Chapter 15
Microbial Community in Anaerobic Digestion System: Progression in Microbial Ecology

Luong N. Nguyen, Anh Q. Nguyen and Long D. Nghiem

Abstract Anaerobic digestion (AD) is a biochemical process that involves four microorganism groups, namely, hydrolyzers, acidogens, acetogens, and methanogens. These groups function in syntrophy and have intra-dependent metabolic pathways. Changes in one group (e.g., over-/underexpressed population and function) can alter this chain of anaerobic process and consequently AD performance. With recent progress in culture-independent techniques, an array of previously unknown and uncultured microorganisms has been recently uncovered in the AD process. Discoveries on the diversity and structure of the AD microbial community can provide new information on digester stability and performance (e.g., biogas production). This chapter provided a critical analysis of the current knowledge on the AD microbial community, focusing on the factors affecting microbial community and the relationship between microbial community and AD performance. Gaining a better understanding of microbial ecology could be the key for greater AD efficiency and biogas production capacity.

Keywords Anaerobic digestion (AD) · Microbial community · Microbial ecology Core microbiome

L. N. Nguyen · A. Q. Nguyen · L. D. Nghiem (✉)
School of Civil and Environmental Engineering, Center for Technology in Water and Wastewater, University of Technology Sydney, Sydney, NSW 2007, Australia
e-mail: DucLong.Nghiem@uts.edu.au

L. N. Nguyen
e-mail: luongngoc.nguyen@uts.edu.au

A. Q. Nguyen
e-mail: qan031@uowmail.edu.au

X.-T. Bui et al. (eds.), *Water and Wastewater Treatment Technologies*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-13-3259-3_15

15.1 Introduction

Anaerobic digestion (AD) has long been used in wastewater treatment plants (WWTPs) to stabilize sewage sludge prior to beneficial reuse or disposal. Recently, there has been a growing emphasis among the water industries and municipalities to achieve sustainability goals by shifting from a sole focus on wastewater treatment to include energy generation and resource recovery. The AD process has then played a vital role in this paradigm shift. The AD process converts sludge into biogas, which mostly contains methane, carbon dioxide, and a nutrient-rich slurry called digestate. Other organic wastes such as food waste, dairy processing waste, agricultural reject, and others have been brought into the AD process (Nghiem et al. 2017). These organic wastes supplement AD with carbon and nutrients and consequently boost biogas production. With this anaerobic co-digestion (AcoD) approach, WWTPs can produce biogas which can be used to produce electricity to offset their energy consumption. AcoD has an additional benefit of reducing the amount of organic waste that is otherwise bound to landfills. Although AD is a mature technology, maintaining a stable and high-performance digester is still a challenging exercise. This is mainly due to the complexity of microorganisms that are involved in the AD process. These microorganisms maintain a syntrophic relationship and depend on each other for their survival and growth (Carballa et al. 2015; Regueiro et al. 2015; Ortseifen et al. 2016; Ju et al. 2017).

A fundamental step to characterize the AD microbial community is the taxonomic and phylogenetic classification of DNA sequence—biomarker of microorganism. The last few decades have seen a revolution in molecular techniques to investigate the small-subunit rRNA sequence (16S rRNA) from simple polymerase chain reaction (PCR) to high-throughput sequencing such as next generation sequencing (NGS). The development of culture-independent techniques has uncovered an abundant array of previously unknown and uncultured microorganisms. Due to these technical advances, the number of investigations on microbial community in the AD process underwent an impressive increase in scientific publications (Carballa et al. 2015; Razaviarani and Buchanan 2014; Gagliano et al. 2015a; Li et al. 2015). NGS has made large advancements in the understanding of the underlying driving force—the microbiome—in AD process. For example, the AD microbial community could be characterized in terms of taxonomic profile and composition. Understanding the connection between microbial community and AD performance can provide intuitive information for optimization of the AD process. This chapter, therefore, provides a state-of-the-art review on the AD microbial community, including the factors affecting microbial diversity and structure and the relationship between microbial community and digester performance.

15.2 Microbial Community—The Driving Force in AD Process

There are four phases in the AD process each is facilitated by a distinctive group of microorganisms (Fig. 15.1). There is also a syntrophic relationship among the very diverse functions of these microorganisms in growth conditions, physiology, metabolic activities, and stress tolerance. The performance of AD is dependent on this complex syntrophic relationship. For example, the main product of the acetogens is acetate, which is also a major carbon and energy source for the methanogens (Fig. 15.1). Corresponding to the four phases in Fig. 15.1, the AD microorganisms are categorized into four groups, namely, hydrolyzers, acidogens, acetogens, and methanogens mainly based on their specific functions in the AD process. These groups of microorganisms are taxonomically divided in the domain of bacteria (hydrolyzers, acidogens, and acetogens) and archaea (methanogens). From the domain, the AD microorganisms are arranged in successive levels of biological classification in the taxonomic hierarchy with the following order: kingdom, phylum, class, order, family, genus, and species. The following section provides a detailed description of each phase in the AD process focusing on different groups of microorganisms and their ecology.

15.2.1 Hydrolyzers

Anaerobic hydrolytic bacteria are widely distributed in various ecosystems such as soils, sewage, rumen of animals, compost, and AD sludge. Hydrolytic bacteria (Phase 1) are the first to react to convert complex organic matter (i.e., carbohydrates, proteins, and lipids) into low molecular weight compounds such as sugar,

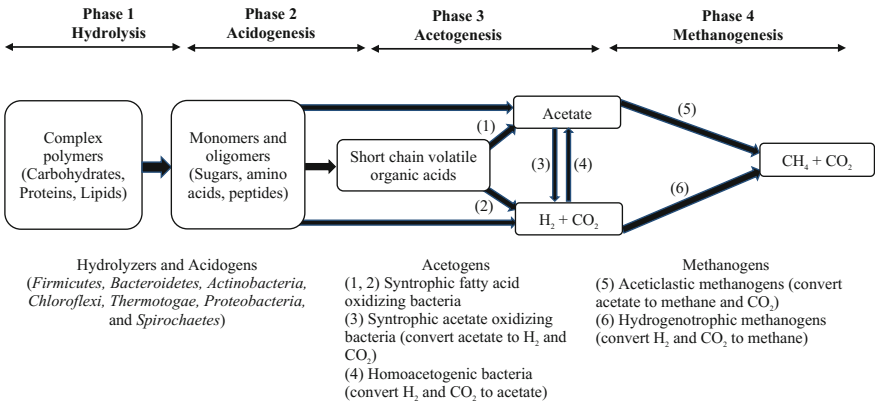

Fig. 15.1 Schematic representation of four phases in the AD process with associated group of microorganisms

amino acids, and peptides (Fig. 15.1). These intermediates serve as food for the next groups of microorganisms in the process chain. Without the initial step by the hydrolytic bacteria, the AD process cannot occur naturally. A functionally stable AD, therefore, contains a healthy portion of hydrolytic bacteria.

Hydrolytic bacteria can be found in a number of different phyla such as *Chloroflexi*, *Thermotogae*, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Spirochaetes*. *Firmicutes* and *Bacteroidetes* are the two most dominant phyla in AD (Fig. 15.2). The abundance of hydrolytic bacteria in AD depends on factors such as type of inoculum, operating temperature, cell retention time (CRT), and substrate characteristics. The abundance of major phylum in the AD process is presented in Fig. 15.2.

As a unique feature of the hydrolytic bacteria, they produce cellulosome, a special multienzyme complex that enables them to secrete different hydrolases such as glucanases, hemicellulases, chitinases, and lihanases. These enzymes enable hydrolytic bacteria to break down a variety of complex organic wastes. Cellulosome was first discovered in 1983 from the *Clostridium thermocellum* in thermophilic AD (Lamed et al. 1983). Since then the role of cellulosome and its presence in the many hydrolytic bacteria were discovered. The cellulosome bridges the connection between bacteria, enzyme, and substrates. It has been demonstrated that the hydrolytic bacteria cannot produce enzymes without cellulosome. The presence of these enzymes has made hydrolytic bacteria important in AD of various organic wastes.

