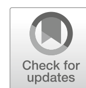
Chapter 7
Microbial Community Dynamics in Anaerobic Digesters for Biogas Production



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Abstract Microbial communities including bacteria and archaea in anaerobic digestion (AD) process play a crucial role in biogas production. In microbial communities, a deep understanding is required related to microbial diversity, composition, abundance, interactions, and its behavior to produce biogas. In addition, their active genes, proteins, and active metabolic products also enhance the

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productivity of AD process. Increased valuable products and biogas production can be carried out by optimizing the process parameters. The optimization can be visually seen by microbial diversity by performing metagenomic, meta-transcriptomic, meta-proteomic, and metabolomic data. However, the abundance and diversity of microbial communities are easily evaluated by high-throughput sequencing and a suitable bioinformatics analysis which are critically important for overall process. This chapter includes the microbial diversity and abundance presented in various bioreactors, process optimization factors and its impact on the biogas production. Thus, metagenomic data-based fermentation or AD can be proposed by integrating the bioinformatic data of microbial communities with their performance on the anaerobic digesters to facilitate the process improvement and higher generation of energy and value-added products.

Keywords Anaerobic digestion · Archaea · Biogas · Bacterial diversity · Methanogens

7.1 Introduction

Bio-methanation is a microbiological process of anaerobic digestion (AD) or degradation of organic materials which causes the production of biogas. It comprises a mixture of methane and carbon dioxide. It mainly occurs in natural environments, such as landfills, rice fields, sediments, and intestinal tracts of animals, where light and inorganic electron acceptors (oxygen, nitrate, sulfate, iron, etc.) are not present or limiting (Hattori 2008). The AD process is a multistep complex process performed by the combined action of three major physiological groups of microorganisms (Hattori 2008): hydrolytic–acidogenic bacteria, syntrophic–acetogenic bacteria, and methanogenic archaea. These fermenting microorganisms decompose the biopolymers (lipids, proteins, nucleic acids, carbohydrates, etc.) to soluble monomers (long-chain fatty acids, glycerol, amino acids, purines, pyrimidines, monosugars, etc.) that are further converted to short chain fatty acids (butyrate, propionate, acetate, etc.), alcohols (ethanol and methanol), hydrogen, and carbon dioxide by the same microbes. Short chain fatty acids and also alcohols are oxidized by proton-reducing syntrophic acetogens to hydrogen, acetate, formate, and carbon dioxide. These end products are ultimately transformed to methane and carbon dioxide by the methanogenic archaea (Thauer et al. 2008).

As shown in Fig. 7.1, AD is a multistep process which includes the number of microbial interrelationships and dependencies. Individual processes are kinetically nonlinear with respect to substrate concentration and inhibitors, and under most circumstances, either hydrolysis or acetolactic methanogenesis is the rate-limiting process. This is not a fixed rule, and under certain conditions (e.g., highly loaded

