CHAPTER
1

Overview of Anaerobic Biotechnology

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> We are convinced . . . that socially compatible and environmentally sound economic development is possible only by charting a course that makes full use of environmentally advantageous technologies. By this, we mean technologies that utilize resources as efficiently as possible and minimize environmental harm while increasing industrial productivity and improving quality of life (United States National Research Council Committee, 1995).

1.1 Anaerobic Biotechnology and Bioenergy Recovery

Environmental pollution is one of the greatest challenges human beings face in the twenty-first century. We are also faced with the consequences of climate change, increased global demand on fossil fuels, energy insecurity, and continuous exploitation of limited natural resources. The traditional approach of pollution control, which focuses on ridding pollutants from a single medium, that is, transformation of pollutants from liquid to solid or gas phases and vice versa, is no longer a desirable option. It has become enormously important to direct research efforts toward sustainable methods that not only alleviate environmental pollution, but also ease the stress on depleted natural resources and growing energy insecurity. The most cost-effective and sustainable approach is to employ a biotechnology option. Anaerobic biotechnology is a sustainable approach that combines waste treatment with the recovery of useful byproducts and renewable biofuels. Widespread application of anaerobic technology could ease increasing energy insecurity and limit the emission of toxic air pollutants, including green house gases to the atmosphere.

Figure 1.1 illustrates the potentials of anaerobic biotechnology in recovery of value-added products and biofuels from waste streams. Carbon, nitrogen, hydrogen, and sulfur from municipal, industrial, and agricultural solid and liquid wastes are converted into value-added resources. These include biofuels (hydrogen, butanol, and methane), electricity from microbial fuel cells (MFCs), fertilizers (biosolids), and useful chemicals (sulfur, organic acids, etc.). The sulfur can be used

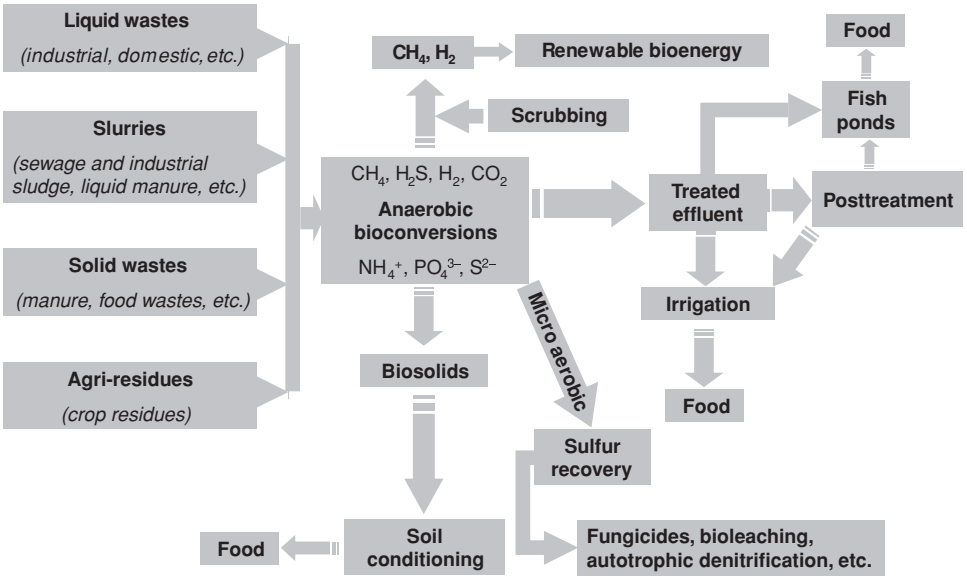

FIG. 1.1. Integrated anaerobic bioconversion processes in recovery of resources from wastes.

as an electron donor for bioleaching of heavy metals or removal of nitrate through autotrophic denitrification. Posttreatment effluent can be lagooned or reused for fish farming, algal production, and irrigation (see Box 1.1).

Box 1.1

Research Need

Due to the concern of endocrine disrupting chemicals (EDCs), e.g., natural steroidal hormones, pharmaceuticals, and personal care products in human/livestock wastes, growing fish, and algae for protein in effluent for human consumption could become a major heath issue. More research is needed to examine the residual levels of EDCs in the effluent and their potential impact on aquatic species.

From the perspective of developing and underdeveloped nations, a wider application of anaerobic biotechnology has even larger implications, as it would fulfill three basic needs: (a) improvement in health and sanitation through pollution control; (b) generation of renewable energy for household activities, such as cooking, lighting, and heating, and running small-scale businesses, for example, poultry farming and silkworm raising; and (c) supply of digested materials (biosolids) as a biofertilizer for crop production. Thus, anaerobic biotechnology

plays a significantly greater role not only in controlling pollution but also in supplementing valuable resources: energy and value-added products. This chapter presents a general overview of anaerobic biotechnology and builds up a foundation for the subsequent chapters.

1.2 Historical Development

The chronological development of anaerobic biotechnology is presented in Table 1.1. The application of anaerobic biotechnology dates back to at least the tenth century, when the Assyrians used it for heating bath water (Ostrem 2004). In 1776, Volta recognized that the anaerobic process results in conversion of organic matter to methane gas (McCarty 2001). The French journal *Cosmos* cited the first full-scale anaerobic treatment of domestic wastewater in an airtight chamber known as "Mouras Automatic Scavenger" in 1881. A septic tank modeled on the Mouras Automatic Scavenger was built in the city of Exeter, England, in 1895 by Donald Cameron. Cameron recognized the importance of methane gas, and the septic tank at Exeter was designed to collect methane for heating and lighting. In 1897, waste disposal tanks at a leper colony in Matunga, Bombay, India, were reported to have been designed with a biogas collection system, and the gas used to drive gas engines (Bushwell and Hatfield 1938).

With the development of a two-stage system known variously as the Travis tank (1904) and the Imhoff tank (1905), the focus shifted from wastewater treatment to settled sludge treatment. With the installation of the first sludge heating apparatus, separate digestion of sludge was reported at the Essen-Rellinghausen Plant, Germany, in 1927 (Imhoff 1938). The separate sludge digestion became immensely popular in larger cities, and the importance of methane gas generation was widely recognized. Methane gas was used for digester heating; it was collected and delivered to municipal gas systems, and it was used for power generation for operating biological wastewater treatment systems. Today, anaerobic digestion is widely adopted for the stabilization of municipal sludge and animal manure, and recovery of useful renewable energy—methane and biosolids.

Due to a failure to understand the fundamental of the process, application of anaerobic biotechnology was limited until 1950. Stander (1950) was the first to recognize the importance of solids retention time (SRT) for successful anaerobic treatment of different wastewaters. This has been the basis for the development of the so-called high-rate anaerobic reactor in which SRT and hydraulic retention time (HRT) were uncoupled. This development led to a wider application of anaerobic biotechnology, particularly for industrial wastewater treatment and biogas recovery.

Some of the widely used high-rate anaerobic treatment processes for industrial wastewater treatment include upflow anaerobic sludge blanket (UASB) reactor, expanded granular sludge bed (EGSB), anaerobic filter, fluidized bed, and hybrid

Table 1.1. Historical development of anaerobic biotechnology.

