplanaceae. These species reduce carbon dioxide and hydrogen and/or utilize formate in the formation of methane. Group 2 consists of 14 species belonging to the family of Methanosarcinaceae. These species utilize acetate, methylamines, and/or methanol. *Methanosarcina barkeri* and *Methanosarcina 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 (group 1) can utilize hydrogen and carbon dioxide as their sole energy source [7], but a 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 [8,9], leading to accumulation of acetic acid to toxic levels. The degradation of acetate to methane is thought to be the rate-limiting step in the overall

conversion of substrate to methane [10–12]. Complex polymers and fats are the exception; here hydrolysis is the rate-limiting step [13].

Coenzymes are specific nonprotein units required for activity of a particular protein. Coenzyme F_{420} [14] and coenzyme M [15] are unique to methanogens; both have potential for use in identification and numeration of methanogens.

8.2 Comparison of Aerobic and Anaerobic Treatments

A general comparison between aerobic and anaerobic treatment processes should be undertaken with caution, as each individual case has peculiarities that may make only certain processes feasible. A broad overview of wastewater criteria directly applicable to aerobic and anaerobic treatments is given in Table 8.1. It should be noted that such comparison is only qualitative and the choice of criteria listed is not at all explicit.

In cases of high-strength industrial wastewater treatment meeting stipulated trade effluent discharge limits, both anaerobic and aerobic processes should be used together for optimal treatment. An anaerobic process is normally designed to remove a majority of the pollutants at the upstream end of the treatment system. The effluent is then polished by an aerobic process so as to meet the discharge standards. Further detailed comparisons of other criteria are addressed in the following sections.

8.2.1 Removal of Pollutants

The primary objective of all processes is pollutant removal. Removal of organics is related to the amount of biodegradable organics in the waste. As discussed earlier, the

Table 8.1 Applicability of Criteria to Aerobic and Anaerobic Treatments

Criterion	Aerobic	Anaerobic
Range of wastewater type	√	
Process stability and control		√
Volumetric loading rates		√
Power input		√
Heat input	√	
Sludge production		√
Nutrient requirements		√
Oxygen requirement		√
Waste removal	√	
Nitrogen removal	√	
Phosphorus removal	√	
Production of valuable by-products		√

√ denotes advantage over the other treatment.
Adapted from P. Vochten, S. Schowanek, W. Schowanek, W. Verstraete, Aerobic versus anaerobic wastewater treatment, In: E.R. Hall, P.N. Hobson (Eds.), Proc. of the 5th Int. Symp. on Anaerobic Digestion, Bologna, Italy, Pergamon Press, Oxford, UK, 1988, pp. 91–104.

total amount of biodegradable substances is nearly the same whether the waste is treated under aerobic or under anaerobic conditions. Although aerobic treatment systems can usually produce a better quality effluent than anaerobic systems, total removals will be more similar as the wastewater becomes more highly concentrated. Removals attained in an anaerobic process alone should be well within effluent quality limits for biodegradable wastes.

8.2.2 Number and Scale of Unit Treatments

Conventional aerobic treatment processes normally require primary sedimentation. This has two disadvantages: an extra vessel and appurtenances are required and the sludge that settles in this vessel is not reduced in volume or degradability. Many anaerobic processes do not normally require primary sedimentation. However, grit removal is mandatory for some biological reactors (particularly those with high solids retention time (SRT)) and desirable to prevent accumulation of inert sludge, which will displace biomass. Grit removal should precede both activated sludge and high-rate anaerobic treatment.

The requirement for equalization is about the same between anaerobic and aerobic processes. When load variations exceed 1:4 it is good practice to provide equalization. It is more difficult to control SRT in suspended growth processes whether they are anaerobic or aerobic. In contrast, fixed-film processes are less susceptible to problems resulting from load variation. For industrial wastes, reactors will usually be larger for an aerobic process compared to an anaerobic process. This can be deduced from an examination of the loading rates reported for the respective processes. Upper loading ranges reported for anaerobic processes are much higher than upper loading ranges for aerobic processes.

Generally, all aerobic and anaerobic processes will require a secondary clarifier or other solids separation device. There are few available data on the settleability of anaerobic reactor effluents compared with aerobic reactor effluents. However, anaerobic processes may require a vacuum degasifier or other means to inhibit gas production before conventional sedimentation. Flotation separation may be used but this also requires an energy input beyond gravity clarifiers.

8.2.3 Chemical Requirements

Common chemical inputs for any biological process are alkalinity or acidity for pH adjustment, buffer capacity, and nutrient addition to satisfy cell synthesis needs of the microorganisms. Alkalinity control agents are the most common agents needed for pH control. Alkalinity addition is quite often the most expensive operation cost of anaerobic systems. In anaerobic digestion the volatile acids/alkalinity ratio should be in the range of 0.3−0.4. The most economical way to minimize alkalinity additions may be to install a pH control system on the reactor itself as opposed to adding excess alkalinity to the influent. Anaerobic processes treating industrial wastes or sludges commonly produce

200−500 mg/L volatile acids. For the range given, 570−1430 mg/L alkalinity as $CaCO_3$ would be required. Aerobic processes do not normally require pH adjustment unless they are designed to convert ammonia to nitrate (nitrification).

As discussed earlier, the nutrient requirement for a given amount of substrate is greater for an aerobic process than for an anaerobic process. Many wastes will contain an adequate amount of nutrients for treatment in either the anaerobic or the aerobic mode. The ratio of carbohydrates and fats to protein is the major controlling factor. Proteins contain significant amounts of nitrogen and phosphorus.

8.2.4 Operational Stability

Anaerobic metabolism is inherently more unstable than aerobic metabolism, and in general, anaerobic treatment is more unstable than aerobic treatment. However, aerobic treatment is not without problems and some of the new anaerobic processes, particularly the fixed-film options, are virtually as stable as conventional aerobic treatment. The upflow anaerobic sludge blanket (UASB) and conventional processes appear to be the least stable anaerobic processes. Nevertheless, this does not mean any of these processes are not viable alternatives.

8.2.5 Sludge Generation

Lower sludge production and lower costs associated with its disposal are a major advantage of anaerobic treatment. Aerobic treatment normally produces much more sludge than anaerobic treatment. Sludge processing for final disposal is energy intensive. It is usually more economical to dewater sludge before transporting it to an approved disposal site, which is often located at a significant distance from the plant site. Gravity or flotation thickening prior to sludge dewatering may be cost-effective. Drying beds, vacuum and pressure filters, and various types of centrifuges are used for dewatering. Except for drying beds, which require large land areas, these dewatering devices consume large amounts of energy. Also, chemical conditioning may be required for effective dewatering.

Nutrient content of aerobic and anaerobic sludges is similar. If an opportunity for sludge resale exists, then the associated income will be greater for an aerobic process because of its larger sludge production. This income will be reduced by costs of processing the larger amounts of sludge before it can be sold. Nutrients not contained in the sludge from an anaerobic process, for the most part, leave in the effluent in soluble form.

8.2.6 Energy Comparison [37]

Energy comparisons are important because energy comprises one of the major operating costs of any treatment process. The lowest temperature for operation of anaerobic reactors is near 10°C, but 20°C is a more conservative practical minimum. The size of the reactor will need to be increased by a factor of about 2 for each 10°C decrease in

operating temperature. For a reactor operating at 35°C, degradable wastes should easily result in a production of 1 m^3 CH_4/m^3 reactor/day. Methane derived from digestion will usually satisfy heating requirements of the waste. Heating the influent, when required, is usually the major energy input into an anaerobic reactor. Conventional and contact reactors also require mixing energy.

Aerobic treatment, except in the case of trickling filters, always requires energy input to pump air or oxygen into the system. Trickling filters use more energy for pumping but, in general, require less energy input than suspended growth aerobic processes. In temperate climates, trickling filters can provide little treatment during winter if they are not enclosed and heated.

The need to recycle, which requires pumping energy, is variable depending on the aerobic or anaerobic process. Fluidized bed reactors definitely require recycling and incur a more significant energy penalty to keep the bed fluidized. UASB and conventional reactors do not require recycling. It may prove more economical to recycle effluent, as opposed to adding chemicals, in upflow anaerobic filters to reduce alkalinity requirements. All aerobic processes require recycling of sludge or liquid. The other major energy input into a treatment process is for solids processing. Less energy is consumed for solids processing in anaerobic systems compared to aerobic systems because of the lower sludge production in anaerobic systems.

The net energy available from an anaerobic process is equal to the chemical energy (methane) produced by the process minus the thermal energy required by the process. The methane yield depends on a number of factors, including waste composition, temperature, hydraulic retention time, and SRT, which dictate the organic and solids loadings. Thermal energy required by the process is the sum of the energy required to heat the wastewater to digester operating temperature and energy needed to replace reactor heat losses to the environment. These heat losses account for only a small percentage, normally less than 10%, of total energy requirements. It is observed that the energy consumption of anaerobic treatments is very dependent on the type of process used and therefore on the type of waste treated. Mixing and pumping energy is the most variable item. Heating energy is directly related to the temperature difference between the reactor and the influent. In addition to the energy consumption factors given, there will be energy costs associated with sludge processing (dewatering) and transport to the ultimate disposal site.

A generalized plot of net specific energy consumption (energy consumed per unit volume of influent flow) versus influent substrate concentration for aerobic and anaerobic processes is shown in Fig. 8.3. Energy consumption for sludge dewatering was incorporated into the data used to plot these lines. Use of a heat exchanger was not considered. Installation of a heat exchanger will favor an anaerobic process operated at temperatures above ambient temperature.

An important observation to be made from Fig. 8.3 is that aerobic processes always involve net energy consumption. In an anaerobic process, the energy required to heat the waste to the reactor temperature is the most significant energy demand; therefore, the temperature difference between the influent and the reactor contents is used as a

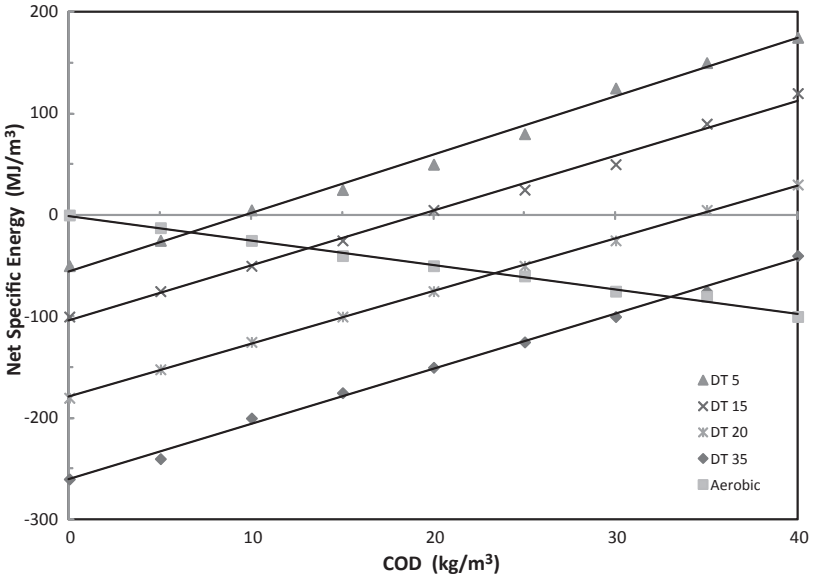

FIGURE 8.3 Energy comparison between aerobic and anaerobic processes. *COD*, chemical oxygen demand; *DT*, temperature difference. *Adapted from R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, Archae Press, Nashville, Tennessee, 1996.*

parameter on the plot. Many industries, particularly the food and beverage industries, produce wastes that are warm. The intersection between the anaerobic process and the aerobic process lines determines the substrate concentration at which net energy consumption is equal for an aerobic and an anaerobic process. At substrate concentrations above this value, anaerobic processes are more favorable for a given temperature difference. Table 8.2 sets forth a concise comparison of the operating features for treating a readily degradable industrial wastewater by either anaerobic or aerobic treatment.

Substantial cost benefits accrue when proper design criteria are met. The favorable reduced synthesis rates of anaerobic processes amount to a reduction in waste biomass accumulation of up to 500 kg/1000 kg COD utilized. Substantially lessened nutrient requirements also provide savings up to US$50/1000 kg COD destroyed, which is the same magnitude of savings derived from having no oxygen transfer requirement (e.g., 1000 kWh

Table 8.2 Anaerobic Versus Aerobic Treatment

- Volumetric organic loading rates 5—10 times higher than for aerobic processes
- Biomass synthesis rates only 5—20% of those for aerobic processes
- Nutrient requirements only 5—20% of those for aerobic processes
- Anaerobic biomass preserved for months or years without serious deterioration in activity
- No aeration energy requirements for anaerobic processes vs. 500—2000 kWh/1000 kg COD for aerobic processes
- Methane production of 12×10^6 BTU/1000 kg COD destroyed

1 BTU = 1.0551 kJ. *COD*, chemical oxygen demand.

Adapted from R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, Archae Press, Nashville, Tennessee, 1996.

Table 8.3 Financial Savings Derived From Anaerobic Treatment

Reduced synthesis rates	\$50/1000 kg COD utilized at \$100/tonne
Lower nutrient requirements	\$50/1000 kg COD destroyed
Electricity savings (no oxygen transfer)	\$50/1000 kg COD removed at \$0.05/kWh
Methane yield energy benefit	\$60/1000 kg COD destroyed at \$5/10^6 BTU

1 BTU = 1.0551 kJ. *COD*, chemical oxygen demand.

Table 8.4 Comparison of Anaerobic Fluidized Bed Versus Aerobic Treatment

Criterion	Fluidized Bed	Aerobic
Reactor volume, m^3	800	6400
Surface area, m^2	80	1400
Energy required, kWh/day	720	8600
Energy cost, US\$/year	15,700	188,200
Methane produced, m^3/day	2000	0
Value of gas, US\$/year	150,000	0
Sludge production, tonnes/year	180	1800

per 1000 kg COD removed equals US\$50/1000 kg of COD for electricity at US\$0.05/kWh). The methane production is also a positive cost benefit of approximately US\$60/1000 kg COD destroyed (e.g., 12×10^6 BTU/1000 kg COD $\times$ US\$5.00/10^6 BTU = US\$60/1000 kg COD). In many smaller installations, the methane production is simply flared because it is uneconomical to invest for its utilization. These significant savings are summarized in Table 8.3.

Table 8.4 lists a comparison of characteristic features of an anaerobic fluidized bed compared with aerobic treatment for an identical wastewater. The comparison is made on a basis that both processes remove 4400 kg biological oxygen demand (BOD)/day.

In the past a misconception has been prevalent that a wastewater must have a very high BOD concentration to be a viable candidate for anaerobic treatment. While anaerobic treatment can accommodate very strong industrial wastewaters, primarily because there are no oxygen transfer or solids flux thickening limitations, the minimum concentration is not controlled by BOD concentration but rather by a number of very important design criteria to be discussed in a later section.

8.3 Merits of Aerobic and Anaerobic Treatment [17]

8.3.1 Aerobic Treatment

Aerobic microbial communities have several specific advantages. They have large free energy potentials, enabling a variety of often parallel biochemical mechanisms to

be operated. These communities are therefore capable of coping with low substrate levels, variable environmental conditions, and multitudes of different chemicals in the influent. They have some very useful capabilities such as nitrification, denitrification, phosphate accumulation, ligninase radical oxidation, etc., which make them indispensable in waste treatment.

In a broad perspective, aerobic treatment has been regarded as simpler and more economical in design and operation. A major asset of aerobic systems is their capacity to handle various sources of wastewaters, especially those with extremely variable composition and toxic pulses. Nevertheless, most aerobic systems remain unable to cope with all treatment requirements. It has been reported that even well-attended aerobic wastewater treatment plants without major shocks or toxic pulses are not meeting the discharge standards around 20% of the operating time [18]. Over the decades, abundant experience from full-scale operations and research and development has helped to improve the applicability of aerobic processes. In recent years, tighter restrictions on sludge disposal site location, air pollution, hazardous waste disposal, and odor control, in addition to other factors, have had a substantial impact on the applicability of aerobic treatment of industrial wastewaters. To manage a green and sustainable scheme for the treatment of wastewaters, the development of improvements that combine high degradation efficiency, lower energy consumption and sludge production, low carbon emissions, and low construction and maintenance costs has become a major priority. State-of-the-art online continuous monitoring devices capable of quantifying the incoming load and possible toxic pulses, and sending signals to the remote operation control system, can be expected. This will undoubtedly further improve the attractiveness of aerobic treatment in general and of the treatment of variable industrial waste streams in particular.

Aerobic wastewater treatment is no longer a matter of removal of the bulk of soluble and particulate organic matter. The removal of nitrogen through nitrification and denitrification has been recognized as a process step of major importance in overcoming problems of eutrophication. Indeed, by careful regulation of the oxygen supply, it is possible to have nitrification at the exterior of the sludge flocs, while denitrification of the nitrate thus prevails in the oxygen-limited interior of the same floc [19,20]. In this fashion, not only the nitrogenous compounds are removed in an elegant way, but also the energy invested in the nitrification step is entirely conserved, because the nitrate ion serves as an alternative electron acceptor for the facultative aerobic microorganisms.

Advancement in nitrification marked the development of the anammox process, denoting the anoxic oxidation of ammonium with nitrite as electron acceptor [38]. The autotrophic growth mode, in combination with the high maintenance requirement due to the slow growth rate, leads to an overall stoichiometry showing a relatively low biomass yield. In 2002, the first full-scale anammox reactor was put into operation at a sludge treatment plant in Rotterdam, the Netherlands [21].

Obviously, nitrogen removal through online-regulated nitrification/denitrification is a great asset of aerobic treatment. The removal of phosphate, based on the special

characteristics of certain aerobic bacteria to accumulate phosphorus, has been experimentally explored for decades [22]. The removal of mineral phosphate from aerobic wastewater treatment appears to pose no major technical problems at the current state-of-the-art applications.

Approaches relating to improvement of the metabolic diversity and affinity of the aerobic microbial community have been attempted. By providing in the mixed liquor matrices on which the microorganisms can colonize, one can obtain a more diverse microbial community, comprising both immobilized and suspended microorganisms. Some approaches along this line are the use of polyurethane foam sponges [23] and powdered activated carbon [24] and the installment of plastic carriers in the activated sludge tank [25].

It must be stressed that current knowledge of the ecology of activated sludge microbial communities is very limited. It was hypothesized that the more varied the composition of the feed, the more diverse the resulting microbial community will be [26]. Current knowledge suggests that, indeed, activated sludge communities are composed of diverse bacteria, actinomycetes, fungi, and protozoa. Hence, their overall genetic pool is very large. As to the aspect of affinity, the aerobic biofilm and activated sludge organisms grow at ambient substrate levels on the order of 0.1−10 mg/L. Insight has become available on the biokinetics at such low substrate levels [27]. This faculty of aerobic microorganisms is also an important asset of aerobic treatment.

8.3.2 Anaerobic Treatment

The anaerobic treatment process offers several advantages over aerobic systems. In anaerobic metabolism, the waste is decomposed by a variety of microorganisms in the absence of molecular oxygen. Under these anaerobic conditions, the anaerobes are capable of converting the organic wastes into methane and carbon dioxide. Unlike aerobic systems, the anaerobic conversion to methane gas yields little energy for the microorganisms. Because the energy available is low, the rate of growth of the anaerobes is relatively slow. The low growth yield signifies that only a small amount of the organics is being synthesized into new cells. As high as 85−95% of the degradable organic portion of a waste can be stabilized by anaerobic conversion to methane gas. Such conversion represents waste stabilization because the methane gas produced readily escapes from the waste stream.

One of the main characteristics of aerobic systems is that the growth rate of the microorganisms is considerably faster as much energy can be secured from the oxidation of organic waste. Consequently, a large portion of the organic matter is used in the synthesis of biomass. The organics converted to biomass have not actually been stabilized, but are simply changed in form. The significant amount of biological solids generated in the aerobic process requires further sludge treatment for ultimate waste stabilization. On the other hand, in anaerobic treatment the problem of sludge disposal is significantly minimized, because only a small portion of the waste is being converted

Table 8.5 Positive Features of Anaerobic Treatment

- Provision of process stability
- Reduction of waste biomass disposal costs
- Reduction of nitrogen and phosphorus supplementation costs
- Reduction of installation space requirements
- Conservation of energy, ensuring ecological and economical benefits
- Minimization of operational attention requirement
- Elimination of off-gas air pollution
- Avoidance of foaming with surfactant wastewaters
- Biodegradation of aerobic nonbiodegradables
- Reduction of chlorinated organic toxicity levels
- Provision of seasonal treatment

to biomass. Biomass yields are typically 10% for anaerobic systems, compared with about 50% for aerobic systems. This results in lower costs for the anaerobic process for sludge treatment and disposal. Because anaerobic treatment does not require oxygen in waste decomposition, the rates of reaction are not limited by oxygen transfer. In addition, there is a notable savings in the energy needed for aeration. Moreover, the combustible end product of methane gas represents an additional source of energy for other operations such as heating and generating electricity.

Over the past decades many installations, embracing a variety of industrial effluents, have demonstrated conclusively the positive features of anaerobic biotechnology in the biotransformation of organic pollutants to methane. These applications will be analyzed in detail, emphasizing the design and operational features that contributed to their success. It will become evident that in using this alternative biodegradation treatment the advantages, as summarized in Table 8.5, far outweigh the disadvantages in the majority of cases studied. The merits of anaerobic processes on specific aspects of wastewater treatment are discussed below.

8.3.2.1 Stable Process and Operation

Process stability (the capacity to achieve efficient pollutant reduction under varying environmental conditions) is provided when an anaerobic facility is designed for efficient biomass immobilization and operated with a reasonable biological safety factor. Such design characteristics are enhanced by the use of granules, fixed films, or membrane reactors and proper attention to satisfying trace metal requirements.

8.3.2.2 Reduction in Biomass Disposal Costs and Space Requirements

Anaerobic biotechnology negates the need for aerobic oxygen transfer with its associated high microbial synthesis characteristics, thus significantly lessening the disposal costs involved with excess biomass synthesis. Consequently disposal costs are often only 10% of those for aerobic processing of the same effluent. Nitrogen and phosphorus requirements are also reduced accordingly. Considerable reduction in space requirement

further increases financial savings. These benefits are accrued in addition to the considerably higher loading rates possible with anaerobic systems, commonly varying from 3 to 32 kg/m^3 day as opposed to the usual load to aerobic facilities of 0.5–3 kg/m^3 day.

8.3.2.3 Conservation of Energy With Concomitant Ecological and Economical Benefits

Anaerobic treatment produces 12×10^6 BTU as CH_4 per 1000 kg of COD converted to CH_4. Because no oxygen transfer is required, the need for the 500–2000 kWh of energy per 1000 kg of oxygen transfer normally required for aerobic treatment is negated, making energy conservation possible with its concomitant ecological and economic benefits. Because approximately 10,000 BTU are consumed in the generation of 1 kWh of electricity, the expenses incurred in generating the $5–20 \times 10^6$ BTU/1000 kg of COD treated necessary for oxygen transfer are eliminated, providing even further energy savings.

8.3.2.4 Minimization of Operation Attention

The two major operator attention requirements for aerobic systems are voided with the choice of anaerobic biotechnology. Because there is no necessity for oxygen transfer, and clarifier failure is not an issue by effectively immobilizing biomass with biofilm or granules, operation attention requirements are thus minimized.

8.3.2.5 Elimination of Off-Gas Air Pollution

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 (for example, acrylic acid and chlorinated solvents). This significant drawback cannot be omitted from the design process of aerobic systems, but is eliminated when anaerobic treatment is utilized.

8.3.2.6 Avoidance of Surfactant Foaming and Degradation of Recalcitrant Substances

Past experience has demonstrated the inability of aerobic biotechnology to biodegrade certain contaminants such as highly chlorinated solvents. Likewise aerobic treatment is inappropriate for processing certain other concentrated industrial wastewater feed stocks such as in pharmaceuticals, beer, and alcohol production.

The often severe foaming of surfactant wastewaters caused by the turbulence and/or bubbling of air involved in the aerobic process may actually preclude the use of air as the basis of the treatment, but nonfoaming biodegradation is possible anaerobically and is thus seen to be of substantial advantage in such cases. Ordinarily approximately 70 volumes of gas are added per volume of wastewater having 2000 mg/L of BOD aerobically, compared to only about 1.6 volumes of gas produced per volume of 2000 mg/L wastewater anaerobically.

8.3.2.7 Reduction of Chlorinated Organic Toxicity

Because chlorinated organics may be biotransformed anaerobically, toxicity levels are dramatically lessened. Dehalogenation was first demonstrated in a nonmethanogenic anaerobic treatment process for pulp and paper mill wastewaters containing organics that were chlorinated during the chlorine-bleaching operations. This feat has since often been repeatedly observed in methanogenic systems.

8.3.2.8 Optimization of Seasonal Treatment

The anaerobic process may be applied to seasonally produced wastewaters, such as winery or sugar operations, which normally produce effluent during only 2—4 months each year. Nevertheless biomass viability is maintained, owing to the unique feature of drastically reduced endogenous decay during starvation.

8.3.2.9 Mitigation of Greenhouse Gas Emissions

An emerging application of anaerobic treatment is associated with the Certified Emission Reduction or Carbon Credit set under the Kyoto Protocol [28]. In addition to contributing to sustainable development with energy recovery in the form of methane, carbon credits can be claimed by application of advanced anaerobic processes in wastewater treatment for mitigating emissions of greenhouse gases [29]. As anaerobic systems are capable of handling high organic loadings concomitant with high-strength wastewater and short hydraulic retention time, they could render many more carbon credits than other conventional anaerobic systems [30]. Looking at the prospects of carbon trading, it may not be an unreasonable expectation that, in the future, wastewater treatment will experience a global shift toward employment of highly efficient granular sludge-based anaerobic processes in maximizing energy production and minimizing greenhouse gas emissions.

8.4 Challenges and New Horizons

8.4.1 Increasing Awareness of Aerobic Treatment Drawbacks

A combination of tighter restrictions on air pollution, hazardous waste disposal, odor control, groundwater contamination, and sludge disposal site location, in addition to other factors, has had substantial impact on the viability of aerobic treatment of industrial wastewaters. In the past, aerobic environmental control processes held a virtual monopoly on the industrial wastewater market. Biomass disposal problems caused by large volumes of refractory biomass and in some cases even the possible long-term contamination of second and third party lands by disposal of waste activated sludge biomass, were largely ignored. Until the enactment of the Clean Air Act in 1994 in the United States, even hazardous volatiles caused by air stripping in aeration reactors were typically allowed. Increasingly restrictive controls are now being placed in many cities around the world on air emissions of volatile organic contaminants from industrial

production, including fugitive emissions from aerobic treatment reactors. More and more establishments have stopped the practice of biomass disposal on second and third party lands or landfills altogether to avoid associated litigation.

Operational problems associated with the activated sludge process (such as chronic bulking with attendant secondary clarification and thickening failure) coupled with foaming problems due to aeration are yet to be resolved in many treatment facilities. Until feasible solutions are found, the present ongoing operational and disposal problems will continue to plague many aerobic treatment plants.

Aerobic treatment may still be the better choice for water quality management under some conditions, such as rapid start-up constraints, dilute and cold wastewaters deficient in alkalinity, low-cost electricity, or an abundance of potential solids disposal sites for the excess biomass produced. Amid the background of today's ecological imperatives, however, which have mandated many new environmental regulations and caused notable shifts in financial advantage, a serious reconsideration of the liabilities of the traditional aerobic methods will favor adoption of anaerobic-based wastewater treatment operations by many industries.

8.4.2 Aerobic Treatment New Horizons

Another factor hampering aerobic wastewater biotechnology is the relatively low biomass density prevailing in the reactor. Owing to the inherent poor settling of fluffy aerobic biomass, the microbial cells in the mixed liquor are constantly subject to washout. Maintaining adequate biomass accumulation in the reactor can thus be challenging. One feasible solution to this problem is to allow the biomass to anchor to a heavy carrier, such as sand particles or plastic media, and to operate the reactor as an upflow fluidized bed. Increased biomass densities can be attained and volumetric loading rates surpassing those of conventional activated sludge can be reached accordingly. The notion of fluidizing the biomass is yet to be accepted. The reasons for this are probably twofold. First, fluidized bed technology increases the complexity of the treatment and involves the need for intensive control: conventional systems are quite simple and controlled only extensively. Second, fluidized bed technology focuses on the rate of removal per unit reactor volume, whereas the major element in aerobic treatment is the quality of the effluent.

Recent advancements marked the development of aerobic granulation in overcoming the problem of biomass washout often encountered in activated sludge processes [31,32]. The novel approach to developing fluffy biosolids into dense and compact granules offers a new dimension for wastewater treatment. Compared with conventional biological flocs, aerobic granules are characterized by well-defined shape and compact buildup, superior biomass retention, enhanced microbial functions, and resiliency to toxicity and shock loading [31]. The application of aerobic granular sludge is currently viewed as one of the promising wastewater treatment innovations [33]. Carucci et al. [34] compared the treatment performances of aerobic granular sludge, sequencing batch

reactor with suspended sludge, and membrane bioreactor. These authors noted that the aerobic granular sludge is the best technology to treat 4-chlorophenol wastewaters in terms of direct comparison on removal rates, system simplicity, land requirement, and start-up times.

Maintaining aerobic granules with adequate structural integrity is one major challenge that hinders practical application of aerobic granulation. The current bottleneck of aerobic granulation development highlights the need for further research in granule stability for full-scale operation. There is a need to explore ways or techniques to develop granules with sustainable integrity. A technological method for cultivating granules of adequate structural stability for storage has been described [35]. Storing granules in a completely dried condition for recultivation was explored by the authors. The granules were recuperated after being dried for 21 days. The granules resumed their original appearance and size upon recultivation. It was reported that COD removal of the dried granules upon recuperation was not affected by the drying. The removal efficiency was comparable with active fresh granules that had not been subject to drying. It appears that drying did not have a notable impact on the granules in terms of morphology and functionality.

8.4.3 Misconceptions About Anaerobic Treatment

To a considerable extent, the use of anaerobic digestion processes has been limited by a number of misconceptions. Many notions on problems associated with anaerobic treatment have stemmed from a lack of understanding of process principles and from improper designs or operations that have resulted in failure. Older pre-1950-designed, low-rate, conventional systems contain design flaws that do not promote stable operation.

A fundamental mistake has been the comparison of efficiencies of anaerobic sludge digestion to aerobic treatment of wastewaters. Aerobic biological treatment efficiency is based primarily on the removal of soluble organics initially present in the waste. Naturally, anaerobic digestion of biological solids produced in aerobic treatment and other solids separated in a primary clarifier is more difficult than treatment of the initial dissolved substrate. This gives rise to the false conception that anaerobic processes are inefficient. Anaerobic processes can be designed to remove degradable soluble substrates from wastewaters as rapidly as or more rapidly than aerobic processes.

The thermodynamic limitations on the low amount of energy that may be obtained from reduced organics by microorganisms under anaerobic conditions also lead to the erroneous notion that anaerobic systems have low removal rates. Aerobic microorganisms are able to extract much larger amounts of energy from a given substrate than that obtained anaerobically. From sugars, aerobes can obtain more than 14 times as much energy as anaerobes. In this sense anaerobes are less efficient than aerobes. However, this does not affect the kinetics of actual treatment systems. From a treatment perspective, there are two major consequences: first, aerobes, being more efficient in this

respect, produce more sludge than anaerobes per unit of substrate processed and second, the chemical energy not captured by the anaerobes results in the formation of methane. Because sludge is usually a disposal problem, its lower production is a desirable feature of anaerobic processes and, furthermore, methane is a fuel. Wastewater becomes a low-energy resource through anaerobic treatment.

Some substances in a wastewater will be susceptible to biological treatment, whereas others will not be affected. The latter are known as refractory or nonbiodegradable materials. The relative amounts of biologically removable and nonremovable substances determine the overall biodegradability of the waste. From a general review of the characteristics of domestic and other wastes in aerobic and anaerobic processes, there is little difference in biodegradability for a given waste under either treatment regime. In other words, removals attainable are approximately the same regardless of the type of biological treatment. Effluent quality from an anaerobic process is usually not as good as that from an aerobic process, but in terms of overall removal the difference is marginal, particularly when high-strength wastes are involved.

Another misconception is that the anaerobic process is unstable. Inadequate mixing in older conventional reactors is a common design flaw. Older conventional reactors are suspended growth systems with mixing supplied by the microorganisms through gas production or by supplemental mechanical power input. Effective mixing is essential in the conventional process to achieve high loading rates and corresponding low liquid retention times.

Poorly mixed or dead (unmixed) zones decrease retention times below design values. This leads to a series of phenomena including reduced treatment and instability and can ultimately result in complete failure. Field evaluations have revealed that more than 50% of the reactor volume in a number of installations with different types of mixing systems was dead space. Furthermore, significant short circuiting, which is flow that passes through the reactor too quickly for treatment, was found.

Although microorganisms involved in anaerobic fermentation are fairly sensitive to their environment, proper design and operation can avoid major problems. It is obvious from this discussion that actual retention times in the conventional process of the past were often significantly below design retention times because of inadequate mixing, a problem for which there are a number of solutions.

Other than instability problems associated with insufficient retention time, low concentrations of the microorganisms in the process also contribute to instability. The new designs inherently promote stability by maintaining large inventories of microorganisms and many of these designs eliminate the need for additional mixing systems.

A common misconception is that anaerobic processes are more sensitive than aerobic processes to toxic substances. This misconception stems from anaerobic digestion's history as a municipal sludge reduction treatment. Sludges generated in sewage treatment concentrate various toxicants, which makes them more difficult to treat by any biological means. A comparison of the LC_{50} for a wide range of chemical classes between aerobic heterotrophs and methanogens showed that there was no significant difference

between the sensitivities of the two groups. There was one exception: the methanogens were more sensitive to chlorinated hydrocarbons and alcohols. Anaerobic processes may handle some toxicants better than aerobic processes. Anaerobic processes generate sulfides that complex or precipitate heavy metals, which are thereby removed as a toxicant.

It is true that the relationship among microbial species in the anaerobic process is more complex than in an aerobic process. There exists more potential for one group, the acid formers, to cause conditions that are unsuitable for the other major group, the methane formers, which have stricter environmental requirements. However, with proper buffering, SRT, and pH control, stable operation can be achieved.

The last fallacy concerns the temperature at which the process must be operated. The methane-forming anaerobes have two optimal temperature ranges depending on the species: one in the range of 33—45°C and the other between 65 and 70°C. Because most digesters have been built for operation near 35°C, there is a general feeling that this temperature must always be used. On the other hand, aerobic systems are almost always designed for ambient temperatures, even though these temperatures are not optimal for aerobes. Aerobic systems are designed to operate at lower temperatures because this is more economical, not because the bacteria perform better at these temperatures.

Maintaining an elevated temperature in an anaerobic process generally requires consumption of the excess energy produced by the process and often requires an external heat source in addition. High temperatures are not always necessary. Many studies have shown that the total amount of methane that can be produced, and thus the treatability of the waste, does not vary with temperature. Reactor loading rates and rate of methane production per unit volume of reactor do vary, but loading rates are still equivalent to, or higher than, loading rates for aerobic systems.

Start-up is also cited as a problem for anaerobic systems. Lower rates of growth for anaerobic bacteria result in start-up times longer than those for aerobic systems. However, start-up should be required only once and there are practical steps that can be taken to minimize start-up times. The problem of start-up is compensated for by the relatively quick recovery time of an anaerobic system that has been shut down for a significant time. It is hard for aerobic cultures to be maintained over long periods of dormancy.

8.4.4 Anaerobic Treatment Challenges and New Horizons

The major challenge of anaerobic treatment is related to the slow growth rate of the methane-producing bacteria. Slow growth rates require a relatively long retention time in the reactor for adequate waste decomposition. The sensitive and delicate nature of the methanogens also limits the rate at which the process can adapt to changing organic loadings, temperatures, or other environmental conditions. It is essential that the microbes have been allowed to acclimatize to the new conditions, especially in starting up the reactors for subsequent satisfactory operation. Longer start-up period is therefore

Table 8.6 Possible Disadvantages of Anaerobic Treatment

- Long start-up requirement for development of biomass inventory
- Insufficient inherent alkalinity generation potential in dilute or carbohydrate wastewater
- Insufficient effluent quality for surface water discharge in some cases
- Insufficient methane generation from dilute wastewaters to provide for heating at 35°C optimal temperature
- Sulfide and odor generation from sulfate feed stocks
- Nitrification not possible
- Greater toxicity of chlorinated aliphatics to methanogens vs. aerobic heterotrophs
- Low kinetic rates at low temperatures
- High NH_4 concentrations (40—70 mg/L) required for maximum biomass activity

needed in the anaerobic process. However, advances in understanding the fundamentals of the biochemistry and microbiology of anaerobic treatment have led to successful applications, which show a great deal of promise in overcoming the limitations associated with the anaerobic process.

Sometimes it would not be practical to use anaerobic treatment, as might be the case in processing low-temperature or dilute wastewaters, insufficient alkalinity wastewaters, or effluents requiring exceptionally low BOD for final discharge regulations. Careful examination of each situation in light of these and other disadvantages listed in Table 8.6 may sometimes dictate aerobic biotechnology as the better choice.

Notwithstanding the many significant and impressive advances in anaerobic microbial process fundamentals and applications over the past few decades, there are areas that require additional attention and development. Perceived as foremost among these are the following:

1. Further elucidation of principal and controlling mechanisms in the anaerobic conversion of complex and relatively recalcitrant substrates, including the microbiology and biochemistry of biotic hydrolytic, fermentative, oxidative, respiratory, acetoclastic, and methanogenic reactions, as well as the abiotic counterparts, either alone or in combination
2. Further determination and prioritization of environmental factors controlling and useful in describing conditions associated with anaerobic microbial treatment process balance or imbalance, including liquid-, solid-, and gas-phase mechanisms and parameters, as well as their individual and collective significance and utility for process development, optimization, and control
3. Translation of theoretical and empirical modeling advances into operationally diagnostic and remedial techniques, including methods to reduce uncertainty and facilitate control of routine process operations with appropriate sensors and instrumentation for evaluation of significant process variables and state conditions
4. Enhanced development of analytical techniques descriptive of biomass structure and viability, including concomitant kinetic, operational, and environmental factors establishing selection and/or dominance, temporal and spatial distribution, and

associated substrate conversion patterns in processes configured or managed by physical, hydrodynamic, or biochemical techniques for phase management and control

5. Extension of the principles and practices of anaerobic microbial treatment processes to emerging and new horizons of investigation and development, including controlled landfills, constructed wetlands, and natural soil and aqueous systems for microbial-mediated remediation, decontamination, and/or detoxification
6. Further development and promotion of standard and consensus nomenclature and notations for clearly identifying and describing anaerobic microbial process types and configurations, indicator parameters and analytical methods, process microbiology and biochemistry, growth and substrate conversion patterns and kinetics, models, and control strategies

Much data already indicate the anaerobic process would be the favorable option in a growing number of industrial wastewater treatment operations. Once the initial start-up and prolonged delay in accumulation of biomass inventory are over, the technology offers inexpensive treatment of many common industrial wastewaters and even more unusual effluents containing low concentrations of chloroform, trichloroethylene, and the other industrial toxicants. New insights into the anaerobic degradation of very different categories of compounds, such as fine and specialty chemicals from the chemical industry, coal and petrochemicals, and textile and dyeing stuff, and into process and reactor technology will lead to very promising new generations of anaerobic treatment systems [36]. These concepts will provide a better efficiency at higher loading rates and are applicable for extreme environmental conditions (e.g., low and high temperatures) and to inhibitory or toxic compounds. Moreover, by integrating the anaerobic process with other biological methods (effluent polishing by aerobic activated sludge and/or biofilm, sulfate reduction, micro-aerophilic organisms) and with physicochemical methods, a complete treatment of the wastewater can be accomplished at very low costs, and at the same time valuable resources can be recovered for reuse. Based on the successful full-scale experience, the anaerobic process is expected to receive wider usage for the treatment of a variety of industrial wastes in the future.

A promising application of anaerobic processes lies with its markedly reduced production of excess sludge. Under anaerobic conditions, more than 90% of the wastewater COD is converted to methane gas as an end product. This energy equivalent is not available for biomass synthesis, thereby considerably lessening both financial and waste biomass disposal site requirements. Increasingly stricter environmental regulations have called for a more stringent sludge disposal in many municipalities; some even have implemented drastic measures such as zero sludge discharge policies to tackle dwindling landfills and rampant illegal sludge dumping. With the significant advantage of decreased excess sludge production, applications of anaerobic processes will be further boosted.

An emerging application of anaerobic systems is associated with the Certified Emission Reduction or Carbon Credit set under the Kyoto Protocol. In addition to contributing to sustainable development with energy recovery in the form of methane, carbon credits can be claimed by application of advanced anaerobic processes in wastewater treatment for mitigating emissions of greenhouse gases [28,30]. Looking at the prospects of carbon trading, it may not be an unreasonable expectation that, in the future, wastewater treatment will experience a global shift toward employment of highly efficient anaerobic processes in maximizing energy production and minimizing greenhouse gas emissions.

8.5 Conclusion

Now that both aerobic and anaerobic wastewater treatments can be considered as having been upgraded to the level of scientific recognition, it is worthwhile to evaluate to what extent both technologies are currently evolving, either as complementary to one another, as they tended to be in the past, or as direct competitors. In the near future, important progress can be expected with regard to the optimal linkage between anaerobic and aerobic processes. In the future, for a growing number of industries, the best choice must be the most environmentally desirable and cost-effective choice; with the applied research now published, anaerobic waste removal coupled with aerobic polishing may well become the solution.

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9

Microbiology and Biochemistry of Anaerobic Treatment

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9.1 Introduction

Anaerobic treatment is widely employed for wastewaters containing large amounts of organic compounds. Anaerobic treatment is considered advantageous for various reasons, such as the low energy cost for operation, less production of sludge, and the possibility of recovering utilizable energy present in the wastewaters in the form of methane. However, the efficiency of the anaerobic treatment process depends not only on operational and environmental parameters such as organic loading rate, hydraulic retention time, solids retention time, pH, temperature, etc., but also on the microbial and metabolic diversity of microorganisms present in these systems. This leads to an interesting question of whether physicochemical parameters or biological components drive the treatment process.

To answer this, it is essential to review the basics a little. Basically, biochemical reactions depend on the type and availability of electron donors and acceptors in the system because the thermodynamics of the reactions and the consequent energy production are dependent on their chemical properties. Almost all kinds of wastewaters contain various types of organic compounds, all of which have the potential to function as substrates or electron donors for the various types of energy-yielding mechanisms. However, in the case of electron acceptors, the presence of large quantities of organic and inorganic compounds naturally eliminates the availability of oxygen as terminal electron acceptor and it is imperative for the system to look for alternative terminal acceptors of electrons. A wide variety of inorganic and metal compounds, viz., iron, nitrates, manganese, sulfates, carbonates, chlorates, arsenic, chromate, etc., are potential final electron acceptors. In an anaerobic process, all of these compounds could be used as electron acceptors depending on their availability and quantities.

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It has to be highlighted here that an important parameter of energy production in biological systems, the redox potential, is defined by the presence and quantities of these various electron acceptors. Stephenson [1] observed that anaerobic microorganisms are redox specialists and that the absence of molecular oxygen does not deter them from harnessing energy from energy-rich substrates. In general, based on the quantities present, nitrates, sulfates, and carbonates are considered the major electron acceptors in anaerobic wastewater treatment systems. The energy produced by these acceptors is in the order nitrate > sulfate > carbonate, because of their oxidation/reduction potentials. Hence, this chapter deals basically with the microbiology and biochemical mechanisms involved with nitrates, sulfates, and carbonates as the final acceptors of electrons.

9.1.1 Microbiology and Biochemistry of Nitrogen Transformation in Anaerobic Wastewater Systems

Nitrogen-containing compounds are some of the most important pollutants in wastewater. Nitrogen has five valence electrons and reduction occurs in the range of −3 to +5. The oxidation states of the principal participants in a treatment plant are the following:

$$\underset{[+5]}{NO_3^-} \leftrightarrow \underset{[+4]}{NO_2} \leftrightarrow \underset{[+3]}{NO_2^-} \leftrightarrow \underset{[+2]}{NO(g)} \leftrightarrow \underset{[+1]}{N_2O(g)} \leftrightarrow \underset{[0]}{N_2(g)} \leftrightarrow \underset{[-3]}{NH_4^+} \quad [9.1]$$

Nitrogen in wastewaters can be present in its oxidized forms such as nitrate (NO_3) and nitrite (NO_2). Nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2), along with the nonionized (NH_3) and ionized (NH_4) forms of inorganic ammonium nitrogen and organic forms of N such as amino acids, amino sugars, urea, uric acid, purines, and pyrimidines, can also be found in wastewater. Their form and concentration depends on the source of the wastewater, level of the pretreatment [2,3], and production and consumption rate of microbial metabolic processes [4].

Several processes, which comprise assimilatory and dissimilatory biological transformations, are necessary for the removal of N from wastewater. Under anoxic conditions, N transformations occur through three principal biological processes (Fig. 9.1): dissimilatory microbial reduction of nitrate to N_2 (denitrification) [5], dissimilatory nitrate reduction to ammonium (DNRA) [6], and anaerobic ammonium oxidation (anammox) [7].

9.2 Denitrification

Denitrification has been recognized as one of the major N removal processes in various environments and can be carried out by many bacteria [8]. The complete denitrification process includes four reduction steps $\left(NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2\right)$ [9]; however, denitrification need not always be carried out completely by a sole microorganism. Some bacteria are able to participate in only some steps, generating a mix of nitrogen species [10–12]. Incomplete denitrification usually produces N_2O, one of the greenhouse gases,

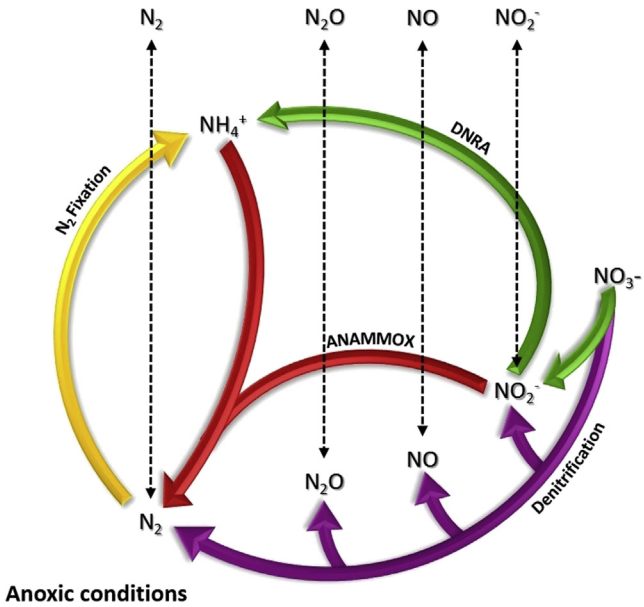

FIGURE 9.1 Nitrogen transformation under anoxic conditions. *Anammox*, anaerobic ammonium oxidation; *DNRA*, dissimilatory nitrate reduction to ammonium.

with a global warming potential 298 times greater than the equivalent amount of CO_2. Emission of this greenhouse gas to the atmosphere from wastewater treatment plants is to be mitigated [13].

A wide array of microorganisms including 40–50 or more genera of bacteria, halophilic archaea, and fungi has been recognized for their denitrification mechanisms [4,5]. Apart from the well-studied heterotrophic denitrification process, which is common in most of the anoxic wastewater treatment processes [3,11,14], autotrophic denitrification occurs too, in combination with oxidation of various compounds such as hydrogen or various reduced-sulfur compounds, such as HS^-, H_2S, S, $S_2O_3^{2-}$, $S_3O_6^{2-}$, or SO_3^{2-}, and with carbon dioxide or bicarbonate as the carbon source. *Thiobacillus denitrificans*, *Sulfurimonas denitrificans*, *Paracoccus denitrificans*, *Thioploca*, and *Beggiatoa* are some of the well-known chemolithotrophic denitrifying bacteria that couple oxidation of inorganic sulfur compounds [15,16]. In addition several halophilic archaea belonging to the Euryarchaeota and several members of the Crenarchaeota are also found to be active denitrifiers. Cabello et al. [17] have described nitrate reduction nitrogen transformation by archaea. Representative bacteria, enzymes, and their functions in the denitrification process are presented in Table 9.1. Except for nitrate reductase, which is localized in the cytoplasm, the rest of the enzymes are encountered in the periplasm.

Jones et al. [30] analyzed the nucleotide sequences of the *nirK*, *nirS*, *norB*, and *nosZ* genes coding for enzymes involved in the denitrification pathway and observed that the evolution of denitrification genes and diversity of microorganisms capable of

Table 9.1 Representative Microorganisms and Enzymes Involved in the Denitrification Process

Enzyme	Gene	Complex/ Subunit	Electron Donor	Function	Microorganisms	References
Nitrate reductase	*narG*	NarGHI	Quinol	Cytoplasmic nitrate reduction	*Paracoccus denitrificans* (α), *Wolinella succinogenes* (ε) *Escherichia coli*	[18] [19]
		NarGHIC	Ubiquinol and periplasmic cytochrome *c*	Cytoplasmic nitrate reduction	*Thermus thermophilus*	[8]
		NarGH	Quinones and periplasmic electron transfer proteins	Nitrate reduction (outside of the cytoplasmic membrane)	*Haloferax mediterranei*, *Haloarcula marismortui*	[8] [20]
	nap	NapA	Quinone through NapB	Periplasmic nitrate reduction	*Desulfovibrio desulfuricans* (δ), *Thiosphaera pantotropha* *W. succinogenes* *Magnetospirillum gryphiswaldense* (α)	[21] [22] [23]
		NapB	Quinone	Periplasmic nitrate reduction	*Rhodobacter sphaeroides* (α), *E. coli* (γ), *P. denitrificans* (α), *Bradyrhizobium japonicum* (α)	[8] [18] [24]
Nitrite reductase	*nirS*	NirS	Monoheme cytochrome *c* or cupredoxin	Nitrite reduction	*Pseudomonas aeruginosa* *Pseudomonas stutzari* *Alcaligenes faecalis*	[8] [25]
	nirK	NirK	Monoheme cytochrome *c* or cupredoxin	Nitrite reduction	*H. marismortui* *B. japonicum* *P. denitrificans*	[25]
Nitric oxide reductase	*nor*	NorB/NorC	Quinol or menaquinol	Nitric oxide reduction (outside of the cytoplasmic membrane)	*P. aeruginosa*, *P. denitrificans*, *P. stutzeri*, *Geobacillus stearothermophilus*	[26] [27]
Nitrous oxide reductase	*nosZ*	Nos	Monoheme cytochrome *c* or cupredoxin, menaquinol	Periplasmic nitrous oxide reduction	*Achromobacter xylosoxidans* (β), *Sinorhizobium morelense* (α), *Pseudomonas fluorescens* (γ) *Agrobacterium tumefaciens*	[28] [18] [29]

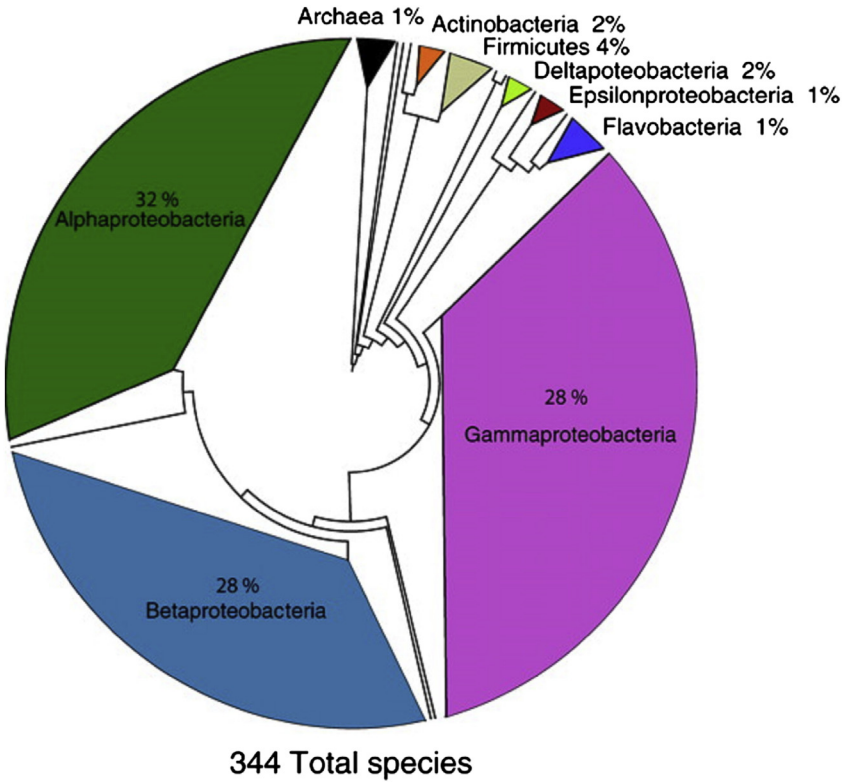

FIGURE 9.2 Phylogeny and distribution of denitrifying microorganisms based on partial sequence alignment of functional denitrification genes [30].

denitrification are not due to horizontal gene transfer, but mainly to gene duplication/divergence and lineage sorting. The phylogenetic distribution of denitrifying microorganisms is presented in Fig. 9.2.

9.3 Dissimilatory Nitrate Reduction to Ammonium

Under nitrogen limitation and rich electron donor conditions, the reduction of nitrate to ammonium is thermodynamically favorable. In contrast, when the NO_3^- concentration is high and there is electron donor limitation, more energy is gained through denitrification [31]. In contrast to denitrification, the DNRA process does not emit N_2 to the atmosphere; this process maintains N in the environment as ammonium.

Ammonium is considered one of the most important nitrogen compounds, as (1) it is preferred as a nutrient by autotrophic bacteria; (2) it is chemically reduced and can be readily oxidized, decreasing the concentration of dissolved oxygen in the water; and (3) the nonionized form, ammonia, is toxic to many forms of aquatic life in low concentrations (>0.2 mg/L) [32].

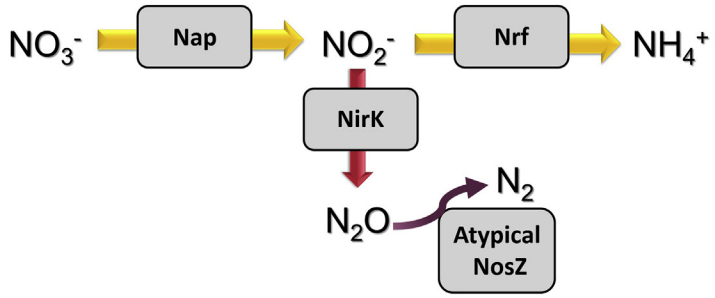

FIGURE 9.3 The dissimilatory nitrate reduction to ammonium (DNRA) (*yellow* (dark gray in print versions)), reduction of nitrate to nitrous oxide (*red* (light gray in print versions)), and atypical nitrous oxide reduction to dinitrogen (*violet* (black in print versions)) pathways that take place in microorganisms carrying out DNRA.

In wastewater treatment processes, many toxic compounds are present, of which ammonia is the most dangerous. Its toxicity depends on the microbial diversity that is present in the treatment and the degree of ionization, in which factors such as pH and temperature are important. If pH and temperature increase, the free ammonia fraction increases, converting the medium to a more toxic one [33]. Wastewater containing sulfides (S^{2-}, HS^{-}, and H_2S) inhibits denitrification and anammox processes [34]. However, in DNRA, they may be employed as electron donors [35].

In DNRA, a periplasmic nitrate reductase complex (NapAB) mainly performs the reduction of nitrate to nitrite. Then, the nitrite is reduced to ammonium without any intermediate by a pentaheme cytochrome *c* nitrite reductase (NrfA) [36]. Some DNRA bacteria encode both *nrfA* and *nirK* genes, whereas others also possess the *nosZ* gen. Thereby, microorganisms carrying out DNRA and containing *nirK* and *nosZ* genes can metabolize N_2O (Fig. 9.3) [13].

A diverse group of microorganisms (Gamma-, Delta-, and Epsilonproteobacteria) has been identified as DNRA bacteria [37]. The *nrf*A gene has been found in *Escherichia coli* [38], *Desulfovibrio desulfuricans* [39], *Wolinella succinogenes* [40], and *Vibrio fischeri* [41]. Although the *nrf*A gen is usually associated with DNRA, some microorganisms such as *Shewanella oneidensis* and *Thioalkalivibrio nitratireducens* employ the octaheme tetrathionate reductase, an enzyme that is able to reduce nitrite to ammonium in a direct way [42,43].

9.4 Anaerobic Ammonium Oxidation

Anammox is a fairly recently discovered mechanism for nitrogen removal. Until 2002, it was considered an unimportant player in the nitrogen cycle [44]. Nevertheless, it was discovered that anammox produces 24–67% N_2 of the total N in sediments [45]. Anammox bacteria are able to produce N_2 under anoxic conditions from oxidation of ammonium using nitrite as electron acceptor (Reaction [9.2]) [46].

$$NH_4^+ + NO_2^- = N_2 + H_2O \quad (\Delta G^{\circ\prime} = -357\ \text{kJ/mol}) \qquad [9.2]$$

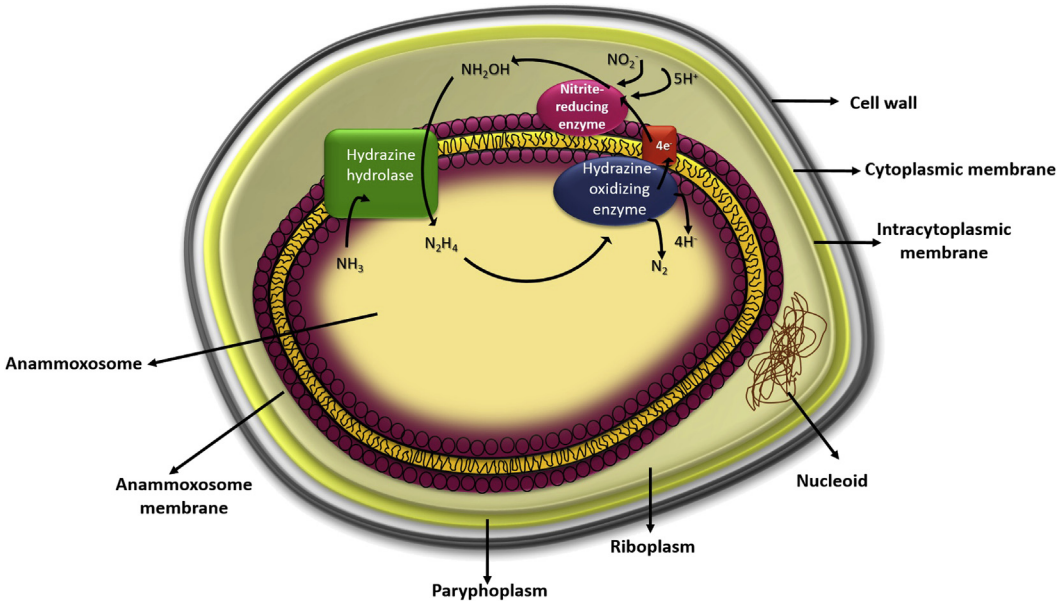

FIGURE 9.4 Enzymes involved in the anaerobic ammonium oxidation process.

This process is realized by the phylum Planctomycete, among four *Candidatus* genera: *Candidatus Brocadia* [47], *Candidatus Kuenenia stuttgartiensis* [48], *Candidatus Scalindua*, and *Candidatus Anammoxoglobus* [49]. The growth of anammox microorganisms is relatively low, with a maximum growth rate (μ_{max}) of 0.065/day and generation time of 10–12 days at 35°C [50].

Anammox bacteria possess an anammoxosome, a bacterial-like "organelle" without ribosomes that comprises 50–70% of the total cell volume (Fig. 9.4) [51].

The anammox mechanism can occur by two possible reactions. In the first, the membrane-bound enzyme complex converts ammonium and hydroxylamine to hydrazine. Then, hydrazine is oxidized in the periplasm, yielding N_2. In the second possible mechanism, the same enzymatic complex carries out the conversion of ammonium and hydroxylamine to hydrazine; however, the electrons generated are transported to the electron transport chain for the reduction of nitrite to hydroxylamine [52].

Two unique enzymes in anammox bacteria are found: Hydrazine hydrolase, which synthesizes hydrazine from nitric ammonium, and hydrazine dehydrogenase, which is able to transport electrons from hydrazine to ferredoxin [53].

9.4.1 Microbiology and Biochemistry of Sulfur Transformation in Anaerobic Wastewater Systems

Sulfur is found in a range of redox states such as sulfide (S^{2-}), elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$), sulfur dioxide (SO_2), sulfite (SO_3^{2-}), dithionite ($S_2O_4^{2-}$), and sulfate (SO_4^{2-}).

Table 9.2 Sulfate Concentration in Wastewaters

Type of Water	Sulfate Concentration (mg/L)	References
Mines	1336	[54]
Food (citric acid production)	2500–4300	[55]
Metallurgic	298–2322	[56]
Landfill	225	[57]
Agricultural runoff	722	[32]
Alcohol production	2900–50,600	[58]
Paper and board	1000–2000	[59]
Oil refineries	40,000–50,000	[60]
Domestic	20–500	[61]
Organic peroxide production	12,000–35,000	[62]
Seafood processing	600–2700	[63]
Rubber processing	500–2000	[64]
Tanneries	2500–3000	[65]
Sulfonated oils	180,000–284,000	[66]
Pharmaceutical	5000	[67]
Textile dyeing	1706–2690	[68]

The amount of sulfate contained in wastewater differs according to its source (Table 9.2). Sulfate concentrations in domestic wastewater vary between 20 and 500 mg/L, but can go up to thousands of milligrams per liter in industrial wastewaters.

In the anaerobic treatment of sulfate-containing wastewater, the most abundant process of sulfate transformation is dissimilative sulfate reduction, which leads to the removal of sulfate. Sulfate-reducing microorganisms play an important role in wastewater treatment because they couple the oxidation of organic and inorganic compounds with the reduction of sulfate to sulfide [69]. Sulfate-reducing bacteria (SRB) readily compete for the available substrates with fermentative bacteria, syntrophic obligate hydrogen-producing bacteria, homoacetogenic bacteria, acetate-oxidizing bacteria, and methanogenic archaea [70].

The sulfide generated can be inhibitory for other groups present in the system. In methanogenic reactors, sulfate reduction is an undesired process because the methane formation is inhibited by the sulfide toxicity and the competition between methanogens and SRB.

9.5 Sulfate-Reducing Bacteria

SRB are anaerobic microorganisms capable of using sulfate or other oxidized sulfur compounds (sulfite, thiosulfate, and elemental sulfur) as final electron acceptors. They mainly reduce sulfate to sulfide and use organic substrates or H_2 as electron donors. It has been reported that they also are capable of use nitrate [71], iron (Fe^{3+}) [72], uranium (U^{6+}) [73], pertechnetate (Tc^{7+}) [74], selenate (Se^{6+}) [75], chromate (Cr^{6+}) [76], and arsenate (As^{5+}) [77] as final electron acceptors.

Based on comparative analysis of 16S rRNA sequences, Muyzer and Stams [78] classified SRB into five and two separate lineages among the domains Bacteria and Archaea, respectively (Fig. 9.5). Earlier, Castro et al. [79] classified them into four subgroups, viz., gram-negative mesophilic SRB, gram-positive spore-forming SRB, thermophilic bacterial SRB, and thermophilic archaeal SRB. The gram-positive spore-forming group is dominated by the genus *Desulfotomaculum*, which is placed in the Clostridia class. This group is known to form heat-resistant endospores [80]. The gram-negative mesophilic group is

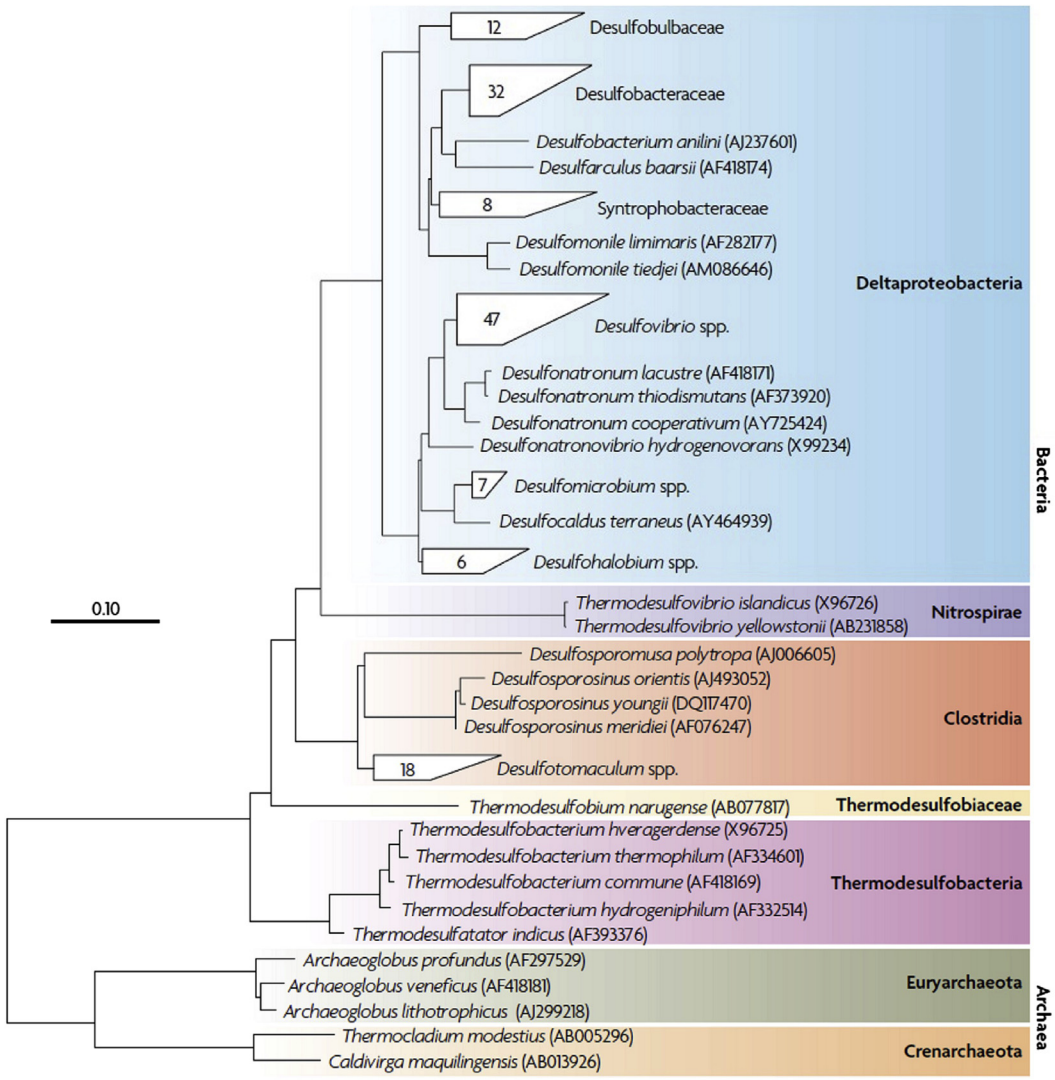

FIGURE 9.5 Phylogenetic lineage of microorganisms with sulfate reduction activity based on the comparative analysis of 16S rRNA sequences [78].

found within the class of Deltaproteobacteria, with following representative genera: *Desulfovibrio*, *Desulfomicrobium*, *Desulfobulbus*, *Desulfobacter*, *Desulfobacterium*, *Desulfococcus*, *Desulfosarcina*, *Desulfomonile*, *Desulfonema*, *Desulfobotulus*, and *Desulfoarculus*. Many of these have been mainly identified in bioreactors treating wastewater, operating at 22–35°C [81]. Thermophilic bacterial SRB are widespread in the families Thermodesulfobactera and *Nitrospira*. *Thermodesulfobacterium commune* and *Thermodesulfovibrio yellowstonii* are common examples of this group [78]. Within the thermophilic archaeal SRB, the species *Archaeoglobus fulgidus* and *Archaeoglobus profundus* have been described. Both microorganisms were isolated from hydrothermal environments and are phylogenetically related to methanogens, especially to members of the *Methanosarcina* genus. They can use the coenzymes methanofuran, methanopterin, and F_{420} for their metabolism [82].

9.6 Fermentation by Sulfate-Reducing Bacteria

Heterotrophic SRB are divided into two categories. In the first, a complete oxidation of organic compounds to CO_2 is performed. In the second, only a partial oxidation occurs, and usually acetate is the end product [83]. In environments where sulfate is low, SRB can grow by fermenting organic compounds such as pyruvate, lactate, and ethanol, producing acetate, carbon dioxide, and hydrogen. This is possible if they grow in syntrophy with methanogens and other hydrogen-consuming microorganisms [84]. One example of this situation is *Syntrophobacter wolinii*, a sulfate-reducing bacterium that, in the absence of sulfate, grows as an acetogen, oxidizing propionate and producing acetate, carbon dioxide, and hydrogen [85]. However, when sulfate is added, it is immediately used as a final electron acceptor. Therefore, SRB can be present in anaerobic environments as sulfate reducers or acetogens [78].

In *Desulfovibrio* species, a cycle was proposed for energy conservation named the "hydrogen cycling model," in which lactate oxidation to acetate allows H_2 production by cytoplasmic hydrogenase (Fig. 9.6). The H_2 produced is diffused across the cytoplasmic membrane and used as electron donor for the reduction of sulfate to sulfide [86].

