Recent Advances of Anaerobic Digestion for Energy Recovery

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Abstract With climate change looming and the unsustainable supply of fossil fuels, the development of renewable and clean energy is urgently required. An often neglected source of clean energy is the organic material contained in waste and wastewater. Millions of tons of solid organic waste and wastewater are generated everyday worldwide. Instead of consuming energy, anaerobic digestion can be applied to treat the generated waste, thus achieving the objective of waste treatment for public health protection and also recovery of renewable methane for heat and power purposes. In this chapter, the benefits of anaerobic digestion will be introduced followed by a discussion on the mechanism and the typical design principles of anaerobic digestion systems. Some of the recent advancement of anaerobic digestion systems such as membrane bioreactors, fluidized bed reactors and co-digestion systems will be presented in the subsequent sections. The state-of-the-art molecular biological tools to monitor and diagnose the microbiology of anaerobic digestion systems will also be discussed. Lastly, the future outlook of anaerobic digestions will be addressed.

Keywords Anaerobic digestion · Waste treatment · Renewable energy · System design · Technology advancement · Molecular tools

Nomenclature

a.c.	Acoustic chemometrics
ABR	Anaerobic baffled reactor
ADM1	Anaerobic digestion model no. 1
AFBR	Anaerobic fluidized bed reactor

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AnMBR	Anaerobic membrane bioreactors
APBR	Anaerobic packed bed reactor
ASBR	Anaerobic sequencing batch reactor
CSTR	Continuous stirred tank reactor
DGGE	Denaturing gradient gel electrophoresis
EGSB	Expanded granular sludge bed
EN	Electronic nose
EPS	Extracellular polymeric substances
ET	Electronic tongue
FLU	Fluorescence spectroscopic
FW	Food waste
GC	Gas chromatographic
HPLC	High-performance liquid chromatographic
IR	Infrared spectroscopic
IWA	The international water association
LCFA	Long-chain fatty acid
MS	Mass spectrometry
NIR	Near infrared spectroscopic
OFMSW	Organic fraction municipal solid waste
PAT	Process analytical technology
PCR	Polymerase chain reaction
PFR	Plug-flow reactor
qPCR	Quantitative polymerase chain reaction
SHW	Slaughterhouse waste
SRB	Sulfate-reducing bacteria
TPAD	Two-phase anaerobic digester
T-RFLP	Terminal restriction-fragment length polymorphism
UAF	Upflow anaerobic filter
UASB	Upflow anaerobic sludge blanket
UV	Ultraviolet spectroscopic
VFAs	Volatile fatty acids
VIS	Visual spectroscopic
VS	Volatile solid
WWTP	Wastewater treatment plant

1 Introduction

The development of anaerobic digestion technology started in the beginning of the 19th century, although aerobic treatment and tertiary treatment were the mainstream treatment process after the Second World War. Nonetheless, anaerobic digestion of

waste has been rapidly developed since the late 1960s and has been used to treat industrial wastewater as well as domestic wastewater for decades (Stronach et al. 1986; Speece 1996).

From a report on solid waste management conducted by the World Bank in 2013, it was estimated that cities currently generate roughly 1.3 billion tonnes of solid waste per year. With the current urbanization trends, this figure is expected to reach 2.2 billion tonnes per year by 2025, accounting for an increase of 70 % from the current level. Organic waste continues to be the largest component in municipal solid waste. The accumulation of solid organic waste is thought to be reaching critical levels in almost all regions of the world, becoming a pressing matter on public health, environmental quality, quality of life, and economic development. Anaerobic digestion can be considered as one of the oldest technologies for stabilization of wastes. There is now a growing interest in this technology to produce bioenergy as a result of increasing demand for energy coupled with the uncertainty surrounding fossil fuels cost. Bioenergy plays an important role in promoting renewable alternatives which is estimated to be the fourth largest energy resource in the world (Chen and Lee 2014).

Anaerobic digestion of wastes covers many aspects. In this chapter, the fundamental aspects including basic principles, microbiological processes, regime and limitation of anaerobic digestion on energy recovery will be introduced. Operational parameters such as acidic and alkaline conditions, occurrence of inhibitory compounds, together with the effect of temperature, are also considered. The design of anaerobic digestion reactor including fundamental design principles, performance enhancement by pretreatment, phase separation and co-digestion are reviewed, with special attention to technological advancement for improved methane recovery. Finally advanced molecular biological tools for system monitoring and the future outlook of anaerobic digestions will also be discussed.

1.1 Application of Anaerobic Digestion

Anaerobic digestion is applicable for a wide range of materials including municipal, agricultural and industrial wastes, and plant residues (Kalra and Panwar 1986; Gallert et al. 1998; Chen et al. 2008). It has a key role in residual waste stabilization for downstream processing. Recently, two new application areas, namely energy generation and production of value-added chemicals have drawn extensive interests (Batstone and Virdis 2014). One of the possible value-added chemicals is the production of volatile fatty acids (VFAs), which is a critical substrate for microorganism involved in the production of biodegradable plastics (Cai et al. 2009) and bioenergy (Lee et al. 2014).

1.2 Benefits of Anaerobic Digestion

Today, fossil fuels are the dominant energy sources meeting over 80 % of the world's energy demand in 2012 (International Energy Agency, France, 2013). The world energy demand was 5.5×10^{20} J in 2010. It is predicted to increase to 6.6×10^{20} J in 2020 and 8.6×10^{20} J in 2040 (Energy Information Administration, U.S. 2013). Nevertheless, fossil fuels are non-renewable and their reserves are limited. Moreover, tremendous amounts of greenhouse gases have been released from fossil fuel consumption driving the incentives of international communities to develop and utilize renewable energy. Of the renewable energy sources such as solar or wind power production, bioenergy becomes increasingly competitive on its own merits, primarily due to the extensive availability of biomass, biomass production technologies and infrastructure, and biomass being the sole feedstock for liquid fuels production. Biogas, a source of bioenergy, is a product of anaerobic digestion of organic substrates, which is one of the oldest processes used for the waste treatment and stabilization of sludge. The production of biogas through anaerobic digestion offers significant advantages over other processes of waste treatment such as (i) producing less residual solid generation in comparison to aerobic treatment, (ii) generating bioenergy in the form of biogas, (iii) yielding a digestate produced with high bioavailability as an improved fertilizer. The biogas formed is generally composed of 48–65 % methane, 36–41 % carbon dioxide, up to 17 % nitrogen, <1 % oxygen, 32–169 ppm hydrogen sulfide, and trace amounts of other gases (Rasi et al. 2007). Carefully designed and engineered anaerobic digestion of organic waste is therefore environmental beneficial in two ways:

(i) Generating of methane which is a kind of the greenhouse gases, in an enclosed reactor to prevent it from entering the atmosphere directly.
(ii) Displacement of energy from fossil fuels by clean bioenergy.

2 Mechanism of Anaerobic Digestion

2.1 Basic Principles of Anaerobic Digestion

Biogas formation is governed by microorganisms and the metabolic activities in the reactor. Typical anaerobic digestion of organic matters occurs in four steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis in which a consortium of microorganisms including fermentative bacteria, acidogenic bacteria, acetogenic bacteria and methanogens are responsible for biogas production from organic materials such as carbohydrate, oils, fats and proteins. The carbohydrates, protein, oils and fats are firstly hydrolyzed into monomeric sugars, amino acids and fatty acids respectively by extracellular enzymes (amylase, lipase, proteolytic enzymes)

produced by fermentative bacteria. Madigan proposed an approximate chemical formula for the mixture of organic materials as $C_6H_{10}O_4$ (Madigan et al. 2009).

The hydrolysis reaction can be written as:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2 \quad (1)$$

In acidogenesis, the hydrolyzed organic compounds denoted as $C_6H_{12}O_6$ are utilized by acidogenic bacteria or acid forming bacteria, which are a group of fast growing bacteria, and generate volatile fatty acids such as acetic acid, propionic acid, butyric acid and valeric acid as well as carbon dioxide, water and hydrogen. Generation of volatile fatty acids can be expressed as:

$$C_6H_{12}O_6 \Leftrightarrow 2CH_3CH_2OH + 2CO_2 \quad (2)$$

$$C_6H_{12}O_6 + 2H_2 \Leftrightarrow 2CH_3CH_2COOH + 2H_2O \quad (3)$$

In acetogenesis, the VFAs except acetic acid are utilized by acetogenic bacteria, which are a group of slow growing bacteria, to produce acetic acid and hydrogen. The acetogenesis can be written as:

$$CH_3CH_2COOH + 2H_2O \Leftrightarrow CH_3COOH + CO_2 + 3H_2 \quad (4)$$

Finally, methanogens utilize acetic acid, ethanol, methanol, hydrogen and carbon dioxide to form methane gas in methanogenesis. Methanogens utilizing acetic acid to produce methane are known as acetotroph while those utilizing hydrogen and carbon dioxide are known as hydrogenotroph. About 70 % of the methane are produced stoichiometrically via the acetate pathway and 30 % are produced via the hydrogen pathway (Siegrist et al. 2002; Madigan et al. 2009). The pathways for methanogenesis can be expressed as:

$$2CH_3CH_2OH + CO_2 \Leftrightarrow 2CH_3COOH + CH_4 \quad (5)$$

$$CH_3COOH + CO_2 \Leftrightarrow CH_4 + 2CO_2 \quad (6)$$

$$CH_3OH + H_2 \Leftrightarrow CH_4 + 2CO_2 \quad (7)$$

$$CO_2 + 4H_2 \Leftrightarrow CH_4 + 2H_2O \quad (8)$$

Figure 1 shows a scheme of the anaerobic digestion pathway, from long-chain organic compounds including proteins, carbohydrates and lipids to the final products, i.e., methane and carbon dioxide (Gujer and Zehnder 1983; Siegrist et al. 2002).