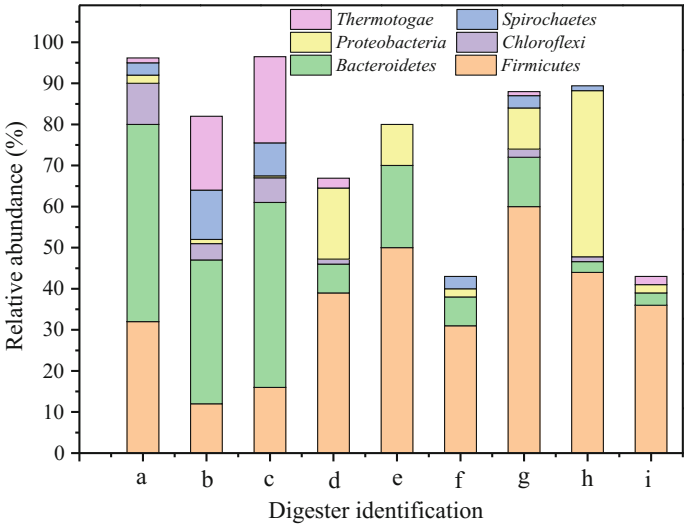

Fig. 15.2 Relative abundance of major phylum in the AD process. Data were extracted from most recent studies, which used high-throughput sequencing technologies to detect their abundance. The digester identification indicates the source of data [a, b, and c] (Guo et al. 2014), [d] (Hanreich et al. 2013), [e] (Li et al. 2013), [f] (Ortseifen et al. 2016), [g] (Rademacher et al. 2012) and [h and i] (Wu and He 2013)

15.2.2 Acidogens

In Phase 2, acidogenic bacteria use the products of hydrolyzers—sugars, amino acids, and peptides—as electron acceptors to generate fermentation products such as formic acid, acetic acid, propionic acid, butyric acid, pentatonic acid, alcohols, CO_2, and H_2 (Fig. 15.1). Acetate, CO_2, and H_2 can be used directly by the methanogens (in Phase 4), while other higher organic acids are subsequently transferred to acetic acids and H_2 by the acetogenic bacteria (Phase 3).

Acidogenic bacteria include facultative and obligate microorganisms. The former can live in both aerobic and anaerobic conditions while the latter is strictly anaerobic. Species of acidogenic bacteria can be found in phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. A few species have been isolated from AD such as *Clostridium* (*Firmicutes*), *Peptococcus* (*Firmicutes*), *Bifidobacterium* (*Actinobacteria*), *Desulfovibrio* (*Proteobacteria*), *Corynebacterium* (*Actinobacteria*), *Bacillus* (*Firmicutes*), *Pseudomonas* (*Proteobacteria*), and *Desulfobacter* (*Proteobacteria*) (Shiratori et al. 2006; Nanninga and Gottschal 1987). These species are especially abundant during high fermentation period, confirming their roles at this phase.

A number of abiotic factors can influence the population of acidogenic bacteria such as digester design, temperature, CRT, and substrate characteristics. Out of these, substrate characteristics (i.e., composition and concentration) are considered to exert the greatest influence. For example, *Desulfovibrio* spp., a sulfate-reducing acidogen, was significantly enriched during AD of high sulfate-containing substrate (Nanninga and Gottschal 1987). Also, *Clostridium* sp., a cellulosic waste degrading bacterium, was found to be dominant in high cellulose substrate (Izquierdo et al. 2010).

15.2.3 Acetogens

Acetogenesis is the third phase of the AD process. In this phase, organic acids such as propionic, butyric, and pentatonic acid are metabolized into acetic acid and H_2 by acetogenic microbes (Fig. 15.1). A number of acetogens belong to the genus *Syntrophomonas* (e.g., *Syntrophobacter wolinii* and *Syntrophomonas wolfei*), which are syntrophic fatty-acid oxidizing microbes. The H_2 production in this process inhibits acetogenic metabolism. The hydrogen partial pressure should be very low so that the thermodynamic conditions become favorable for conversion of volatile fatty acids (VFAs) to acetate (Fig. 15.3). The activity of hydrogenotrophic methanogens is extremely critical to maintain low hydrogen partial pressure in AD (Cazier et al. 2015; Amani et al. 2010). Failure to maintain this interaction is detrimental to the overall AD performance, resulting in VFA accumulation in the system. The success of the acetogenesis determines the biogas production efficiency because 70% of methane is produced through acetate reduction.

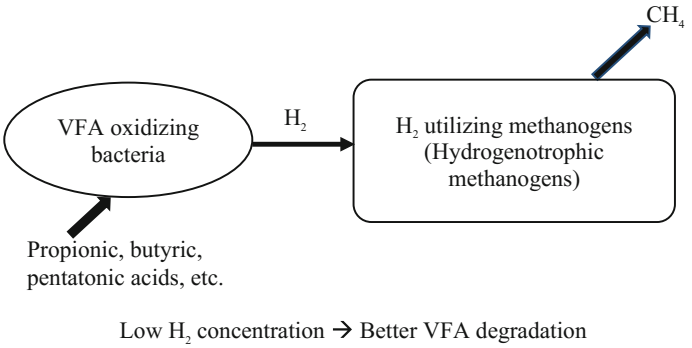

Fig. 15.3 Schematic diagram illustrates the syntrophic acetogenesis

Syntrophic acetate oxidizing (SAO) bacteria can be seen as a stabilizer in the AD process. They play an important role in maintaining the AD stability especially when the system undergoes environmental fluctuations. For example, aceticlastic methanogens, which contribute more than 70% of methane production, are sensitive to high ammonia concentrations, VFAs, heavy metals, and sulfide. Under such conditions, SAO can adapt to dominantly convert acetate to H_2 and CO_2, which are then used by the hydrogenotrophic methanogens for methane production (Sun et al. 2014). This decreases the accumulation of acetate and raises the pH of the digester to support the aceticlastic methanogens. Acetate oxidization by syntrophs has a low conversion rate, and thus altering the operating conditions to support syntrophic growth is necessary. A few SAO microbes are detected from both mesophilic and thermophilic AD. These include *Pseudothermotoga lettingae*, *Thermacetogenium phaeum*, *Syntrophaceticus schinkii*, and some in the phylum of *Spirochaetes* (Westerholm et al. 2010; Hattori et al. 2000; Lee et al. 2015).

Non-syntrophic acetogens are also present in the AD process in the form of homoacetogenic bacteria. This group can utilize H_2 and CO_2 to produce acetate. This reaction is thermodynamically favorable and does not require the presence of methanogens. An example of microbes in this group is the *Clostridium aceticum* that was isolated and characterized as obligately anaerobic (Braun et al. 1981). However, this pathway is less favorable in the AD process and the homoacetogenic bacteria are often outcompeted by hydrogenotrophic methanogens for H_2. To date, the homoacetogenic bacteria have not been elucidated in detail.

15.2.4 Methanogens

The methanogens belong to the archaea domain that is capable of producing methane gas. They are ubiquitously present in anaerobic environment and play important role in the global carbon cycle (Kouzuma et al. 2017). In the AD process,

the methanogens are critical for biogas production. So far, researchers have found 65 methanogenic species and grouped them in five orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales* (Nielsen et al. 2007; Wang et al. 2018). These species are extremely sensitive to oxygen and are registered as slow-growing microbes as they restrict to a limited number of organic compounds for carbon and energy sources (Holmes and Smith 2016).

Based on substrate utilization, methanogens can be sub-divided into three groups: Methylotrophic methanogens that utilize methyl and other one-carbon compounds; Hydrogenotrophic methanogens that utilize CO_2 and H_2; and Aceticlastic methanogens that utilize acetate (Fig. 15.1). Among these archaea, aceticlastic methanogens are responsible for the majority of methane production in AD. This is consistent with the high abundance of the genera such as *Methanosaeta* and *Methanosarcina* (Fig. 15.4) in most of the AD processes. The genus of *Methanosaeta* is strictly aceticlastic methanogens. Chen and He (2015) reported the robustness of *Methanosaeta* genus at high levels of acetate in AD (44 mM). On the other hand, the genus *Methanosarcina* has versatile metabolic pathways and is

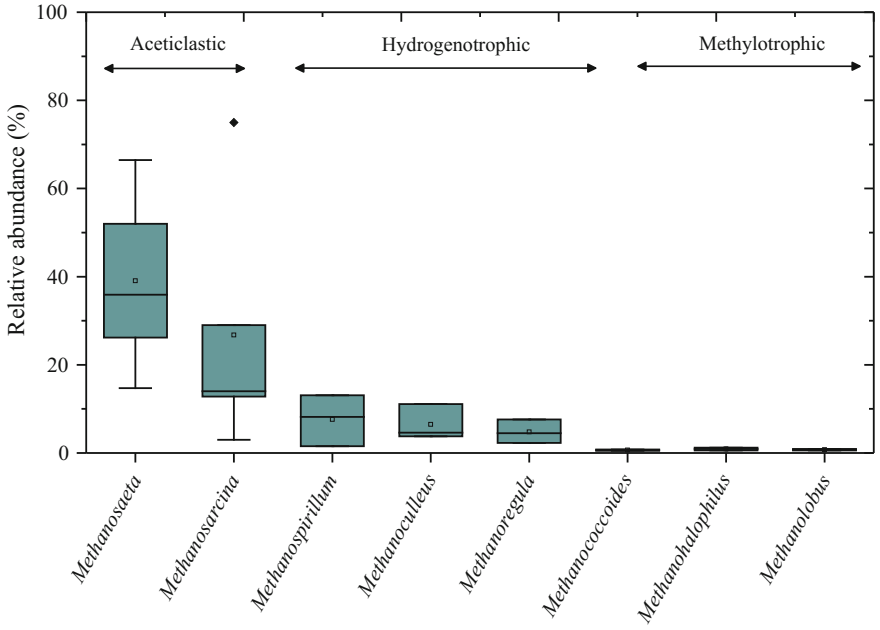

Fig. 15.4 Relative abundance of major methanogen genera. Data were extracted from most recent study that used high-throughput sequencing technology to detect their abundance. The relative abundance was calculated against the total archaea (Chen and He 2015; Guo et al. 2015; Rivière et al. 2009; Jang et al. 2015). The whiskers of the box represent the minimum and maximum values. The bottom and top of the box are the first and third quartiles, respectively, and the line inside the box denotes the median. The solid diamond represents the outliers

relatively tolerant to perturbations (e.g., low pH and high VFAs) (Venkiteshwaran et al. 2017). Compared to aceticlastic methanogens, hydrogenotrophic methanogens occur in AD at lower relative abundance (Fig. 15.4). However, the hydrogenotrophic methanogens are typically tolerant than aceticlastic methanogens under harsh conditions. It has been observed that the methanogenic community shifted from aceticlastic to hydrogenotrophic dominant under perturbed conditions (Lerm et al. 2012; Vanwonterghem et al. 2014). The presence of hydrogenotrophic methanogens, although at relative low abundance, is essential to keep the hydrogen partial pressure low and thus support the acetogenesis of VFAs.