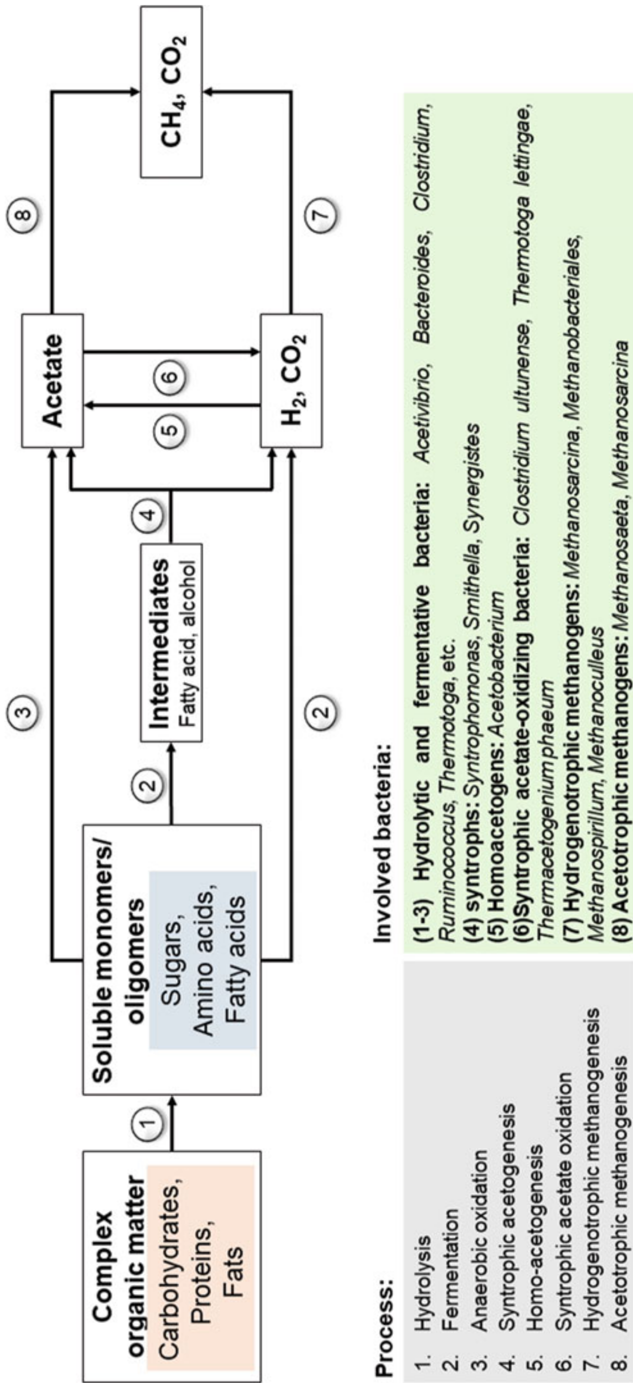

Fig. 7.1 Detailed process flow of anaerobic digestion with involved bacterial communities (Khanal 2011)

glucose-fed systems), a buildup of hydrogen can prevent acetogenesis from occurring (Angelidaki et al. 2011).

7.1.1 Hydrolysis

In AD of complex materials, the term hydrolysis is used to describe a wide range of depolymerization and solubilization processes by which complex polymeric organic compounds are broken down into soluble monomers. Most of these reactions, such as carbohydrate, polypeptide, triglyceride, and nucleic acid hydrolysis, are true hydrolysis processes, while others (e.g., scission of disulfide bonds) are reductive or oxidative bio-transformations (Sevier and Kaiser 2002). The three main primary substrates (biopolymers) for hydrolysis are carbohydrates, lipids, and proteins, which hydrolyze to monosaccharides, long-chain fatty acids plus glycerol, and amino acids, respectively. In waste or feedstreams to anaerobic digesters, materials can either be a mixture of the three substrates. Hydrolysis is widely regarded as the rate-limiting step of degradation of particulate organic matter (e.g., manure, sewage sludge, crop residues, etc.) (Pavlostathis and Giraldo-Gomez 1991). Therefore, the overall rate of the process is determined by the hydrolysis rate of this complex substrate. There are also particular considerations for different primary materials, classified as biofibers, carbohydrates, proteins, and fats. The most common composite feed is waste-activated sludge from sewage treatment plants. This is a mixture of microbial material, decay products, and inert materials originating from the feed. The degradation of decay products and inerts is generally limited in anaerobic processes, and hence the degradability of waste-activated sludge depends heavily on upstream properties (Ekama et al. 2007). Proteins and lipids are generally found together in streams such as originating from meat processing. Protein degradation depends on protein structure, with semi-soluble globular proteins being highly degradable, and fibrous proteins being relatively difficult to hydrolyze (McInerney et al. 2008). Lipids are normally triglycerides, which are hydrolyzed by lipases. Hydrolysis rates of lipids depends less on the chemical properties of the substrate and more on particle size and environmental conditions such as pH and surface tension (Yang et al. 2009).

7.1.2 Fermentation

Fermentation is anaerobic conversion of organic materials in the absence of inorganic electron acceptors such as sulfate, nitrate, or oxygen. Reduction of protons to form hydrogen may take place, but this is generally facultative. This is in contrast to degradation of propionate or butyrate to acetate and hydrogen, a process more properly referred to as anaerobic oxidation. A wide range of substrates can be fermented, including monosaccharides, amino acids, unsaturated fatty acids,

glycerol, and halogenated organics (Madigan et al. 2008). However, the most abundant substrates for fermentation, and a primary route for carbon flux, are monosaccharides and amino acids. Fermentation of amino acids and monosaccharides has common elements, in that both fermentative processes are relatively energy rich and rapid. They have a wide range of operating conditions in terms of pH and oxidation/reduction potential (Batstone et al. 2002; Madigan et al. 2008; Ramsay and Pullammanappallil 2001).