Methane production from anaerobic digestion process in waste treatment is generally limited by the rate of hydrolysis of suspended organic matters. Efficient pretreatment can enhance the ability of bacteria to access the suspended substrate and increase the methane yield. The objective of implementing different types of pretreatment in anaerobic digestion is to enhance the bioavailability of particular

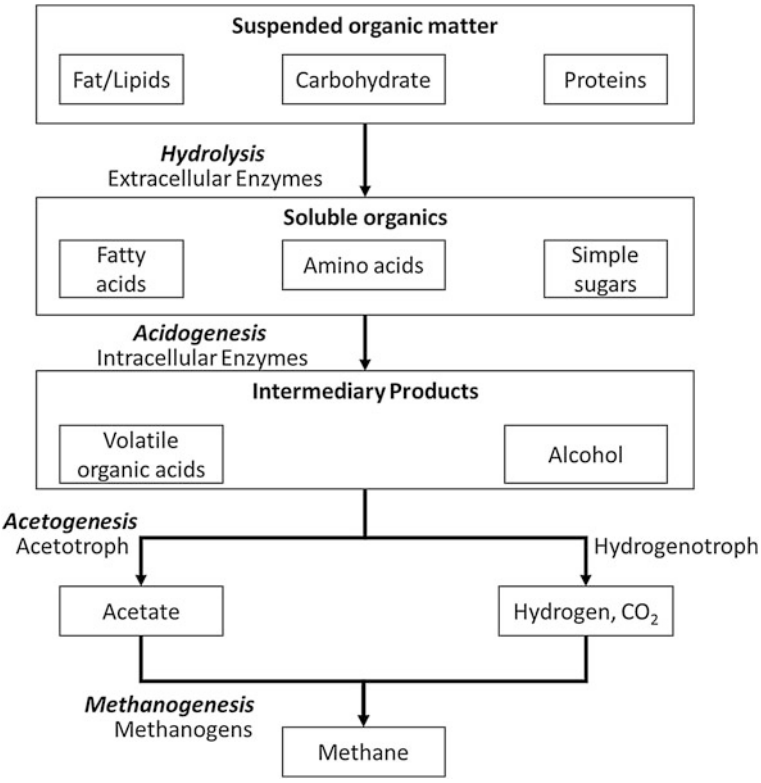

Fig. 1 Scheme of biodegradation steps of complex matter in anaerobic digestion

substrates so that enzymes can more efficiently hydrolyze the substrate. A number of pretreatment methods are discussed in Sect. 4.1. The overall organic matter stabilization can be improved through improvement from pretreatment technology.

Anaerobic digestion of waste is capable of recovering energy from a wide range of feedstock from different sources such as agricultural sector, industrial sector and municipal sector which needs to be (i) biodegradable, (ii) non-woody with low proportion of lignocellulosic material, and (iii) balanced in macro and micro nutrients (Kothari et al. 2014). Therefore, feedstock can range from readily biodegradable wastewater to complex high-solid waste. In order to obtain a higher yield of biogas, anaerobic co-digestion treatment, the simultaneous digestion of two or more substrates, is a feasible option to overcome the drawbacks of single substrate digestion and to improve the process efficiency. Figure 2 shows an overview of various feedstock from different sources. The choice of feedstock is influenced by various interrelated process factors such as reactor design and operation, quality of products, source and mass flux, economic considerations, bacterial physiology and specific purpose (Steffen et al. 2012).

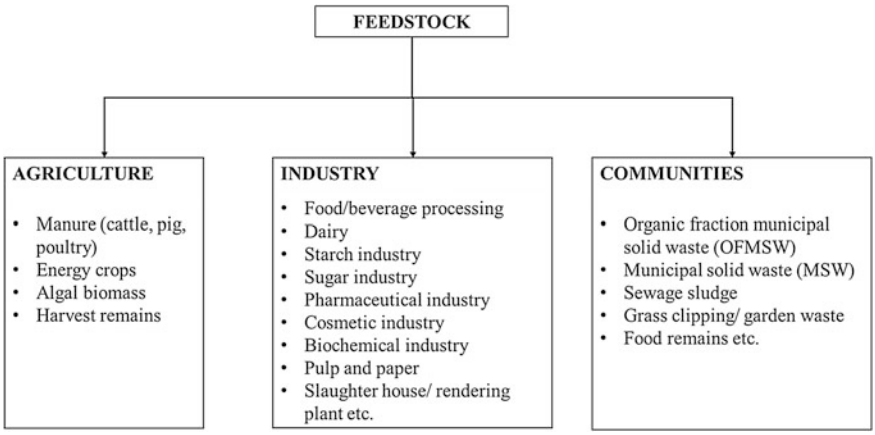

Fig. 2 Categorization of various feedstock from different sources

It should be noted that phase separation can be used for performance enhancement by optimizing the reactor configuration for the different stages of anaerobic processes in separate tanks whereby the conditions are optimized for specific groups of bacteria. The improvement of energy recovery by co-digestion and phase separation in anaerobic digestion are discussed in Sect. 4.2. Anaerobic digestion involves different groups of microorganisms which are highly sensitive to the environment. The operating parameters affecting anaerobic digestion are discussed in detail in Sect. 2.3.

Anaerobic digestion of waste serves the dual purpose of both energy recovery and waste management. Recently, it has been widely applied to municipal solid waste (MSW) to generate energy. Table 1 summarizes the high yield of methane production from anaerobic digestion of municipal solid waste. It should be noted that MSW is classified as a heterogeneous material in which the composition varies widely according to regional differences, climate, extent of recycling, collection frequency, season, cultural practices. Considering the biodegradability of OFMSW, the potential of anaerobic digestion of OFMSW are high as well as the methane yield, and co-digestion of MSW with sewage sludge is also becoming increasingly attractive.

2.2 Microorganism and Microbiological Process

A wide variety of microbial communities have been reported to be involved in the anaerobic digestion process (Fricke et al. 2007; Fantozzi and Buratti 2009). The microbial population distribution is highly dependent on the substrate and product concentration as well as on environmental conditions such as pH, temperature, hydrogen concentration etc. However, knowledge of the microorganism and

Table 1 Literature data of biomass with high yields of methane from municipal solid waste feeds

Feed	Bioreactor	Temp (°C)	HRT (days)	OLR (kg VSm^{-3} d^{-1})	CH_4 yield (m^3 kg^{-1} VS)	CH_4 PR (m^3 m^{-3} d^{-1})	VS_r (%)	Reference
HS-OF MSW 3–5.6 % TS VS = 82–87 % TS	CSTR Laboratory Plant	35	14–20	4	0.430	1.70	NR	Pauss et al. (1984)
HS-OF MSW 6.4 % TS VS = 89.9 % TS	CSTR 3 m^3	33–37	9–25	2.1–6.9	0.390	0.82–2.02	63–69	Cecchi et al. (1986)
SS-OF MSW VS = 88 % TS (Without paper, wood, plastic)	CSTR 3 m^3	35	25	2.1	0.399	NR	69	Mata-Alvarez et al. (1990)
SC-OF MSW 80:20 (% TS basis) VS = 88 % TS	CSTR 2.2 m^3	35	14.5	3.9	0.403	NR	71	Mata-Alvarez et al. (1990)
MS-OFMSW 35 % TS VS = 58.6 % TS	Pilot Plant 500 m^3 Valorga Process	37	15	13.7	0.230	NR	45	Valorga (1985)
Pre-composted MS-OFMSW VS = 43 % TS	CSTR 3 m^3	35	16.2	4.1	0.145	NR	27	Mata-Alvarez et al. (1990)

Remark

Temp Temperature, *HRT* Hydraulic retention time, *OLR* Organic loading rate, VS_a VS added, CH_4 *PR* Methane production rate, VS_r VS reduction
MS-OFMSW Mechanically sorted organic fraction of municipal solid waste, *HS-OFMSW* Hand sorted organic fraction of MSW
SS-OFMSW Source sorted organic fraction of MSW, *SC-OFMSW* Organic fraction of MSW from a separated collection, *NR* Not reported

microbiological processes involved is revealed gradually through the use of modern molecular techniques which complement traditional cultivation process and microscopic identification techniques (Merkel et al. 1999). The development of the modern molecular techniques on microorganisms in anaerobic digestion will be discussed in Sect. 5. The types of microorganism in the four distinct stages of anaerobic digestion are shown in Tables 2 and 3.