AD is a biochemically complex process driven by syntrophic microorganisms. The performance and stability of an AD rely on its microbiome composition and the interactions among microbial groups. Previous studies have demonstrated that variations in the abiotic factors (i.e., digester design and operation) affect the digester performance outcomes but underestimate the change in the microbial community and its relation to the digester performance. In this era of next generation sequencing technologies, it is envisaged that the microbial community profile of many types of AD will be revealed. Detailed connections between microbial community profiles and digester performance can then provide new insights for engineers to better control the AD process. A research roadmap is proposed in Fig. 15.5 to integrate digester microbial community with understanding digester performance and optimization.

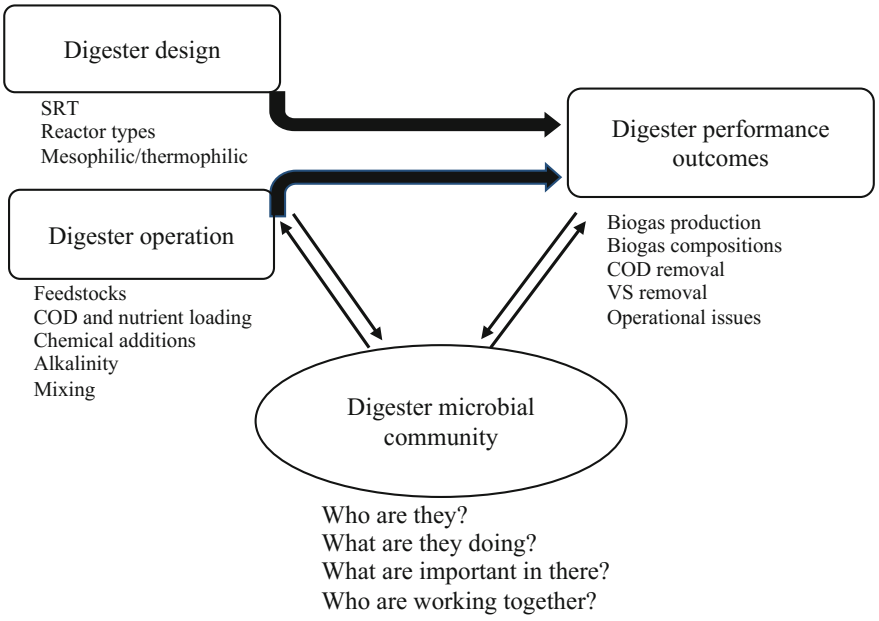

Fig. 15.5 Integrated digester microbial community into the understanding digester performance and control

15.3 Factors Influencing the Microbial Community in AD

The community diversity including richness (i.e., how many species are in a community) and evenness (i.e., how close in numbers each species in a community is) are described using alpha diversity indices. The alpha diversity indices include Observed species, Chao 1, Simpson, and Shannon. Observed species and Chao 1 are used to describe community richness and Simpson and Shannon are used to describe community evenness. The community structure includes the composition and abundance of species at different taxonomical levels (i.e., phylogenetic structure). It is often reflected by the distribution of individuals among species in a community. The differences between communities are measured by the beta diversity. Computational ecology method such as principal coordinate analysis (PCoA), principal components analysis (PCA), and nonmetric multidimensional scaling (NMDS) are used to profile the community structure and to estimate the distance among communities (e.g., dissimilarity). The following sub-sections provide detailed description on the influence of abiotic factors (e.g., temperature, OLR, CRT, and substrate characteristics) and biotic factor (e.g., inoculum source) on the AD microbial community (i.e., diversity and structure).

15.3.1 Temperatures

Mesophilic (30–40 °C) and thermophilic (50–60 °C) are the two common operating conditions in AD. Each condition presents specific advantages and the selection between them is mainly due to a number of factors. Thermophilic AD offers high metabolic rates, high biogas yields, and deactivation of pathogen due to higher operating temperature but its effluent contains high concentration of VFAs especially propionic acid. Mesophilic AD can maintain high organic loading rates but has lower metabolic rate compared to thermophilic AD (Labatut et al. 2014). Generally, thermophilic AD is more susceptible to environmental perturbations than mesophilic AD because the latter has lower community diversity (Moset et al. 2015; Gagliano et al. 2015b; Niu et al. 2015; Shaw et al. 2017). Low diversity of thermophilic AD is consistently reported in literature. For example, Shannon index of thermophilic AD has been reported lower than that of mesophilic AD (6.14 vs. 4.99) (Moset et al. 2015). A twofold decrease in microbial diversity and evenness has been observed when the operating temperature of AD was changed from 37 to 55 °C (Gagliano et al. 2015b). A negative correlation between temperature and all microbial diversity indices has been reported (Lee et al. 2017). The archaeal community of thermophilic AD is also less diverse than that of the mesophilic AD (Niu et al. 2015). An elevated temperature induces a selective pressure on the community resulting in the enrichment of tolerant strains and decrease in diversity. On the other hand, mesophilic AD is relatively resilient to sudden changes in operating conditions. For example, it has been observed that mesophilic AD has

shown resistance to total ammonia nitrogen inhibition of up to 1.6 g/L, whereas thermophilic AD has failed at half dose (Niu et al. 2015). Aceticlastic methanogens has shown extreme sensitivity to sulfide inhibition (e.g., 50% inhibition at 8–17 mg/L), while hydrogenotrophic methanogens were favored (Pender et al. 2004). Generally, a community with high diversity has greater capacity to maintain its stability under perturbances. For this reason, a relative higher diversity and evenness in the mesophilic AD leads to stable performance.

Phylogenic analyses revealed the difference in microbial community structure of mesophilic and thermophilic AD. Clustering analysis (i.e., principal coordinates analysis (PcoA)) revealed a clear separation between mesophilic and thermophilic communities (Carballa et al. 2011), suggesting the different evolution pathways of the community in each condition (Fig. 15.6). Kirkegaard et al. (2017) demonstrated a clear distinction between mesophilic and thermophilic AD communities after surveying 32 full-scale AD in 20 WWTPs in Denmark over a 6-year period. The major phyla were shifted from *Bacteroidetes*, *Proteobacteria,* and *Chloroflexi* to *Firmicutes* and *Synergistetes* dominant in thermophilic AD (Jang et al. 2016). The predominance of the phylum *Firmicutes* was because of their capability to produce diverse enzymes performing hydrolysis, acidogenesis, and acetogenesis. Hydrolytic and fermentative bacteria grow more rapidly at higher temperature and could cause the imbalance between the bacterial and methanogens community's population in thermophilic AD. In thermophilic AD, *Thermotogae* (>60%) was the dominant phylum while *Bacteroidetes* (>47%) was highly expressed in mesophilic AD (Guo et al. 2014). The observed results could be due to the phenotype of *Thermotogae* phylum that can thrive at high temperature.