Hydrogen oxidation is a very important reaction in anaerobic processes. This reaction keeps the H_2 partial pressure below 10^{-3}–10^{-4} atm, making the volatile fatty acids oxidation thermodynamically favorable so that they do not accumulate in the process. The H_2 is produced by obligately hydrogen-producing syntrophic bacteria, and their concentration is kept in balance by methanogenic archaea, homoacetogenic bacteria, and SRB by oxidation of H_2 for their metabolic activity [87].

9.7 Sulfate Reduction Process

The sulfate reduction is performed by two different pathways: the assimilative and the dissimilative (Fig. 9.7). In the assimilative pathway, sulfate is reduced to sulfide for the biosynthesis of sulfur-containing amino acids and enzymes, whereas in the dissimilative pathway, SRB use sulfate as an electron acceptor for energy conservation [88].

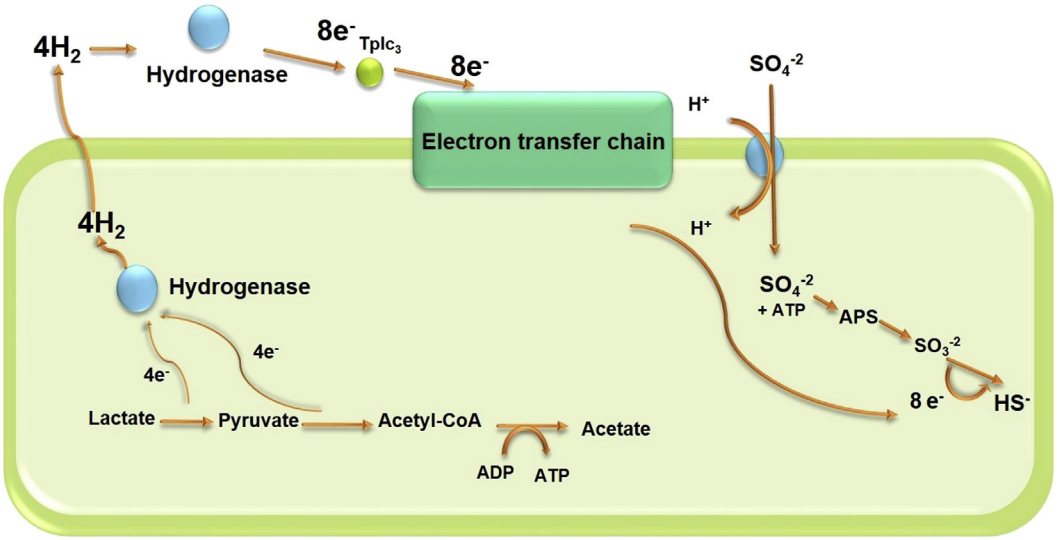

FIGURE 9.6 The hydrogen cycling model in *Desulfovibrio*. *Tplc*$_3$, type I cytochrome c_3.

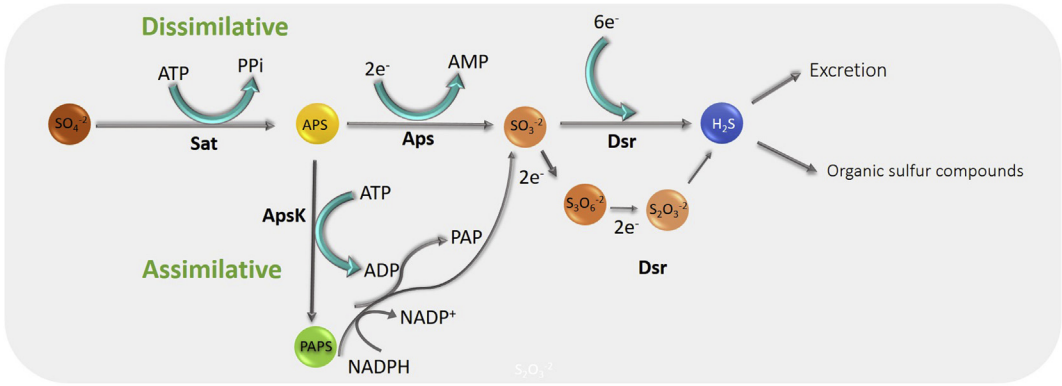

FIGURE 9.7 Dissimilative and assimilative pathways for sulfate reduction. *APS*, adenosine phosphosulfate; *Aps*, APS reductase; *Dsr*, dissimilatory sulfite reductase; *Sat*, ATP sulfurylase. *ApsK*, adenosine phosphosulfate kinase; *PAP*, phosphoadenosyl phosphate; *PAPS*, phosphodenosyl phosphosulfate.

The dissimilative pathway starts with the activation of SO_4^{2-} by ATP sulfurylase (Sat) inside the cell, forming adenosine phosphosulfate (APS) [89]. Then, the APS is reduced by APS reductase to sulfite (SO_3^{2-}) [90]. Finally, the sulfite is subsequently reduced to sulfide (H_2S) in a six-electron reduction by the action of dissimilatory sulfite reductase (Dsr), which is formed by two subunits, α and β, encoded in the *dsr*AB gene [91]. Three molecules of ATP are generated in dissimilative sulfate reduction; however, 2 1/3 molecules are used for the activation of sulfate and its transportation. Thus, in total, 2/3 molecule of ATP is generated in the whole process [88].

Dsr is a very important enzyme in the sulfate reduction process and the genes that encode this enzyme are conserved in all SRB. For this reason, they are used as markers for phylogenetic identification of these microorganisms in industrial wastewater treatments. Pereyra et al. [92] quantified the gene expression of *dsrA* (SRB), *hydA* (fermenters), and *mcrA* (methanogens) to understand the behavior of microbial communities in reactors fed with mine drainage/metal wastewaters. In another study, the *dsrB* gene was used as a molecular marker to identify metabolically active SRB community members and their diversity in lab- and full-scale reactors treating sulfate-rich wastewater [93].

The mechanism of reduction of sulfite to sulfide has been questioned for a long time and many theories about this reduction have been proposed (Fig. 9.8).

Traces of thiosulfate $(S_2O_3^{2-})$ and trithionate $(S_3O_6^{2-})$ have been found during sulfate reduction. For this reason, Kobayashi et al. [95] proposed a trithionate pathway, in which Dsr reduces sulfite to trithionate, which in turn is reduced to thiosulfate by trithionate reductase. Finally, the enzyme thiosulfate reductase reduces thiosulfate to sulfide. Trithionate and thiosulfate reductase have not been found in several sulfate reducers. However, it has been demonstrated that in the sulfate-reducing archaea *A. fulgidus*, Dsr can carry out the reductions of thiosulfate and trithionate [88].

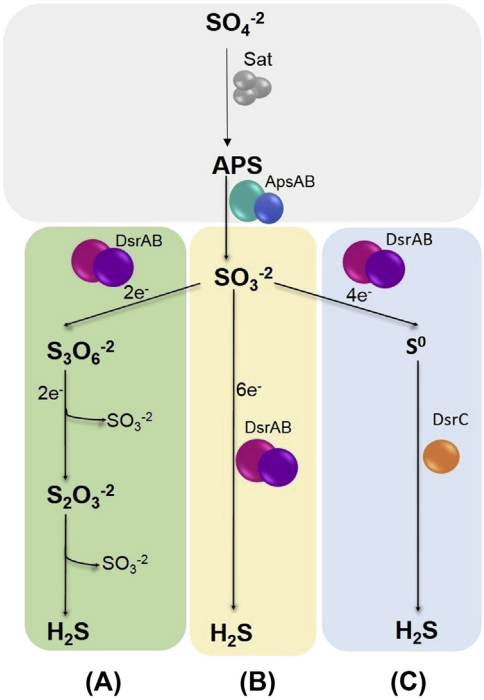

FIGURE 9.8 Mechanisms proposed for sulfite reduction to sulfide. (A) Trithionate pathway. (B) The Rees pathway. (C) Model proposed by Oliveira et al. [94]. *APS*, adenosine phosphosulfate; *Aps*, APS reductase; *Dsr*, dissimilatory sulfite reductase; *Sat*, ATP sulfurylase.

Another proposed mechanism is the Rees pathway. In this theory, the sulfite is directly reduced to sulfide in a six-electron transformation catalyzed by Dsr [96]. On the other hand, Oliveria et al. [94] proposed that a four-electron reduction, instead of six-electron reduction, occurs with the concomitant formation of S^0 as intermediate. This is possible if a subunit DsrC is associated with DsrAB.

9.8 Methanogenesis

Methane production is the last step of many anaerobic digestion processes and the microorganisms involved in this stage belong to the domain Archaea. Compared to the microbial groups that participate in the previous reactions, methanogens are not as phylogenetically diverse as those communities. Also, the number of substrates utilized for methanogenesis is reduced. Ideally, in a methanogenic reactor with good performance, the products generated in acetogenesis (acetate, CO_2, and H_2) are consumed by methanogens [97]. These archaeas produce methane through two different pathways known as the hydrogenotrophic or CO_2-reducing pathway and the aceticlastic pathway [98]. The dominance of either one of these pathways is influenced by the type and composition of substrate, hydrogen concentration, metabolic networks within the reactor, and operational and environmental factors [99].

Methanogens are considered the most sensitive group of anaerobic digestion, and a great number of substances such as ammonia, long-chain fatty acids, heavy metals, aromatic compounds, hydrogen sulfide, and many others, affect their metabolic activity [100]. Additionally, methanogens can also be outcompeted by sulfate-reducing, iron-reducing, denitrifying, and nitrate-reducing bacteria if their electron acceptors are present in abundance [101]. Therefore, if methane is the desired final product, the step of methanogenesis is referred to as the bottleneck [102].

9.9 Methanogens

Methanogens are a diverse group of strictly anaerobic Euryarchaeota that are distinguished by their ability to obtain energy for growth by the biosynthesis of methane. Methanogens can be found in a wide variety of anaerobic environments on earth, such as marine and freshwater environments, biodigesters, rice fields, hydrothermal vents, and inside the gut of living organisms, like ruminants, insects, and even human beings. Their distribution, however, is highly dependent on their adaptation to various temperature, pH, and salinity ranges. These microorganisms are abundant in habitats where electron acceptors such as O_2, NO_3^-, Fe^+, and SO_4^{2-} are depleted because their reductions are thermodynamically more favorable than CO_2 reduction to methane [103–105].

Methanogens use a limited number of substrates (Table 9.3), viz., CO_2 and methyl-group-containing compounds [103].

Table 9.3 Free Energy Obtained From Various Substrates During Methanogenesis

Reaction	ΔG (kJ/mol CH_4)
CO_2 Reduction	
$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	−130
$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$	−120
$CO_2 + 4(\text{isopropanol}) \rightarrow CH_4 + 4(\text{acetone}) + 2H_2O$	−37
Methyl-Group-Containing Groups	
$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	−113
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	−103
$4CH_3NH_2Cl + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_4Cl$	−74
$2(CH_3)_2S + 2H_2O \rightarrow 3CH_4 + CO_2 + 2H_2S$	−49
$CH_3COOH \rightarrow CH_4 + CO_2$	−33

CO_2 is the main substrate and almost all methanogens can reduce this compound to methane with electrons from diverse sources. Hydrogen (H_2) is the main electron donor but others compounds such as formate, CO, and secondary alcohols can also be used. On the other hand, only a limited group of methanogens can utilize methyl-group-containing compounds, of which one methyl group is reduced to methane with electrons from the oxidation of additional methyl groups, H_2, or carbonyl groups. Common methyl-group-containing compounds employed by methanogens include methanol, methylamines, methylsulfides, and acetate [97].

Currently, seven taxonomic orders of methanogens are known: Methanopyrales, Methanococcales, Methanobacteriales, Methanomicrobiales, Methanosarcinales, Methanocellales, and the recent proposed order, Methanomassiliicoccales [97,103, 106,107]. However, only three orders, Methanobacteriales, Methanomicrobiales, and Methanosarcinales, are most commonly found in anaerobic reactors [99]. Members of Methanoccocales are predominant in marine sediments but they are rarely found in wastewater treatments [108]. The order of Methanopyrales includes the only hyperthermophilic species and it is unlikely to be found in anaerobic reactors [109]. Methanocellales and Methanomassiliicoccales represent novel orders and their isolates were found in paddy soils and insect hindguts, respectively [106,110].

Of the three orders frequently found in anaerobic treatments, Methanobacteriales and Methanomicrobiales strictly reduce CO_2 with H_2 to produce methane. They also lack cytochromes and their H_2 threshold is very low. They are usually referred to as "hydrogenotrophic methanogens." On the other hand, members of Methanosarcinales are capable of employing both methyl-group-containing compounds and CO_2-H_2 for methane production; nevertheless, species from this order that are found in anaerobic reactors mainly cleave acetate, reducing the methyl group to methane and oxidizing the carboxyl group to CO_2. They are often called "aceticlastic" or "acetotrophic methanogens." All Methanosarcinales contain cytochromes and methanophenazine and their H_2 threshold is higher [104,111].

Initially, it was assumed that 70% of the methane produced in biodigesters came from the reduction of the methyl group of acetate and the other 30% from CO_2 and H_2 [112]. However, as more studies focused on the composition of methanogenic communities within anaerobic reactors, it was found that CO_2-reducing methanogens predominated these environments. Operational conditions such as organic load rate, substrate type, dilution rate, and agitation, as well as the presence of certain bacterial groups such as obligately H_2-producing syntrophic bacteria, determine the dominance of either hydrogenotrophic or aceticlastic methanogens [108,113–116].

9.10 Methanogenic Pathways

Methanogenesis comprises the last step in the anaerobic decomposition of organic matter and plays an essential role in the global carbon cycle [117]. It also represents an antique pathway for energy conservation [103]. Two major pathways are known for biosynthesis of methane in anaerobic treatments (Fig. 9.2): the CO_2-reducing and the aceticlastic pathways. In the former, also known as hydrogenotrophic, CO_2 is reduced with electrons from H_2, formate, or CO, whereas in the latter, the methyl group of acetate is the one being reduced. Although both pathways implicate different reactions and enzymes, they share the last steps that culminate with methane production. The other pathway known, the methylotrophic one, is often overlooked in anaerobic reactors because methanogens capable of utilizing methanol and methylamines are not found in great numbers in these environments. Also, the methylotrophic pathway shown in Fig. 9.9 is not common for all methanogens capable of employing such substrate.

9.11 CO_2-Reducing or Hydrogenotrophic Pathway

As it indicated in Fig. 9.9, the first step of the CO_2-reducing pathway comprises the binding of CO_2 to methanofuran (MFR) and its reduction to formyl-MFR with electrons donated by ferredoxin. This step is catalyzed by formyl-MFR dehydrogenase and its reaction is highly endergonic owing to the need for reduced ferredoxin. However, it has been demonstrated that the unfavorable reduction of ferredoxin is coupled with the last step of the pathway, the reduction of the heterodisulfide CoM-S-S-CoB [118]. In the next step, the formyl group is transferred to tetrahydromethanopterin (H_4MTP) or its analogue in Methanosarcinales and Methanococcales, tetrahydrosarcinapterin (H_4STP), by the action of formyl-MFR:H_4M(S)PT formyltransferase. The following steps involve the reduction of the formyl-H_4M(S)PT to methyl-H_4M(S)PT, and they are catalyzed by the enzymes CH≡H_4M(S)PT cyclohydrolase, either one of the two methylene-H_4M(S)PT dehydrogenases (Mtd and Hmd), and methylene-H_4M(S)PT reductase [111,117]. The electrons required for the reactions described above originate from oxidation of H_2, CO, or formate, and coenzyme F_{420} functions as the main electron carrier, except in the reaction of dehydrogenation by Hmd, which directly oxidizes H_2 for its reaction without the need of F_{420} [119].

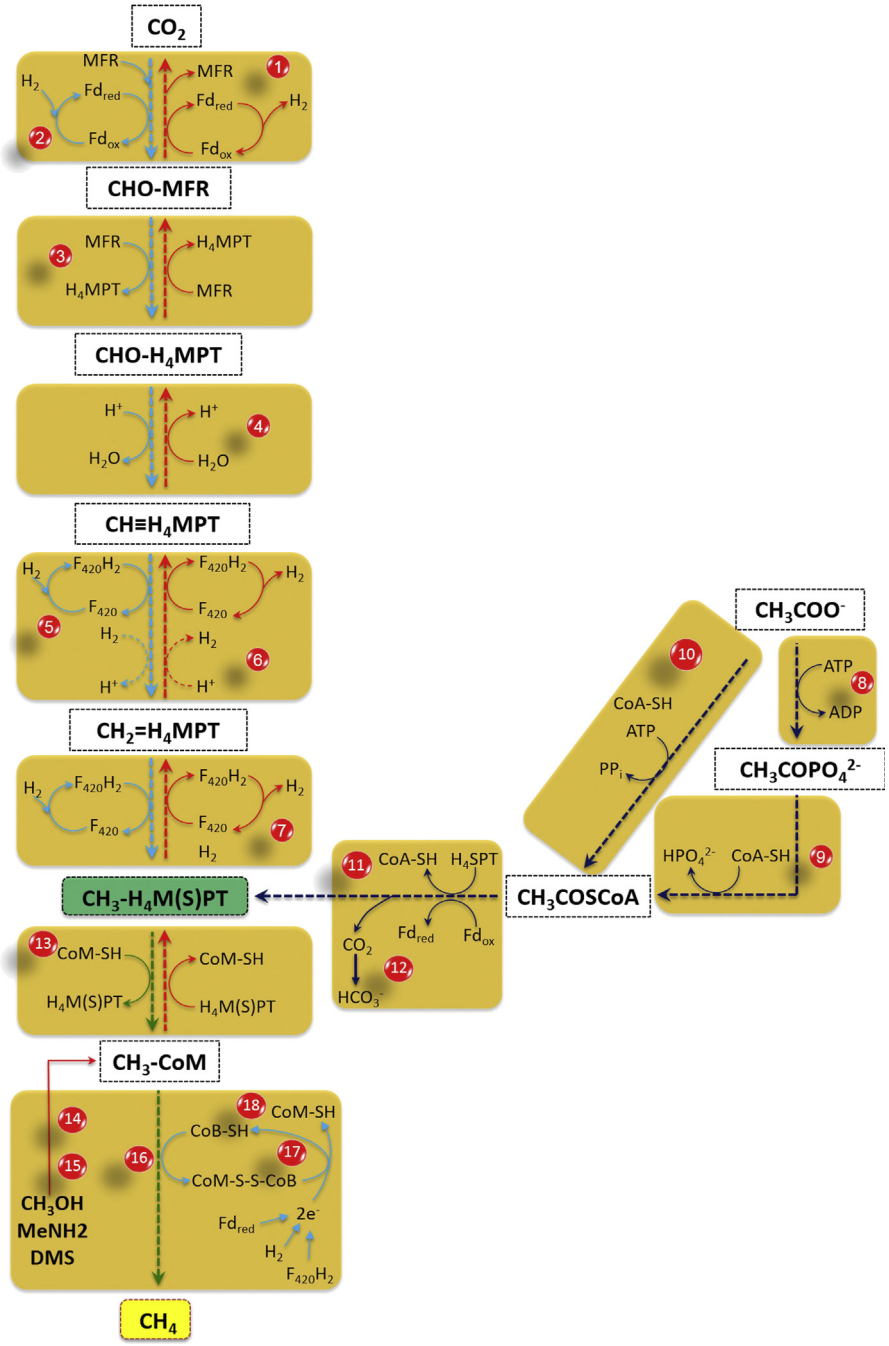

FIGURE 9.9 Methanogenic pathways. *ATP*, adenosine triphosphate; *H_4SPT*, tetrahydrosarcinapterin; *H_4MPT*, tetrahydromethanopterin; *Fd*, ferredoxin; *CoA-SH*, coenzyme A; *CoM-SH*, coenzyme M; *CoB-SH*, coenzyme B; *MFR*, methanofuran; *F_{420}*, coenzyme F_{420}; (1) *Fmd*, CHO-MFR dehydrogenase; (3) *Ftr*, CHO-MFR:H_4M(S)PT formyltransferase; (4) *Mch*, CH≡H_4M(S)PT cyclohydrolase; (5) *Mtd*, CH_2=H_4M(S)PT dehydrogenase dependent on F_{420}; (6) *Hmd*, CH_2=H_4M(S)PT dehydrogenase independent of F_{420}; (7) *Mer*, CH_2=H_4M(S)PT reductase; (8) *Ak*, acetate kinase; (9) *Pta*, phosphotransacetylase; (10) *ACS*, AMP-forming acetyl-CoA synthetase; (11) *CODH/ACDS*, CO dehydrogenase/acetyl-CoA synthase; (12) *Cam*, carbonic anhydrase; (13) *Mtr*, CH_3-H_4M(S)PT:CoM methyltransferase; (14 and 15) *MT1 and MT2*, methyltransferases; (16) *Mcr*, methyl coenzyme M reductase; (17) *Hdr*, heterodisulfide reductase; (18 and 2) *Hdr/Mvh*, heterodisulfide reductase/cytoplasmic F_{420}-nonreducing hydrogenase complex.

9.12 Aceticlastic Pathway

Only two genera are able to utilize acetate as substrate for methanogenesis: *Methanosarcina* and *Methanosaeta*, both belonging to the order Methanosarcinales. The aceticlastic pathway starts with the activation and conversion of acetate to acetyl-CoA; however, both genera achieve that goal employing different mechanisms. In *Methanosarcina* species, activation of acetate is reached by the participation of phosphotransacetylase and acetate kinase as indicated in Fig. 9.2 [120]. In contrast, in *Methanosaeta* species, AMP-forming acetyl-CoA synthetase is the one in charge of conversion of acetate to acetyl-CoA [121]. Cleavage of acetyl-CoA is catalyzed by the CO dehydrogenase/acetyl-CoA synthase (CODH/ACDS), transferring the methyl group to H_4SPT, and oxidizing the carbonyl group to CO_2 with transfer of electrons to ferredoxin [111].

9.13 Common Reactions in All Methanogenic Pathways

The steps following the synthesis of methyl-H_4M(S)PT are shared in both pathways. Methyl-H_4M(S)PT:HS-CoM methyltransferase transfers the methyl group from H_4M(S)PT to coenzyme M (HS-CoM) with the translocation of two Na^+ across the membrane. Finally, in the last step, methyl-CoM is split by the action of methyl coenzyme M reductase. The methyl group is reduced to methane with coenzyme B (HS-CoB) as the electron donor, and this coenzyme along with HS-CoM forms the heterodisulfide CoM-S-S-CoB [98].

Two mechanisms have been proposed for how the C—S bond of methyl-CoM is cleaved. Mechanism I proposes an attack of nickel in cofactor F_{430} on the sulfur atom of methyl-SCoM, producing a methyl radical and CoM-S-NiIIF$_{430}$ [122], whereas mechanism II suggests a nucleophilic attack of nickel generating HS-CoM and methyl-NiIIIF$_{430}$. Studies have shown evidence that favors mechanism II [123—125].

9.14 Energy Conservation by Reduction of the Heterodisulfide

Although methane is actually a waste product, the heterodisulfide CoM-S-S-CoB produced in the last step is important for all methanogens because its reduction by heterodisulfide reductase (Hdr) is coupled to energy conservation. However, there are huge differences in how different species of methanogens conserve energy.

Members of the order Methanosarcinales contain a membrane-bound Hdr that reduces the heterodisulfide with the additional translocation of two protons across the membrane that drive ATP synthesis. Various compounds, depending on the substrate utilized, donate electrons for the reduction of CoM-S-S-CoB. When CO_2 and methyl-group-containing compounds are the substrates employed, H_2 and reduced coenzyme

F_{420} are the electron donors, respectively. When acetate is used for methanogenesis, the ferredoxin reduced in the reaction catalyzed by CODH/ACDS is the one that donates electrons to the heterodisulfide [126].

The mechanism for energy conservation in obligate CO_2-reducing species differs from the one described above because of the absence of cytochromes. Members of the other six orders express a cytoplasmic Hdr that is in close association with the methylviologen-reducing [NiFe] hydrogenase, so reduction of heterodisulfide in these methanogens is not accompanied by proton translocation. In this case, electrons from H_2 are utilized for the reduction of CoM-S-S-CoB and the ferredoxin that is required for the first step of the CO_2-reducing pathway, in a mechanism known as "electron bifurcation." Such strategy is considered an energy conservation mechanism because the free energy generated by an exergonic reaction, i.e., the reduction of the heterodisulfide, drives an endergonic reaction, the reduction of ferredoxin with H_2. If electron bifurcation is not efficient, the ferredoxin must be reduced by other means, which implicates an extra energy input for the methanogens without membrane-bound Hdr [104,118].

9.15 Microbial Interactions in Anaerobic Wastewater Treatment Processes

The metabolic flexibility of microorganisms and the syntrophic associations within the anaerobic processes create a competitive environment in the system. Several microbial groups can compete for substrates as electron donors, and the dominance of one or more groups is influenced by a great number of biochemical and operational factors such as chemical oxygen demand (COD), pH, temperature, and enzymatic parameters, among others. The interactions between methanogens and nitrate- and sulfate-reducing bacteria can be very complex. In general, it is said that nitrogen- and sulfate-reducing bacteria outcompete methanogens when nitrogen-containing compounds and sulfate are present in the wastewater. Under anaerobic conditions, hydrogen is the molecular currency for energy production. Interspecies hydrogen transfer mechanisms by way of syntrophic associations is important for anaerobic energy metabolism in the oxidation of many carbon compounds ($\geq$3). Reactions under anaerobic conditions and the free energies obtained are shown in Table 9.4 and a schematic interaction of various microbial groups is presented in Fig. 9.10.

It has been demonstrated that methanogens and denitrifying bacteria can coexist at high redox potential, establishing syntrophic relations between them. However, metabolites produced during denitrification have a toxic effect on methanogens [127]. Also, denitrifying bacteria can cause a temporary accumulation of SO_4^{2-} and Fe^{3+}, which at the same time allows the activity of sulfate- and iron-reducing microorganisms [128].

Table 9.4 Reactions Involved in Anaerobic Treatments

Equation	ΔG° (kJ/Reaction)
Sulfate-Reducing Reactions	
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	−151.9
$Acetate^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	−47.6
$Propionate^- + 0.75SO_4^{2-} \rightarrow acetate^- + HCO_3^- + 0.75HS^- + 0.25H^+$	−37.7
$Butyrate^- + 0.5SO_4^{2-} \rightarrow 2acetate^- + 0.5HS^- + 0.5H^+$	−27.8
$Lactate^- + 0.5SO_4^{2-} \rightarrow acetate^- + HCO_3^- + 0.5H^+$	−80.2
Heterotrophic Denitrification Reactions	
$5H_2 + 2NO_3^- + 2H^+ \rightarrow 6H_2O + N_2$	−224
$NO_2^- + 4H^+ + 3e^- \rightarrow 2H_2O + 0.5N_2$	−277
$5Acetate^- + 8NO_3^- + 8H^+ \rightarrow 9H_2O + 5CO_2 + 5HCO_3^- + 4N_2$	−797
$5Propionate^- + 14NO_3^- + 14H^+ \rightarrow 17H_2O + 10CO_2 + 5HCO_3^- + 7N_2$	−1398
$5Glucose + 24NO_3^- + 24H^+ \rightarrow 42H_2O + 30CO_2 + N_2$	−2657
$Glucose + 8NO_2^- + 8H^+ \rightarrow 10H_2O + 6CO_2 + 4N_2$	−3144
Autotrophic Denitrification Reactions	
$NO_3^- + H^+ + 2.5H_2 \rightarrow 0.5N_2 + 3H_2O$	−560.3
$3NO_3^- + 5NH_4^+ \rightarrow 4N_2 + 9H_2O + 2H^+$	−297
DNRA Reactions	
$4H_2 + 2NO_3^- + 4H^+ \rightarrow 6H_2O + 2NH_4^+$	−150
$Acetate^- + NO_3^- + 2H^+ \rightarrow CO_2 + HCO_3^- + NH_4^+$	−500
$8Propionate^- + 14NO_3^- + 28H^+ \rightarrow 2H_2O + 16CO_2 + 8HCO_3^- + 14NH_4^+$	−878
$Glucose + 3NO_3^- + 6H^+ \rightarrow 3NH_4^+ + 3H_2O + 6CO_2$	−1767
$Glucose + 4NO_2^- + 8H^+ \rightarrow 4NH_4^+ + 2H_2O + 6CO_2$	−1713
Anammox	
$1.3NO_2^- + NH_3^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2H_2O$	−357
Acetogenic Reactions	
$Propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + H^+ + 3H_2$	+76.1
$Butyrate^- + 2H_2O \rightarrow 2acetate^- + H^+ + 2H_2$	+48.3
In Syntrophic Association With Methanogens	
$Propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + H^+ + 3H_2$	−1.5
$Butyrate^- + 2H_2O \rightarrow 2acetate^- + H^+ + 2H_2$	−31.2
In Syntrophic Association With SRB	
$Propionate^- + 0.75SO_4^{2-} \rightarrow acetate^- + HCO_3^- + 0.75HS^- + 0.25H^+$	−37.7
$Butyrate^- + 0.5SO_4^{2-} \rightarrow 2acetate^- + 0.5HS^- + 0.5H^+$	−27.8
Homocetogenic Reactions	
$4H_2 + 2HCO_3^- + H^+ \rightarrow acetate^- + 4H_2O$	−104.6
$Lactate^- \rightarrow acetate^- + H^+$	−56
Methanogenic Reactions	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−135.6
$Acetate^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31.0

Anammox, anaerobic ammonium oxidation; *DNRA*, dissimilatory nitrate reduction to ammonium; *SRB*, sulfate-reducing bacteria.

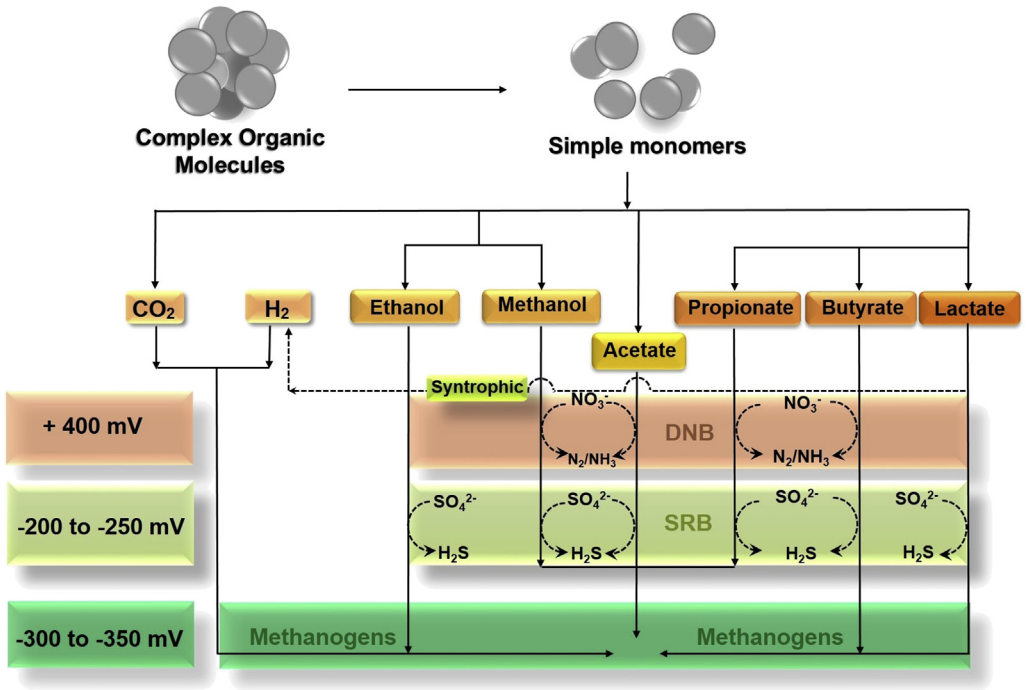

FIGURE 9.10 Schematic interaction of various microbial groups in anaerobic reactors. *DNB*, denitrifying bacteria; *SRB*, sulfate-reducing bacteria.

In contrast, it is said that DNRA enhances methanogenesis [129]. The competition between SRB and methanogens is the most important one because both reactions can be the final step in wastewater treatment. This competition is highly influenced by physicochemical factors, which depend on the origin of the wastewater and kinetic parameters of each group.

A novel process has been proposed for the removal of nitrogen using SRB [130]. It was named sulfate reduction, autotrophic denitrification, and nitrification integrated (SANI). In this process, the COD is removed by SRB in the anaerobic zone. The sulfide produced is transported to the anoxic zone and it is used in the autotrophic denitrification of the nitrate produced. Finally, in the aerobic zone, ammonia is converted to nitrate and recirculated to the anoxic zone [130]. Owing to low growth yields of microorganisms, no excess production of sludge was observed and further, there was high removal of sulfate and nitrogen compounds.

The effect of nitrate on the anaerobic treatment of an industrial sulfate-rich wastewater was investigated using batch cultures [131]. The authors demonstrated that denitrification was the main nitrate reduction pathway under all conditions tested. A lag

phase, caused by a high initial sulfide content, preceded NO_3^- reduction to N_2. During this phase, the methane production was not affected by high nitrate concentrations, whereas sulfate reduction was inhibited by nitrogen oxides. Because sulfide concentrations also dropped, it was suggested that denitrification using sulfide as an electron donor occurred in the system.

In another study, the interactions between methanogenic archaea and nitrate- and sulfate-reducing bacteria were investigated under anoxic incubation of excised rice roots. It was found that nitrate and sulfate addition resulted in the inhibition of methane production. This inhibition was initially attributed to substrate competition for H_2. However, toxicity of N compounds was also observed. Meanwhile, SRB also successfully competed with methanogens for H_2 and retarded the growth of the methanogenic populations [132]. SRB have lower values of K_s and K_m for H_2 and acetate, meaning that they have higher affinities and can outcompete methanogens in environments with low substrate concentrations. Lupton and Zeikus [133] demonstrated such statement evaluating the kinetic parameters of hydrogenases in *Desulfovibrio vulgaris* and *Methanobacterium* strain Ivanov. They obtained a K_m for H_2 of 4 μM, with an enzymatic activity of 2.71 dpm $^3H_2O \times 10^3$/μg cell protein/h for *D. vulgaris*. On the other hand, a K_m for H_2 of 14 μM and a specific hydrogenase activity of 0.38 dpm $^3H_2O \times 10^3$/μg cell protein/h were calculated for *Methanobacterium* strain ivanov. Under these conditions, the free energy change for sulfate reduction was −62.9 kJ/mol and for methanogenesis was −47.4 kJ/mol.

A good understanding of the interactions between the microbial groups in anaerobic wastewater treatments is necessary to establish strategies to improve the performance and efficiency of the process.

9.16 Conclusion

Small-scale anaerobic treatment of wastewater started as early as 1860s by way of a crude version of a septic tank in France by its inventor, Mouras, followed by an improved version in 1895 at Exeter, England. The first anaerobic digester to treat human wastes was installed at Matunga Leper Asylum, Mumbai, India, in 1987, and the biogas produced was used to meet its energetic needs. Since then, the anaerobic treatment process has come a long way and advances in the engineering aspects of the process moved rapidly from 1969 owing to the pioneering work of Young and McCarty on anaerobic filters. However, progress in the knowledge of the microbiology and biochemistry of anaerobic treatment processes is slow because of the difficulties involved in the isolation of pure cultures of anaerobic microorganisms and subsequent maintenance under laboratory conditions. In this chapter the role of nitrogen, sulfate, and carbonate as electron acceptors is explained in terms of transformation of nitrogen and sulfur compounds and methanogenesis; and many other important anaerobic processes such as anoxygenic

photosynthesis, anaerobic oxidation of methane, and the role of other metal acceptors of electrons in oxidation of organic compounds have not been discussed. It can clearly be noted that knowing the microorganisms and their biochemical mechanisms is not enough to understand the processes and their interactions. Although previous outstanding works by various microbiologists on the anaerobic treatment process led to the discovery of many new microorganisms and their biochemical pathways, it still remains a black box. With recent advances in molecular biology and the advent of the "omics" era, we can hope that this black box can soon be decoded and will not only help us know the organisms present in this system, but also what they are doing and under what conditions. A clarity on this microbiology and their biochemical mechanisms will help us improve the efficiency of the process in terms of time, space, and energy recovery.

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10

Anaerobic Bioreactors/Digesters: Design and Development

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10.1 Introduction

The anaerobic process has been widely adopted for waste stabilization and bioenergy production in recent years. Earlier applications of the anaerobic process date back to the 10th century, when biogas was used for heating bath water by the Assyrians [1]. The process became widely popular in the late 18th century for human waste (excreta) digestion [2]. The earlier applications of anaerobic processes were limited to solid and semisolid wastes [3,4]. Advances in the microbiology and biochemistry processes in anaerobic technology have enabled its applications to diverse dilute waste streams [5–8]. Furthermore, developments in process engineering, especially better understanding of digester designs, and innovations have enabled the application of anaerobic processes to treat industrial effluents. One of the advantages of the anaerobic process over other biological waste treatment processes is that it requires a minimal amount of macro-/micronutrients and produces significantly low sludge [9]. Stander (1950) was the first to feature an innovative anaerobic process design that decoupled solids retention time (SRT) from hydraulic retention time (HRT) and addressed the challenges associated with biomass retention and process instability [10]. This chapter covers important considerations in anaerobic treatment of industrial effluents and various anaerobic digester/reactor configurations and examines the design aspects of the anaerobic process for the treatment of industrial effluents.

10.2 Overview of the Anaerobic Process

An efficient anaerobic reactor design requires a thorough understanding of the physiological requirements for diverse microbial groups, namely fermentative/acidogenic and acetogenic bacteria and methanogenic archaea. The fundamental aspects of the

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anaerobic process have been well discussed elsewhere [11] and only a brief discussion is presented here. The anaerobic process is a multistep process mediated by diverse microbial communities in a sequential manner. First, the fermentative microbes excrete hydrolytic enzymes to break the complex carbohydrates into soluble monomers and oligomers, lipids into long-chain fatty acids, and proteins into amino acids. The acidogens then convert soluble molecules into volatile fatty acids (VFAs), alcohols, CO_2, and H_2. Next, the acetogens convert VFAs and alcohols into acetic acid and H_2. Finally the methanogens convert acetic acid and $CO_2 + H_2$ into methane via acetotrophic and hydrogenotrophic methanogenic pathways, respectively. These three microbial groups (fermentative/acidogenic, acetogenic bacteria, and methanogenic archaea) differ in their physiological requirements, and the well-designed reactor ensures the effective growth of these diverse microbial groups. For example, the readily hydrolyzable substrates (e.g., starch) in the anaerobic process create an imbalance between acid and methane formation. An effective solution to this problem is to design a phase-separated system with two distinct phases (i.e., acidogenic and methanogenic phases); the methane formation is prevented in the acidogenic phase through kinetic control (short residence time), pH control (low pH values of 5−6), or a combination of both.

10.3 Important Considerations in Anaerobic Treatment of Industrial Effluents

One important consideration for anaerobic treatment of the industrial effluent is to examine whether the wastewater is biologically amenable. This requires complete characterization of the industrial effluent. The wastewater characteristics govern the types of anaerobic process to be adopted. As indicated earlier, the anaerobic process proceeds through a series of metabolic reactions catalyzed by diverse microbial communities, each with different physiological requirements for its growth. These diverse communities work as a cohort and metabolize the organic matter. It is therefore important to have a reactor design that provides optimal conditions for the growth of diverse microbial communities. Some of these considerations are discussed in the following section.

10.3.1 Biodegradability of Industrial Effluents

Industrial effluents are not all equally biodegradable, even if they have high organic content. Crude parameters such as total organic carbon, biochemical oxygen demand (BOD), chemical oxygen demand (COD), and volatile solids are traditionally used to quantify the organic matter in wastewater. Among these parameters, BOD, the measure of the amount of oxygen required by microorganisms to degrade the organic matter, is the most accurate representation of the biodegradability of wastewater [12,13]. The COD, on the other hand, is the measure of the amount of oxygen required to chemically breakdown the organic matter present in the wastewater. The COD value is always higher than the BOD value because COD includes both biodegradable and nonbiodegradable organic

substances, whereas BOD represents the biodegradable component of organic matter. Although COD or BOD does not provide much information on the composition of organic matter, some information on the biodegradability can be gained by comparing the BOD-to-COD ratio. Industrial effluent with high BOD/COD ratio indicates that the wastewater is biologically amenable, whereas a low BOD/COD ratio indicates that the wastewater is relatively less biodegradable. Usually the wastewater with a BOD/COD ratio of 0.5 or higher is considered biologically amenable [11,14,15]. Some examples of highly biodegradable industrial wastewaters include effluents from the food and beverage industries and biofuel industries that convert starch and sugar into ethanol, whereas effluents from chemical, pharmaceutical, and metal industries are less biodegradable.

10.3.2 Toxic Compounds

Toxic substances may be present in the industrial effluents or they may be generated through the metabolic activities of the microorganisms during anaerobic treatment. Ammonia, heavy metals, halogenated compounds, cyanide, and phenol are examples of the former, whereas ammonia, sulfide, and long-chain fatty acids belong to the latter group. Industrial effluents from seafood processing, molasses fermentation, and distillery slops contain high sulfate (1000 to 6000 mg/L). Anaerobic treatment of such effluents generates sulfide, which could impose toxicity to methanogens. Similarly, ammonia may be present in the effluent or produced during the anaerobic treatment of organic nitrogenous compounds such as protein or amino acids because protein mainly contains 16% nitrogen. Many industrial effluents (e.g., slaughterhouse and dairy processing industry) generate a high concentration of ammonia during anaerobic treatment.

10.3.3 Temperature

Several industries such as brewery, food processing, fermentation, and biofuel generate liquid waste streams at very high temperature. Such effluents need to be cooled down prior to anaerobic treatment because such liquid streams are generally treated at mesophilic and/or thermophilic conditions, the optimal temperature ranges for the methanogens. A strong temperature effect on the maximum substrate utilization rate of microorganisms has been reported in many studies [16]. Most anaerobic studies that examined the effects of temperature showed a strong negative effect on the metabolic activity of mesophilic methanogens at decreasing temperature. The temperature effects on maximum substrate utilization can be described mathematically using the Arrhenius equation. In general, as the operational temperature is lowered, the maximum specific growth and substrate utilization rates decrease [17]. A change in temperature is accompanied by a change in the physical and chemical characteristics of the wastewater. Not only the solubility but also the diffusivity can affect the operating conditions [18]. The solubility of gaseous compounds increases as the temperature decreases [18]. This implies that the dissolved concentrations of CH_4, H_2S, and H_2 will be higher in the effluent operated at lower temperatures than those operated at higher temperatures.

Moreover, the high solubility of CO_2 at low temperatures may lower the reactor pH, which may result in higher dissolved H_2S. The nonionized sulfide is more toxic to methanogens than the ionized form (HS^-).

In the case of a biofilm reactor and granule system, diffusivity is one of the important factors that affect the performance of the anaerobic treatment process. This is because high diffusivity improves the contact between microorganisms and substrate through the liquid film layer [19]. The diffusivity and temperature can be correlated via the following equation [20]:

$$\frac{D_L \mu}{T} = \text{Constant} \qquad [10.1]$$

where D_L is the diffusivity of the solute at infinite dilution (cm^2/s), μ is the viscosity of the solution, and T is the absolute temperature (K)

Comparing two different temperatures, we have:

$$\frac{D_1 \mu_1}{T_1} = \frac{D_2 \mu_2}{T_2}$$

$$D_2 = D_1 \times \frac{\mu_1}{\mu_2} \times \frac{T_2}{T_1}$$

The subscripts 1 and 2 refer to the two different temperatures. The viscosity of liquids also increases as the temperature decreases. Therefore, more energy is required for mixing and the sludge bed becomes more difficult to mix. Hence, diffusivity decreases as the temperature decreases, which increases the viscosity. Biogas production also is reduced at a lower temperature, which further reduces the bed mixing within the reactors.

At higher temperature, digestion proceeds at a much faster rate, requiring smaller tank sizes. The digestion under high-temperature conditions offers advantages like higher metabolic rates and consequently higher specific growth rates. However, the higher death rate of methanogens at thermophilic conditions compared to mesophilic often makes the process highly susceptible to changes in the environmental and operating conditions. The additional heat required to maintain thermophilic conditions offsets the advantage of the reactor system [21,22].

10.3.4 pH

Similar to other biological processes, the pH of incoming feed is important for anaerobic treatment. Methanogens usually prefer a neutral to slightly alkaline pH range (7.0–8.5) [23], whereas acidogens prefer an acidic pH range (6.0–6.5) [24]. Although a neutral pH is ideal for anaerobic treatment, some of the wastewaters, especially those derived from the alcohol (beverage and biofuel) and fermentation industries, have an acidic pH of 4.5–5.5. The anaerobic treatment of such waste streams may require pH adjustment. Such streams, however, are treated without pH adjustment. Moreover, when wastewaters contain proteinaceous matter, the pH is likely to increase owing to the release of ammonia-N. Ammonia-N then reacts with CO_2 generated during the

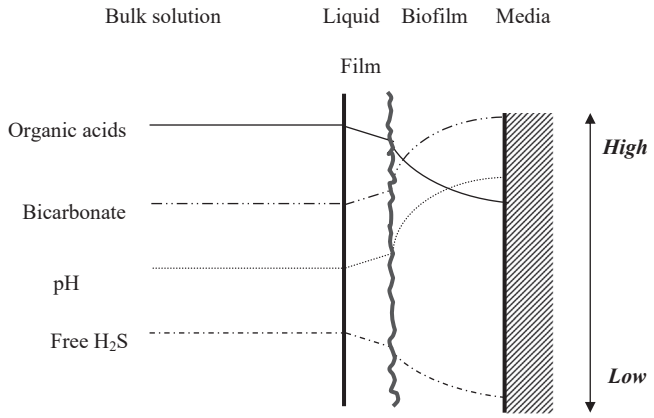

FIGURE 10.1 Profiles of organic acids, HCO_3^-, pH, and free H_2S in an anaerobic biofilm.

biochemical reaction to produce ammonium bicarbonate, which contributes to alkalinity. As a rule of thumb, each mole of organic nitrogen theoretically generates one equivalent of alkalinity [25].

The distribution of ionized and nonionized forms of sulfide and ammonia in the digester is also governed by the pH. It is important to note that the nonionized form is considered to be more toxic than the ionized form. At an acidic pH range, nonionized sulfide (H_2S) predominates the system, whereas nonionized ammonia (NH_3) dominates over the ionized form $\left(NH_4^+\right)$ at high pH.

As shown in Fig. 10.1, there is a localized higher pH within the biofilm, granules, or flocs because of the generation of bicarbonate from methanogenesis [26,27].

10.3.5 Nutrients

It is important that the industrial effluent contain sufficient macronutrients (nitrogen, phosphorus, and sulfur), micronutrients (potassium, calcium, and magnesium), and trace metals (cobalt, iron, manganese, nickel, and molybdenum) for efficient anaerobic treatment. The requirements for macro-/micronutrients are specific to the type of industrial wastewater. Often the macronutrient requirements are estimated based on organic strength of wastewater. As a rule of thumb, for a highly loaded [0.8−1.2 kg COD/(kg volatile suspended solids (VSS) • day)] anaerobic system, the theoretical minimum COD/N/P ratio of 350:7:1 is recommended, whereas for a lightly loaded [<0.5 kg COD/(kg VSS • day)] anaerobic system, the recommended COD/N/P ratio is 1000:7:1 [28].

Methanogens have unique requirements for trace elements, especially Ni, Co, and Mo. Industrial effluent often lacks these elements and needs to be externally supplemented [29,30]. The importance of Ni on the growth of methanogens was first observed by Schonheit et al. [31]. Ni is basically important because it is a structural constituent of factor F430, which is found only in methanogens. Similarly, Co is also important as a structural constituent of vitamin B_{12}, which catalyzes the methanogenic activity. The

higher Co need for these trophic groups is probably due to the involvement of Co-containing corrinoid methyltransferases in the initial step of methanol conversion.

For high-solids substrates, the nutrient requirement is estimated based on the carbon-to-nitrogen (C/N) and carbon-to-phosphorus (C/P) ratios. The desired C/N ratio ranges from 20 to 30, whereas the recommended C/P ratio is around 50.

10.3.6 Organic Loading Rate

One of the major merits of the anaerobic process is its ability to handle a high organic loading rate (OLR). OLR is expressed as mass of organic matter (COD) per unit reactor volume per unit time. Industrial effluents, especially from food processing, fermentation, and agro-based industries, have extremely high organic content. Consequently, an anaerobic process is often used as a pretreatment step to reduce the organic content before they are treated aerobically. The OLR could vary from 10 to over 50 kg COD/m^3 • day.

10.3.7 Types of Treatment System

The treatment system can be classified as an attached growth or a suspended growth. The type of treatment system plays an important role in treating industrial effluents. The attached-growth system is more resistant to toxicity than the suspended-growth system for two reasons, as the attached-growth system can maintain extremely long SRTs at low HRTs and provides a quasi-plug flow hydraulic regime. Other possible reasons for more tolerance in an attached-growth (biofilm) system or granules, especially for an methanogens, could be: (1) a diffusion limitation of toxic substances deep into the biofilm, because MPBs are believed to be predominant at the inner part of the biofilm (or granules) because of their better affinity for adherence to the carriers or for aggregation [32–34], and (2) localized higher pH within the biofilm, granules, or flocs because of the generation of bicarbonate from methanogenesis, which maintains a low free form of compounds (NH_3 and H_2S), as shown in Fig. 10.1 [26,35].

10.3.8 Hydraulic Retention Time and Solids Retention Time

HRT is also known as hydraulic residence time, a measure of the average length of time that industrial effluent (liquid) remains in a bioreactor. Thus, HRT also represents the time organic matter remains in contact with microbes. In the chemical/biological engineering field, the term dilution rate (inverse of HRT) is often used instead of HRT. Higher dilution rate indicates that effluent is fed at a rate faster than the growth rate of microbial cells in a reactor, thereby resulting in washout of cells. SRT represents the time microbes (biomass) stay in the bioreactor. Thus, SRT is a measure of the biological system's capability to achieve specific effluent standards and/or to maintain a satisfactory efficacy of pollutant removal [11]. Long SRT means more stable operation, better toxic or shock load tolerance, and a quick recovery from perturbation. The volumetric OLR in the bioreactor is also determined by the SRT. Because industrial effluent is very dilute, which requires a large flow to be managed in a short liquid residence time,

Table 10.1 Various Approaches to Decoupling Hydraulic Retention Time From Solids Retention Time

Approach	Biomass Retention Mechanism	Anaerobic Digester Type
Biomass immobilization in attached-growth system	Microbes attached to the support media (e.g., plastic, gravel, sand, and activated carbon) to form biofilms	Anaerobic filter; expanded/fluidized-bed reactor
Granulation and floc formation	Microbes agglomerate to form granules and flocs that settle well in the bioreactor	Upflow anaerobic sludge blanket reactor; anaerobic sequential batch reactor; anaerobic baffled reactor
Biomass recycling	Feed with high suspended solids (e.g., wastewater from meat packaging and wood fiber industries) enables microorganisms to attach to solids to form settleable flocs	Anaerobic contact reactor; clarigester
Biomass retention	Membrane integration into an anaerobic reactor retains biomass	Anaerobic membrane bioreactor

decoupling of HRT and SRT becomes essential for efficient treatment. Decoupling allows higher OLR and enables smaller reactor size. There are four approaches for decoupling SRT from HRT, as elucidated in Table 10.1. It is important to note that decoupling is extremely difficult for high solids substrates. Such substrates are often treated in a completely mixed bioreactor in which HRT = SRT and a long detection time is needed for stabilization.

10.4 Anaerobic Bioreactor Design

An anaerobic bioreactor is typically designed on an empirical or experimental basis. Fundamental principles can also be applied for sizing the bioreactor. For a suspended-growth system such as a continuous-stirred tank reactor (CSTR), a mass balance approach is employed, which is discussed in greater detail. One of the important design considerations in anaerobic treatment of industrial effluent is to maintain high biomass, especially methanogens, in the bioreactor. In lieu of the slow growth of methanogens, a central goal of the design consideration, especially for industrial effluent, is to decouple SRT from HRT. Such decoupling maintains an extremely high SRT/HRT ratio and the ultimate goal is to minimize the washout of slow-growing methanogens from the bioreactor. An anaerobic system can be classified into two broad categories, namely suspended-growth and attached-growth systems. In some cases, the anaerobic system is also classified based on different stages (phases), such as acid-phased reactor and temperature-phased reactor. In the former case, two reactors are operated in series: an acidogenic reactor followed by a methanogenic reactor. In the latter case, the mesophilic reactor is operated in series with a thermophilic reactor. A detailed discussion of some of the important bioreactor types and their design is presented in the following section.

10.4.1 Continuous-Stirred Tank Reactor

The CSTR is the most commonly used reactor configuration employed for anaerobic treatment of industrial effluent containing medium to high suspended solids with total solids (TS) content of 0.5% or higher. The contents in the reactor are maintained under completely-mixed conditions by mixing continuously or intermittently using mechanical mixture, biogas sparging, or liquid recirculation. The HRT in CSTRs ranges from 20 to 50 days. In a CSTR, it is almost impossible to decouple SRT from HRT; thus, HRT is equal to SRT. Further, the consistent mixing enables rapid dilution of incoming constituents, which allows CSTRs to handle shock loads and toxic constituents in the waste streams. Fig. 10.2 shows the schematics of a CSTR.

A mass balance approach can be used for sizing a CSTR and to obtain the design parameters, such as HRT and treatment capacity. The mass balance approach takes into account the inputs, outputs, and accumulation of the substrate as discussed below:

$$\begin{aligned}&(\text{Rate of substrate inflow}) + (\text{Rate of substrate degradation})\\ &= (\text{Rate of substrate out}) + (\text{Rate of substrate accumulation})\end{aligned}$$

$$QC_o + r(V_r) = QC_e + \frac{dC}{dt}(V_r) \qquad [10.2]$$

where C_o is the influent substrate concentration (mg/L), C_e is the effluent substrate concentration (mg/L), C is the substrate concentration (mg/L) in the reactor at a given time (t), Q is the substrate flow rate (m^3/s), V_r is the reactor working volume (m^3), and r is the rate constant for biodegradation of substrate (mg/L • s).

There is no accumulation under steady-state conditions. Thus, Eq. [10.2] can be simplified as:

$$QC_o + r(V_r) = QC_e \qquad [10.3]$$

The volume of the CSTR can be obtained as follows.

$$V_r = \frac{Q(C_o - C_e)}{r} \qquad [10.4]$$

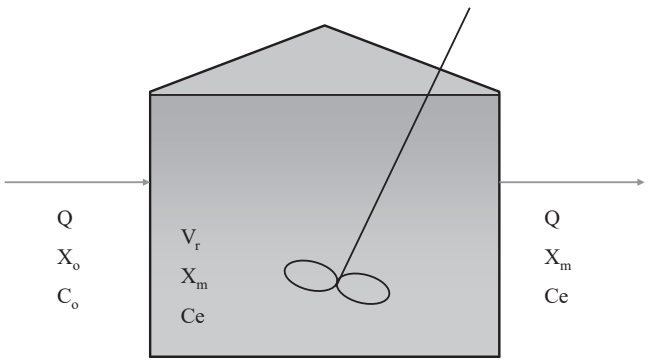

FIGURE 10.2 Schematic diagram of continuous-stirred tank reactor. Adapted from S.K. Khanal, Anaerobic Biotechnology for Bioenergy Production, Wiley-Blackwell, Ames, IA, USA, 2008.

10.4.2 Covered Anaerobic Lagoon

The covered anaerobic lagoon (CAL) is an inexpensive option for treating industrial effluents with TS content of 0.5−3.0%. The CALs are designed as earthen pits constructed with impermeable liners (e.g., clay) at the bottom and sides as shown in Fig. 10.3. A typical CAL uses neither mechanical mixing nor external heating. The SRT in a CAL is longer than its HRT. Because lagoons are operated at ambient conditions, their treatment efficiency is tied to the geographical location and climate. The HRT in CALs can be as long as 3−6 months and the lagoons are sometimes referred to as a waste storage unit. There are several CALs that are designed with a mixing unit and the temperature is controlled to enhance the treatment efficiency. In such cases, the CAL becomes a CSTR and the same design principles as discussed in Section 10.4.1 can be applied.

10.4.3 Anaerobic Contact Reactor

An anaerobic contact reactor (ACR) essentially consists of a CSTR and a downstream settling tank. The settled microbial biomass is recycled back to the reactor as shown in Fig. 10.4. Thus, the ACR configuration maintains a high biomass concentration. Biogas bubbles (CH_4 and CO_2) are removed from the aqueous phase using a degassifier to prevent biomass floating. The ACR is commonly adopted for treating industrial wastewater with high suspended solids (e.g., wastewater from a meat packing plant). The suspended particles in the wastewater attach to the microbes allowing them to settle as flocs in the settling tank. The ACR is a suspended-growth system, and the design approach is very similar to that of a CSTR as discussed earlier except that the biomass is allowed to settle and is recycled in the process.

10.4.4 Upflow Anaerobic Sludge Blanket Reactor

The upflow anaerobic sludge blanket (UASB) reactor is a suspended-growth reactor that maintains very high concentration of microbial biomass by promoting granulation

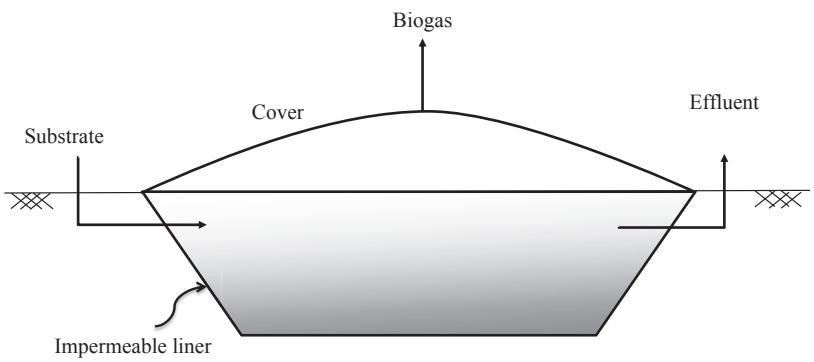

FIGURE 10.3 Schematics of a typical covered anaerobic lagoon.

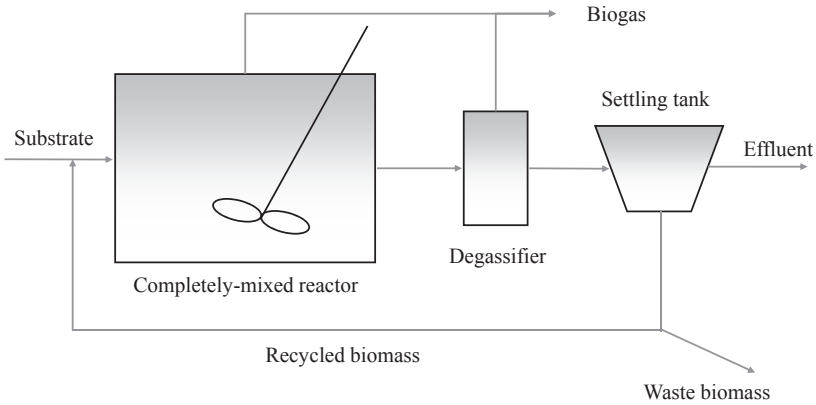

FIGURE 10.4 Schematics of anaerobic contact reactor. Adapted from S.K. Khanal, Anaerobic Biotechnology for Bioenergy Production, Wiley-Blackwell, Ames, IA, USA, 2008.

(Fig. 10.5). The anaerobic granules are 1–3 mm in diameter and dense enough to settle down in the reactor. The biomass concentration in the UASB reactor reaches 50 g/L or higher and thus maintains a very long SRT irrespective of the short HRT of 4–8 h. Many industrial effluents, especially from food processing, agro-based industries, and other carbohydrate-rich industries, promote biomass granulation in UASB reactors. Although originally conceived for industrial wastewater treatment with low solids content, UASB

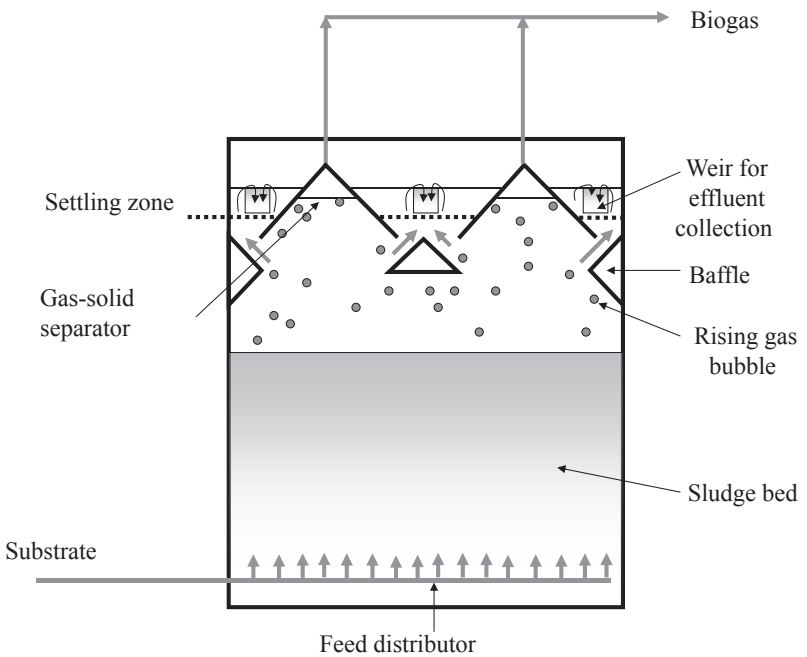

FIGURE 10.5 Schematic diagram of a UASB reactor. Adapted from S.K. Khanal, Anaerobic Biotechnology for Bioenergy Production, Wiley-Blackwell, Ames, IA, USA, 2008.

reactors have also been widely used for bioenergy recovery from a range of high-strength wastewaters.

The substrate is uniformly distributed at the bottom of the reactor where the anaerobic granules come in contact with the organic matter and degrade it. Large and dense granules remain suspended within the sludge bed because of the liquid upflow velocity and rising biogas bubbles. Granules with entrapped gas enter into the gas–solid separator, where the gas bubbles detach as they hit the inclined wall. The granules then slide back into the reactor. The biogas is collected through a gas collection system. The liquid and smaller-size granules enter the settling zone, which is designed in such a way that the superficial upflow velocity decreases significantly as the liquid moves upward (owing to a gradual increase in surface area). This facilitates the settling of small and light granules back into the reactor. The treated effluent is collected in a series of weirs placed at the top of the reactor [11].

There is significant experience in designing UASB reactors. One approach is based on the maximum allowable volumetric OLR to obtain the desired organic removal efficiency. An empirical or theoretical approach can be employed to determine the OLR. In the empirical approach, a series of pilot-scale studies is conducted to obtain the OLR corresponding to maximum organic removal, as illustrated in Fig. 10.6.

The theoretical approach based on specific sludge activity (or specific substrate utilization rate) (U) is discussed here [36]:

$$\text{VOLR} = X_o f_p f_o S_F U \qquad [10.5]$$

where VOLR is the volumetric OLR, X_o is the biomass concentration in the reactor (mg/L), f_p is the contact factor between sludge particles and feed (unitless), f_o is the contact factor between substrate and active biomass (unitless), and S_F is the safety factor (unitless).

Specific sludge activity (U) is given by:

$$U = \frac{k_{max} S}{K_s + S} \qquad [10.6]$$

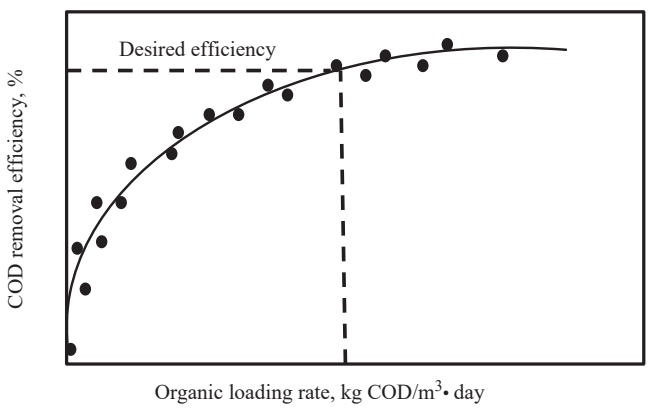

FIGURE 10.6 COD removal efficiency at different organic loading rates.

where k_{max} is the maximum specific substrate utilization rate (kg COD/kg biomass • day), S is the concentration of growth-limiting substrate (mg/L), and K_s is the half-velocity constant (mg/L), k_{max} can also be expressed as μ_{max}/Y_{obs}, where Y_{obs} is the observed yield coefficient.

From Eqs. [10.5] and [10.6], we have the following:

$$\text{VOLR} = X_o f_p f_o S_F \frac{k_{max} S}{K_s + S} \quad [10.7]$$

In Eq. [10.7], the desired X_o can be maintained by controlling the sludge withdrawal rate; S is the effluent soluble COD concentration, which can be set, and k_{max} and K_s are the maximum specific substrate utilization rate (kg COD/kg biomass • day) and the half-velocity constant (mg/L).

The biokinetic parameters of sludge (granules) can be obtained from the literature or determined experimentally. S_F can be chosen based on the design engineer's experience. The "f" factor is governed by the effectiveness of the feed distribution factor. For VOLR exceeding 5−10 kg COD/m^3 • day, the f factor approaches unity [36]. Furthermore, mixing associated with biogas production at higher VOLR also facilitates better contact between the substrate and the biomass. It is important that the superficial velocity (v_a) be maintained below the washout point of the granules. The superficial velocity can be calculated by:

$$v_a = H/\theta \quad [10.8]$$

where H is the reactor height (m) and θ is the HRT (h).

10.4.5 Anaerobic Baffled Reactor

An anaerobic baffled reactor (ABR) consists of a series of baffles, which divide the tank into several compartments (Fig. 10.7). The baffles are arranged in such a way that they force the wastewater to flow over and under the baffles. Microbial biomass accumulates between the baffles forming granular biomass with time. The baffles also prevent short-circuiting and biomass washout from the reactor, thereby enabling a high concentration

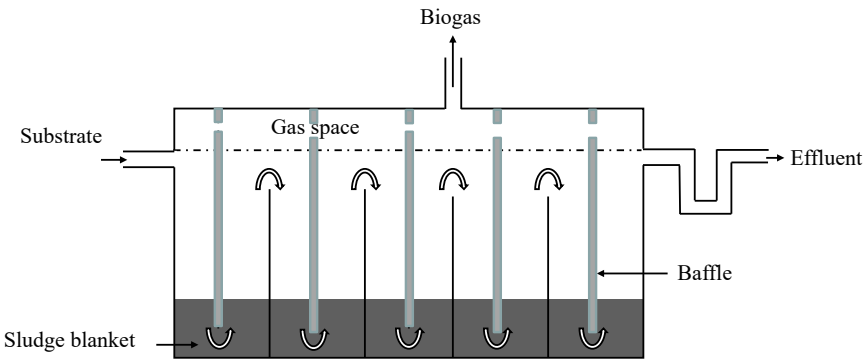

FIGURE 10.7 Schematics of anaerobic baffled reactor.

of microbes in the reactor. Thus, ABR maintains much longer SRT irrespective of HRT. Each chamber in the ABR acts as a CSTR in series and the flow through the ABR resembles a plug flow. The ABR configuration promotes contact between the wastewater and the sludge blanket. If the wastewater contains high suspended solids or particulate matter, the solids may settle down in the first compartment. Industrial effluents with high suspended solids are not suitable for treatment using an ABR.

10.4.6 Anaerobic Sequencing Batch Reactor

The anaerobic sequencing batch reactor (ASBR) provides a unique benefit in treating high-strength industrial effluents with medium solids content (TS 0.5−4%). Because of the sequential operation of the ASBR, a single reactor is operated in batch mode under various cycles as shown in Fig. 10.8. The substrate is fed into the reactor during the "feed" cycle. The organic matter is then degraded by the anaerobes during the "react" cycle. Either mechanical mixture or the biogas is used for mixing the reactor content. The microbial biomass is allowed to settle down in the reactor once the desired degree of treatment is achieved during the "settle" phase. The supernatant is decanted in the "decant" period. The main mechanism of biomass retention in ASBR is bio-flocculation followed by bio-granulation, similar to a UASB reactor.