Anaerobic Technologies	Investigator(s) and Place	Developments in Chronological Order
Discovery of combustible air—methane	A. Volta, Italy	Recognized that anaerobic decomposition of organic matters produces methane (1776)
Mouras Automatic Scavenger	M. L. Mouras, France	Patented in 1881; the system had been installed in the 1860s
Anaerobic filter	Massachusetts Experimental Station, United States	Began operation in the 1880s
A hybrid system—a digester and an anaerobic filter	W. D. Scott Moncrieff, England	Constructed around 1890 or 1891
Septic tank	D. Cameron, Exeter, England	Designed in 1895 with provision for recovery of biogas for heating and lighting
	A. L. Talbot, United States	Designed in 1894 (Urbana); 1897 (Champaign)
Waste disposal tank	Leper colony, Matunga, Bombay, India	Digestion tank with gas collection system (1897)
Travis tank	W. O. Travis	Development of a two-stage system for a separate solid digestion (1904)
Imhoff tank	K. Imhoff, Germany	Modified the Travis tank (1905)
Sludge heating system	Essen-Rellinghausen Plant, Germany	Development of first separate sludge digestion system (1927)
Digester seeding and pH control	Fair and More	Realized the importance of seeding and pH control (1930)
High-rate anaerobic digestion	Morgan and Torpey	Developed digester mixing system (1950)
Clarigester (high-rate anaerobic processes)	G. J. Stander, South Africa	Realized the importance of SRT (1950)
Anaerobic contact process (ACP)	G. J. Schroepfer, United States	Developed ACP similar to aerobic-activated sludge process (1955)
Anaerobic filter (AF)	J. C. Young and P. L. McCarty, United States	Reexamined AF for the treatment of soluble wastewater (1969)
Anaerobic membrane bioreactor (AnMBR)	H. E. Grethlein, United States	An external cross-flow membrane coupled with anaerobic reactor (1978)
	Dorr-Oliver, United States	Developed commercial-scale AnMBR in early 1980s
Upflow anaerobic sludge blanket reactor	G. Lettinga, The Netherlands	Based on his first observation of granular sludge in Clarigester in South Africa (1979)
Expanded-bed reactor	M. S. Switzenbaum and W. J. Jewell, United States	Developed fixed-film expanded-bed reactor (1980)
Anaerobic baffled reactor	P. L. McCarty, United States	Retention of biomass within the baffles (1981)
Trace elements for methanogens	R. Speece, United States	Reported the importance of trace elements for methanogenic activity (1983)
Anaerobic sequential batch reactor (ASBR)	R. Dague and S. R. Pidaparti, United States	Developed ASBR for the treatment of swine manure (1992)

Sources: Lettinga (2001), Liao et al. (2006), McCarty (2001), Pidaparti (1991).

Table 1.2. Applications of anaerobic biotechnology in industrial wastewater treatment.

Types of Industries	Numbers of Plants
Breweries and beverages	329
Distilleries and fermentation	208
Chemicals	63
Pulp and paper	130
Food	389
Landfill leachate	20
Undefined/unknown	76
Total in database	1,215

Source: Franklin (2001). Reprinted with permission.

systems. Table 1.2 shows the different applications of high-rate anaerobic reactors in industrial wastewater treatment worldwide. There will be continued efforts to obtain improved bioreactor design to meet the future needs of environmental protection and resource recovery.

1.3 Importance of Anaerobic Biotechnology in Overall Waste Treatment

Although aerobic processes are widely used worldwide for municipal wastewater treatment, anaerobic processes still play a significant role in overall waste treatment as illustrated in Fig. 1.2.

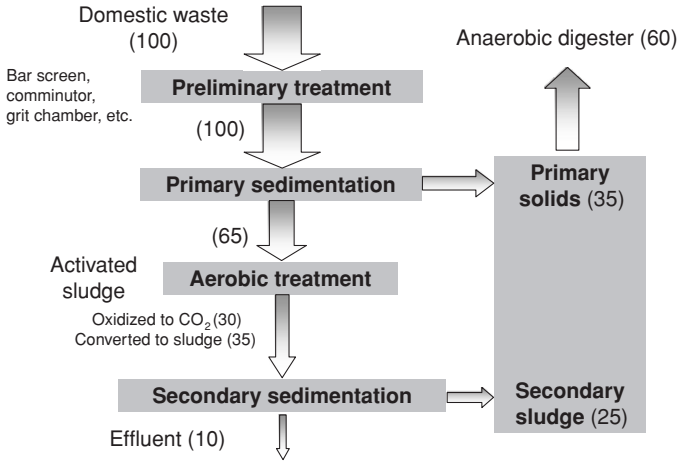

FIG. 1.2. Role of anaerobic biotechnology in overall waste treatment.

During conventional biological wastewater treatment process, preliminary treatment does not reduce biochemical oxygen demand (BOD). This is because particle sizes resulting from preliminary treatment are too large to be measured during a conventional BOD or chemical oxygen demand (COD) analysis. In a typical aerobic biological waste treatment system such as an activated sludge process, the organic pollutants (soluble, colloidal, and/or suspended) are merely transferred from the liquid phase to the solid phase. The solids (primary solids and secondary sludge) account for about 60% of the total influent waste strength, which requires further treatment before final disposal. The fate of the solids and sludge is the anaerobic digester, which reduces their mass and putricibility.

1.4 Definition and Principle of Anaerobic Processes

Anaerobic processes are defined as biological processes in which organic matter is metabolized in an environment free of dissolved oxygen or its precursors (e.g., H_2O_2). Anaerobic process is classified as either anaerobic fermentation or anaerobic respiration depending on the type of electron acceptors.

1.4.1 Anaerobic Fermentation

In an anaerobic fermentation, organic matter is catabolized in the absence of an external electron acceptor by strict or facultative anaerobes through internally balanced oxidation–reduction reactions under dark conditions. The product generated during the process accepts the electrons released during the breakdown of organic matter. Thus, organic matter acts as both electron donor and acceptor. In fermentation the substrate is only partially oxidized, and therefore, only a small amount of the energy stored in the substrate is conserved. The major portion of the adenosine triphosphate (ATP) or energy is generated by substrate-level phosphorylation. Figure 1.3 shows the anaerobic fermentation of glucose to ethanol. It is important to point out that the major portion (two-thirds) of methane is produced through anaerobic fermentation in which acetate acts as both electron

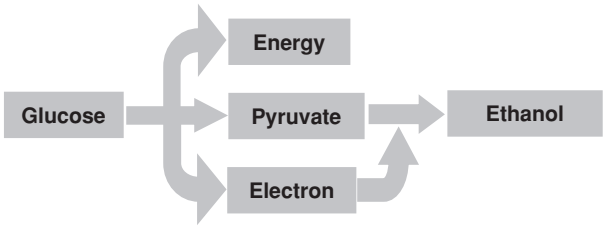

FIG. 1.3. Anaerobic fermentation of glucose to ethanol.

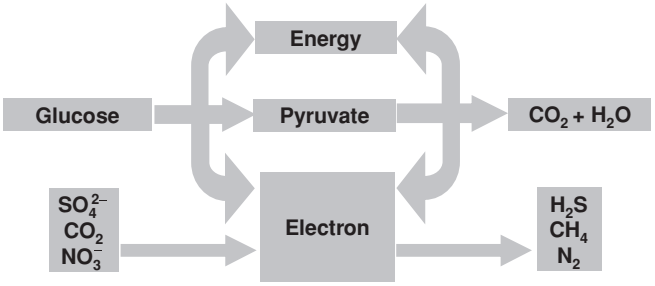

FIG. 1.4. Anaerobic respiration of glucose.

donor and electron acceptor. Methane production through this route is commonly known as acetotrophic (or acetoclastic) methanogenesis. Anaerobic fermentation can be applied for the recovery of both biofuels (e.g., hydrogen and butanol) and biochemicals (nisin and lactic acid) from low-value feedstock.