Monosaccharides: Monosaccharides ferment via the Embden–Meyerhof–Parnas (EMP) or Entner Doudoroff (ED) pathway, and subsequently to C3 products (lactate and propionate) or C2/C4/C6 products (acetate/butyrate/caproate) via acetyl-CoA. C3 products are uncommon, except under overload conditions, and the most common products are acetate, butyrate, and ethanol, with waste carbon converting to carbon dioxide, and excess electrons to hydrogen gas (Rodríguez et al. 2006). There is some dispute as to whether ethanol production is enhanced at low (Ren et al. 1997) or high pH (Temudo et al. 2008).

Amino acids: Amino acids can act as electron acceptor, donor, or in some cases as both (Ramsay and Pullammanappallil 2001). Glutamate fermentation is an example of uncoupled amino acid degradation (Buckel 2001).

7.1.3 Acetogenesis

Acetogenesis refers to the synthesis of acetate, which includes the formation of acetate by the reduction of carbon dioxide and the formation of acetate from organic acids. Hydrogen-utilizing acetogens, previously also termed homoacetogens, are strict anaerobic bacteria that can use the acetyl-CoA pathway as (1) their predominant mechanism for the reductive synthesis of acetyl-CoA from carbon dioxide, (2) terminal electron-accepting, energy-conserving process, and (3) mechanism for the synthesis of cell carbon from carbon dioxide (Drake 1994). These bacteria compete with methanogens for substrates like hydrogen, formate, and methanol.

Organic acids (such as propionate and butyrate) and alcohols (such as ethanol) produced during the fermentation step are oxidized to acetate by hydrogen-producing acetogens. Electrons produced from this oxidation reaction are transferred to protons to produce hydrogen or bicarbonate to generate formate. The term obligate means that the primary substrate cannot be used as alternative electron acceptor, and electrons must be wasted to hydrogen ions or carbon dioxide, with generally unfavorable energetics (Stams and Plugge 2009).

Acetogens that oxidize organic acids obligately use hydrogen ions and carbon dioxide as electron acceptor. Acetogenic bacteria are limited by the unfavorable energetics of the conversion processes (Schink and Stams 2006). Figure 7.1 illustrates the conversion of propionate and butyrate, important intermediates in anaerobic fermentations of complex organic matter, to the methanogenic substrates, acetate and hydrogen. Figure 7.2 shows the involvement of multi-species for the conversion of acid to methane. It is evident that bacteria can only derive energy for

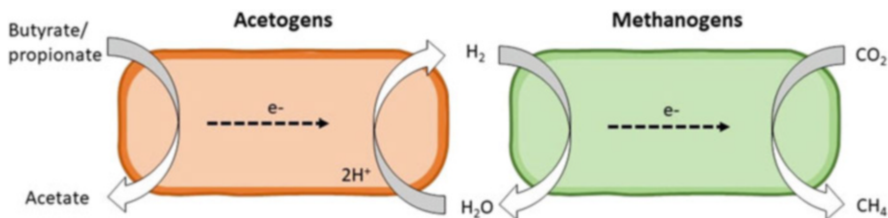

Fig. 7.2 Schematic for electron transfer within inter-bacterial species, i.e., acetogens to methanogens

growth from these conversions if the concentration of the products is kept low. This results in an obligate dependence of acetogenic bacteria on methanogenic archaea or other hydrogen scavengers (e.g., sulfate reducers) for product removal (McInerney et al. 2008; Sousa et al. 2009; Stams and Plugge 2009).

7.1.4 Methanogenesis

Methanogenic bacteria and archaea are responsible for the final step in AD process which is methane formation from acetate and/or from carbon dioxide and hydrogen, formate, alcohols, and methylated C1 compounds (Thauer et al. 2008). These types of organisms are strictly anaerobic microorganisms and their abundance in environment is varied from place to place. It is because of the need of external electron acceptors such as O_2, NO_3, Fe^{3+}, and SO_4^{2-}, which are limited. Common habitats for those archaea are anoxic marine and freshwater sediments, gastrointestinal tracts of ruminants and insects, anaerobic digesters, hot springs, and flooded soils (Nguyen et al. 2019).

Methanogens have a unique metabolism involving a number of unique enzymes and coenzymes (Deppenmeier 2002). The most interesting feature is that none of the methanogenic archaea can utilize energy from substrate level phosphorylation, and ATP is probably generated by a proton motive force and, for hydrogenotrophic methanogens, by a sodium motive force (Boone et al. 1993).

