The Realm of Microorganisms in Biogas Production: Microbial Diversity, Functional Role, Community Interactions, and Monitoring the Status of Biogas Plant

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Abstract Anaerobic digestion is being considered as a sustainable technology to treat organic wastes to reduce contamination and emission of greenhouse gasses and at the same time produce energy in the form of methane. The microbiological process of AD represents the most challenged step during biogas production due to microbial complexity. At the time, at least 11 microbial groups have been described. These populations have been shown unique metabolism and an interspecies interaction because of the limited amount of energy available for growth. The microbial community structure is considered as the core in the success of AD method. Furthermore, to expand AD technology in order to approach an economically feasible process under the concept of biorefinery and not only the advances on engineering processes, the design of new biogas digesters and tools for real-time monitoring for AD are the keys for a successful implementation of this process. In addition, the classification of the microbial community structure and the understanding of the metabolic networks play a crucial role for its development. In this chapter, different aspects of the microbiology of AD of full-scale biogas digesters are discussed with specific focus on the presence of different microbial groups, their activity, and interactions.

Keywords Anaerobic digestion · Microbial communities · Biogas · Microbial networks · Biogas reactors

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1 Introduction

Anaerobic digestion (AD) represents the most prominent worldwide technology to convert organic wastes, such as livestock manures, municipal solid waste, municipal and industrial wastewaters, and agro-industrial residues, into biogas due to an engineered and biochemical process, which involves a series of operational parameters, such as organic loading rate, and the interactions of at least eleven microbial groups (Alvarado et al. 2014). The importance of AD is not only because of its significance in waste management but also because AD offers carbon recovery in the form of methane, which demonstrates to be a sustainable manner to produce clean energy as electricity and heat and as vehicle fuel. Notwithstanding the advances on the engineering processes, the design of new biogas digesters, and tools for real-time monitoring for AD, the microbiology aspect always poses challenges. Microbial community composition analyses, in biogas digesters of several substrates, have been widely reported. However, due to their complexity, these populations have been shown unique metabolism and an interspecies interaction which have not been yet precisely characterized (García-Lozano et al. 2019). This process is still contemplated as the core in the success of AD method. In addition, the classification of the microbial community structure and the understanding of the metabolic networks are crucial to expand the implementation of AD technology in order to achieve an economically feasible process. Moreover, the purpose of this process is presently exploring to include the generation of value-added products, under the concept of biorefinery, not only the energy generation and nutrient recovery (Schnürer 2016). This chapter describes several aspects of the microbiology of AD, including the presence of different microbial groups, their activity and interactions, and the consequent response of the different operational parameters in a full-scale biogas digesters are discussed.

2 Metabolism of Anaerobic Digestion Process

2.1 Anaerobic Digestion: Functional Role

Anaerobic digestion is a chain of interconnected biological reactions in which at least 11 groups of microorganisms, belonging to domain bacteria and archaea, interact in numerous associations where the organic matter, as carbohydrates, proteins, lipids, or more complex compounds, is transformed into biogas (containing ~65% CH_4, 35% CO_2, and trace amounts of H_2S, NH_3, and H_2) and anaerobic biomass. Besides bioenergy production in the form of methane, AD presents several advantages, such as lesser biomass sludge production in comparison to aerobic treatment technologies, elimination of pathogens, the digestate produced is an improved fertilizer, and the reduction of greenhouses gasses (GHG) emissions.

Usually AD is conceptually divided into three or four stages, hydrolysis and/or fermentation, acetogenesis, and methanogenesis. The performance of these processes is carried out by the combined action of hydrolytic-fermentative bacteria, syntrophic acetogenic bacteria, and methanogenic archaea. During the first stage, insoluble and complex polymers (carbohydrates, lipids, proteins, etc.) are hydrolyzed and converted into simple and soluble products (sugars, long-chain fatty acids, glycerol, amino acids, etc.), which are catabolized by fermentative bacteria into alcohol, fatty acids, hydrogen, and carbon dioxide. Subsequent steps involve the oxidation of such alcohols and fatty acids by syntrophic acetogens, forming acetate, H_2, and CO_2. Finally, during methanogenesis, acetate and other methyl-containing C1 compounds are reduced to methane by aceticlastic and methylotrophic methanogens, and CO_2 is reduced by H_2-oxidizing methanogens (Nagamani and Ramasamy 1999).

2.2 *Hydrolysis*

This first step of AD is considered as the rate limiting performed by the microbial decomposition of organic matter (proteins, lipids, and polysaccharides) into soluble small molecules by extracellular enzymes of facultative and obligate anaerobic bacteria (Cazier et al. 2015; Boontian 2014). Substrates are cleaved enzymatically, mainly by the amylases, cellulases, proteases, and lipases excreted by microorganisms (Bajpai 2017). Interaction networks from domains help us to understand the substrate conversion process (Shaw et al. 2017). Usually AD is greater than 10^{16} cells/mL which involves saccharolytic bacteria (~10^8 cells/mL), proteolytic bacteria (~10^6 cells/mL), and lipolytic bacteria (~10^5 cells/mL) (Amani et al. 2010). Proportions of the enzymes excreted from these bacteria and the optimum operation of a biogas plant will depend on the substrate and its degradation characteristics (Weinrich and Nelles 2015). Mostly, substrates employed to start up this stage are wastes like animal waste and lipid-rich wastes from oil industry, pulp-paper processing, wastewaters, animal fat, agricultural waste, or energy crops, which show different microbial communities according to degradation demand (Montañez-Hernández et al. 2018; Tabatabaei et al. 2010; Appels et al. 2011).