1.4.2 Anaerobic Respiration

Anaerobic respiration on the other hand requires external electron acceptors for the disposal of electrons released during the degradation of organic matter (Fig. 1.4). The electron acceptors in this case could be CO_2, SO_4^{2-}, or NO_3^-. Both substrate-level phosphorylation and oxidative phosphorylation generate energy (or ATP). The energy released under such a condition is much greater than anaerobic fermentation.

When CO_2 accepts the electrons released by the organic matter, it is reduced to CH_4 gas. Methane production through this route is known as hydrogenotrophic methanogenesis and accounts for about one-third of total methane production. Some anaerobes such as homoacetogens also use CO_2 as an electron acceptor and reduce hydrogen to acetic acid (Müller 2001). The presence of sulfate in an anaerobic environment diverts part of organic matter toward sulfate reduction by a specialized group of anaerobic bacteria known as sulfate-reducing bacteria (SRB). The release of odorous hydrogen sulfide (H_2S) gas is a characteristic of anaerobic environment in which sulfate acts as an electron acceptor. SRB are mostly obligate anaerobes, although studies have shown that some species of SRB are capable of aerobic respiration.

When NO_3^- acts as an electron acceptor, it is reduced to nitrogen gas. This is a standard biological process for the removal of nitrogenous compounds from wastewater. The process is commonly referred as denitrification or anoxic denitrification. The group of bacteria involved in the process is known as nitrate-reducing bacteria (NRB) or denitrifiers. NRB are usually facultative bacteria, which are capable of aerobic respiration and/or nitrate respiration. The anaerobic environment

Table 1.3. Microbial groups and their preferred electron acceptors and donors, and carbon sources.

Microbes	Electron Acceptor	Electron Donor	Carbon Source
Methane-producing bacteria			
Acetotrophic (or acetoclastic)	Acetate	Acetate	Acetate
Hydrogenotrophic	CO_2	H_2	CO_2
Nitrate/nitrite-reducing bacteria			
Heterotrophic denitrifiers	NO_3^-, NO_2^-	Organic carbon	Organic carbon
Autotrophic denitrifiers	NO_3^-, NO_2^-	S° or H_2	CO_2
Sulfate-reducing bacteria			
Acetotrophic (or acetoclastic)	SO_4^{2-}	Acetate	Acetate
Hydrogenotrophic	SO_4^{2-}	H_2	CO_2
Anaerobic ammonia-oxidizing bacteria	NO_2^-	NH_4^+	CO_2

in which denitrification occurs is sometime known as *anoxic*. Major anaerobic microbes involved in carbon, nitrogen, and sulfur pollution control and the respective electron donors, electron acceptors, and carbon sources are presented in Table 1.3.

From energetic standpoint, oxygen is the most favorable electron acceptor as it releases the greater Gibb's free energy change ($\Delta G^{\circ\prime}$), and hence is favored by microorganisms. In an environment devoid of oxygen, the next best electron acceptor is NO_3^- followed by MnO_2, FeOH, SO_4^{2-}, and CO_2. Findings, however, suggest that fermentation reactions and reductions of SO_4^{2-} and CO_2 may occur almost simultaneously. The affinity of microorganism for the electron acceptor is in the following order (Kiene 1991):

$$O_2 > NO_3^- > MnO_2 > FeOH > SO_4^{2-} > CO_2$$

1.5 Important Considerations in Anaerobic Biotechnology

From both the waste treatment and resource recovery perspectives, it is important to examine some of the important factors that govern the anaerobic bioconversion process. These include organic loading rate, biomass yield, substrate utilization rate, HRT and SRT, start-up time, microbiology, environmental factors, and reactor configuration. The following sections elaborate on these factors.

1.5.1 Volumetric Organic Loading Rate

Anaerobic processes are characterized by high volumetric organic loading rates (VOLRs). High-rate anaerobic reactors such as UASB, EGSB, anaerobic filter, and fluidized bed reactors are capable of treating wastewater at VOLR of 10–40 kg

COD/m^3· day, and on occasion can exceed 100 kg COD/m^3· day in fluidized bed reactors. A high VOLR indicates that more wastewater can be treated per unit of reactor volume. VOLR is one of the most important factors in designing or sizing an anaerobic bioreactor. VOLR is given by the following expression:

$$\text{VOLR} = \frac{C_i Q}{V} \tag{1.1}$$

where C_i is influent wastewater biodegradable COD concentration (mg/L), Q is wastewater flow rate (m^3/day), and V is anaerobic bioreactor volume (m^3).

C_i and Q are known parameters, and VOLR is determined based on on-site pilot-scale testing. For a biological process, the VOLR to the reactor is dependent on several factors, such as the kinetics of pollutant degradation, biomass level in the bioreactor, and types of bioreactor.

1.5.2 Biomass Yield

Biomass yield is a quantitative measure of cell growth in a system for a given substrate. The commonly used term to represent biomass yield is yield coefficient (Y), which is mathematically expressed as:

$$Y = \frac{\Delta X}{\Delta S} \tag{1.2}$$

where ΔX is increase in biomass concentration (mg VSS/L), and ΔS is decrease (consumed) in substrate concentration (mg COD/L).

Of note is the biomass yield per mole of ATP, which totals 10.5 g volatile suspended solids (VSS) for both aerobic and anaerobic processes (Henze and Harremöes 1983). However, when considering the metabolic processes of microorganism, the total aerobic ATP generation is 38 mol, while the anaerobic ATP generation is only 4 mol ATP/mol glucose. This results in a significantly lower biomass yield for the anaerobic treatment process compared to the aerobic process.

Anaerobic degradation of organic matter is accomplished through a number of metabolic stages in a sequence by several groups of microorganisms. This differs from the aerobic treatment process, in which such synergistic relation does not exist. The yield coefficient of acid-producing bacteria is significantly different from that of methane-producing bacteria. The aerobic treatment process gives a fairly constant yield coefficient for biodegradable COD irrespective of the type of substrates. Some common yield coefficients for different processes are presented in Table 1.4.

For an anaerobic system, the yield coefficient depends not only on COD removed but also on the types of substrates being metabolized. Table 1.5 shows the yield coefficients of anaerobic systems under different substrate conditions.

Table 1.4. Yield coefficients.

Process	Yield Coefficient (kg VSS/kg COD)	References
Acidogenesis	0.15	Henze and Harremöes (1983)
Methanogenesis	0.03	
Overall	0.18	
Anaerobic filter (mixed culture) (carbohydrate + protein as substrate)	0.115–0.121	Young and McCarty (1969)
Anaerobic treatment process	0.05–0.15	van Haandel and Lettinga (1994)

Carbohydrate and protein have relatively high yield coefficients, as the two groups of microorganisms (acidogens and methanogens) are involved in the metabolism of the substrates to methane. The overall yield coefficients for these substrates are the sum of individual yield coefficient of acidogens and methanogens. Acetate and hydrogen on the other hand have relatively low yield coefficients as only methanogens are involved in the metabolism of these substrates.

1.5.3 Specific Biological Activity

Specific biological activity indicates the ability of biomass to utilize the substrate. It is usually reported as:

$$\text{Specific substrate utilization rate} = \frac{\text{kg COD}_{\text{removed}}}{(\text{kg VSS} \cdot \text{day})} \tag{1.3}$$

Anaerobic processes have a substrate utilization rate of 0.75–1.5 kg COD/kg VSS · day, which is more than double that of the aerobic treatment process. Henze and Harremöes (1983) also reported substrate removal rate of 1.0 kg COD/kg VSS · day, assuming 50% of the VSS is active. These are quite reasonable rates, as O_2 transfer/diffusion limitation is not an issue in an anaerobic process, unlike an

Table 1.5. Yield coefficients with different substrates.