7.2 Microbial Communities and the Process

In AD process, mainly bacterial and archaeal communities are observed which are mainly responsible for the fermentation or degradation of polymeric substances and its final conversion into methane gas.

7.2.1 Bacteria

Various genuses of Firmicutes such as *Acetovibrio, clostridium, leuconostoc* and *lactobacillus* are generally observed in the AD process. Proteobacteria, *Spirochaetes, Cloacimonetes,* and *Bacteroides* are also most prominent phyla which are presented in the AD process. The major function of these phyla is to convert the polymers into various monomers and monomers to organic acids such as acetic acid, lactic acid, etc. Some bacteria in the AD process are responsible for various other metabolisms, i.e., sulfur reduction, nitrate reduction, metal stress, contaminant reduction, and antibiotic-resistant metabolisms which are not linked or related to the energy or biomethane metabolisms (Angelidaki et al. 2011).

7.2.2 Archaea

Mostly methanogenic microorganisms belong to the Archaea domain, phylum Euryarchaeota. Six phyla of methanogens have been identified, which are: *Methanosarcinales, Methanobacteriales, Methanomicrobiales, Methanococcales*, and *Methanopyrales*. *Methanocellales* (Angelidaki et al. 2011; Sakai et al. 2008). Methanogens are remarkably diverse with respect to cell morphology—from regular and irregular coccoidal cell shape (*Methanococcales, Methanomicrobiales* sp.) to short rods (*Methanobacteriales*, *Methanopyrales*) and long filaments (*Methanosaetacea* sp. within *Methanosarcinales*) (Gao et al. 2019).

7.2.3 AD Process

AD process is comparatively complex which includes more than three types of bacteria or archaea which convert the complex organic matter to methane. The whole process is shown in Fig. 7.3. Initially, hydrolytic bacteria hydrolyze the complex organic matter and then convert into soluble monomers or oligomers. These compounds directly engulfed by the fermentative bacteria which convert into volatile fatty acids and other intermediates. Thereafter, acetogens convert it into acids like acetic acid, lactic acid, propionic acid, etc. Other syntrophic acetate oxidizing bacteria degrade the acids into carbon dioxide and hydrogen gas which is further utilized by hydrogenotrophic methanogens. Sometimes, homoacetogens use carbon dioxide and hydrogen to synthesize the acetic acid which is further degraded by acetomorphic methanogens (Angelidaki et al. 2011; Nguyen et al. 2019; Sun et al. 2016).