2.2.1 Polysaccharide Hydrolysis

Lignocellulosic biomass is mainly found in biodigesters and consists of cellulose (30–56%), hemicellulose (10–27%), and lignin (3–30%). It is worth to mention that lignin is a recalcitrant compound that can limit the hydrolysis rate for biogas production (Sawatdeenarunat et al. 2016; Venkiteshwaran et al. 2016). At present, two types of polysaccharide hydrolysis systems are known: multienzymatic complex systems, called cellulosomes, and free enzymatic systems (Felix and Ljungdahl, 1993). Anaerobic microorganisms produce cellulosomes, fixed on the bacterial cell wall, which bind to the substrate for its hydrolysis. Aerobic microorganisms degrade

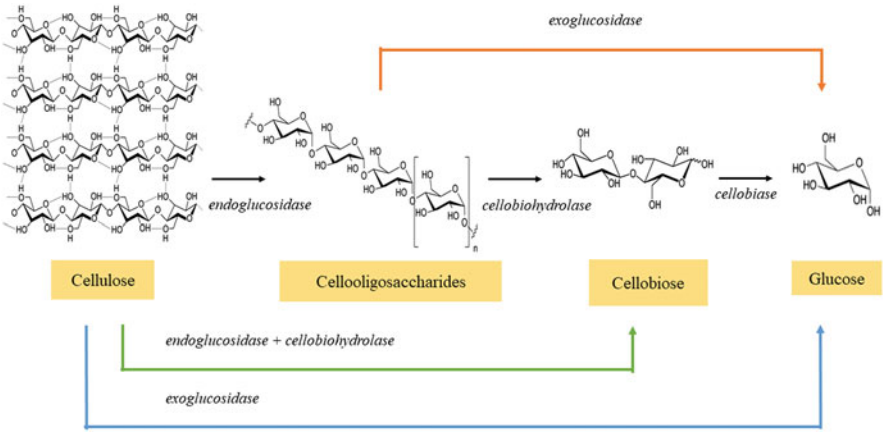

Fig. 1 Schematic representation of enzymatic hydrolysis of cellulose

cellulose, by secreting a set of enzymes, viz., endoglucanase, exoglucanase (cellobiohydrolase), and cellobiases (Fig. 1). Meanwhile, the hydrolysis of starch is performed by a mixture of amylases as α-amylase, α-xylosidase, and β-xylosidase able to hydrolyze amylose and amylopectin (Zhu et al. 2016).

2.2.2 Protein Hydrolysis

As well as carbohydrates and lipids, protein constitutes a major percentage of the organic load in anaerobic sludges and wastewaters. Wastewater and sewage from food processing industries as abattoir, dairy, fish, and vegetables comprise around 40% of protein (Barnett et al. 1994; Ramsay and Pullammanappallil 2001). Proteins are natural nitrogen-rich polymers that are mainly composed of amino acids linked by peptide bonds. Nitrogen provides an essential element for the synthesis of amino acids, protein, and nucleic acids and acts as a strong base when it is converted to ammonia. Physiologically, proteases are released to the extracellular media to cleavage proteins into its constituents, peptides and free amino acids, which are subsequently metabolized to VFAs, CO_2, H_2, NH_4^+, and S_2^-. Proteases are classified principally based on their site of action in two major groups: exoproteases (carboxipeptidases or aminopeptidases) and endoproteases. Further classification is based on the functional group (serine, cysteine, aspartate, or metallo) and optimal pH (acidic, neutral, or alkaline) (Schaechter 2009).

2.2.3 Lipid Hydrolysis

Lipid is the term used to describe fat, oil, and grease contained mostly in wastewater stream and other sources. Lipids are considered as excellent substrates for anaerobic digestion and co-digestion due to the higher methane yield obtained when compared

to proteins or carbohydrates (Yang et al. 2016). Most of lipids in wastes are present as triacylglycerides, a glycerol ester with three long-chain fatty acids (LCFA). During hydrolysis of triacylglycerides, glycerol and LCFA (typically 14 to 24 carbon atoms) are produced by extracellular lipases in order to increase lipid solubility. These enzymes are excreted by acidogenic bacteria, and the further conversion of the hydrolysis products takes place inside the bacterial cells.

2.3 Acidogenesis

The second stage from AD is fermentation, also called acidogenesis, where monomers will be further decomposed by fermentative bacteria into short-chain fatty acids or volatile fatty acids (VFAs). Generally, acetate, butyrate, and propionate (most prevalent VFAs), lactate, valerate, pyruvate, formic acids, CO_2, and/or hydrogen are present as by-products of this stage (Chen et al. 2017; Mani et al. 2016; Ren et al. 2018). During AD, acidogenesis is the quickest step producing precursors of methane. Three main types of fermentation are known: ethanol/acetic acid-type, butyric acid-type, and propionic acid-type. These pathways are determinant to achieve a high performance of methane production, where the major products are butyric and acetic acid (70–90%) (Chen et al. 2015). The performance of the fermentation stage is one of the most attractive strategies for biogas production enhancement in AD process goals, especially on organic wastes (Lu et al. 2018).

2.3.1 Carbohydrates Fermentation

In the absence of methanogens, the major products of sugar fermentation by anaerobic bacteria are acetate, ethanol, H_2, and CO_2. When H_2-utilizing bacteria are active, acetate production is increased. Formerly, for most of microorganisms, fermentation of glucose occurs by the glycolytic pathway, producing pyruvate, or by-products of pyruvate (Fig. 2). Glucose can be fermented to lactate by homofermentative bacteria to lactate or to multiple end products as acetate, formate, butyrate, propionate, valerate, and CO_2, by heterofermentative bacteria. Usually, these microorganisms produce CO_2 and H_2 with the concomitant production of formate, acetate, lactate, and succinate. Commonly, heterofermentative bacteria include *Lactobacillus*, *Microbacterium*, and *Leuconostoc*. The main product of clostridia, eubacteria, fusobacteria, and butivibrios is butyrate, acetate, CO_2, and H_2, while *Clostridium* species can ferment those end products plus others, as acetone. Other anaerobic bacteria, as *Propionibacterium* species, ferment glucose to form CO_2, propionate, acetate, and succinate. Propionate is produced by the partial reversal of Krebs cycle reactions and implies a CO_2 fixation by pyruvate (the Wood-Werkman reaction) which forms oxaloacetate. Subsequently, oxaloacetate is reduced in three steps and then decarboxylated to propionate. In another three-carbon pathway, propionate is formed by a lactyl-SCoA intermediate.