10.4.7 Anaerobic Filter

An anaerobic filter (AF) is a packed-bed attached-growth reactor primarily developed to treat highly soluble wastewater [37]. The two most common configurations of an AF are an upflow AF (UAF; Fig. 10.9A) and a downflow AF (DAF; Fig. 10.9B). In a UAF wastewater flows upward through the medium and the entire filter bed is submerged. Although UAF is a fixed-film reactor, a significant portion of the microbial biomass remains entrapped within the interstices or void spaces between the packing medium. The unattached biomass forms bigger flocs and eventually takes a granular shape

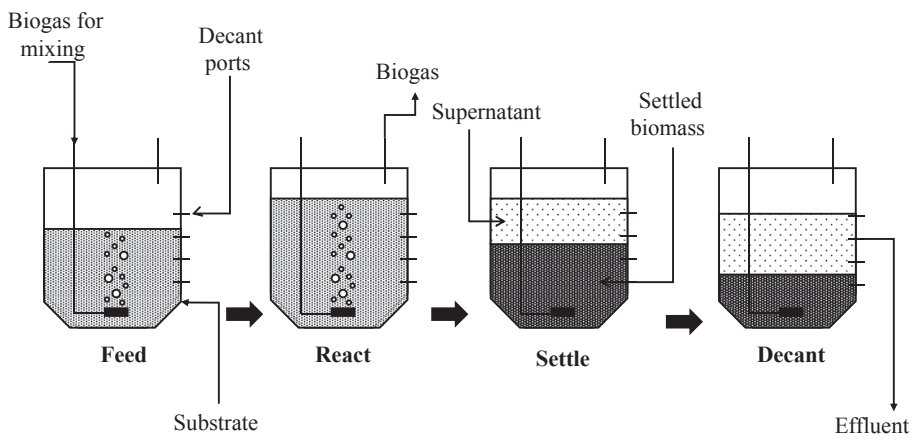

FIGURE 10.8 Schematics of anaerobic sequencing batch reactor. Adapted from S.K. Khanal, Anaerobic Biotechnology for Bioenergy Production, Wiley-Blackwell, Ames, IA, USA, 2008.

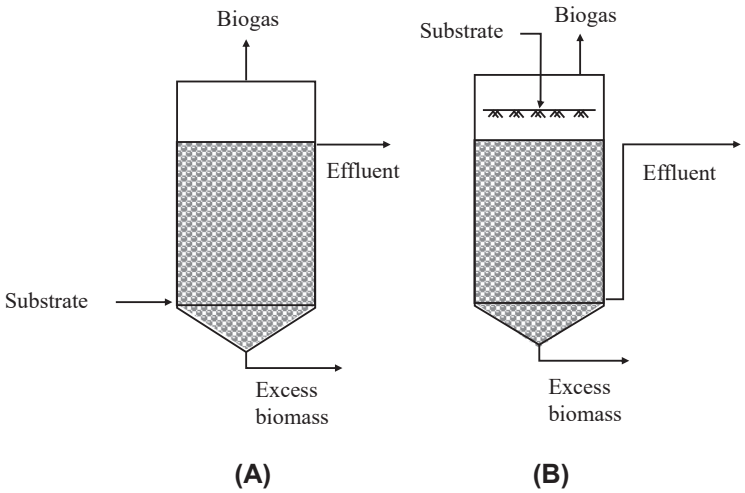

FIGURE 10.9 Schematic diagram of an anaerobic filter (A) upflow anaerobic filter and (B) downflow anaerobic filter. Adapted from S.K. Khanal, Anaerobic Biotechnology for Bioenergy Production, Wiley-Blackwell, Ames, IA, USA, 2008.

because of the rolling action of rising gas bubbles. Thus, unattached biomass contributes significantly to organic removal.

Rocks, gravel, and ceramic tiles were originally employed as packing media in anaerobic filters. Such packing media not only had low specific surface area, but also were associated with serious clogging problems due to low porosity (~40–50%). Currently, light synthetic plastic media of various configurations are employed as packing media. The plastic media usually have a porosity in the range of 80–95% with very high specific surface area of 100 m^2/m^3 or higher.

In a DAF, the wastewater is spread from the top, similar to a trickling filter system. In a DAF, the loosely held biomass washes out from the bed. Thus, DAF represents a true biofilm reactor. Compared to UAF, DAF offers minimal clogging problems and accommodates feed streams with some suspended solids.

A large amount of microbial biomass is retained in an anaerobic filter. Thus, extremely long SRT can be achieved irrespective of HRT. Typically, HRT varies from 0.5 to 4 days with SRT of over 100 days. Excess microbial biomass (sludge) may need to be periodically removed from the bottom of the bioreactor to minimize clogging and short-circuiting. Hydrodynamic conditions play an important role in biomass retention within the void space. The flow regime is often quasi-plug flow.

10.4.8 Expanded-Bed Reactor

An expanded-bed reactor (EBR) is an attached-growth reactor that immobilizes anaerobes on support media such as sand, activated carbon, shredded tire, etc. The support medium bed is expanded by the upflow fluid velocity of the incoming wastewater and recirculation of effluent (if needed) as shown in Fig. 10.10A. The fluid upflow velocity

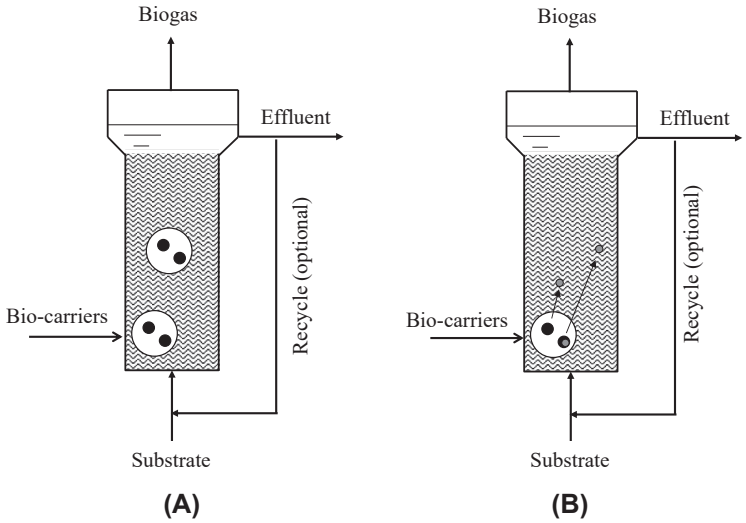

FIGURE 10.10 Schematics of (A) expanded bed reactor and (B) fluidized bed reactor. Adapted from S.K. Khanal, Anaerobic Biotechnology for Bioenergy Production, Wiley-Blackwell, Ames, IA, USA, 2008.

essentially maintains the bed expansion by 15–30%. The EBR poses minimal clogging problems and offers enhanced substrate diffusion within the biofilm. The support media, which tend to remain at the same relative position within the bed, are supported by the fluid upflow velocity and partly through its interaction with adjacent media. The EBR is an ideal reactor configuration for treatment of high-strength industrial effluents, which allows high OLR.

10.4.9 Fluidized-Bed Reactor

A fluidized-bed reactor (FBR) is very similar to an EBR except that the high upflow liquid velocity of 10–25 m/h is maintained in the former to allow the bed expansion by 25–30% of the settled bed volume. Thus, FBR is truly a fixed-film reactor in which the suspended solids including microbes are washed out from the reactor [11]. The biocarriers in an FBR are entirely supported by the fluid upflow velocity, and therefore they move freely in the bed (Fig. 10.10B). Often effluent recycling may be essential to achieve a bed expansion. COD removal efficiency of as high as 94% was reported for slaughterhouse wastewater (with a COD concentration up to 4500 mg/L at an OLR of 27 kg COD/m^3 • day) [38]. Major merits of FBRs are low propensity for clogging and short-circuiting and better substrate diffusion within the biofilm [39].

10.4.10 Anaerobic Membrane Bioreactor

The anaerobic membrane bioreactor (AnMBR) has gained considerable interest for wastewater treatment. The AnMBR essentially retains all the biomass in the reactor without any fear of washout irrespective of HRT using various types of membranes. The

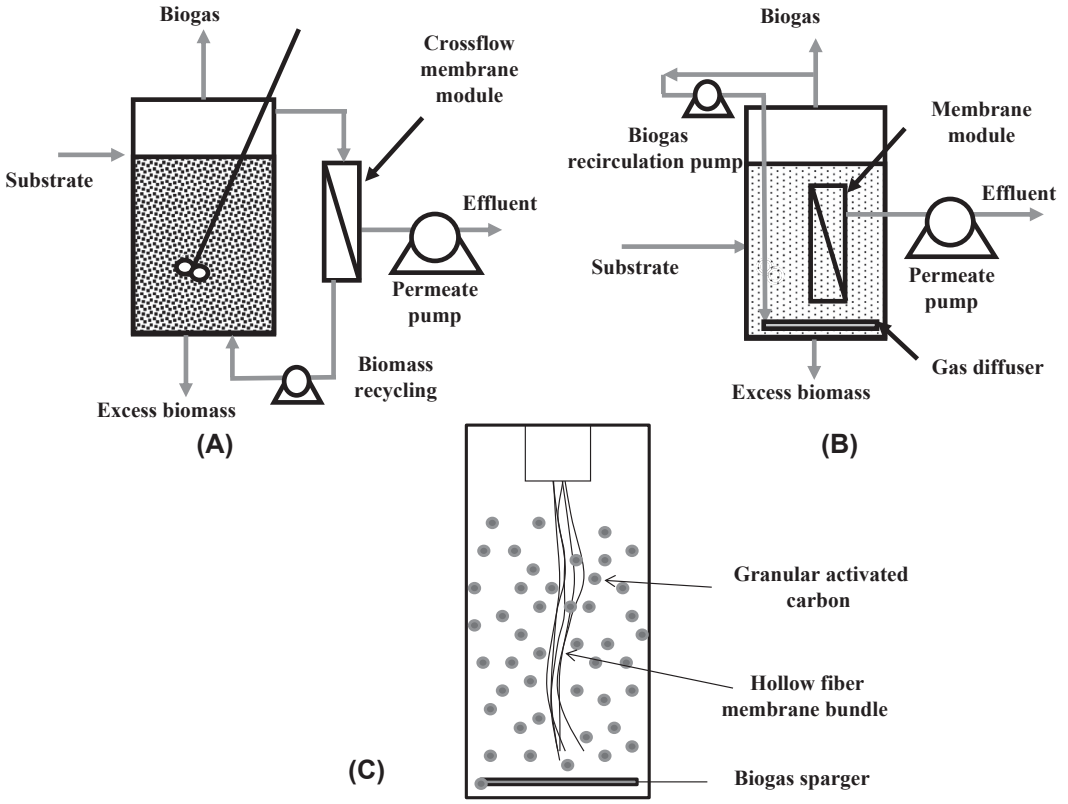

FIGURE 10.11 Schematics of AnMBR (A) External cross flow membrane; (B) Submerged membrane; and (C) Hollow fiber membrane with GAC.

membrane essentially acts as a solid–liquid separation unit, which is able to maintain extremely long SRTs. Some of the merits of AnMBR include (1) superior effluent quality, (2) generation of significantly less sludge, (3) smaller footprint, and (4) ease of process automation. The AnMBR employs either submersible membrane modules (Fig. 10.11A) or external membrane modules (Fig. 10.11B). In submersible modules, the membrane is housed within the bioreactor, which makes it very compact, less energy intensive, and an easy process to control. The major demerit of such modules is that the operation of the bioreactor needs to be completely stopped when repair/maintenance or replacement of the membrane module is required. In an external module, the membrane is placed in an external loop and the retained biomass is recycled back into the reactor. Such module is easy to maintain and clean without affecting the reactor operation [40,41].

The AnMBR has also some disadvantages including high capital and operation costs and membrane fouling, among others. To address the membrane fouling problem, researchers integrated AnMBR with granular activated carbon (GAC) (Fig. 10.11C). The AnMBR employed a hollow fiber membrane submerged in the reactor, and GAC was added in the reactor, acting as both biocarrier and biofilm scrubber from the

surface of the membrane. Such configuration allowed sustained flux with less frequency of membrane cleaning.

10.5 Perspectives

The anaerobic process has been widely adopted for both treatment of high-strength waste streams and bioenergy production because of several inherent merits. Anaerobic processes are often employed as a pretreatment step for industrial effluents to reduce the organic load. One of the critical components of the anaerobic process is the reactor design, the focus of which is to decouple SRT from HRT, especially for liquid waste streams. Some of the strategies for decoupling include biomass immobilization in attached-growth systems, granulation and floc formation, recycling of microbial biomass, and use of membranes for biomass retention. The primary goal of such decoupling is to prevent the washout of slow-growing methanogens from the bioreactor. There is a perpetual need to develop innovative reactor designs that are cost-effective, energy efficient and reliable, and capable of producing superior effluent quality. With the advent of advanced molecular tools, research and development efforts should focus on integrating microbial data in designing and operating an anaerobic reactor. Furthermore, process automation and control should be integrated as a part of the bioreactor design. Thus, both process engineering and process microbiology become critically important for bioreactor design and operation.

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11

Application of Molecular Biological Tools to Monitor Process Efficiency

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11.1 Background

Anaerobic processes such as anaerobic digestion and anaerobic membrane bioreactors are gaining attention in the wastewater treatment industry because of their advantages of significantly lower energy consumption and sludge production compared to conventional aerobic processes and their potential to generate biogas as an energy source. One example is the rapid adoption of the anammox process for nitrogen removal from wastewater. Compared to conventional nitrification/denitrification processes, the anammox process has no chemical oxygen demand (COD) requirement and lower sludge production.

All of these anaerobic processes are microbially mediated, and their efficiency and stability are entirely dependent upon the activity of microorganisms belonging to various functional groups. Microbial communities involved in these engineered environments are often phylogenetically and functionally diverse. Molecular biological tools can help us to identify the key populations capable of carrying out specific metabolic processes and their functions in anaerobic processes.

The traditional biological techniques are culture-dependent. However, the majority of microorganisms in these systems have not yet been cultivated [41]. Moreover, different microorganisms interact with one another through competition and collaboration, which cannot be studied in isolation [53]. Therefore the culture-dependent techniques cannot fully reveal the microbial activity and function in complex microbial environments and the environmental factors affecting them.

To fully understand these communities, novel molecular biological tools combined with visualization methods and chemical analyses are required. This use of complementary techniques will allow the characterization of microorganisms involved, and their interspecies interactions. This information can improve the design and operation of anaerobic processes to fully utilize their potential as effective wastewater treatment and resource recovery processes, and for the generation of high-value products.

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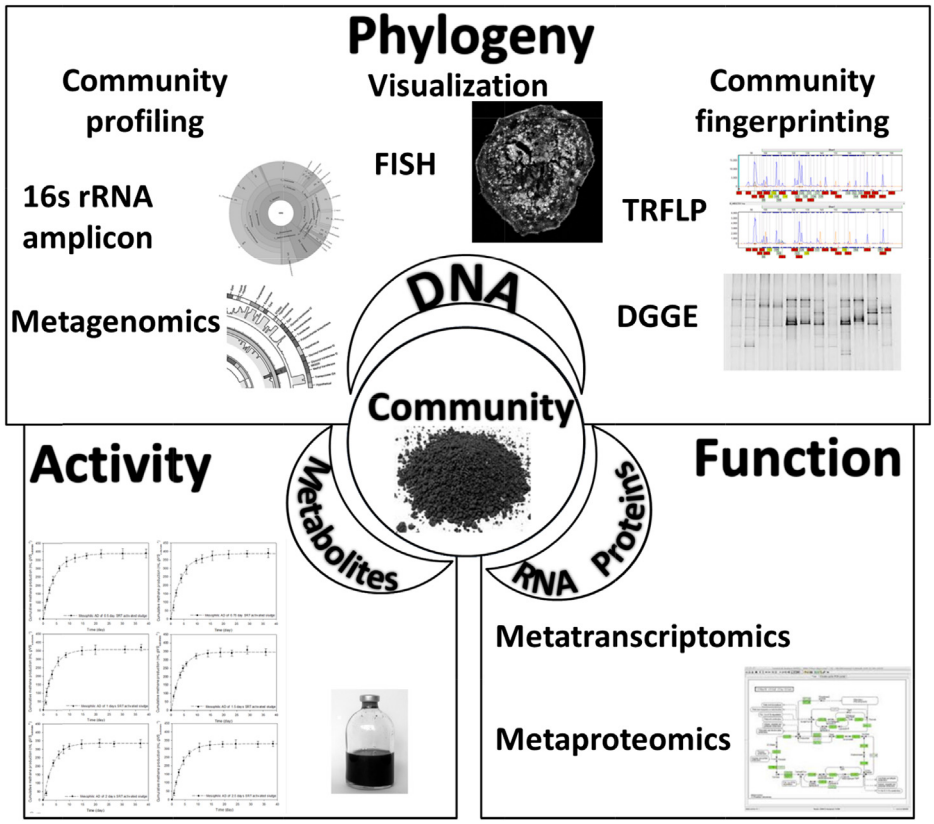

FIGURE 11.1 Overview of the molecular tools for monitoring the microbial communities in the anaerobic wastewater treatment processes. *DGGE*, denaturing gradient gel electrophoresis; *FISH*, fluorescence in situ hybridization; *TRFLP*, terminal restriction fragment polymorphism.

The latest developments of novel molecular techniques for this purpose have been reviewed previously [47]. This chapter focuses on the application of both advanced visualization techniques and emerging molecular methods that can reveal microbial community structure and their function in anaerobic processes (Fig. 11.1).

11.2 Molecular Biological Tools and Applications

11.2.1 Visualization by Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) utilizes labeled oligonucleotide probes targeting the specific position of 16S ribosomal ribonucleic acid (rRNA) in microbes of interest [1]. As a result, fluorescence images can be taken by examining with fluorescence microscopy. Further quantitative analysis can be performed based on the fluorescence intensity and provides relative abundance of microbes of interest. FISH is commonly used as a

visualizing tool to support microbial shifts monitored by other molecular tools or quantification tools to provide the direct abundance shift in microbes of interest.

Another application of FISH in anaerobic systems is spatial profiling. In specific system design, biofilms can form spontaneously with microorganisms that consequently perform various steps of anaerobic digestion. For example, in upflow anaerobic sludge blanket (UASB) reactors, anaerobic granules form with microorganisms that convert the organic matter in the wastewater into methane and carbon dioxide through a series of complex biological reactions including interactions between multiple functional groups. Identification by FISH in conjunction with cryosectioning substantially improves knowledge of the microbial spatial distribution, which may be the key to explaining the complex consequence reactions in anaerobic granules.

Harmsen et al. [14] located a syntrophic propionate oxidizing bacteria related to *Syntrophobacter wolinii* in the middle layer of sucrose-fed and volatile fatty acid-fed granules. Sekiguchi et al. [36], showed a classic FISH image of anaerobic granules with probes targeting different groups of methanogens and two groups of specific clones. This was the first molecular evidence demonstrating the layer structure of anaerobic granules with methanogens and syntrophs accumulating in the inner layers and carrying out the latter steps of anaerobic digestion. They also found a large amount of filamentous green nonsulfur bacteria occupying the outermost layer of thermophilic anaerobic granules, which was presumed to use the primary substrate (sucrose). This finding then further led to the isolation of the novel genus *Anaerolinea* [35], which is an important carbohydrate fermenter in both anaerobic granules [50] and activated sludge [6,20,23]. Batstone et al. [4] and Lu et al. [25] also compared the structures of anaerobic granules treating different types of wastewater and described the differences in the outer layer of granules treating brewery, cannery, and dairy wastewater.

FISH can be fast and accurate in identifying and locating the microbes of interest in anaerobic digesters. However, most detailed FISH studies (down to the genus level) on anaerobic systems are limited to methanogens, which are relatively simple. With a bacterial community in an anaerobic system (e.g., fermenters), specific levels of probes are not available as most bacteria are uncultured or the targeted microbes are diverse (fermenters are spread over 20 genera). Thus, an understanding of the targeted microbes, obtained from other molecular techniques, is essential for the successful application of FISH.

11.2.2 Monitoring by Fingerprinting Techniques

In the 1990s, a set of molecular tools was developed aiming to provide a quick fingerprint (presence and abundance of microbes) of unknown anaerobic treatment systems. 16S rRNA is commonly targeted with these methods, which include denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment polymorphism (TRFLP).

DGGE is based on various denaturation properties of 16S rRNA fragments due to sequence variations, providing a genetic profile representing the microbial diversity and identification by sequencing individual bands. For example, a DGGE band affiliated with *Methanosarcina mazei* was detected only in winery-fed granules, whereas the one affiliated

with *Methanobacterium formicicum* was detected only in brewery-fed granules [22]. Combining specific activity test and DGGE on six full-scale anaerobic digesters, Regueiro et al. [32] illustrated that enhanced hydrolytic and methanogenic activity are associated with high abundance of Bacteroidetes and hydrogenotrophic methanogens.

TRFLP distinguishes the microorganisms based on the restriction site difference closest to a fluorescence-labeled end of an amplified 16S rRNA gene. It has great application in monitoring the composition of the community as well as the community shifting through certain parameters. It is normally used to monitor the microbial community shift between samples and estimates an even and diverse community representing a high-performance lab-scale reactor [8].

Comparing the anaerobic digestion treating different wastewaters, however, it was found that the source of mixed anaerobic cultures plays an important role, as the presence of carbohydrate competitors (such as a sulfate reducer) and shortage of methanogens can lead to low H_2 and methane production [9]. Microbial community shifts after certain stresses or treatments were also frequently monitored by TRFLP. For example, Ike et al. [16] analyzed the microbial community dynamic during start-up of a full-scale anaerobic reactor treating industrial food waste and identified *Actinomyces*/*Thermomonospora* and *Ralstonia*/*Shewanella* as the major fermenters, whereas the archaeal community shifted from acetate-utilizing methanogens toward hydrogen-utilizing after stable performance. Hu et al. [15] also found, during the transformation of methanogenic granules to hydrogenic granules by chloroform, that hydrogen-utilizing methanogens were eliminated and replaced by *Clostridium butyricum*. Lu et al. [26] also monitored the bacterial community shift with pH step changing and illustrated the impact of the precedent pH on the microbial community structure.

Some of the advantages of fingerprinting techniques are that they allow gel-to-gel variation analysis and are suitable for routine analysis of large sample numbers, as they have reproducibility and potential for automatization [39]. However, the actual quantification information cannot be obtained directly from fingerprinting techniques and relative quantification obtained from TRFLP can lead to false information due to the PCR bias and lack of resolution. Other quantification methods such as FISH quantification or quantitative PCR should be applied to support or correct the quantity of each microorganism. The fingerprinting techniques are often used to readily assess the community shift and guide the selection of samples from the time points or locations of interest for further next-generation sequencing analysis [42].

11.2.3 Quantifying by Real-Time Quantitative PCR

Real-time quantitative PCR (qPCR) is specifically designed PCR aiming to quantify targeted groups or individual microbes. Similar to FISH, it utilizes the fluorescent property of either SYBR green or TaqMan probe during the PCR process, with the latter being considered to provide greater target specificity in microbial ecology studies [3]. Absolute quantification (through either digital PCR or standard curve method) and relative quantification (through the comparative CT method) can be performed.

A strong correlation between biogas production rate and methanogen abundance, in particular *Methanosarcina* and *Methanosaeta*, was normally observed by qPCR analysis in anaerobic reactors [40,44]. Thus *Methanosarcina* and *Methanosaeta* were proposed to be bio-indicators of the stability of the process by Traversi et al. [45]. The emergence of hydrogenotrophic methanogen groups as dominant archaeal members was detected by qPCR in long-term operation of psychrophilic anaerobic reactor treating industrial wastewater [27]. Bialek et al. [5] reported a sharp increase in hydrogenotrophic methanogens after the organic loading rate increased from 0.5−2 to 2 kg COD/m^3 day in an expanded granular sludge bed reactor operating at 10°C. Vanwonterghem et al. [46] also utilized qPCR to demonstrate the same trend in biomass concentration during start-up of an anaerobic digester.

Although qPCR is able to quickly access target numbers reflecting total or specific community, the estimation can be rough because of the variation in gene copy number between species [11]. Angly et al. [2] applied gene copy number correction to qPCR and 16S rRNA-based microbial community profiling and reviewed the overestimation of microbial community shift from uncorrected qPCR results.

11.2.4 Identifying by 16S rRNA Clone Library

The 16S rRNA gene clone library is a popular technique for investigating phylogenetic diversity. It is a collection of DNA sequences, usually derived from PCR amplification, inserted into a plasmid vector and cloned into a bacterial host cell (for example *Escherichia coli*). As nearly complete 16S rRNA genes can be sequenced from the clone library, it can provide taxonomy identification to the species level for cultured microorganisms.

Members of the genera *Bacillus* and *Pseudomonas* were commonly detected in a 16S rRNA clone library from different types of granule [21]. Hernon et al. [12] conducted a study using a 16S rRNA clone library on two lab-scale UASBs treating synthetic wastewater containing glucose and sucrose at mesophilic and thermophilic conditions, respectively. They found that Bacteroidetes and Spirochaetes dominated in the mesophilic reactor, whereas Clostridia was the only dominating class in the thermophilic reactor. Alphaproteobacteria were identified in granules treating synthetic powdered skim milk wastewater with the frequently detected clone, which were closely related to *Sphingomonas rhizogenes* [34]. Members of Thermotogae, Synergistetes, Firmicutes, Chloroflexi, and Proteobacteria were observed in anaerobic granules treating syrup wastewater [28]. However, intensive labor and time requirements hamper the application of the 16S rRNA clone library as a monitoring tool in anaerobic systems and it has been replaced by high-throughput next-generation sequencing.

11.2.5 Next-Generation Sequencing Approach

The next-generation sequencing (NGS) approach expands the throughput, scalability, and speed to a new level and is becoming the major molecular technology used to monitor anaerobic systems. It provides the opportunity to sequence small fragments in a parallel fashion with no cloning required. The ability to provide a large set of sequencing

data makes the detection of low-abundance species possible and has an impact on the interpretation of microbiological changes [7].

11.2.5.1 Community Profiling

16S rRNA gene amplicon sequencing through either the Roche 454 or the Illumina platform has become the most common microbial profiling technique for anaerobic digesters [38,48]. Under higher resolution provided by NGS, the impact of operational parameters including temperature [43] and feedstock [54] can be reviewed. Microbial communities of anaerobic digesters are clustered according to the total ammonia concentration, together with free ammonia concentration and digester temperature [10]. Anaerobic digesters processing wheat-based fuel ethanol waste streams contained a dominant core of 42 bacteria within the phyla Firmicutes, Alphaproteobacteria, Actinobacteria, and Chloroflexi. NGS improves our understanding of microbes involved in each anaerobic digestion and also helps us to explain the ability of the community for handling stress. Applying single-stage anaerobic digestion to high-strength food wastewater showed a shift from Chloroflexi dominating in the seed from sewage treatment [29,33] toward a low-diversity community consisting of the phyla Bacteroidetes, Firmicutes, Synergistetes, and Actinobacteria, demonstrating the potential hydrolysis function of these groups [19]. However, with separated mesothermophilic digestion, high abundance of Actinobacteria was detected in both stages with Synergistetes and Firmicutes predominating, respectively [18]. $FeCl_3$ was found to contribute to the improvement of biogas production with *Coprothermobacter* enriched in the digester [51].

Codigestion has become the new field of 16S rRNA gene amplicon sequencing. The relationship between microbes and the amount of substrate loading in codigestion can be investigated. For example, from high to low biodiesel waste glycerin or restaurant grease waste combined with municipal wastewater sludge, methanogens shifted from an acetoclastic to a hydrogenotrophic population with *Candidatus Cloacimonas* dominating under all conditions [30,31]. In the codigestion of crude cheese whey with fruit vegetable waste, the C/N ratio can be optimized and leads to enhanced H_2 with lactic consumers enriched [13].

11.2.5.2 Function Profiling

16S rRNA gene amplicon sequencing can shed some light on metabolic functionality by searching for closely related cultured species [24] or those correlated with operation parameters and performance [48]. Meta-omics studies go beyond this and provide direct potential and actual function identification and dynamics [37].

Metagenomics is the random sequencing of genomic DNA (shotgun approach) directly from a sample, and as PCR is not required, PCR-related bias can thus be eliminated. Potential known function can then be identified and quantified through sequencing results; however, this is mainly hampered by lack of reference genomes [49]. By applying metagenomics to anaerobic digestion, direct evidence showed consistency with previous findings including hydrolysis and fermentation functionality of members in the phyla Firmicutes and Bacteroidetes and syntrophic relationship between

Clostridia and hydrogenotrophic methanogens [17]. It was also concluded that community structure and functional changes over time may be reliably predicted as deterministic processes were shown to guide long-term synchronized population dynamics in a replicated anaerobic digester [47].

Metatranscriptomics is the sequencing of reverse-transcribed mRNA extracted from a microbial community and can show the in situ gene expression. Metatranscriptomic reads will then be mapped against reference genomes or a metagenome from the same bioreactor, so that differential gene expression levels can be compared. Traditional techniques for measuring microbial community gene expression such as microarrays are designed to detect only known sequences. Metatranscriptomics measures the gene expression level without any a priori knowledge of the nucleotide sequence, which increases the range of detection of the metabolically active genes [47]. Metatranscriptomics has been used to analyze microbial communities from an anaerobic digester community [52]. High-level gene expression of the enzymes involved in substrate hydrolysis, acidogenesis, and acetate formation was detected, and also a high transcriptional activity of archaeal populations.

Because meta-omics approaches can directly link microbial populations to specific function processes in the engineering system, an increased application of meta-omics analyses for monitoring anaerobic processes is expected.

11.3 Conclusions

To achieve stable control of the anaerobic process, detailed knowledge of the structure and function relationships within the complex microbial communities is necessary. The monitoring of reactor microbiomes requires many molecular biological tools, including visualization methods and meta-omics approaches. Different tools have different advantages and disadvantages, as shown in Table 11.1. Molecular techniques including

Table 11.1 Summary of the Molecular Biological Tools Used for Monitoring Anaerobic Process and Their Advantages and Disadvantages

Tool	Application	Advantages	Disadvantages
Fluorescence in situ hybridization	Visualization	The only technique that allows in situ access to spatial distribution Fast turnover	Probes are limited to channel and availability Low resolution
Real-time quantitative PCR	Quantification	High specificity Fast turnover Absolute or relative abundance	No identification in total community PCR bias
Community fingerprinting	Monitoring	Fast access to community change	Low resolution Inaccurate identification
DNA clone library	Identification	Nearly complete 16S rRNA gene can be recovered for accurate identification	Time and labor intensive
Meta-omics	Multipurpose	High resolution Multiple sequences and samples can be analyzed in one run	Relatively expensive Bioinformatics intensive Big data mining

spatial profiling, community fingerprinting, qPCR, next-generation high-throughput DNA and RNA sequencing, and meta-omics approaches, whether applied alone or in combination with one another, will allow us to understand and control the anaerobic wastewater treatment processes.

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12

Anaerobic Treatment of Low-Strength Wastewater

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12.1 Introduction

Wastes, both liquid and solid, are produced in every community by anthropogenic activities. The liquid wastes, termed as used water or wastewater, originate from a variety of sources including households, commercial developments, hospitals, institutions, industries, etc. (Fig. 12.1). Because of the diversity of the wastewater sources, the pollutants present in the wastewater also vary considerably. Understanding the constituents of the wastewater is important in determining appropriate treatment methods for wastewater treatment, water reuse, and disposal [1].

The major components of wastewater that flows into the collection system of a community fall under the following four major types: domestic wastewater, which constitutes wastewater from households, commercial, and similar facilities; industrial wastewater from various industries; infiltration/inflow, which is the extraneous water entering the collection system through leaks or cracks in connections; and stormwater, which constitutes runoff from rainfall and snowmelt [1]. Municipal wastewater is considered the collection of wastewaters from all the above-mentioned wastewater types entering the municipal wastewater treatment plant. Stormwater may be conveyed either through separate stormwater sewers or through combined sewers where it is collected and treated along with municipal wastewater. Some industrial wastewaters are treated or pretreated separately in their own facilities before being discharged into a municipal wastewater treatment plant. Significant differences in the physical, chemical, and biological constituents may be observed among different types of wastewaters and across localities and weather patterns. Tables 12.1 and 12.2 list the total suspended solids (TSS), chemical oxygen demand (COD) and biological oxygen demand (BOD), nutrients, and fats, oils, and grease (FOG) concentrations for various types of wastewaters.

The constituents of industrial wastewaters vary highly depending on the type of industry. Industrial wastewaters may contain high concentrations of metals and toxic

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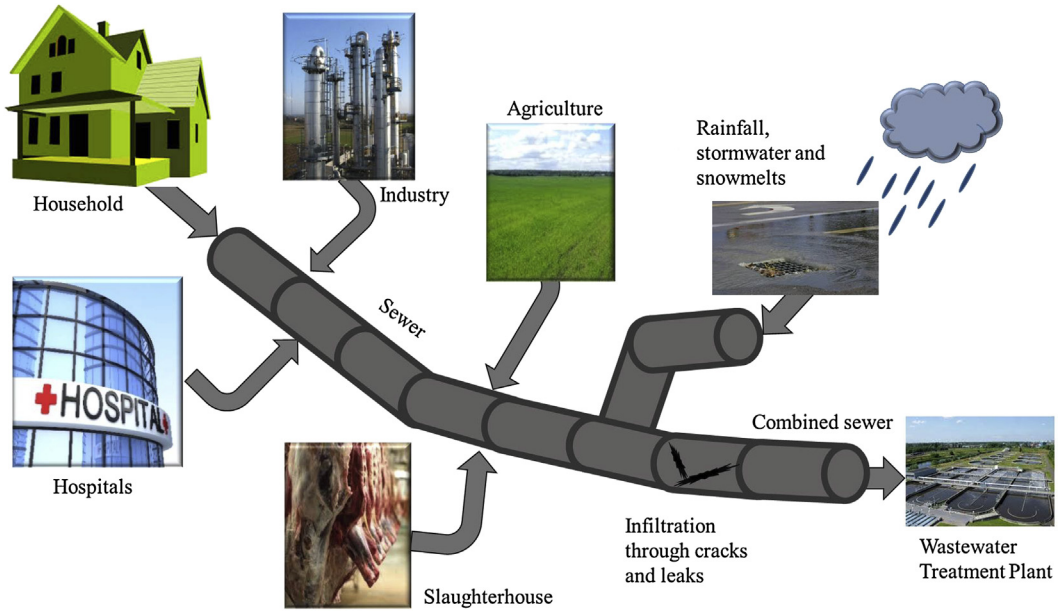

FIGURE 12.1 Various sources of wastewater entering municipal wastewater treatment plants. *Adapted from I.L.C. Drexler, A.L. Prieto, D. Yeh, Wastewater constituents, in: S. Ahuja (Eds.), Comprehensive Water Quality and Purification, Elsevier, Waltham, 2014, pp. 7–29.*

substances specific to the industry and may have extreme physical properties (pH and temperature) depending on the nature of processes used in the industry. Table 12.2 lists some common industries and their associated wastewater compositions.

When choosing wastewater treatment processes, the primary constituents, especially the organic content of the wastewaters, have to be taken into account. There are no standard definitions to classify wastewaters according to strength, and BOD, COD, and FOG concentrations are commonly taken as criteria for the classification of

Table 12.1 Concentrations of Selected Constituents in Various Types of Wastewater

Wastewater	Total Suspended Solids	Biochemical Oxygen Demand (5 days)	Chemical Oxygen Demand Total	Fats, Oils, and Grease	Total Nitrogen
	Concentrations Are Provided in mg/L				
Municipal wastewater	350–1200	100–350	210–740	30–100	20–80
Household wastewater	252–3320 (median 1028)	112–1101	139–1650	16–134	44–189
Storm water	112–1894	12–19	82–178	<1 to <7	3.5
Industrial wastewater	Dependent on industry. See Table 12.2				

Extracted from I.L.C. Drexler, A.L. Prieto, D. Yeh, Wastewater constituents, in: S. Ahuja (Eds.), Comprehensive Water Quality and Purification, Elsevier, Waltham, 2014, pp. 7–29.

Table 12.2 Wastewater Composition in Selected Industries

Industry	Solids Concentration (mg/L) and pH Values	Oxygen Demand (mg/L)	Nutrients Concentration
Mills and tanneries	N.A.	BOD: 1000–2000	Total nitrogen: 200–400 mg N/L
Landfills	pH: 6.5–7.2	BOD: 300–12,000 COD: 1,200–16,000	Total nitrogen: 100–500 mg N/L
Acids, mine drainage (surface mines)	pH: 2.67–7.7 TSS: 4–15,878	N.A.	Ammonia: 0.53–22 mg N/L Sulfate: 22–3860 mg SO_4^{-2}/L
Textiles	TSS: 1560	BOD: 800–1000	Organic nitrogen: 8 mg N/L
Dairy (mixed production)	N.A.	COD: 4000 BOD: 1000–2000	Ammonia: 5–626 mg N/L Nitrate: 0.2–24.4 mg N/L
Slaughterhouse	TSS: 1400	BOD: 500–1000	Organic nitrogen: 300–1005 mg N/L
Breweries	TSS: 500	BOD: 1000–3000	Ammonia: 125 mg N/L Total phosphorus: 10–20 mg P/L
Vegetable canneries	TSS: 1350	BOD: 800–5000	Ammonia: 5–45 mg N/L
Petroleum refining (production)	TSS: 441	N.A.	Ammonia: 0–5000 mg N/L
Commercial laundries	TSS: 1000	COD: 5000	Total phosphorus: 10–50 mg P/L
Photolabs	N.A.	BOD: 400–700	N.A.
Printing houses	TSS: 1180	BOD: 210	N.A.

BOD, Biological oxygen demand; *COD*, chemical oxygen demand; *TSS*, total suspended solids.
Extracted from I.L.C. Drexler, A.L. Prieto, D. Yeh, Wastewater constituents, in: S. Ahuja (Eds.), Comprehensive Water Quality and Purification, Elsevier, Waltham, 2014, pp. 7–29.

low-, medium-, and high-strength wastewaters [2]. The most widely used residential strength testing protocol, NSF Standard 40, defines residential-strength wastewaters as BOD_5 100–300 mg/L and TSS 100–350 mg/L and high-strength wastewaters as "greater than the residential strength" [3]. For wastewaters other than residential wastewaters, the strengths associated with the terms low, medium, and high vary across research papers and wastewater treatment plant design manuals [4–7]. From a comprehensive evaluation of the range of definitions for the strength of wastewaters in the literature, in this chapter we consider wastewaters with COD <2000 mg/L as low strength and those with COD >2000 mg/L as medium to high strength.

This chapter focuses on low-strength wastewaters and their treatment methods. Existing anaerobic treatment methods are discussed with emphasis on energy efficiency, cost effectiveness, and resource recovery capability.

12.2 Low-Strength Wastewater Treatment

In anaerobic processes, the bioconversion of organic matter into methane and biomass occurs anaerobically through bacterial metabolism. The three basic steps involved in an anaerobic process to obtain energy are hydrolysis, fermentation/acetogenesis, and

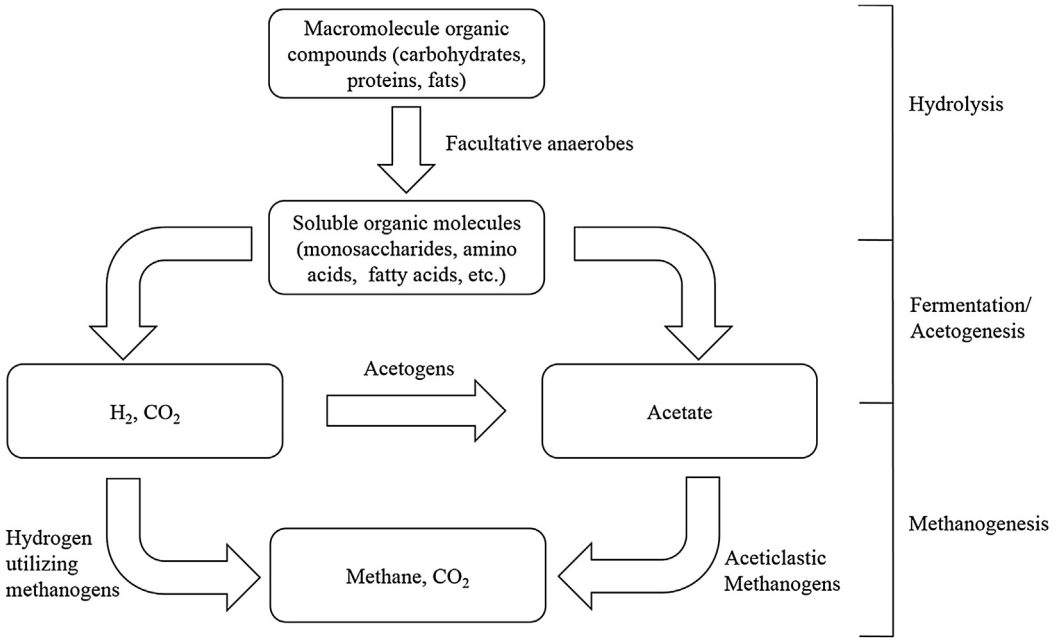

FIGURE 12.2 Metabolic pathways in anaerobic digestion.

methanogenesis (Fig. 12.2) [8]. The first step, hydrolysis, involves the breakdown of high-molecular-weight complex organic compounds into low-molecular-weight simple organic compounds (e.g., monosaccharides, amino acids, fatty acids, etc.) with exo-enzymes. These low-molecular-weight organic compounds then undergo the second step of fermentation in which they are assimilated by bacteria (e.g., acetogens) and are converted into some intermediate compounds such as hydrogen, acetic acids, and other volatile fatty acids (VFAs) such as propionic acid and butyric acid. In the third step, methanogenesis, these intermediate compounds are converted into CO_2, CH_4, and H_2O by a group of microorganisms, such as aceticlastic methanogens and hydrogen-utilizing methanogens [9]. The methanogenesis substrate utilization rate is a rate-limiting step in the anaerobic process and hence it is important to maintain a balance between the fast-growing acetogenic bacteria and the slow-growing methanogens.

Aerobic treatments are usually associated with low-strength wastewaters and anaerobic treatments with medium- to high-strength wastewaters [10]. The advantages of anaerobic treatment in biogas production and energy utilization were realized as early as the 1800s [11], and anaerobic treatments for specific wastewaters (for example, dilute soluble organic wastewater, sugar beet wastewater, potato processing wastewater) have been investigated [12–16]. However, most of the early studies were focused on the anaerobic treatment of medium- to high-strength wastewaters. At higher substrate concentrations, the advantages of anaerobic treatments outweigh the aerobic treatments

Table 12.3 Selection Criteria for Wastewater Treatment Processes

Criterion	Aerobic	Anaerobic
Process stability and control	+	
Power input		+
Heat input	+	
Low nutrient requirements		+
Biogas production and nutrient recovery		+
Waste stabilization		+
Waste sludge production		+
Effluent quality	+	
Process time	+	
Odor	+	

+ Denotes advantage over the other.

Extracted from U. Marchaim, Biogas Processes for Sustainable Development, Food & Agriculture Org., 1992; P.L. McCarty, Anaerobic waste treatment fundamentals, Public Works 95 (9) (1964) 107–112; Y.J. Chan, et al., A review on anaerobic–aerobic treatment of industrial and municipal wastewater, Chemical Engineering Journal, 155 (1–2) (2009) 1–18.

in terms of cost benefits such as lower energy requirement, lower sludge production, and energy recovery from the system and are hence favored for medium- to high-strength wastewaters [7].

With low-strength wastewaters, anaerobic treatment requires longer start-up time to develop the necessary amount of biomass in the reactor and may require heating of the feed wastewater prior to treatment. These disadvantages, along with sensitivity to toxic substances and low effluent quality, limit the use of anaerobic treatments for low-strength wastewater. Table 12.3 summarizes the criteria for selection between aerobic and anaerobic processes for the treatment of various types of wastewaters.

Low-cost anaerobic treatment processes with high effluent quality and energy recovery have become a desired goal, because of the global energy crisis and concerns over climate change [17]. According to the US EPA [18], wastewater treatment plants account for approximately 3% of the total electric load in the United States and the demand for electricity in such plants is expected to grow with the increase in population. A predominant portion of the total energy consumption (65–75%) in a wastewater treatment plant is utilized for plant operations related to aeration and pumping [19]. With the use of anaerobic treatment, aeration costs can be obviated, which would lower the operational cost significantly. In addition, with biogas produced from the anaerobic process, energy self-sufficiency may be achieved [20–22]. By adopting an anaerobic treatment process as shown in Fig. 12.3, a significant reduction in energy use could be attained and, at the same time, biogas may be recovered.

Even though anaerobic digesters and septic tanks for sewage treatment have been in use in developing countries for over 100 years, it was not until the advent of high-rate anaerobic digesters such as anaerobic filters [16], and anaerobic contact processes [23], that research started focusing on anaerobic processes for low-strength wastewater

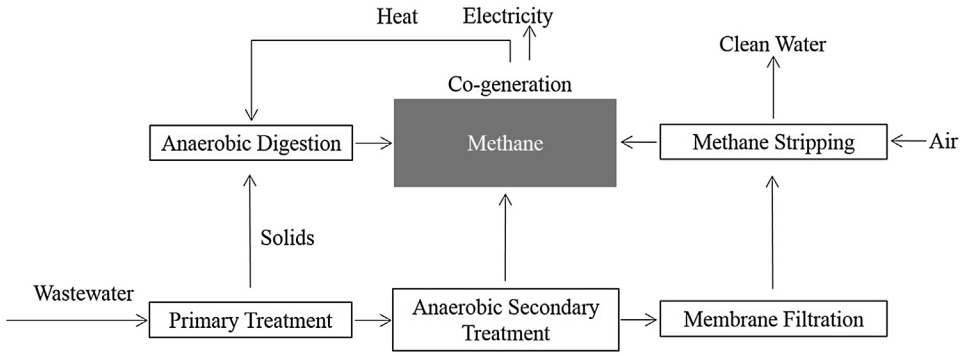

FIGURE 12.3 A hypothetical system for self-sustained complete anaerobic treatment of wastewater. *Adapted from P.L. McCarty, J. Bae, J. Kim, Domestic wastewater treatment as a net energy producer—can this be achieved? Environmental Science & Technology 45 (17) (2011) 7100–7106.*

treatments [7]. Since then, there has been increasing interest in the development of anaerobic processes for low-strength wastewater treatment owing to its lower energy costs and its capability in bioenergy recovery. Studies on the development of energy-efficient anaerobic treatment processes for low-strength wastewaters have focused on lowering the process time, improving the effluent quality, enhancing the energy recovery, and lowering the temperature sensitivity of the processes. The following section focuses on widely investigated anaerobic processes for low-strength wastewater treatment.

12.2.1 Types of Anaerobic Reactors for Low-Strength Wastewater Treatment

Anaerobic reactors can generally be classified as low-rate systems or high-rate systems based on the process capacity. Low-rate anaerobic reactors include waste stabilization ponds, Imhoff tanks, septic tanks, and anaerobic digesters, which have been used for many decades [24]. These processes generally occupy a large space and have very low organic loading rates and very long hydraulic retention times (HRTs) (as high as 40–50 days) [25,26]. These features result in high land/space consumption, which are unfavorable compared to the widely used activated sludge process and other aerobic processes. With the introduction of new anaerobic reactor designs [14,16,23,27], there have been breakthroughs in high-rate anaerobic reactors with high organic loading rates, low HRTs (<10 h), and a resultant low space requirement [26]. The types of high-rate anaerobic reactors available in the literature and the various reactor configurations are shown in Figs. 12.4 and 12.5. Despite the different configurations/designs of different processes/reactors, the biological processes in all types of reactors are similar and follow similar anaerobic metabolic pathways as described earlier (Fig. 12.2).

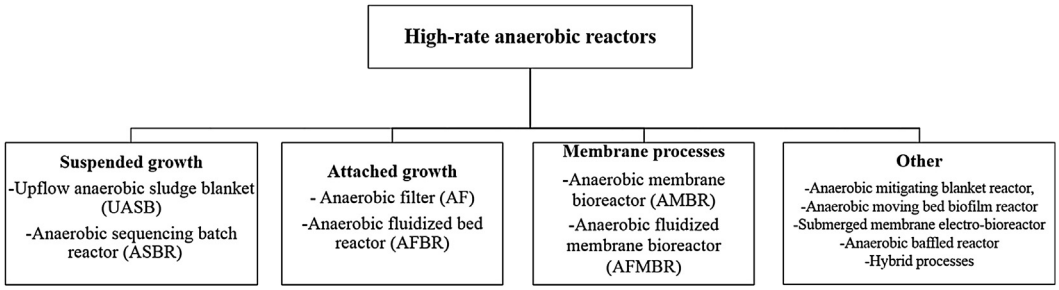

FIGURE 12.4 Types of high-rate anaerobic reactors.

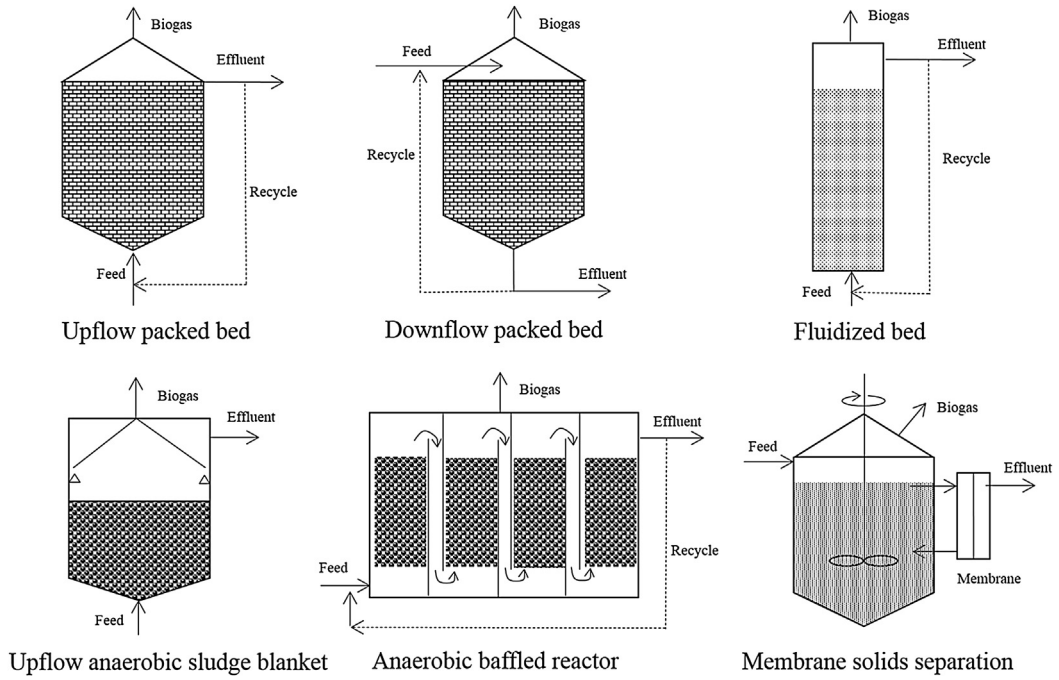

FIGURE 12.5 Various anaerobic reactor configurations. *Adapted from P.L. McCarty, D.P. Smith, Anaerobic wastewater treatment, Environmental Science & Technology 20 (12) (1986) 1200–1206.*

12.2.1.1 Suspended Growth Anaerobic Processes

Suspended-growth anaerobic processes are processes in which microbial aggregations (as sludge flocs or self-immobilized anaerobic granules or both) responsible for treatment are maintained in suspension with appropriate mixing methods. This section explains in detail the two notable high-rate anaerobic processes: upflow anaerobic sludge blanket (UASB) reactor and anaerobic sequencing batch reactor (ASBR).

12.2.1.2 Upflow Anaerobic Sludge Blanket

In the concept of the upflow sludge blanket for anaerobic biological wastewaters treatment, introduced by Lettinga et al. [15], the organic loading rates and HRT are controlled to facilitate the formation of anaerobic granular sludge [26]. A schematic of the UASB reactor is shown in Fig. 12.6.

The UASB reactor essentially consists of a tank with installations for three-phase separation (liquid, solids, and gas) on its upper part. Feed is uniformly distributed from the bottom of the reactor through spaced inlets and passes through the reaction zone where there is an anaerobic sludge bed with anaerobic granules (typically 0.5–2 mm in diameter). These granules are made of multiple microbial species, which are typically grouped in three basic layers (enriched with acidogens, acetogens, and methanogens, respectively) (Fig. 12.7) [9,28]. This organization of microbes has been shown to improve the metabolic pathways across microorganisms and to protect sensitive ones (e.g., methanogens) from the harsh external environment.

The anaerobic granules are agitated from time to time in the sludge bed by the superficial upflow velocity and rising biogas bubbles. Biogas is produced as a result of the anaerobic decomposition occurring during the contact between the granular sludge and the feed wastewater. The rising gas bubbles facilitate adequate mixing through hydraulic turbulence and are separated at the top of the reactor in a three-phase (gas–liquid–solids) separator. At the top of the reactor, biogas and the effluent are collected separately through weirs while the small solid granules settle back to the reactor. The absence of mechanical mixing and the simple design make UASB reactors an inexpensive and attractive treatment option.

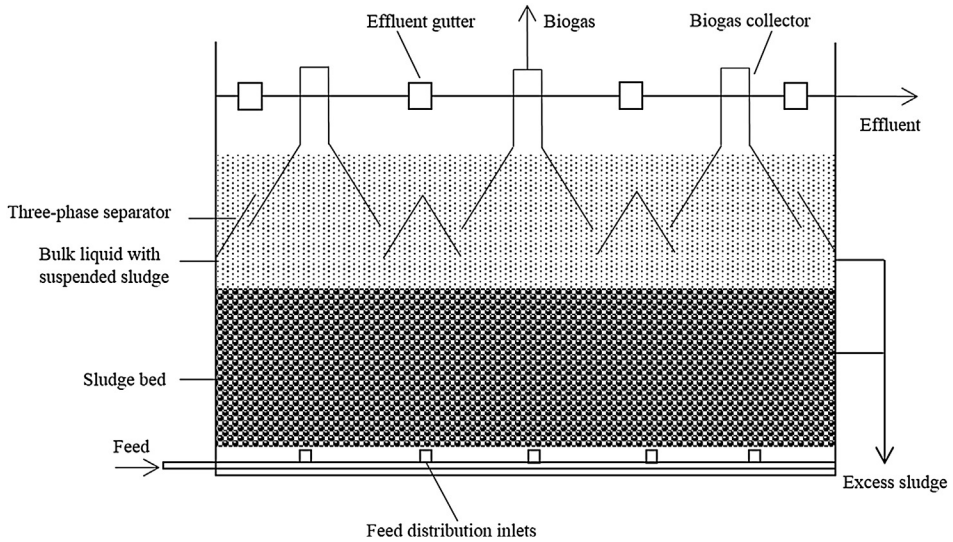

FIGURE 12.6 Schematic representation of an upflow anaerobic sludge blanket reactor.

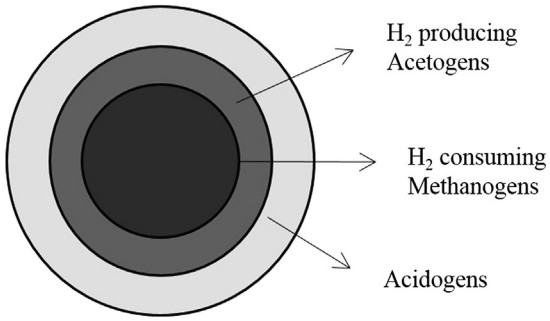

FIGURE 12.7 Agglomeration and grouping of anaerobic bacteria in granules.

Although it was originally developed for medium- and high-strength wastewaters, the UASB showed promising results for the treatment of low-strength wastewaters [6,29–31]. For instance, Kato et al. studied the treatment of low-strength wastewaters (COD ranging from 100 to 2000 mg/L) containing ethanol or whey at 30°C and reported that the treatment efficiency decreased with a decrease in influent COD concentrations. To obtain treatment efficiencies in excess of 85%, the influent COD concentration should be greater than 422 and 630 mg/L for ethanol and whey substrates, respectively [6]. In another study, it was reported that, for a low-strength synthetic wastewater (500 mg COD/L), a UASB can achieve a COD removal of 90–92% at an HRT of 3 h and at temperatures of 20–35°C [32].

Studies were also carried out to investigate the effects of temperature, HRT, and the addition of polymer carriers/additives on the performance of a UASB. By using natural ionic polymer additives, Tiwari et al. studied enhanced granulation in a lab-scale UASB reactor for the treatment of low-strength wastewater (750–850 mg COD/L) at an organic loading rate (OLR) of 1.477 ± 0.118 kg COD/m^3 day. It was found that sludge granulation was enhanced in the presence of additives and that cationic polymer additives showed better performance than anionic polymer additives, although 95–98% COD removal efficiency was achieved in all the reactors with no correlation between enhanced granulation and COD removal efficiency [33]. Another study reported that biomass granulation and higher COD removal efficiency can be achieved with OLR >1.0 kg COD/m^3 day with an inoculum of mixed liquor suspended solids (MLSS) >110 g/L and mixed liquor volatile suspended solids/MLSS ratio <0.3. With OLR <1.0 kg COD/m^3 day, the COD removal efficiency was significantly reduced, even with the same inoculum [34].

Khanh et al. investigated the effects of temperature on low-strength wastewater treatment by UASB reactor using a poly(vinyl alcohol) gel carrier and showed that COD removal was reduced by 50% when the temperature was decreased from 35 to 25°C [35]. The average temperature coefficient was 1.07. Similarly, Rizvi et al. examined the effects of temperature and HRT on the performance of a UASB and found that COD and TSS removal declined with decrease in HRT and temperature [36].

The feasibility of an expanded granular sludge bed (EGSB) reactor, a modification of the UASB with expanded sludge bed and intensified mixing, for low-strength wastewaters was also investigated. Although high treatment efficiency may be achieved in EGSB reactors, owing to the enhanced wastewater–biomass contact, sludge washout and piston flotation at higher OLRs were seen as limitations of the system [37]. Further research was carried out on the treatment of low-strength wastewaters in EGSB reactors [38,39], combined EGSB–UASB reactors [40], membrane-coupled EGSB reactors [41], and EGSB coupled with zeolite bed filtration [42]. Key findings from these studies indicated that higher COD removal efficiency may be achieved by increasing wastewater circulation, thereby enhancing the wastewater–biomass contact [42]; combination of EGSB and UASB resulted in up to 75% energy consumption savings compared to the traditional activated sludge process and offered the advantage of biogas recovery [40]; and the membrane-coupled EGSB reactor showed high feasibility for low-strength wastewater treatment with 85–96% COD removal at temperatures above 15°C [41].

The concentration of the sludge in the reactor acts as a limiting factor for the removal of organic matter in the UASB. Higher sludge concentrations may result in increased escape of sludge from the reactor, thereby reducing the maximum achievable sludge concentration. Additionally, granulation, an important step in UASB operation, can be enhanced when feed water contains high carbohydrates and VFAs, whereas protein and/or FOG-rich wastewaters might lead to fluffy flocs and foaming in the bioreactor.

There have been full-scale UASB plant installations in several cities over the world serving different population sizes [9]. With lower total plant construction costs, lower sludge production, and lower energy consumption compared to conventional activated sludge treatment plants, the UASB has become a highly attractive treatment process for municipal wastewater treatment. With proper design for the capture and utilization of biogas for energy generation, the UASB could serve as an energy self-sustaining treatment process.

12.2.1.3 Anaerobic Sequencing Batch Reactor

The ASBR is a high-rate anaerobic treatment process developed in the early 1990s [43]. The typical operation of an ASBR consists of four steps (Fig. 12.8): (1) a feed step in which the wastewater is fed into the reactor, (2) a reaction step in which the removal of organic matter occurs, (3) a settle step in which the biomass is separated from the water, and (4) a decant step in which the treated wastewater is withdrawn with the biomass retained in the reactor. In the ASBR process, the reactor geometry, the hydraulic mixing pattern and intensity, and the MLSS concentration, as well as the operation temperature, HRT, and OLR, were all hypothesized to affect the treatment performance [44].

Early studies in the ASBR process were carried out on the treatment of swine wastewaters, landfill leachate, and starch wastewaters [45]. Good bio-flocculation, efficient solids separation, long solid retention times (SRTs), and efficient conversion of organic substrates to methane and carbon dioxide were noted as advantages for ASBR. For low-strength wastewaters (400–1000 mg COD/L), ASBR processes were studied at

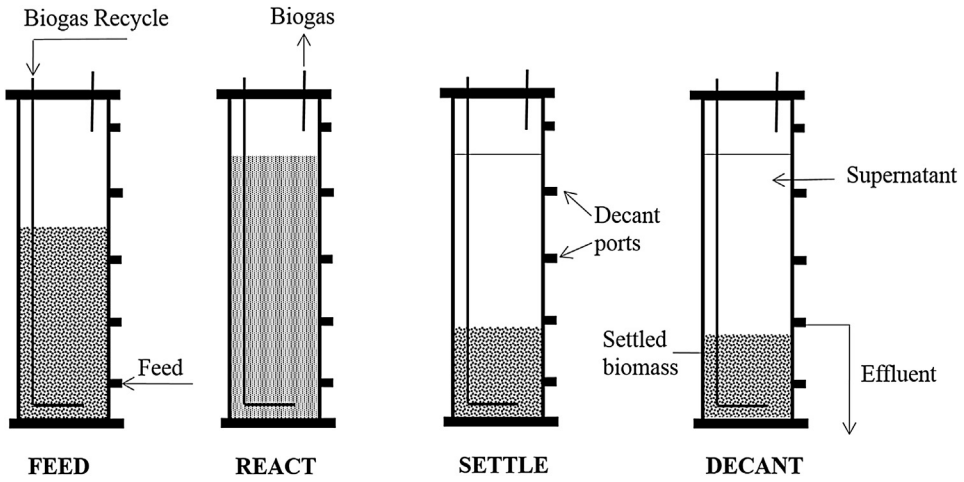

FIGURE 12.8 Operating steps for the anaerobic sequencing batch reactor.

various temperatures (35, 25, 20, and 15°C) and HRTs (12, 16, 24, and 48 h). Even at the lowest temperature (15°C) and at the shortest HRT (12 h), the ASBR was reported to achieve over 80% total COD removal [10,46].

In a study on the influence of agitation rate on the performance of an ASBR for low-strength wastewater (500 mg COD/L) treatment, a kinetic model was developed to determine the optimal mixing regime for ASBR. Maximum removal efficiency, along with relatively good granulation, was achieved at an optimal agitation rate of 50 rpm. Lower agitation rates produced insufficient mixing and very high agitation rate resulted in a negative impact on the granulation of the biomass [47]. Experiments were also conducted on ASBRs with immobilized biomass. The effect of solid-phase mass transfer on the overall performance of the reactor was investigated by varying the size of the carriers from 0.5 to 3.0 cm [48]. It was found that mass transfer was the rate-limiting step for larger carriers (3.0 cm) and influenced the overall reaction. Although the minimum residual substrate concentration increased with the increase in granule size, the overall treatment efficiency was not affected. A two-phase anaerobic system with two ASBRs, hydrolytic–acidogenic and methanogenic reactors in series, was developed for the treatment of low-strength wastewater (approximately 500 mg COD/L) with a high fraction of particulate organic matter (66–68%) (Fig. 12.9). It was reported that the separation of two groups of microorganisms involved in anaerobic digestion improved the overall performance of the process [49].

In another study, an ASBR was used for the treatment of sulfate-rich wastewater with butanol as the carbon source and mineral coal as carrier [50]. Sulfate removal efficiency reached 99% at lower sulfate concentrations (0.25–1.0 g SO_4^{2-}/L) and 71–95% at higher sulfate concentrations (2.0–3.0 g SO_4^{2-}/L). The results demonstrated the feasibility of biological treatment of sulfate-rich wastewaters as a potential application for ASBR.

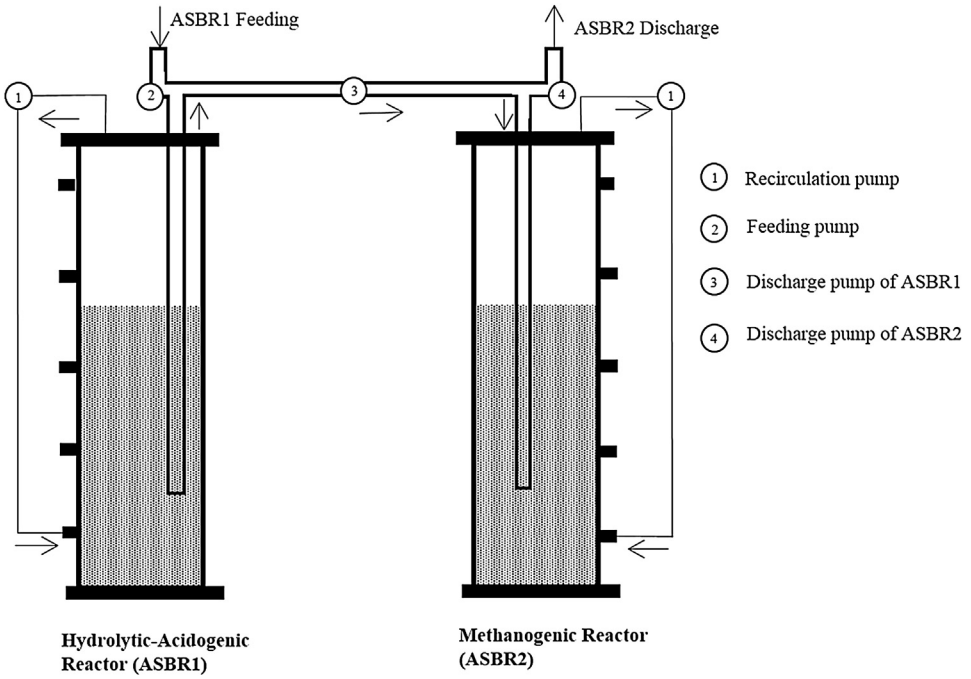

FIGURE 12.9 Two-phase anaerobic sequencing batch reactor (ASBR) system for the treatment of low-strength wastewater with a high fraction of particulate organic matter. *Adapted from A. Donoso-Bravo, G. Ruiz-Filippi, R. Chamy, Anaerobic treatment of low-strength wastewater with a high fraction of particulate matter in an unconventional two-phase ASBRs system, Biochemical Engineering Journal 43 (3) (2009) 297–302.*

Other studies on the use of ASBR for low-strength wastewaters include long-term operation of ASBR at a pilot scale [51] and investigation of the effects of temperature, biomass concentration, and HRT on the performance of the ASBR [46,52]. These studies showed a decrease in COD removal with decreasing temperature, biomass concentration, and HRT. In a review by Zaiat et al. it was suggested that for ASBR to be feasible in industrial applications, optimization of operating parameters and the use of self-immobilized biomass and inert support for biomass immobilization are necessary [53].

Compared to UASB, ASBR does not require feed distribution, nor gas–solid–liquid separation or upflow hydraulic pattern, and is therefore a much simpler design [54]. Kennedy and Lentz found that the performances of UASB reactors and ASBRs were very similar at low and intermediate OLRs. At high OLRs, however, UASB outperformed ASBR in terms of COD removal, biogas production, and VFA accumulation [55].

12.2.2 Attached-Growth Anaerobic Processes

Attached-growth processes are processes in which microorganisms responsible for treatment grow in the form of a biofilm by attaching to some inert medium (such as rocks, ceramic, or plastic fillings). Substrate removal and electron transfer occur within

the biofilm and the overall removal rates are limited by diffusion. Anaerobic biofilm reactors are classified as upflow and downflow reactors, depending on the flow direction of the wastewater [56]. This section discusses two types of anaerobic biofilm processes: anaerobic filters (AFs) (which are packed bed reactors and water flows through the fixed packing) and fluidized-bed reactors (in which fluidization and mixing of the packing material occur).

12.2.2.1 Anaerobic Filters

An AF is the anaerobic equivalent of a trickling filter but operates under flooded conditions where there is no contact of air with the biomass, and the microorganisms in the reactor are strictly anaerobic. The concept of AF was first investigated in 1957 for sewage treatment [57]. The process was largely forgotten until its successful demonstration by McCarty et al., in 1969, for the treatment of protein–carbohydrate wastewater (1500–6000 mg COD/L) at 25°C at OLR of 0.96–3.40 kg/m^3/day. The reactor was constructed with feed wastewater flowing through a column packed with support material for the growth of biofilm. The process showed efficient treatment of wastewater with minimal sludge production [16]. In the subsequent years, more research was conducted on AFs for the treatment of medium- and high-strength wastewaters, in addition to low-strength wastewaters [7]. Fig. 12.10 shows the schematic diagrams of various types of AF reported in the literature depending on feeding mode.

With an HRT of 24 h, Kobayashi et al. studied the treatment of low-strength wastewater (COD average 288 mg/L) using AFs at three temperatures (20, 25, and 35°C). An average COD removal of 73% was achieved with no statistical difference observed at 25 and 35°C [58]. The effects of temperature and HRT on the treatment efficiency of AF were also investigated over the long term (320 days), with temperatures and HRTs ranging from 5 to 30°C and from 7.5 to 30 h, respectively. Treatment efficiency was

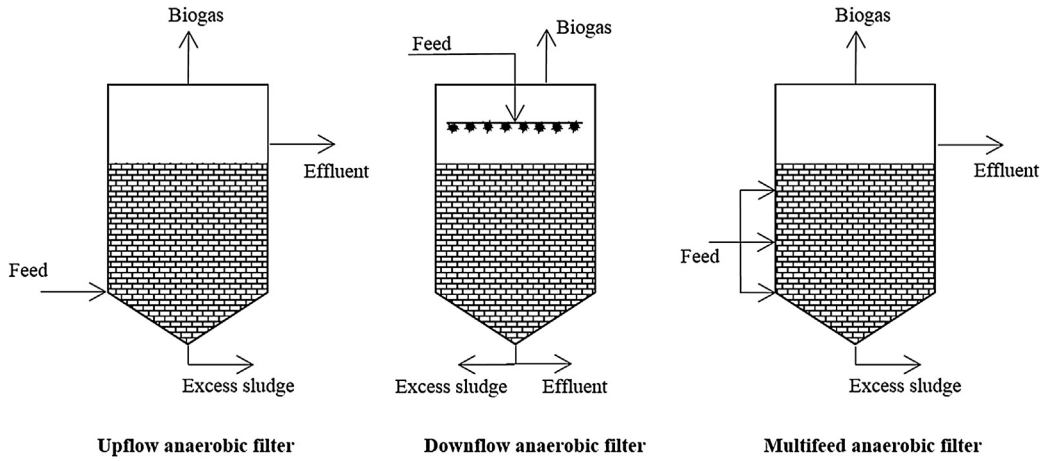

FIGURE 12.10 Schematic diagrams of various types of anaerobic filters depending on the feeding characteristics.

shown to be dependent on both HRT and temperature (i.e., increasing with increased temperature at constant HRT or with extended HRT at constant temperature). A first-order kinetic model for estimating the treatment efficiency based on the experimental results was also developed [59].