Types of Substrates	Yield Coefficient (Y) (kg VSS/kg COD)
Carbohydrate	0.350
Protein	0.205
Fat	0.038
Butyrate	0.058
Propionate	0.037
Acetate	0.032
Hydrogen	0.038

Source: Pavlostathis and Giraldo-Gomez (1991).

aerobic system. Furthermore, by maintaining a high concentration of diversified group of biomass in close proximity through biomass immobilization or granulation, a good balance of syntrophic relation between acidogens and methanogens can be achieved. The significant improvement in specific activity of anaerobic system has been the result of studies conducted by Speece in early 1980s, who reported on the specific nutrient requirements of methanogens.

1.5.4 Hydraulic Retention Time and Solids Retention Time

HRT and SRT are two important design parameters in biological treatment processes. HRT indicates the time the waste remains in the reactor in contact with the biomass. The time required to achieve a given degree of treatment depends on the rate of microbial metabolism. Waste containing simple compounds such as sugar is readily degradable, requiring low HRT, whereas complex wastes, for example, chlorinated organic compounds, are slowly degradable and need longer HRT for their metabolism. SRT, on the other hand, controls the microbial mass (biomass) in the reactor to achieve a given degree of waste stabilization. SRT is a measure of the biological system's capability to achieve specific effluent standards and/or to maintain a satisfactory biodegradation rate of pollutants. Maintaining a high SRT produces a more stable operation, better toxic or shock load tolerance, and a quick recovery from toxicity. The permissible organic loading rate in the anaerobic process is also determined by the SRT. Speece (1996) indicated that HRT is a deciding factor in process design for complex and slowly degradable organic pollutants, whereas SRT is the controlling design parameter for easily degradable organics.

For the slow-growing microorganisms such as methanogens, care must be exercised to prevent their washout from the reactor in order to achieve a longer SRT. Continuous stirred tank reactors (CSTRs) without solid separation and recycling are often prone to failure due to excessive biomass washout unless long HRTs (or SRTs) are maintained. Elevated HRTs require a bigger reactor volume (volume = flow rate $\times$ HRT), which is costly. An early attempt to maintain a long SRT irrespective of HRT was the use of the clarigester or anaerobic contact process, where the anaerobic sludge was allowed to settle in the settling tank and was then returned back to the reactor.

A wide variety of high-rate anaerobic reactors have been able to maintain extremely high SRTs due to biomass immobilization or agglomeration. Such systems operate under short HRTs without any fear of biomass washout. The first full-scale installation of a UASB reactor in the Netherlands has adequately demonstrated that anaerobic treatment is possible with an HRT as short as 4 h, up to an organic loading rate of 16 kg COD/$m^3 \cdot$ day (Lettinga et al. 1980). The empirical HRTs for different anaerobic systems to achieve the same degree of treatment are presented in Table 1.6.

Table 1.6. HRTs of anaerobic systems needed to achieve 80% COD removal efficiency at temperature >20°C.

Anaerobic System	HRT (h)
UASB	5.5
Fluidized/expanded bed	5.5
Anaerobic filter	20
Anaerobic pond[a]	144 (6 days)

Source: van Haandel and Lettinga (1994). Reprinted with permission.
[a] BOD removal efficiency.

1.5.5 Start-Up Time

Start-up is the initial commissioning period during which the process is brought to a point where normal performances of the biological treatment system can be achieved with continuous substrate feeding. Start-up time is one of the major considerations in anaerobic processes because of the slow growth rate of anaerobic microorganisms, especially methanogens, and their susceptibility to changes in environmental factors. Anaerobic treatment systems often need quite a long start-up time, which may weaken their competitiveness with aerobic treatment systems that have a relatively short start-up time of 1–2 weeks. The start-up time could be reduced considerably if the exact microbial culture for the waste in question is used as a seed. Under such a situation the generation time of the microorganisms is greatly reduced. A start-up time of 2–4 months is quite common at a mesophilic temperature range (37°C). Periods exceeding a year may be needed under thermophilic conditions (55°C), due to the high decay rate of biomass. The start-up time also depends on the initial biomass inventory (i.e., the initial amount of seed placed in the reactor). The more seed used, the shorter the start-up time. Loading rates and environmental factors such as pH, nutrient availability, temperature and oxidation–reduction potential (ORP) should be maintained within the limits of microbial comfort during the start-up.

1.5.6 Microbiology

The microbiology of the anaerobic treatment system is much more complicated than that of the aerobic one. An anaerobic process is a multistep process in which a diverse group of microorganisms degrades the organic matter in a sequential order resulting a synergistic action (see Fig. 2.1). The stability of an anaerobic treatment system is often debated, mainly due to the fragile nature of microorganisms especially methanogens to the changes in environmental conditions such as pH, temperature, ORP, nutrients/trace metals availability, and toxicity. When an anaerobic treatment system fails because of lack of proper environmental factors

or biomass washout from the reactor, it may take several months for the system to return to a normal operating condition because of an extremely slow growth rate of methanogens.

1.5.7 Environmental Factors

It has been pointed out earlier that anaerobic processes are severely affected by the changes in environmental conditions. Anaerobic treatment system is much more susceptible than the aerobic one for the same degree to deviation from the optimum environmental conditions. The successful operation of anaerobic reactors, therefore, demands a meticulous control of environmental factors close to the comfort of the microorganisms involved in the process. The effect of environmental factors on treatment efficiency is usually evaluated by the methane yield because methanogenesis is a rate-limiting step in anaerobic treatment of wastewater. Hence, the major environmental factors are usually governed by the methanogenesis. Brief descriptions of the important environmental factors are outlined here.

1.5.7.1 Temperature

Anaerobic processes, like other biological processes, strongly depend on temperature. The anaerobic conversion of organic matter has its highest efficiency at a temperature 35–40°C for mesophilic conditions and at about 55°C for the thermophilic conditions (van Haandel and Lettinga 1994). Anaerobic processes, however, can still operate in a temperature range of 10–45°C without major changes in the microbial ecosystem. Generally, anaerobic treatment processes are more sensitive to temperature changes than the aerobic treatment process.

1.5.7.2 Operating pH

There are two groups of bacteria in terms of pH optima, namely acid-producing bacteria (acidogens) and methane-producing bacteria (methanogens). The acidogens prefer a pH of 5.5–6.5, while methanogens prefer a range of 7.8–8.2. In an environment where both cultures coexist, the optimal pH range is 6.8–7.4. Since methanogenesis is considered as the rate-limiting step, where both groups of bacteria are present, it is necessary to maintain the reactor pH close to neutral.

1.5.7.3 Oxidation–reduction potentials

Morris (1975) reported that to obtain the growth of obligate anaerobes in any medium, the culture ORP value should be maintained from −200 to −350 mV at pH 7. It is well established that methanogens require an extremely reducing

environment, with redox potentials as low as −400 mV (Archer and Harris 1986; Hungate 1969).

1.5.7.4 Nutrients and Trace Metals

All microbial-mediated processes require nutrients and trace elements during waste stabilization. A question may arise how nutrients and trace elements are involved in waste stabilization. In fact nutrients and trace metals are not directly involved in waste stabilization; but they are the essential components of a microbial cell and are thus required for the growth of an existing microbial cell and synthesis of new cell. Besides, nutrients and trace metals also provide a suitable physicochemical condition for optimum growth of microorganisms. It is important to note that if the waste stream in question does not have one or more of the important nutrients and trace elements, the waste degradability is severely affected. This is because of inability of microbial cell to grow at optimum rate and to produce new cells.