2.3 Operational Parameters Affecting Anaerobic Digestion

The ultimate methane production is influenced by a number of operational parameters in the anaerobic digestion reactor such as temperature, type of feedstock, pH level, retention time, C/N ratio, VFA concentration etc. Maximum methane production takes place when optimum range of these operational parameters is chosen. The optimum range of these parameters is reviewed in this section.

2.3.1 Temperature

Microorganisms in anaerobic digestion are very sensitive to temperature changes which affect hydrogen and methane production, and the decomposition of organic materials. There are three possible ranges of temperature in which the process can be carried out (psychrophilic, mesophilic and thermophilic) as shown in Table 4. Chae et al. studied the effects of temperature and temperature shock on the biogas yield from anaerobic digestion (Chae et al. 2008) and reported that methane content increased with increasing digestion temperature, but only to a small extent. Temperature shocks from 35 °C to 30 °C and 30 °C to 32 °C led to a drop in the biogas production rate. No lasting damage was observed from the digestion performance after recovery.

Thermophilic anaerobic digesters often manifest chronically higher VFAs concentration than those found in mesophilic anaerobic digesters (Kim et al. 2002). Therefore, the optimal conditions for anaerobic digestion to reduce energy consumption may be thermophilic hydrolysis/acidogenesis and mesophilic methanogeneis which is consistent with a two-phase anaerobic digestion process. This arrangement uses a mesophilic reactor as a polishing stage, eliminating the drawbacks of the thermophilic process. However, thermophilic conditions are applied in most of the large-scale centralized biogas co-digesters (Kothari et al. 2014). Digestion of organic urban wastes using thermophilic and mesophilic processes has also been studied by researchers and they found that thermophilic process is a more realistic and viable option as the added amount of heat required for thermophilic operations can be offset by the higher gas production yields and rates (Parkin and Owen 1986; De Baere 2000; Kim et al. 2002; Kuo and Lu 2004).

Table 2 Characteristics of typical hydrolytic bacteria and acidogenic bacteria in anaerobic digestion (Amani et al. 2010)

Type	Substrates	Products	Typical species	Reference
Hydrolytic bacteria	Proteins	Amino acids, sugars	*Clostridium sp.,* *Proteus vulgaris,* *Peptococcus sp.,* *Bacteroides sp.,* *Bacillus sp.,* *Vibrio sp.,*	Kim et al. (2009) Fang et al. (2009) Westlake et al. (1967) Ochoa-Reparaz et al. (2008) Chang et al. (2008) Parvez et al. (2008)
	Carbohydrates	Sugars	*Clostridium sp.,* *Acetivibrio cellulolyiticus,* *Staphylococcus sp.,* *Bacteroides sp.,*	Chong et al. (2009) Khan (1980) Ziagova et al. (2009)
	Lipids	Higher fatty acids, alcohols, amino acids	*Clostridium sp.,* *Micrococcus sp.,*	Jo et al. (2008) Tuleva et al. (2009)
		Sugars	*Staphylococcus sp.,*	
Acidogenic bacteria	Amino acids	Valerate, isovalerate, propionate, butyrate	*Lactobacillus sp., Eschericia coli*	Kim et al. (2009) Li et al. (2003)
		Acetate, H_2, Higher fatty acids	*Staphylococcus sp.,* *Bacillus sp.,* *Pseudomonas sp.,* *Micrococcus sp.,* *Eubacterium limosum,* *Clostridium sp.,* *Zymomonas mobiliz*	Kalyani et al. (2009) Hur and Rafii (2000) Fritsch et al. (2008) Wilkins (2009)
	Sugars	CO_2, H_2, formate, acetate, butyrate	*Eubacterium sp.,*	Kalyani et al. (2009)
		CO_2, H_2, formate, acetate, ethanol, lactate	*Eschericia coli*	
		Formate, acetate, ethanol, lactate	*Bifidobacterium sp.,*	Cheikhyoussef et al. (2009)
		Acetate	*Acetobacterium sp.,*	Bainotti et al. (1996)
	Fatty acids	Valerate, isovalerate, propionate, butyrate, acetate, H_2	*Clostridium sp.,*	
	Alcohols		*Syntrophomonas wolfei*	

Table 3 Characteristics of typical acetogenic bacteria and methanogens in anaerobic digestion (Amani et al. 2010)

				Optimum growth condition	
Type	Substrates	Products	Typical species	pH	Temp. (°C)
Acetogenic bacteria	Butyrate	Acetate	*Syntrophobacter wolinii*	NR	35–40
		H_2/CO_2, formate	*S. fumaroxidans*	NR	35–40
	Propionate	H_2/CO_2, formate	*Syntrophomonas wolfei*, *Pelotomaculum thermopropionicum*, *P. schinkii*	NR	35–40 50–60† 32–37
		Butyrate, acetate	*Smithella propionica*	NR	35–40
	H_2, CO_2	Acetate	*Clostridium aceticum*	NR	30–37
Methanogens	Acetate	CH_4, CO_2	*Methanothrix soehngenii*, *Methanosaeta concilii*, *Methanosarcina acetivorans*	7.4–7.8 7.1–7.5 6.5–7.5	35–40 35–40 35–40
	H_2, CO_2	CH_4	*Methanobacterium bryantii*, *M. thermoautotrophicum*, *M. alcaliphilum*, *Methanobrevibacter arboriphilus*, *Methanococus jannaschii*, *Methanolacinia paynteri*, *Methanospirillum hungatei*, *Methnanoplanus endosymbiosus*, *M. olentangyi*, *Methanothermus fervidus*	6.9–7.2 7.2–7.6 8.1–9.1‡ 7.8–8.0 5.0–7.0 6.6–7.2 NR 6.6–7.2 NR 6.5	37–39 65–70† 37 30–37 83§ 40 30–37 40 30–37 83§

(continued)

Table 3 (continued)

				Optimum growth condition	
	Formate, H_2, CO_2	CH_4, CO_2	*Methanobacterium formicicum,*	6.6–7.8	37–45
			Methanobrevibacter smithii,	7.0	37–39
			M. ruminantium,	7.0	37–39
			Methanococcus voltae,	6.5–8.0	35–40
			M. deltae,	NR	37
			M. maripaludis,	6.5–8.0	35–40
			M. thermolithoautotrophicus,	6.5–8.0	65[†]
			Methanoplanus limicola,	7.0	40
			Methanogenium cariaci,	6.2–6.6	20–25
			M. marisnigri,	6.8–7.3	20–25
			M. olentangyi,	NR	37
			M. tatii,	7.0	37–40
			M. thermophilicum,	6.5–7.2	55–60[†]
			M. bourgense,	6.3–6.8	35–42
			Methanocorpusculum aggregans	6.4–7.2	35–37

Remarks [†]Theromophilic; [‡]Alkaliphilic; [§]Hyper-thermophilic
NR Not reported

Table 4 Classification of anaerobic digestion by operating temperature

Type	Operating temperature	Reference
Psychrophilic (or cryophilic)	10–20 °C	Sutter and Wellinger (1987)
Mesophilic	30–40 °C	Bolzonella et al. (2005), Zhang et al. (2014)
Thermophilic	55–70 °C	Buhr and Andrews (1977)
Ambient/seasonal temperature	Temperature changes in the surrounding environment (Typically 15–25 °C)	Yusuf and Ify (2011)
Hyperthermophilic	65–70 °C	Lee et al. (2009)

2.3.2 Acidic and Alkaline Conditions

Methanogens are extremely sensitive to pH, while fermentative microorganisms are generally less sensitive and can function in a wider range of pH between 4.0 and 8.5 (Hwang et al. 2004). A range of pH values suitable for anaerobic digestion has been reported by various researchers, but the optimal pH for methanogenesis has been found to be around 7.0 (Huber et al. 1982; Yang and Okos 1987; Khalid et al. 2011). The growth rate of fermentative bacteria is faster than those of methanogens, leading to the accumulation of acids in the digesters. Two main strategies for rectifying the low pH due to acid accumulation: (i) stopping the feed and allowing enough time for the methanogenic population to reduce the concentration of VFAs inside the system; and (ii) addition of bases to raise pH and provide additional buffering capacity. Another strategy suggested by Shah is that drastic reduction of pH could be prevented by the addition of another feed at a suitable ratio with the main feed (Shah et al. 2015) as practices in co-digestion.