Reyes et al. used a multistage AF packed with waste tire rubber for low-strength wastewater treatment and showed that short HRTs are sufficient to achieve high removal efficiency. With three-stage AF, >60% BOD removal was achieved with HRT as low as 8 h [60]. Bodik et al. investigated the use of an upflow AF for low-strength wastewater (100–250 mg COD/L) treatment at various temperatures (9–23°C) and HRTs (4–46 h). A 46–92% COD removal was achieved depending on the temperature and HRT [61]. In other studies, at a low temperature (13°C), up to 71% total COD removal was achieved, with 60% of the removed COD continuously converted to methane [62,63].

In a study examining the effects of HRT, temperature, and effluent recycling on the treatment efficiency of AF for the treatment of rural domestic sewage (average COD of 209.69 mg/L), it was reported that gas production dropped significantly with decrease in HRT and temperature. The highest COD removal and gas production were obtained at an HRT of 3 days, with an effluent recycle ratio of 2:1 and operation temperature of 30°C [64].

The main disadvantages of AF include the need for carriers to support the attached growth, long start-up time, low reduction of pathogens, and high risk of clogging. The cost of the carriers may be reduced by using natural materials such as rocks instead of synthetic plastic media. Pretreatment of the wastewater to remove suspended solids and frequent washing of the filters is necessary to prevent and minimize clogging. With its low operating costs and long service life, AF is used as the main treatment unit in some small- and medium-sized municipalities and for polishing applications in large treatment plants [9].

12.2.2.2 Anaerobic Fluidized-Bed Bioreactor

The application of fluidized-bed reactors for anaerobic wastewater treatment dates back to the 1970s [65]. The use of anaerobic fluidized-bed bioreactor (AFBRs) for low-strength wastewaters was initially introduced as an attached-growth expanded-bed bioreactor (EBR) (Fig. 12.11) [66]. The system relies on biomass attachment to the carriers as bio-agglomerations [67]. The agglomeration bed is expanded under high upflow velocity of the feed with effluent recirculation. The EBR process has been reported to be highly efficient in the treatment of low-strength wastewater (COD $\leq$600 mg/L) at short HRTs (less than 6 h), low temperatures (10 and 20°C), and high OLRs (up to 8 kg COD/m^3 day). Subsequent studies supported these findings in the treatment of domestic and food-manufacturing wastewaters [68–70].

The configuration of an AFBR (Fig. 12.12) is similar to that of an EBR but with an extremely high upflow velocity (10–25 m/h) and a bed expansion of 25–300% of the settled bed volume, compared to 15–30% bed expansion in an EBR. This minimizes clogging and short-circuiting and, at the same time, maintains high biomass

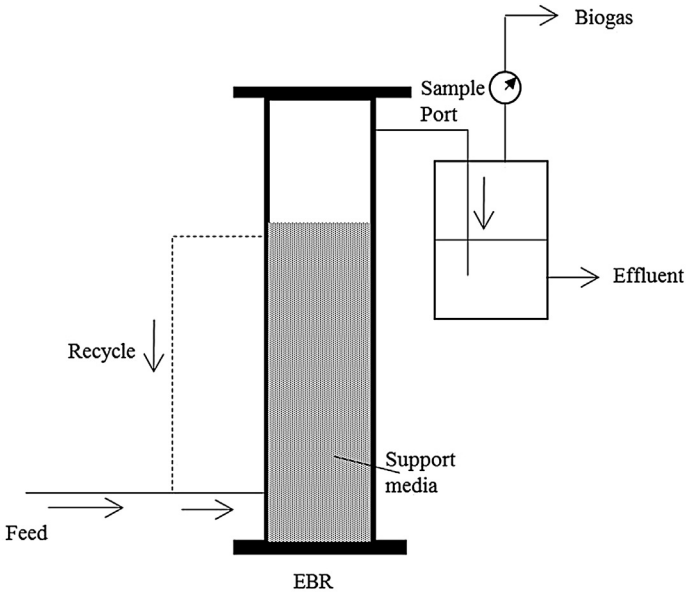

FIGURE 12.11 Schematic diagram of the first expanded bed bioreactor installation. *Adapted from M.S. Switzenbaum, W.J. Jewell, Anaerobic attached-film expanded-bed reactor treatment. Journal (Water Pollution Control Federation) (1980) 1953–1965.*

concentrations and long SRTs. At such high upflow velocities, the biocarriers are supported entirely by the upflow liquid velocity and thus have the ability to move freely in the reactor, in contrast with the EBR, in which the granules are supported partly by fluid flow and partly by contact with adjacent granules [26]. Shin et al. studied the effect of the influent dissolved oxygen (DO)/COD ratio on the performance of an AFBR and reported that influent DO adversely affected the performance of the reactor, with inhibition in methanogenic activities. The study highlighted the necessity for influent DO control for a successful application of AFBR in low-strength wastewater treatment [17]. Studies on the treatment of textile wastewater using AFBR suggested that there was a need for the addition of glucose as an external carbon source for color removal, which might be a concern in practical applications [71,72].

AFBR has the advantages of improved mixing, which prevents short-circuiting and the formation of dead zones inside the reactor, thus resulting in reduced space requirements. However, long start-up time, high pumping costs used to maintain the fluidized bed, and the need of a biocarrier medium are the limitations of AFBR.

12.2.3 Anaerobic Membrane Processes

12.2.3.1 Anaerobic Membrane Bioreactor

In recent years, the anaerobic membrane bioreactor (AnMBR), which combines anaerobic treatment and membrane separation, has sparked considerable interest

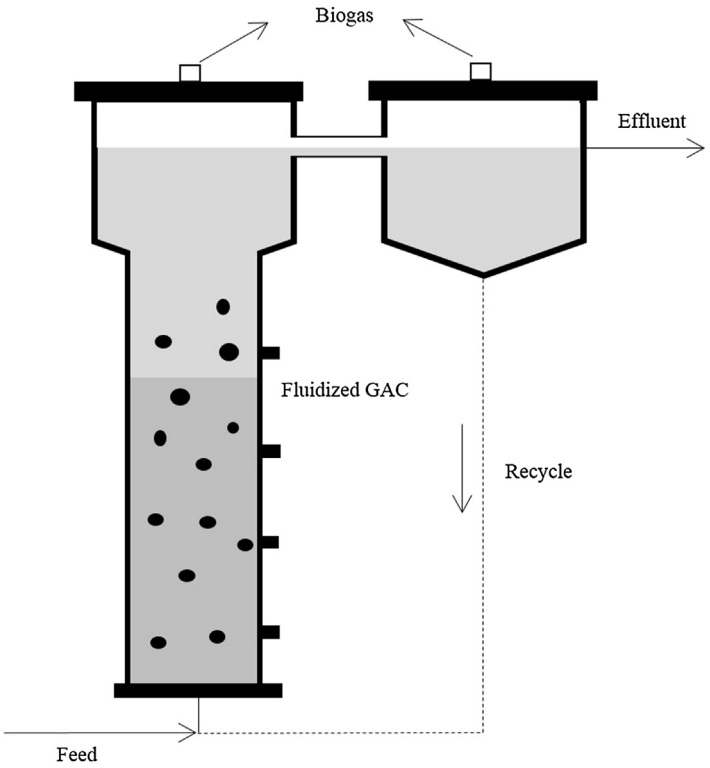

FIGURE 12.12 Schematic diagram of an anaerobic fluidized-bed bioreactor with fluidized granular activated carbon (GAC) as microbe supports.

in the wastewater research and industry communities. Since its introduction, there has been intensive research in the process development and commercial installations of AnMBR [73,74]. Of >250 peer-reviewed AnMBR research papers, 100 were published between 2007 and 2013 [75]. The advantages of AnMBR over conventional anaerobic processes in terms of compact process configuration, low sludge production, and better effluent quality make it an appealing alternative for wastewater treatment.

Several review papers on AnMBRs have been published focusing on their applications for different types of wastewater [74,76], including industrial wastewater [77] and municipal wastewater [78,79]; on operational parameters [80]; and on the membrane fouling issues [75].

The basic configurations of an AnMBR are shown in Fig. 12.13. The performance of an AnMBR may be affected by not only the operational conditions (such as shearing intensity, water flux, operation mode, temperature, SRT, HRT) and the physical and chemical characteristics of the sludge and the membranes, but also the configurations of the membrane modules [79].

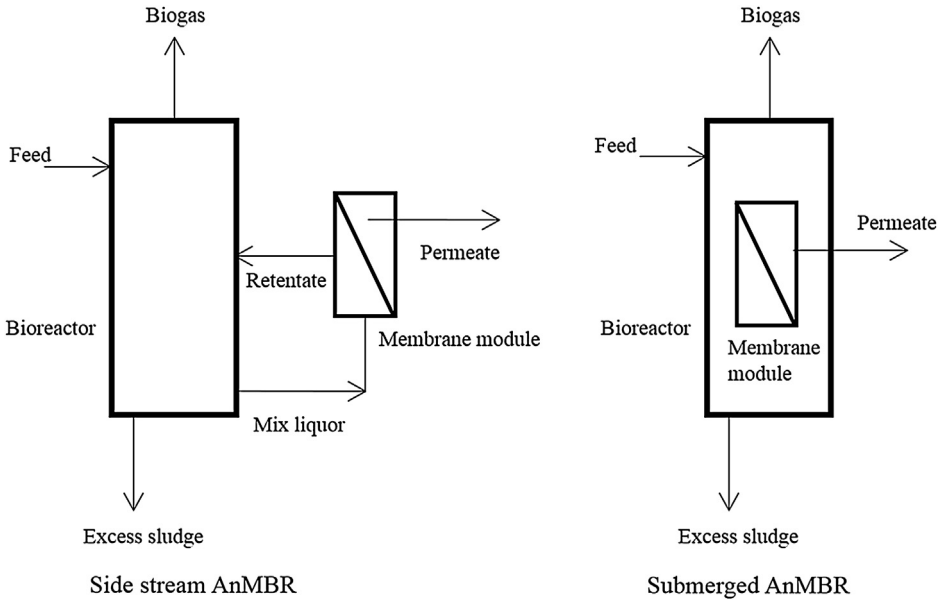

FIGURE 12.13 Schematic diagram of sidestream and submerged anaerobic membrane bioreactor (AnMBR) configurations.

The possibility of integrating various anaerobic bioreactors including UASB, continuous stirred tank reactor (CSTR), EGSB, and fluidized-bed bioreactor (FBR) with membrane separation as a discharge unit for enhanced and optimal process performance has been investigated [79]. A 2015 study on the use of a forward-osmosis membrane in a submerged AnMBR for low-strength wastewater treatment showed >96% removal of organic carbon, nearly 100% of total phosphorus, and 62% of ammonia nitrogen, which suggests a higher removal efficiency than conventional AnMBRs [81].

Compared to the other anaerobic processes, AnMBRs offer many advantages including total biomass retention, better effluent quality, lower sludge production, and a smaller footprint. However, low flux, membrane fouling, and high capital and operational costs limit its extensive use [75]. Further work on the development of low-cost membranes with minimal fouling and optimization of the process configuration and operation conditions, as well as investigation on the combination of AnMBR with other anaerobic processes, is needed for AnMBR to be an economically advantageous alternative for wastewater treatment.

12.2.3.2 Anaerobic Fluidized-Bed Membrane Bioreactor

The anaerobic fluidized-bed membrane bioreactor (AFMBR) is a relatively new process that combines an AFBR with an internally submerged membrane (Fig. 12.14). In the two-stage system, the AFMBR treats the effluent from the AFBR with granular activated carbon (GAC) as carrier in both reactors. Membranes are placed directly in the AFMBR.

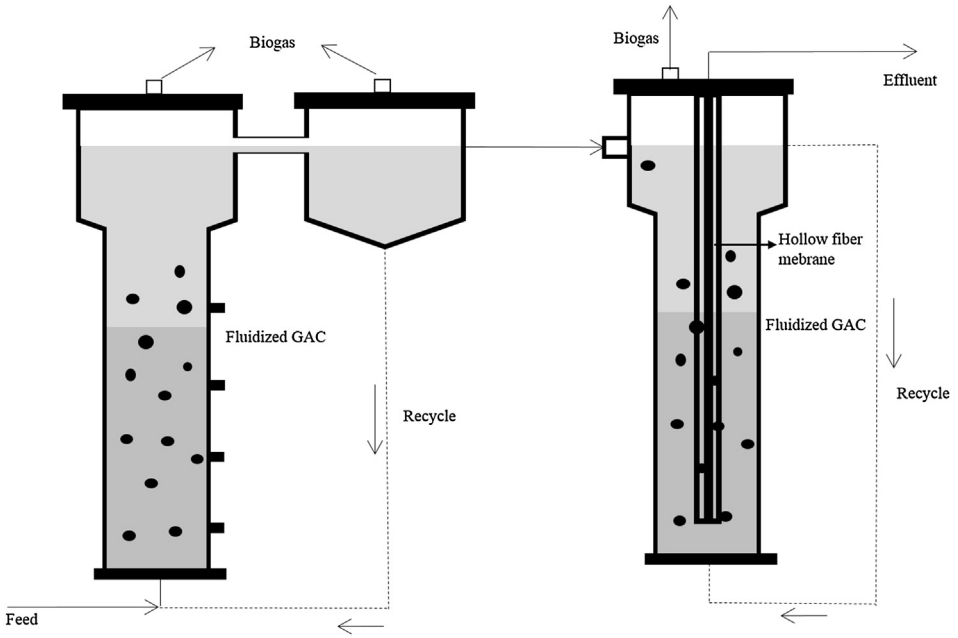

FIGURE 12.14 Schematic diagram of two-stage anaerobic fluidized-bed membrane bioreactor. *GAC*, granular activated carbon.

When the system was fed with synthetic influent wastewater (513 mg COD/L) and operated at 35°C with 2.0- to 2.5-h HRT for the AFBR and 2.2-h HRT for the AFMBR, an overall COD removal of 99% was achieved with minimal fouling due to the scouring effects of the fluidized GAC on the membrane surface [82].

A similar concept was used in the development of an integrated anaerobic fluidized-membrane bioreactor (IAFMBR) with GAC carrier. The design consists of a reactor with an outer loop that performs as an AFBR and an inner loop that serves as an AFMBR. The performance of the IAFMBR was investigated using synthetic wastewater (300 mg COD/L) at various HRTs (8, 6, and 4 h), and 75.8%, 73.6%, and 54.1% COD removal was achieved, with the conversion of 45.2%, 53.1%, and 43.8% of COD to methane. It was also found that transmembrane pressure (TMP) increased more rapidly at shorter HRT when the GAC dosage was kept constant [83]. The effects of temperature on the performance of the IAFMBR process were also investigated. Decreased removal efficiencies with accelerated membrane biofouling were observed with a decrease in temperature [84]. Bae et al. compared the performance of an IAFMBR with a two-stage AFMBR for the treatment of low-strength wastewater (200 mg COD/L). Similar COD removal (93–96%) was achieved in both systems at total HRTs of 2.2–3.3 h. Both reactors exhibited similar TMP decrease, bulk liquid suspended solids, and extracellular polymeric substances (EPS) and soluble microbial products (SMP) concentrations, suggesting that IAFMBR may be a promising alternative to the two-stage AFMBR system [85]. In a pilot-scale test of a two-stage AFMBR

for the treatment of domestic wastewater (average influent concentration of 424 mg COD/L), COD removal of 94% and 90% during summer and winter was reported, respectively, with the average effluent COD consistently less than 23 mg/L. It was also found that the energy required for the system operations was drawn from the primary and secondary methane production and could be further reduced through hydraulic modifications [86].

Although anaerobic membrane processes are capable of achieving high COD removal and biomass retention, their application in wastewater treatment is limited by membrane fouling and scaling. An AFMBR with GAC carriers was shown to be effective in preventing membrane fouling as well as in achieving high biomass retention and organic removal. However, increased capital and operational costs resulting from GAC addition and reactor fluidization would need to be considered.

12.2.4 Other High-Rate Anaerobic Treatment Processes

Apart from the commonly used high-rate anaerobic reactors discussed above, there are several other processes/reactor configurations conceived for the treatment of low-strength wastewater. The designs of these novel processes/reactors and their treatment performance, advantages, and limitations are discussed in this section.

12.2.4.1 Anaerobic Baffled Reactors

Anaerobic baffled reactors (ABRs) typically consist of a series of vertical baffles with upflow and downflow chambers through which wastewater is forced to flow to achieve phase separation and enhanced treatment (Fig. 12.15). To a certain extent, ABR may be described as a series of UASB reactors [87]. First developed for the treatment of medium-strength wastewaters [27], there are only a few reports on ABR for low-strength

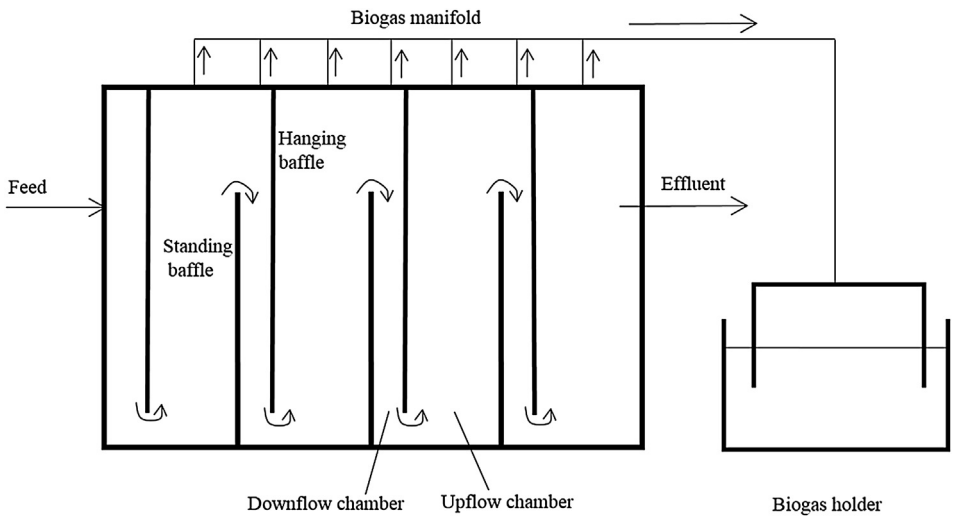

FIGURE 12.15 Schematic diagram of an anaerobic baffled reactor.

wastewater treatment [88]. Although the use of ABRs is limited by the lack of clear and unified design guidelines and long process start-up time, it offers advantages in achieving good solids retention, low bed bypass, and stable reactor performance, especially in its ability to compartmentalize acidogenesis and methanogenesis along the reactor chambers, thereby providing the most favorable growth conditions for different groups of microorganisms [89,90]. These advantages of ABR can be exploited for wastewater treatment under extreme environmental conditions such as severe hydraulic and organic shock loads, intermittent feeding, and temperature changes and for the treatment of refractory wastewaters.

12.2.4.2 Anaerobic Migrating Blanket Reactor

The anaerobic migrating blanket reactor (AMBR) was developed as a compartmentalized, continuously fed, staged reactor in which the wastewater flow is periodically reversed on a regular basis [91]. Advantages offered by AMBRs include smaller biomass migration rates and minimized short-circuiting. The feasibility of AMBR for low-strength wastewaters (600 mg/L COD) at low temperatures (15°C) was examined with total COD removal of 59% at 4-h HRT. It was also observed that effective granular retention promoted the organic removal over the period of operation [92]. The AMBR is an attractive option for the treatment of low-strength domestic and industrial wastewater at low temperature because of its high biomass retention and process stability under fluctuating influent flow.

12.2.4.3 Microbial Fuel Cells

Microbial fuel cells (MFCs) are a relatively new and leading edge technology, which uses anaerobic oxidation for the conversion of chemical energy in biodegradable organic compounds into electricity [93]. A typical MFC for energy recovery from wastewater consists of an anodic chamber for the oxidation of the organic matter and a gas-porous air cathode for the reduction of atmospheric oxygen [94]. Electricity production from domestic wastewater through MFCs has sparked broad interest, with many studies reporting on the development of this process [95,96]. However, scale-up tests have identified many bottlenecks and limitations in the process, including low COD removal and electricity conversion [97]. It has been suggested that for the successful implementation of MFCs for medium- to low-strength wastewater treatment, a clear understanding of the fluid dynamics within the anodic chamber and the adaptation of microbial communities during the start-up is important.

12.2.5 Hybrid Processes

Whereas many of the high-rate anaerobic reactors have proven to be efficient in wastewater treatment and energy recovery, stand-alone anaerobic systems are often insufficient to meet the effluent discharge standards. This highlights the need for hybrid systems, which combine two anaerobic processes or couple anaerobic–aerobic

processes. Some of the combined systems such as the membrane-coupled EGSB, UASB, CSTR, and FBR were mentioned earlier in the chapter. This section discusses research that focuses on other hybrid installations for the treatment of low-strength wastewater.

12.2.5.1 Two-Stage Microbial Fuel Cell—Anaerobic Fluidized-Bed Membrane Bioreactor

A novel two-stage lab-scale process combining MFC and AFMBR was developed to overcome the inadequate effluent quality of MFCs [98]. With low-strength wastewater (210 ± 11 mg/L COD) as influent, an overall COD removal of 92.5% was achieved with the production of 0.0197 kWh/m^3 electrical energy at room temperature (25°C). The electricity output was marginally more than the amount of electrical energy required for the system operation. This result suggested that MFC—AFMBR may be effectively used to treat domestic wastewater with high effluent quality and low-energy requirements.

12.2.5.2 Anammox Coupled With Various Anaerobic Reactors

Anammox (anaerobic ammonium oxidation) is a microbiologically mediated, energy-efficient alternate nitrogen removal process [99]. The bacteria responsible for anammox oxidize ammonium under anoxic conditions, with nitrite as electron acceptor, to produce nitrogen gas [100]. The application of anammox in low-strength wastewater treatment has been studied in many high-rate anaerobic reactors such as UASB [101], AF [102], and ASBR [103]. As an autotrophic nitrogen removal process, anammox is particularly suitable for the treatment of wastewaters with high ammonium concentration and low organic content. The low growth rate of anammox bacteria results in low biomass yield and less sludge production. This, however, also renders the retention and enrichment of anammox bacteria key to the successful implementation of the process. Additionally, there is a need for further studies to extend the application of anammox under ambient temperature and for wastewaters with relatively lower ammonium concentrations [102].

12.2.5.3 Membrane Distillation and Anaerobic Moving-Bed Biofilm Reactor

The feasibility of using membrane distillation (MD) for the post-treatment of effluent from an anaerobic moving-bed biofilm reactor (AMBBR) process has been evaluated [104]. The AMBBR effluent was distilled using a polyvinylidene difluoride membrane with a transmembrane temperature of 20°C. It was suggested that the biogas obtained from the AMBBR process could be converted into heat energy, which in turn could be used as the driving force for the MD process. Although this combination (AMBBR—MD) was shown to be an energy-efficient process, the effluent leaving the system requires ammonia stripping as a post-treatment to comply with the total nitrogen discharge limits.

12.2.6 Coupled Anaerobic—Aerobic Systems

Despite the advantages of anaerobic treatment processes in terms of cost-effectiveness and energy recovery, effluent quality in most of the anaerobic processes for low-strength

wastewaters do not meet discharge standards. On the other hand, aerobic treatment systems, which perform well in terms of higher removal efficiency and better process stability, unfortunately impose financial constraints and are less energy efficient. Sequential anaerobic–aerobic systems can serve as a viable alternative by exploiting the advantages of both systems in a more cost-effective way [105]. Such systems show great potential for energy recovery in the anaerobic pretreatment step as well as high overall treatment efficiency due to the use of the aerobic post-treatment step [106]. Integration of the anaerobic–aerobic systems in a single bioreactor has garnered much attention because of its compactness and minimal sludge production. However, there is a lack of large-scale implementation of most of the integrated systems and further research is needed for the evaluation of their performance on larger scales [107].

12.3 Summary and Research Needs

With the escalating energy crisis and concerns about climate change, there is an increasing need for the development of high-performance, self-sustainable wastewater treatment processes. Anaerobic processes, owing to their low energy consumption and capacity for bioenergy recovery, are gaining increased attention in wastewater treatment research and application. The developments in high-rate anaerobic treatment processes, such as UASB, ASBR, ABR, etc., provide more efficient alternatives for anaerobic wastewater treatment. However, applications in low-strength wastewaters are still limited, mainly because of the low influent substrate concentration and the resultant reduced system efficiency and biogas production, which pose problems needing further investigation/developments. The emerging AnMBR shows better effluent quality, low sludge production, and a smaller footprint compared to other anaerobic processes, thus making it a promising alternative for low-strength wastewater treatment. Nonetheless, membrane fouling is a major problem, which needs to be solved for real applications. Microbial electrochemical technologies such as MFCs, which accomplish direct biological conversion of chemical energy in organics into electricity, may provide promising alternatives in making wastewater treatment a net energy producer. However, at the current stage, the high system cost and low energy conversion efficiency, especially when working with low-organic wastewaters, still hinder its applications. More research is needed regarding its system efficiency, scalability, system lifetimes, and reliability.

Anaerobic processes are promising alternatives to aerobic treatment. However, as they are not capable of complete wastewater treatment, especially in the removal of nutrients, anaerobic processes can rarely be used as stand-alone treatments. A reasonable positioning of anaerobic processes in wastewater treatment should be as a pretreatment measure to accomplish maximal preliminary conversion/removal of pollutants with minimal energy inputs and to recover the bioenergy contained in the wastewaters as far as possible. Their combinations with minor aerobic treatment are still necessary to meet a final discharge standard, with minimized energy input for aerobic

treatment necessary to further polish the anaerobically treated wastewaters. Together with the development/implementation of suitable nutrient recovery processes for additional resource recovery, all of these would make sustainable and self-sufficient wastewater treatment a feasible goal to be achieved.

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13

High-Strength Wastewater Treatment Using Anaerobic Processes

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13.1 Introduction

Anaerobic digestion is well used for treating high-strength organic matter including wastewaters. This technology is via a multistep process involving a number of serial and parallel reactions. This process is carried out by various microorganisms mainly in three reaction stages: hydrolysis, acidogenesis, and methanogenesis (Fig. 13.1). In the hydrolysis stage, complex organic matter is hydrolyzed into monomers such as glucose and amino acids by the action of extracellular enzymes produced by hydrolytic bacteria. In the acidogenesis stage, the hydrolyzed monomers are converted into volatile fatty acids (VFAs) and alcohols. The higher VFAs, such as propionic and butyric acids, are further converted into acetic acid and H_2/CO_2 by acetogenic bacteria. Finally, acetic acid and H_2/CO_2 are converted into methane by methanogens [64,65]. From the viewpoint of utilizing gaseous and liquid metabolite products for various purposes, dark "fermentation" is well used to describe this anaerobic process, including hydrogen fermentation, methane fermentation, and ethanol fermentation.

Both hydrogen and methane are main biogas biofuels and they are mainly used in fuel cells and internal combustion engines, respectively, for electricity generation. Methane production is a well-used technology for a century in anaerobically digesting sewage sludge, wastewater sludge, agricultural wastes, and animal manures. Two-stage ($H_2 + CH_4$) production has been shown to have 8–43% higher energy recovery than one-stage (CH_4) production [114]. However, fermentative biohydrogen production research has a history of only about 1.5 decades as regards high-strength wastewaters. Therefore, this chapter focuses on the anaerobic treatment of high-strength wastewaters for biohydrogen production.

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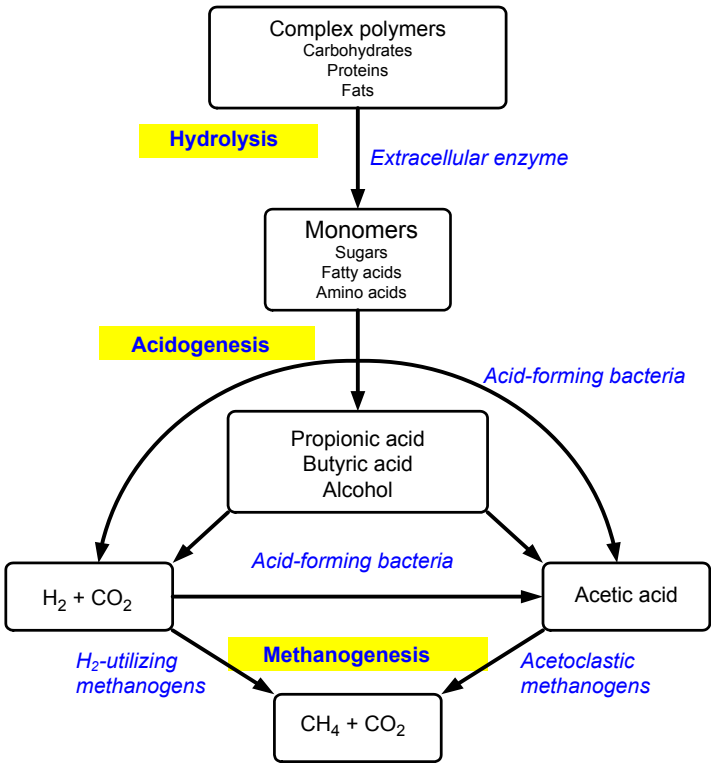

FIGURE 13.1 Schematic of the anaerobic digestion process [65].

It is clear from Fig. 13.1 that to maximize the production of hydrogen biogas, the final step of anaerobic digestion, i.e., the methanogenesis, must be blocked. The bioactivity of methanogens in the digester has to be suppressed. This could be achieved by a heat treatment on seed sludges because methanogens are sensitive to heat, whereas hydrogen-producing bacteria are not [8,45,94]. Other methods to inhibit methanogenesis in a digester include (1) operating at short hydraulic retention time (HRT) to wash out the slow-growing methanogens and (2) operating at an acidic environment to inhibit pH-sensitive methanogens [7,57].

The main reaction stoichiometries of the anaerobic degradation of glucose during acidogenesis are summarized in Table 13.1. The theoretical maximum H_2 yield of complete oxidation of 1 mol glucose is 12 mol H_2, but it is not a thermodynamically favorable reaction under standard conditions. However, the theoretical maximum H_2 yield should be 4 mol H_2 per mole glucose associating with acetate as the single metabolic product of anaerobic digestion.

Nowadays, about 90% of hydrogen is produced by thermochemical and electrochemical methods, such as steam re-forming and electrolysis of water [15]. A large number of microorganisms, including significantly different taxonomic and physiological types, can produce molecular hydrogen from various feedstocks. Biological

Table 13.1 Reaction Stoichiometries of the Anaerobic Degradation of Glucose During Acidogenesis [40]

Reaction	Stoichiometry	ΔG (kJ/reaction)
Complete oxidation of glucose	$C_6H_{12}O_6 + 12H_2O \rightarrow 12H_2 + 6HCO_3^- + 6H^+$	+3.2
Acetate production	$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COO^- + 4H_2 + 2HCO_3^- + 4H^+$	−206.3
Butyrate production	$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COO^- + 2H_2 + 2HCO_3^- + 3H^+$	−254.8
Ethanol production	$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2OH + 2HCO_3^- + 2H^+$	−235.0
Acetate and ethanol production	$C_6H_{12}O_6 + 3H_2O \rightarrow CH_3COO^- + CH_3CH_2OH + 2H_2^+$ $2HCO_3^- + 3H^+$	−215.7
Lactate production	$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOO^- + 2H^+$	−198.1
Butanol production	$C_6H_{12}O_6 + H_2O \rightarrow CH_3CH_2CH_2OH + 2HCO_3^- + 2H^+$	−280.5
Propionate production	$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COO^- + 2H_2O + 2H^+$	−359.0
Valerate production	$C_6H_{12}O_6 + H_2 \rightarrow 2CH_3CH_2CH_2CH_2COO^- + HCO_3^- + H_2O + 2H^+$	−330.9
Acetogenesis	$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + H_2O$	−104.6
Acetogenesis	$C_6H_{12}O_6 \rightarrow 3CH_3COO^- + 3H^+$	−310.6
Acetate fermentation to H_2	$CH_3COO^- + 4H_2O \rightarrow 4H_2 + 2HCO_3^- + H^+$	+104.6
Butyrate fermentation to H_2	$CH_3CH_2CH_2COO^- + 10H_2O \rightarrow 10H_2 + 4HCO_3^- + 3H^+$	+257.3

hydrogen production processes can be classified as follows: (1) direct bio-photolysis, (2) indirect bio-photolysis, (3) photofermentation, (4) water–gas shift reaction, (5) dark fermentation, and (6) microbial fuel cell. Each approach has distinct advantages and disadvantages with challenging technical barriers to practical applications. Integration of dark fermentation and photofermentation into two stages is a very efficient technology to effectively convert wastewaters into biohydrogen with a net energy gain and no generation of acids in the effluent.

Various solid wastes and wastewaters rich in organic contents have attracted considerable attention because of advantages such as high organic loading possibilities, low nutrient requirements, and positive net energy gain. Thus the exploration of solid wastes and wastewater as substrates for H_2 production with concurrent wastewater treatment is an attractive and effective way of tapping clean energy from renewable sources in a sustainable approach. This provides dual environmental benefits in the direction of wastewater treatment along with sustainable bioenergy generation.

13.2 Characteristics of High-Strength Wastewaters

Biofuel feedstock has been classified into first-, second-, and third-generation categories. The first-generation biofuel feedstock are traditionally food-related, such as corn for ethanol and vegetable oil and animal fats for biodiesel, with consumption of a lot of heat and increases in food prices. Wastewaters belong to the second-generation biofuel feedstock [91]. Various organic wastewaters have attracted substantial attention in bioenergy production because of advantages such as high organic loading potentials, low nutrient supplies, simultaneous wastewater treatment, and positive net energy production.

13.2.1 Food Industrial Wastewater

The food industry uses large amounts of water for many different purposes including cooling, cleaning, as a raw material, as sanitary water for food processing, transportation, cooking and dissolving, as auxiliary water, etc. In principle, the water used in the food industry may be used as process and cooling water or boiler feed water. Characteristics of the effluent consist of large amounts of suspended solids, nitrogen in several chemical forms, fats and oils, phosphorus, chlorides, and organic matter. Generally, the BOD (biochemical oxygen demand) and COD (chemical oxygen demand) of food industry wastewater are 10 or even 100 times higher than those of domestic wastewater [27].

The treatment of condensed molasses fermentation solubles (CMS), with 320−350 g COD/L, is a troublesome problem for a glutamate manufacturing factory. However, CMS contains high carbohydrate and nutrient contents and is an attractive and commercially potential feedstock for bioenergy production. This molasses wastewater contains various nutrients and other fermentation products such as microbial proteins, amino acids, organic acids, vitamins, and coenzymes [44]. Beverage factory wastewater (BFW) is also a kind of high-strength wastewater. High COD (760−860 g COD/L) and high carbohydrate (610−670 g carbohydrate/L) in BFW are the preferred organic carbon source for anaerobic fermentation microorganisms [42].

13.2.2 Livestock Industrial Wastewater

Livestock are domesticated animals raised in an agricultural setting to produce commodities such as food, fiber, and labor. Livestock wastewater contains high strengths of COD, BOD_5, color, nitrogen, phosphorus, and suspended solids. Many countries have paid attention to the treatment of livestock wastewater [46]. Generally wastewater from large-scale livestock and poultry farms is treated in their own wastewater treatment plants so as to satisfy the corresponding discharge standards. However, the discharge of large quantities of treated water containing low levels of chemical constituents may still cause an excessive input of nutrients in a receiving water body [110]. Several treatment processes are therefore used to treat livestock wastewater. Among these are anaerobic digestion, aerobic digestion (e.g., the autothermal thermophilic aerobic digestion process), anaerobic−aerobic digestion (e.g., anaerobic−aerobic sequencing batch reactor), and aerobic digestion−chemical treatment [46].

The characteristics of livestock wastewater vary highly depending on the amount of water used to clean the stable and the kind of pits used to collect the slurries, animal feeding habits, and zone climatology, as well as the number of animals on the farm; their health state; the feed composition; the means used for cleaning, washing, and disinfection; and the sort of drugs used for animal treatment and prevention of diseases [21]. Table 13.2 shows that the characteristics of livestock wastewaters from cattle and pig farms include high concentrations of soluble and undissolved organics (COD 641−12,570 mg/L, BOD_5 324−9550 mg/L, total suspended solids 115−8828 mg/L, total nitrogen 84−1279 mg/L, total phosphorus 2−380 mg/L, pH 6.0−8.4).

Table 13.2 Characteristics of Livestock Wastewaters

	Source	
Parameter[a]	**Cattle Farm [39,85,74]**	**Pig Farm [21]**
pH	8.2–8.8	6.0–8.4
Chemical oxygen demand	1446–2840	641–12,570
Biochemical oxygen demand	1090–1190	324–9550
Total suspended solids	470–2340	115–8828
Total nitrogen	445–1279	84–1138
Total phosphorus	78–380	2–27

[a]All parameters are in mg/L except pH.

13.2.3 Municipal Wastewater

Municipal wastewater is characterized by low organic strength and high particulate organic matter content [23]. However, it has high aeration costs and generates large amounts of residual sludge after an activated sludge process, which is the most widely used to treat this wastewater. Thus, the main conceptual limitation of an activated sludge process is high biomass yield implying the use of energy (O_2) to transform biodegradable dissolved or suspended organic matters into settleable cell sludge that is often partially converted into biogas using anaerobic digestion [23]. Table 13.3 shows that high concentrations of pollutants in municipal wastewater would be produced after the treatment. The anaerobic process has been applied for reducing the residue sludge in municipal wastewater plants. Moreover, the biogas energy could be recycled after anaerobic digestion and used to heat the digestion by biogas boiler.

13.3 Process Configurations

All process configurations are viable candidates for many wastewaters but none can ensure every advantage. Similarly, no particular bacterium is the panacea for all

Table 13.3 Characteristics of Municipal Wastewater and Its Sludge

	Source	
Parameter[a]	**Municipal Wastewater [9]**	**Municipal Wastewater Sludge [79]**
pH	7.50–8.07	5.5–5.8
Chemical oxygen demand	101–254	41,000–75,000
Total suspended solids	–	19,000–62,000
Total nitrogen	30.6–72.4	1126–1362
Total phosphorus	1.10–5.60	–

[a]All parameters are in mg/L except pH.

metabolism needs. A process cannot be expected to be appropriate in all site locations or for all feedstock; thus the design of a "side-by-side" comparison evaluation of various configurations requires great care [84]. The bioreactor component is of critical importance to the overall success of an anaerobic treatment process. In general, two basic bioreactor configurations are used for achieving anaerobic biomass immobilization and sustaining the desired biochemical reactions: suspended growth and granulation systems.

13.3.1 Reactor Type

13.3.1.1 Suspended-Growth System

The anaerobic biomass is suspended and mixed with the liquid or biogas from the bioreactor using external pumps or propeller mixers or produced biogas to provide a good mixing environment. However, collisions and coalescing of rising biogas bubbles with settling biomass particles in a gravity clarifier would increase the possibility of biomass washout. Consequently, the maximum potential biomass concentration attainable in the bioreactor would be limited. A number of alternatives can be adopted to enhance biomass separation and concentration. Fig. 13.2 shows the typical reactor configurations used for anaerobic suspended-growth digestion systems.

13.3.1.1.1 ANAEROBIC CONTINUOUSLY STIRRED TANK REACTOR

A continuously stirred tank reactor (CSTR) (Fig. 13.2A) digestion system is a common continuous hydrogen production model. This system's complete mixing operation allows intimate contact between the substrate and the biomass microorganisms, as well as effective pH and temperature control. Many reports used CSTR to produce biohydrogen by anaerobic fermentation technology. Azbar et al. [4] reported a high hydrogen yield (HY) (22 mmol H_2/g COD) in a CSTR system fed with cheese-processing wastewater at a pH of 5.5. A high hydrogen production rate (HPR) of 9.5 L/L/day was also obtained at a constant pH of 5.5 in a CSTR system using CMS (40 g COD/L) feedstock [44]. A CSTR also

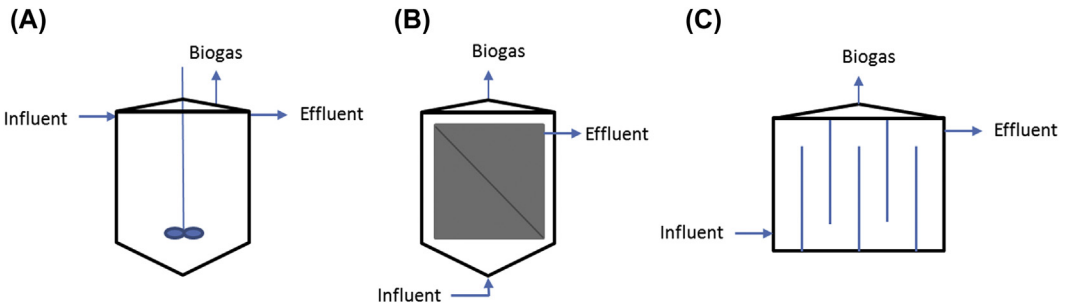

FIGURE 13.2 Typical reactor configurations used for anaerobic suspended-growth digestion systems. (A) Continuously stirred tank reactor. (B) Anaerobic membrane bioreactor. (C) Anaerobic baffled bioreactor.

had been applied in anaerobic hydrogen production from other kinds of organic wastewaters such as coffee drink manufacturing wastewater [36], olive pulp water [41], purified terephthalic acid [113], sugar beet wastewater [32], and sugary wastewater [92].

A modified CSTR named an intermittent CSTR (I-CSTR) was used to enhance hydrogen production. The I-CSTR was developed to decrease washout problems in CSTRs and it was operated with a fill-and-draw process to avoid slurry substrates clogging the tube. The I-CSTR was successfully used to ferment kitchen waste to produce biohydrogen efficiently at mesophilic or thermophilic conditions [48,51,52,100].

13.3.1.1.2 ANAEROBIC MEMBRANE BIOREACTOR

The membrane-controlled anaerobic bioreactor consists of a suspended-growth, completely mixed anaerobic bioreactor that is followed by an ultrafiltration unit with multiple membrane modules. Membrane bioreactor processes have attracted significant scientific and industry attention over the past few decades. The bioreactor content is continuously pumped through membrane modules where a concentrated biomass stream and a clarified effluent stream (permeate) are produced. The concentrated biomass stream is recycled back to the anaerobic bioreactor to maintain a desired biomass holdup as well as to mix the bioreactor content. The permeate flow in excess of the feed rate is recycled to maintain a constant liquid level in the bioreactor [82]. Moreover, excess biomass is wasted directly from the bioreactor. In particular, use of the anaerobic membrane bioreactor (AnMBR) (Fig. 13.2B) has increased significantly because it has the ability to treat concentrated wastewaters and simultaneously produce biogas. The AnMBR was applied to treat pharmaceutical wastewater [69], slaughterhouse wastewater [34], municipal wastewater [101], liquid dairy manure [99], sulfate-rich urban wastewater [78], and so on.

13.3.1.1.3 ANAEROBIC BAFFLED BIOREACTOR

Baffles are installed in an anaerobic baffled bioreactor (ABR) to divide the bioreactor into a series of chambers (Fig. 13.2C). The wastewater flow is directed through these chambers in an upflow mode such that the biomass can accumulate in the chambers similar to the sludge blanket in upflow anaerobic sludge blanket (UASB) bioreactors. Mechanical mixers are sometimes provided in the chambers to keep the biomass in suspension. Because the bioreactor is divided into a series of reaction stages, the flow pattern is similar to that in a plug-flow bioreactor. The ABR is operated at HRTs in a range of 6–24 h with biomass concentrations varying from 4 to 20 g volatile suspended solids/L. This type of bioreactor has been used to produce hydrogen from tapioca wastewater [90], for codigestion of municipal food waste and kitchen wastewater [87], and to cultivate anaerobic ammonium oxidation (anammox) bacteria to remove nitrogen in wastewater [35].

13.3.1.2 Granulation System

High biomass concentrations, up to a certain degree, could increase organic loading and treatment efficiency of bioreactors. Granulation technology is key for increasing the

biomass concentration in an anaerobic bioreactor. The theories on anaerobic sludge granulation reviewed in this chapter are organized in three groups, namely physical, microbial, and thermodynamic approaches, which are considered the main factors responsible for granule formation [31]. (1) Physical theories: In this granulation approach, the phenomenon is explained in terms of the consideration of physical conditions prevailing in the reactor. Liquid and gas upflow velocities, suspended solids in the effluent or seed sludge, and attrition and removal of excess sludge from the reactor are considered the factors responsible for granulation. (2) Microbial theories: The theories explain sludge granulation mainly based on the characteristics of certain microorganisms. In this approach, the physical factors mentioned above are often also integrated. The observation of granular characteristics, namely granule structure and correspondent microbiology, coupled to the conditions prevailing in the reactor (hydrodynamics, substrate and intermediate concentration profiles along the reactor, etc.) is the basis of the theories presented. (3) Surface thermodynamics as the determining factor in granulation is presented in the next section.

Retention strategies affect both the quality and the quantity of the biomass in hydrogen and methane fermentation. Sludge granulation is the most effective means of increasing biomass concentration and retention in a bioreactor, improving H_2 production by altering the microbial community structure [103]. Granulation is a complex process involving physicochemical, biological, and hydrodynamic mechanisms, in which microbial composition, extracellular polymeric substances, and hydrodynamics play important roles. Granulation might be enhanced by several means, including (1) addition of cations, e.g., Al^{3+}, Ca^{2+}, Fe^{3+}, or Mg^{2+}, which can reduce the repulsive forces acting between the negatively charged bacteria cells; (2) addition of polymers, which form bridges between the cells (Fig. 13.3); and (3) proper reactor design and operating strategy [58].

13.3.1.2.1 UPFLOW ANAEROBIC SLUDGE BLANKET AND EXPANDED GRANULAR SLUDGE BED BIOREACTOR

A UASB is typically constructed with influent pumped from the unit bottom and flowing upward through a blanket of sludge where pollutants are degraded by granule sludge (Fig. 13.4A). A UASB has a unique gas–liquid–solid separator for separating biogas from sludge granules and effluent. The remarkable design of a gas–solid–liquid phase separator serves two key functions but uses just a single reactor, namely maximizing biomass retention without an external clarifier. The UASB technology is well accepted because of its high organic removal efficiency, simplicity, low capital and operating cost, and low footprint requirement. Moreover, it is characterized by low sludge production and low energy input [88]. UASB bioreactors have been used extensively in laboratory- or pilot-scale studies and are effective in treating organic wastes and converting them into hydrogen. Lee and Chung [47] reported that a pilot-scale two-stage hydrogen/methane fermentation plant generated 3.9 H_2 L/m^3/day with a hydrogen content of 60%t at HRT 21 h. This plant had a hydrogen fermenter (acidogenesis tank) with a working volume of

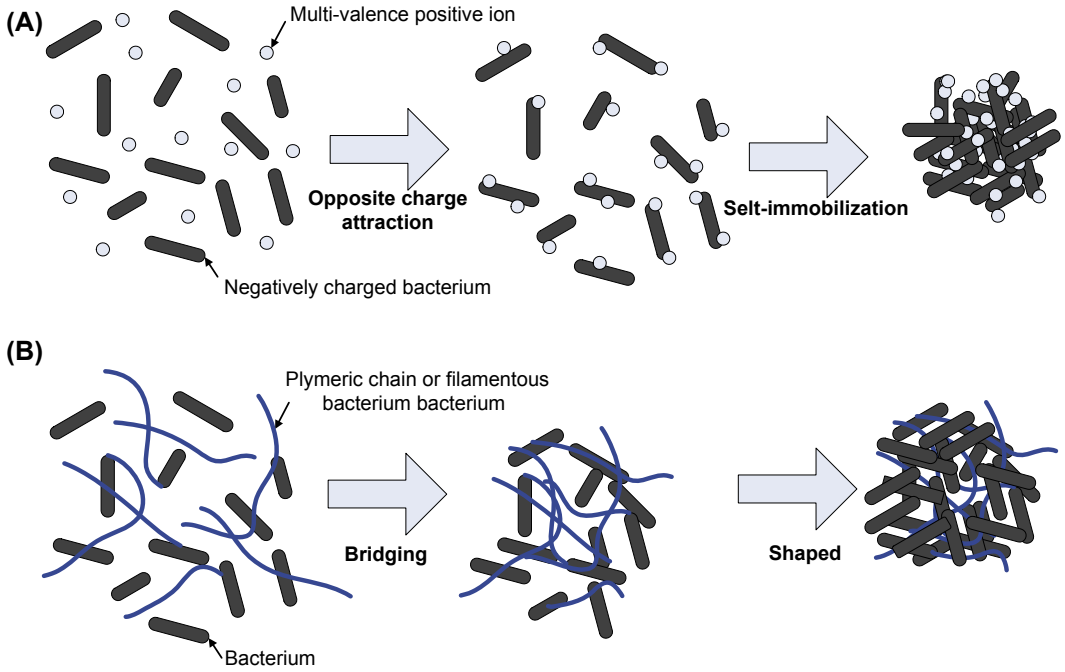

FIGURE 13.3 Granulation models. (A) Multivalence positive ion bonding. (B) Polymer bonding [62].

500 L for the first stage and a methane fermenter (methanogenesis tank) with a working volume of 2300 L. Moreover, a precipitation tank (working volume 100 L) was used to collect the hydrogen fermenter effluent and a storage tank (1000 L) was used to equalize the precipitation tank supernatant, which was used as a substrate for methane fermentation.

The expanded granular sludge bed bioreactor is able to overcome the shortcomings of the UASB system [88] with biomass growth in a granular form similar to UASB granules. The process is especially suitable for treating wastewater containing compounds that are toxic in high concentrations and that can be degraded only in low concentrations. It is also possible to operate the reactor as an ultrahigh-loaded anaerobic reactor (to 30 g COD/L/day) for applications in some industries (e.g., brewing, yeast, sugar, corn ethanol production, etc.) [88].

13.3.1.2.2 ANAEROBIC FLUIDIZED-BED BIOREACTOR

The fluidized-bed bioreactor has the merits of improved movement and preventing short-circuiting and dead-zone formation inside the reactor. Using granular activated carbon as carrier in an anaerobic fluidized-bed bioreactor (AnFBR) provides the extra advantages of large surface area for biomass attachment and the absorption of toxic pollutants [53]. Mustafa et al. [67] used an AnFBR (Fig. 13.4B), which utilized zeolite particles as the carrier medium, to treat municipal wastewater to achieve high COD

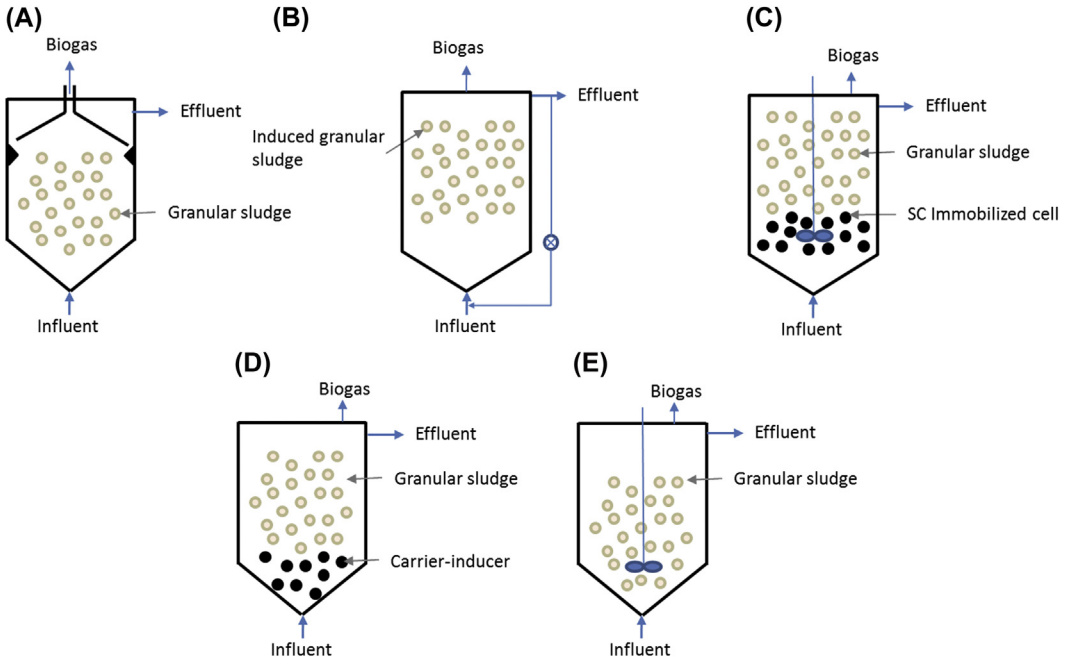

FIGURE 13.4 Typical reactor configurations used for anaerobic granulation fermentation systems. (A) Upflow anaerobic sludge blanket and expanded granular sludge bed bioreactors. (B) Anaerobic fluidized-bed bioreactor. (C) Continuously stirred anaerobic bioreactor. (D) Carrier-induced granular sludge bed reactor. (E) Agitated granular sludge bed reactor.

removal and VSS destruction efficiencies of 85% and 88%, respectively, at an HRT of 8.9 days and organic loading rate (OLR) of 4.2 kg COD/m^3 day. Andalib et al. [2] developed a newly integrated anaerobic fluidized bed with a circulating fluidized bed to treat high-strength wastewater containing 10,700 mg COD/L and 250 mg NH_3-N/L over 6 months with COD removal of 99.7%, nitrogen removal of 84%, and a very low sludge yield of 0.017 g VSS/g COD. An AnFBR was also applied to produce bioenergy [5].

13.3.1.2.3 CONTINUOUSLY STIRRED ANAEROBIC BIOREACTOR

A three-phase continuously stirred anaerobic bioreactor (CSABR) that consisted of a column 10 cm in diameter and 12.8 cm in height and a working volume of 1 L (Fig. 13.4C) was reported to have high HPR [103]. A CSABR seeded with silicone-immobilized sludge was tested for high-rate fermentative H_2 production at HRT of 0.5 h using a sucrose substrate (30–40 g COD/L) to give a high HPR of 362 L/L/day (14.7 mol/L/h) and an optimal HY of 3.5 mol H_2/mol sucrose. The formation of self-granulated sludge during short-HRT operation was crucial to the high-rate H_2 production. Sludge granulation might also trigger a change in bacterial community resulting in

a twofold increase in specific HPR. Denatured gradient gel electrophoresis analysis showed a bacterial dominance of *Clostridium pasteurianum* [58].

13.3.1.2.4 CARRIER-INDUCED GRANULAR SLUDGE BED REACTOR

A carrier-induced granular sludge bed reactor (CIGSBR) (Fig. 13.4D) was developed to improve biomass retention and mass-transfer efficiency [114] with calcium ion (5.4−27.2 mg/L) supplementation to enhance (threefold increase) the granules' mechanical strength. Ca^{2+} addition led to a high biomass concentration and a fivefold increase in HPR (up to 122 L/L/day). Two reflux strategies were utilized to enhance the mass-transfer efficiency of the CIGSBR. Liquid reflux enhanced the HPR by 2.2-fold at an optimal liquid upflow velocity of 1.09 m/h, giving a peak biomass concentration of 22 g VSS/L. Gas reflux at rates of 1.0−1.49 m/h gave similar HPR and reduced the biomass concentrations to 2−7 g VSS/L. These strategies were effective for a stable and efficient H_2 production for 100 days [114].

13.3.1.2.5 AGITATED GRANULAR SLUDGE BED REACTOR

An agitated granular sludge bed reactor (AGSBR) (Fig. 13.4E) having a working volume of 1 L and a paddle (agitation, 15 rpm) was operated with an initial addition of 1 g/L powdered activated carbon as microbial carrier. This AGSBR was fed with starch wastewater and gave a peak HPR of 48 L/L/day at pH 6.0 at HRT of 0.5 h with a total sludge density of 45−48 g VSS/L [115].

13.3.2 Integration Process

13.3.2.1 Dark-Fermentative Hydrogen and Methane Production

Dark fermentation is a promising technology to produce bioenergy from organic materials. A two-phase H_2/CH_4 fermentation process has been developed during the past decades. To efficiently convert biowastes and wastewaters into bioenergy, pretreatment of the feedstock is necessary before applying a two-phase fermentation process. For lignocellulosic materials, breaking the lignin seal, the feedstock size and structure, and the modifying chemical composition of the biomass are important [70]. The pretreatment process includes biological, physical, chemical, and physicochemical methods. A combination of these methods has also been studied [1]. Furthermore, a phase-separator unit such as the activated sludge method might be applied to remove effluent COD to meet a discharge standard.

The Green Energy Development Center, Feng Chia University, Taiwan, has successfully developed high-rate hydrogen production technologies with granular microflora. The advanced granular bioreactors used are CSABR, CIGSBR, and AGSBR, which are operated at quite short HRTs (0.5−1.5 h), attaining high biomass concentrations [58]. Some pilot-scale studies on synthetic and raw wastewaters have been reported [55,59,60]. Novel two-phase hydrogen and methane production technology and its operation have been established at Feng Chia University. This technology was

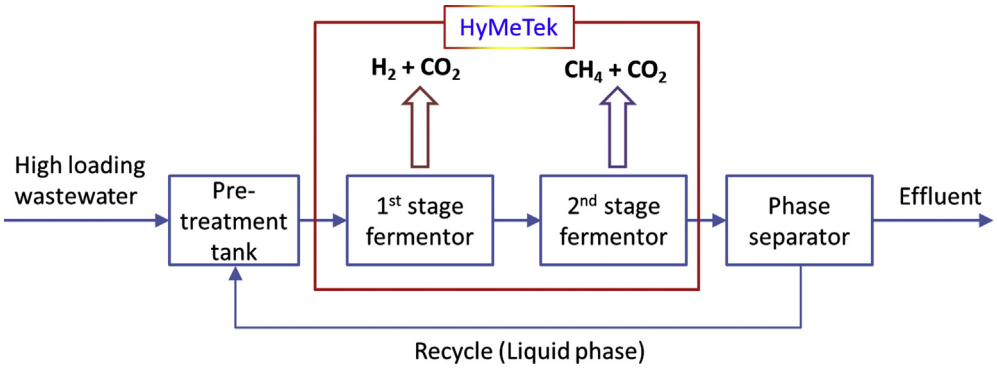

FIGURE 13.5 Scheme of the Innovative Hydrogenesis and Methanogenesis Technology (HyMeTek) for high-strength-loading wastewater treatment.

named "Innovative Hydrogenesis and Methanogenesis Technology (HyMeTek)" (Fig. 13.5). To commercialize this HyMeTek technology, a verification scale of the HyMeTek system including hydrogen (reactor volume, 2 m^3) and methane (50 m^3) fermenters was built in a food industry plant located in Taoyuan, Taiwan.

13.3.2.2 Photofermentative Hydrogen and Dark-Fermentative Methane Production

Sequential dark fermentation and photofermentation of organics is a promising method of producing renewable biogas. During dark fermentation, sugars are converted to H_2, CO_2, and short-chain organic acids with a theoretical maximum HY of 4 mol H_2/mol hexose, when all sugars are fermented to acetate, CO_2, and H_2 [75]. The effluent of the dark fermentation is used as the substrate for photosynthetic bacteria during the second photofermentative step, in which short-chain organic acids are assimilated to H_2 when light is present, producing maximally 4 and 6 mol of H_2 per mole of acetate and lactate, respectively. Because of this, the combined two-step process has a theoretical maximum HY of 12 mol H_2/mol hexose [75].

Hyvolution has been granted in the 6th European Union Framework Programme on Research, Technological Development and Demonstration and scheduled from 2006 to 2010 (Fig. 13.6) [12]. Biological hydrogen production (BHP) includes dark fermentation (WP 2) and photofermentation (WP 3). Biomass was treated (WP 1) to hydrolyze the cellulosic material to sugar. Sugar is converted to H_2 and CO_2 in the gas phase and organic acids in the liquid phase by anaerobic bacteria in the first stage. The organic acids are used as the feedstock for photofermentation bacteria to produce H_2 and CO_2 in the second stage. Therefore, the theoretical maximum hydrogen production yield of 12 mol H_2/mol hexose might be obtained in this two-stage BHP.

13.3.2.3 Microbial Electrolysis Cell and Dark-Fermentative Methane Production

Bioelectricity can be generated sustainably in microbial fuel cells (MFCs) during wastewater treatment [20,29,61]. In an MFC, microbes convert the chemical energy

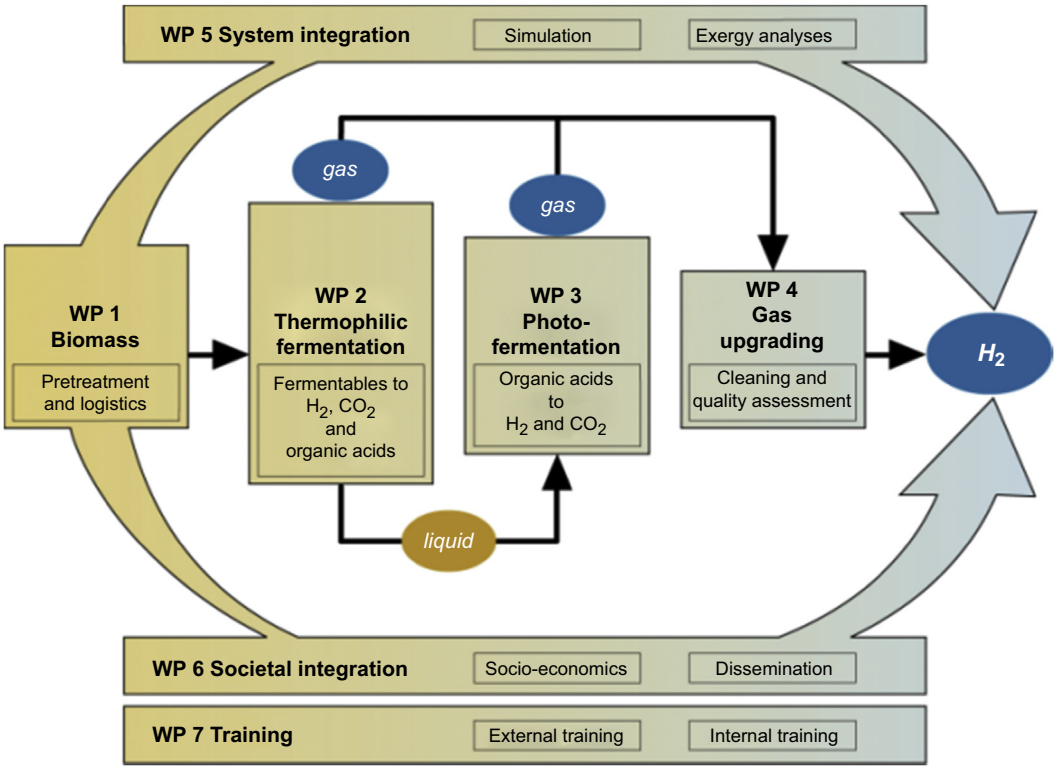

FIGURE 13.6 Hyvolution: An integrated approach for nonthermal hydrogen production, which covers the whole chain from biomass to hydrogen, including societal integration for implementation in society [12].

stored in organic compounds into electricity [30]. Anaerobic microorganisms grow on the anode by oxidizing organic substrates and release electrons to the anode electrode. The electrons are then transported to the cathode electrode via a wire and an external load. At the cathode released electrons reduce oxygen, iron, manganese, or permanganate or are taken up by a biological electron acceptor. Thus, a closed circuit is formed and electrical current is produced (Fig. 13.7). This approach has dual advantages of simultaneously reducing wastewater pollutants and producing bioenergy. The electricity generation efficiency is affected by the MFC architecture, electrode design, and exoelectrogenic cultures [63].

In addition to photofermentation, organic acids such as acetate in the effluent of an anaerobic H_2 fermentation system can be fed into another system to extract energy in the form of H_2 from a microbial electrolysis cell (MEC) or electricity from an MFC or CH_4 from an anaerobic digester. However, additional energy is required for the second stage of these processes, for instance, light for photofermentation or electricity for MECs [26]. Moreover, the electrons from an MFC can support the MFC to reduce energy consumption (Fig. 13.8).

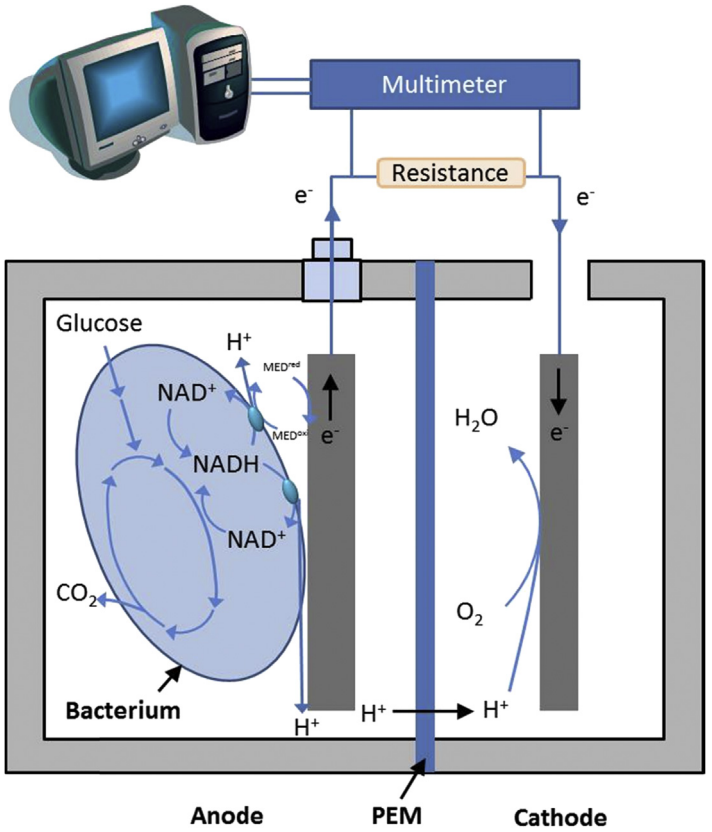

FIGURE 13.7 Scheme of a two-chamber microbial fuel cell.

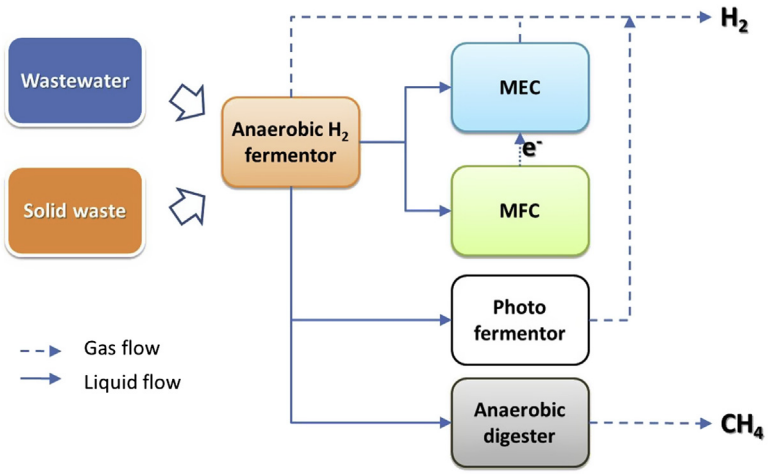

FIGURE 13.8 Possible two-stage systems for complete conversion of substrate. *MEC*, microbial electrolysis cell; *MFC*, microbial fuel cell.

13.4 Environmental Factors Affecting the Anaerobic Treatment Process

Biohydrogen and methane production performance strongly relates to operation strategy and main process parameters (including feedstock treatment, substrate concentration, pH, HRT, temperature, and reactor type). Many reviews [24,49,54,70] have summarized the optimum values of these parameters, with most of the studies focusing on lab-scale systems. The indexes for identifying high biogas production efficiency are HY or methane production yield (defined as the biohydrogen or methane production per unit weight of substrate, mol H_2/g COD or mol CH_4/g COD) and HPR or methane production rate (defined as the biohydrogen or methane production per unit working volume per day, L/L/day). Some reports investigated the operation strategies for a two-phase fermentation process using pilot-scale fermenters, and those are summarized next.

13.4.1 Feedstock Pretreatment

High-strength wastewaters might contain lignocellulosic compounds. Pretreatment of lignocellulosic materials for enhancing digestion efficiency is widely used. Pretreatment aims to decompose the lignin structure and disarrange the crystalline structure of cellulose to enhance enzyme accessibility to the cellulose during the hydrolysis step [1]. The key properties for a low-cost and advanced pretreatment process are (1) high yields for multiple crops, site age, and harvesting time; (2) highly digestible pretreated solids; (3) no significant sugar degradation; (4) minimum amount of toxic compounds; (5) biomass size reduction not required; (6) operation in reasonably sized and moderately priced reactors; (7) nonproduction of solid-waste residues; (8) effectiveness at low moisture content; (9) attainment of high sugar concentration; (10) fermentation compatibility; (11) lignin recovery; and (12) minimum heat and power requirements [116].