1.5.7.5 Toxicity and Inhibition

Anaerobic microorganisms are inhibited by the substances present in the influent waste stream and by the metabolic byproducts of microorganisms. Ammonia, heavy metals, halogenated compounds, and cyanide are examples of the former, while ammonia, sulfide, and volatile fatty acids are examples of the latter. It is interesting to point out that many anaerobic microorganisms are also capable of degrading refractory organics (Stronach et al. 1986) that otherwise might be considered toxic. In some cases, toleration is manifested by acclimation to toxicants. These observations provide a considerable cause for optimism about the feasibility of anaerobic treatment of industrial wastewaters that contain significant concentrations of toxic compounds (Parkin and Speece 1982).

1.5.8 Reactor Configuration

Selection of a proper reactor configuration is of prime importance in anaerobic processes. The relatively low biosynthesis rate of methanogens in an anaerobic system demands special consideration for reactor design. The selection of reactor types is based on the requirement of a high SRT/HRT ratio, so as to prevent the washout of slow-growing methanogens.

The treatment performance of the selected reactors is, therefore, mainly dependent on their capability to retain biomass, thus maintaining a high SRT/HRT ratio.

Another approach for reactor configuration selection is based on required effluent quality. Because of relatively high half-saturation constants (K_s) for anaerobic microorganisms, CSTRs may not be suitable, as immediate dilution of the waste

leads to low concentrations of organic matters, but still too high to meet the effluent discharge standards, which are below the range of anaerobic degradation. Under such circumstances, a staging or plug flow type reactor would be more beneficial.

1.6 Merits of Anaerobic Biotechnology

Anaerobic biotechnology is becoming immensely popular due to its potential to produce renewable biofuels and value-added products from low-value feedstock such as waste streams. In addition, it provides an opportunity for the removal of pollutants from liquid and solid wastes more economically than the aerobic processes. These merits are illustrated in the following sections.

1.6.1 Recovery of Bioenergy and Biofuels

1.6.1.1 Biomethane Production

Methane gas is a major byproduct of anaerobic degradation of organic solid and liquid wastes. Methane gas has an energy content of 55,525 kJ/kg at 25°C and 1 atm (CRC Handbook of Chemistry and Physics 1996). With one-third conversion efficiency of heat energy into electrical energy, the electricity generation is 5.14 kWh/kg CH_4 [55,525 × (1/3) × (1/3,600)]. The methane energy generation is calculated as follows:

Stoichiometrically, 1 kg of COD releases about 15.625 mol (or 0.35 m^3 at standard temperature and pressure (STP)) of methane gas (see Example 1.1). Thus, 1 kg COD is needed to produce 15.625 mol (or 0.25 kg) of methane. The electrical energy generated from methane is 1.29 kWh/kg $COD_{removed}$ ((5.14 kWh/kg CH_4) × (0.25 kg CH_4/kg COD)).

Example 1.1

How much methane gas could be generated through complete anaerobic degradation of 1 kg COD at STP?

Solution

Step 1: Calculation of COD equivalent of CH_4

It is necessary to calculate the COD equivalent of methane by considering its complete oxidation, as shown in the following chemical equations:

$$\begin{aligned} &CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \\ &16\ g \quad 64\ g \\ &\rightarrow 16\ g\ CH_4 \sim 64\ g\ O_2\ (COD) \\ &\rightarrow 1\ g\ CH_4 \sim 64/16 = 4\ g\ COD \end{aligned} \quad (1)$$

Step 2: Conversion of CH_4 mass to equivalent volume

Based on ideal gas law, 1 mol of any gas at STP occupies a volume of 22.4 L.

$$\begin{aligned} &\rightarrow 1 \text{ mol } CH_4 \sim 22.4 \text{ L } CH_4 \\ &\rightarrow 16 \text{ g } CH_4 \sim 22.4 \text{ L } CH_4 \\ &\rightarrow 1 \text{ g } CH_4 \sim 22.4/16 = 1.4 \text{ L } CH_4 \end{aligned} \quad (2)$$

Step 3: CH_4 generation rate per unit of COD removed

From Eqs (1) and (2), we have:

$$\begin{aligned} &\rightarrow 1 \text{ g } CH_4 \sim 4 \text{ g COD} \sim 1.4 \text{ L } CH_4 \\ &\rightarrow 1 \text{ g COD} \sim 1.4/4 = 0.35 \text{ L } CH_4 \\ &\text{or } 1 \text{ kg COD} \sim 0.35 \text{ m}^3 \text{ } CH_4 \end{aligned} \quad (3)$$

So, complete anaerobic degradation of 1 kg COD produces 0.35 m^3 CH_4 at STP.

1.6.1.2 Biohydrogen Production

In anaerobic fermentation, hydrogen is produced during acidogenic phase. The consumption of hydrogen by hydrogenotrophic methanogens is prevented by proper process control, such as pH and heat treatment (Khanal et al. 2006). From a global environmental perspective, production of hydrogen from renewable organic wastes represents an important area of bioenergy production. Using glucose as a model substrate, the hydrogen production can be represented by the equations (Miyake et al. 1984):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad \Delta G^\circ = -184 \text{ kJ}$$

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2 \quad \Delta G^\circ = -255 \text{ kJ}$$

Most of the studies on hydrogen production have been primarily confined to the laboratory scale. The low yield of hydrogen through anaerobic fermentation alone has long been a major challenge to engineers and scientists. Liu and Fang (2003) reported a maximum hydrogen yield of about 3.76 mol H_2/mol sucrose using acidogenic granular sludge at an HRT of 13.7 h, temperature of 26°C, and pH of 5.5 in a CSTR. Kataoka et al. (1997) studied continuous hydrogen production in a chemostat using a pure culture of *Clostridium butyricum* SC-E1 with glucose as an organic substrate at an HRT of 8 h, temperature of 30°C, and pH of 6.7. The authors reported the maximum hydrogen yield of 1.3–2.2 mol H_2/mol glucose. The hydrogen production potential of cellulose was investigated using two types of natural inocula: anaerobically digested sludge and sludge compost in batch cultures at 60°C (Ueno et al. 1995). The authors reported a hydrogen yield of

0.9 and 2.4 mol/mol hexose for anaerobic digested sludge and sludge compost as inocula, respectively. More research is needed to improve the hydrogen yield for commercial viability.

1.6.1.3 Butanol Production

Butanol is also a potential substitute for fossil fuel and is considered a superior fuel to ethanol for several reasons: more favorable physical properties, better economics, and safety. In addition, the butanol eliminates the need for engine modification that has been running on gasoline. Butanol is produced by fermentative bacteria including *Clostridium acetobutylicum* (Qureshi et al. 2006) and *Clostridium beijerinkii* (Formanek et al. 1997). The ratio of acetone, butanol, and ethanol (ABE) is 3:6:1, with butanol being the major fermentation byproduct. The ABE fermentation consists of two distinct phases: acidogenesis and solventogenesis. The solvent production particularly, butanol, takes place during the solventogenesis and is directly correlated to the spore-forming ability of the culture (Long et al. 1984). Low butanol yield through fermentation coupled with cheap petroleum feedstock is the major impediment to the widespread development of butanol fuel. Environmental Energy Inc., Blacklick, OH, claimed that the use of fibrous bed bioreactor along with their patent process could produce 2.5 gal butanol per bushel of corn (http://www.butanol.com/). Carbohydrate-rich waste stream could serve as an ideal feedstock for butanol production. Hydrogen gas is another byproduct of butanol fermentation and can also be recovered as a renewable energy.