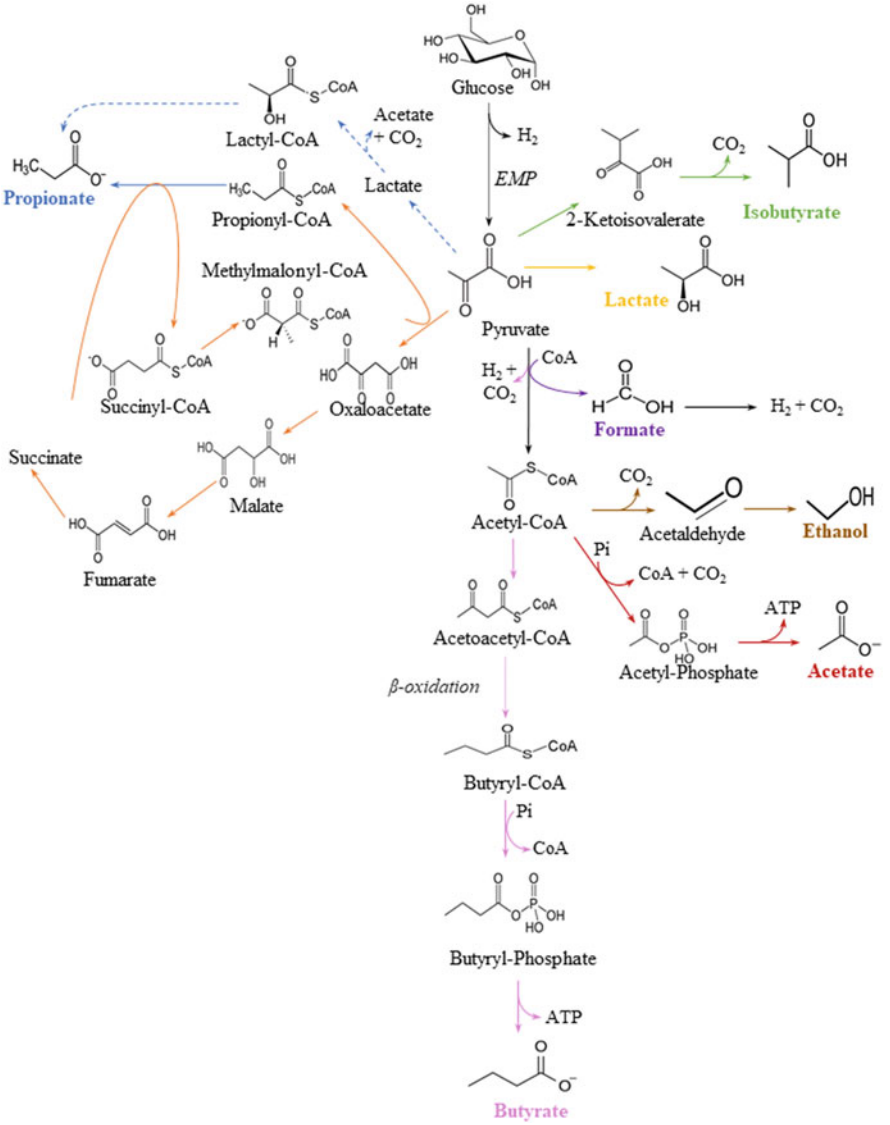

Fig. 2 Fermentative pathways occurring in anaerobic digestion for bacteria, and the major end products formed from glucose. EMP: Embden-Meyer Pathway. Orange line: Reversal Krebs cycle. Blue dotted line: Acrylate pathway

2.3.2 Amino Acid Fermentation

Amino acid can be fermented anaerobically by two principal ways: a pair of amino acids can be decomposed through the Stickland reaction, or one single amino acid can be degraded by H_2-utilizing bacteria. The end products of fermentation include

short-chain and branched-chain organic acids, NH_3, CO_2, and small amounts of H_2 and sulfur-containing compounds (Ramsay and Pullammanappallil 2001). The Stickland reaction implies one amino acid, used as electron donor, while another amino acid acts as an electron acceptor. This reaction produces 0.5 mole of ATP per mole of amino acid transformed, and their utilization may be linked to the oxidative deamination step and/or the decarboxylation step (Andreesen et al. 1989). The alternative pathway to the Stickland reaction proceeds when hydrogen partial pressure is sufficient low, releasing hydrogen as electrons (Schnürer 2016). It is worth to mention that the oxidative deamination reactions are endergonic under standard conditions. Thus, the reaction cannot proceed unless the reducing equivalents produced are taken up via interspecies hydrogen transfer by methanogens, sulfate reducers, or acetogens and by another amino acid in the Stickland reaction or in the reduction of acetate to butyrate (Örlygsson et al. 1995).

2.3.3 Glycerol Fermentation

As mentioned earlier, most of glycerol present in biodigesters is a product of lipid hydrolysis plus LCFA. Glycerol is a source of carbon and energy, and its uptake can occur by active or passive transport (Holst et al. 2000). Anaerobic fermentation of glycerol can be carried out by a reductive or an oxidative pathway (Biebl et al. 1999).

The reductive pathway leads to 1,3-propanediol production by means of glycerol dehydration. The oxidative pathway leads to glycerol dehydrogenation to produce phosphoenolpyruvate which can in turn be converted to propionate by several decarboxylations, or it can be converted to pyruvate. Thus, pyruvate can be then be fermented in simpler compounds, depending of the microorganism and the environmental conditions, such as 2,3-butanediol, lactate, butyrate, n-butanol, ethanol, acetate, formate, hydrogen, and carbon dioxide (Siles et al. 2009).

2.4 Acetogenesis and Syntrophy

Obligate anaerobic bacteria that synthesize acetyl-CoA by the reductive acetyl-CoA or Wood-Ljungdahl pathway, for energy and cell carbon obtaining from CO_2, are called acetogens; acetogenic bacteria that produce acetate as sole end product are called homoacetogen (Drake 1994). The pathway consists in the reduction of 2 moles of CO_2 to 1 mole of acetate by 8 protons (Hattori 2008). In addition, the participant key enzyme for the pathway is the acetyl-CoA synthase (ACS) (Müller and Frerichs 2013). Thereby, the aforementioned statements separate acetogens from those microbial groups that produce acetate as an end product of fermentation (Schuchmann and Müller 2016). Acetogens versatility is demonstrated by their wide variety of useful substrates, i.e., sugars, $CO_2 + H_2$, C_1 compounds, dicarboxylic acids, and alcohols (Müller and Frerichs 2013). In addition, electron acceptors such as nitrate, nitrite, thiosulfate, and fumarate can be used by acetogens. However,

repression of acetyl-CoA pathway takes place (Müller and Frerichs 2013). In anaerobic digestion, acetogenic bacteria contribute in the formation of acetate as a precursor for CH_4 production by aceticlastic methanogenesis. Therefore, their presence in anaerobic digesters benefits the process.