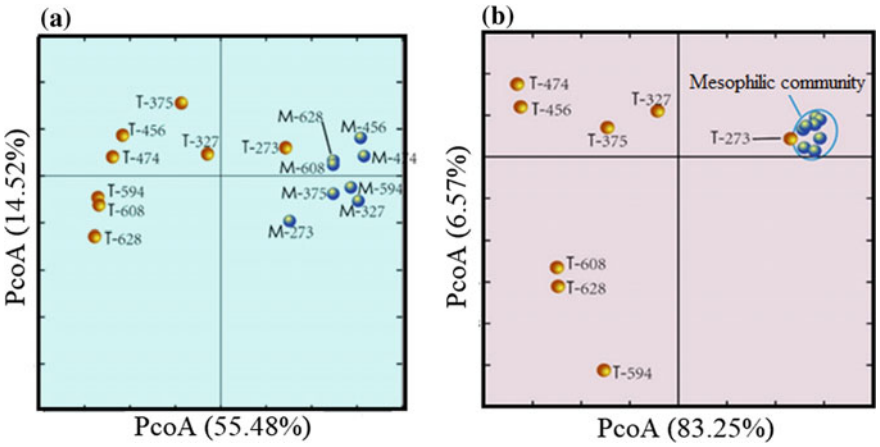

Fig. 15.6 An example of the PcoA plot of **a** archaeal and **b** bacterial communities, indicating the different microbial community structures in mesophilic and thermophilic AD. The data are from Ghasimi et al. (2015)

Different operating temperatures also induced the development of distinct methanogenic community. Mesophilic AD was dominated by the families of *Methanotrichaceae*, *Methanocorpusculaceae*, *Methanoregulaceae,* and *Methanomassiliicoccaceae*, whereas *Methanobacteriaceae* and *Methanomicrobiaceae* were prominent in thermophilic AD (Shaw et al. 2017). This also suggested that the aceticlastic methanogens are dominant in mesophilic AD and hydrogenotrophic methanogens are preferable in thermophilic AD (Ghasimi et al. 2015). Shaw et al. (2017) detected the well-known association between the hydrogen-producing bacteria such as *Ruminococcaceae* and *Prevotellaceae* and hydrogenotrophic methanogens *Methanomicrobiaceae* in thermophilic AD. The shift of methanogenic community from aceticlastic to hydrogenotrophic methanogens at high temperature is currently unclear and future research is required. Overall, temperature is one of the most significant factors determining the microbial community diversity and structure.

15.3.2 Organic Loading Rate

Organic loading rate (OLR) can have a profound impact on the microbial community diversity and structure. Microbial community diversity often decreases under high OLR (Jang et al. 2016; Kundu et al. 2013). Aceticlastic and hydrogenotrophic methanogens are especially affected under this condition. For instance, the underrepresentation of aceticlastic and hydrogenotrophic methanogens was observed under the increase of OLR from 2.22 to 6 kg COD/m^3d in both mesophilic and thermophilic AD (Kundu et al. 2013). On the other hand, the phylum *Firmicutes* is often overexpressed under high OLR. For example, the population of *Firmicutes* increased from 4 to 48.4% when OLR changed from 2.7 to 7.2 kg COD/m^3d. Two- and threefold increase in the abundance of *Firmicutes* was observed in AD of glycerol, fat oil, and grease with high OLR (Ferguson et al. 2016). The phylum *Firmicutes* were significantly enriched when OLR is increased from 2.74 to 4.12 kg VS/m^3d (Sun et al. 2017). Bacteria of phylum *Firmicutes* are capable of degrading VFAs to acetic acid. Under a high OLR, VFA productions are accelerated which would favor the growth and reproduction of *Firmicutes*. Accordingly, it has been indicated that the increase of *Firmicutes* was correlated with deterioration in methane production, suggesting that the *Firmicutes* abundance could be an indicator of process overloading and fluctuated performance.

After the phylum *Firmicutes*, *Bacteroidetes* is the second most enriched under increased OLRs. *Bacteroidetes* spp. is mainly involved in hydrolysis and acidogenesis in AD. An elevated OLR provides substrates for growth. Prevalence of *Bacteroidetes* was observed in AD of macroalgae biomass associated with high protein content due to OLR increase (Sun et al. 2017). The profound abundance of *Bacteroidetes* could lead to the high production of VFAs in the digester (Regueiro et al. 2015).

Phyla such as *Proteobacteria*, *Synergistetes*, *Spirochaetes*, *Chloroflexi*, *Thermotogae*, *Actinobacteria,* and *Planctomycetes* show variable patterns in response to the OLR changes. OLR increase could suspend the growth of *Proteobacteria* and *Chloroflexi* phylum from 23.8 to 5.4% and 14.5 to 2.5%, respectively (Chen et al. 2014). The abundance of *Proteobacteria* increased from 6.7 to 14.5% when there is an increase in OLR from 1.37 to 2.74 kg VS/m^3d but dropped to 1.9% at OLR of 4.12 kg VS/m^3d (Sun et al. 2017). Similarly, *Synergistetes* was significantly suspended at 300% increase in OLR. The varied reaction of different phyla to change in OLRs reflects the wide array of microorganisms in AD with different tolerance levels to environmental pressures.

Methanogenic community shows different degrees of disturbances by OLR stress. *Methanosarcina* increased from 2.9 to 22% under overloading condition, whereas *Methanosaeta* decreased by approximately 50% (Fig. 15.7) (Razaviarani and Buchanan 2014). Also, the methanogenic community shifted from *Thermoplasmata* (24.4%), *Thermoprotei* (18.0%), and *Methanobacteria* (30.8%) to *Thermoplasmata* (70.4%) and *Methanomicrobia* (16.8%) (Chen et al. 2014) at high OLR. Lerm et al. (Lerm et al. 2012) revealed the enrichment of hydrogenotrophic methanogens (*Methanospirillum hungatei* and *Methanoculleus receptaculi*) under high OLR. However, in some cases, a stable methanogenic community has been reported. High-solid AD of municipal sewage sludge under OLR range of 3.4–5.0 g VS/Ld does not affect methanogenic community and methane yield (Gómez et al. 2011). It is suggested that there exists a critical level of OLR increase. Overall,

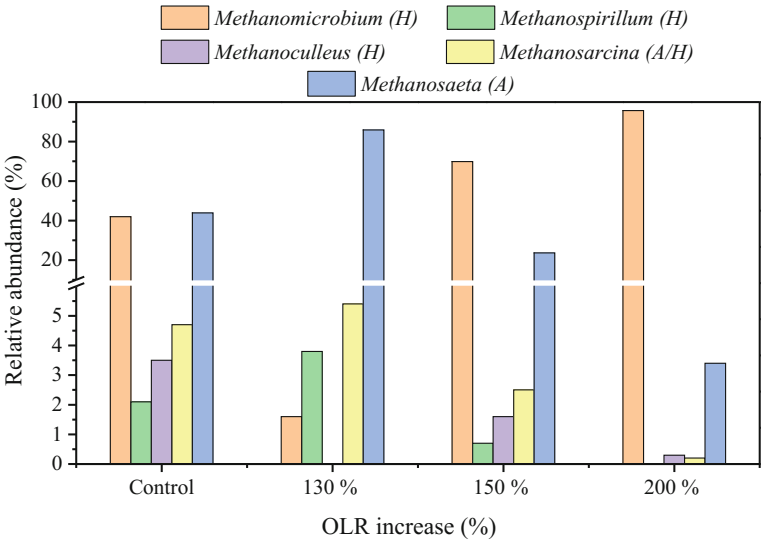

Fig. 15.7 Dynamic changes of methanogenic genera abundance under the different OLR increase (%). The data were extracted from Razaviarani and Buchanan (2015). The letters in the parentheses H and A indicate the hydrogenotrophic and aceticlastic methanogens, respectively. The relative abundance of each taxon was calculated against the total methanogenic population

maintaining the OLR of AD is highly recommended to ensure the stability of microbial community and system performance. In case of changing OLRs (i.e., addition new substrates and co-digestion), detailed evaluations on the maximum OLR at which the resilience of AD community could be maintained are required.

15.3.3 Cell Retention Time

CRT is an important operational parameter (Appels et al. 2008). Determination of approximate CRT has an important value on operational points. Overly long CRT could reduce the digester capacity while too short CRT could decrease the treatment efficiency because of biomass washout (Amani et al. 2010; Vanwonterghem et al. 2015).

Low CRT could result in the reduction of microbial community diversity in AD. However, no report on the change of community diversities could be retrieved from the literature at this stage. A few studies reported the shifts in the microbial community structure under different CRTs. The population of phylum *Bacteroidetes* was significantly enriched from 12.5 to 22% when CRT was decreased from 20 to 5 days (Lee et al. 2011). The enrichment of *Bacteroidetes* is consistent with the increase in OLR as lower CRT means higher OLR into the digester. This observation further reconfirms that there is favorable growth of phylum *Bacteroidetes* under high substrate levels. Meanwhile, loss of the bacteria in the phylum *Chloroflexi* occurred when CRT was decreased from 20 to 4 and 5 days. The *Chloroflexi* sp. is capable of degrading persistent organic compounds but they need longer time for the degradation.

In comparison to the bacterial community, methanogenic community is profoundly affected by low CRT due to its slow growth rate. CRT of below 5 days is insufficient for a stable digestion due to the accumulation of VFAs and washout of methanogens (Appels et al. 2008). Accumulation of propionate was observed in a digester operating at CRT of 8 days due to the washout of syntrophic propionate oxidizers (Vanwonterghem et al. 2015). Similarly, the *Archaea* gene copies significantly decreased under short CRT, indicating the washout of methanogens (Lee et al. 2011). It is recommended that CRT cutoff should be above 10 days (Ju et al. 2017; Appels et al. 2008) for a stable performance. This value is subject to change depending on substrate complexity.