2.3.3 Inhibitory Compounds

It is desirable to control inhibitory or toxic materials to achieve higher efficiency or a more economical operation of anaerobic digestion process performed by removal of toxic materials from waste stream or by dilution of the waste to below the toxicity threshold in the systems. Precipitation is commonly employed to remove the toxic materials from the systems.

Ammonia toxicity

Ammonia is an essential nutrient for the growth of microorganisms involved in anaerobic digestion but also acts as an inhibitor at high concentration. Fermentation of nitrogen-containing materials such as urea and proteins releases ammonia-nitrogen largely in the ionized form (NH_4^+). The toxic unionized form (NH_3)

increases with increasing pH in the system as the *pKa* value of ammonia is 9.3 (Koster and Lettinga 1984). Free ammonia is more toxic to methanogens than ionized ammonium (NH_4^+) because it is more readily diffusible through the cell membrane, causing proton imbalance, and/or potassium (K^+) deficiency, while ionized ammonium may just inhibit the methane synthesizing enzyme directly (Gerardi 2006). Another reason why ionized form of ammonia is less inhibitory than the free form is that the hydroxide ion produced can react with carbon dioxide to form bicarbonate, which increases the buffering capacity of the anaerobic reactor, making the process less susceptible to pH fluctuations when the production rates of acetogenic bacteria and methanogens differ.

Sulfide toxicity

A number of industrial wastes from petrochemical plants, tanneries, viscose rayon factories and coal gasification for electricity production generate sulfate-containing waste streams. Sulfidogens or sulfate-reducing bacteria (SRB) play a significant role in anaerobic digestion, which reduce sulfate to sulfide in the reactor under certain condition. Sulfide generated may be inhibitory to anaerobic digestion by (i) inhibiting methanogens, (ii) reducing rate of methanogenesis, and (iii) decreasing the quantity of methane produced by competing for the available carbon and/or hydrogen source. Inhibitory effect of sulfide in anaerobic digestion can be separated into two parts: competition for substrates between sulfate-reducing bacteria and methanogens directly and inhibition of methane formation by sulfide ions in the system. Competition between sulfate-reducing bacteria and methanogens in sulfate-containing waste streams for acetate as their common primary substrate can significantly affect the methane production efficiency.

The optimum conditions for anaerobic metabolic activity proposed by researchers are summarized in Table 5.

Table 5 Optimum conditions for anaerobic metabolic activity

Parameters	Optimum conditions	Reference
Temperature	Mesophilic range (35–40 °C) Thermophilic (50–65 °C)	Van Haandel and Lettinga (1994) Arsova (2010)
pH	6.3–7.8	Wang et al. (2012)
Carbon to nitrogen ratio (C/N ratio)	25–30	Ghosh and Pohland (1974)
Volatile fatty acid (VFA)	2000–3000 mg/L	Eastman and Ferguson (1981)
Organic loading rate (OLR) and nutrient concentration	Varies according to the substrate and inoculum	–

3 Design of Anaerobic Digestion Processes

Anaerobic digestion composes a broad family of processes which can be classified according to:

(a) their feedstock input mode: batch and continuous processes;
(b) single-step, double- or multiple steps; and
(c) geometry of the main treatment unit: vertical and horizontal unit.

3.1 Design Principles of Anaerobic Digestion System

Reactors of anaerobic digestion often operates under heterogeneous system whereby three phases namely: solid phase (sludge), liquid phase (wastewater) and gaseous phase (biogas) present simultaneously. The oldest and simplest type of anaerobic digester is not equipped with any mixing or heating, thus a long digestion period of 30–60 days is required. Some degree of natural mixing occurs inside the reaction tank due to bubbling of gas generated and thermal convection currents created from the digestion processes. Due to the lack of proper mixing, stratification usually occurs in four zones: (i) scum layer, (ii) supernatant layer, (iii) layer of digesting biosolid and (iv) layer of digested biosolid. A schematic diagram of this type of anaerobic digesters without mixing is given in Fig. 3. The accumulated biosolid at the bottom of the reaction tank is periodically discarded.

Different variations of anaerobic digestion have evolved over the years to improve the degradation performance including high-rate digestion and phase separated digestion. Figure 4 shows the schematic diagram of a typical high-rate digestion. The characteristic features of high-rate anaerobic digestion including

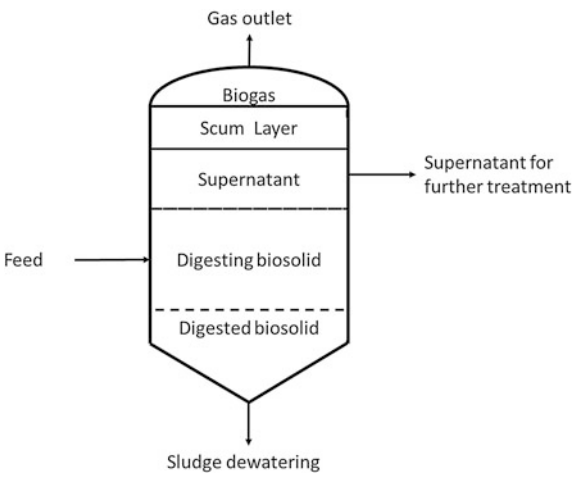

Fig. 3 Schematic diagram of low rate anaerobic digestion without mixing and heating

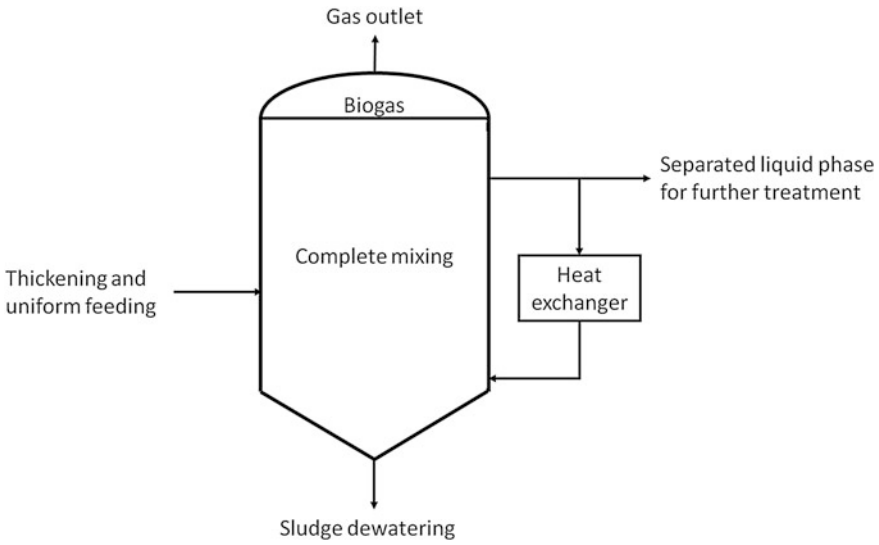

Fig. 4 Schematic diagram of high-rate anaerobic digestion

heating, auxiliary mixing, thickening and uniform feeding are introduced to the reactor design to create a uniform environment for microbial growth in order to improve stability and efficiency of biodegradation processes.

Determination of reaction tank volume is the first important consideration in designing an anaerobic digestion system. Various methods have been used for sizing of digestion tank including (i) per capita basis, (ii) solids loading, (iii) solids retention time, (iv) volatile solids destruction and (v) gas production (Turovskiy and Mathai 2006).

Anaerobic digestion reactors are mostly cylindrical or egg shaped. Vertical cylindrical digestion tanks are widely used in the United States, with diameter from 6 to 38 m, typically made of concrete although steel tank design are also common in smaller tank size. Tank floors are usually conical with slopes of varies between 1:3 and 1:6 to facilitate the accumulation and withdrawal of digested sludge from the low point in the centre of the tank. Egg-shaped digestion tanks are originated in Germany to eliminate grit accumulation by the steeply sloped bottom and to avoid scum accumulation by small liquid surface area at the top.

Another variation in the design of anaerobic digestion processes is on the solid content in the reactors. Content of solid in the reactor affects the reactor volume and treatment process. The percentage of total solids in the digester can be categorized into low solid content (LS) (<15 %), medium solid content (MS) (15–20 %) and high solid content (HS) (20–40 %) (Fernández et al. 2008; Cao and Pawlowski 2012; Raposo et al. 2012). Wet systems are low solid AD which are applied to liquid waste streams with total solids content typically less than 15 % while dry systems are high solid AD which handle stackable feedstock with total solid contents typically higher than 30 % without any addition of external liquids.