There are other bacteria that are in the presence of hydrogen-scavenger microorganisms, such as hydrogenotrophic methanogens, which act in syntrophic relationship to obtain energy. Syntrophic acetate oxidation (SAO) process consists in the oxidation of acetate, by syntrophic acetate-oxidizing bacteria (SAOB), to produce H_2 and CO_2, available substrates for hydrogenotrophic methanogens to form CH_4 (Sun et al. 2014). It is believed that acetate oxidation is carried out by the reversible reactions of the Wood-Ljungdahl pathway (Müller and Frerichs 2013). The process of transferring reducing equivalents (such as H_2) from bacteria to archaea is called interspecies electron transfer (Stams and Plugge 2009). Oxidation of acetate is a highly endergonic reaction under standard conditions ($\Delta G^{0'} = +104.6$ kJ/mol) (Hattori 2008). Therefore, it requires low H_2 partial pressure (<10 Pa) that can be obtained by the activity of hydrogenotrophic methanogens (Schink 1997). Coupling of both pathways results in an overall exergonic reaction ($\Delta G^{0'} = -31.0$ kJ/mol). However, this small energy released is shared by both microorganisms, explaining their slow growth rate (Hattori 2008).

2.5 Methanogenesis

Methanogenesis is the final stage of anaerobic digestion, where the biological formation of methane is performed by methanogens, an obligate anaerobic archaeon. Methanogens use three main substrates to obtain energy. The first type of substrate is CO_2; most of the methanogens are capable to reduce CO_2 to methane by electrons from H_2, but also other electron donors such as formate, secondary alcohols such as 2-propanol, 2-butanol, and even ethanol might be used by methanogens. The oxidation of these last compounds occurs partially generating ketones and acetate. The second substrate is compounds that contain methyl groups, such as methanol and amines. The last group corresponds to acetate.

Methane biosynthesis occurs through two main pathways known as hydrogenotrophic or CO_2-reduction and aceticlastic. In the CO_2-reduction pathway, formate (Hungate et al. 1970; Archer and Harris 1986) or H_2 is oxidized, and CO_2 is reduced to CH_4, whereas in the aceticlastic pathway, acetate is cleaved with the carbonyl group oxidized to CO_2 and the methyl group reduced to CH_4 (Ferry 2011). Although both routes differ in terms of reactions and enzymes, the last step that corresponds to the production of methane and the formation of heterodisulfide is common in both pathways. The reduction of CO_2 to CH_4 reaction sequence starts with a two-electron reduction of CO_2 and methanofuran (MFR) to formyl-MFR where the formyl group is bound to the amino group of the coenzyme. The formyl group is then transferred to the N^5 of tetrahydromethanopterin (H_4MPT); the formyl-H_4MPT thus generated cyclizes to the methenyl-H_4MPT, which is reduced in two

steps to the methyl-H_4MPT. Finally, the methyl group is transferred to the thiol group of coenzyme M. The methyl thioether formed is reduced to CH_4 in the final step of the pathway (Hedderich and Whitman 2013).

In methanogenesis from methanol, the methyl group enters the C1 pathway at the level of coenzyme M and is reduced to methane. The electrons for this reduction are obtained from the oxidation of an additional methyl group to CO_2 using the reverse of the steps of the reductive C1 pathway (Hedderich and Whitman 2013). During growth on acetate, the methyl (C-2) carbon of acetate is reduced to methane using electrons obtained from the oxidation of the carboxyl (C-1) carbon of acetate. In this metabolism, the methyl group enters the C1 pathway at the level of methyl-H_4MPT (Hedderich and Whitman 2013).

Around 70% of the methane synthesized by methanogens in a full-scale biogas plant comes from the acetoclastic pathway, while the remaining percentage comes from the CO_2-reduction. However, phylogenetic studies recognize the CO_2-reduction pathway as the oldest since some of the specific enzymes for this pathway are not distributed in other microorganisms. In contrast, the enzymes required for the acetoclastic pathway are also found in some acetogenic and fermentative bacteria, suggesting that the appearance of this pathway occurred much later than hydrogenotrophic pathway (Bapteste et al. 2005).

3 Microbial Composition in Full-Scale Biogas Digesters

3.1 Hydrolytic Bacteria

The implementation of polysaccharides, as substrate for carbon source, is generally used on biogas plants. Mostly, lignocellulosic-rich substrates are feedstock in high-capacity bioreactor and present as energy crops, agricultural residues, animal manure, and food waste as a sustainable source (Koch et al. 2010; Ziganshin et al. 2013). Microorganism presence found in biogas plants for the hydrolytic anaerobic process varies on the type of reactor as is shown in Table 1. However, mostly the phyla of *Proteobacteria* (within *Deltaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Alphaproteobacteria* classes) are present in the initial phase where *Clostridiales* (*Clostridium*, *Ruminococcus*, *Butyrivibrio*, *Acetivibrio*, and *Eubacterium*), *Thermoanaerobacterales* (*Caldicellulosiruptor*), *Fibrobacteres* (*Fibrobacter*), *Spirochaetales* (*Spirochaeta*), *Tissierellia* (*Anaerococcus*) orders are involved and some archaea started to appear, as well as in the hydrolytic stage (Cirne et al. 2007; Manyi-Loh et al. 2013; Narihiro and Sekiguchi 2007). A study reported by Tian et al. (2017) observed the order of *Bacteroidales* accounted for the 30% of the total prokaryote population; in this order of microorganisms, the family of *Marinilabiaceae* accounted the 85% of the order. Thus, *Bacteroidales* were predicted as the microorganisms able to degrade biopolymers, including xylan, and also reported to degrade chitin in anaerobic conditions. Nonetheless, *Bacteroidales* was identified by their abundance and its role in anaerobic digestion of cellulose and hemicellulose.