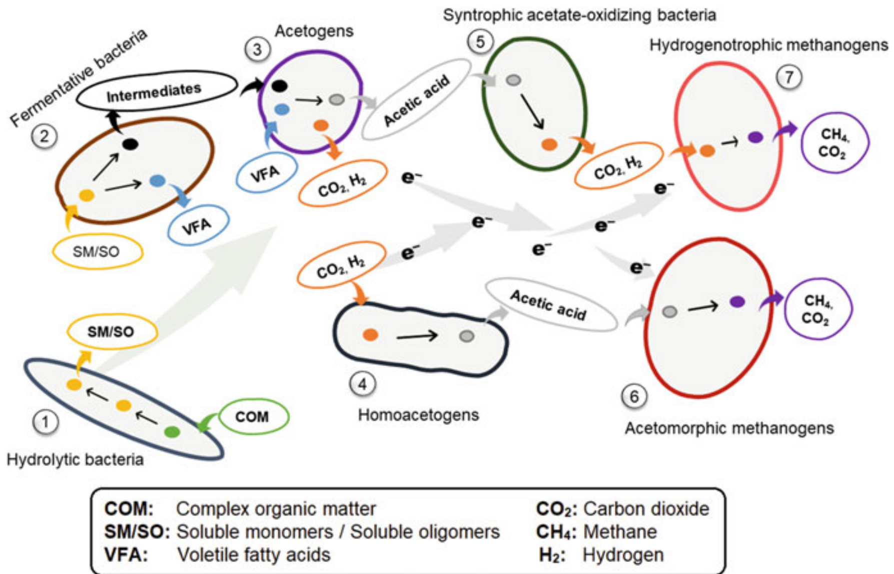

Fig. 7.3 AD process is conducted from complex organic matter to methane synthesis via a series of bacteria and intermediate process, wherein process is started from 1 to 6 intermediate process. (1) conversion of organic complex matter to soluble monomers/oligomers by hydrolytic bacteria, (2) conversion of soluble monomers/oligomers to volatile fatty acids by fermentative bacteria, (3) conversion of intermediate metabolites and volatile fatty acids to acetic acid, CO_2 and H_2 by synotrophs or acetogens, (4) conversion of CO_2 and H_2 to acetic acid by homoacetogens, (5) conversion of acetic acid to CO_2 and H_2 gases by syntrophic acetate oxidizing bacterial, (6) conversion of acetic acid to CH_4 and CO_2 by acetomorphic methanogens, and (7) conversion of CO_2 and H_2 to CH_4 and CO_2 gases by hydrogenotrophic methanogens

7.3 Bioinformatics Analysis

Microbial communities of anaerobic digester can be analyzed via metagenomic and bioinformatics approaches which include a series of steps (Fig. 7.4) (Zhang et al. 2019).

7.3.1 Sample Collection

Sample collection can be carried out from various AD processes which is mainly dependent on the different feedstock. The most dominant feedstock are food waste (>30%), sludge waste (22.8%), manure (20.3%), agricultural and horticultural waste (15.2%) (Suhartini et al. 2014; Zhang et al. 2019).

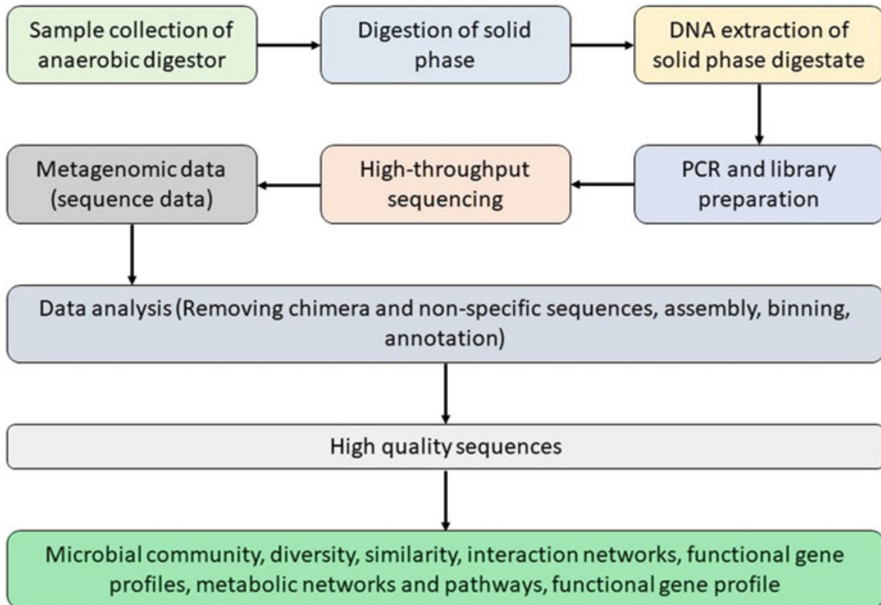

Fig. 7.4 Flowchart of microbial community analysis of anaerobic digesters which is carried out using metagenomic and bioinformatics approaches

7.3.2 DNA Extraction, PCR, and Library Preparation

DNA extraction is a prerequisite for performing bioinformatics analysis of metagenomic data of microbial communities. Prior to DNA extraction, the digestate should be centrifuged for 10 min and the supernatant should be decanted. Then, a certain amount (0.3–0.5 g) of wet weight sludge material is used to extract the DNA of microbial communities. Thereafter, PCR can be done using 16S rRNA or specific primers which are provided by the distributor. After obtaining the PCR products, they are cloned into specific vectors and the vector library has been prepared using vector cloning methods (Ju and Zhang 2015; Zhang et al. 2019).

7.3.3 Sequencing

Next-generation sequencing (NGS) technologies are competent to perform qualitative and quantitative analysis of microbial communities from different environments. The analysis is carried out fast and is cheap. The most frequently used DNA sequencing method is based on the Roche GS FLX454 pyrosequencing platform (Sun et al. 2016). However, some NGS techniques are based on Illumina sequencing platform (Zhang et al. 2017), ABI SOLiD™ short-read DNA sequencing

platform (Sträuber et al. 2016), and ABI analysis reagents coupled with Applied Biosystems 3130xl (Zhang et al. 2019).