Single-stage low solids (SSLS) wet anaerobic digestion processes have been used for decades in the stabilization of sludge. The feedstock is conditioned to the appropriate solid content (10–15 %) by adding process water in the wet anaerobic digestion reactor with internal mixing to obtain homogeneity. The predominant reactor of wet anaerobic digestion is continuously stirred tank reactor (CSTR) with mechanical stirring to avoid stratification of the substrate inside the reactor. Short circuiting may be experienced in CSTR. Large amount of water consumption is needed to be mixed with the feedstock to obtain the low solid content, which can be acquired from treated supernatant.

High solid anaerobic digestion has been claimed to be more advantageous than low solid anaerobic digestion for several reasons, such as smaller reactor volumes, lower energy requirement for heating, higher biogas yield from undiluted wastes and less material handling (Duan et al. 2012). However, dry streams may suffer some drawbacks. They usually require proper preconditioning of the feedstock material, including substrate treatment and mixing with structure material, and special loading and unloading techniques. The content inside the digester may not be totally mixed, leading to lower methane yields than the wet systems. Different types of single-stage high solids (SSHS) dry anaerobic digestion processes have been developed and are in use commercially in Europe such as Dranco, Kompogas, and Valorga processes. The Dranco process developed in Belgium is a true dry-process for treatment of organic fraction of MSW, which is characterized by its design of feeding from the top, collection of digested biosolid at the bottom of the reactor and no internal mixing mechanism with total solid content at about 30–40 % (Cho et al. 2013). The Kompogas process developed in Switzerland takes place in plug flow in a horizontally cylindrical steel tank with total solid content at about 23 % (Hartmann and Ahring 2006). The Valorga process developed in France is a semi-dry mesophilic process in which mixing of waste with recycled process water takes place with total solid content of 30 % (Fernández et al. 2008). In additional to the improvement of reactor tank design in anaerobic digestion, the technological advancement in process design such as pretreatment, phase separation, co-digestion and biomass immobilization are discussed in Sect. 4.

4 Technology Advancement for Improved Methane Recovery

4.1 Pretreatment for Digestion Enhancement

Most researchers reported that the rate-limiting step for complex organic substrates is the hydrolysis step in an anaerobic digestion process (Valo et al. 2004; Izumi et al. 2010; Rafique et al. 2010; Bordeleau and Droste 2011; Fdez-Guelfo et al. 2011; Ma et al. 2011). Different pretreatments are utilized for anaerobic digestion such as mechanical (ultrasound, high pressure and lysis), thermal (<100 °C, >100 °C), chemical (ozonation, alkali, acids), microwave, ultrasonic, electric pulses, wet

oxidation, freeze/thaw and biological treatment to increase the bioavailability of complex organic matters to microbes. Pretreatment methods to improve performance of anaerobic digestion have been the focus of many research studies over the last 30 years (Holm-Nielsen et al. 2009; Pilli et al. 2011) and the improvement of anaerobic digestion in terms of increasing methane generation and solid reduction are well known advantages of pretreatments.

The pretreatment effects are complex and generally linked to substrate characteristics and pretreatment mechanisms. Carlsson et al. have examined the effect of substrate pretreatment on anaerobic digestion (Carlsson et al. 2012), namely particle size reduction, solubilization, formation of refractory compounds, biodegradability enhancement and loss of organic materials for different substrate categories including wastewater treatment plant (WWTP) residues, organic waste from households, energy crops/plant residues, waste from food industry and manure. It is reported that thermal and ultrasonic pretreatments are predominantly applied on anaerobic digestion of WWTP residues, chemical and thermal pretreatment have been applied to less frequently studied substrates such as energy crops/harvesting residues, organic waste from food industry and manure, whereas mechanical and thermal pretreatments are commonly applied to organic fraction of municipal solid waste (OFMSW). Another focus on pretreatment methods is their ability to enhance anaerobic digestion process in terms of efficiency, energy balance, environmental sustainability as well as capital, operational and maintenance costs (Ariunbaatar et al. 2014).

4.1.1 Mechanical Pretreatment

Mechanical pretreatment is used to reduce both the particle size and crystallinity of lignocellulosic materials through a combination of chipping, grinding or milling processes, in order to increase the specific surface area and reduce the degree of polymerization of substrate (Sun and Cheng 2002). Smaller particles increase the surface area available to the microorganisms, resulting in increased bioavailability to bacteria and improved anaerobic degradability. Particle size reduction can accelerate the hydrolysis and acidogenesis processes as well as the production of soluble organic materials such as VFAs, resulting in a higher organic loading in the anaerobic digester. However excessive size reduction may result in higher solubilization and in turn excessive VFAs accumulation, leading to a decrease in methane production. The power requirement of mechanical pretreatment is relatively high depending on the final particle size and the substrate characteristics. In particular, the recalcitrant nature of cell walls of green waste makes mechanical pretreatment energy intensive (Izumi et al. 2010).

Ultrasonic disintegration is one type of mechanical pretreatments in which ultrasonic treatment acts to disrupt the cell structure and floc matrix of the substrate. There are two key mechanisms associated with ultrasonic treatment: (i) cavitation, which is favoured at a low frequency, and (ii) chemical reactions due to the formation of free radicals at a high frequency (Carrère et al. 2010). According to the studies by Show and co-workers, the optimal range of solid content for sonication

lies between 2.3 and 3.2 % TS (Show et al. 2007). If the solid concentration of feedstock is too high, increased viscosity hinders cavitation bubble formation. The threshold specific energy ranges from 1000 to 16,000 kJ kg^{-1} TS with sludge as substrate although biogas production increases with energy input (Salsabil et al. 2009).

4.1.2 Thermal-Alkaline Solubilization Pretreatment

Alkaline treatment is one commonly used chemical treatment in anaerobic digestion in which there are two major reactions: (i) solvation and saphonication inducing swelling of solids to increase the specific surface area of the substrate; followed by (ii) simultaneous reactions of saponification and neutralization of various acids formed by degradation of the particulates leading to an increase in COD solubilization (Kim et al. 2003). Alkaline treatment is relatively effective in sludge solubilization, with the order of efficacy being NaOH > KOH > $Mg(OH)_2$ and $Ca(OH)_2$. Alkaline pretreatment by sodium hydroxide at relatively low dosage levels is effective in solubilizing municipal waste activated sludge at ambient temperature. Mouneimne et al. demonstrated that high concentration of Na^+ and K^+ may cause subsequent inhibition of anaerobic digestion (Mouneimne et al. 2003). Alkaline treatment is normally combined with thermal treatment. Waste solubilization and biodegradability improve with alkali dosage and temperature (Kim et al. 2003) Thermal-alkaline pretreatment usually proceeds at temperature lower than thermal hydrolysis alone and could result in a higher biogas production with a higher methane content.

4.1.3 Oxidative Pretreatment (Ozonation)

Oxidative pretreatment by ozonation is another chemical pretreatment method. It is the most widely used chemical method which does not lead to accumulation of salt and no chemical residues remain in the systems as compared to other chemical pretreatment methods (Carrère et al. 2010). Ozone is a strong oxidant which decomposes into radicals and reacts with organic substrates directly and indirectly. The direct reaction depends on the structure of the reactant whereas the indirect reaction is based on the hydroxyl radicals. Several studies have shown an optimal range of ozone dosage for the enhancement of anaerobic biodegradability such as 0.1 g O_3 g^{-1} COD (Weemaes et al. 2000), 0.2 g O_3 g^{-1} TSS (Yeom et al. 2002), and 0.15 g O_3 g^{-1} TSS (Bougrier et al. 2007). Ozonation has been combined with anaerobic digestion as a pretreatment (Weemaes et al. 2000; Yeom et al. 2002; Bougrier et al. 2007) or post-treatment with recycling back to the anaerobic digester (Battimelli et al. 2003; Goel et al. 2003).

Ariunbaatar et al. compared the efficiency of various pretreatment methods for enhancing the anaerobic digestion of OFMSW and food waste (FW) in terms of biogas production, VS reduction and COD solubilization as listed in Table 6.