Table 1 Microorganisms predominant identified in anaerobic digestion from different types of reactors, with relative order abundance (%) and OTUs of species within the orders

Order	Species related to order	OTUs	Order abundance %	Type of reactor	References
Erysipelotrichales	*Erysipelotrichaceae*	NA	<0.1–2%	Wet fermentation BGP CSTR	(Sundberg et al. 2013)
Clostridiales	– *Pelotomaculum isophthalicicum* – *Ruminococcus* – *Acetivibrio cellulolyticus* – *clostridium thermocellum* – *Pelotomaculum thermopropionicum* – *Pelotomaculum isophthalicicum* – *Heliobacillus mobilis*	42 NA NA NA 110 270 1078	15–18%	– Wet and dry fermentation BGP – UASB – Continuous anaerobic digester – Full-scale thermophilic and mesophilic – CSTR	(Sundberg et al. 2013; Stolze et al. 2015; Lee et al. 2012; Sun et al.2015; Doloman et al. 2017)
Spirochaetales	– *Treponema sp.* – *Spirochaeta cellobiosphila* – *Spirochaeta halophila*	NA 6 36	>16%	– Wet and dry fermentation BGP – Full-scale thermophilic and mesophilic	(Stolze et al. 2015; Lee et al. 2012; Sundberg et al. 2013)
Bacteroidales	*Prevotella sp.*	NA	08–44%	– Wet fermentation BGP – CSTR – UASB	Ziganshin et al. 2013; Sun et al. 2015; Doloman et al. 2017)
Cytophagales	*Fulvivirga kasyanovii* *Sediminitomix flava*	11 62	NA	Full-scale thermophilic and mesophilic	(Lee et al. 2012)
Burkholderiales	*Curvibacter delicatus* *Acidovorax defluvii*	46 280	NA	Full-scale thermophilic and mesophilic	(Lee et al. 2012)

Thermoanaerobacterales	*Caldanaerobius fijiensis*	68	NA	Full-scale thermophilic and mesophilic	(Lee et al. 2012)
Synergistales	*Anaerobaculum mobile* *Treponema primitia*	453 396	1.9%	Full-scale thermophilic and mesophilic UASB	(Lee et al. 2012; Doloman et al. 2017)

UASB upflow anaerobic sludge blanket reactor, *CSTR* continuous stirred-tank reactor, *BGP* biogas plant

Generally, the main microorganisms in anaerobic digesters involved in *protein hydrolysis* are from the order *Bacteroidales*, *Clostridiales*, *Fusobacteriales*, *Selenomonadales*, and *Lactobacillales* (Amani et al. 2010). *Clostridiales* and *Bacteroidales* are recognized as the main contributors in polymer hydrolysis and fermentation steps. In biogas plants (BGPs), these versatile orders are capable of hydrolyzing a wide range of substrates, including carbohydrates, lipids, and proteins. Previous metagenomic studies in BGPs have demonstrated its dominance ranging from 15 to 84% of the total microorganisms (Schlüter et al. 2008; Sundberg et al. 2013). More recently, its prevalence is shown in mesophilic and thermophilic biogas plants fed with lignocellulosic wastes (agricultural) and animal manure (Table 2).

Nonetheless, other works have acknowledged groups as *Spirochaetales* and *Bacillales* participating in protein degradation specifically of maize silage either with pig or chicken manure (Ortseifen et al. 2016; Stolze et al. 2015) and *Candidatus Cloacamonas* as the main protein degrader phylum from a BGP treating dairy manure (Li et al. 2014). Particularly wastes from food or ethanol fermentation have shown an increased abundance of specific groups of microorganisms. *Thermotogales* (10.4%) dominated a BGP treating food waste wastewater, while *Coprothermobacterales* (68.2%) showed a marked dominance in a mesophilic farm-scale digester treating brewery and swine wastes (Cho et al. 2017; Lee et al. 2016).

As seen in Table 2, serine and metalloproteases seem to have an important role generally in anaerobic bacteria. *Clostridiales*, *Bacteroidales*, and *Coprothermobacterales* encode for both enzymes, while other groups of microorganisms synthetize mostly for serine proteases. Both proteases are ubiquitously found in prokaryotes, and its mechanism depends on the active site which include a nucleophilic serine amino acid (serine proteases) or generally requires zinc or cobalt (metalloproteases) (Hedstrom 2002; Rawlings and Barrett 1995). However, little is known about the role of proteases in anaerobic digestion. Hence, a deeper insight is still necessary in order to known which specific proteases are participating in AD and to understand the dynamic changes within the community treating specific kind of substrates.

On the other hand, when treating lipids, *Firmicutes* and *Bacteroidetes* are crucial for the performance of the biodigester (Salama et al. 2019). Syntrophic β-oxidizing bacteria from microbial consortium as *Proteobacteria* and *Syntrophomonas* sp. from *Clostridiales* order have been reported as FOG degraders. Moreover, within the *Proteobacteria* phylum, *Rheinheimera* sp. and *Bacillus* sp. can digest FOG under anaerobic conditions and decrease LCFA deposition (Klaucans and Sams 2018). Studies on reactor treating palmitate and oleate revealed a predominance of *Clostridiaceae* and *Syntrophomonadaceae* from *Clostridiales* (Alves et al. 2009).

It is well-known that long-chain fatty acid (LCFA) oxidation on AD is performed through the path of β-oxidation, where coenzyme A is utilized for LCFAs conversion into acetate and hydrogen (Rasit et al. 2015). Studies have reported that FOG biodegradability has high potential biogas production on methane yielding (~1200 L CH_4/kg VS) on full-scale wastewater treatment plants (WWTPs) (Shen et al. 2015). Lipid degradation is critical for the effective degradation of food waste to produce biogas; also lipids are considered as a good substrate to produce renewable energy at an industrial level (Ziels et al. 2016). A study by He et al. (2018) presented an

Table 2 Principal orders involved in protein hydrolysis identified in biogas plants