7.3.4 Sequence Analysis

Raw NGS reads are obtained after acquisition of the metagenomic data. The pretreatment of raw sequences is an overly critical step to attain high quality reads for downstream analysis. Various bioinformatics tools have been developed to pretreat the sequences which are Trimmomatic software (Campanaro et al. 2016), ACE Pyrotag Pipeline (Ho et al. 2014), HMMER (Azizi et al. 2016), MG-RAST (Wirth et al. 2012), ChimeraSlayer (Martínez et al. 2014), etc. The sequence pretreatment generally includes (1) removing adapters and linkers, (2) excluding chimeras and replication, and (3) demultiplexing of barcoded samples and quality control. UCHIME is the most cited tool to check and remove chimeras from the raw sequences while MOTHUR and QIIME (http://qiime.org/) are currently the two most frequently used platforms to denoise the metagenome data(Zhang et al. 2019).

7.3.5 OUT Clustering Analysis

After cleaning the sequences, all sequences are aligned using sequence aligners such as MOTHUR (Martínez et al. 2014), INFERNAL aligner (Cardinali-Rezende et al. 2016), and ClustalW (Zhang et al. 2019). Subsequently, the aligned sequences are clustered into operational taxonomic units (OTUs) with average neighboring clustering algorithm via Usearch software or various sequence classifiers like RDP Bayesian Classifier (Cardinali-Rezende et al. 2016), UCLUST-RDP classifier (Pope et al. 2013), and MEGA/MEGA5 (Cardinali-Rezende et al. 2016; Rudakiya et al. 2019).

7.3.6 Diversity Analysis

For investigating the biological richness/diversity of microbial communities in various biogas-producing systems, the OTUs-based alpha diversity analysis in terms of Chao1 richness estimator (Chao1), abundance coverage-based estimator of species richness (ACE), Shannon-Weaver diversity index (Shannon), and Simpson diversity index (Simpson) can be performed using the MOTHUR package (Zhang et al. 2019), Rsoftware package with VEGAN library (Oksanen et al. 2007), and the RDP Pipeline (Cardinali-Rezende et al. 2016). Straightforward calculation using the given equations to rapidly estimate biological diversity of microbial communities is a big advantage for various diversity indices. However, the diversity

indices like Simpson index and Shannon index combine richness and evenness components into a simple index through a single measure, leading to a loss of relative roles of other different variables such as potential economic, ecological, and social importance of individual species (Barrantes and Sandoval 2009).

7.3.7 Taxonomic Composition Analysis

Taxonomic composition analysis is one of the most frequently used bioinformatics analyses for anaerobic microbial communities. Generally, this analysis can be performed through two steps: (1) database comparison and filtration and (2) taxonomic classification of sequences. Particularly, sequences are firstly filtered through a BLAST search against the sequence database which are SILVA database, EzTaxon database, GenBank NT/NR database, and RDP database (Zhang et al. 2019).

7.3.8 Statistical Analysis

Based on a brief survey of the literature concerning anaerobic microbial community analysis, the most common multivariate analysis techniques include principal component analysis (PCA), principal coordinate analysis (PCoA), non-metric multi-dimensional scaling (NMDS), redundancy analysis (RDA), and canonical correspondence analysis (CCA) (Barrantes and Sandoval 2009; Khanal 2011; Sun et al. 2016). The similarity of these multivariate techniques is that each one is essentially regarded as an ordination analysis, with a basic aim of depicting similar objects near to each other and dissimilar objects further apart from each other (Ramette 2007). Generally, these techniques are classified into two groups: unconstrained ordination analysis (PCA, PCoA and NMDS) and constrained ordination analysis (RDA and CCA) based on the types of used data sets and computing methods.

7.4 Factors Affecting AD Process

To operate the AD process effectively, various environmental as well as operational factors are important for the diversity and abundance of microbial communities. Important factors such as feedstock characteristics (composition, C:N ratio, particle size, total solids, etc.), process monitoring (pH, temperature, inhibitors, etc.), type of bioreactor (suspended growth anaerobic digester, attached growth anaerobic digester, solid-state anaerobic digester, household anaerobic digester), and process management (batch or continuous process) are important to achieve higher methane production

(Nguyen et al. 2019; Rocamora et al. 2019). These factors are discussed as follows in brief.