Table 6 Efficiency of pretreatment for enhancing anaerobic digestion of OFMSW and FW

				Result			
Substrate	Pretreatment type	Pretreatment condition	Type of AD system	COD solubilization	VS reduction	CH_4 generation	Reference
OFMSW (source-sorted)	Mechanical	Disc screen	Thermophilic batch		80.6 %	338 ml CH_4/g VS	Davidson et al. (2007)
	Mechanical	Screw press	Thermophilic batch		63.2 %	354 ml CH_4/g VS	
	Mechanical	Shredder with magnetic separation	Thermophilic batch		63 %	289 ml CH_4/g VS	
OFMSW (source-sorted)	Mechanical	Disc screen	Thermophilic batch			428 ml CH_4/g VS	Hansen et al. (2007)
	Mechanical	Screw press	Thermophilic batch			461 ml CH_4/g VS	
	Mechanical	Shredder with magnetic separation	Thermophilic batch			487 ml CH_4/g VS	
OFMSW	Mechanical	Rotary drum	Thermophilic batch			457–557 ml CH_4/g VS	Zhu et al. (2009)
OFMSW (Synthetic)	Thermal hydrolysis process (THP)	Thermophilic pre-hydrolysis	Thermophilic continuous (2-stage)	81.5 % removal	95.7 %	2 times higher biogas production	Ueno et al. (2007)
OFMSW	Chemical	Alkaline	NA	11.5 % higher		150 ml CH_4/g VS	López Torres and Espinosa Lloréns (2008)

(continued)

Table 6 (continued)

				Result			
OFMSW	Thermal hydrolysis process (THP)	Pre-hydrolysis at 55 °C	Mesophilic continuous		47.5–71.6 %	299–418 ml CH_4/g VS	Schmidt and Ahring (1993)
OFMSW	Ultrasonic	Sonication at 20 kHz for 30-60 min	Mesophilic batch	60 % higher		24 % higher than untreated	Cesaro and Belgiorno (2014)
OFMSW (Synthetic)	Thermal hydrolysis process (THP)	Mesophilic and thermophilic pre-hydrolysis	Mesophilic and thermophilic continuous (2-stage)			341 ml CH_4/g VS	Escamilla-Alvarado et al. (2012)
OFMSW and corn stalk	Freeze/thaw	Freeze explosion followed by thermophilic pre-hydrolysis	Thermophilic			520 ml CH_4/g VS, 104 ml H_2/g VS	Kvesitadze et al. (2012)
FW	Thermal hydrolysis process (THP)	Semi-aerobic and anaerobic pre-hydrolysis	Mesophilic continuous	95 % removal		500 ml CH_4/g VS	Kim et al. (2000)
FW	Thermal hydrolysis process (THP)	Thermophilic pre-hydrolysis	Mesophilic		61.3 %	280 ml CH_4/g VS	Kim et al. (2004)
FW	Thermal hydrolysis process (THP)	Mesophilic pre-hydrolysis	Mesophilic continuous (2-stage)			9–13 % higher than mesophilic and thermophilic AD respectively	Verrier et al. (1987)
FW	Thermal hydrolysis process (THP)	Mesophilic pre-hydrolysis	Mesophilic continuous			546 ml CH_4/g VS, 65 ml H_2/g VS	Wang and Zhao (2009)

(continued)

Table 6 (continued)

				Result			
FW	Thermal hydrolysis process (THP)	Thermophilic pre-hydrolysis	Mesophilic continuous			464 ml CH_4/g VS, 205 ml H_2/g VS	Chu et al. (2008)
FW with polyactide	Thermal hydrolysis process (THP)	Hyper-thermophilic/thermophilic pre-hydrolysis	Thermophilic—Temperature phased AD			15–18 % higher than conventional thermophilic digester	Wang et al. (2011)
FW	Electric pulse	400 pulses with electroporation	Mesophilic continuous			20–40 % higher than untreated	
FW	Freeze/thaw	Frozen/thaw and pre-hydrolysis for 7 days	Mesophilic continuous	10 % higher		23.7 % higher than untreated	Stabnikova et al. (2008)
	Freeze/thaw	Frozen/thaw and pre-hydrolysis for 12 days	Mesophilic continuous	4 % higher		8.5 % higher than untreated	Carlsson and Anox Kaldnes(2008)

4.2 *Phase Separation and Co-digestion of Anaerobic Digestion*

4.2.1 Phase Separation

The prospects of phased anaerobic digestion of waste are extremely promising to achieve increased stability, higher loading capacities and greater process efficiencies than single-stage systems (Shuizhou and Zhou 2005). The advantages of two-phase anaerobic digestion (TPAD) have been extensively documented (Ghosh and Pohland 1974; Ghosh et al. 1985). Efficiency improvement of anaerobic digestion can be brought about by either digester design modification or advanced operating techniques.

Anaerobic digestion occurs in four steps, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis as discussed above. The degradation process of organic matters in anaerobic digestion can be separated into two phases, (i) the "acid fermentation" phase or acidogenesis, leading to the production of intermediate products predominated by volatile organic acids such as acetic acid, propionic acid, butyric acid and valeric acid; and (ii) the "methane fermentation" phase or methanogenesis, resulting in the conversion of the intermediate products to stable end products mainly methane and carbon dioxide. The two phases in anaerobic digestion differ in bacterial populations, digestion rate, environmental requirements, degradation process and products. In two-phase anaerobic digestion system each phase can be controlled at the best environmental conditions in separate reactor.

Recently, various reactor configurations and substrates are being applied to two-phase anaerobic digestion as shown in Table 7. In order to accelerate the acidogenesis and methanogenesis processes in TPAD, the two separate reactors may be applied in various high rate anaerobic reactors such as upflow anaerobic sludge blanket (UASB)—UASB system (Fongsatitkul et al. 1995), continuous stirred tank reactor (CSTR)—upflow anaerobic filter (UAF) system (Held and Wellacher 2002), hybrid reactor (Yalcin et al. 2008), CSTR—anaerobic fluidized bed reactor (AFBR) system (Yu et al. 1999), two-phase plug-flow reactor (PFR) (Liu and Ghosh 1997; Liu 1998), and anaerobic packed bed reactor (APBR) (Tatara and Yamazawa 2004).

Two-phase anaerobic processes have been applied to treat many kinds of wastewater and solid wastes from difference sources such as distillery (Shin et al. 1992), landfill leachate (Agdag and Sponza 2005), coffee (Kida et al. 1994), cheese whey and dairy (Yilmazer and Yenigün 1999), starch (Demirel and Yenigün 2002), fruit and vegetable solid (Yu et al. 1999; Pavan et al. 2000), food (Shin et al. 1992), pulp and paper (Rintala and Puhakka 1994), olive mill (Borja et al. 1998), abattoir (Banks and Wang 1999), dye (Talarposhti et al. 2001), primary and activated sludge and solid (Bhattacharya et al. 1996).

Phase separation of anaerobic process has a number of major advantages (Shuizhou and Zhou 2005) including (i) isolation and optimization of potential rate-limiting steps; (ii) improvement of reaction kinetics and stability through pH

Table 7 Performance on two-phase anaerobic digestion with various kinds of feedstock

Feedstock	Reactor configuration	System HRT (days)	System organic loading rate (OLR) (kg COD/m^3.d)	Performance	CH_4 yield	Reference
Distillery wastewater	UASB-UASB	–	16.5–44.0	80 % COD removal	16.5 l/l COD. d	Shin et al. (1992)
Coffee waste	–	–	–	70 % COD removal	0.3 m^3/kg waste	Kida et al. (1994)
Cheese whey	CSTR-UAF	4–7	–	–	0.55 m^3/kg COD	Yilmazer and Yenigün (1999)
Abattoir waste	–	4–12	1.4–7.0	–	0.3 m^3/kg COD	Banks and Wang (1999)
Olive mill	–	–	2.3–2.4	–	0.36 m^3/kg COD	Borja et al. (1998)
Dye waste	APBR-APBR	3–5	0.25–1.0	90 % color removal	–	Talarposhti et al. (2001)
Fruit and vegetable	TPAD (mesophilic-thermophilic)	12	–	–	0.6 m^3/kg TVS	Pavan et al. (2000)
Primary and mixed primary-activated sludge	TPAD (mesophilic-mesophilic)	10	–	43 % VS removal	0.11 m^3/kg VS	Ghosh and Taylor (1999)

control, resistant to shock loading, selection of faster-growing microorganisms; and (iii) potential for detoxification in first phase. However, application of phase separation of anaerobic digestion has encountered certain barriers such as (i) disruption of syntrophic relationships; (ii) requirement of experience engineers and operators; (iii) uncertainty of linkage between reactor configuration and substrate types which determine the amenability of feedstock to two-phase anaerobic digestion.

4.2.2 Co-digestion

Mono-digestion (anaerobic digestion of a single substrate) usually suffers from its limitations in the cases of (i) low organic loads of sewage sludge; (ii) low organic loads and high nitrogen concentrations in animal manures; (iii) relatively high concentration of heavy metals in organic fraction of municipal solid waste (OFMSW); (iv) seasonal substrates such as crops and agro-industrial wastes; (v) potential inhibitors of methanogenic activity in slaughterhouse waste (SHW) such as the presence of high concentration of nitrogen and long-chain fatty acids (LCFA). Anaerobic co-digestion, i.e., simultaneous digestion of two or more substrates, is a feasible option to overcome the drawbacks of mono-digestion and to improve economic feasibility of anaerobic digestion (Mata-Alvarez et al. 2014).