Order	Biogas plant	Substrate	Methane yield	Functional role	Abundance	References
Clostridiales	Full-scale mesophilic digester (34–35 °C)	Low substrate loading of sewage sludge	7000 m^3/d with a methane content of 61.26%	It is the most abundant order in anaerobic digesters, encodes principally metalloproteases	7.03%	(Świątczak et al. 2017)
	Industrial-scale biogas plants (37–38 °C)	CD1: Slaughterhouse waste CD2: Thin stillage CD4: Grass, wheat based stillage	CD1, 319 ± 24 CD2, 307 ± 54 CD4, 348 ± 24 (methane potential)		CD1, 4.3% CD2, 3.8% CD4, 56.3%	(Sun et al. 2016)
	Thermophilic pilot plant digester (56 °C)	Poultry litter	57.7 ± 2.2% methane of the total biogas		46%	(Smith et al. 2014)
Bacteroidales	Mesophilic pilot-BGP (36–38 °C)	Above ground biomass of Jerusalem artichoke	50–60% methane content	*Bacteroidales* secretes carboxypeptidases, metalloproteases, serine proteases, and ATP-dependent	ND	(Ciccoli et al. 2018)
	Thermophilic BGP (54 °C)	Maize silage, barley, cattle manure, and pig manure	0.62 m^3_{biogas} kg^{-1}_{VS} d^{-1} with a methane content of 53-54%.		4.5% of bacterial domain	(Maus et al. 2016)
Coprothermobacterales	Mesophilic farm-scale digester (35 °C) during a period of starvation	Sludge from brewery and swine wastewater and sewage waste	0.1–0.3 m^3/m^3/d with a methane content from 20-60%	Encoding mostly metalloproteases and serine proteases	68.2%	(Cho et al. 2017)
Bacillales	Mesophilic BGP3 (40 °C)	Maize silage and pig manure	528.5 l/kg oDM	Encoding for serine proteases	0.07 FPKM (fragment per kilobase)	(Ortseifen et al. 2016)
Thermotogales	Thermophilic full-scale anaerobic digester (58.5 °C)	Food waste-recycling wastewater (34% of protein)	ND	Encodes serine proteases	10.4%	(Lee et al. 2016)

(continued)

Table 2 (continued)

Order	Biogas plant	Substrate	Methane yield	Functional role	Abundance	References
Spirochaetales	Mesophilic BGP (40 °C)	Dry fermentation (DF): Maize silage, green rye, chicken manure. Wet fermentation (WF): Maize silage, pig manure	DF, 350.5 l/kg oDM WF, 417.8 l/kg oDM	From genus *Treponema* encodes serine proteases	DF, 0.5% WF, 1.7%	(Stolze et al. 2015)
C. Cloacamonas	Mesophilic mixed plug-flow loop reactor (37 °C)	Dairy manure	60% of methane	Encodes all the machinery for protein degradation including proteases and peptidases	20–27%	(Li et al. 2014)

TAN Total ammonia nitrogen, *ND* Not determined
*Genus. #Phylum.

organic loading rate for stable biogas production of 0.5–1.5 g VS^{-1} $days^{-1}$ using cooking oil skimmed from food waste as the only carbon source, where *Anaerovibrio* (lipid hydrolysis bacteria) hydrolyze triglycerides to produce glycerol and fatty acids. This increased from 9.3 to 40% in a relative high concentration of lipids with the highest value of 2.0 g VS L^{-1} $days^{-1}$, while the genus of *Syntrophomonas* increased to ~29%, playing significant roles in the mesophilic anaerobic digestion.

3.2 Fermentative Bacteria

Biogas reactors have been tested in different manners during monosacharides fermentation, sucha as ADM1 model, with lactate suggesting that *Clostridiales* is a butyrate-producing bacterium predominantly, and other microorganisms were found *Propionibacteriales* synthetizing propionate, *Lactobacillales* (*Carnobacterium* sp.), a lactic acid bacteria, and *Synergistales* (*Lactivibrio alcoholicus*) a lactate-degrading bacteria (Satpathy et al. 2016). On thermophilic biogas plants, the order of *Petrogales*, *Defluviitoga tunisiensis*, and *Desulfotomaculum australicum* are described as lactic acid degraders, also contained acidogenic/acetogenic bacteria belonging to the *Clostridiales*, *Tissierellales*, and *Bacillales* orders (Table 3) (Maus et al. 2016).

When a high rate of *amino acid fermentation* occurs, high amounts of NH_3 and ammonium (NH_4^+) are produced, mostly when treating a proteinaceous-rich feedstock as animal wastes as slaughterhouse waste, dairy manure, animal manure, and aquaculture sludge and wastes from food industry and households. In AD, high concentrations of NH_3 are toxic to some microorganisms inhibiting cytosolic enzymes, as well as NH_4^+ which can be intracellular accumulated modifying the

Table 3 Main orders involved in monosaccharides and other fermentative substrates in a thermophilic biogas plant

Order	Strain	Substrate	Acid formed
Clostridiales	▪ *Clostridium kluyveri* DSM-555. ▪ *Clostridium cochlearium* JCM 1396. ▪ *Sporanaerobacter acetigenes* DSM-13106. ▪ *Desulfotomaculum guttoideum* JCM-11016.	▪ Succinate ▪ Glucose ▪ Glucose ▪ Ethanol	▪ Acetic acid. ▪ Acetic, butyric and propionic acid. ▪ Acetic acid. ▪ Acetic acid.
Petrotogales	▪ *Dendrosporobacter quercicolus.* ▪ *Selenomonas bovis* WG.	▪ Lactic acid ▪ Glycerol	▪ Acetic and propionic acid. ▪ Lactic, propionic acids, and succinate.
Bacillales	▪ *Bacillus thermoamylovorans* BHK67. ▪ *Soehngeria saccharolytica* DSM-12858.	▪ Glucose ▪ Lactic acid	▪ Acetic and propionic acid. ▪ Acetic acid.

pH and K^+ concentration causing process instability. Hence, an overproduction of ammonia can inhibit the whole process of AD due to that protein hydrolysis is faster than carbohydrate or lipid hydrolysis (Andreesen et al. 1989).

Although several studies have demonstrated ammonia-tolerant bacteria population by high methane yields, the fraction of NH_3 relative to the total (NH_3 + NH_4^+)-nitrogen (TAN) should be monitored (Hansen et al. 1993). TAN concentration of 0.68 g L^{-1} does not affect the methanogenic activity at mesophilic conditions. However, a range between 1.5 and 3 g L^{-1} of TAN is inhibitory, and a TAN concentration $>$ 4 g L^{-1} fully inhibits AD (Angelidaki and Ahring 1993; Hansen et al. 1993).