Several pretreatment technologies have been developed during the past decades: biological, physical, chemical, and physicochemical methods. Combinations of these methods have also been studied [1]. Table 13.4 lists the advantages and drawbacks of these pretreatment methods.

13.4.2 Loading Rate

The allowable level of loading rate is one of the most important advantages of an anaerobic process. Because there are no oxygen transfer limitations in an anaerobic system and no biomass thickening limitations with proper biomass immobilization, loading rates can be much higher than for aerobic treatment [84]. Factors controlling allowable loading rates in anaerobic treatments are: (1) viable biomass concentration that can be retained in the bioreactor, (2) mass transfer between the incoming wastewater and the retained biomass, (3) biomass proximity for metabolism of H_2 mediate,

Table 13.4 Benefits and Drawbacks of Treatments Used for Hydrolysis of Solid Feedstocks [1]

Reactor Type	Benefits (+) and Drawbacks (−)	References
BIOLOGICAL	+Degrades lignin and hemicelluloses +Low energy consumption −Low rate of hydrolysis	[14,25]
PHYSICAL		
Milling/extrusion	+Reduces cellulose crystalline −High power and energy consumption	[3]
CHEMICAL		
Alkali	+Increases cellulose digestibility and lignin solubilization effectively −Loss of fermentation sugar −Inhibitory compounds production	[71]
Ozonolysis	+Reduces lignin content +Does not imply generation of toxic compounds −High cost of large amount of ozone needed	[22]
Organosolvation	+Causes lignin and hemicellulose hydrolysis −High cost −Solvents need to be drained and recycled	[111]
Concentrated acid	+High glucose yield +Ambient temperatures −High cost of acid and needs to be recovered −Reactor corrosion problems −Formation of inhibitors	[13,37,68]
Diluted acid	+Fewer corrosion problems than concentrated acid +Less formation of inhibitors −Generation of degradation products −Low sugar concentration in exit stream	[18,14,38,109]
PHYSICOCHEMICAL		
Steam explosion	+Causes lignin transformation and hemicellulose solubilization +Cost-effective +Higher yield of glucose and hemicellulose in the two-step method −Generation of toxic compounds −Partial hemicellulose degradation	[16,33,50]
Liquid hot water	+Does not require rapid decompression and employs any catalyst or chemical +Hemicellulose solubilization −Low degradation products	[6]
Ammonia fiber explosion	+Increases accessible surface area +Low formation of inhibitors −Not efficient for raw materials with high lignin content −High cost of large amount of ammonia	[89,105]
CO_2 explosion	+Increases accessible surface area +Cost-effective +Do not imply generation of toxic compounds −Does not affect lignin and hemicelluloses −Very high pressure requirements	[112]
Wet oxidation	+Efficient removal of lignin +Low formation of inhibitors +Minimizes energy demand (exothermic) −High cost of oxygen and alkaline catalyst	[76]

(4) ease of metabolism of organic pollutants, (5) operation temperature, (6) toxicity level in the wastewater, (7) elevated K_s, (8) operation pH, and (9) reactor configuration/staging [84].

Higher substrate concentrations can enhance hydrogen production efficiency, but substrate or product inhibition would occur when the substrate loading exceeded a threshold level. Substrate concentration and the optimization operation conditions affect anaerobic fermentation for biohydrogen production (Table 13.5). The suitable substrate concentrations for biohydrogen production from wastewaters are lower than 40 g COD/L but higher HYs might be obtained at lower substrate concentrations (Table 13.5). A maximum HY of 25 mmol/g COD (612 mL/g COD) was obtained with a very low vinasse concentration (0.25 g COD/L) in a batch system [19]. Moreover, a high HY value (237 mL/g volatile solids (VS)) in solid waste fermentation was obtained from thermal chemically treated rice straw (3 g/L) in a batch system [6].

HRT is an important operation parameter in anaerobic treatment. Table 13.6 reveals that shorter HRTs (<10 h) result in higher HYs (>245 mL/g COD) and HPRs (>3 L/L/day). However, exceptions have been found. For example, using cheese whey wastewater (47 g COD/L) in a CSTR could give a high HY of 22 mmol/g COD (539 mL/g COD) at a long HRT of 3.5 days [4]. Moreover, HPR is OLR-dependent; OLR can be controlled either by increasing the substrate concentration or by shortening the HRT. Generally, increasing the substrate concentration and OLR leads to an increase in HPR in continuous anaerobic systems fed on wastewaters. Lin and Lay [58] reported that at OLR of 1920 g COD/L/day (HRT 0.5 h) using 40 g COD/L of sucrose feedstock gave a high HPR of 362 L/L/day for a CSABR system. An HPR of around 8 L/L/day was obtained from brewery wastewater (6.05 g COD/L) and palm oil effluent (100 g COD/L) using batch systems [10]. At an OLR of 320 g COD/L/day (HRT 3 h), a high HPR was obtained in a CSTR system using CMS (40 g COD/L) [44]. In contrast to HPR, a high OLR might reduce the HY from a metabolic shift to a solventogenic phase (e.g., ethanol), which is unfavorable for hydrogen production [103]. An OLR of 62.5 g VS/L/day (HRT 9.6 h and 25 g glycerol/L) was shown to give an HPR of 6.9 L/L/day in a continuous system using a UASB system [80].

13.4.3 pH

A pH range of 6.5–8.2 favors methane production via anaerobic digestion. pH values above or below this range markedly reduce the methane production rate. Methanogenesis occurs at pH 6.0 and even lower at reduced rates but the bicarbonate alkalinity does not buffer well under such conditions, and this characteristic tends to result in considerable instability [84]. Moreover, *Methanosarcina mazei*, a commonly observed methanogen, is reported to be able to operate at a pH range lower than that of other species of methanogens.

pH control is crucial to dark-fermentative hydrogen production because of its effect on hydrogenase activity and metabolic pathways. When the pH of a fermentation medium is

Table 13.5 Anaerobic Biohydrogen Production Processes at Various Substrate Concentrations Using High-Strength Wastewater Feedstock

Wastewater	Culture Type	Seed Sludge	Substrate Conc. (g COD/L)		HY (mol H_2/mol hexose)	HPR (L/L/day)	References
			Range Studied	Optimal			
Apple processing	Batch	Soil	9	–	4.08 mmol/g COD	2.16	[93]
Brewery	Batch	AS	2–12	6.05	6.11 mmol/g COD	8.58	[81]
Cereal	Batch	AS	8.92	–	0.24	–	[72]
Condensed molasses fermentation solubles (CMS)	Batch	Coculture 1	10–160	50	1.78	1.92	[28]
CMS	Batch	AS	10–160	40	1.5	2.39	[102]
Confectionery processing	Batch	Soil	6.5	–	6.94 mmol/g COD	0.24	[93]
Distillery effluent	Batch	Coculture 2	10	–	2.76	1.56	[95]
Olive mill	Batch	ADS	68.1	–	0.54 mmol/g COD	0.07	[17]
Palm oil effluent	Batch	*Clostridium butyricum* EB6	100	–	1.30 mmol/g COD	8.27	[10]
Potato processing	Batch	Soil	20	–	5.71 mmol/g COD	5.04	[93]
Preserved fruit soaking solution	Batch	AS	1.24–6.2	3.72	3.72	–	[43]
Probiotics	Batch	ADS	2–8	5.0	1.8	–	[83]
Dairy	ASBR	AM	2.4–4.7	4.7	–	0.03	[96]
Distillery	ASBR	ADS	9.6	–	–	5.15	[97]
Cheese processing	CSTR	ADS	5.0–7.0	7.0	3.21 mmol/g COD	1.00	[107]
Cheese whey	CSTR	ADS	21–47	47	22.00 mmol/g COD	1.5	[4]
Coffee drink manufacturing	CSTR	AS	20	–	0.20	0.34	[36]
CMS	CSTR	AS	40	–	0.9	9.50	[44]
Olive pulp water	CSTR	ADS	17.8–19.6	19.6	2.8	0.48	[41]
Purified terephthalic acid	CSTR	ADS	4.0	–	19.29 mmol/g COD	0.79	[113]
Sugar beet	CSTR	ADS	10	–	1.7	–	[32]
Sugary	CSTR	Sludge compost	31.85	–	2.52	4.85	[92]
Citric acid	UASB	AB	5.0–19.2	19.2	0.84	0.72	[106]
Coffee drink manufacturing	UASB	AS	20	–	0.96	4.64	[36]
Rice winery	Upflow reactor	AS	14–36	34	2.14	3.81	[108]

Coculture 1, coculture of *Clostridium sporosphaeroides* F52 and *Clostridium pasteurianum* F40; coculture 2, coculture of *Clostridium freundii* 01, *Enterobacter aerogens* E10, and *Rhodopseudomonas palustris* P2. *AB*, anaerobic bacteria; *ADS*, anaerobic digest sludge; *AM*, anaerobic mixed microflora; *AS*, anaerobic sewage sludge; *ASBR*, sequencing batch reactor; *COD*, chemical oxygen demand; *CSTR*, continuously stirred tank reactor; *HPR*, hydrogen production rate; *HY*, hydrogen yield; *UASB*, upflow anaerobic sludge blanket.

Table 13.6 Anaerobic Biohydrogen Production Processes at Various HRTs Using High-Strength Wastewater Feedstock

Wastewater	Culture Type	Seed Sludge	HRT (h)		HY (mol H_2/mol hexose)	HPR (L/L/day)	References
			Range Studied	Optimal			
Dairy	ASBR	AM	24	–	–	0.03	[96]
Distillery	ASBR	ADS	24	–	–	5.15	[97]
Cheese processing	CSTR	ADS	12–24	24	3.21 mmol/g COD	1.00	[107]
Cheese whey	CSTR	ADS	24–84	84	22.00 mmol/g COD	1.5	[4]
Coffee drink manufacturing	CSTR	AS	6–12	6	0.20	0.34	[36]
CMS	CSTR	AS	3–24	3	0.9	9.50	[44]
Olive pulp water	CSTR	ADS	7.5–30	7.5	2.8	0.48	[41]
Purified terephthalic acid	CSTR	ADS	6	–	19.29 mmol/g COD	0.79	[113]
Sugar beet	CSTR	ADS	14.2	–	1.7	–	[32]
Sugary	CSTR	Sludge compost	0.5–72	0.5	2.52	4.85	[92]
Citric acid	UASB	AB	8–48	12	0.84	0.72	[106]
Coffee drink manufacturing	UASB	AS	4–8	4	0.96	4.64	[36]
Rice winery	Upflow reactor	AS	2–24	2	2.14	3.81	[108]

ASBR, sequencing batch reactor; *COD*, chemical oxygen demand; *CMS*, condensed molasses fermentation solubles; *CSTR*, continuously stirred tank reactor; *HRT*, hydraulic retention time; *HPR*, hydrogen production rate; *HY*, hydrogen yield; *UASB*, upflow anaerobic sludge blanket.

too low, either the metabolic activity of the H_2-producing bacteria would be inhibited or there would be a shift in the metabolic pathway resulting in cessation of hydrogen generation. A high HPR can be obtained for dark fermentation using wastewaters with a slightly acidic environment (<pH 7.0) in batch and continuous systems [54]. pH 5.5 and 6.5 efficiently ferment wastewaters and solid waste to produce H_2. For example, an initial cultivation pH of 5.5 resulted in a maximum HY (25 mmol/g COD) when using vinasse for batch biohydrogen production and a high HY (22 mmol/g COD) in a CSTR system fed on cheese-processing wastewater [4]. pH 5.5 also resulted in a maximum HPR in a CSTR system using CMS feedstock (40 g COD/L) [44]. Other high HPR values of 8.3–8.6 L/L/day were also obtained at initial cultivation pH values of 5.5 and 6.05 using palm oil effluent [10] and brewery wastewater [81] (Table 13.7).

13.4.4 Temperature

The anaerobic process is more sensitive to temperature variation than aerobic processes. Conversion of acetate to CH_4 is more temperature-dependent than that of acetate-forming

Table 13.7 Anaerobic Biohydrogen Production at Various pH Values Using High-Strength Wastewater Feedstock

Wastewater	Culture Type	Seed Sludge	pH		HY (mol H_2/mol hexose)	HPR (L/L/day)	References
			Range Studied	Optimal			
Apple processing	Batch	Soil	6.1	—	4.08 mmol/g COD	2.16	[93]
Brewery	Batch	AS	4—8	5.95	6.11 mmol/g COD	8.58	[81]
Cattle	Batch	SS	4.5—7.5	5.5	12.41 mmol/g COD	0.34	[86]
Cereal	Batch	AS	6.0	—	0.24	—	[72]
Chemical wastewater and domestic sewage	Batch	AM	6.0[i]	—	1.25 mmol/g COD	—	[98]
Condensed molasses fermentation solubles (CMS)	Batch	Coculture 1	7.0[i]	—	1.78	1.92	[28]
CMS	Batch	AS	4.0—8.0[i]	6.0[i]	1.5	2.39	[102]
Confectionery processing	Batch	Soil	6.1	—	6.94 mmol/g COD	0.24	[93]
Distillery effluent	Batch	Coculture 2	5.2—7.0	—	2.76	1.56	[95]
Domestic sewage	Batch	ADS	5.5	—	6.01 mmol/g COD	0.16	[19]
Glycerin	Batch	ADS	5.5	—	6.03 mmol/g COD	0.19	[19]
Lagoon	Batch	AS	6.0	—	0.51	—	[72]
Olive mill	Batch	ADS	6.8	—	0.54 mmol/g COD	0.07	[17]
Palm oil effluent	Batch	*Clostridium butyricum* EB6	5.0—8.5	5.5	1.30 mmol/g COD	8.27	[10]
Potato processing	Batch	Soil	6.1	—	5.71 mmol/g COD	5.04	[93]
Preserved fruit soaking solution	Batch	AS	4.0—8.0[i]	6.0[i]	3.72	—	[43]
Probiotics	Batch	ADS	4.5—7.0	5.5	1.8	—	[83]
Vinasse	Batch	ADS	5.5	—	24.97 mmol/g COD	0.60	[19]
Dairy	ASBR	AM	4.56—6.28	—	—	0.03	[96]
Distillery	ASBR	ADS	5.2—7.0	—	—	5.15	[97]
Cheese processing	CSTR	ADS	4.79	—	3.21 mmol/g COD	1.00	[107]
Cheese whey	CSTR	ADS	5.5	—	22.00 mmol/g COD	1.5	[4]
Coffee drink manufacturing	CSTR	AS	5.5	—	0.20	0.34	[36]
CMS	CSTR	AS	5.5	—	0.9	9.50	[44]

Table 13.7 Anaerobic Biohydrogen Production at Various pH Values Using High-Strength Wastewater Feedstock—cont'd

Wastewater	Culture Type	Seed Sludge	pH		HY (mol H_2/mol hexose)	HPR (L/L/day)	References
			Range Studied	Optimal			
Olive pulp water	CSTR	ADS	4.8–5.0	4.9	2.8	0.48	[41]
Purified terephthalic acid	CSTR	ADS	6.0	–	19.29 mmol/g COD	0.79	[113]
Sugar beet	CSTR	ADS	5.2	–	1.7	–	[32]
Sugary	CSTR	Sludge compost	6.8	–	2.52	4.85	[92]
Citric acid	UASB	AB	7.0	–	0.84	0.72	[106]
Coffee drink manufacturing	UASB	AS	5.5	–	0.96	4.64	[36]
Rice winery	Upflow reactor	AS	4.5–6.0	5.5	2.14	3.81	[108]

Coculture 1, coculture of *Clostridium sporosphaeroides* F52 and *Clostridium pasteurianum* F40; coculture 2, coculture of *Clostridium freundii* 01, *Enterobacter aerogens* E10, and *Rhodopseudomonas palustris* P2. *ADS*, anaerobic digest sludge; *AM*, anaerobic mixed microflora; *AS*, anaerobic sewage sludge; *ASBR*, sequencing batch reactor; *COD*, chemical oxygen demand; *CSTR*, continuously stirred tank reactor; *HPR*, hydrogen production rate; *HY*, hydrogen yield; *SS*, sewage sludge; *UASB*, upflow anaerobic sludge blanket.

biomass. A lowered temperature might cause an increase in volatile acid concentrations because acidogens are less affected than methanogens in metabolic rate. This VFA increase potentially can exceed the buffer capacity with a corresponding drop in pH. Thus a temperature decrease can have drastic repercussions on a process operation [84].

Fermentative hydrogen production by mixed cultures has been performed mostly under mesophilic (20–40°C) and thermophilic (50–60°C) conditions with only a few studies on extreme thermophilic (65–75°C) conditions. Cultivation temperatures ranging from 23 to 60°C showed that HY and HPR increased along with the temperature increase in both batch and continuous systems. A high HY was obtained when dark fermentation of vinasse was carried out at 25°C in a batch mode operation [19] (Table 13.8). To develop biohydrogen production technology, it is very important to operate the system at lower temperatures that would have positive energy gain and safe maintenance and monitoring. However, for certain wastewaters like textile industry effluent, with a temperature around 70–80°C, the hydrogen production system might need to be operated under thermophilic conditions. Moreover, thermophilic digestion has been proposed for agricultural wastes because it is easy to maintain a high activity for cellulosic enzymes [49].

13.5 Net Energy Gain Analysis of Wastewater to Bioenergy

To develop an energy-efficient biohydrogen production system, it is necessary to evaluate the net energy gain (NEG). Such analysis is an integral feature of energy economics that is calculated as the difference between the energy input to harvest an energy source

Table 13.8 Anaerobic Biohydrogen Production Processes Operated at Various Temperatures Using High-Strength Wastewater Feedstock

Wastewater	Culture Type	Seed Sludge	Temperature (°C)		HY (mol H_2/mol hexose)	HPR (L/L/-day)	References
			Range Studied	Optimal			
Apple processing	Batch	Soil	23	—	4.08 mmol/g COD	2.16	[93]
Brewery	Batch	AS	25—45	36	6.11 mmol/g COD	8.58	[81]
Cattle	Batch	SS	30—55	45	12.41 mmol/g COD	0.34	[86]
Cereal	Batch	AS	30	—	0.24	—	[72]
Chemical wastewater and domestic sewage	Batch	AM	29	—	1.25 mmol/g COD	—	[98]
Condensed molasses fermentation solubles (CMS)	Batch	Coculture 1	35	—	1.78	1.92	[28]
CMS	Batch	AS	35	—	1.5	2.39	[102]
Confectionery processing	Batch	Soil	23	—	6.94 mmol/g COD	0.24	[93]
Distillery effluent	Batch	Coculture 2	26—39	—	2.76	1.56	[95]
Domestic sewage	Batch	ADS	25	—	6.01 mmol/g COD	0.16	[19]
Glycerin	Batch	ADS	25	—	6.03 mmol/g COD	0.19	[19]
Lagoon	Batch	AS	30	—	0.51	—	[72]
Olive mill	Batch	ADS	35	—	0.54 mmol/g COD	0.07	[17]
Palm oil effluent	Batch	*Clostridium butyricum* EB6	30—55	37	1.30 mmol/g COD	8.27	[10]
Potato processing	Batch	Soil	23	—	5.71 mmol/g COD	5.04	[93]
Preserved fruit soaking solution	Batch	AS	35	—	3.72	—	[43]
Probiotic	Batch	ADS	37	—	1.8	—	[83]
Vinasse wastewater	Batch	ADS	25	—	24.97 mmol/g COD	0.60	[19]
Dairy	ASBR	AM	28	—	—	0.03	[96]
Distillery	ASBR	ADS	28	—	—	5.15	[97]
Cheese processing	CSTR	ADS	35—38	—	3.21 mmol/g COD	1.00	[107]
Cheese whey	CSTR	ADS	55	—	22.00 mmol/g COD	1.5	[4]
Coffee drink manufacturing	CSTR	AS	35	—	0.20	0.34	[36]
CMS	CSTR	AS	35	—	0.9	9.50	[44]

Table 13.8 Anaerobic Biohydrogen Production Processes Operated at Various Temperatures Using High-Strength Wastewater Feedstock—cont'd

Wastewater	Culture Type	Seed Sludge	Temperature (°C)		HY (mol H_2/mol hexose)	HPR (L/L/-day)	References
			Range Studied	Optimal			
Olive pulp water	CSTR	ADS	35	–	2.8	0.48	[41]
Purified terephthalic acid	CSTR	ADS	35	–	19.29 mmol/g COD	0.79	[113]
Sugar beet	CSTR	ADS	32	–	1.7	–	[32]
Sugary	CSTR	Sludge compost	60	–	2.52	4.85	[92]
Citric acid	UASB	AB	35–38	–	0.84	0.72	[106]
Coffee drink manufacturing	UASB	AS	35	–	0.96	4.64	[36]
Rice winery	Upflow reactor	AS	20–55	55	2.14	3.81	[108]

Coculture 1, coculture of *Clostridium sporosphaeroides* F52 and *Clostridium pasteurianum* F40; coculture 2, coculture of *Clostridium freundii* 01, *Enterobacter aerogens* E10, and *Rhodopseudomonas palustris* P2. *ADS*, anaerobic digest sludge; *AM*, anaerobic mixed microflora; *AS*, anaerobic sewage sludge; *ASBR*, sequencing batch reactor; *COD*, chemical oxygen demand; *CSTR*, continuously stirred tank reactor; *HPR*, hydrogen production rate; *HY*, hydrogen yield; *SS*, sewage sludge; *UASB*, upflow anaerobic sludge blanket.

and the amount of energy gained from such harvest [77]. However, dark fermentation has been operated at temperatures higher than ambient temperatures to get a high yield but without considering the NEG, which is indirectly proportional to the cultivation temperature. HY is generally reported in terms of moles hydrogen produced per mole feedstock used. The NEG defined above can be estimated from the reported yields via the following equation [77],

$$E_N = \frac{YCkV(\mathrm{LHV}) \times 10^{-3} - V\rho_w c_p (T_f - T_a)}{VC} \tag{13.1}$$

where E_N is the net energy gain (kJ/kg), Y is the hydrogen production (mL) per unit feedstock (g COD for wastewater and g VS for solid waste), C is the feedstock concentration (g COD/L for wastewater and g VS/L for solid waste), k is the COD equivalent of the wastewater feedstock (g feedstock/g COD), V is the liquid volume in the reactor (L), LHV is the lower heating value of hydrogen (120,000 kJ/kg), T_f is the fermentation temperature, T_a is the ambient temperature, ρ_w is the density of water (1 kg/L), and c_p is the specific heat of water (4.2 kJ/kg K). Here, T_a was set equal to the standard ambient temperature of 25°C.

Generally the operations performed at ambient temperature have positive energy gain because less energy is used in maintaining a high operating temperature. Based on the NEG calculation, vinasse (140.39 kJ/g COD) and glycerin wastewater (68.65 kJ/g COD) and domestic sewage (51.84 kJ/g COD) are reported to have high positive NEGs, with an HY of 245 mL H_2/g COD (Table 13.9 and Fig. 13.9).

Table 13.9 Net Energy Gain Parameters and Analysis Results for Hydrogen Production From Wastewater Feedstock

Feedstock	*Y* (mL H_2/g COD)	*C* (g COD/L)	T_f (K)	Net energy gain (kJ/g COD)	References
Apple processing WW	100	9	23	1.91	[93]
Brewery WW	150	6.05	36	−6.17	[81]
Cattle WW	304	1.32	45	−60.47	[86]
Cereal WW	0.24	2.75	29	−5.81	[72]
Cheese processing WW	79	7	38	−1.63	[107]
Cheese whey WW	539	47	55	3.49	[4]
Chemical WW and DSW	31	2.75	29	−4.28	[66]
Citric acid WW	0.84	19.2	38	0.61	[106]
Glycerin WW	148	0.25	25	68.6	[19]
Condensed molasses fermentation solubles	0.9	40	35	0.71	[44]
Coffee drink WW	0.96	20	35	1.62	[36]
Confectionery processing WW	170	6.5	23	4.25	[93]
Distillery WW	2.76	10	39	−2.01	[95]
Domestic sewage	147	0.25	25	51.84	[19]
Lagoon WW	0.51	1.67	30	−6.91	[72]
Olive mill WW	13.2	68.1	35	−0.18	[17]
Olive pulp water	2.8	19.6	35	2.86	[41]
Palm oil effluent	32.0	100	37	0.06	[10]
Potato processing WW	140.0	20	23		[93]
Preserved fruit soaking solution	3.72	3.72	35	−3.25	[43]
Probiotic WW	1.8	5	37	−4.47	[83]
Purified terephthalic acid	19.29	4	35	22.01	[113]
Rice winery WW	2.14	34	55	0.58	[108]
Sugar beet WW	1.7	10	32	2.97	[32]
Sugary WW	2.52	31.85	60	−0.15	[92]
Vinasse WW	611.0	0.25	25	140.40	[19]

C, feedstock concentration; *COD*, chemical oxygen demand; *DSW*, domestic sewage; T_f, fermentation temperature; *WW*, wastewater; *Y*, hydrogen production per unit feedstock.

13.6 Future Prospects

Traditionally, high-strength organic wastewaters are managed from the viewpoint of environmental protection to reduce their strength via anaerobic digestion to meet discharge standards. However, based on the requirements of resource utilization and low carbon technology for waste/wastewater treatment, a shift from treatment to energy production in wastewater management is necessary. Anaerobic digestion has the characteristics of generating energy-containing products (H_2/CH_4). Some attractive prospects are proposed.

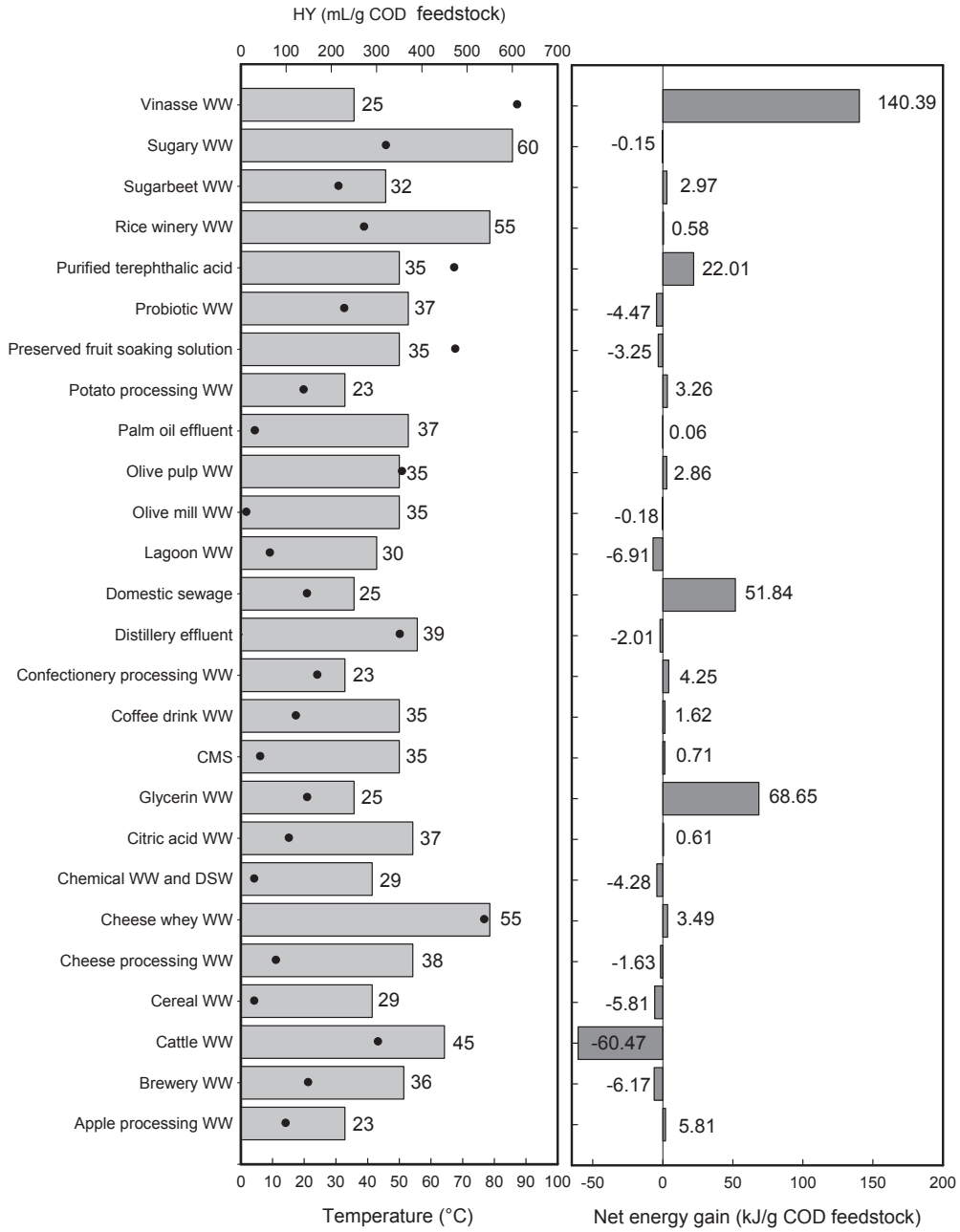

FIGURE 13.9 Net energy gain analysis for wastewater feedstock. *COD*, chemical oxygen demand; *CMS*, condensed molasses fermentation solubles; *DSW*, domestic sewage; *HY*, hydrogen yield; *WW*, wastewater.

13.6.1 Concept of Bioenergy and Bioresources Center for Wastewater Management

A biorefinery produces fuels, power, heat, and value-added chemicals from biomass by integrating biomass conversion processes, which concept is based on today's petroleum refinery to produce multiple fuels and products [73]. The main challenges for commercializing the biogas production technology are energy efficiency and cost benefits. The concept of a "bioenergy and bioresource center" is suggested for the commercial biogas production technology through a biorefinery idea (Fig. 13.10). In a sewage treatment plant transformed into a bioenergy and bioresources center, three divisions of bioenergy generation, fermentation, residue utilization, and bioresource recycling, are suggested. They are detailed as follows.

13.6.1.1 Biogas Generation Division

Using anaerobic fermentation on waste materials (such as agricultural waste, industrial wastewater, food waste, and waste sludge) to produce hydrogen and methane gases is a low-cost bioenergy production technology. Other renewable energies such as wind and solar can be integrated to improve the total energy efficiency of a biogas production process. Hydrogen, methane, and carbon dioxide are the main products in this division. Methane is usually combusted in a combustion engine to produce electrical power and heat. It also can be upgraded to remove carbon dioxide and feed it to the local natural

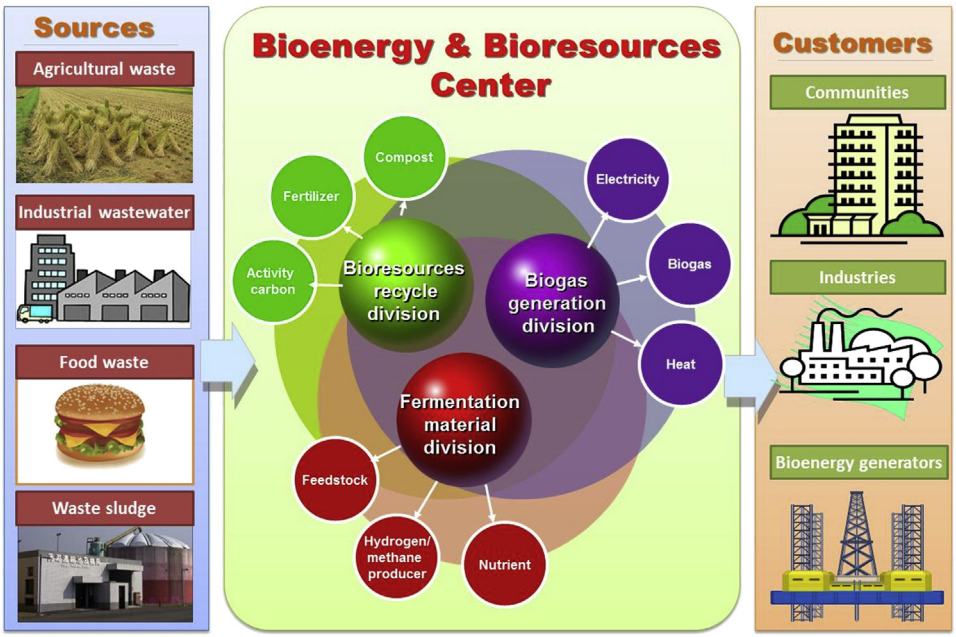

FIGURE 13.10 Concept of a bioenergy and bioresources center.

gas grid [104]. Methane can also be re-formed into hydrogen, which can be converted to electricity and heat via fuel cells and internal combustion engines. Note that carbon dioxide can be obtained through purification from the biogas and is a useful and profitable chemical.

13.6.1.2 Fermentation Material Division

Active hydrogen/methane producers and suitable-composition feedstock are important to anaerobic fermentation. Fermentation material mixed with wastes and wastewaters can improve biogas (hydrogen and methane) production efficiency at a bioenergy factory. For example, carbohydrate-rich feedstock could mix with low carbon/nitrogen ratio materials for hydrogen production.

13.6.1.3 Bioresource Recycling Division

There are some hydrogen fermentation by-products that could be recovered as valuable bioresources such as compost and fertilizer. These products elevate the economic benefits of biogas production.

13.6.2 Potential Locations for Constructing Organic Wastewater-Based Bioenergy Production Systems

There are many potential locations for constructing high-strength organic wastewater-based bioenergy production systems.

13.6.2.1 Biohydrogen System From Industrial Wastewater

The most feasible way to commercialize the biohydrogen–bioenergy generation from wastewater is an on-site system such as factories and communities that produce sufficient high-strength organic wastewaters (Fig. 13.11). Integrating the biohydrogen process with a conventional wastewater treatment process has many advantages such as improving environmental compatibility of the wastewater treatment process and

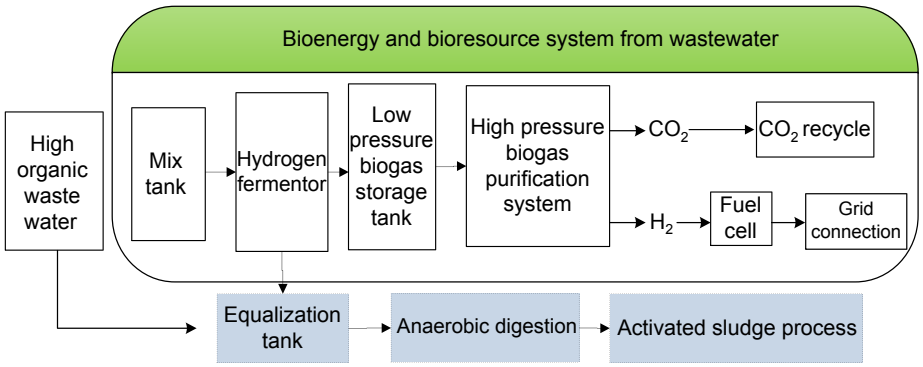

FIGURE 13.11 Concept of an on-site bioenergy and bioresource system using high-strength organic industrial wastewater.

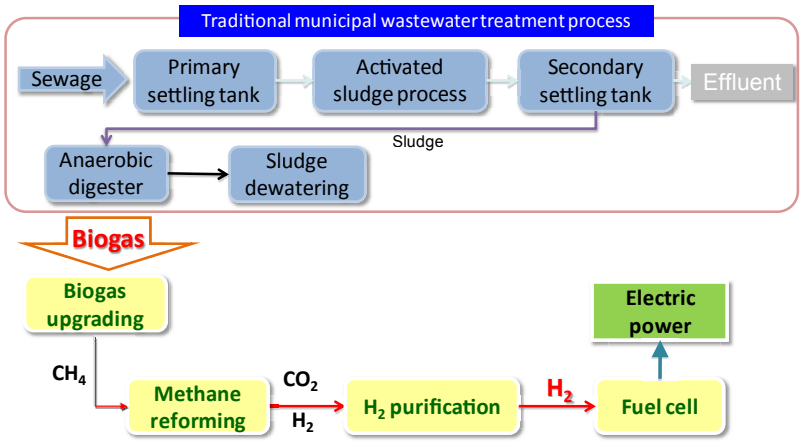

FIGURE 13.12 Concept of a bioenergy center for sewage.

lowering the wastewater treatment cost by increasing COD removal efficiency, reducing sludge production, and generating clean bioenergy. The produced biohydrogen can be fed into a boiler fuel to reduce fossil fuel utilization or converted into electricity by fuel cell for on-site usage. Moreover, the CO_2 produced could be collected and utilized to gain additional benefits for the factory by reducing capital investment.

Moreover, following the goal of elevating the bioenergy capacity, for example, a "water resource recycling center" (originally, sewage treatment plant) in Taiwan could be expanded to a "bioenergy center for sewage," which has multiple functions for municipal sewage treatment and bioenergy generation (Fig. 13.12).

13.6.2.2 Biohydrogen-Based Sustainable Green Energy House

Another biohydrogen energy application is to establish a feasible model of a biohydrogen energy-based sustainable house [117]. The hydrogen-based house confirms the concept of sustainable green energy design by performing the stages of energy production, storage, distribution control, load applications, and recycling and reuse. To increase the efficiency of total energy recovery and to reduce the COD of an organic effluent for discharging into a community sewer system, an anaerobic digester is coupled to a dark hydrogen fermentation process to produce methane using dark fermentation effluent as the substrate at the sustainable green energy house [11]. There are also several alternative feedstocks for bioenergy production in the sustainable green energy houses, such as kitchen waste, convenience store dairy waste, fruit and vegetable market waste, tofu factory waste, and sewage sludge. Support systems in a green energy sustainable house include: (1) a biohydrogen/methane chamber, (2) hydrogen storage/methane tanks, (3) a hydrogen supply system, (4) fuel cells, (5) a power distribution panel, and (6) building power load. A hydrogen filling facility must be added if

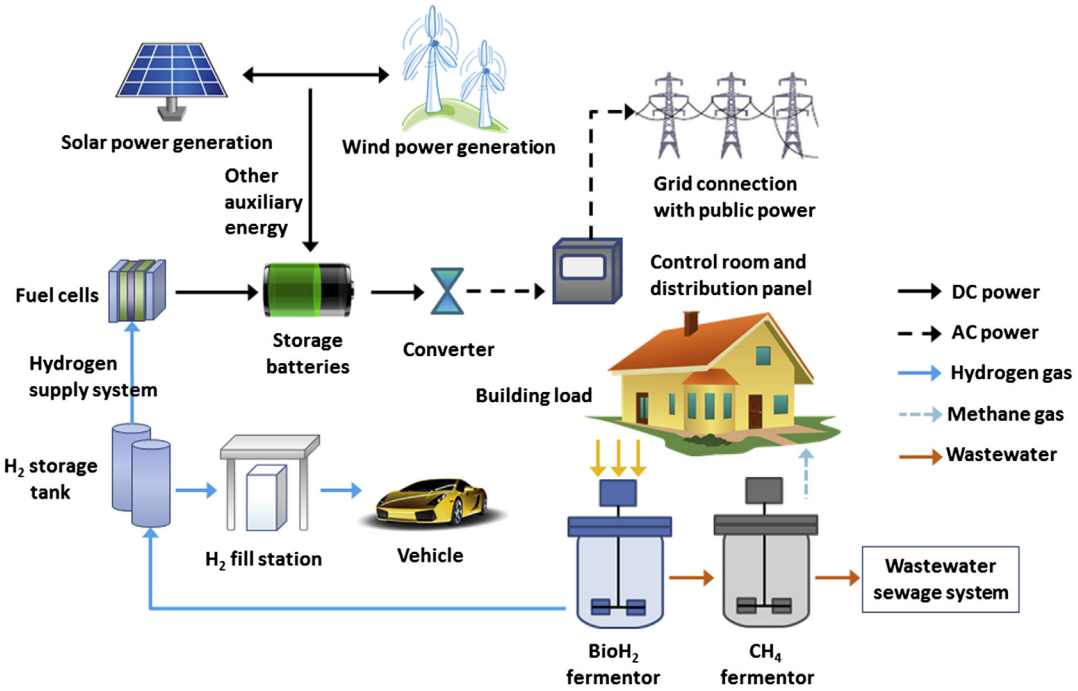

FIGURE 13.13 Concept of a biohydrogen-based sustainable green energy house [11].

hydrogen fuel cell vehicles are used in a future hydrogen energy society. The biohydrogen/methane production chamber should contain a substrate tank, a nutrient tank, a hydrogen production fermenter, a gas–liquid separator, a hydrogen purification device, and a methane fermenter [11]. This biohydrogen-based sustainable green energy house demonstrates a feasible solution for developing a self-sustainable community utilizing biohydrogen and methane as the major energy sources (Fig. 13.13).

13.6.3 Biohydrogen Utopia

Hydrogen is a promising energy carrier, which can be converted into electricity via fuel cell with high efficiency. The clean characteristics give hydrogen an important role in solving global climate change problems and thus a "hydrogen economy" is proposed. Environmentally friendly green hydrogen can be obtained by dark fermentation using nonfood feedstock of waste organic materials. To integrate the up- and downstream technologies of biohydrogen and to evaluate the feasibility of the green hydrogen economy, declaring a hydrogen society scenario and constructing a feasible hydrogen energy technology development road map are the key steps to accelerating the realization of the hydrogen economy (Fig. 13.14) [56].

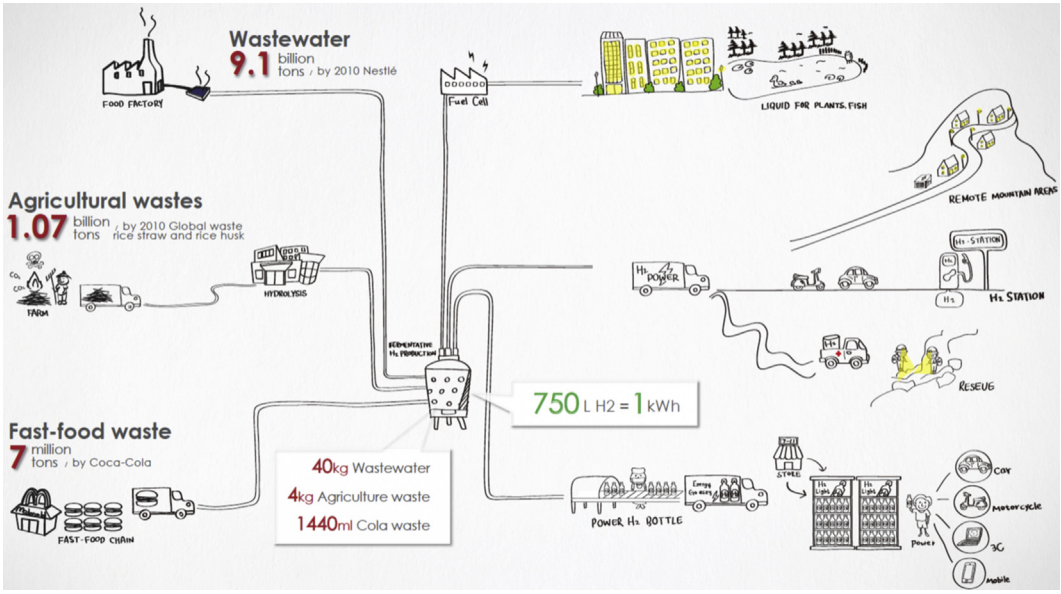

FIGURE 13.14 Concept of "Biohydrogen Utopia" [56].

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14

Dechlorination in Wastewater Treatment Processes

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14.1 Introduction

Dechlorination is a process by which some or most of the chlorine is removed. In the case of wastewaters, it is done for wastewater effluents after chlorine has been applied to remove pathogenic organisms that were not inactivated/removed during the wastewater treatment process or to remove the remaining ammonia in the effluent. The residual chlorine in the wastewater is considered toxic to aquatic life and hence a limit for total residual chlorine in wastewater of <0.01 mg/L is applied in many jurisdictions [1]. The toxicity mainly arises from chloramines [2,3], which are formed during chlorination of organic nitrogen-containing compounds or ammonia, which are abundant in wastewater effluents [4]. Additional challenges arise as these organic chloramines are much more difficult to dechlorinate than free chlorine and are persistent in natural water [75].

Toxicity in wastewater effluents could arise from chlorinated organic compounds and pharmaceuticals that enter into wastewater treatment plants with the influent and are not removed or are converted to less dangerous forms by the wastewater treatment processes [5]. These compounds are rarely monitored and controlled and may induce endocrine disruption or other ecological effects.

Chlorine could be added before any wastewater treatment begins (prechlorination) or during the biological process itself (intermediate chlorination), but dechlorination is not needed to reduce the chlorine level. Prechlorination is used to control odor, which is mostly associated with hydrogen sulfide in the influent wastewater [6]. Intermediate chlorination is used to control the undesirable growth of filamentous microorganisms, by applying chlorine to return sludge. Chlorine can also be used to control algae growth on clarifier weirs and wastewater filters. Most of the chlorine applied before the final effluent is fully lost in reactions with reducing agents in the water.

Toxicity of chlorinated effluents in San Francisco Bay was first reported by Esvelt et al [7,64]. This study proved that chlorinated effluents were more toxic to aquatic life than

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unchlorinated effluents. The studies also showed that a dechlorinated effluent was less toxic than either the chlorinated or the unchlorinated effluent. Dechlorination became an important process since then. Dechlorination is achieved by the addition of a reducing chemical. Most common chemicals used to dechlorinate wastewater treatment plant effluents are based on four-valent sulfur S(IV) such as sulfur dioxide (SO_2) or aqueous solutions of sulfite compounds.

A study reported later showed the importance of the amount of residual chlorine in the effluent [1,8].

- In areas receiving waste waters treated continuously with chlorine, total residual chlorine should not exceed 0.01 mg/L, for the protection of more resistant organisms only, or 0.002 mg/L, for the protection of most aquatic organisms.
- In areas receiving intermittently chlorinated wastes (power plants), total residual chlorine should not exceed 0.2 mg/L for a period of 2 h/day, for more resistant species of fish, or exceed 0.04 mg/L for a period of 2 h/day, for trout or salmon. If free chlorine persists, total residual chlorine should not exceed 0.01 mg/L for a period of 30 min/day for areas with populations of trout and salmon.

Practically, this means zero total chlorine residual in the effluent. These requirements are difficult to meet and to reliably measure. In complying with this requirement, water utilities tend to apply a higher than stoichiometric amount of dechlorinating agents, causing significant issues in terms of oxygen depletion and pH modification downstream of effluent disposal [9].

In addition, when the water has to be reused or recycled, additional considerations are needed depending on the intended use. For example, if water needs to be conserved and distributed for recycling purposes such as for toilet flushing, irrigation, and washing clothes, then there needs to be disinfectant residual to minimize the risk of microbial contamination [10]. If the right amount of chlorine is added, with sufficient time to ensure minimum CT [concentration (mg/L) $\times$ time (min)] requirement to meet microbiological criteria, then there is no need to dechlorinate the water, as the presence of disinfectant residual is essential for preventing bacterial regrowth, accidental consumption in case of dual pipe connection to residences [11], or depletion of dissolved oxygen encouraging sulfate-reducing bacteria, which eventually create malodor and discoloration [12]. If superchlorination is practiced, the dechlorination has to be applied carefully to control the concentration of chlorine residual at the desirable level.

Wastewater could also be recycled for cooling purposes, but that requires a low dissolved solids concentration, which is often achieved by desalination by reverse osmosis (RO) as successfully used in the Kwinana Industrial Estate, Western Australia [13]. Other applications, such as concrete mixing, may be sensitive to sulfate concentration, so the use of sulfur dechlorination needs to be closely considered not to interfere with concrete properties [14].

Aquifer recharge and direct potable reuse application require much more stringent requirements, needing RO treatment or advanced oxidation processes. All these processes are not always foolproof and require additional considerations [5], for example,

N-nitrosodimethylamine (NDMA) is formed during oxidation of wastewater constituents and is persistent [15].

For the benefit of the reader, various aspects, including chlorination, are also reviewed.

14.2 Chlorine Chemistry

Chlorine is used as a disinfectant to control waterborne diseases and pathogenic organisms in water and in the wastewater treatment process [16]. Chlorine is used in wastewater treatment for odor control, septicity control, and activated sludge bulk control and cyanide destruction [6]. Here only a summary of chlorine chemistry sufficient for the understanding of the chapter is given. For a detailed account the reader is referred to White [1].

14.2.1 Generation

Chlorine is mostly added as a gas. However, the addition of other chlorinated compounds such as sodium hypochlorite solution and dry calcium hypochlorite is also practiced [17]. The use of sodium hypochlorite solution is limited by its decomposition at higher temperature and formation of unwanted compounds (chlorite ions or chlorate) [18].

There are a number of ways to produce chlorine gas, such as electrolysis of alkaline brine (sodium chloride) or hydrochloric acid, the reaction between sodium chloride and nitric acid, and the oxidation of hydrochloric acid [16]. Practically all chlorine these days is produced by electrolysis of NaCl according to the following equation:

$$2NaCl + 2H_2O \rightarrow Cl_2 + H_2 + 2NaOH$$

In electrolysis chlorine production, the material of the electrode is critical and a lot of research has been done on cost-efficient methods [19,20]. Most suitable materials for chlorine production would have high selectivity, easy availability, low cost, mechanical and chemical stability, and a desirable health safety record [21]. Nanocrystalline titanium dioxide (TiO_2) thin-film electrodes have been proposed as practical for use in industrial production [20].

14.2.2 Important Chlorine Reactions

When chlorine is added to the water it reacts according to the following equation:

$$Cl_2 + H_2O \rightarrow HOCl + HCl \qquad [14.1]$$

Hypochlorous acid (HOCl) acts as a potent oxidizing agent and immediately begins to react with numerous organic and inorganic compounds found in the water. The hypochlorous acid dissociates into hydrogen ions (H^+) and hypochlorite ions in the reversible reaction:

$$HOCl \rightleftharpoons H^+ + OCl^- \qquad [14.2]$$

Hypochlorous acid is a weak acid with a pK_a of 7.53 at 25°C [22]. Hypochlorous acid, the key disinfecting agent, is much more effective (about 80 times) than hypochlorite ion (OCl^-). Owing to its dissociation constant, HOCl predominates at a pH below 7.53, and hypochlorite ion at pH above 7.53.

In wastewater, chlorine reacts with a variety of components including bacteria, viruses, ammonia nitrogen, organic nitrogen, hydrogen sulfide, tannins, cystine, uric acid, humic acid, pickle liquor, cyanides, and phenols [23]. In plant effluents, a substantial amount of ammonia nitrogen exists in the form of either ammonia (NH_3) or ammonium ion (NH_4^+), and the relative abundance is dependent on pH and temperature. The pK_a value being 9.24, when the wastewater pH is around 7 and chlorine is dosed, it reacts with ammonium forming chloramines:

$$HOCl + NH_4^+ \rightleftharpoons NH_2Cl\ (\text{monochloramine}) + H_2O + H^+ \quad [14.3]$$

$$HOCl + NH_4^+ \rightleftharpoons NHCl_2(\text{dichloramine}) + H_2O + H^+ \quad [14.4]$$

$$HOCl + NH_4^+ \rightleftharpoons NCl_3(\text{nitrogen trichloride}) + H_2O + H^+ \quad [14.5]$$

If the pH drops below 7, dichloramine ($NHCl_2$) begins to form, and at a much lower pH, nitrogen trichloride (NCl_3) is produced [1,24–26]. Dichloramine and trichloramine also form when the chlorine-to-ammonia molar ratio exceeds 1 at a stable pH of 8 for monochloramine.

Among all of these chloramine types, monochloramine is the only useful disinfectant. $NHCl_2$ and NCl_3 are too unstable to be useful and are highly malodorous [22].

In addition to the stated chloramine types, organochloramines, which do not have a practical germicidal effect, always occur in waste water chlorination owing to the presence of organic nitrogen compounds:

$$HOCl\ +\ R\text{-}NH_2 \rightarrow R\text{-}NHCl\ (\text{organic chloramine}) \quad [14.6]$$

In the chlorination process, chlorine may form a bond with carbon in dissolved natural organic matter (DNOM) and produce various disinfection by-products (DBPs) [23]. Reaction with DNOM follows parallel first-order decay with two components, namely fast- and slow-reacting agents [27,67]), defined by the equation

$$\frac{dC_{Cl}}{dt} = -k_F \times C_{Cl} \times C_F - k_S \times C_{Cl} \times C_S \quad [14.7]$$

where C_{Cl}, C_F, and C_S represent concentrations of chlorine, fast-reacting agents, and slow-reacting agents, respectively, and k_F and k_S are decay-rate coefficients of fast- and slow-reacting agents. To solve this complex differential equation, Jabari Kohpaei and Sathasivan [28] proposed an analytical solution to make it easy for researchers and utility operators to adopt the technique.

When chlorine reacts with water it forms several unintended DBPs. Trihalomethanes (THMs) and haloacetic acids (HAAs) were the first chlorine halogenated DBPs reported [29]. Furthermore, haloacetonitriles, haloketones, chlorophenols, chloropicrin, chloral hydrate,

and cyanogen chloride were also identified later [22]. Inspite of the ability to form such compounds the measured amounts in the effluent are low enough to cause concern ([68]; Asano, 1993).

Full mineralization can be achieved for some organic compounds by chlorine according to the following equations:

$$C_5H_7O_2N + 10HOCl \rightarrow 4CO_2 + HCO_3^- + NH_4^+ + 10H^+ + 10Cl^- + H_2O \quad [14.8]$$

$$C_5H_7O_2N + 10OCl^- \rightarrow 4CO_2 + HCO_3^- + NH_4^+ + 10Cl^- + H_2O \quad [14.9]$$

Chlorine reacts with other inorganic species such as ferrous (Fe^{2+}), manganous (Mn^{2+}), and nitrite (NO^{2-}) ions and sulfide (S^{2-}). These ions are oxidized to ferric (Fe^{3+}), manganic (Mn^{4+}), nitrate $\left(NO_3^-\right)$, or S or H_2SO_4 species, respectively [23]:

$$2Fe^{2+} + Cl_2 \rightarrow 2Fe^{3+} + 2\,Cl^- \quad [14.10]$$

$$2Mn^{2+} + Cl_2 \rightarrow 2Mn^{4+} + 2Cl^- \quad [14.11]$$

$$H_2O + NO_2^- + Cl_2 \rightarrow NO_3^- + 2HCl \quad [14.12]$$

In dilute aqueous solutions, the production of colloidal sulfur or sulfates occurs from the reaction of chlorine with sulfides, which depends on the pH, temperature, and ratio of chlorine to sulfides:

$$HOCl + H_2S \rightarrow S\downarrow + HCl + H_2O \quad [14.13]$$

$$H_2S + 4HOCl \rightarrow H_2SO_4 + 4HCl \quad [14.14]$$

Bromide, if present in the effluent, is also rapidly oxidized by chlorine into bromine, which can also act as a disinfectant [23].

Similar to chlorine, chloramine reacts with organic and inorganic compounds and chloramine decays with time [30,66]). However, chloramine is a weak oxidant and reacts slowly compared to chlorine and hence takes more time to chemically decay. In addition to chemical decay, microbes including nitrifiers can decay chloramine [31] as much as 10 times the chemical decay [32]. Such decay is reported in drinking water supply systems, but in wastewater chlorination/chloramination such mechanisms have not been reported. It is likely that such mechanisms could exist at the point where wastewater effluents are disposed.

14.2.3 Breakpoint Chlorination

To achieve a strong disinfection effect within the short retention time often available in wastewater effluents, free chlorine should be formed, implying that chlorine should be added to destroy all forms of inorganic chloramines. Breakpoint chlorination is the reaction of excess chlorine with ammonia, i.e., a molar ratio of $Cl_2/NH_3 > 1.5$. When chlorine is added to water, first it reacts with ammonia to form chloramine (Eq. [14.3]). Additional chlorine reacts with chloramine, finally oxidizing it to nitrogen (Eq. [14.15]).

Evidence of breakpoint chlorination can be noted when the amount of chlorine is reduced despite the addition of chlorine. Detailed breakpoint chlorination chemistry is described in standard textbooks:

$$2NH_2Cl + HOCl = N_2 + 3HCl + H_2O \quad [14.15]$$

14.2.4 Disinfection Kinetics

14.2.4.1 Chick's Law

The primary purpose of chlorine addition is disinfection of pathogenic microbes present in the effluent. Harriette Chick in 1908 described the reduction in bacteria with respect to chlorine dose and time of exposure [16]. When a monoculture of microorganisms is exposed to a concentration of disinfectant, the reduction in microorganisms follows a first-order reaction:

$$\frac{dN}{dt} = -k \times N \quad [14.16]$$

or

$$N = N_0 e^{-kt} \quad [14.17]$$

where N is the number of microorganisms (N_0 is the initial number of microorganisms), k is the disinfection constant (s^{-1}), and t is the contact time (s).

14.2.4.2 Chick–Watson Model

In 1908, Herbert Watson modified Chick's model and proposed his description,

$$C^n \times t = K_r, \quad [14.18]$$

where C is the concentration of disinfectant (mg/L), n is the empirical constant (—), t is the time (s), and K_r is the empirical value for a percentage of inactivation (e.g., 99%).

In practice, the CT value for the most difficult pathogen to disinfect is used. The chosen difficult pathogen is *Giardia lamblia*. Standard tables are available, for example, in [6].

14.3 Chemicals Used in Dechlorination

Chlorine is used in many industrial and municipal waters to disinfect, oxidize, or bleach. However, a chlorine residuals are toxic to certain fish and other aquatic life even at very low levels, water can be discharged to receiving waters only after all the chlorine (<0.01 mg/L total Cl) has been removed. Such a low concentration of chlorine cannot be achieved without dechlorination.

Dechlorination is practiced to reduce the toxicity of chlorine by removing the free and combined chlorine residual remaining after chlorination. It is also reported that

dechlorination diminishes the genotoxicity of chlorinated secondary effluent [33]. Sulfur dioxide gas is the most commonly used dechlorinating agent. Here sulfur 4^+ is oxidized by chlorine to sulfate sulfur 6^+. Other compounds containing sulfur(IV), such as sodium sulfite, sodium bisulfite, or sodium metabisulfite, can be substituted for sulfur dioxide. An additional sulfur compound used for dechlorination is thiosulfate, but it is not converted to sulfate in the first stage, but to tetrathionate, which introduces complexity. All dechlorination compounds are reviewed in [34].

14.3.1 Sulfur Dioxide

Sulfur dioxide (SO_2) is a nonflammable, colorless gas, which may be liquefied to a colorless liquid with a specific pungent odor [69]. The main difference between chlorine and SO_2 is the lower vapor pressure of SO_2 (at 21°C the SO_2 vapor pressure is ~240 kPa) compared with chlorine (at 21°C the chlorine vapor pressure is ~620 kPa). The problems associated with low vapor pressure include low withdrawal, which does not occur to the same extent when chlorine is handled. Nonetheless, the high solubility of SO_2 (120 g/L) compared with chlorine (7 g/L) makes it easier to dissolve in water [1].

Sulfur dioxide is stable and nonflammable in the gas or liquid phase. It is exceptionally corrosive in the presence of any moisture, as is chlorine. Therefore, special materials are used for storage of sulfur dioxide.

The initial reaction when SO_2 is added to water is shown in Eq. [14.19]:

$$SO_2 + H_2O \rightarrow H_2SO_3 \quad [14.19]$$

SO_2 dissolves in water rapidly, forming sulfurous acid. Chlorine residual species react with the sulfurous acid:

$$HOCl + H_2SO_3 \rightarrow HCl + H_2SO_4 \quad [14.20]$$

$$NH_2Cl + H_2SO_3 + H_2O \rightarrow NH_4Cl + H_2SO_4 \quad [14.21]$$

$$NHCl_2 + 2H_2SO_3 + 2H_2O \rightarrow NH_4Cl + HCl + 2H_2SO_4 \quad [14.22]$$

$$NCl_3 + 3H_2SO_3 + 3H_2O \rightarrow NH_4Cl + 2HCl + 3H_2SO_4 \quad [14.23]$$

According to these equations all of the chlorine species can be dechlorinated with SO_2. The production of H_2SO_4 can affect the alkalinity and pH of the dechlorinated water. As water is sufficiently buffered, there is no need to consider the compensation for pH. There is a chance that excess sulfur dioxide can consume dissolved oxygen in a receiving water source:

$$SO_2 + H_2O + \frac{1}{2}O_2 \rightarrow H_2SO_4 \quad [14.24]$$

However, this reaction is very slow and does not contribute to significant reduction of the dissolved oxygen concentration in natural waters.

As mentioned, sulfur dioxide is toxic and a person exposed to a dose of SO_2 usually experiences only acute irritation, which is alleviated in open air. Lower concentrations of SO_2 can cause coughing, sneezing, burning of the eyes, and a sensation of suffocation [69].

14.3.2 Sulfite Compounds

There are four sulfur compounds that are used as alternatives to SO_2 dechlorination: sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), sodium metabisulfite ($Na_2S_2O_5$), and sodium thiosulfate ($Na_2S_2O_3$). These sulfite compounds are used for dechlorination where SO_2 is not practical to use, for example, when storage of compressed or liquefied gas is not desirable [1].

14.3.2.1 Sodium Sulfite

Na_2SO_3 is a soluble sodium salt that is available as a white powder or as crystals. Because of its hygroscopic nature, it is difficult to handle in the dry form and often is delivered as a solution. Dechlorination with the addition of Na_2SO_3 [or any other sulfur(IV) compound] decreases the genotoxicity in the chlorinated secondary effluent containing ammonia and other nitrogen compounds [33]. Like in other S $^{(1V)}$ compounds the sulfur is oxidized by chlorine or chloramines to sulfate:

$$Na_2SO_3 + Cl_2 + H_2O \rightarrow 2NaCl + H_2SO_4 \quad [14.25]$$

14.3.2.2 Sodium Bisulfite

$NaHSO_3$ is a white powder or granular compound, which can be dissolved in water. The solution is commonly dosed for removal of free or combined chlorine in the dechlorination process. The reaction with chlorine is presented in Eq. [14.26]:

$$NaHSO_3 + Cl_2 + H_2O \rightarrow NaHSO_4 + 2HCl \quad [14.26]$$

This reducing agent can reduce dissolved oxygen if dosed in large excess. Availability in tablet form is the main advantage of using $NaHSO_3$, which has an approximately 1 year shelf life under good storage conditions [34]. $NaHSO_3$ is reported as an irritant to the eyes, skin, mucus membranes, and respiratory tract, mainly due to the release of sulfur dioxide [72].

14.3.2.3 Sodium Metabisulfite

$Na_2S_2O_5$ is also a good reducing agent, which is available as crystals, powder, or solution [1,34]. $Na_2S_2O_5$ creates problems when storing, transporting, and handling, as again it tends to release SO_2 in contact with the air, which contains carbon dioxide. The reaction with chlorine produces the strong acids HCl and H_2SO_4, which may affect the pH of the treated water [34]:

$$2Na_2S_2O_3 + 2Cl_2 + 3H_2O \rightarrow 2NaHSO_4 + 4HCl \quad [14.27]$$

Overexposure to $Na_2S_2O_5$ could be highly toxic and can cause oral and esophageal burns if it enters the body (Grotheer et at., 2014).

14.3.2.4 Sodium Thiosulfate

$Na_2S_2O_3$ is not commonly used as a plant-scale dechlorinating agent because its full reaction with chlorine is slow and highly pH dependent [1] before the thiosulfate is fully converted to sulfate. The first and relatively fast reaction is to tetrathionate:

$$2Na_2S_2O_3 + Cl_2 \rightarrow 2NaCl + Na_2S_4O_6 \quad [14.28]$$

Subsequently tetrathionate is slowly oxidized to sulfate. The advantage of thiosulfate is that it is relatively stable and nontoxic [1]. The overall reaction between thiosulfate and chlorine is shown in Eq. [14.29]:

$$Na_2S_2O_3 + 4Cl_2 + 5H_2O \rightarrow 2NaHSO_4 + 8HCl \quad [14.29]$$

Apart from this advantage, the use of SO_2 generates handling problems, which necessitate expensive storage and containment facilities [9]. Prior to dechlorination with bisulfite solids, it must be dissolved into solution, and frequent heating of the solution is essential to prevent freezing in colder months [1].

14.3.3 Activated Carbon

The use of activated carbon in the dechlorination process is a very attractive and effective technique, but it is very expensive (EPA, 2000). Such process is used in dechloraminating water before dialysis in hospitals [73]. In this method, the residual chlorine or chloramine is eliminated by oxidizing the surface of the activated carbon, which is different from the adsorption phenomena of removing the organic compounds [35].

The reaction between free chlorine and activated carbon is

$$C + HOCl \rightarrow CO^* + HCl \quad [14.30]$$

where CO* indicates surface oxide on carbon.

Alternatively, the reaction can progress to carbon dioxide according to Eq. [14.31]:

$$C + 2Cl_2 + 2H_2O \rightarrow 4HCl + CO_2 \quad [14.31]$$

Part of the carbon is permanently destroyed during this process [1,34].

Based on these equations up to 12 g of carbon can be consumed by 71 g of chlorine. Obviously, only a small proportion of granular activated carbon (GAC) can be consumed before the pores of the GAC start to crumble. Therefore, destroyed carbon must be removed as fines or the whole bed replaced with new carbon to maintain the function of the system [63].

This process does not change the mineral content of the water (Na^+ and SO_4^{2-}) and does not deplete the dissolved oxygen level, which occurs when sulfur-based dechlorination processes are used.

Activated carbon can be used as a powdered form and watery slurry or most frequently in the form of a GAC column. The limitation of the process is that it has limited

effectiveness for reduction of chloramines and there can be an increase in bacteria growing on the activated carbon surface. Because of these limitations the activated carbon has only limited application as a dechlorination process even when application of a GAC column is fairly simple and does not require sophisticated control strategy.

14.3.4 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is an alternative chemical for dechlorination. However this is not frequently used because it is dangerous to handle [69]. It has an advantage where excess SO_2 is toxic to aquatic life and imposes an oxygen demand on the receiving water source. In the receiving water, there is usually a sufficient amount of iron and manganese hydrated oxides, which rapidly decompose any excess hydrogen peroxide to oxygen and water.

H_2O_2 reacts rapidly with free chlorine available in the system in which the pH is more than 7. Therefore, H_2O_2 can be effectively used to dechlorinate effluents from caustic/chlorine odor scrubbers. Even if there is no upper limit to the pH, 8.5 is considered a desirable pH to provide a rapid reaction [6].

$$Cl_2 + H_2O_2 \rightarrow O_2 + 2HCl \qquad [14.32]$$

In the majority of cases the produced oxygen can be dissolved in water and is beneficial for the receiving waters.

Reaction of H_2O_2 with combined chlorine is very slow compared to the free chlorine reaction. Accordingly, most municipal wastewater effluent solutions that contain ammonia cannot be dechlorinated with H_2O_2 [1].

14.4 Fate of Chlorinated Compounds in Wastewater Treatment Plants

14.4.1 Chlorinated Organic Compounds in Wastewater

Large numbers of chlorinated organic compounds are identified in water bodies receiving domestic and industrial wastewater effluents. Some of these compounds, such as polychlorinated biphenyls (PCBs), are endocrine-disrupting and/or toxic and have been well studied for many years. The presence of some chlorinated organic compounds, such as the pharmaceutical diclofenac, triclosan, 4,4′-dichlorocarbanilide, and the pesticides clomazone and 4-hydroxychlorothalonil, has been investigated. Table 14.1 summarizes the reported chlorinated organic compounds in influent and effluent wastewater reported from several countries.

Aquaculture industry wastewater discharges chlorinated contaminants such as hexachlorobenzene, mirex, and chlordane into the environment. Petroleum industry wastewater discharges some chlorinated contaminants such as PCBs, and pulp and paper industry wastewater discharges chloroform and dioxin.

Table 14.1 Fate of Some Chlorinated Contaminants in Wastewater Treatment Plants (Activated Sludge Process)

Compound	Wastewater	Influent Concentration	Effluent Concentration	Country	References
Diclofenac	25% industrial, 75% domestic	691 ± 18 ng/L[a]	481 ± 6 ng/L[a]	Luxembourg	Majewsky et al. [36]
	Domestic	470–1920 ng/L[b]	310–930 ng/L[b]	Switzerland	Buser et al. [37]
	Mostly domestic	188 ± 63 ng/L[c]	147 ± 76 ng/L[c]	Norway	Plósz et al. [38]
	20% industrial, 70% domestic	1600 ± 500 ng/L[c]	410 ± 260 ng/L[c]	Greece	Samaras et al. [39]
	Domestic	1220 ± 510 ng/L[c]	800 ± 390 ng/L[c]	Greece	
Triclosan	25 facilities in 18 US states	4.1 ± 1.1 μg/L[d]	0.07 ± 0.04 μg/L[d]	USA	Heidler and Halden [40]
	50% industrial, 50% domestic	1.2 ± 0.08 μg/L[a]	0.051 ± 0.008 μg/L[a]	Germany	Bester [41]
	Both industrial and domestic	1.52–4.43 μg/L[e]	0.49–0.56 μg/L[e]	USA	Katz et al. [42]
	20% industrial, 70% domestic	1.56 ± 0.43 μg/L[c]	0.11 ± 0.03 μg/L[c]	Greece	Samaras et al. [39]
	Domestic	1.42 ± 0.31 μg/L[c]	0.13 ± 0.08 μg/L[c]	Greece	
Triclocarban	25 facilities in 18 US states	4.2 ± 0.8 μg/L[d]	0.23 ± 0.08 μg/L[d]	USA	Heidler and Halden [40]
	2% industrial, 98% domestic	6.1 ± 2.0 μg/L[c]	0.17 ± 0.03 μg/L[c]	USA	Heidler et al. [43]
4,4′-Dichlorocarbanilide	25 facilities in 18 US states	90 ± 40 ng/L[d]	40 ± 10 ng/L[d]	USA	Heidler and Halden [40]
Diuron	25% industrial, 75% domestic	318 ± 209 ng/L[a]	132 ± 173 ng/L[a]	Luxembourg	Majewsky et al. [36]
Terbuthylazine	25% industrial, 75% domestic	24 ± 103 ng/L[a]	34 ± 94 ng/L[a]	Luxembourg	Majewsky et al. [36]
2-Methyl-4-chlorophenoxyacetic acid	25% industrial, 75% domestic	108 ± 150 ng/L[a]	59 ± 171 ng/L[a]	Luxembourg	Majewsky et al. [36]
Fipronil	25 facilities in 18 US states	30 ± 10 ng/L[d]	30 ± 10 ng/L[d]	USA	Heidler and Halden [40]

[a]Mean values ± day-to-day standard deviation of samplings of a single wastewater treatment plant (WWTP). [b]Range of concentrations found from a total of five sampling dates from three WWTPs. [c]Mean ± standard deviation from one WWTP. [d]Median ± 95% of 25 WWTPs. [e]Range of concentrations from a single WWTP.