1.6.1.4 Biodiesel Production from Biogas

The biogas generated during anaerobic digestion of organic waste can be converted into liquid fuel—biodiesel. The biogas is first converted into liquid methanol using a thermal catalytic process. Biodiesel or methyl ester is then produced by transesterification of fats or oil with methanol in the presence of a base catalysis (e.g., sodium or potassium hydroxide). Smithfield's Circle Four Swine farm in Southwestern, Utah, USA, is running two full-scale anaerobic digesters under mesophilic condition. The biogas produced will be used for in situ methanol production, which is currently under construction. The produced methanol will be shipped to Texas for biodiesel production. Some of the important features of biogas/biodiesel process are outlined in Box 1.2.

1.6.1.5 Electricity Generation Using Microbial Fuel Cell

An MFC is a device that directly converts biochemical energy stored in the carbohydrate and other organic matter in wastewater into electricity. An MFC contains two chambers, consisting of an anode and cathode similar to hydrogen fuel cell,

Box 1.2

Number of hogs: 144,000 head
Total feed flow rate: ~872 m^3/day (230,400 gal/day)
Hydraulic retention time: ~30 days
Feed total solids content: ~3–4%
Volatile solids destruction rate: 55–64%
Biogas production: ~11,300–14,100 m^3/day (400,000–500,000 ft^3/day)
Methanol production potential: ~15–19 m^3/day (4,000–5,000 gal/day)

separated by a proton (cation) exchange membrane (PEM). The organic matter is oxidized by anaerobic microbes in the anode chamber and electrons are released. These electrons are then transferred to the anode (positively charged terminal) and flowed to the cathode (negatively charged terminal) through a conductive material such as a resistor or to an external load. The electrons in the cathode combine with protons that diffuse through the PEM and oxygen (from air). The oxygen is reduced to water. In the MFC, the driving force is the redox reaction of substrates (wastewater) mediated by anaerobic microorganisms. Thus, MFC research has a potential to treat the wastewater and produce electricity. MFC studies, however, have been mainly confined to the laboratory-scale level, and their full-scale application is still on the far horizon.

1.6.2 Recovery of Value-Added Products

1.6.2.1 Recovery of Acetic Acid

Miller and Wolin (1995) reported production of high concentration of acetate (0.33 M) from cellulose substrate by a coculture of cellulolytic bacterium and a reductive acetogen that yields acetate from H_2 and CO_2. In a conventional anaerobic process, acetate is produced by homoacetogens from H_2 and CO_2, and with proper control of environmental conditions, acetate production can be enhanced. The control strategies include pH, redox potential, periodic depletion of hydrogen via nitrate addition, and addition of other substances, such as protein, bile salts, or by varying feed composition variation (Verstraete and Vandevivere 1999).

1.6.2.2 Production of Nisin and Lactic Acid

Nisin is a bacteriocin produced commercially by fermentation using lactic acid bacteria (LAB), primarily *Lactococcus lactis*. Nisin is of considerable interest because of its increasing use as a natural food preservative against a wide range of gram-positive

pathogens. Waste streams from soy-processing (soy whey), cheese-processing (cheese whey), corn-processing, and other food-processing industries serve as an ideal feedstock for nisin production. These waste streams are nutritionally rich, containing protein, carbohydrate, phosphorus, and numerous trace elements needed for the growth of *L. lactis*. The LAB are also able to produce lactic acid during anaerobic fermentation.

1.6.3 Waste Treatment

1.6.3.1 Less Energy Requirement

Aerobic treatments are energy-intensive processes for the removal of organic matter, requiring 0.5–0.75 kWh of aeration energy for every 1 kg of COD removed (van Haandel and Lettinga 1994). Anaerobic treatments need no air/O_2 supply. The aeration energy requirement is calculated based on the following consideration:

For the removal of 1 kg COD, 0.5–0.75 kg O_2 is required during a conventional aerobic treatment process. The higher end of the range can be explained by the O_2 requirement for endogenous respiration. The energy input for the transfer of O_2 into liquid for most aerators is in the order of 1 kWh/kg O_2.

The aeration energy requirement is:

$$= \frac{1 \text{ kWh}}{\text{kg } O_2} \times \frac{0.5\text{–}0.75 \text{ kg } O_2}{\text{kg COD}}$$
$$= 0.5\text{–}0.75 \text{ kWh/kg COD}$$

The reader should bear in mind that the use of anaerobic treatment provides a net financial gain through energy generation from methane gas, as well as savings realized through the elimination of energy inputs required for aeration. The energy balance between anaerobic and aerobic treatment processes is shown in Box 1.3.

1.6.3.2 Less Biomass (Sludge) Generation

Aerobic wastewater treatment process, especially activated sludge process, generates considerable amounts of sludge. Biological oxidation of every kilogram of soluble BOD produces 0.5 kg of sludge as depicted in Fig. 1.5. The cost of treatment and disposal of sludge accounts for 30–60% of the total operational costs in a conventional activated sludge process.

Anaerobic treatment processes, on the other hand, utilize more than 90% of the biodegradable organic matter (COD) for methane production, with only 10% or less converted to biomass, as illustrated in Fig. 1.6.

Sludge production in an anaerobic process, as depicted in Figs 1.5 and 1.6, is <20% of the aerobic treatment process. Furthermore, the anaerobic sludge is well stabilized and needs no further treatment other than dewatering for final disposal.

Box 1.3

Compare the energy balance between aerobic and anaerobic processes for treating a food-processing wastewater with the following characteristics:

Wastewater flow rate:	10 MGD (~37.85 m^3/day)
Wastewater soluble chemical oxygen demand:	10,000 mg/L
Influent temperature:	20°C

The anaerobic reactor will be operated under mesophilic condition (35°C).

Anaerobic process:

(a) Energy generation from methane gas

Methane yield = 0.35 m^3/kg COD at STP

COD loading rate = 10,000 mg/L (10^{-6} kg/10^{-3} m^3) × 37.85 m^3/day
= 378.5 kg COD/day

Total methane generation = 0.35 m^3/kg COD × 378.5 kg COD/day
= 132.5 m^3/day

The net heating energy content of methane = 35,846 kJ/m^3 (at STP)

Thus, the total net energy content of methane = 35,862 kJ/m^3 × 132.5 m^3/day
= 4.75 × 10^6 kJ/day

(b) Energy need for temperature increase from 20 to 35°C

Heat energy needed = 37,850 kg/day × ((35–20)°C) × (4,200 J/kg °C)
= 2.38 × 10^6 kJ/day

Aerobic process:

Aeration energy requirement = (0.75 kWh/kg COD) × (3,600 s/h) × (378.5 kg COD/day) = 1.02 × 10^6 kJ/day

Energy	Anaerobic Treatment	Aerobic Treatment
Methane gas (kJ/day)	4.75 × 10^6	—
Energy for reactor heating (kJ/day)	−2.38 × 10^6	—
Aeration energy (kJ/day)	—	−1.02 × 10^6

Note: Anaerobic treatment provides a net energy gain, whereas aerobic process requires energy input. If the costs of sludge handling, treatment, and disposal are included in this calculation, anaerobic process will result even higher net energy gain.

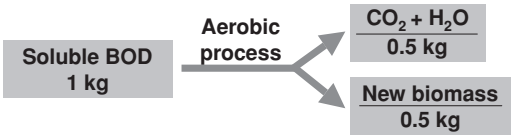

FIG. 1.5. Fate of organic matter in an aerobic process.

1.6.3.3 Less Nutrients (N and P) Requirement

Owing to the lower biomass synthesis rate during the anaerobic process, the nutrient requirements are considerably lower, with the anaerobic process requiring just 20% of the nutrients required for the aerobic process.