15.3.4 Substrate Characteristics

In recent years, concern about climate change and energy security has renewed the interest in AD as a platform for renewable energy production for organic wastes (Nghiem et al. 2017). Arrays of organic wastes have used the AD process for biogas production enhancement. Although the benefits of AD of organic wastes have been

well documented, little is known about the effect of different substrates on AD microbial community. Thanks to the development of high-throughput sequencing technology, more studies have focused on revealing the microbial community diversity and structure in AD. This section provides a summary of such studies performed within the last 5 years on some of the most common substrates.

Lipid-rich substrates. Lipid-rich substrates are from various sources such as dairy industry, food processing industry, slaughterhouses, restaurant oil trap, and vegetable oil/fat refineries. The anaerobic hydrolysis of lipids produces mainly long-chain fatty acids (LCFA). Although this process occurs rapidly, the subsequent step of LCFA oxidation via β-oxidation process is slow. Due to the mismatch between LCFA production and consumption, AD of lipid-rich substrates influences the microbial community, specifically the methanogens.

Co-digestion of lipid-rich substrates such as fat oil and grease with sewage sludge (SS) increased both bacterial and archaeal richness (Yang et al. 2016; Ziels et al. 2016). Adding 10% (VS) of fat, oil, and grease (FOG) into the digestion of SS increased the community richness from 6.2 to 8.5 (Simpson index) (Amha et al. 2017). Bacterial community structure reacts faster than the methanogenic community does in response to the addition of fat, oil, and grease. For example, the population of bacteria involved in the hydrolysis and acidification was higher in the AcoD of fat, oil, and grease compared to the mono-digestion of SS (Fig. 15.8). The complementarity between two substrates results in the better nutrient balance and provides the greater opportunity for microbial growth over the mono-digestion. The phylum *Firmicutes* was enriched in the AcoD of fat, oil, and grease and SS from 22.9 up to 56.5, 32.6, and 10.4% with fat, oil, and grease addition increase of 1, 2, and 3 g VS, respectively. Fatty-acid oxidizing bacteria *Syntrophomonas* was significantly enriched within the bacteria community from 3 to 14% after FOG addition (Fig. 15.8a) (Ziels et al. 2016).

Significant changes in the methanogenic community were observed in the AcoD of fat, oil, and grease. Hydrogenotrophic *Methanospirillum* was enriched from 1.3 to 34% (Fig. 15.8a). Another strictly aceticlastic methanogen, *Methanosaeta*, was also out dominated in the fat, oil, and grease co-digestion (Ziels et al. 2016). The enhancement of these genera was positively correlated with biogas production, suggesting their roles in the AD of fat, oil, and grease. The Bray–Curtis dissimilarity values between the control and fat, oil, and grease digester were significantly different, suggesting the shift of the community (Fig. 15.8b). Similarly, Yang et al. (2016) demonstrated the dominance of *Methanosaeta*—a genus of *Methanosarcinales* order—in AcoD of fat, oil, and grease and SS. The abundance was positively correlated with the biogas production in the redundancy analysis (RDA).

Changes in the microbial community were found to be similar in the AD of pig/cattle slaughterhouse wastes. The enrichment of fatty-acid oxidizing bacteria such as *Syntrophomonas* sp., *Coprothermobacter* sp., and *Anaerobaculum* sp. occurred under digestion of pig/cattle slaughterhouse wasters (Palatsi et al. 2011). Palatsi

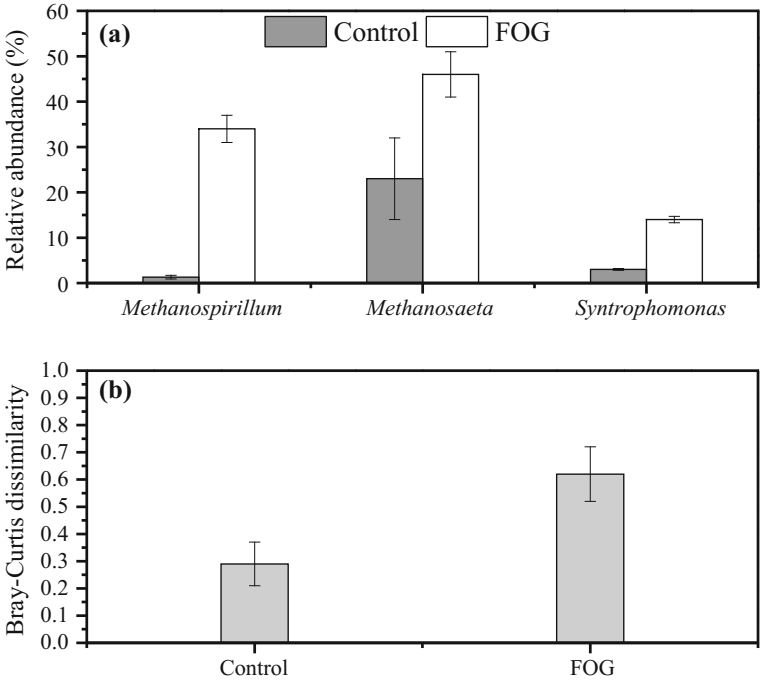

Fig. 15.8 The relative abundance of three major genera, which showed significant enriched under FOG digestion (**a**). The Bray–Curtis dissimilarity indicated the shift of methanogenic community under FOG co-digestion (**b**). Data were extracted from **a** and **b** Ziels et al. (2016)

et al. (2011) demonstrated the syntrophy between the *Syntrophomonas* and *Methanosarcina* in the digester feeding with high lipids content wastes.

Carbon-rich substrates. Addition of carbon-rich and nutrient-rich substrates in AcoD has demonstrated increased biogas production due to their complementary effects (Nghiem et al. 2017; Wickham et al. 2018). The addition of carbon-rich substrates also affects the digester community. AcoD of food waste strongly affects the microbial community diversity and structure. A gradual decline in community richness and evenness was observed upon a stepwise increase of food waste (Xu et al. 2017). Shannon index decreased from 9.42 in control to 5.21 in digester with 57% food waste addition (OLR %) together with the disappearance of 3787 Observed species. Addition of food waste induced profoundly the growth of substrate-favorable groups in the digester. The microbial community diversity and evenness decreased with food waste addition and the degree of decrease was proportional with the increased food waste ratio (Jang et al. 2016).

Two bacterial phyla, *Firmicutes* and *Actinobacteria,* showed the most distinctive change under the addition of food waste. The phylum *Firmicutes* outcompeted

others with an increase of abundance from 14.5 to 56.5% at 40% food waste addition (OLR %), but their abundance decreased to 10.4% when added food waste at 57%. On the other hand, *Actinobacteria* population gradually increased with the increase of FW addition. Their population peaked at 56%. The results suggest that bacteria of phylum *Actinobacteria* are favorable to high organic loading stress. In another study of AcoD food waste and waste activated sludge, the phylum *Bacteroidetes* increased significantly from 10.6% (control) to 39.8, 46.3 and 45.8% with food waste addition of 25, 50, and 75% (VS ratio), respectively (Jang et al. 2016). It was suggested that the substrate characteristics and mixing ratio were highly related to the development of various phyla in different studies.

Adding food waste caused the dominant population of the methanogenic community to shift from *Methanosarcina* to *Methanosaeta*. The abundance of *Methanosaeta* increased from 3.7 to 36.1%, indicating their tolerance under high OLR. Of note, the bacterial community was affected more by excessive food waste addition in comparison to the methanogenic community. This suggested the high tolerance of methanogenic community to high food waste levels, but the reasons behind this remain unclear (Xu et al. 2017).

Lignocellulose-rich substrates. Plant-based or "lignocellulosic" substrates such as grass silage, pulp and paper mills wastes, hay, bagasse, and agricultural residues are considered the most abundant raw materials for biogas production in the AD process (Anwar et al. 2014; Shrestha et al. 2017). Lignocellulose is a matrix of biopolymers including cellulose, hemicellulose, and lignin. The AD of lignocellulose-rich substrates is limited mainly due to the complex chemical structure (Shrestha et al. 2017). Cellulose is a crystalline microfibril that is insoluble and difficult to degrade. The microfibrils are attached to hemicellulose, which is a polymer of various sugars. Lignin is crosslinked with cellulose and hemicellulose providing a rigid structural support to the biopolymer matrix. To achieve the desired performance, the AD of lignocellulosic substrates often required pretreatment and co-digestion.

Only a few reports on the microbial community in the AD of lignocellulosic substrate are available. The microbial community is less diverse in the AD of lignocellulosic substrates probably due to the substrate recalcitrant that limits the growth of many microorganisms. The community in the AD treatment of xylose, xylan, and cellulose was less diverse than that of the AD treatment of food waste (Wilkins et al. 2015). The phylogenetic structure of the AD process treatment of lignocellulosic substrates mainly contains the phyla *Firmicutes* and *Bacteroidetes* (Azman et al. 2015). The phylum *Firmicutes* accounted for 97% of the total in AD of waste papers (Tsavkelova et al. 2018). The population of phyla *Firmicutes* and *Bacteroidetes* was above 65% in four different digesters treatment of corn stover (Liu et al. 2018). These observations suggest the role of bacteria in the phyla of *Firmicutes* and *Bacteroidetes* in the hydrolysis of lignocellulosic substrates.