14.4.2 Harmful By-products Formation/Reduction during the Activated Sludge Process

Chlorination is a widely used disinfection method in conventional wastewater treatment processes, in which by-products formation is identified as a major drawback. Bedner and MacCrehan [44] investigated the reactions of the amine-containing drugs fluoxetine and metoprolol during the chlorination and dechlorination processes used in the activated sludge process. They observed the reactions of both compounds with chlorine during the disinfection process to produce *N*-chloramine. They also studied the reactivity of the *N*-chloramines with sulfite to simulate dechlorination, which is often employed in wastewater treatment and they confirmed the reactions. Katsoyiannis and Samara [45] investigated the fates of several persistent chlorinated organics in the conventional activated sludge treatment process and concluded that most chlorinated compounds are not eliminated by the process (Table 14.2). They selected a wastewater treatment plant (WWTP) that serves about 1 million residents and treats 120,000−150,000 tons/day of raw wastewater, which consists of 5−10% industrial wastewater. The treatment process included screening, grit removal, and primary sedimentation without the use of chemical coagulants, conventional activated sludge treatment, and effluent disinfection using chlorine. The dechlorinating chemical was not mentioned.

Importantly, they detected isobenzan in the samples collected from the treatment unit operations but not in the influent wastewater. Endrin levels in the influent wastewater also showed lower concentrations than the levels observed in the samples collected from the treatment unit operations (Table 14.2). The results of this study suggest the possibility of the synthesis of isobenzan and endrin during the wastewater treatment using the activated sludge process.

Despite a widespread investigation of THMs and HAA as chlorination disinfectant by-products in tap water, limited studies have investigated the THM and HAA levels in the effluents of WWTPs. Tang et al. [46] investigated THM and HAA levels in the effluent of eight WWTPs using biological treatment processes. The average (range) THM and HAA levels reported in this study were 277 (130−500) and 494 (300−710) μg/L, respectively. They also reported a close correlation between the efficiency of the treatment process and DBP formation. As volatile compounds, THMs easily escape to the air while in the natural environment and HAA is easily metabolized by the microbes [74].

Treatment of nitrogen-rich wastewater raises concerns regarding the formation of haloacetonitriles (HANs), such as HANs and haloacetamides. As a chemical class, the HANs are more toxic than regulated carbon-based DBPs, such as THM and HAA. The toxicity of HANs may become a health concern because of the increased use of alternative disinfectants, such as chloramines, which may enhance the formation of HANs, which may induce acute genomic DNA damage [47]. HANs are by-products of water chlorination and may form in vivo from the reaction of residual chlorine with endogenous compounds such as amino acids. According to Krasner et al. [48], the concentration of HANs in WWTP effluents after chlorine addition was undetectable to 12 μg/L (median and 75th percentile

Table 14.2 Concentration of Chlorinated Organic Compounds in Various Stages at the Thessaloniki Wastewater Treatment Plant [45]

Chlorinated Organic Compound	Raw Wastewater (After Grit Chamber)	Primary Sedimentation Tank	Secondary Sedimentation Tank	Sludge From Primary Sedimentation Tank	Activated Sludge	Final Sludge
	Mean	Mean	Mean	Mean	Mean	Mean
	ng/L	ng/L	ng/L	ng/g (dw)	ng/g (dw)	ng/g (dw)
Isobenzan	ND	0.35	0.23	1.9	6.1	15
Endrin	1.8	ND	2.8	5.6	ND	ND
Hexachlorobenzene	20	9.1	1.7	11	13	6.8
Quintozene	60	35	14	20	30	20
α-Endosulfan	51	34	2.7	28	3.5	6.4
α-Hexachlorocyclohexane	39	26	6.2	11	9.2	5.0
β-Hexachlorocyclohexane	26	8.8	6.3	1.1	21	8.2
γ-Hexachlorocyclohexane	1.4	1.3	0.57	3.1	2.0	10
Aldrin	10	ND	ND	ND	ND	ND
Isodrin	ND	ND	ND	ND	ND	ND
Dieldrin	27	20	8.9	45	19	15
Heptachlor	46	11	6.4	13	43	40
Heptachlor-exo-epoxide	330	170	25	240	200	270
Heptachlor-endo-epoxide	ND	ND	ND	ND	ND	ND
p,p′-Dichlorodiphenyl-dichloroethylene	12	1.6	0.23	13	24	27
Hexachlorobutadine	ND	ND	ND	ND	ND	ND
Dichlobenil	ND	ND	ND	ND	ND	ND
p,p′-DDD	22	17	6	67	8.3	78
p,p′-DDT	6.9	ND	ND	ND	ND	ND
PCB-28	4.8	4.5	3.3	5.6	11	6.8
PCB-52	390	220	110	115	300	160
PCB-101	260	130	45	150	120	91
PCB-118	15	12	5.9	18	13	30
PCB-153	14	10	0.98	17	9.8	22
PCB-138	11	4	2.9	15	10	22
PCB-180	340	250	74	140	150	210
ΣPolychlorinated biphenyls	1000	630	250	460	620	550

PCB, polychlorinated biphenyl.

levels of 0.3 and 0.8 μg/L, respectively), and chloropicrin was present from undetectable levels to 0.6 μg/L. However, they detected most of the HANs and all of the chloropicrin before chlorine addition at these WWTPs (after chlorine addition, the median and 90th percentile increases in HANs were 0.3 and 2.8 μg/L, respectively).

Some organic compounds are not fully degraded in the conventional wastewater treatment process and some of the organic compounds can escape through the secondary treatment process owing to inefficiency of the treatment process. Those organics may react with chlorine in the disinfection process yielding chlorine-containing organic

compounds [49], some of them persistent are and toxic. In the early 1970s, Barnhart and Campbell [50] also reported that persistent organic chemicals readily react with chlorine to produce chlorine-containing persistent organic compounds. Benzenoid compounds with chloro groups are possible end products, which are more resistant to microbial degradation if discharged into the environment.

The potent carcinogen NDMA is a commonly reported DBP in conventional WWTPs [75]. NDMA is produced by reacting monochloramine and organic nitrogen-containing precursors in the disinfection process [65]. Monochloramine is formed when water containing ammonia is dosed with chlorine (Eq. [14.3]). There are several hypothesis suggested for the formation of NDMA during the disinfection of secondary wastewater. Some researchers have hypothesized that NDMA formation during wastewater treatment is attributable to the presence of nitrite [52]. The pathway suggested by Mitch and Sedlak [51] for the formation of NDMA includes slow formation of 1,1-dimethylhydrazine by the reaction of monochloramine and dimethylamine followed by its rapid oxidation to NDMA and other products, including dimethylcyanamide and dimethylformamide. Other pathways also led to NDMA formation during chlorination, such as the reaction of sodium hypochlorite with dimethylamine. The authors also observed strong pH dependence in the proposed pathway. Mitch and Sedlak [51] measured the NDMA formation after extended chlorination of samples from conventional and advanced WWTPs. They reported that the dissolved NDMA precursors were always present in primary and secondary effluents of municipal WWTPs. Biological treatment effectively remove the known NDMA precursor dimethylamine, lowering its concentration to levels that could not produce significant quantities of NDMA upon chlorine disinfection. However, biological treatment was less effective at removing other dissolved NDMA precursors, even after extended biological treatment. The RO process generally used to reclaim wastewater for potable use is known not to be effective in removing NDMA. They suggested the following strategies for the prevention of NDMA formation during wastewater chlorination.

- Include ammonia removal by nitrification to preclude chloramine formation during chlorine disinfection.
- Eliminate dimethylamine-based polymers.
- Use filtration and RO to remove particle-associated precursors and dissolved precursors.

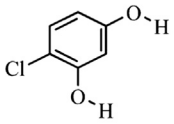
14.5 Impacts of Compounds in Chlorinated Effluents

14.5.1 Impact on Aquatic Life

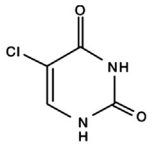
The introduction of chlorinated organics into the aquatic environment is of great environmental concern because of their potential toxicity to various aquatic

organisms. Only a few chlorinated toxic organic compounds have been studied so far and limited toxicology knowledge is available for most of the chlorinated organics. To fully understand the effects of various chlorinated compounds, which are constantly newly produced and introduced into the aquatic environment, each compound should be investigated individually and it requires an enormous amount of resources and effort. This chapter is limited to some compounds for which the effects are known.

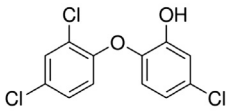
Gehrs et al. [53] reported that 5-chlorouracil and 4-chlororesorcinol, which are among the constituents of chlorinated effluents, decreased the hatchability of crab eggs at concentrations as low as 1 ppb.



4-chlororesorcinol



5-chlorouraciltriclosan



Triclosan

Some of the chlorinated organic compounds are endocrine disrupters and the aquatic environment is more sensitive to them than mammals. For example, triclosan is a commonly detected chlorinated hydrocarbon in aquatic ecosystems, as it is only partially removed during the wastewater treatment process (Table 14.1). Sorption, biodegradation, and photolytic degradation mitigate the availability of triclosan to aquatic biota; however, the by-products, such as methyltriclosan and other chlorinated phenols, may be more resistant to degradation and have higher toxicity than the parent compound. The continuous exposure of aquatic organisms to triclosan, coupled with its bioaccumulation potential, has led to detectable levels of the antimicrobial in a number of aquatic species. Research suggests that there is strong evidence that aquatic species such as algae, invertebrates, and certain types of fish are very sensitive to triclosan, which alters reproductive and developmental cycles in some fish [54].

Some chlorinated organic compounds are very stable and bioaccumulative. High levels of persistent chlorinated organic compounds have been reported particularly in aquatic mammals. Anderson and DeFoe have observed behavior changes of some aquatic animals when they are exposed to endrin [55]. Endrin is a chlorinated organic compound, which was first produced as an insecticide, as well as a rodenticide and pesticide. However, some researchers have reported that the level of endrin is increased along the conventional wastewater treatment process (Table 14.2) [45], suggesting some processes in conventional wastewater treatment can form endrin and it could be avoided if understood.

14.5.2 Impact on Public Health

Most of the chlorinated organic compounds exhibit various toxic effects to people, such as endocrine dysfunction, developmental impairment, birth defects, reproductive dysfunction and infertility, immunosuppression, and cancer, even at extremely low doses [56]. Therefore, it is extremely dangerous to discharge treated wastewater with chlorinated organic compounds to environmental water bodies.

NDMA is a commonly reported by-product of wastewater chlorination. Measurements made in an effluent-dominated river suggest that although NDMA may be removed after wastewater effluent is discharged, wastewater-derived NDMA precursors could persist long enough to form significant concentrations of NDMA in drinking water treatment plants that use water originating from sources that are subjected to wastewater effluent discharges [57].

NDMA is very harmful to the liver of humans [58]. People who were intentionally poisoned on one or several occasions with unknown levels of NDMA in beverage or food died of severe liver damage accompanied by internal bleeding. Limited literature data are available to explain the correlation between NDMA and human cancer; however, a number of animal studies have suggested a strong positive correlation. Animals that ate food, drank water, or breathed air containing high levels of NDMA over a period of days or several weeks also developed serious, noncancerous, liver disease. When rats, mice, hamsters, and other animals ate food, drank water, or breathed air containing lower levels of NDMA for periods more than several weeks, liver cancer and lung cancer as well as noncancerous liver damage occurred. The high-level short-term and low-level long-term exposures that caused noncancerous liver damage and/or cancer in animals also usually resulted in internal bleeding and death. Based on the results of animal studies, it is reasonable to expect that exposure to NDMA by eating, drinking, or breathing could cause cancer in humans. Mice that were fed NDMA during pregnancy had offspring that were born dead or died shortly after birth. However, it is not known whether NDMA could be the cause of death of human babies whose mothers are exposed during pregnancy [59].

Long-term chronic exposure to some chlorinated organic compounds such as endrin and isobenzan may result in various nonspecific symptoms, including headaches, nausea, fatigue, muscle twitching, and visual disturbances. In addition, chronic exposure to these agents may be associated with the development of blood dyscrasias, including aplastic anemia and leukemia in humans.

14.6 Regulations Surrounding Dechlorination

After dechlorination, it is essential to meet the treated wastewater quality guidelines before discharging into the environment. National or provincial treated wastewater quality guidelines are available for discharge, which are decided considering various

factors, such as location of the plant (urban, rural, or coastal), possible usage of discharged water (drinking, agricultural, industrial, and recreational), receiving water body (ocean outfall such as in Sydney needs less treatment, but an inland water body used for other beneficiary uses needs more stringent requirement), etc. Three main quality parameters are relevant for chlorination and dechlorination processes, which are microbiological criteria (total coliforms, fecal coliforms, *Escherichia coli*, enterococci, etc.), chlorine (total chlorine, free chlorine), and chlorinated organic compounds and by-products.

The main purpose water quality guidelines is to protect aquatic life. When the effluent limitations based on technology are not sufficient to protect aquatic life of the receiving waters, the limitations based on water quality criteria must be used to define the pollutant concentration in the discharged water.

The water quality standards set the maximum permissible limit of the pollutant in the discharged water to minimize the risk to aquatic life in the receiving water. For example, in 1986 the US Environmental Protection Agency (US EPA) established criteria for total residual chlorine for discharge wastewater based on the acute and chronic toxicity effects on aquatic life. Considering the acute exposure, the maximum chlorine level is set at 19 μg/L to minimize the risk to aquatic life. To meet this criterion, the 1-h average chlorine concentration should not exceed 19 μg/L more than once every 3 years on the average. In the chronic toxicity criteria, the maximum allowable chlorine concentration in freshwater is 11 μg/L. To meet this criterion, the 4-day average chlorine concentration should not exceed 11 μg/L more than once in 3 years on average. For marine water, the levels are defined as 13 and 7.5 μg/L under acute and chronic toxicity criteria, respectively. These guidelines are used to calculate the allowable chlorine concentrations to protect aquatic species [60]. In all states of the United States, the above guidelines are used to calculate the allowable discharge total residual chlorine level in treated wastewater. However, in Canada the guideline value for the calculation is 2 μg/L [60].

To verify the compliance of water quality standards, samples are collected from the water body after the mixing zone and analyzed for the total residual chlorine. The mixing zone is that the portion of water body adjacent to an effluent outfall where effluent water and environmental water are mixed and diluted.

Discharge wastewater quality guidelines are available for limited chlorinated organic compounds. For example the maximum permissible limit of pesticides and PCBs is fixed to 120 ng/L in New South Wales, Australia. However, the guideline values are hard to find for some harmful by-products of wastewater chlorination and dechlorination, such as NDMA and pharmaceutical compounds. Table 14.3 presents some of the guidelines for wastewater chlorination and dechlorination in Australia. Watson et al. [62] suggested a bioassay based on the toxicology and ecosystem impact of compounds present in the wastewater effluent as opposed to the measurement of individual compounds. The US EPA has introduced the whole effluent toxicity test (WET), which is similar to what Watson et al. [62] suggested.

Table 14.3 Quality Parameters Relevant for Wastewater Chlorination and Dechlorination in Australia [61]

Quality criterion	State	Guideline	Comment
Coliforms, thermotolerant	Tasmania	200 cfu/100 mL	Interim emission license for existing plants discharging to freshwaters
Coliforms, thermotolerant	Tasmania	1000 cfu/100 mL	Interim emission license for existing plants discharging to marine waters
Fecal coliforms	Queensland	150 cfu/100 mL	Discharge to marine waters
Escherichia coli	Victoria	14 org/100 mL	Discharge must not cause bacteriological quality to exceed in surface waters used for shellfish
Enterococci	South Australia	33 cfu/100 mL	For discharge into surface waters used for primary contact recreation
Total chlorine	Victoria	0.1 mg/L	Level required after dechlorination prior to discharge to surface waters
Free chlorine	Queensland	<0.7 mg/L	Discharge to marine waters
Chlorinated organic compounds	Victoria	Not specified	Effluent requires monitoring for chlorinated organic compounds. Toxicity tests may be required, if dechlorination is practiced.

14.7 Conclusion

Dechlorination is an essential process if a wastewater is chlorinated before being discharged to the natural environment. Toxicity to aquatic organisms and the public arises mostly from the formation of chlorinated organic compounds during chlorination or from pharmaceutical and toxic compounds introduced from industrial and domestic activities and not removed by conventional biological processes. Dechlorination aims to remove the toxicity arising from chloramines, more specifically organochloramines arising from chlorine reacting with organic amines. Of the numerous organic compounds formed or introduced, the impact of only a limited number of compounds is known, meaning much more research into their impact and formation is required.

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15

Anammox Processes

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15.1 Brief History of Anammox Bacteria and the Reaction

Ammonia can be oxidized by microorganisms under aerobic and anaerobic conditions. Aerobic oxidation of ammonia (Eq. [15.1]) by ammonia-oxidizing bacteria (AOB) was discovered before the 19th century, whereas anaerobic ammonia oxidation (anammox) (Eq. [15.2]) by anaerobic AOB (or anammox bacteria) was discovered in early 1990 [1,2]. The discovery of the anammox process led to the realization that there is another pathway for ammonia conversion to nitrogen gas apart from the conventional route, i.e., nitrification–denitrification.

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \ (\Delta G = -235 \ \text{kJ/mol}) \quad [15.1]$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \ (\Delta G = -357 \ \text{kJ/mol}) \quad [15.2]$$

There were few postulations before 1990 that suggested ammonia oxidation under anaerobic conditions. Hamm and Thompson [3] were the first scientists to predict anaerobic ammonia oxidation in the ocean. In 1965, Richards proposed ammonia oxidation with nitrate under anoxic conditions [4]. However, these studies received little attention because of the lack of proof of the existence of anammox bacteria. Broda [5] did thermodynamic calculations and predicted that two kinds of lithotrophs were missing in nature. He suggested that these missing lithotrophs could oxidize ammonia to nitrogen gas using either nitrate or nitrite as an electron acceptor under anaerobic conditions [5]. In 1995, Mulder et al. [2] observed that ammonia disappeared at the expense of nitrite in a pilot plant denitrifying fluidized-bed reactor (FBR) at Gist-Brocades (The Netherlands) treating wastewater from a yeast factory under anoxic conditions. A clear production of nitrogen gas in the pilot plant was also observed by Mulder. The term “anammox” was given by Arnold Mulder [1]. van de Graaf et al. [6] used labeled $^{15}NH_4^+$ and $^{14}NO_2^-$ as tracers in an FBR and observed $^{14-15}N_2$ gas as the dominant end product. Based on this labeling experiment the authors confirmed that anammox bacteria use nitrite as an electron acceptor instead of nitrate in the anammox

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reaction. In 1998, Strous et al. [7] used a mass balance approach to calculate the stoichiometry of the anammox process. Eq. [15.3] shows the stoichiometry calculated by Strous et al. [7]:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 0.066CH_2O_{0.5}N_{0.15} + 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad [15.3]$$

The first anammox bacterium, one of the missing lithotrophs predicted by Broda in 1977, was identified as a new planctomycete and named *Candidatus* "*Brocadia anammoxidans*" [8,9]. Eleven anammox bacterial species have been identified so far in the order Brocadiales. The cell structure and culture conditions of anammox bacteria are also known now. The anammox process has been successfully applied to treat various ammonia-rich wastewaters in pilot- and full-scale treatment plants.

15.2 Anammox: Metabolism, Stoichiometry, and Biodiversity

15.2.1 Metabolism

Anammox is the oxidation of ammonium with nitrite as the electron acceptor and dinitrogen gas as the product. The process is mediated by obligately anaerobic chemolithoautotrophic bacteria that form a monophyletic cluster inside the Planctomycetales, one of the major divisions of the Bacteria. So far, 11 species have been detected and enriched from the biomass of sewage treatment plants and the most populous species are *C.* "*Brocadia anammoxidans*," *Candidatus* "*Kuenenia stuttgartiensis*," *Candidatus* "*Scalindua wagneri*," and *Candidatus* "*Scalindua brodae*." In addition, *Candidatus* "*Scalindua sorokinii*" was detected in the anoxic water column of the Black Sea, providing the first direct evidence for anammox bacteria in the natural environment. Anammox bacteria have a cell compartment known as the anammoxosome, which is the site of anammox catabolism. The lipid bilayer membrane surrounding this anammoxosome contains unusual lipids, so-called "ladderane" lipids, concatenated cyclobutane moieties that either are ether and/or ester linked to the glycerol backbone or occur as free alcohols (e.g., Fig. 15.1, structures II to IV). The other membranes of anammox bacteria contain lipids typical of planctomycetes in general: iso, normal, and midchain methyl hexadecanoic acids (e.g., Fig. 15.1, structure I).

15.2.2 Stoichiometry and Biodiversity

The anammox reaction is a thermodynamically favorable process for autotrophic bacteria to derive energy by coupling the oxidation of NH_4^+ to the reduction of NO_2^- [5]. This biochemical pathway involved in the microbial N cycle was discovered initially in a wastewater system in a laboratory reactor [6] and also has important implications for global N transformation because the bacteria have been found in freshwater wetland [10], agricultural soil [11], polluted wetland [12], coastal mangrove [13], subtropical mangrove [13,14], oil reservoir [15], and ocean [16,17]. They are believed to contribute

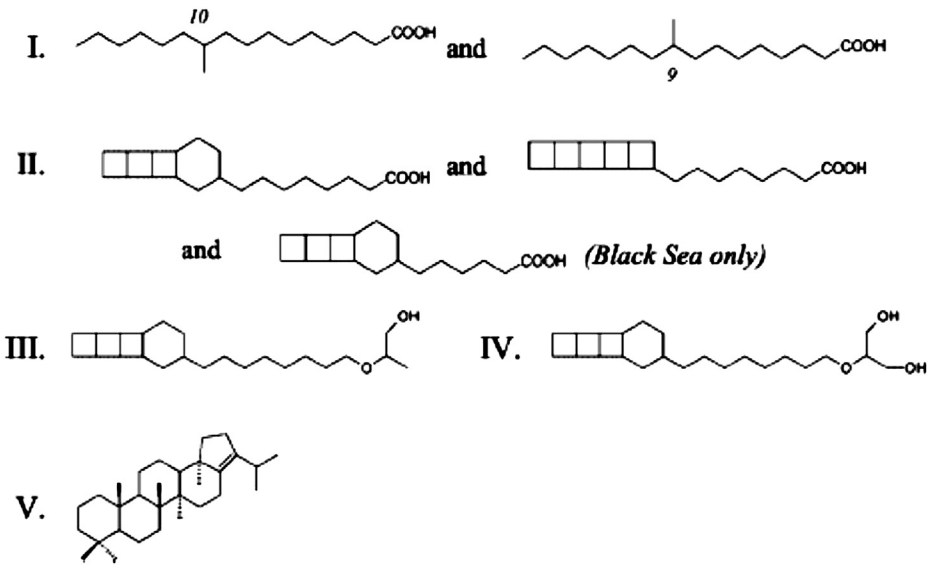

FIGURE 15.1 Structure of anammox lipids, i.e., branched fatty acids (I), ladderane fatty acids (II), ladderane glycol ether (III), ladderane glycerol ether (IV), and hop-17(21)-ene (V), present in the enrichment culture of *Candidatus "Brocadia anammoxidans."*

>50% of the N_2 released from oxygen-minimum zones of the oceans. In addition, the community composition of anammox bacteria shows a clear pattern of responding to anthropogenic influence from coastal ecosystems to the pristine South China Sea [17].

The biochemical reaction stoichiometric relationship was investigated with the nitrogen-15 isotope pairing technique (NIPT), a well-established ^{15}N method used in the study of denitrification previously [18]. The detailed methodology for IPT application in sediments where anammox and denitrification coexist is available [19]. In the experimental procedures, samples are properly treated and incubated for a relatively short term (e.g., 24 h) in parallel with various ^{15}N-labeled inorganic species: (1) $^{15}NH_4^+$ alone, (2) a mixture of equal amounts of $^{15}NH_4^+$ and $^{14}NO_2^-$, and (3) $^{15}NO_2^-$ alone. After incubation and termination of the reaction, N_2 produced under each treatment can be measured on an isotope mass spectrometer for ^{15}N concentration. The first incubation is used as a control to detect any oxidation of ammonium without the addition of nitrite, and the second is used to measure the anammox activity, where the production of $^{29}N_2$ stoichiometrically is a direct confirmation on the coupling between the oxidation of ammonium $(^{15}NH_4^+)$ and the reduction of nitrite $(^{14}NO_2^-)$ through the anammox process. The third incubation is to estimate the relative contributions of both anammox and denitrification collectively, where the production of $^{29}N_2$ and $^{30}N_2$ indicates the activities of anammox and denitrification, respectively [20,21]. Experimental details of the NIPT procedures by Risgaard-Petersen et al. [19] were further modified by Ward et al. [22]. As of this writing, NIPT is the only accepted method to quantify the rate and contribution of anammox processes in various environmental samples. NIPT was used

to estimate the relative contribution of the anammox reaction to the overall total N_2 production in marine sediment [23]. However, new evidence on the reduction of $^{15}NO_3^-$ to $^{15}NO_2^-$ and then to $^{15}NH_4^+$ under selective conditions suggests that denitrification may be partitioned to the anammox reaction [24]. In addition, NIPT provides indisputable information on the specific process involved, but unfortunately neither abundance nor the organism responsible for the anammox process is available from the analysis [25].

Another new isotope labeling approach for detecting the presence and activity of anammox bacteria is stable isotope probing (SIP) with $^{13}CO_2$ and/or ^{15}N-labeled inorganic N species as a substrate for incorporation into the microbial biomass under incubation conditions. SIP is a powerful technique when combined with current available molecular methods in revealing the active anammox community and composition and the associated microbial activity in environmental samples [26]. This method depends on the effective incorporation of an isotope-enriched substrate that is enriched in a heavier stable isotope, such as ^{13}C or ^{15}N, which allows the identification of the active population of microorganisms through selective recovery and analysis of isotope-enriched cellular biomolecules, such as DNA, RNA, proteins, and phosphorus lipid fatty acids [26,27]. SIP has been successfully applied in the study of the microbial nitrogen cycle, e.g., ammonia-oxidizing archaea and AOB in soils [28–30]. In these investigations, $^{13}CO_2$ and $^{14}N/^{15}N$ were used as substrates for active microbial utilization in the soil samples, and then total DNA, RNA, or mRNA was extracted after a period of incubation for analysis of the active microbial communities and microbial abundance. Using the relevant molecular techniques direct evidence could be obtained on the active microbial groups and their abundance [28,29]. In research on anammox bacteria, ^{14}C-labeled substrates were also used for fluorescence in situ hybridization (FISH)–microautoradiography analysis to confirm the chemolithoautotrophic biochemical pathway carried out by anammox bacteria [7,31], and no other related reports are available on this group of bacteria. Therefore, SIP coupling with an array of molecular techniques (see below) is a promising approach for the detection and quantification of anammox bacteria in the ecosystem.

15.3 Molecular Biological Techniques for Identification and Quantification

The first discovered anammox bacterium, named *C.* "*Brocadia anammoxidans*," was from a bioreactor enrichment culture and physically purified using the Percoll gradient centrifugation procedure [9]. The anammox bacteria show complex cellular structures and chemical composition of the membrane lipids and a cellularly distinguishable compartment called the anammoxosome in which the biochemical anammox reaction takes place, with the unique ladderane lipids in the membrane to maintain the biochemical reaction [9]. As of this writing, only five anammox genera are recognized on

a global scale, namely *Brocadia* [9,32], *Kuenenia* [33,34], *Scalindua* [35,36], *Anammoxoglobus* [37], and *Jettenia* [38]. New species are discovered from oil fields [15], pristine ocean sediments [16], and coastal mangroves [17]. They show clear distribution patterns along salinity and anthropogenic gradients [17], but the available anthropogenic N seems to dictate the distribution more significantly than the salinity [10,12,17]. From the information available currently, *Scalindua* dominates in open ocean and pristine freshwater ecosystems almost exclusively [17], indicating the distribution pattern is dependent upon the anthropogenic pollution and impacts.

Three main categories of approaches have been used to detect anammox bacteria in natural environments and wastewater treatment systems since 2005: activity measurements by the NIPT, analysis of anammox bacteria-specific lipids for biomass, and a suite of nucleic acid-based molecular techniques from PCR amplification to quantitative PCR (qPCR) and reverse-transcription (RT)-PCR of anammox bacterial gene biomarkers. Several reviews have discussed the detection methods for anammox bacteria to some extent [25,39–42] and the information from these techniques has improved our general understanding about the distribution of anammox bacteria in various niches and their community composition.

Anammox bacteria, different from all other known prokaryotes, have special lipids in their cellular membrane surrounding the anammoxosome within the cell [43,44]. These membrane ladderane lipids contain cyclobutane/cyclohexane ring structures, which make the anammoxosome membrane highly impermeable compared to other known nonanammox bacterial membranes [43,45]. Because the unique lipids are found only in anammox bacteria at the moment, the ladderane lipid is an indicator and a biomarker for the presence of anammox bacteria in environmental samples and its concentration is directly related to the biomass [35,43]. Furthermore, ladderane lipids are predominately enumerated as the core lipid derivatives [35,47,48], but occur as intact ladderane glycerophospholipids (ladderane IGPs) within cells with high abundances [49]; thus the ladderane IGPs, such as C_{20}-[3]-ladderane monoalkyl ether phosphocholine, may reflect living biomass more accurately than ladderane core lipids. Because of this, they are more specific biomarkers for viable anammox bacteria [50]. The detection and quantification of ladderane lipids are used not only to infer the presence of anammox bacteria in environmental samples [9,43,46], but also to assess the presence of anammox bacteria associated with some geological events [48].

To analyze ladderane lipids, samples containing anammox bacteria are first extracted with methanol, methanol/dichloromethane, and dichloromethane substantially. The extracts are methylated with B3/methanol after the solvent is removed and then the fractions are separated by a small silica column using ethyl acetate as the eluent. After that, the obtained fraction is sialylated with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) in pyridine convert alcohols in tetramethylsilane (TMS) ethers for further analysis by gas chromatography (GC) and GC/mass spectrometry (MS) [43]. Various chemicals contained in the ladderane lipids, such as fatty acids, glycerol diethers, glycerol ether esters, and *sn*-2-glycerol monoether, would be analyzed through these

procedures [43,46]. In 2006, a high-performance liquid chromatography/atmospheric pressure chemical ionization–MS/MS method allowing determination of low levels of ladderane lipids in complex matrices (e.g., sediments) was developed with success, and this technique could detect as low as about 35 pg ladderane lipids, which improved the method resolution greatly compared to the GC/MS method [51]. Although the lipids analysis provides a powerful tool to detect anammox bacteria, the following deficiencies still exist when using this technique on environmental samples: (1) the extraction procedures are complicated; (2) a high volume of sample is required, such as the seawaters and sediment, because of the low abundance of anammox bacteria; (3) difficulties are encountered in purification when dealing with sediment materials because of other contaminants, such as humic acid and fulvic acid; and (4) these lipids may also be present in nonliving organic matter and thus may not necessarily indicate the presence of metabolically active anammox bacteria without error [25], which limits the quantification of active anammox bacteria populations with high confidence and precision.

15.3.1 Molecular Techniques

The foundation of microbiology is pure culture of microorganisms from various environments so that further in-depth investigations on biochemistry, physiology, and genetics can be performed on them under laboratory conditions. This practice was effective in the early phase of developing microbiology into an independent area of research and study by many pioneers in the field, but it has run into difficulties with more environmental samples being examined and more new microorganisms being targeted for isolation into pure cultures, because $<1\%$ of the natural populations of microorganisms can be isolated into pure culture and investigated with our current technical capabilities [52]. This phenomenon has been widely recognized and other approaches have been developed to reveal the community of microbial populations, without isolation into pure culture, using phospholipids as biochemical markers and the latest DNA/RNA-based molecular markers for specific groups of microorganisms [52]. This has advanced our understanding of microbial ecology and ecophysiology tremendously because many previously unknown microorganisms have been revealed and their activity and functions can now be further investigated with additional techniques, SIP and/or DNA/RNA-based probing. In addition, the more recent developments in metagenomics and pyrosequencing allow more in-depth investigation of microbial community and abundance, with more detailed information on the microorganisms in a sample being recovered.

Because anammox bacteria have not been isolated in pure culture, a wide range of culture-independent methods, specifically DNA/RNA-based molecular techniques [53], are the being used and are popular approaches for detecting and analyzing this group of microorganisms in various environmental samples [25]. Because anammox bacteria are generally in low abundance and activity in natural ecosystems, their detection through PCR amplification has been a challenging task in many studies, and biases are also

involved throughout the experimental procedures, from extraction of DNA from samples to PCR primer specificity [53]. For molecular techniques, the available ones include PCR-based methods, quantification by qPCR and RT-qPCR, and FISH-based techniques, which are discussed below.

15.3.2 PCR-Based Technique

PCR amplification of DNA templates in samples with specific PCR primers and subsequent phylogenetic analysis of the amplified DNA sequences is the method of choice in detecting any previously known or unknown or unculturable microorganism in environmental samples [52]. For anammox bacteria, this method is the most widely used and most efficient, and it has successfully identified the presence of anammox bacteria in a wide range of samples from sediments to wastewater [13,16,17,53,54]. In addition to the commonly used 16S rRNA gene for PCR amplification, gene biomarkers associated with metabolic reactions in the anammoxosome have also been used, e.g., the *nirS*, hydrazine hydrolase, and hydrazine synthase genes, with very good performance, especially in light of wastewater samples by the 11 pairs of PCR primers tested [17].

15.3.2.1 16S rRNA Gene

The most commonly used phylogenetic biomarker for studying microbial communities is the 16S rRNA gene. According to available information, 16S rRNA gene sequence analyses have shown that all known anammox bacteria form a monophyletic clade within the phylum Planctomycetes [1]. Most of the studies detecting anammox bacteria in natural assemblages so far are based on 16S rRNA genes [1,17,40]. However, the initial difficulty in detection of anammox bacteria by the PCR method is that the anammox bacterial 16S rRNA genes are not amplified very well with the universal bacterial primers [25,55], and extraction procedures also play a critical role in optimal amplification [56]. This is due to a high 16S rRNA gene sequence divergence that occurs among various genera of the anammox bacteria (<87.1% identity), and there are no general PCR primers for the genera of anammox bacteria [40,41], especially in some environments where anammox bacteria constitute only a very small fraction (<1%) of the whole community [42]. Some selective PCR primer sets intended for anammox bacteria from natural environments have very low specificity, and as a result nonanammox bacteria, such as *Vibrio* species, can be amplified from coastal marine sediment [55]. Sample associated bias is another issue because anammox bacterial DNA cannot be extracted equally or more efficiently with the commercially available extraction kits and because of this, the extraction procedure cannot yield the most anammox bacteria from the samples when the relative population of anammox bacteria is low [56]. Sequential extraction of the same sample with multiple steps can yield more meaningful information on both the community and the abundance after modifications are made in the extraction steps. This revision allows a better understanding of the anammox distribution in natural samples that contain a low population density of this group of bacteria.

15.3.2.2 Functional Genes

In addition to the PCR amplification of the 16S rRNA gene, widely used in detection of anammox bacteria, functional gene biomarkers provide a more accurate account of the presence of anammox bacteria. Based on the anammox reactions, four core catalytic proteins are promising candidates for new PCR primer design: nitrite and nitrate reductases, hydrazine hydrolase, and hydrazine dehydrogenase [34]. Hydrazine dehydrogenase (HZO), also called the hydroxylamine oxidoreductase-like protein (HAO), targets three different clusters of HAO/HZO proteins [57,58], but only HZO cluster 1 is considered the most suitable biomarker for anammox bacteria phylogenetic analysis. As of this writing, the *hzo* gene has been successfully used to detect anammox bacteria from various environmental samples, including wastewater treatment plants [17,58–60], mangrove sediment [55,61], estuaries [61,62], coastal and deep ocean sediments [16,55,62,63], hydrothermal vents [62], and oil reservoirs [15]. They collectively indicate that the *hzo* gene is a competitive functional biomarker for anammox bacteria detection because it gives a higher resolution for the community structure of anammox bacteria than that given by 16S rRNA genes [55].

Another functional biomarker with application in anammox bacteria detection is the CD1 nitrite reductase (*nirS*) gene, and a PCR primer set designed for the amplification of the *nirS* gene of *Candidatus Scalindua* sp. is available [64]. With this primer set, the transcription levels of anammox *nirS* genes in the Peruvian upwelling zone were reported, correlating to activity rather than the presence of anammox bacteria alone in this area [55,64]. In addition, results indicated that these *Scalindua nirS* genes were fairly diverse, but all clustered with the *nirS* gene present in the *Candidatus Scalindua* genome assembly (73–93% nucleotide sequence identity) and two sequences obtained from the Arabian Sea; however, they were clearly different from the typical denitrifiers' *nirS* genes (<63% sequence identity), indicating that *Scalindua nirS* can be a candidate functional gene biomarker for anammox in environmental samples [64].

A hydrazine hydrolase-based PCR primer has become readily available and the PCR performance has been in general most acceptable [17,65]. PCR primers of *hzs* gene are favorable for detecting anammox bacteria in wastewater and also other natural samples [17].

15.3.2.3 Quantitative PCR

QPCR, quantifying anammox bacterial 16S DNA or RNA or specific functional gene copies, is also an effective and suitable estimation of the anammox bacteria in various samples for comparison and understanding their relative contribution to N removal. Anammox bacteria can be quantified in wastewater treatment reactors [66], wastewater treatment plants [17], marine oxygen minimum zones [64], marine sediments [55,61,67], and agricultural soils [11]. PCR amplification of a unique functional gene as target significantly increases the detection efficiency from samples with low anammox bacteria (<1%) [42]; therefore, the recent progress in anammox bacterial genomics and biochemistry and physiology will identify reliable functional genes for such use.

By combining with the RT-PCR of the functional genes, such as *hzo* and *Scalindua nirS*, the activity of anammox bacteria could also be assessed through the qPCR technique [60,64,68]. However, the abundance of anammox bacteria using qPCR might not match very well with the results of FISH counts [67]. Reasons for this mismatch are due to different detection efficiencies with different probes used and primers for the various anammox species; for example, detection of cells by FISH can be a challenge because of its detection limit, whereas qPCR can be hampered by organic matter in the samples [67,69,70]. Despite this uncertainty, qPCR estimating anammox bacterial RNA or DNA gene abundance is still an effective and powerful tool for quantification analysis of anammox bacteria when RT-qPCR is performed.

15.3.3 FISH-Based Technique

FISH is a useful tool for culture-independent in situ identification of the target bacteria in environmental samples. Many investigations have used the FISH technique to collect both qualitative and quantitative data on anammox bacteria in environmental samples [25]. For this widely used technique on anammox bacteria, the specificity of the fluorochrome-labeled DNA oligonucleotide probe is one of the most important factors for successful application in practice [71,72]. Most of the available probes used in FISH detection target the 16S rRNA genes of anammox bacteria, but a probe for the 23S rRNA gene has also been developed, i.e., the L-*-Amx-1900-a-A-21 [73]. However, the high divergence (<87.1% similarity) among genera of anammox bacteria prevents the discovery of new anammox species [40,74]. The FISH technique has been widely used to assess the abundance of anammox bacteria in various environmental samples, which has provided a quantitative distribution of anammox bacteria globally [75,76]. Probe signal intensity can be enhanced by polynucleotide FISH [77] and catalyzed reported deposition FISH [78,79], or by minimizing probe penetration problems and increasing hybridization efficiencies with different probe chemistries, nucleic acid FISH [80], and locked nucleic acid FISH [81]. Integration of FISH with other approaches can be carried out to gain more insight into the metabolic activity of anammox bacteria and extend the applications of FISH as a powerful technique.

To learn more about the in situ activity of anammox bacteria, the ISR between16S and 23S rRNA genes has been targeted with fluorescently labeled oligonucleotide probes and quantitative FISH experiments with cells of *C.* "*Brocadia anammoxidans*" [73]. Intergenic spacer regions targeted FISH (ISR-FISH) FISH has great potential in monitoring activity changes in enriched cultures of anammox bacteria and ecosystems [25,82]. However, one disadvantage associated with this technique is that the ISR sequence lacks evolutionary pressure, which might result in two strains of the same species showing very different sequences. FISH with microautoradiography (FISH−MAR) has been developed by linking the uptake of radiolabeled substrates, such as [^{14}C]acetate, [^{14}C]butyrate, [^{14}C]bicarbonate, and ^{33}Pi, with specific organisms in a complex environmental sample so to obtain insight into the microbial community

structures and functions simultaneously [83]. For detection of anammox bacteria, FISH–MAR with radiolabeled $^{14}CO_2$ is very meaningful to demonstrate the chemolithoautotrophic pathway active in anammox bacteria [7,31]. In cocultures of aerobic and anaerobic ammonium oxidizers, FISH–MAR is also successfully used to measure the uptake of $^{14}CO_2$ [25].

The experimental procedures for higher resolution and elimination of interference from the sample matrix need performance improvement to fit a wide range of environmental samples, and better anammox-specific probe sets are also required for further specific evaluation. FISH has been proven to be a powerful technique for environmental detection of anammox bacteria, which not only obtains qualitative and quantitative basic information, but also provides new insights into the metabolic activity of anammox bacteria.

15.4 Other Methods for Anammox Process Identification

In ecological/bioreactor systems, bacteria communicate with one another by a system called quorum sensing (QS), which regulates gene expression in response to fluctuations in the cell population density. The bacteria having a QS system produce quorum signaling molecules and then release them to their surroundings. At a certain cell population density the signal concentration reaches a threshold value and the binding of signal molecules as well as regulatory proteins activates the QS-regulated genes to activate relevant phenotypes. The acyl homoserine lactones (AHLs) are the signal molecules in gram-negative bacteria. The anammox bacteria are gram-negative organisms that can release AHLs into the system. In 2015, it was found that anammox bacteria release three AHLs named C_6-HSL, C_8-HSL, and C_{12}-HSL [84]. Moreover, it was understood that the addition of C_6-HSL could increase the anammox activity and growth rate, whereas C_8-HSL was found to promote anammox activity and C_{12}-HSL decreases the anammox activity. Therefore, QS-based expression can be used to identify the metabolism of anammox bacteria.

On the other hand, the usage of simple stoichiometry of various processes occurring in a reactor system and their coupling along with the analysis of crucial intermediates is one of the possible ways to identify the occurrence of the anammox process in the system. The FISH technique using a 16S rRNA-targeted oligonucleotide probe like Amx820 can confirm the presence of anammox species in the system, and the activity of the species can be ensured by monitoring the physicochemical parameters such as nitrogen species consumption. The overall characteristics of the anammox process are quite remarkable, especially considering the production of the intermediate N_2H_4, which is used as a rocket fuel and constitutes an intermediate in the production of explosives and pesticides. Although anammox catabolism takes place within the ladderanes, in some instances, N_2H_4 and NH_2OH are added to speed up the anammox process. Therefore, the analysis of the presence of crucial intermediates such as N_2H_4 and NH_2OH can confirm the activity of anammox species in the reactor system. On the other

hand, the addition of these two compounds and their stability/consumption can also reflect the anammox activity in the reactor system.

Using the stoichiometric relationships, the quantity of nitrogen consumed in partial nitrification/anammox/denitrification can be modeled considering the reactor a black box [85]. However, before modeling it is essential to confirm the sequence of processes occurring in the reactor system, i.e., partial nitrification, anammox, denitrification, sulfidogenesis, and/or their combination. For a better anammox activity to occur it is essential that a partial nitrification activity has to be preceded or the influent should have enough nitrite. To model the system it is essential to consider (1) the effect of partial nitrification before anammox and denitrification and the nitrite production in partial nitrification (i.e., the molar ratio of $NH_4^+ - N$:$NO_2^- - N$ produced in the system), (2) the molar ratio of $NH_4^+ - N$:$NO_2^- - N$ consumed by the anammox system and subsequently the molar ratio of $NO_3^- - N$ produced in the system, (3) the molar ratio of $NO_3^- - N$ utilized in denitrification, and (4) the quantity of organic matter consumed [soluble chemical oxygen demand (COD) consumed] and the organic matter composition in wastewater (based on the elemental analysis of wastewater). Vega De Lille et al. [86] developed an ammonium estimator to simulate the anammox process that occurs in a sequencing batch reactor (SBR). The system pH was correlated with concentrations of ammonium, nitrite, and nitrate and also used to calibrate an ordinary partial differential equation (ODE). Subsequently, the simulated data from the ODE was fed into an artificial neural network (ANN) to train the network. Following the training period and validation, the simulated ANN was used to predict the ammonium concentration and anammox activity from online pH measurement alone. In the past, similar strategies were adopted to control continuous-flow nitrification−denitrification SBRs using online oxidation-reduction potential (ORP) and pH measurements combined with the ANN model [87]. However, further anammox studies using alternative parameters, i.e., conductivity/ORP, and modeling them using ANNs will reduce the amount of experimental work required in monitoring and controlling the anammox systems.

15.5 Reactors for Anammox Process Development

The reactor system is the heart of any bioprocess development, including the anammox process. It is a key factor that determines the start-up and stable operation of the anammox process. Extremely slow growth rate and low biomass yield of anammox bacteria are responsible for the long start-up time of the anammox process. For example, the first full-scale anammox reactor in Rotterdam, The Netherlands, took more than 3 years for start-up, which is undesirable for widespread application of the anammox process. Several reactors, such as SBR, FBR, upflow biofilter (UBF), upflow anaerobic sludge blanket (UASB), membrane bioreactor (MBR), airlift reactor, and rotating biological contactor, have been used for the development of the anammox process. The SBR, UASB, and fixed-bed reactors are among the three most widely used and proven to be the most efficient reactors for the cultivation of anammox bacteria (Table 15.1; [88]).

Table 15.1 Lab-Scale Applications of the Anammox Process in Treating Real Wastewater Reported Since 2004

Process	Stages in Nitrogen Removal	Wastewater	Reactor Type	Reactor Volume (Anammox) (L)	$NH_4^+ - N$ Removal Efficiency (%)	References
OLAND	Single	Digested sludge dewatering wastewater	MBR	1.5	82	[89]
Anammox	Single	Landfill leachate	UASB	4.46	87.5	[90]
PN—anammox—oil infiltration systems	Two	Landfill leachate	Upflow fixed-bed biofilm reactor	36	60	[91]
PN—anammox	Two	Livestock manure digester liquor	UASB	3	79.2	[92]
Short-cut nitrification reactor—anammox	Two	Landfill leachate	UASB	8.5	93	[93]
UASB-MBR-SHARON-anammox configuration	Two	Landfill leachate	CSTR	2.3	78	[94]
SNAD	Single	Optoelectronic wastewater	SBR	2.5	>85	[95]
CANON	Single	Pretreated swine slurry	SBR	1.5	78	[96]
CANON	Single	Swine digester liquid	SBBR	6.5	—	[97]
Anammox	Single	Monosodium glutamate industrial wastewater	SBR	2.2	69—74	[98]
SNAD	Single	Digester liquor of swine wastewater	SBR	5	96	[99]
CANON	Single	Optoelectronic wastewater	SBR	18	98	[100]
PN—anammox	Two	Old landfill leachate	Anammox hybrid reactor	5	>90	[101]

CANON, completely autotrophic nitrogen removal over nitrite; *CSTR*, continuous-stirred tank reactor; *MBR*, membrane bioreactor; *SBBR*, sequencing batch biofilm reactor; *SBR*, sequencing batch reactor; *SNAD*, simultaneous partial nitrification, anammox, and denitrification; *UASB*, upflow anaerobic sludge blanket; *PN*, partial nitrification; *OLAND*, oxygen-limited autotrophic nitrification-denitrification, *SHARON*, single reactor system for high activity ammonium removal over nitrite.

In the case of an SBR, efficient biomass retention, homogeneous distribution of reactants and products over the reactor, and long-term reliable operation make it the most suitable reactor for anammox process development [7]. The doubling time of anammox bacteria is 11 days in an SBR [7] compared to 30 days in an FBR [102]. SBR has been efficient in treating a very high strength $\left(\sim 3800\ \text{mg}\ NH_4^+ - N/L\right)$ wastewater [100]. A problem of biomass flotation due to the production of gas bubbles (N_2, N_2O, and NO) has been observed in SBRs. The biomass flotation can be minimized by using granular sludge or carriers to immobilize anammox bacteria as biofilm. Fernandez et al. [103] used high inorganic salt concentrations to form granular sludge in one SBR, whereas zeolite was used as a carrier to immobilize anammox sludge in another SBR. They observed that biomass washout was significantly reduced in both SBRs.

Various types of biomass carriers such as porous nonwoven fabric [104], novel acrylic resin material [105], polyethylene sponge strips [29] and spheres [100], spherical plastic [106], bamboo charcoal [106], polyvinyl alcohol–sodium alginate gel beads [107], and waste activated sludge spheres [108] have been used to immobilize anammox bacteria as a biofilm on the surface of carriers in SBRs and other reactors. Though the above methods are effective in minimizing the biomass flotation in SBRs, process parameters such as shock loading and fluctuations in the influent can provoke the flotation and biomass washout [109,110]. Complete biomass retention is feasible in an MBR. The development of the anammox process in MBRs has been studied by a few authors [89,110–112]. Shorter start-up time of the anammox process has been achieved in MBRs compared to SBRs [111,112]. The major limitations of the MBR system include high operational cost due to membrane fouling and high energy consumption.

The UBF, a kind of fixed-bed reactor, uses porous media such as polyester porous nonwoven strips [113], fibrous plastic media [114], hollow bamboo balls [115], etc., to immobilize the biomass. In a comparative lab-scale study, the UBF was proved a better reactor system than the SBR for anammox process development as it offers the following advantages over SBR: (1) short start-up time and (2) better stability against shock loadings [114]. The doubling time of anammox bacteria has been effectively reduced from 11 days in an SBR to between 4.3 and 7.4 days in a UBF [115]. Tsushima et al. [113] observed the maximum nitrogen removal rate of 24.0 kg-N/m^3 day in a lab-scale UBF. The maximum nitrogen loading rate (NLR) attained in a UBF was 34.5 kg-N/m^3 day with nitrogen removal efficiency of >98% [115]. The biomass flotation problem can also be avoided by using a UASB, which has a solid–liquid separator to separate gas, water, and biomass. Moreover, biomass forms compact granules in a UASB, which further supports biomass retention in the reactor [116]. Higher removal rates can be achieved in a UASB compared to an SBR and other reactor types. The highest nitrogen removal rate of up to 76 kg-N/m^3 day and specific anammox activity of up to 5.6 kg-N/kg of volatile suspended solids (VSS)/day have been achieved in a UASB [117]. It is also efficient in treating low-strength wastewater $\left(< 20\ \text{mg}\ NH_4^+ - N/L\right)$ under low temperatures [118].

15.6 Coupling Anammox With Other Processes

15.6.1 Anammox With Partial Nitrifiers and Heterotrophic Denitrifiers

The goal of anammox is the removal of nutrients, i.e., nitrogen in the form of $NH_4^+ - N$ and $NO_2^- - N$. Therefore, under certain conditions the application of the anammox process is limited, for example, anaerobic digester effluent treatments. The supernatant from anaerobic digester effluents consists of high COD/biological oxygen demand (BOD) and $NH_4^+ - N$. The removal of COD/BOD is not possible with anammox and, at the same time, a complex COD/BOD (the digester effluents are of mixture of many compounds and not always limited to formate, acetate, and propionate) can inhibit the growth of anammox. Moreover, as per the metabolic reaction of anammox (Eq. [15.3]), nearly 89% of nitrogen is removed as $NH_4^+ - N$ and $NO_2^- - N$ whereas 11% of nitrogen is released in the form of $NO_3^- - N$. Under such stringent disposal limits, complete nitrogen removal is impossible with the nitrifying/anammox biomass. A complete organic and nitrogen removal from any source could be achieved by coupling anammox and denitrification. Moreover, the adaptation of simultaneous partial nitrification, anammox, and denitrification (SNAD) cultures in a single reactor could be helpful for achieving the goal of energy-neutral or energy-generating wastewater treatment systems with the capability of producing clean water (Fig. 15.2). Therefore, the development of a system with anammox and denitrification is really useful, and can also offer complete organics and nitrogen removal from wastewater(s).

The essential conditions/parameters required for establishing a fairly good interaction between anammox and denitrification/partial denitrification in a single reactor system are: (1) a suitable reactor system with optimized hydraulic and sludge retention times and (2) optimized dissolved oxygen, pH, temperature, alkalinity, and limiting substrate concentrations (carbon, NH_4^+, NO_3^- and NO_2^-). UASB, SBR, FBR, and fluidized-membrane bioreactor are the most suitable reactor configurations for the stable establishment of anammox and denitrification processes [119]. In addition to the

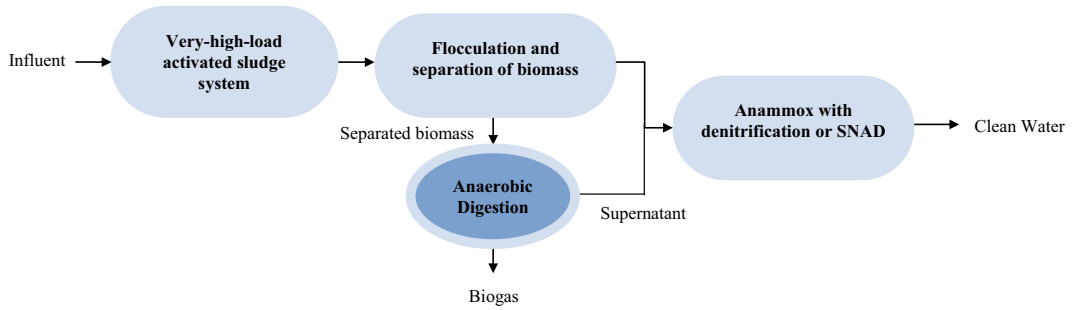

FIGURE 15.2 Concept for complete organic carbon (C) and nitrogen (N) removal in a sewage treatment system by combining anammox with denitrification or simultaneous partial nitrification, anammox, and denitrification (SNAD).

selection of reactor configurations, it is essential to know the optimum/favorable growth conditions of anammox and denitrifying microorganisms.

15.6.2 Anammox With Sulfidogenesis

Effluents from food processing, semiconductor, chemical, and pharmaceutical industries contain both ammonia and sulfate as pollutants. Sulfate is usually removed separately by sulfate-reducing bacteria under anaerobic conditions, which is time-consuming and further increases the treatment cost. Fdz-Polanco et al. [120] predicted a novel process for simultaneous anaerobic ammonium oxidation and sulfate reduction by a sulfate-dependent anammox process (Eq. [15.4]). The reaction is catalyzed by a new autotrophic Planctomycete bacterium named *Anammoxoglobus sulfate* [121]. The bacterium is capable of oxidizing ammonia to nitrite using sulfate as electron acceptor [121]. The sulfate-dependent anammox process was further confirmed in an anaerobic attached-growth bioreactor treating ammonia- and sulfate-rich synthetic wastewater [122]. The bacterial strain responsible for simultaneous removal of ammonia and sulfate in this reactor was isolated and named as ASR [123]. The isolated strain ASR is related to *Bacillus benzoevorans* based on electron microscopy, physiological tests, and 16S rDNA phylogenetic sequence analysis [123]. Rikmann et al. [124] also developed this process in a moving-bed biofilm reactor and UASB reactor using synthetic wastewater. The feasibility of the sulfate-dependent anammox process in treating real wastewaters needs to be tested.

$$2NH_4^+ + SO_4^{2-} \rightarrow N_2 + S_0 + 4H_2O \ (\Delta G = -46 \ \text{kJ/mol}) \qquad [15.4]$$

15.7 Current Trends and Success in Anammox Applications

Anammox bacteria are ubiquitous and widely distributed in ecosystems including both surface and subsurface, freshwater and marine, natural wetlands, and artificial systems of wastewater treatment plants. This indicates their adaptability and evolution from the very beginning; the early anammox cells with the capability of coupling NH_4^+ and NO_2^-/NO_3^- to form N_2 may have utilized the available inorganic N at low concentration and then evolved to those adapted to higher concentration of inorganic N and also assimilation of low-molecular-weight organic acids. The five known genera of anammox bacteria show a very clear phylogenic distinction in that the *Scalindua* genus is apparently distantly related to the other four genera. Such relationship is also correlated to their tolerance or adaptability to available inorganic N in the culture medium, in that *Scalindua* prefers a low concentration of inorganic N, e.g., open oceans [17] and freshwater wetlands [10,12] without anthropogenic influence, whereas the other four grow actively in wastewater treatment plants and coastal wetlands and rivers where pollution by wastewater and surface runoff is apparent [17]. The evolutionary

relationships among the anammox bacteria can provide key information on the driving force for the biological change in this group of microorganisms. Such information may enlighten us on climate change or anthropogenic impacts on the planet Earth.

Laboratory-scale applications of anammox in treating various types of ammonia-rich wastewaters such as landfill leachate, optoelectronic industrial effluents, anaerobic digester effluent, etc., have been successfully reported (Table 15.1). Both two-stage (partial nitrification–anammox) and single-stage [CANON (completely autotrophic nitrogen removal over nitrite) and SNAD] anammox processes have been used for ammonia removal (Table 15.1). Several full-scale anammox reactors are successfully operated around the world. Most of these full-scale anammox reactors are in Austria, China, Taiwan, Japan, The Netherlands, and the United States [85,125]. The first full-scale anammox reactor (70 m^3) designed by Paques BV was started up in Rotterdam, The Netherlands, in 2007 and it treats up to 750 kg-N/day. The start-up time was 3.5 years for this first full-scale reactor [126], whereas a second reactor was started up in a year. The first Asian full-scale plant was built in Japan and took only 2 months for the start-up [125]. In Taiwan, the anammox process was first observed in a full-scale landfill-leachate treatment plant in 2009 [85]. The anammox sludge taken from this full-scale treatment plant was used to establish the anammox process in three different leachate treatment plants in Taiwan with average leachate flows of 304, 208, and 500 cubic meters per day (CMD) by Leaderman & Associates. Ni and Zhang [125] compiled a list of full-scale anammox plants implemented worldwide by Paques. Current applications of the anammox process mainly focus on the areas described in the next sections.

15.7.1 Application of Anammox Process at Moderate Temperature and Lower Ammonia Concentration

As of this writing most of the anammox reactors are operated at a higher temperature range (≥30°C) and treat high-strength wastewater. The optimum range for the growth of anammox bacteria is between 30 and 40°C [9,127]. The activities of the metabolic enzymes of anammox bacteria reduce as temperature decreases from the optimum value. Therefore, nitrite uptake by anammox bacteria ceases at lower temperature, and its accumulation in the reactor causes process inhibition [128]. Several reports suggest that the nitrogen removal rate of anammox reactors significantly decreases when temperature decreases from ≥30 to ≤20°C [129,130]. Reactor operation in wastewater treatment plants at higher temperature (≥30°C) is not economical, whereas operation at lower temperatures (≤20°C) is challenging. On the other hand, few wastewater streams such as municipal wastewater have low ammonia concentration $\left(\leq 50\ \text{mg}\ NH_4^+ - N/L\right)$.

A few successful attempts using synthetic wastewater have been made to operate an anammox reactor at lower temperature and lower ammonia concentrations. For example, Hendrickx et al. [131] successfully started up a gas lift reactor with synthetic wastewater containing 69 mg-N/L $\left(NH_4^+ - N/L + NO_2^- - N/L\right)$ as influent at 20°C. Ma et al. [118] studied low-strength wastewater $\left(< 20\ \text{mg}\ NH_4^+ - N/L\right)$ treatment in a

UASB reactor under 16 and 30°C. The authors achieved 2.28 kg-N/m^3 day nitrogen removal rates at 16°C. Hu et al. [132] studied the feasibility of nitrogen removal from synthetic municipal wastewater in a two-staged SBR (partial nitrification–anammox) at lower temperature. The reactor could achieve more than 90% ammonia removal at 12°C.

15.7.2 Simultaneous Removal of Ammonia and Methane

The end products of anaerobic digestion are ammonia and methane. Methane is a renewable source of energy and it can be collected for electricity production in the gas phase. However, recovery of dissolved methane is difficult and it slowly releases into the environment and contributes to the greenhouse effect [133]. Therefore, it is desirable to remove dissolved methane along with ammonia from the digester effluent. A new process named nitrite-dependent anaerobic oxidation of methane (n-damo) has been developed. This n-damo process is catalyzed by *Candidatus* "*Methylomirabilis oxyfera*" bacteria [133]. Studies [133,134] suggest the feasibility of coculturing n-damo and anammox bacteria for simultaneous removal of ammonia and methane in the near future.

15.8 Future Directions for Anammox Research and Application

More and more interest in developing anammox reactors has emerged in recent years. Because of the decreased oxygen demand, COD requirement, and sludge production, anammox can be considered as a sustainable process for nitrogen removal from wastewater. Potential designs are based on an anoxic anammox reactor coupled with a partial nitrification process such as SHARON [135,136] or anammox plus partial nitrification simultaneously appearing in a biofilm reactor such as the CANON/OLAND processes [137–139] or aerobic deammonification [140–142]. Implementation of anammox in urban wastewater treatment would lead to a significantly increased sustainability of these systems [136]. The problem for the introduction of the anammox process is the very slow growth rate of the bacteria (making running pilot plants time-consuming) and the complex interactions in anammox–nitrifying biofilms, which need further investigation. Moreover, efficiently retaining the biomass is another new area of research in bio-environmental engineering; especially it is much needed in handling slow-growing biomasses like anammox bacteria. One of the methods of retaining the biomass is by immobilizing it in a supporting medium, for example, gel beads, sponge and polyurethane-foam, etc.

Many researchers have reported the successful immobilization of anammox bacteria on polyvinyl alcohol (PVA) [143], sodium alginate (SA) [65], a mixture of PVA and SA [144,145], polyethylene glycol [146], and PVA–SA gel beads [107]. The reactors operated with immobilized anammox biomass have shown excellent nitrogen removal rates;

however, the biomass in the inner layers of the attachment have shown less contribution toward the nitrogen removal rates. In addition, most of the immobilized biomass studies have been carried out at laboratory scale. The optimal thickness of attachment, hydrodynamic behavior, and durability of the immobilized biomass for real-time and long-term operation need to be explored, which may possibly be the critical and crucial anammox research areas in the near future. On the other hand, combining anammox, denitrification, and sulfidogenesis in a single reactor can be useful for removing nitrogen, organic carbon, and sulfate simultaneously, instead of removing each pollutant in a sequential chain of treatment units. The establishment of anammox, denitrification, and sulfidogenesis in a single reactor is more complicated considering the difficulty in the determination of favorable operating conditions including the ORP of the system and the ratios of NH_4^+:NO_2^-, COD:NO_3^- and COD:SO_4^{2-}. Additionally, the competition for nitrite by anammox, denitrification and sulfidogenesis could create intricacy in the coupling of these processes. However, further research on the optimization of the biochemical routes of these processes can advance the wastewater treatment process.

The presence of methanogenic archaea has been observed in many wastewater treatment systems such as activated sludge processes and granular systems, including anammox systems. Gonzalez-Martinez et al. [147] confirmed the presence of methanogens in various full-scale anammox wastewater bioreactors. The highest archaeal population was observed in the DEMON system and the least in a CANON bioreactor. The taxonomic composition of the archaeal communities in the reactors was closely affiliated to *Methanosaeta* sp. and the presence of a higher archaeal population has the tendency to reduce the autotrophic nitrogen removal efficiency in anammox reactors. The presence of *Methanosaeta* sp. in the granular biomass of anammox systems confirms the presence of some steps of methane as well as nitrogen cycles. The anaerobic methane oxidation with mediation of nitrite and nitrate as an electron acceptor coupled with anammox metabolism is possible as discussed in an earlier section. However, more detailed investigation into full-scale anammox reactors is required to better understand the role of archaea and their effects on nitrogen elimination.

The other potential areas of anammox research include (1) the effects of the presence of aromatic hydrocarbons and other micro/emerging pollutants on anammox activity and nitrogen removal rate, (2) the type of extracellular polymeric substance (EPS) secreted from the anammox organisms and their impact on the survival of other organisms of interest in nitrogen removal, and (3) the development of sensor-based systems/devices for effective monitoring and control of anammox and anammox-coupled systems.

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16

Treatment of Recalcitrant Waste

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16.1 Introduction

With the rapid increases in the world's population and industrialization, the world is confronting an acute problem of controlling wastes generated from several manufacturing processes. Global increases in energy consumption and stringent wastewater discharge requirements have prompted researchers and industrialists to look into wastewater treatment processes that should be sustainable and energy effluent [1]. To take these aspects into account, anaerobic digestion with net zero energy consumption is recognized as the most suitable option for recalcitrant wastewater treatment. Anaerobic digestion uses a microbial consortium present in wastewaters to convert organic contaminants into other degradable products. It is one of the simplest ways of harvesting energy because biogas production is also accompanied by anaerobic digestion. Biogas, a mixture of hydrogen and methane, is a renewable source of energy and can be used in combined heat and power generation plants for the production of heat and electricity by combustion engines [2].

The overall conversion of biodegradable organic solids to end products such as biogas is believed to take place in three different stages [3]: (1) hydrolysis of complex and insoluble molecules to simple and soluble products, (2) production of acetic acid and hydrogen through acetogenesis, and (3) methane production through methanogenesis. Therefore, an intimate contact between microorganisms and organic compounds is important for the successful application of anaerobic digestion to recalcitrant wastewater treatment.

Considering the aforementioned aspects, the efficiency of the anaerobic treatment process depends on several factors such as the biodegradable nature of organic contaminants, activity of the microbial consortium, rate of mass transfer, and configuration and type of anaerobic digester. Of all these aspects, the most challenging factor is the extent of the biodegradable nature of recalcitrant wastewater. It accentuates the importance of wastewater characterization. However, for quantitative and qualitative assessment of any wastewater, it is important to identify the industrial processes that make a significant contribution to wastewater discharge. This will not only simplify the

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characterization process but also make the process selection criteria simpler. Considering this aspect, the next section of this chapter discusses various sources of recalcitrant wastewater and waste stream characterization in detail. Later, the discussion is extended to various anaerobic treatment processes with their potential of resource recovery.

16.2 Recalcitrant Wastewaters

Human, animal, industrial, and household wastes are recognized as the principal sources of recalcitrant wastewaters. Characterized by high chemical oxygen demand (COD) and low biochemical oxygen demand (BOD)/COD ratio, these wastewaters contain high concentrations of nonbiodegradable compounds such as polymers, toxicants, and synthetic organic contaminants. Depending on the production source, wastewaters are divided into two main categories: (1) municipal wastewater and (2) industrial wastewater.

Municipal wastewater, also known as domestic wastewater, is usually collected through sewage systems as a discharge of residential and commercial zones. It comprises 99.9% water and 0.1% suspended and dissolved organic and inorganic solids. Carbohydrates, soaps, detergents, proteins, fats, and lignin are the substances that are commonly present in domestic wastewaters. On the other hand, industrial wastewater, characterized by a wide range of recalcitrant organic compounds, is always a major concern when it comes to environmental protection. In general, industries generate large volumes of wastewaters, which vary in composition depending on the type of process [4]. Therefore, their characteristics are much more complicated than those of domestic wastewaters. Industrial wastewaters normally contain a wide variety of organic compounds that cannot be quantified or identified individually. Depending on the type of industrial process, industrial effluents contain a mixture of various pollutants with different compositions. Commonly present pollutants include suspended solids, biodegradable organic matter, pathogens, priority pollutants, recalcitrant organics, and dissolved inorganic and heavy metals [5].

Depending on the type of industrial process and contaminants, industrial wastewaters are classified as organic and inorganic wastewater. Inorganic industrial wastewaters are produced from coal, steel, nonmetallic minerals, commercial enterprises, and metal surface processing industries. On the other hand, organic industrial wastewater contains organic wastes produced from pharmaceutical manufacturing factories, leather tanneries, textile factories, the oil refining industry, the metal processing industry, pulp and paper manufacturing plants, and fermentation factories [6]. Waste from these industries is usually produced in the form of liquids, solids, or gases. Furthermore, depending on the type of industry, manufacturing processes, and chemicals, the composition of industrial effluents varies from one industry to another. This accentuates the importance of understanding industrial processes and process streams in detail to determine the feasibility of any wastewater treatment process. Therefore, the next section of this chapter discusses in detail the manufacturing processes, production streams, and

characterization of selected industrial wastewaters. Furthermore, the characteristics of the wastewaters generated from various industries are summarized in Table 16.1.