Carbohydrate-fermenting bacteria usually degrade proteins in a process energetically favorable. Many studies have shown proteolytic bacteria from the genus *Clostridia*, which also play an important role in amino acid fermentation (de Vladar 2012). In fact, *Clostridia* species only carry out Stickland reaction using all amino acids and producing δ-aminovalerate, α-aminobutyrate, or γ-aminobutyrate as intermediates in the fermentation (Mead 1971). As shown in Table 4 several orders have been grouped as including the order *Clostridiales*. However, other groups as *Synergistales*, *Thermotogales*, and *Thermoanaerobacterales* have been found in biogas plant treating agricultural wastes, food waste wastewater, and sewage sludge, and they have been recognized to degrade several amino acids to produce propionate and/or acetate (Lee et al. 2016; Maus et al. 2016; Świątczak et al. 2017).

Nonetheless, a phylum lately recognized as protein degrader and amino acid fermenter is *Candidatus Cloacamonas acidaminovorans* belonging to WWE1 candidate division, which encode all the machinery for protein degradation and derive most of the carbon and energy from amino acid fermentation (Pelletier et al. 2008). *C. Cloacamonas* has been found in great abundance in mesophilic BGPs, mainly digesting agricultural wastes and animal manure (Stolze et al. 2015, 2015; Sun et al. 2016). However, this phylum was more abundant (28.6%) in a mesophilic-thermophilic lagoon-type reactor treating pig manure and several wastes (Pampillón-González et al. 2017). In spite of proteinaceous feedstock are usually no recommended for biogas production considering the increased risk of inhibition by ammonium (Kragl and Aivasidis 2005), several studies had led to reach an adaptation of the microbial community to protein-rich biomass which can be appropriate to sustainable biogas production (Kovács et al. 2015, 2013).

Anaerobic digestion of *glycerol* as sole source or in co-digestion with other organic materials has been widely explored (Viana et al. 2012). However, both ways showed clear limitations mainly associated (1) to the presence of toxic compounds as LCFAs and inorganic salts of chloride and sulfates and (2) to the high chemical oxygen demand of glycerol. Despite of such disadvantages, microbial communities are able to adapt to high salinity, achieving promising methane potentials in anaerobic reactors treating only glycerol. Various works have shown that methane potential values are near to the theoretical methane production potential for glycerol (0.426m^3 CH_4/kg glycerol), making glycerol a challenge (Kolesárová et al. 2011; Siles et al. 2009; Yang et al. 2008).

Table 4 Principal orders involved in amino acid fermentation identified in biogas plants to reach an adaptation of the microbial community to protein-rich biomass which can be appropriate to sustainable biogas production (Kovács et al. 2015; Kovács et al. 2013)

Order	Biogas Plant	Substrate	Methane yield	Tan (g/l)	Functional role	Abundance	References
Clostridiales	Sewage treatment plants (STP, 37–40 °C)	STP03: Municipal and textile wastes STP07: Municipal, food, brewing and slaughterhouse wastes STP08: Municipal waste	STP03, 0.20 STP07, 0.80 STP08, 0.28 (m^3 biogas/m^3 fermenter volume/day)	ND	Only carry out Stickland reactions. All species use proline as main substrate forming acetate as end product	0.015–0.16% (OBP54 class *Clostridia*)	(Buettner and Noll 2018)
	Thermophilic BGP (54 °C)	Maize silage, barley, cattle manure, and pig manure	0.62 $m^3_{biogas}\ kg^{-1}vs\ d^{-1}$. Methane content, 54%	ND		36.5%	(Maus et al. 2016)
Synergistales	Full-scale mesophilic digester (34–35 °C)	Low substrate loading of sewage sludge	7000 m^3/d with a methane content of 61.26%	ND	*Synergistales* able to degrade most of the amino acids	4.61%	(Świątczak et al. 2017)
Thermotogales and Thermoanaerobacterales	Full-scale anaerobic	Food waste-recycling	ND	2–4.3	*Thermoanaerobacterales* produce acetate and propionate while	*Thermoanaerobacterales*, 7.9%	(Lee et al. 2016)

(continued)

Table 4 (continued)

	digester (58.5 °C)	wastewater (34% of protein)			*Thermotogales* produce acetate. All from a mix of L-alanine, L -serine, L-threonine, L-cysteine, L- glutamate, and L-methionine		
	Thermophilic BGP (54 °C)	Maize silage, barley, cattle manure, and pig manure	0.62 m^3_{biogas} kg^{-1}_{VS} d^{-1}. Methane content, 54%	ND		*Thermotogales*, 7.1% *Thermoanaerobacterales*, 0.22%	(Maus et al. 2016)
**Candidatus Cloacamonas*	Mesophilic-thermophilic (35–55 °C) AD reactor	Pig manure and wastes	318.6–543.9 m^3/day with 67% of CH_4	0.64	*Cloacimonetes* degrade only proline, alanine, aspartate, glutamate, lysine, and asparagine, as source of energy and generate CO_2 and H_2 as end products Other groups: *Marinimicrobia* and *Fusobacteriales* capable of ferment glutamate by five different pathways	*Cloacimonetes* 28.6%	(Pampillón-González et al. 2017)
	Industrial-scale biogas plants (37–38 °C)	CD3: Slaughter-house waste	347 ± 15 (methane potential)	CD3, 2.6		*Cloacimonetes*, 18% *Marinimicrobia*, 8.2%	(Sun et al. 2016)
	Mesophilic BGPs (2 and 3, 40 °C)	B2: Maize silage, grass, poultry/pig/cattle manure B3: Maize silage, pig manure	B2, 336.68 l/kg oDM B3, 276.93. l/kg oDM	B2, 2.32 B3, 3.15		*Cloacimonetes*, B2, 8% B3, 8–12% *Fusobacteriales*, B3, 15–18%	(Stolze et al. 2016)

	Mesophilic BGP (40 °C)	Dry fermentation (DF): Maize silage, green rye, chicken manure. Wet fermentation (WF): Maize silage, pig manure	DF, 350.5 l/kg oDM WF, 417.8 l/kg oDM	DF, 2.25WF, 2.85		*Cloacimonetes*, DF, 0.01% *Synergistales* WF, 0.4% DF, 0.1%	(Stolze et al. 2015)