In process monitoring factors, pH and temperature of AD process, total solids, volatile fatty acids, alkalinity, organic acid concentration, chemical oxygen demand, C:N ratio, hydrogen and ammonia gas content are the major factors. For AD process, optimal conditions are mainly affected the microbial communities, wherein pH (6.8–7.4), temperature for mesophilic (35–40 °C) and thermophilic (50–60 °C), total solids for solid state (15–30%) and liquid state (<15%), total volatile fatty acids (50–250 mg/L), acetic acid (<1000 mg/L), propionic acid (<250 mg/L), total alkalinity (1500–3000 mg/L), ratio of volatile fatty acid and alkalinity (0.1–0.2), carbon to nitrogen ratio (20–40), total ammonia nitrogen (50–1000 mg-N/L), hydrogen gas content (<100 ppm), and ratio of chemical oxygen demand to nitrogen to phosphorous (350:7:1) should be within the range (Nguyen et al. 2019).

7.5 Microbial Communities of Anaerobic Digesters

Microbial communities of different AD process are described as follows:

Anaerobic digester treating the conventional and vacuum toilet flushed blackwater exhibited the methanation rates were 0.23–0.29 and 0.41–0.48 g CH_4-COD/g feed COD and the COD removal rates were 72% and 89%, respectively (Gao et al. 2019). Archaeal genera in the inoculum and reactors were different in composition which are *Methanospirillaceae, Methanoculleus, Methanospirillum,* and *Methanogenium*. The enriched bacteria were linked with high ammonia conditions, including *Porphyromonadaceae, Fibrobacteraceae, Ruminococcaceae, Bacteroidaceae, Clostridiales,* etc. (Gao et al. 2019).

Anaerobic digester treating the steam exploded food showed lower methane yield, however more organic acids like lactic acid, propionic acid, and acetic acid were produced (Svensson et al. 2018). Microbial community such as Tenericutes (42%) dominated in frequently fed digester but Firmicutes (31%) was most abundant in the Daily Fed Digester (Svensson et al. 2018).

Microbial community of anaerobic mono-and co-digesters treating food waste and animal waste is described by Koo et al. (2019). *Methanobacterium beijingense, Methanobacterium petrolearium, Methanoculleus bourgensis, and Methanoculleus receptaculi* are the major species found in the anaerobic digester. In digester, 32 bacterial genera had relative abundance >0.5% which accounted for 77.1% of total reads. *Fastidiospila* (16.4 ± 5.2%), *Petrimonas* (14.7 ± 5.1%), *Rikenellaceae* RC9 gut group (11.1 ± 6.2%), *Candidatus Cloacamonas* (4.7 ± 9.5%), *Christensenellaceae* R-7 group (3.5 ± 0.9%), *Proteiniphilum* (3.3 ± 2.3%), and *Sedimentibacter* (3.0 ± 1.0%) were dominant bacteria in anaerobic digester.

Microbial communities of mesophilic anaerobic digesters treating food wastewater or sewage sludge were shown by Lee et al. (2018). Major methane producing communities observed are *Methanoculleus* (78.6 ± 17.0%), *Methanobacterium* (7.3 ± 13.6%), *Methanomassiliicoccus* (4.1 ± 3.9%), *Methanomethylophilaceae*

(1.8 ± 3.1%), *Candidatus Methanoplasma* (2.6 ± 3.6%), uncultured *Methanosarcina* (1.2 ± 4.7%), and *Methanimicrococcus* (0.9 ± 2.0%) in anaerobic digester (Lee et al. 2018).

Microbial communities of mesophilic and thermophilic anaerobic digesters treating food waste-recycling wastewater were shown by Kim et al. (2018). In the mesophilic digester, *Fastidiosipila*, *Petrimonas*, *vadinBC27*, *Syntrophomonas*, and *Proteiniphilum* were dominant bacterial genera; they may contribute to hydrolysis and fermentation. In the thermophilic digester, *Defluviitoga*, *Gelria,* and *Tepidimicrobium* were dominant bacteria; they may be responsible for hydrolysis and acid production. In the mesophilic digester, dominant methanogens changed from *Methanobacterium* (17.1 ± 16.9%) to *Methanoculleus* (67.7 ± 17.8%) due to the increase in ammonium concentration. In thermophilic digester, dominant methanogens changed from *Methanoculleus* (42.8 ± 20.6%) to *Methanothermobacter* (49.6 ± 25.9%) due to the increase of pH (Kim et al. 2018).