15.3.5 Inoculum Sources

The effect of inoculum sources on the AD process has been demonstrated in a number of studies (Gu et al. 2014; Ventorino et al. 2018; Han et al. 2016; Liu et al. 2017). Gu et al. (2014) compared six different inocula including digested dairy manure, digested swine manure, digested chicken manure, digested municipal sludge, and anaerobic granular sludge. All the inocula were used in the batch digester treatment of rice straw with the same inoculum-to-substrate ratio of 1:1 (VS content). Digested dairy manure was found to be the best inoculum among six different inocula. Liu et al. (2017) observed significant differences in methane production when compared to three inocula (digested municipal sludge, digested stillage, and digested manure) in a biomethane potential test with inoculum and substrate (cellulose) ratio of 4:1 VS content.

Inoculum is microbial source for the AD process. The AD microbial community resembles their respective source of inoculum. Han et al. (2016) used four inocula from stillage, manure, paper milling, and wastewater sludge digesters. All the inocula were used for the digestion of cellulose. The results indicated similar compositions of inoculum and digester microbial community. However, the microbial community diversity decreased in the digester in comparison to the inoculum. On the other hand, De Vrieze et al. (2015a) observed that the digester community has higher richness than inoculum community. The reason is probably due to the variation in the substrates in different studies.

Liu et al. (2017) observed the effect of inoculum only at the initial state (one cell retention time). Over time, the digester performance and digester community were comparable among four different inocula sources. The authors suggested that under a long operation period, substrate characteristics and operation condition driven the digester performance and community structure rather than the inoculum sources. De Vrieze et al. (2015a) also observed that the microbial community evolved toward a similar composition in five digesters initially inoculated with five different inocula.

The effect of inoculum source on the digester community resilience has also been reported (De Vrieze et al. 2015a). De Vrieze et al. (2015a) investigated the resilience of digester inoculated with five different inocula under stress conditions (i.e., high total ammonia nitrogen). The inocula included digested potato waste (I), digested mix maize, lipid and fruit waste (II), digested mix maize and manure (III), digested municipal sludge (IV), and a mixture of abovementioned inocula (V). The results indicated that the effects of total ammonia nitrogen were inoculum dependent. The digesters inoculated with I and II maintained their methane production function, whereas methane production deteriorated in the digester with inocula III, IV, and V at the maximum total ammonia nitrogen tested. This observation is because microbes in inoculum I and II have higher level of adaptation to total ammonia nitrogen. The results suggested that the use of selected or enriched inoculum for typical substrates or operating conditions could enhance process stability.

15.4 Connecting Microbial Community to AD Performance

15.4.1 Microbial Community Diversity and AD Performance

Microbial community diversity indices (i.e., richness and evenness) could probably indicate process performance. A more richness and evenness community indicates the presence of more species that could enhance community resilience toward perturbations (Wittebolle et al. 2009; McCann 2000; Regueiro et al. 2012). On the other hand, less diverse community is susceptible to changes probably due to the high level of specialization (Regueiro et al. 2012). However, to date, the linkage between community diversity and AD performance remains unclear. Indeed, the extent influence of microbial community diversity on AD performance has not been determined. So far, results in the literature are still inconsistent. Venkiteshwaran et al. (2017) showed no correlation between digester performance (i.e., CH_4 production), community richness, and evenness. Similarly, Li et al. (2015) compared the community diversity at stable and deteriorated stages and showed no differences in diversity indices between two stages. Their results suggested that diversity indices were not sensitive for process status indication. This limitation is probably because the diversity indices are statistical data to describe the community diversity without consideration of its compositions (Li et al. 2015; Dearman et al. 2006). No correlation between Shannon index (i.e., evenness) and methane yields has been reported (Fig. 15.9) (Jang et al. 2016; Xu et al. 2017). On the other hand, some studies have claimed that the microbial community evenness relates with the digester function (Lee et al. 2017; Carballa et al. 2011). Carballa et al. (2011) demonstrated that the digester with higher evenness in the bacterial community achieved a higher biogas production. Lee et al. (2017) observed a positive correlation between bacterial evenness and COD removal. Wittebolle et al. (2009) reported that the initial community richness and evenness were the key factors to preserve the community function under perturbation conditions, although this study was not done with the AD process. Due to the inconsistency, richness and evenness index need to be carefully considered as process indicators. More data from the future studies, especially on those that use high-throughput sequencing technologies, are needed to unmask potential trends. It is also suggested that future studies should focus on the methanogenic community given that it has lower diversity in comparison to bacterial community. Until then, the finding of the community diversity and digester performance relationship can be used to indicate proactive AD performance.

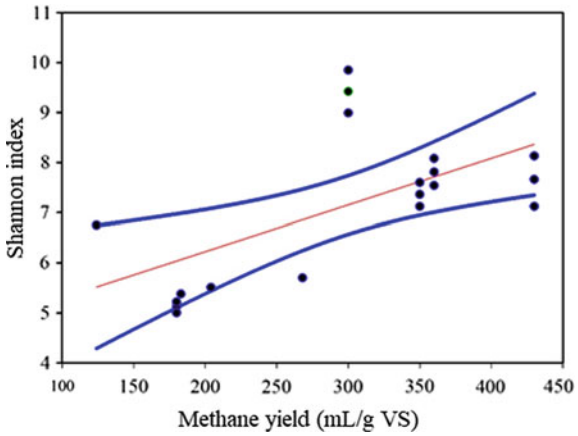

Fig. 15.9 A linear regression analysis of Shannon index and methane yield with ±95% confidence intervals. The data retrieved from Jang et al. (2016) and Xu et al. (2017). The plot (R^2 = 0.37) indicates diversity index that cannot be used to predict digester performance. The red line and the space between two blue curves are the linear regression and the boundary of ±95% confidence intervals, respectively

15.4.2 Microbial Community Structure and AD Performance

Information on the relationship between AD microbial community structure and its function has gained much attention recently as they can be applied to potentially engineered AD with superior functions (Werner et al. 2011) or to indicate process stability (de Jonge et al. 2017).

The AD microbial community naturally shows degrees of variation at constant operating conditions. In other words, there is a degree of variation in community population in a functionally stable community. Variation (i.e., presence and/or variation of specific organisms) due to changes in environmental variables must be larger than naturally occurring changes. Previous sections in this chapter have defined a list of genera that showed significant variation under changes in environmental variables. These include phyla of *Firmicutes*, *Bacteroidetes,* and *Actinobacteria* (hydrolytic and fermentative bacteria), *Syntrophomonas* and *Synergistetes* (acetogenic and syntrophic acetate oxidizing) and *Methanosaeta*, and *Methanoculleus* (methanogens). Consequently, a few studies have reported the linkage between microbial community structure and AD performance.

Analyzing the microbial community compositions at stable and deteriorative stage, Li et al. (2015) revealed the correlation between microbial community structure and process stability. Syntrophic fatty oxidizing and acid producing bacteria outcompeted other bacteria at the deteriorative stage. The mismatch between acid production and consumption were accounted for system deterioration. Regueiro et al. (2012) observed that hydrolytic and methanogenic activities linked

with the high abundance of *Bacteroidetes* and *Archaea*. Lignocellulose-degrading microorganism population was correlated with biogas production in the AcoD of food waste and wheat straw (Shi et al. 2018). Yang et al. (2016) provided the details positive correlations between the *Methanosaeta* and biogas production. The hydrogenotrophic *Methanobacteriales* correlated with biogas production in 29 full-scale digester studies, confirming their role in maintenance of digester function (De Vrieze et al. 2015b). Negative correlation between community structure and AD performance has also been reported. High level of VFAs led to the reduction of syntrophic acetogenic bacteria (Peng et al. 2018). Ziels et al. (2016) suggested to track the syntrophic LCFA-degrading bacteria abundance to regulate the loading rate of fat, oil, and grease into the AD. Understanding the linkages between community structure and AD performance provide estimation of thresholds at which the function and resilience of the AD process are maintained.

15.5 Summary and Future Outlook

This chapter reviews recent literature to provide new insights into microbial ecology in the AD process. The information include microbial community driven the AD process, factors influencing the microbial community diversity and structure, and the linkages between microbial community and AD performance. The available studies suggest that the community diversity and structure are different among digesters. This may be due to the greater variety of abiotic factors such as temperature, OLR, CRT, substrate characteristics, and in biotic factor such as inoculum sources among digesters. Despite these variations, some common observations from this chapter are the following:

(i) Bacteria in the phyla of *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* dominate the AD microbial community.
(ii) The population of bacteria in the phyla of *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* varies profoundly when the AD process experiences a changing condition.
(iii) Altering the operating conditions (e.g., increased OLR and adding co-substrates) positively affects the microbial community diversity and structure beyond a threshold.
(iv) The methanogenic community is more susceptible to environmental variables in comparison to the bacterial community. The reason is mainly due to the higher diversity and functional redundancy of the bacterial community.