Researchers found that the improvement of methane production is mainly a consequence of the increase in the organic loading rate (OLR) rather than synergisms between the primary substrate and co-substrate (Mata-Alvarez et al. 2011). Different kinds of mixtures can be considered and used in co-digestion as long as the blend ratio and types of co-substrate favor synergisms, dilute inhibitory compounds, optimize methane production and does not disrupt digestate quality. Typically, the decisions on the ratio between the primary substrates and co-substrates have been simplified to optimize the C/N ratio. The primary substrates like animal manures are characterized by high buffer capacities and a low C/N ratio while the co-substrates like agro-industrial waste and OFMSW are normally characterized as a high C/N ratio and low buffer capacity (Astals et al. 2012; Wang et al. 2012). However, the optimized combination in the mixture also requires consideration of other parameters such as macro and micronutrients equilibrium, pH and alkalinity, dilution of inhibitory compounds, amounts of biodegradable organics and dry matter (Hartmann et al. 2002).

4.3 Biomass Retention

Reactors of anaerobic process can be categorized according to how the biomass is retained in the system and the type of biomass in the system. Bacteria grow in the reactor liquid as flocculent or granular sludge in suspended growth reactors. Granular sludge exhibits higher activity rates and settling velocity that reduce the reactor volume required and allowing higher organic loading rates to the systems.

The most robust configurations for suspended growth anaerobic reactors are UASB (upflow anaerobic sludge blanket) and EGSB (expanded granular sludge bed).

4.3.1 UASB System

With the widespread industrial application of UASB reactors, increasing attention is focused on the granulation of anaerobic sludge (Fang 2000). Biomass is retained as granular matrix or blanket as suspension in the reactors. The advantages of granulation includes the establishment of a regular, thick and well-built microbial structure that is ready to operate with different transport phenomena, high biomass retention time leading to a high loading rate and better removal efficiencies, appropriate settleability, resistance to high OLR and toxicity shock (Speece 1996). The operation of UASB reactors may be limited by a number of factors including (i) inadequate retention of viable biomass for treating specific types of wastes that is not able to cultivate granular sludge, (ii) granule disintegration or wash-out of hollow granules, (iii) occurrence of fluffy granules, and (iv) scaling by inorganic precipitate.

4.3.2 EGSB System

EGSB systems are not equipped with an internal settler as in the conventional UASB, but with an advanced liquid-solid separation device. The main features of the EGSB reactors are: (i) high design organic loading rates; (ii) very small surface area; (iii) tall reactor system; and (iv) high upflow velocity. Engineering anaerobic sludge granules is a new area of research that targets at expanding the catabolic capabilities of the sludge.

4.3.3 Attached Growth Reactors

Attached growth reactors make use of either fixed film or carried media for the bacteria to grow and attach. Attached-growth systems comprise of fixed-film reactors and fluidized bed reactors involving immobilization of microbial biomass on inert media. In fixed film processes, bacteria reside on static support surface such as plastics rings, rocks, media modules or membrane modules. In fluidized bed processes, suspended carrier media such as sand, provide attachment surfaces in the reactors.

Hybrid anaerobic reactors are popular in recent development which take advantages of both suspended and attached growth processes in a single reactor. An example of hybrid anaerobic reactor design combine UASB as the lower section and upflow anaerobic filter as the upper section in a single reactor (Abdullah et al. 2005). The advantages of hybrid anaerobic reactors include (i) development of granular or flocculent sludge bed in the reactor, leading to an increased biomass

inventory, (ii) suitability for treating wastes where granular sludge formation is difficult, and (iii) increasing process stability and removal efficiency.

4.3.4 Membrane Bioreactors

Efficient liquid-solids separation is the basis of any anaerobic high-rate reactor system for waste treatment. Anaerobic membrane bioreactors (AnMBRs) are emerging alternatives for UASB reactors. With the presence of the inert supportive media for bacterial growth, membrane bioreactors can achieve outstanding effluent quality (<20 ppm organics), and COD and solid removal (up to 99 and 100 % for domestic wastewater respectively) (Smith et al. 2012) with the advantages of (i) possible operation at approximately infinite SRT to reach very low effluent substrate concentrations, (ii) allowing the growth of slow-growing micro-organisms, and (iii) possible treatment of recalcitrant compounds. However, membrane bioreactors usually suffer the drawbacks of high pressure physical separation causing disruption to microbial communities and subjected to membrane fouling and scaling with typical precipitates such as calcium carbonate.

4.4 Reactor Configuration

Reactor configurations of anaerobic digester can be divided into conventional anaerobic digesters and high-rate anaerobic digesters. The first conventional anaerobic digester was used in 1881 to liquefy the solid components of sewage. In 1955, anaerobic contact process was developed to treat soluble organics and dilute wastewaters (Hassan et al. 2013). A variety of new bioreactor designs have been developed in recent years which facilitate a significantly high rate of reaction for the treatment of waste (Bouallagui et al. 2003; Mumme et al. 2010; Xing et al. 2010). High rate anaerobic reactors include completely mixed anaerobic digester, anaerobic contact process, anaerobic sequencing batch reactor (ASBR), anaerobic packed bed or anaerobic filter, anaerobic fluidized bed and expanded bed reactors, upflow anaerobic sludge blanket (UASB) reactor and anaerobic baffled reactors (ABR) (Barker et al. 1999). Through the development of innovative high-rate reactor designs, anaerobic treatment can now challenge the cost of aerobic treatment for many wastewater treatment applications (Malina Junior and Pohland 1992). Ward et al. reported that an anaerobic bioreactor should be designed in a way that allows a continuously high and sustainable organic loading rate with a short hydraulic retention time and has the ability to produce the maximum level of methane (Ward et al. 2008). Reactors can be classified into the following categories (i) batch and continuous process, (ii) single-phase, and (iii) multi-phase reactors. Reactor shape must also take into consideration, both mixing and heat transfer.

In addition to basic reactor design, mixing of the contents in anaerobic digesters are required to ensure efficient transfer of particulate organic material for active

microbial biomass, to release gas bubbles trapped in the reactors and to prevent sedimentation of denser particulate materials. The mixing pattern may be intermittent, which is determined by the type of reactor, type of agitator used and the total solid contents of the feedstock (Burton and Turner 2003). Recirculation of biogas in the reactor or hydraulic mixing by recirculation of digestate with pump is commonly used to prevent the need of moving parts within the reactors. A certain degree of mixing is necessary but excessive mixing conditions can reduce biogas production (Gomez et al. 2006). It has been postulated that propionate-oxidizing bacteria and methanogenic archaea live in close proximity in granules with H_2 and formate as electron carriers. Excessive agitation can disrupt the granule structure, reducing the rate of oxidation of fatty acids and leading to digester instability (McMahon et al. 2001). Extracellular polymeric substances (EPS) are a combination of proteins and carbohydrates which are responsible for the formation of granules (Liu et al. 2004). An increase in mixing decreased the amount of EPS found, suggesting that minimal mixing produced larger anaerobic granules as greater quantities of EPS are required to maintain the granule structure (Ong et al. 2002). Mixing with biomass support media could be an important area in optimizing reactor configuration of anaerobic digester. Biomass support media provides an anchorage for the granular microbial communities and allows a high-shear type of mixing to increase solubility of COD without disruption to the microbial communities.

4.5 Process Control and Monitoring

Despite decades of academic and industrial research efforts, the complex anaerobic digestion processes are far from being understood in detail. Many anaerobic digestion plants are merely relying on a few simple-to-measure parameters mainly due to the conservative design of the over-sized reactors to guarantee process robustness, which gives a poor indication of the state of the biological process. Furthermore, unintentional organic loading, accidental addition of toxic substrates, process interruptions and lack of raw material quality control are believed to be one of the main limitations for effective process operation (Hjort-Gregersen et al. 1996; Holm-Nielsen et al. 2008; Nielsen and Angelidaki 2008; Kaparaju et al. 2009a, b). Introducing reliable monitoring and control technology would allow anaerobic digestion plants to be operated closer to their effective capacity limit instead of wasting reactor volume due to conservative design rules.

Process Analytical Technologies (PAT) is one of the recent advances in process monitoring in anaerobic digestion which allows complex bioconversion processes to be monitored and deciphered to a new level of reliability and effectiveness using spectroscopic and electrochemical measurement principles together with chemometric multivariate data analysis. Research efforts has been put in reviewing the potential application of PAT, Theory of Sampling (TOS) and chemometric data analysis within the field of anaerobic digestion monitoring (Madsen et al. 2011).