Methane production was observed during the bioconversion of rice straw which is shown by Wachemo et al. (2019). Major bacterial communities observed *Enterobacteriaceae, Clostridiaceae1, Prevotellaceae,* and *Peptostreptococcaceae.* Archaeal *Methanosaeta* is dominant among all digester samples. However, the *Methanobacterium* is predominant (34.88–59.40%) in samples obtained at late stages of AD period (Wachemo et al. 2019).

Maximum methane generation rates in dry anaerobic co-digestion of rice straw and cow manure were found to increase by 30.5 ± 2.2%, 20.5 ± 1.9%, and 18.7 ± 1.8% in the reactors with limonite concentrations of 1%, 5%, and 10%, respectively. Hydrogen-consuming methanogenesis by *Methanofollis* was dominant in the reactors digesting the rice straw and cow manure with added limonite (Xu et al. 2019).

Microbial communities of thermophilic digester treating the dairy manure were shown by Lv et al. (2013). Three temperatures (50, 55, and 60 °C) were tested on the thermophilic digester and 50 °C was found to be the optimal temperature for overall performance with 31% VS removal and 0.22 L methane/g VS fed. *Methanobacterium* and *Methanoculleus* were the most predominant methanogen genera which produced the methane gas (Lv et al. 2013).

7.6 Biogas Applications

Biogas produced from AD process comprises methane and carbon dioxide. In addition to this, some traces of moisture, hydrogen sulfide, ammonia, nitrogen, and hydrogen gas are detected. In biogas cleaning, majority of unwanted gases like hydrogen sulfide, hydrogen, nitrogen, and moisture have been eliminated using various methodologies wherein scrubber and other porous materials have been used. Biogas upgrading is the enhancement of the methane content by reducing the carbon dioxide content. After biogas upgradation, methane content is above 90%. The biogas has many different applications depending on its quality. Cleaned

biogas (CH_4 50–75%, $H_2S < 1000$ ppm) is suitable for cooking, burning in boiler, or generating electricity and heat via CHP units (Nguyen et al. 2019). Upgraded biogas, often termed biomethane, is more than 95%. It can be injected into natural gas grid ($H_2S < 4$ ppm) or converted into CNG as transportation fuel ($H_2S < 16$ ppm). Biogas can also be reformed to produce syngas (mixture of H_2 and CO_2), which can be converted into methanol or various liquid fuels using different catalysts via Fischer–Tropsch process (Munasinghe and Khanal 2011).

7.7 Concluding Remarks

Microbial communities involved in AD process are most phylogenetically and functionally diverse among different environments. In the last 15 years, substantial research on the microbial communities of various anaerobic digester is conducted and concluded that certain methanogen communities produce methane, acetogens and fermentative bacteria produce acid, and other bacteria assist in the bio-methanation process. Day by day, many new applications of bioinformatics technology and tools are emerged, which offer the potential analysis that can be benefited for the biogas industries (Zhang et al. 2019). In addition to metagenomics, other omics branches strengthen the microbial diversity data which is also essential for further and in-depth application of AD process. This use of complementary techniques will allow the simultaneous identification of phylogeny, interspecies interactions and function, and improve the operation of anaerobic digesters to fully utilize their potential as an effective waste management strategy and resource recovery process, and for the production of high-value products (Vanwonterghem et al. 2014). However, research is still limited to the bacterial communities and abundance. Majority of research are focused on the identification of the communities and metabolites which does not show the actual potential of metagenomics. The research should focus more on the following areas:

(a) More and precise databases should be created for bacterial identification, so that identification of bacterial and archaeal communities is error free. It also helps to identify the bacteria/archaea as majority (above 90%) of them belong to the unidentified group.
(b) More precise sequencing techniques should be evolved to identify the bacteria error free with less nucleotide sequence.
(c) More statistical tools and techniques should be created to validate the data obtained by sequencing.
(d) Utilization of omics approach or integration of multi-omics approach should be mandatory to obtain perfect and balanced data of AD system (Vanwonterghem et al. 2014).
(e) Isolation and screening of genes and proteins which are responsible for potential industrial application should be explored.

(f) Extraction and purification of industrially important molecules generated during biogas production should be explored.
(g) After identification of bacteria, more research is needed to integrate these molecular methods to develop the molecular microbiology for industrial applications.

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