16.2.1 Textile Industry

The textile industry is one of the important economic sectors and always serves as the backbone of the economy of developing and underdeveloped countries. It is a diverse and complex industry, comprising variable processes such as washing, scouring, bleaching, mercerizing, dyeing, and finishing to produce textile-related products such as clothing, carpeting, draperies, upholstery, linens, towels, and much more [28]. Characterized by intense consumption of chemicals, water, fuel, and energy, the textile industry has emerged as one of the biggest threats to the environment [29]. Depending on the dyeing process and type of textile product, the amount of water consumption varies from one industry to another. For instance, the wet processing industry consumes a large amount of water and high concentration of chemicals. Approximately 500 to 300,000 L of water is utilized in a typical cotton industry to produce 1 kg of textile product [30]. As a result, large volumes of highly toxic wastewater are produced. It is estimated that about 1000−3000 m^3 of wastewater is produced after processing about 12−20 tons of textiles per day [31].

In addition to water consumption, the textile industry also consumes a large quantity of chemicals and dyes. Approximately 700,000 tons of dyes are available on the market and two-thirds of the total dyestuff is consumed in textile dyeing processes. The type and the amount of dye consumed vary depending on the type of manufacturing process. Reactive dyes, direct dyes, naphthol dyes, indigo dyes, acid dyes, dispersed dyes, basic dyes, and direct dyes are the major classes of dyestuffs used in textile industries [32]. With a worldwide consumption of 178,000 tons of dyes per year, reactive dyes are the most commonly used dyes in cotton industries [31,33]. In the textile industry, dyes, colorants, surfactants, salts, and other chemicals are also used to increase the cloth resistivity toward physical, chemical, and biological agents [34,35]. The contribution of these reagents, along with textile dyes, makes the wastewater complex in nature and produces toxic and nonbiodegradable compounds when discharged into the environment.

As previously mentioned, textile processing involves various processes that produce a vast amount of liquid, solid, and gaseous waste. The sequence in which textile processes take place is sizing, desizing, scouring, bleaching, dyeing, and finishing [36]. Therefore, the composition of wastewaters generated depends on the type of individual process, the type of textile products, and the chemicals used. For instance, compared to sizing and desizing, large volumes of wastewaters are produced in bleaching, dyeing, and scouring. In the sizing operation, synthetic agents such as polyvinyl alcohol, polyacrylates, and carboxymethyl cellulose are used. The effluent generated in this process is usually small in volume but highly concentrated in terms of COD, BOD, and total suspended solids (TSS) [28]. In addition, the usage of starch in the desizing process contributes up to 50%

Table 16.1 Characterization of Selected Recalcitrant Wastewaters From Various Industries

Source	COD	BOD	TOC	TSS	pH	N	P	Other parameters	Refs
	g/L	g/L	g/L	g/L					
Olive oil	117	34.4	–	8.9	5.46	1.58	0.84	Glucose, 12 g/L; reducing sugar, 26; TS, 11.4%; TV, 9.3; VSS, 6.5 g/L; residual oils, 9.2 g/L	[7]
	97.6	85	23	–	4.85	–	–	Oil grease, 0.766 g/L; total phenol, 4.023 g/L	[8]
	63	38	26.8	16.8	5.56	0.69	–	TS, 41.4 g/L; acidity (mg/L $CaCO_3$), 6.636 g/L; Cl^-, 1.150 g/L; oil and grease, 5 g/L	[9]
	150	37.5	–	34.7	4.8	0.95	3.8	Soluble COD, 150 g/L; TS, 52.16 g/L; alkalinity, 1.550 g/L; total phenol, 8.9 g/L	[10]
Dairy industry	10.25	4.84	–	5.802	8.34	0.663	0.153	Chlorides, 0.616 g/L	[11]
	2–68.6	1.2–40	–	0.3–59	4–11	0.065–1.12	0.009–0.5	VS, 0.33–2.6 g/L; fats and grease, 0.3–9.44 g/L	[12]
Meat industry	2.8–3	1.4–1.6	–	2.2–2.5	6.7	–	–	Fixed solids, 1.2–2.4 g/L; VS, 1.05–1.132 g/L; turbidity, 1000–1200 NTU	[13]
Slaughterhouse	2–6.2	1.3–2.3	–	0.85–6.3	6.3–6.60	0.07–0.24	0.015–0.04	Acidity, 0.9–1.78 g/L; oil and grease, 0.04–0.6 g/L; turbidity, 90–130 NTU; VSS, 0.66–5.25 g/L; fixed suspended solids, 0.34–1.4 g/L	[14]
	13.381	–	–	7.0	–	0.059	0.034	VS, 6.2 g/L; FOG, 5.953 g/L; VFA, 0.566 g/L; TKN, 0.294 g/L	[15]

Winery wastewater	0.32 −49.105	0.203 −22.418	0.041 −7.363	0.066–8.6	2.5–12.9	0.01 −0.415	0.28	TS, 0.748–18.332 g/L; total phenolic compounds, 1.450 g/L	[16]
Palm oil	77–82	20–23.4	–	26.25–28.92	4.25 −4.48	0.4–0.49	–	Soluble COD, 37.5–40 g/L; VFA (acetic acid), 8.5–10 g/L; total VS, 32.5–38.7 g/L; oil and grease, 3.167–5.193 g/L	[17]
	15–100	10.5–43.75	–	5–54	3.4–5.2	0.18–1.4	1.281 −1.928	TS, 11.5–79; total VS, 9–72; oil and grease, 0.13–18; color (ADMI), >500	[18]
Paper and pulp industry	0.95 −38.588	0.14–13.08	–	0.037 −23.319	4.2–11.6	0.002 −0.35	–	TS, 1.16–51.583; color (Pt-Co), 0.0166–4.667	[19]
Paint industry	10–17	–	–	9.5	7.6	–	–	Dissolved COD, 7.43 g/L; color, gray; VSS, 5.42 g/L	[20]
Bamboo industry	14–86	0.180	0.597	0.045	8.46	0.114	–	Turbidity, 56 NTU; NH_3-N, 0.102 g/L; color (Abs-575), 1.27	[21]
Petroleum wastewater	15–51	7.8	6	–	6.16	0.06	–	VFA, 2.3 g/L; alkalinity, 0.7 g/L; C/N, 109/1	[1]
	55–60	30–32	–	0.02–0.3	2.5–2.7	0.05 −0.212	0.102 −0.227	VFA, 93–95 g/L; total acidity, 45–46 g/L; phenol, 0.36 g/L; oil and grease, 0.012–0.013 g/L	[22]
	15	–	49.5	0.3	6.12	0.046	–	Total VFA, 2.210 g/L; VS, 0.46 g/g; C/N, 107/1	[23]
Tannery industry	12–23	0.8–4.0	–	6–31	6–8.2	–	–	Suspended solids, 6–31 g/L; ammonium, 0.12–0.25 g/L; chlorides, 2–7 g/L; sulfides, 0.03–0.13 g/L	[24]
Coking mill	16	5.45	4.39	0.712	9.1	–	–	Phenol, 1.65 g/L; oil and grease, 0.00473 g/L; turbidity, 691 NTU	[25]
	2–3	0.6–0.8	–	–	6.5–7.5	0.1–0.15		Total phenols, 0.05–0.15 g/L; alkalinity, 0–4 mmol/L	[26]
Cosmetics industry	7.9–11.8	–	–	1.57–1.80	7.03 −7.18	–	–	Soluble COD, 5.2–7.8 g/L; VSS, 1.30–1.55 g/L; fats and oils, 1.42–2.0 g/L	[27]

BOD, biochemical oxygen demand; *COD*, chemical oxygen demand; *FOG*, fat, oil, and grease; *TKN*, total Kjehldahl nitrogen; *TS*, total solids; *TSS*, total suspended solids; *VFA*, volatile fatty acids; *VS*, volatile solids; *VSS*, volatile suspended solids.

of the total BOD in woven fabric processing. Furthermore, the presence of oils, fats, waxes, minerals, and grease is not desirable because they interfere with the dyeing and finishing process. These impurities can be removed in the scouring process by using water containing scouring agents or solvents, but intense chemical consumption usually produces wastewater with high COD, BOD, and toxic solids content. In the textile industry, the bleaching process produces wastewater with high solids content. It is produced as a result of high consumption of chemicals such as sodium hypochlorite, hydrogen peroxide, optical brighteners, and some auxiliary compounds. In the textile dyeing process, there is always a portion of unfixed dyes that gets washed out without being attached to the textile fiber [37]. Therefore, high concentrations of dyes are always present in textile effluents. Also, a large amount of water is required in the rinsing and dyeing process to remove unfixed dyes, which contributes to the generation of an enormous amount of wastewater. Unfixed dyes cause health issues such as hemorrhage, ulceration of the skin, nausea, and dermatitis. In addition, they also prevent photosynthesis and reduce reoxygenation [38].

Considering the aforementioned discussion, it is already established that the characteristics of textile effluents vary depending on the type of textile product and the chemicals used. The available literature shows that untreated textile wastewaters can be characterized by BOD, COD, color, suspended solids, dissolved solids, and heavy metals [39–46]. Based on previous studies, the textile industry discharge is highly nonbiodegradable as depicted in Table 16.2. Typical textile wastewaters contain high levels of COD, color content associated with residual dyes, a wide range of pH, and low biodegradable salt content [37,42]. In addition, textile effluents contain trace metals like Cr, As, Cu, and Zn, which can harm the environment. The presence of highly nonbiodegradable substances is also indicated by the BOD/COD ratio of the wastewater, which is around 1:4. The wastewaters from textile dyeing facilities are difficult to treat because of the

Table 16.2 Characteristics of Typical Untreated Textile Wastewater

Parameter	Range
Chemical oxygen demand (mg/L)	150–12,000
Biochemical oxygen demand (mg/L)	80–6000
Total suspended solids (mg/L)	15–8000
Total dissolved solids (mg/L)	2900–3100
pH	6–10
Temperature (°C)	35–45
Color (Pt-Co)	50–2500
Sodium (mg/L)	70
Nitrogen (mg/L)	70–80
Oil and grease (mg/L)	10–30
SO_4 (mg/L)	600–1000
Chlorine (mg/L)	1000–6000

large amounts of dyestuffs, surfactants, and additives, which are generally nonbiodegradable. These recalcitrant compounds are not easily amenable to chemical or biological treatment. These are the reasons wastewaters released from textile plants seldom meet the discharge standard [37].

16.2.2 Pharmaceutical Wastewater

The pharmaceutical industry is one of the major complex industries and it generates toxic industrial wastes that can affect the environment and human health adversely [47]. A high-quality water supply is a very important raw material in the pharmaceutical industry for production, material processing, and cooling processes [48]. Pharmaceutical manufacturing consists of manufacturing, extraction, processing, purification, and packaging of biological products, medicinal chemicals, botanical products, and other pharmaceutical products. This industry is characterized by a diversity of products, processes, and wastewater quantity and quality [49].

It is almost impossible to characterize each and every product waste from the pharmaceutical industry because it comprises a variety of processes. These processes include chemical synthesis, fermentation, and extraction and produce various products depending on the type of raw material used [49]. Based on the raw material used and waste produced, pharmaceutical manufacturing can be divided into five major subcategories [28]: (1) fermentation plants, (2) synthesized organic chemicals plants, (3) fermentation/synthesized organic chemicals plants, (4) natural/biological product extractions, and (5) drug mixing, formulation, and preparation plants. The pharmaceutical industry uses an array of complex batch-type processes and technologies for its product manufacturing [50]. As a result, pharmaceutical effluents with different characteristics and quantities are generated throughout the year based on the seasonal use of many raw materials. The general characteristics of raw pharmaceutical wastewater are measured in terms of COD, BOD, and TSS as depicted in Table 16.3.

The chemical synthesis process is a multistep process that produces a mother liquor that contains unreacted reactants, products, by-products, and residual products in the organic solvent base [51,52]. Other products generated may include acids, bases, halides, nitrates, sulfates, cyanides, and metals. The fermentation plant also generally produces

Table 16.3 Composition of Pharmaceutical Wastewaters [49]

Parameters	Chemical Process Wastewater
Chemical oxygen demand (mg/L)	2000–3000
Biochemical oxygen demand (mg/L)	1200–1700
pH	6.5–7.0
Total alkalinity (mg/L)	50–100
Total suspended solids (mg/L)	300–400
Phenols (mg/L)	65–72

extremely strong and highly organic wastes that are difficult to treat and are frequently inhibitory to biological systems. The wastewater from the fermentation plant contains a large amount of unreacted raw materials such as the nutrient broth, metal salts, starch, nitrates, and phosphates. They have high COD, BOD, and TSS with pH values ranging from 4 to 8. Moreover, the synthetic organic chemical plant also generates wastewater streams typically consisting of cooling waters, condensed steam still bottoms, mother liquors, crystal end-product washes, and solvents that are nonbiodegradable and toxic. In addition, the biological production plant produces approximately 180,000 gal/day of wastewater. This wastewater contains animal manure, animal organs, baby fluid, blood, fats, egg fluid, egg shells, spent grains, and several other industrial wastes. These wastes are very high in BOD, COD, total solids, colloidal solids, toxicity, color, and odor. The BOD/COD ratio of this wastewater is around 0.66, which indicates the presence of highly nonbiodegradable organic compounds. On the other hand, the natural and biological extraction process produces waste streams with low BOD, COD, and TSS, with relatively neutral pH values. The pH values range from 6 to 8, as natural materials (plant and animal) are used to extract the active pharmaceutical ingredient. Finally, the drug formulating process consists of mixing, pelletizing, encapsulating, and packaging. This process produces slightly acidic wastewater with high organic strength and relatively low suspended solids, which exhibits a degree of toxicity.

Treatment of effluents from pharmaceutical industries is also becoming a challenge. The effluents are generated in small volumes but are high in nonbiodegradable recalcitrant compounds [53]. Much attention has been given to the presence of pharmaceutically active compounds (PhACs) in the water. About 3000 substances have been registered in the European Union for pharmaceutical purposes [54]. After their use, most of the PhACs are released into the aquatic environment as effluents from municipal sewage treatment plants. Pharmaceutical origins are often partially eliminated during the wastewater treatment. Most of them are complex organic chemicals that are resistant to biological degradation [55]. Pharmaceutical wastes have also reached drinking water and are in bank-filtered water [56]. Literature studies show that drugs and their metabolites are widely distributed in surface waters. Pharmaceutical wastewaters have been examined thoroughly and characterized as having high BOD, COD, total dissolved solids, and TSS concentrations and extremely variable pH values [57]. Meeting the national standards has been a constant struggle for pharmaceutical wastewater. This is caused by the varying wastewater compositions and fluctuating pollutant concentrations.

16.2.3 Petroleum Refinery Wastewater

The petroleum industry is one of the largest industries, processing crude oil into a wide range of petroleum products. These products include gasoline, fuel oil, heating oil, and petrochemicals [58]. The global demand for petroleum products is increasing. This is causing more usage of water for petroleum refinery activities leading to the generation of a significant volume of petroleum wastewater. Approximately 3.5–5 m^3 of highly

polluted wastewater per ton of crude oil is produced through the petroleum manufacturing process. Wastes generated from the petroleum industry include produced water, drilling muds, and tank bottom sludge.

The oil refining process is a complex operation that involves separation of crude molecular constituents, molecular cracking, molecular rebuilding, and solvent finishing steps to produce petroleum-derived products. The refining process is a major step, which generates highly recalcitrant wastewater, which contains phenol, suspended solids, oil, ammonia, sulfides, chlorides, dissolved materials, and other hydrocarbons [59]. After crude oil is separated from natural gas, it is transported to refineries and processed into petroleum-derived products. Refineries range from small to giant complex units that process 150−600,000 barrels of crude oil per day. The refining processes can generally be divided into separation, conversion, and chemical treatment processes, as shown in Fig. 16.1 [60].

Intermediate and finished products of crude oil are stored in tanks to give adequate supplies for primary fractionation runs, equalizing process flows, and providing feedstock for intermediate processing units [61]. Tank storage is also used to store final products prior to shipment in adjustment to market demands. Generally, the operating schedules permit sufficient detention time for settling of water and suspended materials. The wastewater derived from the storage of crude oils and products is mainly free oil, emulsified oil, and suspended solids. This waste is high in COD and low in BOD. On the other hand, finished products are alkaline and the refinery processes generate wastewater with high BOD. The wastewater also contains tetraethyl lead. Tank cleaning can also contribute to large amounts of oil, COD, and suspended solids and a small amount of BOD. In addition to this, catalytic cracking produces wastewater, which contains oil, sulfides, phenols, cyanides, and ammonia that produce alkaline wastewater with high BOD and COD concentrations [62].

According to a US EPA survey, it can be concluded that more than 150 processes used in petroleum refineries generate large quantities of hazardous wastewaters containing

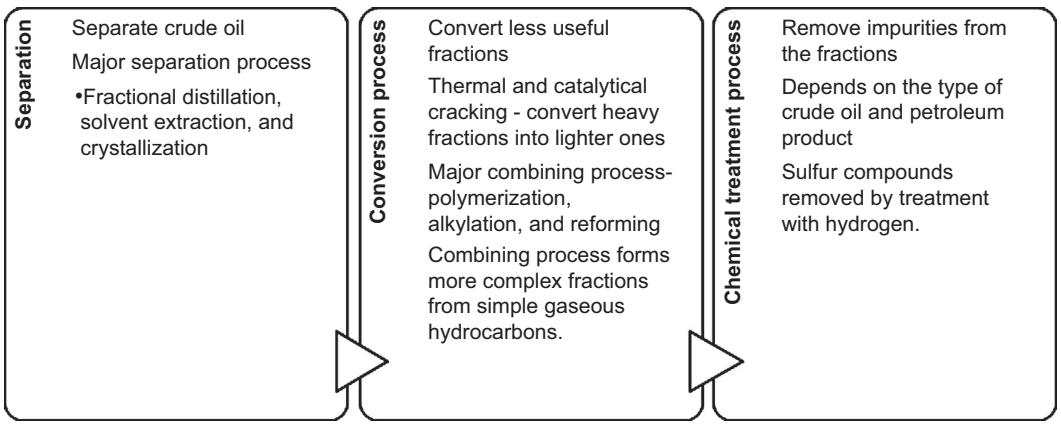

FIGURE 16.1 Schematic diagram of the refining process.

similar constituents. The amounts and types of waste generated in a refinery depend on a variety of factors such as process configuration, crude capacity, refining processes, and crude source [63]. Based on a recent available figure of crude oil exploration, an estimated amount of 33.6 million barrels per day of wastewater is generated. The major wastes are wastewater treatment plant sludge, spent caustics, sulfur, and spent catalysts. Wastewater from refineries contains approximately 200−600 mg/L COD levels, 20−200 mg/L phenol, 1−100 mg/L benzene, 0.1−100 mg/L chrome, and 0.2−10 mg/L lead [28]. Major wastes in this refinery are wastewater treatment plant sludge (dewatered by pressure filtration), spent catalysts, and spent clay filter media. Phenolic derivatives are the major pollutant causing significant threat to the environment and human health because of their toxicity, stability, and poor biodegradability [64]. In the face of strict global regulations and legislation on pollution control, there is a dire the need for an efficient water treatment technology.

16.2.4 Landfill Leachates

Landfill leachates are known to contain large amounts of organic and inorganic compounds such as heavy metals, humic-derived constituents, ammonia−nitrogen, and chlorinated organic and inorganic salts [65,66]. Treatment of landfill leachates is a major concern because of the volume of wastewater discharge and the properties of these wastewaters. Leachates are formed when water percolates through the dumped wastes and takes up the organic and inorganic products through physical extractions and hydrolytic and fermentative processes [65]. Leachates have distinct characteristics. Their composition is based on the deposited wastes' composition, their physicochemical characteristics, and the volume of water that supports microbial activity [67,68]. Previous studies have shown that the variability of organic, inorganic, and heavy metal contents is strongly influenced by the age of leachate [68]. Young leachates that are formed within 2 years of deposition of wastes hold more organics and have lower molecular weight (BOD/COD) compared to old leachates [66]. Organics are partially degradable and the fulvic acid substances are of relatively high molecular weight with persistent characteristics in older leachates. This results in lower biodegradability with a low BOD/COD ratio [67,69].

In addition to leachate age, there are several factors that influence the composition and quality of landfill leachates. Some of these are moisture and adsorption capacity of the waste, topography of the landfill site, the techniques of landfilling, and the quality of the waste. As an effect of these factors, the quality of the leachate is rather complex and it is also difficult to predict the volume and quality of a leachate at a given landfill.

According to the latest available data, more than 200 organic compounds have been identified in leachates. Of these, 35 substances, including chlorobenzene, dichlorobenzene, styrene, naphthalene, toluene, ethylbenzene, and trichloro-, tetrachloro-, and pentachloro phenols, are recognized to have a hazardous impact on the environment. Therefore, to predict the overall impact of these compounds, leachate is also characterized

Table 16.4 Characterization of Landfill Leachate [70–72].

Parameter	Range
Chemical oxygen demand (g/L)	2.8–28
Biochemical oxygen demand (g/L)	1.04–11.3
pH	8–8.9
Total suspended solids (g/L)	0.85–5.84
Nitrogen (g/L)	0.002–3.199
Phosphorus (g/L)	0.011–0.018
Chloride (g/L)	1.95–3.65
Alkalinity (g/L	4.04–22.1
Ammonia (g/L)	0.75–0.84

in terms of toxicity measurement. Toxicity is categorized as acute or chronic depending on short-term and long-term exposures to living species. Furthermore, landfill leachate also contains a wide variety of heavy metals. The composition of these metals varies depending on the source. However, quantification of data is essential prior to the selection of any treatment process. For this purpose, Table 16.4 presents the characteristics of landfill leachate that will be helpful in the selection of treatment process.

16.3 Anaerobic Wastewater Treatment Technologies With Resource Recovery

The essence of any biological process to treat wastewater depends on the activity of the microorganisms and the intimate contact between the bacterial community and the organic contaminants. Therefore, the configuration of the wastewater treatment unit plays a fundamental role in the success of anaerobic digestion. Considering these aspects, anaerobic wastewater treatment systems are usually classified into conventional and high-rate anaerobic systems. The subclassification of these two groups is described in Fig. 16.2.

16.3.1 Conventional Wastewater Treatment Systems

Anaerobic digestion is a simple and straightforward way to harvest energy (in the form of biogas) from organic compounds present in wastewaters [73]. The biogas produced has high calorific value and is recognized as a renewable source of energy. Conventionally, anaerobic digestion is carried out in anaerobic sludge digesters [74], ponds [75], septic tanks, and anaerobic lagoons [15]. Characterized by low volumetric organic loads, slow reaction rates, partial decomposition of the organic fraction, large footprints, and poor gas capture, these processes are not suitable for treating industrial wastewaters [75,76].

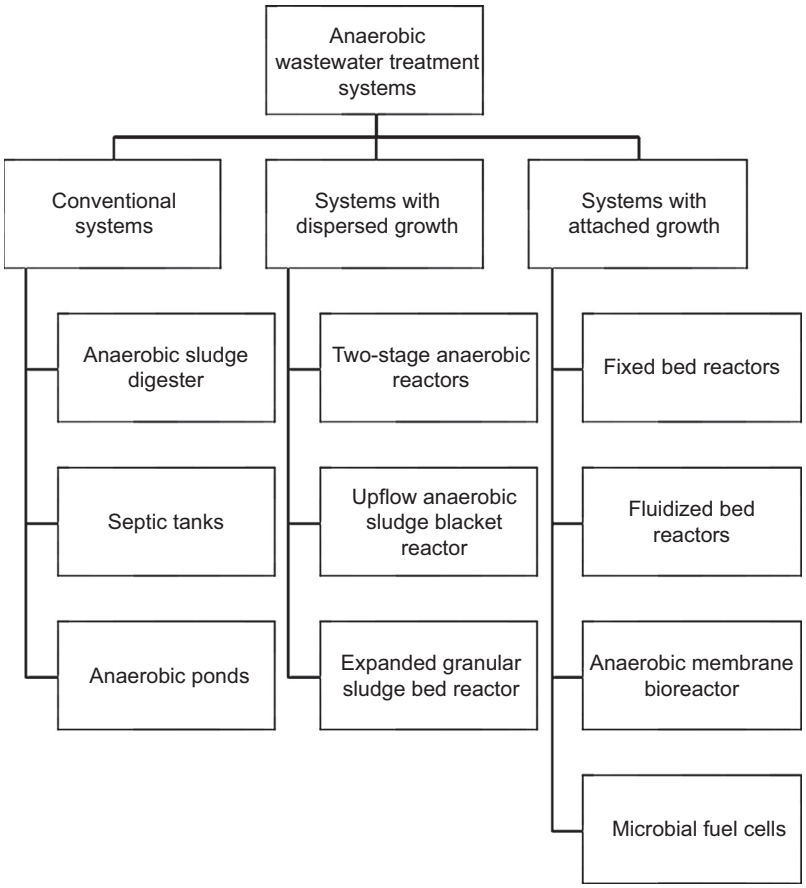

FIGURE 16.2 Classification of anaerobic treatment systems.

For instance, septic tanks are known for treating limited amounts of wastewater and are suitable for treating lower organic concentrations such as domestic or sewage wastewater. These systems can hardly remove 15–40% of BOD and retain 50–80% of solids [77]. This kind of treatment cannot guarantee the elimination of pathogens and results in poor yields of methane gas. On the other hand, anaerobic ponds with the potential of treating highly concentrated organic loads (slaughterhouse, brewery, and dairy industry) have long hydraulic retention times. Therefore, these reactors are classified as low volumetric organic load reactors [78]. A significant improvement in the performance of conventional anaerobic digestion systems can be achieved by an anaerobic sludge digester. Anaerobic sludge digesters are generally used for the stabilization of primary and secondary sludge with simultaneous energy production in the form of biogas [79]. The biogas produced comprises 60–70% of methane and is used in combined heat and power generation plants for the production of heat and electricity by combustion engines [2]. Furthermore, the sludge produced, as a result of anaerobic digestion, can also

be used as a potential source of bioenergy [76]. Therefore, anaerobic sludge digesters play a key role in improving the economic balance of wastewater treatment plants. Depending on the demands of particular wastewater treatment plants and available technology, two types of anaerobic sludge digesters exist:

1. Low-rate anaerobic digester
2. High-rate anaerobic digester

16.3.1.1 Low-Rate Anaerobic Digesters

Conventional anaerobic sludge digesters are known as low rate anaerobic sludge digesters, with a long digestion time of 30–60 days. These digesters perform two simultaneous functions, i.e., stabilization by anaerobic digestion and separation of digested solids from supernatant in a single vessel. Therefore, low-rate digesters are not efficient and they demand intense mixing of anaerobic biomass and excess sludge. In practice, the mixing requirements of a low-rate anaerobic digester are fulfilled by recirculation of biogas, which in fact fails to fulfill the demand of large-scale wastewater treatment plants. Therefore, as a result of poor mixing and heating, different zones are developed within a reactor. These include: (1) digesting sludge, (2) supernatant, (3) scum layer, and (4) gas [74]. A schematic representation of the zones formed in a low-rate anaerobic sludge digester is shown in Fig. 16.3. As a result of sludge stratification, 50% of the digestion volume is used in the digestion process. Therefore, to achieve a highly stabilized sludge, a large volume is required [80]. However, large-volume reactors fed with low

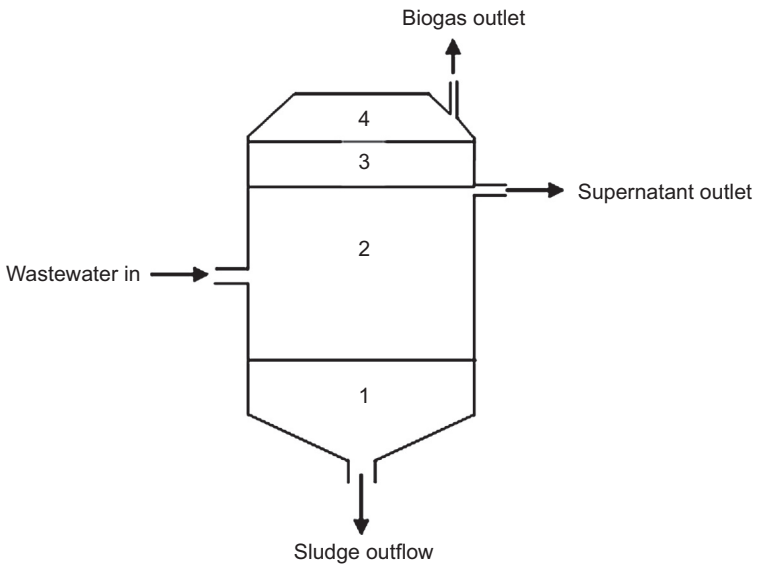

FIGURE 16.3 Schematic representation of a low-rate anaerobic digester with different zones: (1) digesting sludge, (2) supernatant, (3) scum layer, and (4) gas.

organic content produce less biogas, which is not able to fulfill the heating and mixing requirements of anaerobic digestion. Thus, to achieve high yields of biogas, small-volume reactors are employed with low organic content and sludge with high solid content. However, poor mixing and low mass-transfer rates in low-rate anaerobic digesters result in poor performance of the small reactors. According to Liao and Li [80], sludge with more than 12% solid content hardly produces biogas. Keeping all these aspects in view, high-rate anaerobic digesters have been introduced as a modification of low-rate anaerobic digesters. High-rate anaerobic digesters are a major improvement in conventional treatment technologies because the entire volume of the digester is effectively utilized for digestion, whereas phase separation is carried out in a separate tank.

16.3.1.2 High-Rate Anaerobic Digesters

A high-rate anaerobic digester, also known as a two-stage anaerobic sludge digester, comprises a vessel equipped with a mechanical stirrer and a secondary tank. The secondary tank is used for phase separation and sludge storage. Therefore, it is not equipped with any mixing device. If the secondary tank is fitted with a floating cover, it can also be used to store biogas [81]. The primary tank is equipped with mixing and heating devices, which help provide a uniform mixing of the feed sludge. The sludge is completely mixed and thickened, and thus the entire reactor volume is utilized for efficient digestion. As a result, the tank volume is greatly reduced and process stability and sludge stabilization are improved at the same time [76]. Once the system attains the steady-state condition, sludge should be fed continuously to maintain the conditions and reduce shock loadings. In the secondary tank, solids reduction and biogas production are very small and in most cases, the supernatant contains high concentrations of solids and small gas bubbles [11]. This limiting factor is linked to the poor digestion in the primary digestion tank [11]. In such case, recycling of the supernatant to the primary tank will be an extra load on the sludge in the digester. It is, thus, recommended to replace the secondary tank with some solid—liquid separators. Because phase separation in these units is better, the recirculation of supernatant will not cause problems in anaerobic digestion [81].

16.3.2 Systems With Dispersed Growth

16.3.2.1 Two-Stage Anaerobic Reactors

Anaerobic digestion is mainly employed to reduce solids content and pathogens from wastewater while recovering a renewable source of energy in the form of methane gas. In this regard, methanogenic bacteria play a fundamental role in stabilizing the solid and liquid content of the wastewater being treated [82]. However, accumulation of highly concentrated volatile fatty acids at high organic loading rate or short hydraulic retention time may result in a decrease in pH if a single-chamber configuration is used [83]. A decrease in pH is not desirable because it results in the termination of the

anaerobic digestion process before the methanogenesis process takes place. In addition, a short hydraulic retention time may result in complete washout of the methanogenic bacteria [82].

Recognizing the difference between the acid and the methane formers, it is suggested to maintain the controlled stabilization by phase separation of the two groups and culturing in isolated environments [84]. In this way, an optimum environment can be provided to both groups of the bacteria with controlled substrate loading rates to improve process efficiency. One of the ways to attain these conditions is the use of two-stage processes. The two-stage anaerobic process comprises two sequential steps of hydrogen and methane production. In the first stage acetogenic bacteria convert the substrates such as carbohydrates to hydrogen, carbon dioxide, and fatty acids. The gaseous product exits the reactor and fatty acids enter the second stage, in which they are further degraded by methanogenesis to produce methane and carbon dioxide [85].

A two-stage anaerobic digester offers two advantages. First, because of the balance between volatile fatty acid production and their consumption, higher rates of methane are attained. Second, the hydrogen produced has high energy yields of 122 kJ/g, which is 2.75 times higher than that of hydrocarbons and is a renewable energy source [86]. Therefore, a two-stage anaerobic digestion with the potential of both methane and hydrogen production can increase the energy recovery efficiency of the wastewater treatment system. Nevertheless, the overall efficiency of the process is significantly affected by several factors such as microbial community, organic loading rate, hydraulic retention time, and temperature. For instance, a 40% increase in methane production can be achieved when a two-stage anaerobic system is operated at a hydraulic retention time of 24 days and organic loading rate of 4.5 kg/m^3 [87]. This implies that hydraulic retention time and organic loading rate conditions can significantly affect the energy recovery efficiency of the two-stage anaerobic system. According to developments in the two-stage anaerobic digestion system, a 50% increase in the hydrogen yields was obtained when the system was operated under thermophilic conditions [88]. In another study, a maximum 35% COD reduction was attained when COD loading rates were maintained at 90 kg/m^3 day. However, a further increase in COD loading rate resulted in the reduction of COD removal efficiency, which showed the importance of optimizing the two-stage anaerobic process [85]. Some examples of the two-stage anaerobic process are summarized in Table 16.5. From the examples listed in Table 16.5, it is noticeable that the two-stage anaerobic process possesses shorter hydraulic retention time and the process performs differently under different conditions. For instance, Maspolim et al. [82] compared the COD removal efficiency of both single and two-stage anaerobic digestion under different hydraulic retention times (HRTs). The authors found that in the case of a single-stage digester the COD removal efficiency was decreased from 39% to 26.3% when HRT was reduced from 30 to 12 days. However, for a two-stage anaerobic digestion process a 42.3% COD reduction was attained when the HRT was maintained at 12 days [82].

Two-stage anaerobic digesters are available in different reactor combinations. The most frequently used combination is the continuously stirred tank reactor for hydrogen production and the upflow anaerobic sludge blanket reactor (UASB) for methane production. In one example, hydrogen and methane at production rates of 3.63 and 1.75 m^3/m^3 day were achieved at volatile solid loading rates of 11.9 kg/m^3 day [98]. Furthermore, Cooney et al. [99] reported that methane production in a two-stage system was more stable and effective than in the one-stage process. Like a single reactor, a two-stage system is severely affected by the variation in operating parameters. This is also obvious from the literature provided in Table 16.5. Therefore, more data related to operating conditions and energy production potential should be collected to establish an optimized operating range for the two-stage anaerobic digestion.

16.3.2.2 Upflow Anaerobic Sludge Blanket

Conventional anaerobic digestion processes are inefficient for treating high-strength organic waste. In this regard, UASBs have emerged as an efficient treatment technology. Short retention times, low operation cost, ability to treat highly concentrated organic waste, propensity to promote intimate contact between microorganisms and organic contaminants, and energy recovery in the form of biogas are a few aspects that make UASBs a high-rate anaerobic treatment system [100,101].

An upflow sludge blanket reactor comprises a large cylindrical vessel. The process consists of upflow of wastewater through a dense sludge bed with high microbial activity [102]. The solids profile within the reactor varies from dense and granular particles in the sludge bed zone (bottom section) to a dispersed and light sludge close to the sludge blanket zone (top section). In a UASB, the upward direction of the influent promotes intense mixing within the systems, which results in conversion of organic matter in all reaction areas (bed and sludge blanket). The wastewater enters at the bottom and leaves at the top of the reactor through an internal settling tank. A gas and solids separation device located below the settling tank maintains the favorable conditions for particle sedimentation for particles that stray from the sludge blanket, allowing them to return to the digestion section instead of leaving the system [74].

During the start-up, the upflow direction of wastewater results in immature granulation of sludge, which results in long start-up times. Therefore, granular sludge from another the facility is usually introduced to reduce the start-up time to less than a day [103].

The sustainability of UASBs lies in the production of biogas. Wastewater with high sulfate content causes a competition between sulfur-reducing bacteria and methane-producing archaea (MPA) for reducing carbon sources. This results in a decrease in methane production and failure of the treatment process. Thus, a balance between COD and sulfate is essential for successful operation of anaerobic digestion. In the literature, diverse values have been reported for optimized COD/SO_4^{2-} ratios. The reported values vary from 0.67 to 10 for successful operation of anaerobic digestion in UASBs [104,105].

Table 16.5 Summary of Literature on the Two-Stage Anaerobic Process

Wastewater	Optimum Conditions	Output	References
Municipal wastewater (primary and secondary sludge)	OLR COD = 3.5 g COD/L day; OLR VS = 2.1 g VS/L day; HRT = 12 days; $T = 35°C$	COD (%) = 42.3; VS (%) = 35.5; CH_4 = 20.6 L/day; Biogas composition: CH_4: 64–67% CO_2: 30–34%	[82]
Anaerobic digester sludge from anaerobic digesters of wastewater treatment plant	OLR = 1.83 g TVS/L day; COD = 28.9 g/L day; TS = 58.3 g/L; HRT = 24 days	Without prethermal treatment: Biogas production: 16 L/day; CH_4: 67.7%; TS(%) = 65.7%; COD (%): 42.9%; SCOD (%): 67.9%	[89]
Anaerobic digester sludge from anaerobic digesters of wastewater treatment plant	OLR = 1.83 g TVS/L day; COD = 28.9 g/L day; TS = 58.3 g/L; HRT = 24 days	Preheated at 55°C for 12 h: Biogas: 21.7 L/day; COD (%): 53.1; TS (%): 68.2; SCOD (%): 91.4	[89]
Mixture of olive oil, cheese whey, and liquid cow manure	Acidogenic reactor: HRT: 0.75 day; OLR: 126.67 kg COD/m^3 day; TS: 73.52 g/L; VS: 63.52 g/L; TCOD: 95 g/L; SCOD: 58.70 g/L Methanogenic reactor: 3.37 kg COD/m^3 day	Acidogenic reactor: Biogas: 4.90 L/L_R day; H_2 (L/L_R day): 1.72; H_2 (%): 35.45% Methanogenic reactor: biogas: 0.84 L/L_R day; CH_4: 0.5 L/L_R day; SCOD: 51.97%; TS (%): 26.11; VS (%): 47.51	[90]
Mixture of sorghum, cheese whey, and liquid cow manure	Acidogenic reactor: HRT: 0.5 day; OLR (COD): 171.60 kg COD/m^3 day; OLR (VS): 115.62 kg COD/m^3 day Methanogenic reactor: HRT: 16 days; OLR (COD): 7.15 kg COD/m^3 day; OLR (VS): 3.87 kg COD/m^3 day	Acidogenic reactor: Biogas: 5.69 L/L_R day; H_2: 2.14 L/L_R day; VS: 18.99% Methanogenic reactor: Biogas: 1.52 L/L_R day; CH_4: 0.9 L/L_R day; VS: 42.32%; TCOD: 83.86%; SCOD (%): 85.09	[91]
Pelletized grass	Acidogenic reactor: pH: 5.5; T: 35°C; HRT: 18 h Methanogenic reactor: pH: 7; T: 35°C; HRT: 11.25 days	Acidogenic reactor: H_2: 5.2 L/day; yield: 6.7 L H_2/g VS Methanogenic reactor: CH_4: 33.98 L/day	[92]
Cassava wastewater	Acidogenic reactor: pH: 5.5; T: 55°C; OLR (COD): 90 kg COD/m^3 day	Acidogenic reactor: Biogas: 5.5 L/day; COD (%): 35; H_2: 2.2 L/day	[88]

Continued

Table 16.5 Summary of Literature on the Two-Stage Anaerobic Process—cont'd

Wastewater	Optimum Conditions	Output	References
	Methanogenic reactor: *T*: 55°C; OLR (COD): 15 kg/m^3 day	Methanogenic reactor: Biogas: 22 L/day; COD (%): 72; CH_4: 16 L/day	
FPW and OFMSW	Acidogenic reactor: pH: 5.5; *T*: 36°C; OLR (COD): 4.5 g COD/L day; HRT: 5 days Methanogenic reactor: *T*: 55°C; HRT: 15 days	COD (%): 86.6%; CH_4: 1.23 L/L day; CH_4 yield: 0.44 L CH_4/g VS added; SCOD: 90%; TS: 62%	[93]
Mesophilic anaerobic digester wastewater/vegetable waste	OLR: 1.7 g VS/L day (3.4 g COD/L day); *T*: 37°C; pH: 6.7	Biogas: 0.97 L/L day; CH4: 0.22 L/L day; COD (%): 67.6%	[94]
Skim Letex serum	Acidogenic reactor: *T*: 55°C; HRT: 36 h; OLR: 25.3 g VS/L day; pH: 5.5 Methanogenic reactor: HRT: 9 days; pH: 7.4—7.9	Acidogenic reactor: H_2: 59.2 L/g VS; COD: 20—30% Methanogenic reactor: CH_4: 6.41 L CH_4/L-SLS; COD (%): 62%	[95]
Brewery wastewater/starfish/ municipal wastewater	OLR: 4 g COD/L day; HRT: 1 day	CH_4: 296 mL CH_4/g COD; COD (%): 44	[96]
Anaerobic sludge from primary methane digester/raw thin stillage	Acidogenic reactor: OLR: 6 g COD/g VSS; pH: 5.5; HRT: 5 h Methanogenic reactor: pH: 7	Acidogenic reactor: H_2: 1974 mL; H_2 production rate: 62 mL/h Methanogenic reactor:	[97]

[AU1]

**COD*, chemical oxygen demand; *FPW*, food processing industry; *HRT*, hydraulic retention time; *OFMSW*, organic fraction of municipal waste; *OLR*, organic loading rate; *SCOD*, soluble chemical oxygen demand; *TCOD*, total chemical oxygen demand; *TS*, total solids; *TVS*, total volatile solid; *VS*, volatile solids.

Furthermore, sulfate-reducing bacteria cannot compete with MPA if acetate is present at an influent COD/SO_4^{2-} ratio of 2−16 [106].

One of the advantages of using a UASB is the formation of a compact granular sludge structure. This is because it helps protect the microorganisms from toxic substances present in wastewaters containing biological toxic compounds [107]. However, granular formation in UASBs is highly sensitive to fats and moderately sensitive to organic content. Therefore, wastewater should be characterized and pretreated (if necessary) before being introduced into a UASB [15].

The UASB process is recommended because it is characterized by low energy requirement, less sludge formation, and low maintenance cost. However, the treated effluent from a UASB seldom meets wastewater disposal standards [108]. Application of a UASB for low-strength wastewater is impeded by the long start-up times, granular erosion, shock loading, and low yields of biogas production [101]. Therefore, continuous innovations in the field of anaerobic wastewater treatment are in demand because the success of these systems lies in the application of a relatively high loading rate while maintaining long Solid Retention Time (SRT) at relatively short HRT [109].

16.3.2.3 Extended Granular Sludge Bed Reactor

Upflow anaerobic sludge bed reactors show poor performance for low-strength wastewaters. Furthermore, sludge flotation and washout of active biomass under highly loaded conditions result in the operational failure of the anaerobic process [100]. In this regard, the extended granular sludge bed reactor (EGSB), a modified form of the UASB, can effectively treat highly concentrated industrial wastewater.

The EGSB shares most of its features with UASBs except that the upflow velocity of inlet wastewater results in the expansion of the granular sludge. The granular sludge is retained within the reactor and is kept expanded because of the high upflow velocity. The increased upflow velocity is attained by either using tall reactors or recycling the effluent. As a result, a good contact between organic compounds and sludge is maintained to segregate small inactive suspended particles from the sludge blanket.

In anaerobic wastewater treatment technology, EGSBs are preferable over UASB anaerobic systems. Unlike UASBs, EGSBs can efficiently treat highly concentrated industrial wastewater because of their ability to dilute wastewater by recirculating the treated effluent, improving substrate diffusion at the liquid granular interface and segregating small inactive suspended particles from the sludge blanket [109,110]. Furthermore, the use of high upward velocities requires a large cylindrical vessel of 20 m in height and this results in a significant reduction of the area required and makes it feasible for small treatment plants [74].

EGSBs are inherently designed for the efficient removal of soluble effluents, because high surface velocity of liquids cannot remove particulate organic materials. To solve this problem, a two-step anaerobic system comprising a UASB in series with an EGSB has been proposed [109]. The advantage of this combined system is that removal of

suspended solids, partial hydrolysis, and acidification take place in the first step and dissolved COD is converted into methane gas in the second stage. In this way, highly concentrated wastewater enriched with solid particles can efficiently be treated at a short HRT of 5 h.

16.3.3 Systems With Attached Growth

16.3.3.1 Fixed/Fluidized-Bed Reactors

The anaerobic digestion process is characterized by high HRTs of several hours to a few days. High HRTs are not desirable and require large reactors and high investment cost for proper operation [111]. In addition, low HRT results in poor substrate utilization and low biogas production, mainly caused by low microbial activity [112]. Altogether, these factors result in poor start-up of the anaerobic digester, which may cause inefficient removal of soluble nutrients, prolonged acclimation periods, and poor microbial proliferation [113]. Studies discussing anaerobic digestion have shown that a variety of carrier material can be used as a stationery packed bed to facilitate bacterial attachment or keep them in their interstices. Thus, the biofilm formed has a long residence time and dense population of microorganisms. Frequently used materials include sand, waste tires, zeolite, and glass beads [114]. Fixed-bed anaerobic reactors show a high potential for treating high-concentrations organic waste in comparison to other commonly used reactors. This is also evident from the study conducted by Zhao et al. [114] in which a six times increase in organic loading rate resulted in an increase in biogas production from 2.1 (6.7 kg/m^3 day) to 13.22 L/L day (35 kg/m^3 day).

In a few studies, it has been identified that high concentrations of ammonia may result in digester upset or its operational failure. Under high concentrations, ammonia passively diffuses into bacterial cells, leading to proton imbalance, and interferes with the metabolic enzymes of microorganisms [115]. The methanogenic activity has been reported to drop to zero at high nitrogen concentrations of 5800−6000 mg/L. To avoid this, ammonia adsorption materials such as bentonite, activated carbon, and zeolite are recommended to use as the fixed bed [115]. Based on the adsorption characteristics of the bed, free ammonia can easily be removed from the reactor. Furthermore, immobilization of bacteria on the bed interstices promotes stable proliferation of the microorganisms.

Furthermore, by comparing the performance of horizontal tubular fixed-bed bioreactors and simple bioreactors, H_2 production rates were 703 mL/L h, which was 2.3 times higher than that achieved in a simple bioreactor [116]. Nevertheless, application of a fixed-bed anaerobic reactor for treating high solid concentrations is challenging. Accumulation of biomass may lead to blockage or formation of hydraulic short-circuits, which may result in an increase in energy requirements and overall pressure drop within a reactor [74]. To avoid this, a uniform mixing or continuous agitation of bed particles is required. In this regard, fluidization of bed particles using high fluid velocity can help avoid blocking of bed material.

Fluidized-bed systems have several advantages over fixed-bed reactors. For example, fluidized-bed reactors have been reported to perform well with lower HRTs of about 2−4 h, whereas fixed-bed reactors need longer HRTs, assuming constant organic loading rates (OLRs) for both systems [117]. Furthermore, high biomass concentrations, mass-transfer rates, high biomass retention times, low biomass yield, and sludge production are a few advantages that make them capable of treating a broad spectrum of wastewaters including both readily and hardly biodegradable organic contaminants [118,119].

Considering the potentials of anaerobic fluidized-bed reactors, they can be used for treating both soluble and suspended wastewater. Anaerobic fluidized-bed reactors treating textile wastewater with CODs of 800−1200 mg/L and very low concentrations of TSS (<10 mg/L) can achieve 90% COD removal efficiency at HRT of 12−72 h [118]. In anaerobic fluidized-bed reactors, the treatability of a highly suspended solid stream is challenging owing to the long-term impact of suspended inert solids and probability of the biomass washing out. Nevertheless, Andalib et al. [119] treated high-strength thin stillage and primary sludge with a Total Chemical Oxygen Demand (TCOD) of 130,000 and 42,000 mg/L, respectively, and high TSS concentrations of 47,000 and 34,000 mg/L, respectively. As a result, around 88% TCOD and 80% TSS removal efficiencies were obtained with a net methane production of 0.31 and 0.25 L/g_{COD}, respectively [119].

To sum up, fluidization overcomes the operating problems such as bed clogging and high pressure drop that would be faced if a fixed-bed reactor were used [120]. Furthermore, a high circulation rate and expansion of small granular particles ensure a very large surface area for the growth of a uniform biofilm around each particle. Therefore, maximum contact between the bacterial consortium and the organic contaminants can be made for efficient anaerobic wastewater treatment. To date, volumetric loading rates as high as 20−30 kg COD/m^3 day have been reported with COD removal efficiencies of 70−90% and CH_4 yields of 0.31 L/g_{COD}. Unlike advanced anaerobic wastewater treatment systems, fluidized-bed reactors are also capable of treating low-strength wastewaters because they provide an efficient mass transfer between substrate and biofilm with low clogging tendency and long SRT [121].

16.3.3.2 Anaerobic Membrane Bioreactor

Anaerobic digestion is one of the most important processes for maintaining both economic and energy sustainability in wastewater treatment plants [122]. Nonetheless, poor effluent quality, large footprints, long start-up times, slow microbial growth rates, and poor biomass retention are a few factors that challenge the wide application of these systems [123].

Start-up times, sludge reduction, biogas production, and organic removal efficiencies have significantly been improved by using advanced treatment systems such as UASB, fluidized beds, and high-rate anaerobic digesters. However, biomass retention is an aspect that has largely limited the efficiency of these systems. Because of poor settling properties and low production rates of biomass, anaerobic bioreactors are unable to provide sufficient retention time for the methanogens, resulting in slow

growth rates and loss of biomass to the effluent [123]. However, a complete retention of biomass and microorganisms can be achieved by integrating membranes with an anaerobic digester, forming an anaerobic membrane reactor (AnMBR). AnMBR technology combines the advantages of anaerobic treatment and membrane bioreactor technology. Here, membranes help to retain solid particles within the reactor, resulting in high biomass concentrations, better control of solid retention times, high OLRs, and excellent effluent quality [124]. In such a scenario, methanogens are able to proliferate without being washed out of the reactor. A UASB coupled to an external ultrafiltration membrane has been employed for treating municipal wastewater. At the lowest HRT of 7 h, the system was able to achieve 87% COD removal efficiency and 0.18−0.23 Nm^3 CH_4/kg $COD_{removal}$ at a Volumetric Loading Rate (VLR) of 2.2 kg COD/m^3 day [125]. According to a 2014 study, a 64% increase in biogas production was observed with a 44% increase in OLR. In this study, 10.7 L H_2/L day was also obtained when thermophilic conditions were maintained at an OLR of 125.4 kg COD/m^3 day [126]. Notwithstanding the advantages and improvements, membrane fouling is a substantial challenge in advancing anaerobic membrane bioreactors. Soluble microbial products (SMPs), also called soluble extracellular polymeric substances, play a fundamental role in membrane fouling. These are produced as a result of microbial activity [127]. It has also been recognized that the activity of these microbes to produce SMPs increases at elevated temperatures. Therefore, thermophilic conditions accelerate the release of both extracellular polymeric substances (EPSs) and protein-to-carbohydrate ratio in bound to EPSs, which results in a 5−10 times increase in membrane resistance [128].

Membrane fouling is an inevitable phenomenon and results in an increase in both operational and energy cost of the process. In membrane bioreactors, membranes are either submerged within the reactor or placed external to it. External configuration is not recommended because high cross-flow velocity is used to reduce fouling, which may disturb the microbial activity [129]. However, for submerged membranes, gas scouring is generally used to reduce membrane fouling. Gas scouring is energy extensive and accounts for 47% of the total operational cost [130]. Other strategies to mitigate membrane fouling include the use of additives such as activated carbon, coagulants, and quorum quenchers [131]. One of the common features of using these additives is their ability to remove microbial macromolecules, which are the most dominant contributors toward membrane fouling. Alternatively, hydrolase can also be used to remove or break down these macromolecules, i.e., SMPs or EPSs. An approximately two to three times reduction in membrane resistance was observed when 100 mg/mL of protease was used as an enzymatic material [131].

AnMBRs are generally used to treat high-strength wastewater. However, the efficacy of an AnMBR for low-strength wastewater is rather limited because of the dilute organic load, slow proliferation of methanogens, and limitation of membrane rejection [132]. Therefore, it is recommended to use the membrane in combination with a UASB so that highly concentrated organic contaminants can be degraded efficiently.

The coupling of a membrane with an anaerobic digester is highly encouraged because the membrane helps to retain the biomass and microbes within the system. Membranes in the submerged configuration are a recommended option. This is because much less energy is consumed under submerged conditions, and with fewer cleaning procedures, membrane fouling can be reduced for better process efficiency [123].

16.3.3.3 Microbial Fuel Cells

The microbial fuel cell (MFC) is a sustainable and green technology. It is intensively studied as an alternative method for energy-efficient wastewater treatment [133]. MFCs comprise anode and cathode compartments separated by a proton-exchange membrane. Organic contaminants in wastewater are oxidized by microbes in the anode compartment, resulting in the formation of electrons and protons. Protons and electrons enter the cathode compartment through the separator membrane and external circuit. Here, these are oxidized by oxygen or some other electron acceptor to produce water or hydrogen peroxide through a four- or two-electron oxygen reduction reaction [134,135]. Based on the position of the membrane, MFCs are classified into two main categories: (1) single chamber and (2) dual chamber. In the dual-chamber configuration, anode and cathode chambers are physically separated by a proton/cation-exchange membrane [136,137] or salt bridge [138] as shown in Fig. 16.4A. However, the single-chamber configuration mainly comprises a single compartment with the cathode forming one wall of the cell, such that one of its sides faces water and the other faces air [139], as presented in Fig. 16.4B.

Being in the developmental stage, this technology is facing several technical and economic challenges such as lower power densities [140], low coulombic efficiencies [137], high internal resistances [141], longer start-up times [142], type of configuration [143,144], selection and cost of anode and cathode materials, microbial community [145,146], and high cost of membrane [147]. Among all, the most challenging are the high cost of cathode and membrane materials. Separator membranes account for 38% of the capital cost and together they cover up to 90% of MFCs [148]. The use of carbonaceous materials as cathode has already ousted the use of expensive material such as platinum [139,140,149,150]. Nevertheless, the use of a membrane is still challenging owing to both cost and high internal resistance of MFCs.

Considering the potential of MFCs for power production followed by wastewater treatment, their commercialization has become a global quest. Currently, studies are being carried out to improve the performance of MFCs in terms of power and COD removal efficiencies. The scope of these studies include selection of the specific microconsortium, modification of anode and cathode material, and replacement of costly proton-exchange membrane, i.e., Nafion, with some cheaper materials.

Considering the potential of MFCs for wastewater treatment simultaneous with power production, the economic feasibility of MFCs is important to ensure their scaled-up applications. Nafion 117 is very costly, around \$1400/m^2, which is even more expensive compared to a simple cation-exchange membrane, which costs around \$80/m^2 [151].

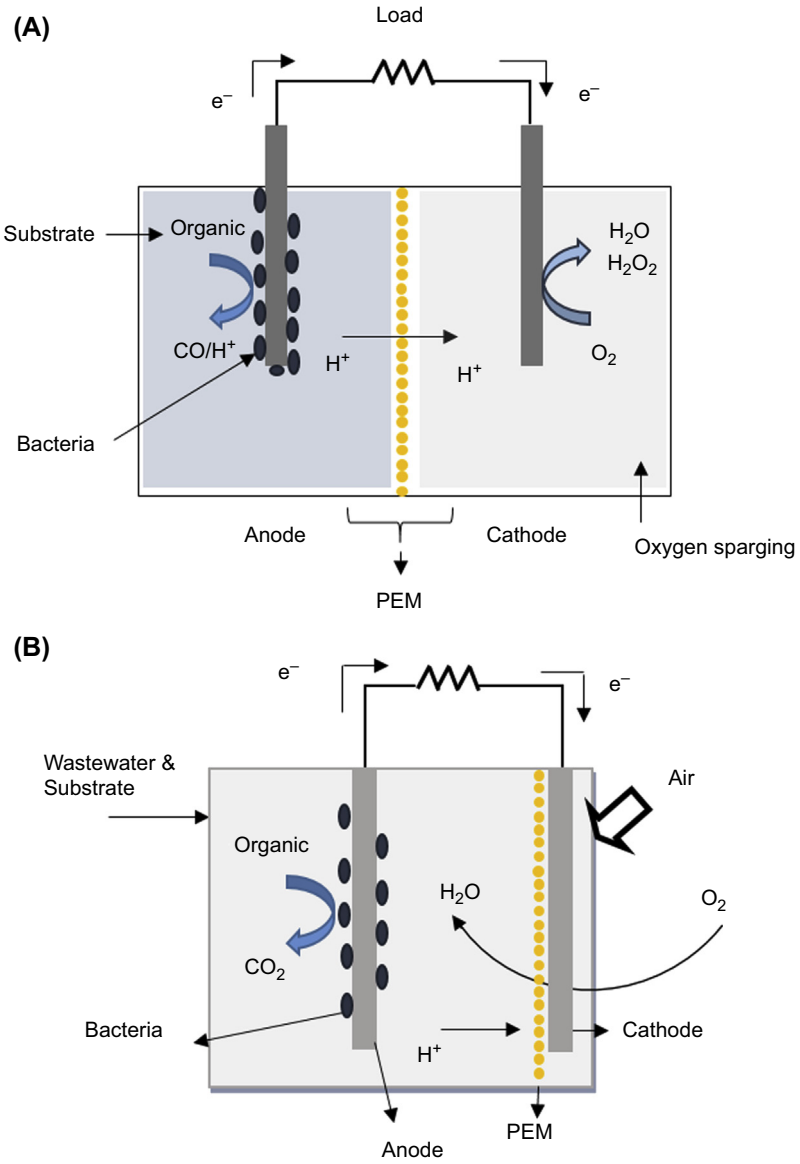

FIGURE 16.4 Schematics of (A) a dual-chamber WBMFC and (B) a single chamber wastewater based microbial fuel cell (WBMFC). *PEM*, proton-exchange membrane.

Therefore, materials such as ultrafiltration membranes [152], J-cloth [153], microfiltration membranes [154], glass fiber [155], Zirfon [156], fumasep [156], and nylon [157] have been successfully investigated based on their pore size, internal resistance, ionic conductivity, and oxygen and substrate crossovers. Using these materials, researchers are able to reduce the cost of the membrane to $\$4/m^2$, which shows the feasibility of MFCs for industrial wastewater treatment.

With an understanding of a correlation between the performance and the surface chemistry of electrodes, attempts have been made to improve the performance of MFCs by treating the anode and cathode surfaces. As of this writing, ammonia and heat treatments of carbonaceous materials have been identified as the most economical and practical methods for large-scale implementation. The maximum power that has been achieved is 1970 mW/m^2 with start-up times of 2.5 days [158].

Since 2005, there has also been a growing interest in in situ production of H_2O_2 in MFCs in view of its potential for both wastewater treatment and power production. It is estimated that commercial-grade H_2O_2 costs \$300−\$590 per ton [140]. Synthesis of hydrogen peroxide has been witnessed experimentally by a few authors using simple and low-cost graphite electrodes [137,139,145]. This is because the two-electron oxygen reduction reaction involves incomplete conversion of oxygen at the cathode surface [137]. As of this writing, a maximum of 196.50 mg/L of H_2O_2 has been produced simultaneous with a COD removal efficiency of 87% and coulombic efficiency of 29% [159]. Furthermore, in another study a maximum of 25.13 mW/m^2 of power was produced with simultaneous production of 78 mg/L of H_2O_2 [137].

Considering the aforementioned discussion, it is concluded that MFCs offer an integrated solution for wastewater treatment, as the power produced can be used for the aeration in the cathode chamber. Furthermore, the potential to produce H_2O_2 has also ensured a sustainable solution for chemical oxidation of recalcitrant wastewaters through advanced oxidation techniques. In its early stages, the MFC is facing several technical and economic challenges, but still it offers a green and sustainable solution for recalcitrant wastewater treatment with simultaneous power production.

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17

Removal of Toxic Component of Wastewater by Anaerobic Processes

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17.1 Classification of Toxic Compounds in Industrial Wastewater

During the industrial manufacturing process, various types of raw materials, intermediate products, and wastes are introduced into water. Therefore, industrial wastewater produced by each sector has its own characteristics as to the mixture of pollutants. Among these substances, some are toxic to the ecosystem and cause inhibition of biological treatment processes. Because of the various characteristics of industrial wastewater, it is important to know the unique features of the particular industrial wastewater before the treatment can be designed. Generally, the chemical characteristics of toxic compounds in industrial wastewater can be classified as inorganic or organic.

17.1.1 Inorganic Toxic Compounds in Industrial Wastewater

Inorganic industrial wastewater is mainly produced from the coal and steelworks industry, nonmetallic minerals industry, and metal surface processing industry. The wastewaters from these industries usually contain large amounts of suspended solids, which can be removed by a sedimentation process. The sedimentation process can also be aided by chemical flocculation using flocculation agents and organic polymers. Below are some of the toxic inorganic compounds commonly found in industrial wastewater.

17.1.1.1 Heavy Metals

Heavy metals of the greatest concern in the treatment of wastewaters are copper, cadmium, iron, lead, zinc, mercury, chromium, and silver, as they are highly toxic, nonbiodegradable, and bioaccumulative in living organisms [79]. The main sources of heavy metals are tanning, petroleum refining, chemical manufacturing, electroplating, mining, textile industry, fertilizer plants, photographic process industry, battery manufacturing, metal and steel working and finishing, and landfill leachates.

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Table 17.1 Heavy Metals Found in Major Industries [12]

Industry	Ag	As	Cd	Cr	Cu	Hg	Pb	Ni	Zn
Pulp and paper mills				X	X	X	X	X	X
Organic chemicals	X	X	X	X		X	X		X
Alkalis, chlorine		X	X	X		X	X		X
Fertilizers	X	X	X	X	X	X	X	X	X
Petroleum refining	X	X	X	X	X		X	X	X
Steel works		X	X	X	X	X	X	X	X
Aircraft plating, finishing	X		X	X	X	X		X	
Flat glass, cement				X					
Textile mills				X					
Tanning				X					
Power plants				X					

Wastewaters containing heavy metals derived from various industrial activities must be finally discharged into the environment. As heavy metals are extremely harmful to human health and living organisms, the treatment of wastewaters for the removal of heavy metals is therefore critical [59]. Industries producing or discharging heavy metals are summarized in Table 17.1.

Cadmium may be discharged from metal smelting and refining and also mining industries. Factories manufacturing cadmium products such as batteries, coatings, and plastics also contribute to the cadmium in wastewater. Cadmium has chemical characteristics similar to those of zinc; both metals frequently undergo geochemical processes together. Both metals are found in water in the oxidation state of +2. Acute cadmium poisoning in humans causes adverse effects such as high blood pressure, liver and kidney failure, and damage of testicular tissue and red blood cells [101].

Lead is an extremely toxic heavy metal, the removal of which from wastewater is critical in specific industries. Lead poisoning can cause neurological disorders, elevated body blood pressure, anemia, and gastrointestinal diseases in the human body. These adverse effects were observed even at very low concentrations (0.01−5.0 mg/L) [99]. Lead contamination originates from the discharge of untreated wastewaters from electroplating, printing pigments, textile and fuel industries, mining, battery manufacturing, explosives manufacturing, automotive industry, and building construction. Industrial wastewaters contain a wide concentration range of soluble Pb^{2+}, which has significant variations in type and also in specific sources [146]. According to the US EPA environmental regulations, the toxicity threshold level for Pb^{2+} is 5.0 mg/L in wastewaters [3,58].

Mercury is among the most toxic heavy metals. It exists in three forms: elemental mercury (Hg^0, metallic mercury, and mercury vapor), inorganic mercury (Hg^+ and Hg^{2+}), and organic mercury, such as methyl mercury (CH_3Hg, or MeHg) and ethyl mercury (C_2H_5Hg). Hg^0 is oxidized in air to its inorganic forms (Hg^+ and Hg^{2+}) and released to soil or into rivers, lakes, and oceans during rain events. Inorganic mercury, derived from industrial discharge and from contaminated water, is biomethylated to

MeHg primarily by sulfate-reducing bacteria. MeHg is bioaccumulated to high concentrations in shellfish, predatory fish, and sea mammals by the liver, brain, kidney, and muscle [17,34]. For the industry application, metallic mercury is used as an electrode in the electrolytic generation of chlorine gas, in laboratory vacuum apparatuses, and in other applications, e.g., thermometers, barometers, and pressure-sensing devices. Organic mercury compounds used to be widely applied as pesticides, particularly fungicides. However, fungicides containing mercury are no longer in use.

17.1.1.2 Cyanides

Cyanide ion, CN^-, is probably the most important compound among the various inorganic species in wastewater. Cyanide, a highly toxic substance, exists in water as HCN, a weak acid. The cyanide ion has a strong affinity for many metal ions, forming relatively less toxic ferrocyanide, $Fe(CN)_6^{4-}$, with iron(II), for example. Cyanide is widely used in the metal processing and electroplating industries, tanning industry, gold-processing industry, and specialized laboratories. It is also one of the main gas and coke scrubber effluent pollutants from gas works and coke ovens [101].

17.1.1.3 Ammonia

Ammonia is produced from the decay process of nitrogenous organic wastes. It is also a building block for the synthesis of many pharmaceuticals and is used in many commercial cleaning products. Ammonia is both caustic and hazardous. Because the pK_a of the ammonium ion, NH_4^+, is 9.26, most ammonia in water exists in the form of NH_4^+ rather than NH_3 [101].

17.1.1.4 Sulfides

Hydrogen sulfide (H_2S) is a colorless, toxic, and corrosive gas that occurs naturally during wastewater treatment processes through bacterial action on organic matter under anaerobic conditions, particularly in biological reactors [132]. H_2S aggravates environmental and economic problems in a variety of sectors such as chemical plants, paper mills, textile mills, tanneries, and the petrochemical industry. Sulfides present in aqueous solution are also responsible for stress corrosion cracking of steel, which is also known as sulfide stress cracking. In general, the concern of H_2S in air lies in its toxicity and unpleasant odor. H_2S has a noxious odor even at trace-level concentrations, with a low odor threshold that ranges from 0.5 to 300 ppb, although 18 ppb can be considered the standard concentration detectable by the human nose [33,40].

17.1.2 Organic Toxic Compounds in Industrial Wastewater

Organic toxic compounds in industrial wastewater originate from those chemical industries and large-scale chemical works that use organic substances for chemical reactions. The organic substances used can have numerous origins and properties. The organic substances can be eliminated only by special pretreatment of the wastewater,

followed by biological treatment [124]. Most organic toxic compounds in industrial wastewater are produced by the following industries and plants:

- Pharmaceuticals, cosmetics, organic dyestuffs, glues and adhesives, soaps, synthetic detergents, and pesticides and herbicides manufacturing factories
- Tanneries and leather factories
- Textile factories
- Cellulose and paper manufacturing plants
- Oil refinery industries
- Brewery industries
- Metal processing industry

Major hydrocarbons are mostly aliphatic, polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs), and these hydrocarbons are widespread contaminants in rivers and lagoons. Hydrocarbons may originate from the incomplete combustion of organic matter, including biomass and fossil fuels (pyrolytic source), from the spillage of petroleum or refinery products (petrogenic source), or from continental higher plants [70].

17.1.2.1 Chlorinated Aliphatic Hydrocarbons

Chlorinated aliphatic hydrocarbons (CAHs) are human-made organic compounds, typically manufactured from naturally occurring hydrocarbon constituents, such methane, ethane, and ethene, and chlorine. CAHs have been used extensively as industrial solvents, dry-cleaning agents, and degreasers [50]. They are common soil and groundwater contaminants in industrial areas of the world [10]. The most prevalent chlorinated contaminants are tetrachloroethene, trichloroethene, dichloroethene, and vinyl chloride. The presence of all CAHs in the environment is of major concern as they are either known or suspected carcinogens. Their complex physical properties combined with the heterogeneity of the subsurface has resulted in the technical difficulty in their remediation [10].

17.1.2.2 Chlorinated Hydrocarbons/PCBs

PCBs have been generally detected in water, sediments, and bird and fish tissue. There are 209 configurations of PCBs formed or made by substituting between 1 and 10 Cl atoms onto the biphenyl aromatic structure [101].

Commercial PCB mixtures were used in a wide variety of applications, including dielectric fluids in capacitors and transformers, heat transfer fluids, hydraulic fluids, lubricating oils, and as additives to some epoxy paints, carbonless copy paper, adhesives, sealants, and plastics. Their commercial application was based largely on their chemical stability, including low vapor pressure, low flammability, and desirable physical properties, including electrical insulating properties [51].

PCBs are persistent and bioaccumulative chemicals that are resistant to degradation and could accumulate in the environment, animals, and humans. PCBs provoke a wide

range of toxic effects including adverse impacts on genetic material and the reproductive system. PCBs are classified as probable human carcinogens by the International Agency for Research on Cancer (IARC) [25].