Table 8 Short-chained VFAs in sample matrices of manure and wastewater sludge

IUPAC nomenclature	Formula	CAS#	MW (Da)	bp (°C)	pK_a
Ethanoic acid	CH_3COOH	64-19-7	60.1	117.9	4.76
Propanoic acid	CH_3CH_2COOH	79-09-4	74.1	141.2	4.87
n-Butanoic acid	$CH_3CH_2CH_2COOH$	107-92-6	88.1	163.8	4.83
2-Methylpropanoic acid	CH_3CHCH_3COOH	79-31-2	88.1	154.5	4.84
n-Pentanoic acid	$CH_3(CH_2)_3COOH$	109-52-4	102.1	186.1	4.83
2-Methylbutanoic acid	$CH_3CH_2CHCH_3COOH$	116-53-0	102.1	177	4.80
3-Methylbutanoic acid	$CH_3CHCH_3CH_2COOH$	503-74-2	102.1	176.5	4.77

Table 9 Four main classes of reviewed analytical modalities collectively known as PAT in AD process monitoring

Main class	Reviewed analytical modalities
Spectroscopic	Fluorescence (FLU) Peck and Chynoweth (1992), Infrared (IR) Steyer et al. (2002), Near Infrared (NIR) Nordberg et al. (2000), Hannsson et al. (2002), Holm-Nielsen et al. (2007), Holm-Nielsen et al. (2008), Raman, Visual (VIS), Ultraviolet (UV) Redondo et al. (2008), Rudnitskaya and Legin (2008), Buczkowska et al. (2010)
Electro-chemical	pH, Redox potential, Electronic tongue (ET), Electronic nose (EN)
Chromatographic	GC, GC headspace, HPLC Pind et al. (2003), Boe et al. (2005), Diamantis et al. (2006)
Other	Acoustic chemometrics (a.c.) Nacke et al. (2005), Mass spectrometry (MS), Microwaves Lomborg et al. (2009), Titration Feitkenhauer et al. (2002), Lahav and Morgan (2004)

The use of multivariate sensor technologies and electrochemical arrays is encouraged as many studies have shown promising results in both laboratory-scale and pilot-scale. Many authors have suggested VFAs as control parameters as these acids are indicative of the activity of methanogenic consortia. A number of analytical methods have been developed for quantification of relevant VFAs for anaerobic digestion process monitoring. Short-chained VFAs commonly present in sample matrices such as manure and wastewater sludge are listed in Table 8 (Hill and Holmberg 1988; Christiansen et al. 1995; Nielsen et al. 2007). The application of numerous monitoring techniques for quantifying these parameters has been reported in the literature. An overview of available techniques is provided in Table 9.

4.6 Mathematical Models

Two most widely used models for anaerobic digestion are the Anaerobic Digestion Model no. 1 (ADM1) developed by a task group for the International Water Association (IWA) and Siegriest Model (Siegrist et al. 2002). The two models are

constructed with different approaches: Siegriest model parameters are based on experiments, whereas the ADM1 uses review consensus (Batstone 2006).

Lyberatos and Skiadas (1999) gave an extensive review on modelling for anaerobic digestion. They pointed out that some important factors describing the behavior of anaerobic digesters should be evaluated and taken into account from a modelling point of view including digester startup conditions, degree of acclimation to the feedstock, hydraulic loading, organic loading, biogas production per unit volume, concentration of inhibitors, availability of nutrients, cation concentrations, types and concentration of solids in the feedstock. Anaerobic digestion is a complex system of biochemical and physical processes. Due to its complexity, it has traditionally been treated as a black box system and optimization has been based on experience or trial-and-error methods. As experiments of anaerobic digestion are expensive and time-consuming, modelling can provide a useful tool for process understanding and optimization (Kothari et al. 2014).

5 Advanced Molecular Biological Tools for System Monitoring

Anaerobic digestion is carried out by a mixture of different *Bacteria* and *Archaea* living in a microbial community. The microbial community is generally considered complex as hundreds of different types of organisms are involved in the process and these organisms are also interacting among themselves. In order to optimize the yield of biogas and for trouble shooting purposes in case of a process upset, a comprehensive view on the composition and metabolic functions of the organisms in the system is warranted. In the past decade, a number of advanced molecular tools targeting the DNA, RNA and proteins of microbial cells have become available to provide detailed biological information on the microbial community and it is now possible to move beyond the traditional 'black box' approaches of operating an anaerobic digester. An overview of these tools is described in this section.

5.1 Low-throughput Methods

Given the high microbial diversity present in anaerobic digestion, culture-dependent methods to analyze a microbial community are not practical and not feasible. Hence, culture-independent methods are required. In the 1990s and early 2000s, polymerase chain reaction (PCR) targeting the 16S rRNA gene of *Bacteria* and *Archaea* with universal primers followed by clone library and Sanger sequencing was a popular method to identify the organisms present in anaerobic digesters (Chouari et al. 2005). Typically, a few hundred clones are randomly picked and sequenced as the method is labor intensive and expensive. Because a relatively small number of clones can be analyzed, clone library method can only capture the dominant

populations and the organisms that are present at a low relative abundance are usually not captured. Other methods that are suitable to identify the dominant populations include terminal restriction-fragment length polymorphism (T-RFLP) (Ike et al. 2010) and chemical or temperature denaturing gradient gel electrophoresis (DGGE) (Bialek et al. 2012). The aforementioned methods are semi-quantitative where the relative proportion of the taxa is determined. When absolute quantification is required to determine the concentration of a specific population in an anaerobic digester, quantitative PCR (qPCR) can be applied and qPCR has the advantage that the quantification range spans a few orders of magnitude, making it possible to quantify the low and high abundant organisms such as different methanogens (Goberna et al. 2010). The application of these molecular methods to a single sample can provide a snapshot of the microbial community, but when multiple samples at different time points and under different conditions are analyzed, the shift in composition of the microbial community can be revealed.

5.2 High-throughput Methods

The advent of sequencing technology in the past few years has revolutionized the ability to analyze microbial communities, providing both breadth and depth in coverage of information. Furthermore, the cost per DNA base has decreased and robotic instruments have automated many procedures in the lab, making the analysis less labor intensive. First, it was the emergence of the next-generation sequencing platforms by 454 Life Sciences that can generate a few hundred million bases per run and long read length up to 450 bp. Later, the sequencers developed by Illumina (Solexa) have further increased throughput to as much as a few hundreds gigabases per run with a shorter read length ($\sim$125 bp). With the Illumina platforms, the number of reads that can be obtained per sample to analyze the composition and structure of a microbial community can range from a few thousands to tens of thousands, which is substantially more than a clone library analysis (Sundberg et al. 2013). With this sequencing depth, both the dominant and minor members of the community can be identified, which represents substantial improvement over previous methods as the minor members could also be functionally important. In addition to targeting the 16S rRNA gene, high-throughput sequencing has also been applied to analyze functional genes such as the methyl coenzyme M reductase (*mcrA*) gene that is ubiquitously present in all methanogens for catalyzing the last step of methane generation (Ellis et al. 2012; Wilkins et al. 2015).

Targeting taxonomic and/or functional genes as biomarkers can identify the organisms present. However, in order to determine the metabolic functions of these organisms, shotgun metagenomic sequencing of the microbial community can be performed to determine the gene content present in these organisms. Hampered by the lower throughput of previous sequencing platforms, a gene-centric approach was usually taken in early metagenomic studies where the goal is to simply identify the metabolic functions present (Li et al. 2013; Wong et al. 2013). Recently, with

the increase in the throughput of sequencers, acquiring a substantial quantity of reads per sample is possible, enabling a genome-centric approach in metagenomic sequencing where genes are placed in a genomic framework (Sekiguchi et al. 2015). A genome-centric approach offers the advantages that the metabolic capability of an organism and its interactions with other members in the community can be better deciphered.

Building on the metagenomic sequencing results, two complementary approaches, namely metatranscriptomics and metaproteomics, can be further applied to query the dynamics and expression of genes under different conditions in anaerobic digesters. Metatranscriptomics make use of high-throughput sequencing to analyze the expressed RNA (Zakrzewski et al. 2012), while metaproteomics utilize advanced mass-spectrometry to analyze the expressed proteins (Hanreich et al. 2013; Lü et al. 2014). Metagenomics are useful to determine what organisms are present and what biochemical functions these organisms possess. However, under what conditions these organisms are active and what metabolic functions are executed cannot be easily interpreted from the metagenomic data. Therefore, metatranscriptomics and metaproteomics are useful tools to provide detailed information on the activity of the organisms in a digester. The combination of metagenomics, metatranscriptomics and metaproteomics is generally referred to as 'omics' methods and these innovative molecular biology tools can help microbiologists and engineers to better diagnose anaerobic digesters.

6 Future Outlook

Climate change, waste treatment and renewable energy are pressing issues facing society in the 21st century. Anaerobic digestion of organic materials can address all these issues simultaneously. Given that anaerobic digestion is a mature technology, the deployment of this technology in large centralized scale or small decentralized scale is expected to gain widespread use in the near future. Further optimization and enhancement of the engineering coupled with knowledge in the microbiology will certainly further improve the robustness and performance of anaerobic digestion. Without a doubt, the outlook of anaerobic digestion is promising and this technology will play an important role in our society.

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