1.6.3.4 Higher Volumetric Organic Loading Rate

A higher organic loading rate is not recommended for aerobic treatment processes primarily due to the following:

1. Limited O_2 supply/transfer rate, especially in fixed-film reactors, such as a trickling filter and a rotating biological contactor.
2. Limitation related to the maintenance of high biomass concentrations due to poor settleability, especially in the activated sludge process.

Anaerobic treatment processes are not limited by O_2 transfer capability, and extremely high concentrations of biomass can be maintained in high-rate reactors such as UASB, anaerobic filters, and expanded/fluidized bed reactors. Therefore, loading rates 10–20 times higher for anaerobic treatment processes are possible. The completely mixed anaerobic digesters are the exception in this case, where a maximum concentration of solid/biomass in the reactor is governed by the adequate mixing requirement.

1.6.3.5 Space Considerations

Since a relatively high biomass concentration is maintained in an anaerobic system compared to an aerobic one, large volumetric organic loading rates can be applied.

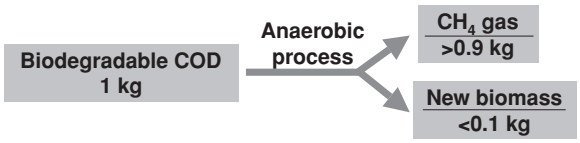

FIG. 1.6. Fate of organic matter in an anaerobic process.

The application of a higher loading rate, therefore, requires a smaller reactor volume, reducing land requirements for the anaerobic treatment units.

1.6.3.6 Ability to Reduce Concentrations of Refractory Organics

With proper acclimation, many of the previously identified refractory organics such as carbon tetrachloride, chloroform, trichloroethane, tetrachloroethane, and polychlorinated biphenyl have been successfully transformed to a lower chlorine functionality by anaerobic microorganisms. These byproducts can then be further degraded by aerobic bacteria to nontoxic end products (Petersen and Samual 1998).

1.6.3.7 Odor Control

Anaerobic treatment largely proceeds in a closed reactor to avoid oxygen contact with the anaerobic biomass and to collect the produced biogas. This prevents the emanation of malodorous compounds, especially hydrogen sulfide.

1.7 Limitations of Anaerobic Process

Although the anaerobic process has many inherent benefits as reported earlier, it is not a panacea for the treatment of all types of wastewaters. Some of the limitations of anaerobic treatment system are outlined here.

1.7.1 Long Start-Up Time

Low sludge yield is deemed one of the major advantages of anaerobic treatment systems. The flip side is that low sludge yields require longer start-up times to attain a given biomass concentration. Start-up times can be reduced by maintaining a higher biomass inventory during the reactor start-up.

1.7.2 Long Recovery Time

If an anaerobic treatment system is subjected to disturbances, due to either biomass washout, toxic substances, or shock loading, it may take a longer time for the system to return to the normal operating condition. However, the extent of such effect could be alleviated by using high-rate anaerobic reactors, such as UASB, anaerobic filter, anaerobic membrane bioreactor (AnMBR), and anaerobic sequential batch reactor, which maintain relatively high SRTs.

1.7.3 Specific Nutrients and Trace Metal Requirements

Anaerobic microorganisms have very specific nutrient requirements. Trace amounts of iron, nickel, and cobalt are essential for optimum growth of methanogens. Municipal wastewater usually contains sufficient amounts of micronutrients and trace metals. However, industrial wastewater often lacks such micronutrients and trace metals, and requires external supplementation. Speece (1996) reported that failure of many anaerobic reactors prior to the 1970s may have been due to lack of understanding of the micronutrient requirement of methanogens.

1.7.4 More Susceptible to Changes in Environmental Conditions

Anaerobic microorganisms, especially methanogens, are more prone to changes in environmental factors such as temperature, pH, and redox potentials. Thus, treatment of low-temperature wastewater requires heating to bring the temperature to an optimum level. Wastewater with a low pH or low alkalinity generation potential, such as dilute wastewater or carbohydrate-rich wastewater, may require alkalinity supplementation to maintain optimum pH. Moreover, an anaerobic reactor operating at the thermophilic temperature is more likely to fail due to changes in environmental conditions than one operating at a mesophilic condition. It is important to note that the degree of susceptibility could be reduced by maintaining a high biomass concentration through the use of high-rate anaerobic reactors.

1.7.5 Treatment of High-Sulfate Wastewater

Anaerobic treatment of high-sulfate wastewater poses considerable challenges to engineers. The presence of sulfate reduces the methane yield due to substrates (such as hydrogen and acetate) diversion to sulfate reduction. In addition, methanogens are inhibited by the presence of sulfide produced by sulfate reducers. The hydrogen sulfide also lowers the quality of the biogas as fuel. Finally, hydrogen sulfide is extremely corrosive gas and produces an objectionable odor. The author has successfully developed an online sulfide control method for the treatment of such wastewaters (Khanal and Huang 2006).

1.7.6 Effluent Quality of Treated Wastewater

The minimum substrate concentration (S_{min}) from which microorganisms are able to generate energy for their growth and maintenance is much higher for an anaerobic treatment system than the aerobic one. Owing to this fact, the anaerobic process may not be able to degrade the organic matter to a level meeting discharge limits required by many environmental agencies for ultimate disposal. Thus, in

many cases, the anaerobically treated effluent may require posttreatment before final disposal.

1.7.7 High Protein- and Nitrogen-Containing Wastewater

Proteins are not completely degraded during anaerobic treatment. The partial degradation of proteins produces amines that impart a foul smell. Little information exists on anaerobic degradation of amines (Verstraete and Vandevivere 1999). Similarly, nitrogen concentrations remain unchanged during anaerobic treatment, as reducing equivalents necessary for denitrification are removed. Thus, in anaerobic treatment, only the forms of nitrogen are changed; that is, organic nitrogen is simply transformed to inorganic ammonia or ammonium, depending on pH. However, recent findings suggest that NH_4^+ can be anaerobically oxidized to N_2 in the presence of NO_2^-, as shown by the following biochemical reaction:

$$NH_4^+ + HNO_2 \rightarrow N_2 + 2H_2O$$

The above process is commonly referred as *an*aerobic *amm*onia *ox*idation (ANAMMOX).

1.7.8 Meticulous Attention

A successful operation of an anaerobic treatment system requires careful attention. Attention is needed especially on the availability of trace metals, nutrients, and alkalinity; avoidance of toxic chemicals, volatile fatty acids accumulation, shock loadings, air exposure, and sludge washout; and maintenance of proper environmental conditions, for example, temperature, pH, and ORP. Such attention is quite often crucial during the start-up phase. Poor attention to these details may lead to complete failure of anaerobic reactors.

Example 1.2

A UASB reactor has been employed to treat leachate from an acidogenic fermentation unit in a two-phase anaerobic digestion of food waste at 20°C. The leachate flow rate is 2,000 L/day with mean soluble COD of 7,000 mg/L. Calculate the maximum methane generation rate in m^3/day. What would be the biogas generation rate at 85% COD removal efficiency with 10% of the COD removed diverted to biomass? The mean methane content of the biogas is 80%.

Solution

Maximum methane generation rate:

The complete degradation of organic matter in the waste could only lead to maximum methane generation, which is also regarded as theoretical methane generation rate.

$$\therefore \text{Total COD removed} = \frac{(7,000 \times 10^{-6})}{(10^{-3})} \times (2,000 \times 10^{-3}) \text{ kg/day}$$
$$= 14 \text{ kg/day}$$

From Eq. (3) in Example 1.1, we have:

1 kg COD produces 0.35 m^3 CH_4 at STP
14 kg COD produces $\sim 0.35 \times 14 = 4.9$ m^3 CH_4/day at STP

$$\text{Total COD removed} = \frac{(7{,}000 \times 10^{-6})}{(10^{-3})} \times (2,000 \times 10^{-3}) \times 0.85 \text{ kg/day}$$
$$= 11.9 \text{ kg/day}$$

As 10% of the removed COD has been utilized for biomass synthesis, the remaining 90% of the removed COD has thus been converted to CH_4 gas.