Understanding the connection between microbial community and AD performance can provide intuitive information for optimization of the AD process. Future interactions between microbial ecologists and environmental engineers in combination with the availability of new methods to characterize microbial community could offer opportunities to integrate microbial community and performance into a

unified picture. This information could be used to design, maintain, and operate a more efficient AD.

References

Amani T, Nosrati M, Sreekrishnan TR (2010) Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects—a review. Environ Rev 18:255–278

Amha YM, Sinha P, Lagman J, Gregori M, Smith AL (2017) Elucidating microbial community adaptation to anaerobic co-digestion of fats, oils, and grease and food waste. Water Res 123:277–289

Anwar Z, Gulfraz M, Irshad M (2014) Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. J Radiat Res Appl Sci 7:163–173

Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. Prog Energy Combust Sci 34:755–781

Azman S, Khadem AF, van Lier JB, Zeeman G, Plugge CM (2015) Presence and role of anaerobic hydrolytic microbes in conversion of lignocellulosic biomass for biogas production. Crit Rev Environ Sci Technol 45:2523–2564

Braun M, Mayer F, Gottschalk G (1981) *Clostridium aceticum* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. Arch Microbiol 128:288–293

Carballa M, Smits M, Etchebehere C, Boon N, Verstraete W (2011) Correlations between molecular and operational parameters in continuous lab-scale anaerobic reactors. Appl Microbiol Biotechnol 89:303–314

Carballa M, Regueiro L, Lema JM (2015) Microbial management of anaerobic digestion: exploiting the microbiome-functionality nexus. Curr Opin Biotechnol 33:103–111

Cazier EA, Trably E, Steyer JP, Escudie R (2015) Biomass hydrolysis inhibition at high hydrogen partial pressure in solid-state anaerobic digestion. Bioresour Technol 190:106–113

Chen S, He Q (2015) Persistence of *Methanosaeta* populations in anaerobic digestion during process instability. J Ind Microbiol Biotechnol 42:1129–1137

Chen Z, Wang Y, Li K, Zhou H (2014) Effects of increasing organic loading rate on performance and microbial community shift of an up-flow anaerobic sludge blanket reactor treating diluted pharmaceutical wastewater. J Biosci Bioeng 118:284–288

de Jonge N, Moset V, Møller HB, Nielsen JL (2017) Microbial population dynamics in continuous anaerobic digester systems during start up, stable conditions and recovery after starvation. Bioresour Technol 232:313–320

De Vrieze J, Gildemyn S, Vilchez-Vargas R, Jáuregui R, Pieper DH, Verstraete W, Boon N (2015a) Inoculum selection is crucial to ensure operational stability in anaerobic digestion. Appl Microbiol Biotechnol 99:189–199

De Vrieze J, Saunders AM, He Y, Fang J, Nielsen PH, Verstraete W, Boon N (2015b) Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome. Water Res 75:312–323

Dearman B, Marschner P, Bentham RH (2006) Methane production and microbial community structure in single-stage batch and sequential batch systems anaerobically co-digesting food waste and biosolids. Appl Microbiol Biotechnol 69:589–596

Ferguson RMW, Coulon F, Villa R (2016) Organic loading rate: a promising microbial management tool in anaerobic digestion. Water Res 100:348–356

Gagliano MC, Braguglia CM, Gianico A, Mininni G, Nakamura K, Rossetti S (2015a) Thermophilic anaerobic digestion of thermal pretreated sludge: role of microbial community structure and correlation with process performances. Water Res 68:498–509

Gagliano MC, Braguglia CM, Gallipoli A, Gianico A, Rossetti S (2015b) Microbial diversity in innovative mesophilic/thermophilic temperature-phased anaerobic digestion of sludge. Environ Sci Pollut Res 22:7339–7348

Ghasimi DSM, Tao Y, de Kreuk M, Zandvoort MH, van Lier JB (2015) Microbial population dynamics during long-term sludge adaptation of thermophilic and mesophilic sequencing batch digesters treating sewage fine sieved fraction at varying organic loading rates. Biotechnol Biofuels 8:171

Gómez E, Martin J, Michel FC (2011) Effects of organic loading rate on reactor performance and archaeal community structure in mesophilic anaerobic digesters treating municipal sewage sludge. Waste Manage Res 29:1117–1123

Gu Y, Chen X, Liu Z, Zhou X, Zhang Y (2014) Effect of inoculum sources on the anaerobic digestion of rice straw. Bioresour Technol 158:149–155

Guo X, Wang C, Sun F, Zhu W, Wu W (2014) A comparison of microbial characteristics between the thermophilic and mesophilic anaerobic digesters exposed to elevated food waste loadings. Bioresour Technol 152:420–428

Guo J, Peng Y, Ni B-J, Han X, Fan L, Yuan Z (2015) Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing. Microb Cell Fact 14:33

Han S, Liu Y, Zhang S, Luo G (2016) Reactor performances and microbial communities of biogas reactors: effects of inoculum sources. Appl Microbiol Biotechnol 100:987–995

Hanreich A, Schimpf U, Zakrzewski M, Schlüter A, Benndorf D, Heyer R, Rapp E, Pühler A, Reichl U, Klocke M (2013) Metagenome and metaproteome analyses of microbial communities in mesophilic biogas-producing anaerobic batch fermentations indicate concerted plant carbohydrate degradation. Syst Appl Microbiol 36:330–338

Hattori S, Kamagata Y, Hanada S, Shoun H (2000) *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. Int J Syst Evol Microbiol 50:1601–1609

Holmes DE, Smith JA (2016). Biologically produced methane as a renewable energy source. In: Sariaslani S, Michael Gadd G (eds) Advances in applied microbiology, vol 97. Academic Press, pp 1–61 (Chapter 1)

Izquierdo JA, Sizova MV, Lynd LR (2010) Diversity of bacteria and glycosyl hydrolase family 48 genes in cellulolytic consortia enriched from thermophilic biocompost. Appl Environ Microbiol 76:3545–3553

Jang HM, Kim M-S, Ha JH, Park JM (2015) Reactor performance and methanogenic archaea species in thermophilic anaerobic co-digestion of waste activated sludge mixed with food wastewater. Chem Eng J 276:20–28

Jang HM, Ha JH, Kim M-S, Kim J-O, Kim YM, Park JM (2016) Effect of increased load of high-strength food wastewater in thermophilic and mesophilic anaerobic co-digestion of waste activated sludge on bacterial community structure. Water Res 99:140–148

Ju F, Lau F, Zhang T (2017) Linking microbial community, environmental variables, and methanogenesis in anaerobic biogas digesters of chemically enhanced primary treatment sludge. Environ Sci Technol 51:3982–3992

Kirkegaard RH, McIlroy SJ, Kristensen JM, Nierychlo M, Karst SM, Dueholm MS, Albertsen M, Nielsen PH (2017) The impact of immigration on microbial community composition in full-scale anaerobic digesters. Sci Rep 7:9343

Kouzuma A, Tsutsumi M, Ishii SI, Ueno Y, Abe T, Watanabe K (2017) Non-autotrophic methanogens dominate in anaerobic digesters. Sci Rep 7:1510

Kundu K, Sharma S, Sreekrishnan TR (2013) Changes in microbial communities in a hybrid anaerobic reactor with organic loading rate and temperature. Bioresour Technol 129:538–547

Labatut RA, Angenent LT, Scott NR (2014) Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance and stability? Water Res 53:249–258

Lamed R, Setter E, Bayer EA (1983) Characterization of a cellulose-binding, cellulase-containing complex in *Clostridium thermocellum*. J Bacteriol 156:828–836

Lee I-S, Parameswaran P, Rittmann BE (2011) Effects of solids retention time on methanogenesis in anaerobic digestion of thickened mixed sludge. Bioresour Technol 102:10266–10272

Lee S-H, Park J-H, Kim S-H, Yu BJ, Yoon J-J, Park H-D (2015) Evidence of syntrophic acetate oxidation by Spirochaetes during anaerobic methane production. Bioresour Technol 190:543–549

Lee J, Shin SG, Han G, Koo T, Hwang S (2017) Bacteria and archaea communities in full-scale thermophilic and mesophilic anaerobic digesters treating food wastewater: key process parameters and microbial indicators of process instability. Bioresour Technol 245:689–697

Lerm S, Kleyböcker A, Miethling-Graff R, Alawi M, Kasina M, Liebrich M, Würdemann H (2012) Archaeal community composition affects the function of anaerobic co-digesters in response to organic overload. Waste Manage 32:389–399