17.1.2.3 Polycyclic Aromatic Hydrocarbons

The PAH compounds are also classified as priority pollutants by the US EPA [72]. PAHs are one of the most widespread organic pollutants. They are found in fossil fuels (oil and coal) and in tar deposits. They are also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense (e.g., in engines and incinerators or when biomass burns in forest fires). Most PAHs are not soluble in water and persist in the environment [27]. PAHs have been identified as carcinogenic and mutagenic, as well as teratogenic.

17.1.2.4 Phenols

Phenolic compounds are also classified as priority pollutants by the US EPA [72]. Phenol was first extracted from coal tar, but today is produced on a large scale from petroleum. Phenol is a versatile precursor to a large collection of drugs, particularly aspirin, and also many herbicides and pharmaceutical drugs. Phenols are also present in the effluents of various industries such as the coal conversion, pharmaceutical, petroleum refining, petrochemical, steel, paper, textile, food, and pesticide industries [106]. Phenol and its vapor are corrosive to the eyes, the skin, and the respiratory tract. Prolonged skin contact with phenol may cause dermatitis, and inhalation of phenol vapor may cause lung edema [16]. Long-term exposure to the substance may cause harmful effects to liver and kidney.

17.1.2.5 Nitrophenols

Nitrophenol compounds are classified as priority pollutants by the US EPA [72]. Nitrophenols are highly toxic, inhibitory, and persistent organic compounds. Nitrophenols are common by-products of many industries manufacturing pesticides, dyes, and pharmaceutical products. They are also one of the most challenging contaminants to remove from wastewater streams [98]. In the United States, the maximum allowable concentration of nitrophenols is 20 μg/L [24]. It is therefore essential for industries to have an efficient treatment system to reduce the nitrophenol compounds in industrial wastewater.

17.1.2.6 Nitroanilines

p-Nitroaniline (PNA), a nonbiodegradable organic compound, is used extensively in the manufacturing process of pharmaceutical products, dye, and polymers. PNA is classified as hazardous because of its chemical stability and toxicity once it contaminates the water. It is considered a high-risk compound to human health and aquatic microorganisms even at very low concentrations [86,148]. Prolonged exposure to aniline compounds can result in damage to human DNA [87]. Hence, it is vital to remove PNA to reduce its harmful and adverse effects to human health.

17.1.2.7 Formaldehyde

Formaldehyde, a highly reactive chemical compound, is often discharged into the wastewater of construction, textile, wood processing, furniture, and pharmaceutical industries [135]. Formaldehyde is a water-soluble compound, which can diffuse into many tissues rapidly, react with various macromolecules such as proteins and nucleic acids, and cause DNA–DNA, protein–DNA, and protein–protein cross-links [94,96,127,151]. Therefore, this compound has a toxic effect on all organisms, and the IARC [66] has classified formaldehyde as a human carcinogen that causes nasopharyngeal cancer and probably leukemia.

17.2 Anaerobes Involved in Removal of Various Toxic Compounds

It has been widely reported that both inorganic and organic toxic compounds from industrial wastewaters can be effectively removed through the anaerobic process. Anaerobic microorganisms, also named as anaerobes, are the key factor in the anaerobic degradation process. They can effectively degrade the majority of toxic compounds for cell metabolism through various metabolic pathways. Generally, toxic inorganics, especially heavy metal ions, can be transformed into immobile forms and be removed from wastewater by sulfate-reducing bacterial populations, whereas for toxic organics, successful detoxification usually relies on the breaking down of chloride covalent bonds or aromatic rings, which can be achieved by various groups of anaerobes. Fig. 17.1 presents representative anaerobic degradation pathways for some typical toxic compounds in industrial wastewater.

17.2.1 Heavy Metals Removal

Heavy metals can be biologically transformed from toxic and mobile forms into less toxic and immobile forms [73,138]. The dominant mechanism of heavy metals removal in anaerobic bioreactors is precipitation in the form of sulfides. Two steps are involved in the removal by precipitation [105]: (1) Under anaerobic conditions, hydrogen sulfide is produced by sulfate-reducing bacteria (SRB) utilizing an organic carbon source as the electron donor and sulfate as the electron acceptor. The reaction is expressed as $2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S$. (2) The produced hydrogen sulfide reacts with dissolved cationic heavy metals (M^{2+}) to form metal sulfide precipitates following the equation $H_2S + M^{2+} \rightarrow MS\downarrow + 2H^+$. A variety of heavy metals such as Cu^{2+}, Zn^{2+}, Ni^{2+}, and Cr^{6+} could be removed by SRB via this method [26,73]. An isolate of marine SRB designated as isolate TKW was found to reduce Cr^{6+} to Cr^{3+} [26]. In addition, many anaerobes have been found to utilize inorganic arsenic (As) as either electron donor or electron acceptor for anaerobic respiration. Yamamura and Amachi [150] summarized a wide variety of As(III) oxidizers, such as *Alkalilimnicola ehrlichii* sp. nov. and *Ectothiorhodospira* sp. PHS-1, and As(V) reducers, such as *Chrysiogenes arsenatis*,

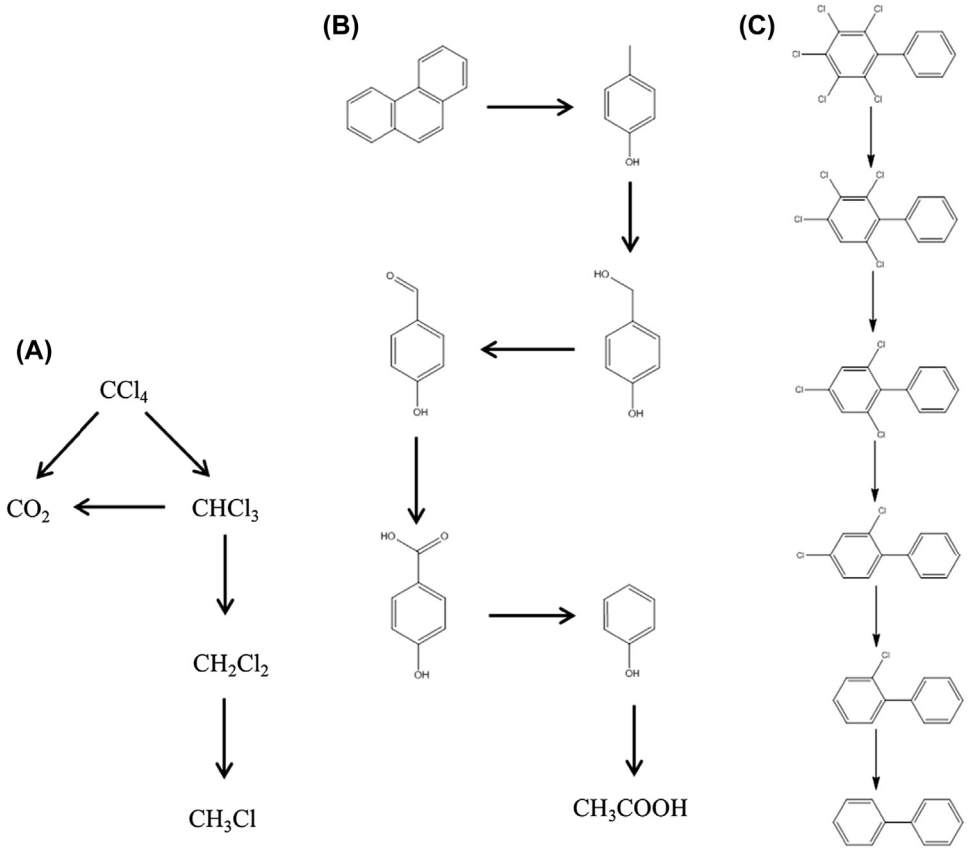

FIGURE 17.1 Representative pathways for anaerobic biodegradation of toxic compounds. (A) Chlorinated aliphatic hydrocarbons [49]. (B) Polycyclic aromatic hydrocarbons [137]. (C) Polychlorinated biphenyls [104].

Bacillus spp., *Desulfitobacterium* spp., *Shewanella* sp. ANA-3, *Geobacter* spp., and *Anaeromyxobacter* sp. PSR-1.

17.2.2 Chlorinated Hydrocarbons Removal

17.2.2.1 Chlorinated Aliphatic Hydrocarbons Removal

The major attenuation mechanism for the chlorinated hydrocarbons in natural ecosystems is biological transformation [46]. Numerous studies have revealed that CAHs could be degraded by a variety of anaerobic microbes via oxidative [42,48], fermentative [14,69,89], and reductive [41,74,85] dehalogenation processes. According to the energetic feasibility, oxidative and fermentative pathways are more favorable for anaerobic microorganisms than reductive pathways [67]. However, reductive dehalogenation could also be the dominant pathway under the prevailing environmental conditions [57].

17.2.2.1.1 OXIDATIVE DEHALOGENATION

Several pure cultures have been isolated and found capable of oxidative dehalogenation of CAHs. Egli et al. [48] observed transformation of trichloromethane to CO_2 by a cell suspension of *Acetobacterium woodii* via an oxidative dehalogenation process when they investigated degradation of tetrachloromethane by three strictly anaerobic bacteria, *A. woodii*, *Desulfobacterium autotrophicum*, and *Methanobacterium thermoautotrophicum*. Dijk et al. [42] reported that 2-chloroethanol could be degraded to CO_2 with NO_3^- as the electron acceptor via anaerobic oxidation by the pure bacterial culture designated as *Pseudomonas stutzeri* strain JJ. The reaction equations involved are $C_2H_5ClO + 2HNO_3 \rightarrow 2CO_2 + HCl + N_2 + 3H_2O$ and $C_2H_5ClO + 5HNO_3 \rightarrow 2CO_2 + HCl + 5HNO_2 + 2H_2O$. The observation indicates that denitrifying bacteria might also be applied in bioremediation of contaminating CAHs. In addition, anaerobic microbial oxidation of CAHs was observed in mixed cultures with various electron acceptors [43,57,141].

17.2.2.1.2 FERMENTATIVE DEHALOGENATION

Traunecker et al. [136] first reported that Strain MC, which was gram-positive and strictly anaerobic, was capable of metabolizing chloromethane via a fermentative pathway. Strain MC either utilized chloromethane and CO_2 according to the reaction $4CH_3Cl + 2CO_2 + 2H_2O \rightarrow 3CH_3COO^- + 7H^+ + 4Cl^-$ or utilized chloromethane and CO according to the reaction $CH_3Cl + CO + H_2O \rightarrow CH_3COO^- + 2H^+ + Cl^-$. A detailed scheme of chloromethane conversion to acetate was provided in a later study [95]. However, Strain MC was not able to utilize dichloromethane (DCM) as a carbon source. A strictly anaerobic bacterium named *Dehalobacterium formicoaceticum*, isolated by Mägli et al. [89], was capable of utilizing DCM as a source of carbon and energy. The fermentation followed the equation $3CH_2Cl_2 + CO_2 \rightarrow 2HCOOH + CH_3COOH + 6HCl$. Two hypothetical pathways for the metabolism of DCM were proposed and were further investigated by Mägli et al. [88]. The diversity of *Dehalobacter* spp. was expanded by Justicia-Leon et al. [69]. They also observed a *Dehalobacter* sp. utilizing DCM as the sole substrate in an enrichment culture derived from river sediment. Furthermore, their results demonstrated that *Dehalobacter* metabolism is not restricted to organohalide respiration.

17.2.2.1.3 REDUCTIVE DEHALOGENATION

The mechanisms of reductive dehalogenation under anaerobic conditions can be divided into cometabolic and metabolic conversion [126]. Cometabolism is a set of reactions bringing about merely a fortuitous modification of a compound by enzymes or cofactors that normally catalyze other reactions and is not energetically useful for the microorganisms [64]. Enzymes or metal-ion-containing tetrapyrroles were incorporated as cofactors to catalyze the dehalogenation process [126]. Picardal et al. [110] reported that an anaerobic iron-reducing bacterium, *Shewanella putrefaciens*, was able to catalyze the reductive dehalogenation of tetrachloromethane under anaerobic conditions. No DCM, chloromethane, or methane was produced and trichloromethane was the only

product identified during the process. Egli et al. [49] tested five anaerobic bacterial species for the enzymes involved in the transformation of tetrachloromethane. Cultures of the SRB *D. autotrophicum* could transform 80 μM tetrachloromethane to trichloromethane and a small amount of DCM in 18 days, whereas the acetogens *A. woodii* and *Clostridium thermoaceticum* could degrade 80 μM tetrachloromethane completely within 3 days.

On the other hand, metabolic conversion was found in halorespiring bacteria, which coupled the reductive dehalogenation reaction with specific, high-affinity biocatalysts to microbial growth [126]. *Dehalobacter restrictus* was found to utilize tetrachloroethene and trichloroethene as electron acceptors and only H_2 as the electron donor in an anaerobic respiration process [63,149], whereas it was found to reductively dechlorinate 1,1,1-trichloroethane to 1,1-dichloroethane and chloroethane [133].

17.2.2.2 Polychlorinated Biphenyls Removal

The major role of anaerobes in the biodegradation of PCBs is dechlorination [13,140]. Most of the identified PCB-dehalogenating microorganisms belong to the phylum Chloroflexi. In general, the rate and extent of PCB-dechlorinating were closely related to the degree of chlorination, whereas dechlorination patterns depended on the bacterial species [140]. For example, bacterium *o*-17, within a deep branch of Chloroflexi and with 89% similarity to *Dehalococcoides ethenogenes*, was identified for the first time as a PCB-dechlorinating anaerobic microorganism by Cutter et al. [36]. Bacterium *o*-17 showed the ability to remove single-flanked *ortho*-PCB chlorines. However, strain DF-1, another dechlorinating anaerobic microorganism with a high similarity to bacterium *o*-17, can dechlorinate only congeners with double-flanked chlorines [55]. In addition, Fagervold et al. [53] reported that three Chloroflexi phylotypes (SF1, SF2, and DEH10) were responsible for dechlorinating PCBs to unflanked tetra- and trichlorobiphenyls. Their results also showed that some phylotypes within the genus *Dehalococcoides* were capable of reductive dechlorination. However, as of this writing, the identified PCB-dechlorinating anaerobic microorganisms are still limited.

In general, anaerobes reported to play key roles in the dehalogenation process are summarized in Table 17.2.

Table 17.2 Anaerobes Involved in Chlorinated Hydrocarbons Removal

Category	Anaerobes Involved in the Dehalogenation Process
Oxidative dehalogenation	*Acetobacterium woodii* [48]; *Pseudomonas stutzeri* strain JJ [42]
Fermentative dehalogenation	Strain MC [136]; *Dehalobacterium formicoaceticum* [89]
Reductive dehalogenation	*Shewanella putrefaciens* [110]; *Desulfobacterium autotrophicum* [49]; *A. woodii* [49]; *Clostridium thermoaceticum* [49]; *Trichlorobacter thiogenes* [38]; *Dehalobacter restrictus* [133]

17.2.3 Aromatic Compounds Removal

17.2.3.1 Benzene Removal

Anaerobic benzene biodegradation can occur under methanogenic and nitrate/sulfate/iron-reducing conditions [35]. Several strains involved in benzene degradation have been reported. Ulrich and Edwards [139] enriched nine distinct anaerobic cultures involved in the degradation of benzene. Within these cultures, five bacterial 16S rRNA sequences (Benzene mineralizing consortium clone SB-21, *Dehalospirillum multivorans*, and three uncultured bacteria) and four archaeal 16S rRNA sequences (Enrichment culture clone E31B1, *Methanobacterium formicicum*, and two uncultured archaea) were identified. Sakai et al. [119] postulated that bacterium Hasda-A is responsible for the initial steps of benzene degradation under methanogenic conditions. In addition to methanogens, some nitrate/sulfate/iron-reducing enrichment cultures were also associated with benzene degradation. Coates et al. [30] first reported two *Dechloromonas* strains, RCB and JJ, capable of complete degradation of benzene to CO_2 anaerobically with nitrate as the electron acceptor. Herrmann et al. [62] investigated benzene degradation under sulfate-reducing conditions and found that a *Cryptanaerobacter*/*Pelotomaculum*-related phylotype contributed to the first steps of benzene degradation, resulting in the release of hydrogen, acetate, and some low-molecular-weight fermentation products. Oka et al. [107] also identified a sequence closely related to clone SB-21, a member of the family Desulfobacteraceae, playing a key role in benzene degradation. Under iron-reducing conditions, benzene as the sole carbon and energy source was biodegraded although no isolate was obtained [75]. Kunapuli et al. [75] hypothesized that members of *Clostridium* primarily oxidized benzene. In addition, Anderson et al. [2] found that iron-reducing microorganisms closely related to *Geothrix fermentans* could also play a role in benzene degradation.

17.2.3.2 Polycyclic Aromatic Hydrocarbons Removal

In general, the rate of PAH degradation is inversely correlated with the number of benzene rings [21,31]. Many studies have reported the anaerobic degradation of two- and three-ring PAHs. Among them, naphthalene is the simplest PAH. Several studies have shown that naphthalene was degraded under sulfate-reducing conditions [28,153]. Three sulfate-reducing strains (NaphS2, NaphS3, and NaphS6) capable of degradation of naphthalene were isolated [56,102]. Strains NaphS3 and NaphS6 were Deltaproteobacteria and closely related to strain NaphS2 from North Sea sediment. Degradation of naphthalene was also reported under nitrate-reducing conditions. Mihelcic and Luthy [97] first reported completed degradation of 7 mg/L naphthalene in 45 days under denitrification conditions. Later, Rockne et al. [117] isolated three pure naphthalene-degrading cultures, designated NAP-3-1, NAP-3-2, and NAP-4. Nitrate-dependent transformation of 70–90% of added naphthalene was achieved by

Table 17.3 Anaerobes Involved in Aromatic Compounds Removal

Aromatic Compound	Anaerobes Involved in Degradation
Benzene	Clone SB-21 [139]; *Dehalospirillum multivorans* [139]; clone E31B1 [139]; *Methanobacterium formicicum* [139]; *Dechloromonas* strains RCB and JJ [30]; Hasda-A [119]
Polycyclic aromatic hydrocarbons	NaphS2 [56,102]; NaphS3 [56,102]; NaphS6 [56,102]; NAP-3-1 [117]; NAP-3-2 [117]; NAP-4 [117]

NAP-3-1 and NAP-4. Other electron acceptors such as ferric iron and manganese were also reported to associate with naphthalene degradation [29,78]. The detailed mechanism involved in anaerobic naphthalene degradation was explained by Meckenstock and Mouttaki [92]. In addition, phenanthrene, a larger PAH, was degraded under nitrate and sulfate-reducing conditions [90,153]. The pathways of phenanthrene degradation under sulfate-reducing conditions were proposed [137]. However, limited biodegradation of naphthalene and phenanthrene by methanogenic consortia was found [90], although methanogenic degradation was feasible from a thermodynamic point of view [45]. Anthracene, another larger PAH, was degraded under methanogenic conditions [145]. The bacterial genera *Bacillus*, *Rhodococcus*, and *Herbaspirillum* might have links with methanogenic degradation of anthracene. On the other hand, only a few studies have shown limited evidence that PAHs with more than three rings (e.g., pyrene) could be degraded under anaerobic conditions [1,21,81,82,116]. Nevertheless, no responsible microorganism has been isolated or reported.

Overall, anaerobes found to degrade aromatic compounds are documented in Table 17.3.

17.2.4 Anaerobes Involved in Other Toxic Compounds Removal

Other than the aforementioned compound groups, anaerobes are also capable of breaking down other organics such as phenol, toluene, nitrophenols, etc. Regarding the degradation pathway, for example, the initiation steps during anaerobic degradation of aromatic compounds usually include the oxidation of functional groups (i.e., $-CH_3$ to $-COOH$) on the aromatic rings and further replacement of them (i.e., $-NH_2$, $-NO_2$, $-COOH$, etc.) by hydroxyl groups to form phenols. Subsequently, these phenols can be degraded following the pathways as discussed in Section 17.2.3. In addition, there are some inorganics such as cyanide that have been reported to be removed by the activity of anaerobes, but the underlying mechanism remains unclear as of this writing [23]. The microbial strains related to other toxic compounds removal are summarized in Table 17.4.

Table 17.4 Anaerobes Involved in Other Toxic Compounds Removal

Toxic Compound	Anaerobes Involved in Degradation
Phenol	*Bacillus cereus* [6]; *Ralstonia eutropha* [80]; *Halomonas* sp. strain PH2-2 [61]; iron-reducing organism GS-15 [84]
Toluene	Iron-reducing organism GS-15 [84]; *Dechloromonas* strain RCB [19]; *Desulfosporosinus* [134]
Nitrophenols	*Bacillus pantothenticus* [131]; *Bacillus aminovorans* [131]; *Arthrobacter chlorophenolicus* A6 [118]; *Arthrobacter* 4Hβ [154]; *Arthrobacter* sp. HY2 [113]
Cyanide	*Klebsiella oxytoca* [23]

17.3 Modern Applications of Anaerobic Techniques for Toxic Compounds Removal

The anaerobic treatment of toxic compounds in wastewater has become a viable technology in recent years due to the rapid development of high-rate bioreactors. This section focuses on the documented application of anaerobic systems for toxic compounds removal.

17.3.1 Upflow Anaerobic Sludge Blanket

The upflow anaerobic sludge blanket (UASB) has become popular and commonly used for wastewaters from high-strength industries such as the food and beverage and agricultural industries, in which the pollutants are mostly carbohydrates [54], formaldehyde [144], PAHs [103,155], and phenolic compounds [111,123]. The UASB is a methanogenic digester that evolved from the anaerobic clarigester and uses an anaerobic process while forming a blanket of granular sludge, which is suspended in the reactor [125]. The UASB has been proven to be effective for medium- and high-strength wastewater within a wide range of hydraulic retention times, from 3 to 48 h [121]. Anaerobic granules in the UASB display unique physical and chemical characteristics, such as compact structure, good flocculation, and settling ability, that allow production of a good quality effluent. Many studies have been done on the removal of toxic compounds by UASB with different operating conditions, and performances achieved are provided in Table 17.5.

17.3.2 Expanded Granular Sludge Bed

The expanded granular sludge bed (EGSB) reactor is a modification and variant of the traditional UASB concept for anaerobic wastewater treatment, which is also inoculated with granular sludge, but the hydrodynamic conditions are different from those of the UASB. Higher upward-flow velocity in the EGSB could improve the mixing and enhance the wastewater–sludge contact [111]. As a result, the sludge bed of an EGSB is more expanded and it is possible to apply much higher upflow velocities than in a UASB in the settler; thus it requires a smaller volume and also a smaller footprint than the UASB

Table 17.5 Operating Conditions and Performance of Upflow Anaerobic Sludge Blanket for Toxic Compounds Removal

Toxic Compound	Type of Wastewater	Volume (L)	HRT (h)	OLR (kg/m^3 day)	Influent (mg/L)	Removal (%)	Application	References
Phenol	Coal gasification	1	24	2.5	545	40–41	Lab scale	[147]
	Synthetic coal	15.5	7.9	0.74–1.72	752	68–95	Lab scale	[114]
	Synthetic	2.8	12	–	1260	>97	Lab scale	[54]
2,4-Dichlorophenol	Synthetic	2.2	3.87	18.7	10	62–64	Lab scale	[128]
	Synthetic	5.4	48	1.9	100	75	Lab scale	[111]
Pentachlorophenol	Synthetic	6	144	0.4	1	>99	Lab scale	[47]
4-Chloro-2-nitrophenol	Synthetic	7	8–30	–	30	90.3–94.6	Lab scale	[130]
2-Chloriphenol	Synthetic	3	6–16	1.9–5.3	30	88.3–96.5	Lab scale	[91]
3,4,5-Trimethoxybenzaldehyde	Manufacture	6	36–48	3–24	498–556	96.8–98.1	Lab scale	[81,82]
Polycyclic aromatic hydrocarbons	Heavy oil refinery effluent	4.2	24	–	0.27	54	Lab scale	[155]
		2.2	24	0.5	10.33	>99	Lab scale	[103]
Total petroleum hydrocarbon	Refinery effluent	2.2	24	0.5	1520	>99	Lab scale	[103]
Formaldehyde	Synthetic	0.1	14.9	2.32–6.03	50–2000	>95	Lab scale	[144]
Nitrophenol	Synthetic	12.5	12–30	4.02–4.6	30.1	89.7–96	Lab scale	[71]

HRT, hydraulic retention time; *OLR*, organic loading rate.

system. Van Lier [142] reported that 34% of all anaerobic systems sold worldwide in 2002–2007 were UASB systems and 52% were EGSB reactors. However, limited knowledge with respect to the removal of toxic compounds using the EGSB still hampers the applicability of anaerobic treatment for industrial wastewater. Removal of selected toxic compounds by the EGSB is provided in Table 17.6.

17.3.3 Anaerobic Baffled Reactor

The successful application of the anaerobic process for the treatment of industrial wastewaters with specific target toxic compounds is critically dependent on the development and application of a high-rate anaerobic bioreactor. The anaerobic baffled reactor (ABR) has been developed as a promising system for industrial wastewater treatment, as the ABR systems are extensively applied in various types of wastewater such as *p*-nitrophenol-containing wastewater [76], azo dye-containing wastewater [11], nitrobenzene-containing wastewater [77,83], and synthetic tannery wastewater containing sulfate and chromium(III) [9]. Removal of toxic compounds by ABR with various

Table 17.6 Operating Conditions and Performance of the Expanded Granular Sludge Bed for Toxic Compounds Removal

Toxic Compound	Type of Wastewater	Volume (L)	HRT (h)	OLR (kg/m^3 day)	Influent (mg/L)	Removal (%)	Application	References
2,4-Dichlorophenol	Synthetic	5.4	48	1.9	100	84–95	Lab scale	[111]
2,4,6-Trichlorophenol	Synthetic	3.5	48	2.5	50	30	Lab scale	[32]
	Synthetic	5.4	–	7.5	1318	83–99	Lab scale	[112]
Phenol	Alcohol	3.5	12	5	1000	65–85	Lab scale	[120]
Formaldehyde	Chemical factory	220,000	1.8	–	10,000	>93	Full scale	[156]
Amoxicillin	Antibiotic	1.47	20	–	19.7–214.7	53.2–79.8	Lab scale	[93]

HRT, hydraulic retention time; *OLR*, organic loading rate.

Table 17.7 Operating Conditions and Performance of the Anaerobic Baffled Reactor for Toxic Compounds Removal

Toxic Compound	Type of Wastewater	Volume (L)	HRT (h)	OLR (kg/m^3 day)	Influent (mg/L)	Removal (%)	Application	References
p-Nitrophenol	Synthetic	28.8	249	0.289	10–700	82–99	Lab scale	[76]
2-Clorophenol	Synthetic	13.7	24	1.1	200	99.3–99.9	Lab scale	[100]
Chromium(III)	Synthetic	8	–	0.274	50	>99	Lab scale	[9]
Azo dye	Synthetic	19	48		20–2000	81–97	Lab scale	[109]
Nitrobezene	Synthetic	28.8	249	0.3	30–700	>99	Lab scale	[77]
	Synthetic	12.8	24	–	8.3–79.7	94–99	Lab scale	[83]

HRT, hydraulic retention time; *OLR*, organic loading rate.

operating conditions is provided in Table 17.7. The ABR is a reactor that uses a series of baffles to force the organic pollutant-containing wastewater to flow under and/or flow through the baffles as it passes from the inlet to the outlet [8], which could cause the bacteria in the ABF to gently rise and settle, because of the flow characteristics and gas production, and move down the reactor at a slower rate. The ABR is considered a robust system, but the sludge and effluents still need further treatment to be reused or discharged properly. There are some advantages of using the ABF, such as resistance to organic and hydraulic shock loads, no electrical energy requirement, low operating costs and sludge production, and longer service life [8].

17.3.4 Other Anaerobic Processes

As discussed in the previous sections, the most commonly used anaerobic high-rate processes for removal of toxic compounds are UASB and EGSB because the granular-based technology is feasible and economical. However, the complex compositions of

industrial wastewaters could have a negative impact on the sludge granulation process, which would lead to the loss of biomass. Therefore, many other hybrid and new anaerobic processes have become the focus of research in conjunction with toxic compounds removal to improve on the existing anaerobic techniques; examples are the anaerobic migrating blanket reactor, upflow anaerobic fixed-bed reactor, and anaerobic hybrid reactors. Several examples of anaerobic processes for treatment of various types of wastewater containing various toxic compounds are listed in Table 17.8.

17.3.5 Combined Process for Toxic Compounds Removal

Anaerobic processes have been proven to achieve simultaneous reduction of organic pollutants and various toxic compounds during industrial wastewater treatment. However, owing to the lower redox potential of anaerobic conditions compared with aerobic conditions, some groups of toxic compounds (e.g., aromatic amines) cannot be effectively degraded anaerobically. In addition, the vulnerability of anaerobic treatment under highly fluctuating industrial wastewater environments also hinders its full-scale application. Therefore, several types of combined processes are being attempted to enhance the removal efficiency of toxic compounds as well as the system long-term stability.

17.3.5.1 Combined Anaerobic–Aerobic Process

The combined anaerobic–aerobic process is known as operationally and economically advantageous for treatment of high organic strength industrial wastewater, because it possesses benefits such as high organic loading capacity, high process stability, high overall treatment efficiency, energy recovery (i.e., CH_4), and low energy consumption [18]. The combinations of various anaerobic and aerobic reactors have been widely applied for treating industrial wastewaters including palm oil mill effluent, pharmaceutical wastewater, pulp and paper industry effluent, and food processing wastewater [20]. Regarding toxic compounds removal, the combined process is still preferred because of its complete degradation ability. For instance, azo dyes are aromatic organics containing an azo group (−N═N−) and other substituents such as nitro ($-NO_2$), amino ($-NH_2$), chloro (−Cl), methyl ($-CH_3$), and hydroxyl (−OH) [122]. These dyes are usually recalcitrant to microbial decomposition, and therefore neither anaerobic nor aerobic treatment could be employed alone for efficient removal. Nevertheless, Jonstrup et al. [68] reported a complete degradation of azo dyes through a sequential anaerobic–aerobic process. The findings were supported by other studies, in which the azo bond can be first broken down under an anaerobic step (rarely occurs under aerobic conditions), and the degradation of the remaining aromatic amine group (hardly degraded under anaerobic condition) is further achieved in the subsequent aerobic step [15,39].

17.3.5.2 Combined Physicochemical–Anaerobic Process

Despite the economical burden, physicochemical means are still being applied in the treatment of hardly and nonbiodegradable pollutants in wastewater. Popular

Table 17.8 Operating Conditions and Performance of Various Anaerobic Processes for Toxic Compounds Removal

Toxic Compound	Type of Wastewater	Anaerobic Process	Volume (L)	HRT (h)	OLR (kg/m^3 day)	Influent (mg/L)	Removal (%)	Application	References
Phenol	Synthetic coal gasification	AHR	15.5	7.9–36	0.74	752	77–99	Lab scale	[114]
	Coal gasification	AnMBBR	9	3	–	12.07	82.4	Lab scale	[65]
2,4-Dichlorophenol	Synthetic	UAFB	2.5	60	–	10–200	5–99.6	Lab scale	[5]
2,4,5-Trichlorophenol	Synthetic	HAIB	2.5	24	–	2–13	99	Lab scale	[7]
2,5-Dichlorophenol	Synthetic	HAIB	1.99	24	1.1	8	>99	Lab scale	[37]
Pentachlorophenol	Coal gasification	AnMBBR	9	3	–	3.87	93.6	Lab scale	[65]
	Synthetic	AMBR	13.5	249	0.89	10	64.6 –99.9	Lab scale	[129]
Formaldehyde	Paint industry	UAFB	1.12	10–24	0.18–3.61	8400–8545	41–99	Lab scale	[115]
	Synthetic	HAIB	0.8	12	–	1156	>95	Lab scale	[108]
Tylosin	Pharmaceutical	UASR	11	96	1.86	20–200	95	Lab scale	[22]
Petroleum hydrocarbons	Petroleum-contaminated	UAnFB	5	24	–	100–800	93.7 –99.9	Lab scale	[60]
		SnBR	5	24	–	100–800	87.7 –99.7	Lab scale	[60]

AHR, anaerobic hybrid reactor; *AMBR*, anaerobic migrating blanket reactor; *AnMBBR*, anoxic moving-bed biofilm reactor; *HAIB*, horizontal-flow anaerobic immobilized biomass; *HRT*, hydraulic retention time; *OLR*, organic loading rate; *SnBR*, sequential anoxic batch reactor; *UAFB*, upflow anaerobic fixed-bed reactor; *UAnFB*, upflow anoxic fixed-bed bioreactor; *UASR*, upflow anaerobic stage reactor.

physicochemical approaches mainly include the Fenton reaction, advanced oxidation process, electrocoagulation process, bioelectrochemical process (microbial fuel cell or microbial electrolysis cell), and adsorption process (e.g., activated carbon). In the combined physicochemical–anaerobic process, the physicochemical step can either be applied as a pretreatment to enhance biodegradability by cleaving reluctant chemical bonds in the toxic compounds or used as a polishing step so that the final effluent can meet the discharge requirements. A number of studies have reported the successful application of the combined physicochemical–anaerobic process for removal of toxic compounds in industrial wastewater [4,44,52,143,152].

17.4 Conclusions

This chapter reviews the removal of toxic compounds in industrial wastewater through the anaerobic process. Various anaerobic bacterial groups have been found to effectively degrade most of these toxic compounds (e.g., heavy metals, phenols, PCBs, CAHs, PAHs, etc.) via different degradation pathways. Three types of high-rate anaerobic bioreactors, UASB, EGSB, and ABR, are widely applied in toxic compounds removal from various types of industrial effluents. In addition, combined processes such as anaerobic–aerobic processes and physicochemical–anaerobic processes are advantageous for enhancing overall removal efficiencies.

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18

By-products of Anaerobic Treatment: Methane and Digestate From Manures and Cosubstrates

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18.1 Introduction

Anaerobic digestion is a widely used and arguably the most sustainable technique to recover the energy and nutrient content from manure, crop residuals, and other organic wastes while minimizing environmental emissions during agricultural production. Concerns about energy and food security, emission of greenhouse gases to the atmosphere as well as nutrients to the aquatic environment have spurred significant scientific interest in anaerobic digestion for biogas and organic fertilizer production.

The anaerobic digestion process occurs naturally in the environment, where microbes in an oxygen-free (anaerobic) condition metabolize and degrade biodegradable organic materials. Examples of these natural anaerobic digestion processes can be found in swamps, sediments, and the gut of ruminant animals. In an engineering system, as illustrated in Fig. 18.1, anaerobic digestion converts manure, crop residuals, and other biodegradable organic wastes in a controlled manner into biogas and nutrient-rich digestion residue (i.e., digestate). The produced methane-rich biogas can be used to generate heat and electricity, as transport fuel, for direct injection into the natural gas supply network, and even as a precursor for bioplastic production. The digestate, which contains most of the nutrients from the original raw materials, can also be utilized for agricultural production. In the context of sustainable farming, the integration of anaerobic digestion of manure and crop residuals with agricultural activities is essential for energy and nutrient management. However, the extent to which biogas and digestate can be used beneficially depends significantly on their quality. Thus, in this chapter, specific emphasis is given to biogas and digestate utilization.

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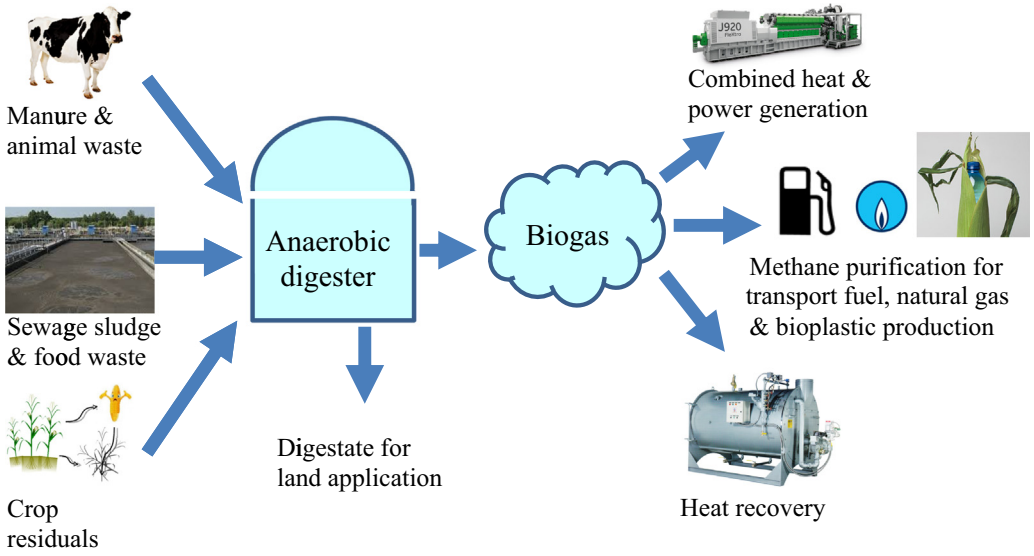

FIGURE 18.1 Energy and nutrient recovery from manure, crop residuals, and biodegradable organic wastes by anaerobic digestion and possible utilization of the produced biogas and digestate.

During anaerobic digestion, a consortium of anaerobic microbes degrades the organic material input in four sequential stages, namely, hydrolysis, acidogenesis, acetogenesis, and methanogenesis. A brief description of each of these stages is included here to provide the necessary context for further discussion of biogas and digestate utilization.

In the hydrolysis stage, hydrolytic microorganisms hydrolyze polymer materials to form monomers, such as amino acids and glucose. These monomers are subsequently converted to H_2, CO_2, and short-chain fatty acids such as acetic, propionic, and butyric acids in the acidogenic stage. In the acetogenic stage, syntrophic acetogenic bacteria metabolize volatile fatty acids (VFAs) to produce precursors for the methanogenic fermentation. Finally, CH_4 is formed from either acetate or CO_2 and H_2 by methanogenic bacteria in the methanogenesis stage [32,35,39].

The principles of anaerobic digestion are seemingly simple; however, process optimization is a rather challenging task. A specific group of bacteria is responsible for each of these stages. In addition, whereas the primary desirable final product is methane gas, numerous other intermediate products are also produced. The accumulation of some of these intermediate products such as ammonia and VFAs may disrupt the final conversion of acetate, CO_2, and H_2 into methane gas. In other words, these four stages must work in tandem to achieve a stable process. It is also noteworthy that hydrolysis is often the rate-limiting step in anaerobic digestion. On the other hand, biogas quality (i.e., percentage of methane in biogas and the occurrence of trace gases) is governed mostly by the methanogenesis stage.

18.2 Factors Affecting Anaerobic Digestion

There are several factors that can affect the anaerobic degradation of biodegradable materials and, hence, biogas and digestate quality. The most important factors are summarized in Fig. 18.2 and are briefly described below.

18.2.1 Operating Conditions

Key operating conditions that can influence the performance of an anaerobic digester are temperature, pH, and alkalinity. Anaerobic digestion performance depends strongly on temperature. Biogas formation can occur over a wide range of temperature from as low as 10°C to over 100°C, corresponding to psychrophilic (<20°C), mesophilic (20–40°C), and thermophilic (>40°C) conditions [31]. Because biogas yield under psychrophilic conditions is negligible, in most cases, the digesters are operated under either mesophilic or thermophilic conditions. Biogas yield by thermophilic digestion is considerably higher than that by mesophilic digestion because methanogen growth is more amenable to high temperature [5,6]. Thermophilic digestion also offers higher pathogen removal efficiency compared to mesophilic digestion [8]. It is, however, noteworthy that mesophilic species outnumber thermophiles, and they are also more tolerant to changes in environmental conditions than thermophiles. In addition, thermophilic digestion is prone to the buildup of ammonia and VFAs, which can disrupt the anaerobic process [2]. Thus, mesophilic digestion is more stable than its thermophilic counterpart.

Anaerobic bacteria are also sensitive to temperature changes. Therefore, it is essential to maintain a stable temperature for the growth of anaerobic microbes [6]. Temperature fluctuation of only 2°C [37] could lead to inactivation of anaerobic bacteria, resulting in a decrease in biogas production. Process failure has been reported at temperature changes in excess of 1°C per day [2].

pH is another key operating parameter that governs the performance of an anaerobic digester. In the early stages (i.e., hydrolysis, acidogenesis, and acetogenesis), pH decreases because of the formation of organic acids. Once the methanogenesis step occurs, pH may increase slightly because of the production of ammonia [35]. Inhibition of

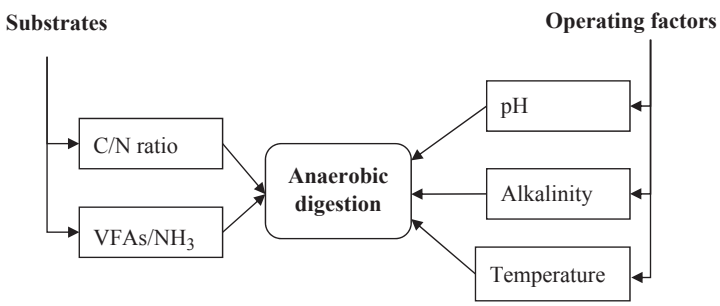

FIGURE 18.2 Factors influencing anaerobic digestion performance.

CH_4-forming bacteria can occur below pH 6 [6]. The pH inside a digester is an important factor governing the growth of anaerobic microbes, particularly methanogens, through its impact on enzyme activity. This is because each group of microorganisms may have their own optimum pH range. Methanogenic bacteria are most suited to a pH range between 6.5 and 7.8 [31], whereas acid-forming bacteria can function in a wider pH range from 4.0 to 8.5 [17] but prefer a pH of 5.5−6.5 [20,37]. In practice, it is necessary to keep the pH close to neutral because methanogenesis is the yield-limiting step. Lime addition is a common technique to overcome pH reduction.

Alkalinity refers to the buffering capacity of the digester against any changes in pH. In the anaerobic digestion process, alkalinity originates from the degradation of organics in the form of CO_2, bicarbonate, and ammonia [17]. The equilibrium of CO_2 and bicarbonate resists any changes in pH, allowing for stable operation despite the production of VFAs and other organic acids during hydrolysis, acidogenesis, and acetogenesis [3].

18.2.2 Substrate Composition

VFAs are important intermediate products and their production governs the stability of the anaerobic digestion process. VFAs are the precursors for the subsequent production of methane gas. However, excessive VFA concentration is a leading cause of process failure due to a reduction in pH below the optimum range of 6.5 and 7.8 [4]. The upper limit for VFA concentration to obtain a stable performance was reported at about 13,000 mg/L [36]. Additionally, a reduction in chemical oxygen demand (COD) removal efficiency has been observed with increased VFA production [31]. In the acetogenic stage, VFA accumulation can lead to a decrease in pH, which directly inhibits the growth of methanogens. If inhibition persists, acetogens will predominate in digesters. As discussed, the addition of buffering is an effective solution because this can resist pH drop and maintain sufficient VFA concentration for subsequent reactions [37]. Whereas acetic acid is the key substrate for methanogenesis, propionic and butyric acids are inhibitory to methanogenic bacteria. Appropriate regulation of VFA, especially butyric acids, has been shown to stabilize the overall system [37].

Ammonia is produced from the breakdown of nitrogen-containing organics, mainly from protein and urea during anaerobic digestion. Ammonia is an inhibitory substance to the anaerobic digestion process [7]. At pH above its pK_b value of 9.3, ammonia exists in its neutral form (free ammonia or NH_3), soluble in water. Given its neutral state and small molecular structure, free ammonia could easily penetrate through cell walls, causing pH imbalance and enzyme malfunction [7]. This inhibition in general has been conclusively observed in the methanogenesis stage. Koster and Lettinga [22] showed that along with an increase in ammonia concentrations in the range of 4051−5734 mg NH_3-N/L, acidogenic populations in the granular sludge were hardly affected, whereas the methanogenic population lost 56.5% of its activity. In regard to CH_4 production, ammonia has a stronger impact on aceticlastic than on hydrogenotrophic methanogens. In general, it is recommended that the free ammonia concentration be kept to below

80 mg/L although the anaerobic digestion process can tolerate a much higher total ammonia concentration (including ammonium and free ammonia) without any inhibitory effects [5].

The buildup of VFAs and ammonia in the digester can be regulated by selecting a substrate with the appropriate carbon/nitrogen stoichiometry. Indeed, the C/N ratio is a common parameter that has been thoroughly investigated by numerous anaerobic digestion studies. The C/N ratio can be defined as the relative amount of organic carbon measured by COD and nitrogen present in the feedstock. The changes in the specific CH_4 yields and CH_4 content consistently comply with the C/N ratio. A low nitrogen content would lead to the inhibition of anaerobic digestion because anaerobic microbes need an adequate amount of nitrogen for their growth, whereas organic carbon is considered a sole source for anaerobic activity. Increase in pH can result from a low C/N ratio. On the other hand, a high C/N ratio can directly result in a rapid conversion of nitrogen and low biogas production. It has been established that the optimum C/N ratio is in the range of 20−30 [29,41].

18.3 Feedstocks for Anaerobic Digestion

Animal manure is probably the most widely used substrate for anaerobic digestion applications worldwide. A primary objective used to be the stabilization of manure for beneficial use as fertilizer. In recent years, the economic value of biogas production has added further benefit to manure treatment by anaerobic digestion. In principle, all manures and animal wastes can be directed to anaerobic treatment. However, depending on their quantities and characteristics, as well as the plant design, they can be digested either alone or in conjunction with digestion of other raw materials (codigestion). The methane production potential of manures differs between manure types and depends on animal feeding and housing solutions, total solids content, and the bedding materials. Manure is a good base material for biogas plants as it contains all the nutrients required by the anaerobic bacteria and has a high buffering capacity. On the other hand, the high nitrogen content of (especially poultry) manure may require specific pretreatment (e.g., dilution with water) or codigestion with another nitrogen-lean substrate to achieve a suitable C/N ratio.

Plant biomass is another common substrate for anaerobic digestion. Unlike manure, biomass utilization is driven mostly by the economic value of biogas and the need to codigest with manure for a balanced C/N ratio. Common plant biomass substrates are energy crops and/or crop residues. They should be harvested within their growing period as fresh, green plants and used immediately for anaerobic digestion. It is possible to store plant biomass for a continuous supply of substrate to the digester. However, the drier and more straw-like the plant is, the less biogas it produces [1,34]. In suboptimal storing conditions, crops may be partly degraded, which usually decreases their biomethane potential. Thus, storage should be optimized for preventing such degradation.

Ensiling and paling are recommended storing methods for most crop materials. Ensiling (e.g., maize) under anoxic conditions without any preservatives has been reported to even increase the biomethane potential by 25%, possibly because of the formation of organic acids (lactic acid) which serve as a precursor for methane production [1]. Some examples of methane yields during continuous anaerobic digestion with manure and codigestion of manure and crop residuals (Table 18.1) illustrate the significant energy potential of crop materials compared to digesting manure alone. Even a small addition of crops increases the methane yield significantly.

Sewage sludge, municipal organic wastes, and certain industrial (particularly food processing) wastes are organic rich and thus can also be used for biogas production. The economic driver in this case is mostly in terms of gate fee (or commercial charge) to dispose of these materials. The characteristics of these materials vary in many ways. For example, the characteristics of the organic fraction of municipal solid waste vary depending on collection method (e.g., source separation or mechanically sorted), collection sites (e.g., restaurants, school canteens, hospitals, or residential housing), and time of year (e.g., amount of gardening waste) [37]. The characteristics of sewage sludge are also variable depending on the wastewater treatment process and the wastewater origin. By-products from food processing offer good raw materials for biogas plants, but may have an elevated concentration of nitrogen, sulfur, or phosphorus that may entail some unintended impact on the process. Overall, despite the significant economic benefit from gate fees, the anaerobic digestion of sewage sludge, municipal organic wastes, and industrial wastes represents a considerable risk associated not only with process stability but also with the quality of biogas and digestate.

Codigestion of two or more substrates is a pragmatic way to increase biogas production and overcome inhibition. Codigestion with organic wastes (e.g., municipal solid wastes and food processing wastes) can also increase profitability via gate fees for receiving and treating these waste materials. There could also be a synergetic effect by mixing different materials [42]. Codigestion can, however, affect the process

Table 18.1 Operational Data of Various Anaerobic Bioreactors Used for Different Types of Agricultural Waste [1,23,24,27,31,37,40]

Substrate	Manure/Crop Ratio	HRT (days)	OLR (kg VS/m^3 day)	CH_4 Yield (m^3/tVS Added)
Cattle manure		0.5–140	0.117–7.3	93–382
Swine manure		0.9–113	0.9–15.4	22–360
Maize				312–410
Wheat				390
Grass silage				306–372
Cattle manure + grass silage	93:7	54	2.7	240
Swine manure + rye/maize	43–50:50–57	75–130	2.11–4.25	360–400

HRT: hydraulic retention time; *OLR*: organic loading rate; *tVS*: total volatile solids; *VS*: volatile solids.

requirements through legislation. For example, if manure is to be digested with food waste, hygienization may be required. There may also be national legislation that regulates the use of the produced biosolids. In Australia, if sewage sludge is used as a cosubstrate for mesophilic anaerobic digestion, the digestate can be classified as biosolids class C and is restricted to land application for nonedible crops or forestry away from any protected water catchment. On the other hand, in Europe, products from anaerobic digestion from a farm-scale or farm-cooperative biogas plant can be utilized on the owners' land without any hygienization.

18.4 Biogas Quality and Purification

18.4.1 Biogas Quality

Biogas mainly consists of methane (40–75%) and carbon dioxide (15–60%). There are also several other trace gases that usually make up less than 2% in volume. These include hydrogen sulfide (H_2S), ammonia (NH_3), hydrogen (H_2), and nitrogen (N_2). In addition, biogas is usually saturated with water vapor, and may contain organic silicon compounds.

Depending on the level of biogas purification, it can be utilized for a range of applications from heating and cooking to power generation, transport fuel, natural gas supply, and even raw materials for the petroleum industry (Fig. 18.3). The economic value of biogas utilization increases substantially along these applications; however, it is also proportional to the cost of biogas purification. In fact, owing to the high cost and complexity of biogas purification, biogas from small-scale installations can be used only for heating and cooking.

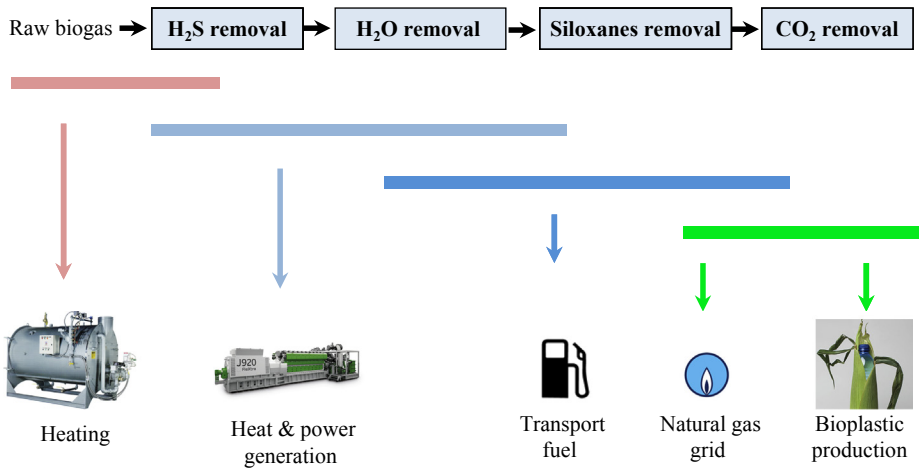

FIGURE 18.3 Biogas purification and utilization.

Table 18.2 Biogas Impurities and Their Impacts on Subsequent Usage

Impurity	Possible Impact
CO_2	Reduction in the calorific value
Water vapor	Corrosion in compressors, gas storage tanks, and engines Accumulation of water in pipes Condensation and/or freezing due to high pressure
H_2S	Oxidation to corrosive gases (e.g., SO_2) causing corrosion in compressors, gas storage tanks, and engines
NH_3	Correction in compressors, gas storage tanks, and engines when combined with water
Siloxanes	Formation of abrasive materials (SiO_2 and microcrystalline quartz) causing excessive wear and tear and scaling of spark plugs and equipment

For applications other than heating and cooking, the quality of biogas has to be improved. In large installations, biogas can be utilized in a combined heat and power (CHP) engine for the production of thermal energy and electricity. If not adequately removed, some of the above-mentioned impurities can damage the CHP engine (Table 18.2). In addition, by removing CO_2 from biogas to increase the energy content, natural gas and transport fuel can be derived from biogas. It is also possible to use the purified methane gas as a raw material for the production of bioplastics and a range of petroleum-substitute products.

Depending on the final biogas utilization option, CO_2 and impurities such as H_2S, water vapor, NH_3, particulate matter, and halogenated compounds have to be removed from the biogas. When biogas is combusted, H_2S reacts with oxygen to form SO_2 and SO_3, which are extremely corrosive. SO_2 also lowers the dew point in the stack gas leading to the condensation of water vapor. If not adequately removed, water vapor in biogas can condense and combine with NH_3, SO_2, and SO_3 to form a corrosive solution. Organic silicon compounds (or siloxanes) can also be present in biogas and can cause severe damage to CHP engines. During the combustion process, these organic silicon compounds are oxidized to silicon oxide, which deposits at spark plugs, valves, and cylinder heads, abrading the surfaces and eventually causing damage to the engine. Damages to microturbine engines due to excessive abrasion caused by silicon oxide have also been widely reported. CO_2 removal is required only when pure methane is desirable and will be used as natural gas standard, vehicle fuel, or raw material for bioplastic production.

18.4.2 Factors Influencing Biogas Quality

Certain components of biogas, including CH_4, CO_2, H_2O, and NH_3, occur naturally regardless of the feedstock characteristics. On the other hand, the composition of the feedstock is a key factor governing the occurrence of H_2S and siloxanes in biogas. High H_2S concentration in biogas can be attributed to a sulfur-rich substrate (e.g., food waste). Similarly, siloxane concentration in biogas can also be attributed to the silicon content of

the substrate. High siloxane concentration in biogas can be expected if the organic fraction of municipal solid waste is used as feedstock. An obvious strategy is to remove these materials from the feedstock. However, when financial benefit from gate fees is a major driver for the inclusion of these materials in the feedstock, other options to control biogas quality can be considered.

Another notable approach is to develop in situ techniques to control and reduce the formation of H_2S during the digestion process. These include ferric or ferrous addition to the feedstock to sequester sulfide in the form of iron sulfide, thus preventing the release of H_2S into biogas. A potentially cost-effective technique is to reduce H_2S formation during anaerobic digestion by micro-aeration [10−16,21]. This involves the introduction of a minute amount of oxygen or air uniformly into an anaerobic digester to create a condition that is prohibitory to H_2S formation but not methane formation. Nghiem et al. [28] have demonstrated that this desirable condition can be defined in terms of the oxidation−reduction potential in the digester of −320 to −270 mV. Although the effectiveness of micro-aeration for controlling H_2S in biogas has been demonstrated by a number of laboratory-scale investigations [10−16,21], its practical application for full-scale installation has not been realized yet. One exception is a short-term full-scale demonstration of micro-aeration to reduce H_2S concentrations in biogas reported by Jenicek et al. [19].

18.4.3 Biogas Purification

Purification is the most common approach to improve the quality of biogas for subsequent utilization. Technologies widely used for removing biogas impurities are briefly discussed below.

18.4.3.1 H_2S Removal

Several techniques can be used to remove H_2S from biogas. They include adsorption using iron oxides or activated carbon, wet scrubbing, biological scrubbing, and membrane separation.

H_2S can react readily with iron oxide to form iron sulfide [38]. The process is often referred to as an “iron sponge” because rust-covered steel wool may be used to form the reaction bed. Steel wool, however, has a relatively small surface area, which results in low binding capacity for the sulfide. Thus, wood chips impregnated with iron oxide have been used as the preferred reaction bed material because they have a larger surface-to-volume ratio. Iron oxide can also be coated on the surface of pellets made from red mud, a waste product of the Bayer process for aluminum production from bauxite.

H_2S can also be removed by activated carbon adsorption. Before entering the activated carbon bed, a small volume of air (about 5% w/w) is added to the biogas. Oxygen reacts with H_2S to form elementary sulfur, which is subsequently adsorbed onto the activated carbon. The best efficiency can be obtained at pressures of 700−800 kPa and temperatures of 50−70°C. This temperature is easily achieved through heat generation

during compression. Regeneration can be performed with hot nitrogen (inert gas) or steam. Sulfur is vaporized and, after cooling, liquefied at approximately 130°C. However, because of the low commercial value of sulfur and the low cost of the activated carbon, it is usually replaced rather than regenerated.

Wet scrubbing of H_2S can be based either on physical or on chemical processes. Physical wet scrubbing involves dissolving H_2S in a solvent (usually water), whereas chemical wet scrubbing involves dissolving H_2S in water followed by a chemical reaction to enhance the kinetics of the process. As some of the most common approaches, NaOH or $FeCl_2$ is added to water to create a basic solution that is readily reactive to H_2S. Compared to physical wet scrubbing, chemical wet scrubbing has a much lower water demand and is much more effective. H_2S removal from biogas in the range of 90−100% can be readily achieved with chemical wet scrubbing.

Biological scrubbing also involves the dissolution of H_2S into water saturated with O_2. In this technique, the aerated water is circulated countercurrently to the flow of biogas. Sulfur-oxidizing bacteria such as *Thiobacillus* are ubiquitous in the environment. They oxidize H_2S into elementary sulfur. The contact tower is airtight so that the biogas is in contact only with the oxygenated water and not ambient air. Biological scrubbing is effective and can remove up to 99% of H_2S from biogas.

H_2S can be removed from biogas using a semipermeable membrane that is highly permeable to H_2S (and CO_2) but not CH_4. In addition, supported liquid membranes can also be used. This consists of a microporous hydrophobic membrane separating the gas from the liquid phase. The molecules from the gas stream, flowing in one direction, diffuse through the membrane and will be absorbed on the other side by the liquid, flowing in countercurrent. Although it is technically feasible, membrane separation of H_2S is expensive and thus has not been used for commercial applications.

18.4.3.2 Water Vapor Removal

Raw biogas is usually saturated with water and the water content is temperature dependent. At 35°C, the saturated water vapor content is about 5%. The condensation of water on a metal surface can cause severe corrosion damage. Thus, water removal is essential for the utilization of biogas as a transport fuel or natural gas. Compressed natural gas vehicle fuel standards require a dew point (temperature at which water vapor condenses to form liquid water) of at least 10°C below the 99% winter design temperature for the local geographic area at atmospheric pressure [30]. Pipeline quality standards require a maximum water content of 100 mg/m^3. Water can be removed from biogas by physical separation and chemical drying.

Physical separation through refrigeration condensation is probably the simplest, most cost-effective, and most widely used technique for water removal from biogas. This method can lower the dew point to only about 1°C because of ice crystal formation on the heat exchanger surface. A lower dew point can be achieved by compressing the biogas before cooling and then later expanded to the desired pressure. The condensed water droplets are entrapped and removed.

Water vapor can also be removed using chemical desiccants (such as silica, alumina, and triethylene glycol). These techniques are usually applied at elevated pressures. When silica, alumina, or their mixture is used, the biogas is pressurized and led through a column filled with these desiccants. Two columns can be used in parallel. One column is used to absorb water while the other is being regenerated. Regeneration is achieved by evaporating the water through decompression and heating. Absorption of water using triethylene glycol can be achieved in a similar manner. The spent glycol is also regenerated by heating at 200°C. A low dew point of about −15°C (at atmospheric pressure) can be achieved using silica, alumina, or triethylene glycol as desiccant. Chemical drying is, however, more expensive and thus less commonly used than physical separation.

18.4.3.3 Siloxane Removal

Siloxanes are silicones containing Si−O bonds and organic radicals (methyl, ethyl, and other organic groups). During the combustion process, they are converted into abrasive microcrystalline quartz particles that can cause severe damage to metal components of internal combustion engines or microturbines. Engine manufacturers often limit the content of siloxanes in fuel to less than 0.03 mg/m^3 for microturbines and 28 mg/m^3 for internal combustion engines [9,30].

Siloxanes can be removed by absorption using silica gel or activated carbon. These adsorbents are also sensitive to water. Therefore, a dehumidification step must be performed before siloxane is removed. Heat treatment at about 250°C can be used to regenerate silica gel and activated carbon. The regeneration efficiency of silica gel (>95%) is higher than that of activated carbon. However, given the more affordable cost of activated carbon, it is still the most common adsorbent for siloxane removal.

18.4.3.4 CO_2 Removal

The removal of CO_2 is necessary to increase the energy content of biogas. Techniques developed for CO_2 removal from flue gas can also be used for biogas upgrade. They include chemical scrubbing using amines, pressure swing adsorption, and membrane separation. Amines (e.g., monoethanol amine, diethanol amine, and diglycol amine) can be used as absorbents for CO_2. In this technique, CO_2 removal is usually conducted using an adsorption column and a desorption column. The adsorption of CO_2 into an amine solution is facilitated at high pressure and low temperature (<50°C) as:

$$RNH_2 + H_2O + CO_2 \rightarrow RNH_3^+ + HCO_3^-$$

CO_2 stripping can then be achieved by decreasing the pressure and increasing the temperature (>115°C) as:

$$RNH_3^+ + HCO_3^- \rightarrow RNH_2 + H_2O + CO_2$$

Pressure swing adsorption uses a column filled with a molecular sieve, typically activated carbon, silica gel, alumina, or zeolite. These adsorbents retain CO_2 while allowing CH_4 to pass through. The CO_2 molecules are adsorbed loosely in the cavities of

the molecular sieve. It is a cyclic batch process in which adsorption is carried out at a relatively higher pressure (around 8 bars) and desorption (regeneration) at a lower one. The pressure swing adsorption process can be used only for dry gas. Thus, water removal is necessary prior to CO_2 stripping. Pressure swing adsorption can be operated either on the basis of equilibrium or kinetic selectivity, depending on the residence time in the column. For separation based on equilibrium selectivity, the more strongly adsorbed components of a gas mixture are retained within the column, whereas the effluent contains the less strongly adsorbed species. In the case of separation based on kinetic selectivity, the faster diffusing species are retained by the adsorbent and the high-pressure product is concentrated in slower-diffusing components. The off-gas from pressure swing adsorption still contains a significant amount of CH_4 and thus must be flared (or used for a boiler) to prevent the release of CH_4 into the atmosphere.

Membrane separation is based on the selective permeability of CO_2 and CH_4. Separation can occur when the transport of CH_4 through the membrane is higher than that of CO_2. In generation, the applied pressure for membrane separation is in the range of 20–36 bars. Although several different polymeric and inorganic membranes are available for CH_4/CO_2 separation, cellulose acetate is the most widely used membrane material for this application. After a single pass, raw biogas can be upgraded to about 92% CH_4. It is noteworthy that the off-gas is rich in CO_2 but still contains 10–25% CH_4. Thus, it must be flared or used for a boiler to prevent the release of CH_4 into the atmosphere.

18.5 Digestate Quality and Utilization

After anaerobic treatment, the obtained digestate is consistent, homogeneous, and rich in both phosphorus and nitrogen [18,25,26]. Most of the volatile organic matter of the initial feedstock has been converted into biogas, making the digestate biologically stable. In comparison to raw manure, because the nitrogen content remains the same, the ratio of carbon and nitrogen in digestate makes it more suitable as a fertilizer. A higher amount of total nitrogen is present as soluble and readily available ammonium for plants, further increasing the fertilizing value of the digestate. Codigestion of manure with other cosubstrates can improve the fertilizer value further. For example, codigestion of crop residuals and manure produces a better phosphorus-to-nitrogen ratio in the digestate than manure digestion alone. Over 50% of the total nitrogen in crop residuals is converted to ammonium (which is readily available for plant uptake) during the process. By contrast, the conversion of nitrogen in manure to ammonium is only 20–30%.

An even more crucial aspect of digestate utilization is the reduction of pathogenicity due to anaerobic treatment. Manure, certain organic wastes, and even crop residuals contain a range of pathogenic vectors that can cause adverse effects to both human health and the environment. Mesophilic or thermophilic anaerobic digestion can destroy or inactivate pathogens. The degree of destruction increases as the process temperature and residence time increase. The anaerobic process can also degrade or destruct vermin,

weed seeds, and certain hazardous compounds, such as phthalates and polycyclic aromatic hydrocarbons. In addition, as the anaerobic digestion process degrades compounds causing foul odors, the use of the digestate is more acceptable also in the vicinity of settlements than that of raw manure.

More targeted fertilizer products can also be made via inclusion of other technological processes after the digestion. Possible technologies include mechanical separation, pelletization, ammonia stripping, and phosphorus crystallization. The products from these processes may closely resemble inorganic fertilizers as they usually aim at separating and concentrating the nutrient of digestates. Examples of such products include ammonia water, ammonium sulfate, and struvite.

Land application is arguably the most beneficial approach to managing the digestate from anaerobic digestion of manure and other cosubstrates. Digestate from manure is an excellent biofertilizer and can replace synthetic fertilizer with regard to the supply of nitrogen and phosphorus. The use of digestate as liquid fertilizer is, however, not completely risk free [18]. It is essential to recognize and consider several potential adverse impacts of digestate spreading.

The pathogenic activity of digestate is lower than that of raw manure but remains significant. Thus, subsurface injection of digestate is recommended for public safety. To reduce the risk of spreading pathogenic agents to other farms, digestate must be pasteurized if applied to a different farm. On the other hand, digestate from a farm-scale or farm-cooperative anaerobic treatment plant can be utilized on the owners' land without any further treatment. When food waste is used as a cosubstrate, further treatment of the digestate may be required prior to land application.

Most of the biologically available nitrogen content in the digestate is in the form of ammonia. Because ammonia can be volatile, particularly at high pH, appropriate digestate storage in covered storage tanks is necessary to minimize ammonia evaporation. Covered storage also reduces methane emission from the digestate. Appropriate coverage also eliminates unwanted dilution from rain water. Ammonia evaporation is proportional to the storage temperature. Thus, provision of shading to keep the temperature low is also essential to prevent nitrogen loss through ammonia evaporation.

The prevention of ammonia evaporation is essential not only given the fertilizer value of nitrogen, but also to prevent harmful environmental effects of ammonia [25,26]. Indeed, ammonia evaporation and the resulting acidifying effects on the environment are the most significant environmental hazard from manure. Ammonia emissions from digestate also occur through field application. Because ammonia evaporation during storage is preventable, most of the ammonia loss is through on-field applications. Thus, digestate injection into the soil is recommended to minimize ammonia evaporation. In addition to ammonia evaporation prevention, injection into the soil, or at least instantaneous mulching, can also direct the nutrients where they are needed, i.e., in the root zone of the crops, and reduce nutrient runoff. The digestate from anaerobic digestion plants using manure or a mixture of manure and other cosubstrates is a sludge, which can be spread as such on fields using the same machinery as for liquid manure (Fig. 18.4).

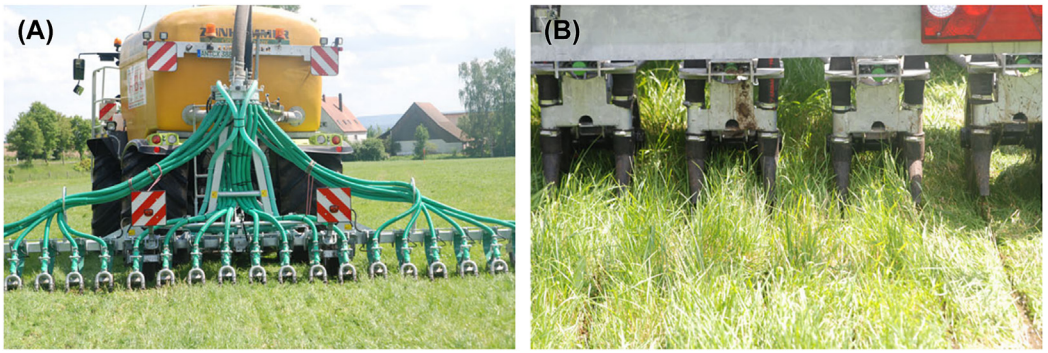

FIGURE 18.4 (A) Equipment to apply liquid fertilizer including manure digestate. (B) Subsurface injection to avoid ammonia loss [26].

The accumulation of a range of micropollutants commonly used in husbandry and the industry due to on-farm nutrient recycling is also an emerging and noteworthy issue [18]. The concentrations of these micropollutants such as antibiotics and growth hormones in raw manure are low and are typically in the range of up to several micrograms per liter. As these micropollutants are often poorly removed by anaerobic treatment [33], they are ubiquitous in digestate from manure. Further scientific investigations are recommended to shed light onto the accumulation of micropollutants particularly when their exact ecological effects at concentrations frequently found in anaerobic digestate (submicrogram per liter) are still to be ascertained.

18.6 Conclusion

Anaerobic digestion is a practical and cost-effective approach for biogas production and nutrient recovery from manure and other biomass. The obtained biogas can be utilized for a range of applications. However, gas purification is still a major bottleneck in biogas utilization for energy production and other forms of beneficial use. Although there are a full range of biogas purification technologies, allowing even for the production of bioplastic materials from raw biogas, they remain expensive and too complex for a farm-scale operation. Digestate from manure is an excellent biofertilizer and can be applied using the same equipment designed for liquid fertilizer. Consideration should also be given to digestate utilization particularly when manure is codigested with other cosubstrates or the digestate is used on a different farm.

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