COD utilized for CH_4 generation $= 11.9 \times 0.9$ kg/day $= 10.71$ kg/day

From Eq. (3) in Example 1.1, we have:

1 kg COD produces 0.35 m^3 CH_4 at STP
10.71 kg COD produces $0.35 \times 10.71 = 3.75$ m^3 CH_4/day at STP
At 20°C, the CH_4 gas generation $= 3.75 \times (293/273) = 4.02$ m^3/day

The biogas generation rate $= 4.02/0.80 = 5.03$ m^3/day

References

Archer, D. B., and Harris, J. E. 1986. Methanogenic bacteria and methane production in various habitats. In *Anaerobic Bacteria in Habitats Other Than Man*, edited by E. M. Barnes and G. C. Mead, pp. 185–223. Blackwell Scientific Publications.

Bushwell, A. M., and Hatfield, W. D. 1938. *Anaerobic Fermentation.* Bulletin No. 32, State Water Supply.

CRC Handbook of Chemistry and Physics, 76th edn (1995–1996), edited by D. R. Lide. CRC Press, Boca Raton, FL, USA.

Formanek, J., Mackie, R., and Blaschek, H. P. 1997. Enhanced butanol production by *Clostridium beijerinckii* BA101 grown in semidefined P2 medium containing 6 percent maltodextrin or glucose. *Appl. Environ. Microbiol.* 63:2306–2310.

Franklin, R. J. 2001. Full-scale experience with anaerobic treatment of industrial wastewater. *Water Sci. Technol.* 44(8):1–6.

Henze, M., and Harremöes, P. 1983. Anaerobic treatment of wastewater in fixed film reactors—a literature review. *Water Sci. Technol.* 15:1–101.

Hungate, R. E. 1967. A roll tube method for cultivation of strict anaerobes. In *Methods in Microbiology*, edited by J. R. Norris and D. W. Ribbons, pp. 117–132. Academic Press, New York, USA.

Imhoff, K. 1938. Sedimentation and digestion in Germany. In *Modern Sewage Disposal*, edited by L. Pearse, p. 47. Lancaster Press, Lancaster, PA, USA.

Kataoka, N., Miya, K., and Kiriyama, K. 1997. Studies on hydrogen production by continuous culture system of hydrogen-producing anaerobic bacteria. *Water Sci. Technol.* 36(6–7):41–47.

Khanal, S. K., Chen, W.-H., Li, L., and Sung, S. 2006. Biohydrogen production in continuous flow reactor using mixed microbial culture. *Water Environ. Res.* 78(2):110–117.

Khanal, S. K., and Huang, J.-C. 2006. Online oxygen control for sulfide oxidation in anaerobic treatment of high sulfate wastewater. *Water Environ. Res.* 78(4):397–408.

Kiene, R. P. 1991. Production and consumption of methane in aquatic systems. In *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethane*, edited by J. E. Rogers and W. B. Wiliams, pp. 111–146. American Society of Microbiology.

Lettinga, G. 2001. Digestion and degradation, air for life. *Water Sci. Technol.* 44(8):1567–1576.

Lettinga. G., Velsen, A. F. M., Hobma, S. W., De Zeeuw, and Klapwijk, A. 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. *Biotechnol. Bioeng.* XXII:699–734.

Liao, B. Q., Kraemer, J. T., and Bagley, D. M. 2006. Anaerobic membrane bioreactors: Applications and research directions. *Crit. Rev. Environ. Sci. Technol.* 36:489–530.

Liu, H., and Fang, H. H. P. 2003. Hydrogen production from wastewater by acidogenic granular sludge. *Water Sci. Technol.* 47(1):153–158.

Long, S., Jones, D. T., and Woods, D. R. 1984. The relationship between sporulation and solvent production in *Clostridium acetobutylicum* P262. *Biotechnol. Lett.* 6(8):529–534.

McCarty, P. L. 2001. The development of anaerobic treatment and its future. *Water Sci. Technol.* 44(8):149–156.

Miller, T. L., and Wolin, M. J. 1995. Bioconversion of cellulose to acetate with pure cultures of ruminococcus albus and a hydrogen-using acetogens. *Appl. Environ. Microbiol.* 61:3832–3835.

Miyake, J., Mao, X. Y., and Kawamura, S. 1984. Photoproduction of hydrogen by a co-culture of a photosynthetic bacterium and *Clostridium butyricum*. *J. Ferment. Technol.* 62:531–535.

Morris, J. G. 1975. The physiology of obligate anaerobiosis. *Adv. Microb. Physiol.* 12:169–246.

Müller, V. 2001. Bacterial fermentation. In *Encyclopedia of Life Sciences*, pp. 1–7. Nature Publishing.

Ostrem, K. 2004. *Greening Waste: Anaerobic Digestion for Treating the Organic Fraction of Municipal Solid Wastes*. M.S. thesis, Earth Resources Engineering, Columbia University.

Parkin, G. F., and Speece, R. E. 1982. Attached versus suspended growth anaerobic reactors: Response to toxic substances. *Water Sci. Technol.* 15(8–9):261–289.

Pavlostathis, S. G., and Girolda-Gomez, E. 1991. Kinetics of anaerobic treatment: A critical review. *Crit. Rev. Environ. Sci. Technol.* 21(5–6):411–490.

Petersen, J. N., and Samual, Y. B. 1998. The effect of oxygen exposure on the methanogenic activity of an anaerobic bacterial consortium. *Environ. Prog.* 17(2):104–110.

Pidaparti, S. 1991. *Sequential Batch Reactor Treatment of Swine Manure at 35°C and 25°C*. Master's thesis, Iowa State University, Ames, IA, USA.

Qureshi, N., Li, X.-L., Hughes, S., Saha, B. C., and Cotta, M. A. 2006. Butanol production from corn fiber xylan using *Clostridium acetobutylicum*. *Biotechnol. Prog.* 22(3):673–680.

Speece, R. E. 1996. *Anaerobic Biotechnology for Industrial Wastewater Treatments*. Archae Press, Nashvillee, TN, USA.

Stander, G. J. 1950. Effluents from fermentation industries. Part IV. A new method for increasing and maintaining efficiency in the anaerobic digestion of fermentation effluents. *Journal of the Institute of Sewage Purification*, Part 4, 447.

Stronach, S. M., Rudd, T., and Lester, J. N. 1986. *Anaerobic Digestion Processes in Industrial Wastewater Treatment.* Springer-Verlag, Berlin, Germany.

Ueno, Y., Kawai, T., Sato, S., Otsuka, S., and Morimoto, M. 1995. Biological production of hydrogen from cellulose by nature anaerobic microflora. *J. Ferment. Bioeng.* 79:395–397.

van Haandel, A. C., and Lettinga, G. 1994. *Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate.* John Wiley & Sons, Chichester, England.

Verstraete, W., and Vandevivere, P. 1999. New and broader applications of anaerobic digestion. *Crit. Rev. Environ. Sci. Technol.* 28(2):151–173.

Young, J. C., and McCarty, P. L. 1969. The anaerobic filter for waste treatment. *J. Water Pollut. Control Fed.* 41(5):R160–R173.