Li A, Chu YN, Wang X, Ren L, Yu J, Liu X, Yan J, Zhang L, Wu S, Li S (2013) A pyrosequencing-based metagenomic study of methane-producing microbial community in solid-state biogas reactor. Biotechnol Biofuels 6:3

Li L, He Q, Ma Y, Wang X, Peng X (2015) Dynamics of microbial community in a mesophilic anaerobic digester treating food waste: relationship between community structure and process stability. Bioresour Technol 189:113–120

Liu T, Sun L, Müller B, Schnürer A (2017) Importance of inoculum source and initial community structure for biogas production from agricultural substrates. Bioresour Technol 245:768–777

Liu C, Wachemo AC, Tong H, Shi S, Zhang L, Yuan H, Li X (2018) Biogas production and microbial community properties during anaerobic digestion of corn stover at different temperatures. Bioresour Technol 261:93–103

McCann KS (2000) The diversity–stability debate. Nature 405:228

Moset V, Poulsen M, Wahid R, Højberg O, Møller HB (2015) Mesophilic versus thermophilic anaerobic digestion of cattle manure: methane productivity and microbial ecology. Microb Biotechnol 8:787–800

Nanninga HJ, Gottschal JC (1987) Properties of *Desulfovibrio carbinolicus* sp. nov. and other sulfate-reducing bacteria isolated from an anaerobic-purification plant. Appl Environ Microbiol 53:802–809

Nghiem LD, Koch K, Bolzonella D, Drewes JE (2017) Full scale co-digestion of wastewater sludge and food waste: bottlenecks and possibilities. Renew Sustain Energ Rev 72:354–362

Nielsen HB, Uellendahl H, Ahring BK (2007) Regulation and optimization of the biogas process: propionate as a key parameter. Biomass Bioenergy 31:820–830

Niu Q, Takemura Y, Kubota K, Li Y-Y (2015) Comparing mesophilic and thermophilic anaerobic digestion of chicken manure: microbial community dynamics and process resilience. Waste Manage 43:114–122

Ortseifen V, Stolze Y, Maus I, Sczyrba A, Bremges A, Albaum SP, Jaenicke S, Fracowiak J, Pühler A, Schlüter A (2016) An integrated metagenome and -proteome analysis of the microbial community residing in a biogas production plant. J Biotechnol 231:268–279

Palatsi J, Viñas M, Guivernau M, Fernandez B, Flotats X (2011) Anaerobic digestion of slaughterhouse waste: main process limitations and microbial community interactions. Bioresour Technol 102:2219–2227

Pender S, Toomey M, Carton M, Eardly D, Patching JW, Colleran E, O'Flaherty V (2004) Long-term effects of operating temperature and sulphate addition on the methanogenic community structure of anaerobic hybrid reactors. Water Res 38:619–630

Peng X, Zhang S, Li L, Zhao X, Ma Y, Shi D (2018) Long-term high-solids anaerobic digestion of food waste: effects of ammonia on process performance and microbial community. Bioresour Technol 262:148–158

Rademacher A, Zakrzewski M, Schlüter A, Schönberg M, Szczepanowski R, Goesmann A, Pühler A, Klocke M (2012) Characterization of microbial biofilms in a thermophilic biogas system by high-throughput metagenome sequencing. FEMS Microbiol Ecol 79:785–799

Razaviarani V, Buchanan ID (2014) Reactor performance and microbial community dynamics during anaerobic co-digestion of municipal wastewater sludge with restaurant grease waste at steady state and overloading stages. Bioresour Technol 172:232–240

Razaviarani V, Buchanan ID (2015) Anaerobic co-digestion of biodiesel waste glycerin with municipal wastewater sludge: microbial community structure dynamics and reactor performance. Bioresour Technol 182:8–17

Regueiro L, Veiga P, Figueroa M, Alonso-Gutierrez J, Stams AJM, Lema JM, Carballa M (2012) Relationship between microbial activity and microbial community structure in six full-scale anaerobic digesters. Microbiol Res 167:581–589

Regueiro L, Lema JM, Carballa M (2015) Key microbial communities steering the functioning of anaerobic digesters during hydraulic and organic overloading shocks. Bioresour Technol 197:208–216

Rivière D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach J, Li T, Camacho P, Sghir A (2009) Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. The ISME J 3:700

Shaw GT-W, Liu A-C, Weng C-Y, Chou C-Y, Wang D (2017) Inferring microbial interactions in thermophilic and mesophilic anaerobic digestion of hog waste. PLoS ONE 12:e0181395

Shi X, Guo X, Zuo J, Wang Y, Zhang M (2018) A comparative study of thermophilic and mesophilic anaerobic co-digestion of food waste and wheat straw: process stability and microbial community structure shifts. Waste Manage 75:261–269

Shiratori H, Ikeno H, Ayame S, Kataoka N, Miya A, Hosono K, Beppu T, Ueda K (2006) Isolation and characterization of a new *Clostridium* sp. that performs effective cellulosic waste digestion in a thermophilic methanogenic bioreactor. Appl Environ Microbiol 72:3702–3709

Shrestha S, Fonoll X, Khanal SK, Raskin L (2017) Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: current status and future perspectives. Bioresour Technol 245:1245–1257

Sun L, Müller B, Westerholm M, Schnürer A (2014) Syntrophic acetate oxidation in industrial CSTR biogas digesters. J Biotechnol 171:39–44

Sun M-T, Fan X-L, Zhao X-X, Fu S-F, He S, Manasa MRK, Guo R-B (2017) Effects of organic loading rate on biogas production from macroalgae: performance and microbial community structure. Bioresour Technol 235:292–300

Tsavkelova E, Prokudina L, Egorova M, Leontieva M, Malakhova D, Netrusov A (2018) The structure of the anaerobic thermophilic microbial community for the bioconversion of the cellulose-containing substrates into biogas. Process Biochem 66:183–196

Vanwonterghem I, Jensen PD, Ho DP, Batstone DJ, Tyson GW (2014) Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. Curr Opin Biotechnol 27:55–64

Vanwonterghem I, Jensen PD, Rabaey K, Tyson GW (2015) Temperature and solids retention time control microbial population dynamics and volatile fatty acid production in replicated anaerobic digesters. Sci Rep 5:8496

Venkiteshwaran K, Milferstedt K, Hamelin J, Fujimoto M, Johnson M, Zitomer DH (2017) Correlating methane production to microbiota in anaerobic digesters fed synthetic wastewater. Water Res 110:161–169

Ventorino V, Romano I, Pagliano G, Robertiello A, Pepe O (2018) Pre-treatment and inoculum affect the microbial community structure and enhance the biogas reactor performance in a pilot-scale biodigestion of municipal solid waste. Waste Manage 73:69–77

Wang P, Wang H, Qiu Y, Ren L, Jiang B (2018) Microbial characteristics in anaerobic digestion process of food waste for methane production—a review. Bioresour Technol 248:29–36

Werner JJ, Knights D, Garcia ML, Scalfone NB, Smith S, Yarasheski K, Cummings TA, Beers AR, Knight R, Angenent LT (2011) Bacterial community structures are unique and resilient in full-scale bioenergy systems. Proc Natl Acad Sci 108:4158

Westerholm M, Roos S, Schnürer A (2010) S*yntrophaceticus schinkii* gen. nov., sp. nov., an anaerobic, syntrophic acetate-oxidizing bacterium isolated from a mesophilic anaerobic filter. FEMS Microbiol Lett 309:100–104

Wickham R, Xie S, Galway B, Bustamante H, Nghiem LD (2018) Anaerobic digestion of soft drink beverage waste and sewage sludge. Bioresour Technol 262:141–147

Wilkins D, Rao S, Lu X, Lee PKH (2015) Effects of sludge inoculum and organic feedstock on active microbial communities and methane yield during anaerobic digestion. Front Microbiol 6:1114

Wittebolle L, Marzorati M, Clement L, Balloi A, Daffonchio D, Heylen K, De Vos P, Verstraete W, Boon N (2009) Initial community evenness favours functionality under selective stress. Nature 458:623

Wu Y-R, He J (2013) Characterization of anaerobic consortia coupled lignin depolymerization with biomethane generation. Bioresour Technol 139:5–12

Xu R, Yang Z-H, Zheng Y, Zhang H-B, Liu J-B, Xiong W-P, Zhang Y-R, Ahmad K (2017) Depth-resolved microbial community analyses in the anaerobic co-digester of dewatered sewage sludge with food waste. Bioresour Technol 244:824–835

Yang Z-H, Xu R, Zheng Y, Chen T, Zhao L-J, Li M (2016) Characterization of extracellular polymeric substances and microbial diversity in anaerobic co-digestion reactor treated sewage sludge with fat, oil, grease. Bioresour Technol 212:164–173

Ziels RM, Karlsson A, Beck DAC, Ejlertsson J, Yekta SS, Bjorn A, Stensel HD, Svensson BH (2016) Microbial community adaptation influences long-chain fatty acid conversion during anaerobic codigestion of fats, oils, and grease with municipal sludge. Water Res 103:372–382