TAN Total ammonia nitrogen, *ND* Not determined
*Phylum

Many microorganisms are able to metabolize glycerol in aerobic conditions; nevertheless, few are able to do it anaerobically. Species from the order *Enterobacteriales*, *Clostridiales*, *Lactobacillales*, *Bacillales*, and *Burkholderiales* have been reported to ferment glycerol in 1,3-propanediol and ethanol (Varrone et al. 2013; Yazdani and Gonzalez 2007; Zhou et al. 2017). More recently, sludge from brewery and glycerol used to methane production in a shock loading consortia acclimation showed that species from order *Thermotogales*, *Lactobacillales*, and *Clostridiales* were strongly dependent on the glycerol feeding system (Vásquez and Nakasaki 2016). Microbial dynamicity on glycerol fermentation has been also evaluated in anaerobic reactors overloaded with lipids, demonstrating a predominant order of *Selenomodales*, *Lactobacillales*, *Clostridiales*, and *Bacteroidales* (De Francisci et al. 2015). Lately, only one work has analyzed the enrichment of ammonia oxidation bacteria as *Candidatus Brocadia caroliniensis* from a full-scale process treating anaerobic digester effluent with the addition of glycerol. This worked attributed greatly the order *Brocadiales*, a partial transformation capability of glycerol (Park et al. 2017).

3.3 Acetogens and Syntrophic Acetate Oxidizers

Acetogens are mainly found in three phyla *Firmicutes*, *Acidobacteria*, and *Spirochaetes*. Nevertheless, most of them are inside of the first phylum and belong to *Clostridia* class (Scherer et al. 2018), as it can be observed in Table 5. In the study of St-Pierre and Wright (2014), the mentioned phyla were found in three full-scale digesters fed with cow manure as the main substrate. *Firmicutes* phylum was the most diverse and predominant in all digesters, the same occurred with *Clostridia* class. In contrast, the presence of *Negativicutes*, another class were acetogens can be found, was almost null (0.1% from *Firmicutes* reads). Interestingly, the dominant pathway for methane production affected *Clostridia* presence, showing less abundance when hydrogenotrophic methanogenesis prevail.

In an anaerobic digester fed with excess activated sludge, *Clostridium*, *Eubacterium*, *Thermoanaerobacter*, *Moorella* (all from *Clostridia* class) and *Treponema* (from *Spirochaetia* class) were the dominant acetogenic genus, but just the first two were among the top 50 in abundance. Furthermore, the prevalence of genes involved in the Wood-Ljungdahl pathway (i.e., acetate kinase and phosphate acetyltransferase) confirmed the constant formation of acetate and its role as precursor for CH_4 production; this latter was observed by the higher abundance of *Methanosaeta* (26.2% from total reads of methanogens) and *Methanosarcina* (12.8%) genera over hydrogenotrophic methanogens (*Methanospirillum*, 13.1%; *Methanoculleus*, 11.1%; *Methanoregula*, 7.6%) (Guo et al. 2015). A similar outcome was reported by Zhang et al. (2009) after the implementation of a Focused-Pulse treatment in a WWTP for biosolids removal enhancement. Here, microbial populations suffered a shift that caused the loss of hydrogenotrophic methanogens dominance against aceticlastic methanogenesis. In addition, an acetogenic group

Table 5 Taxonomical groups belonging to acetogenic bacteria from *Firmicutes* (1), *Acidobacteria* (2), and *Spirochaetes* (3) phylum (Müller and Frerichs 2013; Schink 1994; Ragsdale and Pierce 2008; complemented with NCBI taxonomic information)

Class	Order	Family	Genera and species
1.Clostridia	Clostridiales	*Clostridiaceae*	*Caloramator fervidus* *clostridium aceticum* *Oxobacter pfennigii* *Natronincola histidinovorans* *Tindallia californiensis*
		Eubacteriaceae	*Acetobacterium woodii* *Eubacterium limosum*
		Lachnospiraceae	*Acetitomaculum ruminis* *Marvinbryantia formatexigens* *Syntrophococcus sucromutans*
		Peptostreptococcaceae	*Acetoanaerobium noterae*
	Halanaerobiales	*Halobacteroidaceae*	*Acetohalobium arabaticum* *Natroniella acetigena*
	Thermoanaerobacterales	*Thermoanaerobacteraceae*	*Moorella glycerini* *Moorella thermoacetica* *Thermacetogenium phaeum* *Thermoanaerobacter kivui*
Negativicutes	*Selenomonadales*	*Sporomusaceae*	*Sporomusa ovata*
2.Holophagae	*Holophagales*	*Holophagaceae*	*Holophaga foetida*
3.Spirochaetia	*Spirochaetales*	*Spirochaetaceae*	*Treponema primitia*

called *Treponema primitia* was favored by the shift and increased its abundance from 7.1 to 11.5% (from *Spirochaetes* reads) even it is phylum reads decreased (18.8 to 13.2%), supporting acetate production.

As we have seen in digesters with cow manure as substrate, acetogens can also be found in reactors treating poultry or pig manure. Furthermore, their presence is not limited by awkward conditions such as the predominance of hydrogenotrophic methanogens. For example, in a pilot-scale digester exclusively fed with poultry manure, *Firmicutes* dominated bacterial abundance with 76%. Within it, *Clostridia* was composed of *Clostridiales* (64%) and *Thermoanaerobacterales* (11%). Two OTUs in *Clostridia* probably belonged to the last-mentioned order because they had a close similarity to *Moorella glycerini* and *Moorella thermoacetica*. Furthermore,

Table 6 Identified SAOB in *Firmicutes* (1) and *Thermotogae* (2) phylum (Hattori 2008; Maus et al. 2016; Ruiz-Sánchez et al. 2018; Westerholm et al. 2016; complemented with NCBI taxonomic information)

Class	Order	Family	Genera and species
1.Tissierellia	*Tissierellales*	*Tissierellaceae*	*Clostridium ultunense*
Clostridia	*Thermoanaerobacterales*	*Thermoanaerobacteraceae*	*Thermacetogenium phaeum*
		Thermoanaerobacterales family III. *Incertae sedis*	*Syntrophaceticus schinkii*
2.Thermotogae	*Thermotogales*	*Thermotogaceae*	*Thermotoga lettingae* *Thermotoga maritima*

1.6% of total OTUs abundance belonged to *Negativicutes* class and possibly to acetogenic microorganisms (Smith et al. 2014). However, aceticlastic methanogenesis did not prevail in the reactor, explaining the limited abundance of acetogens. The same prevalence of hydrogenotrophic methanogens was observed in another pilot-scale digester reported by Liu et al. (2009); pig manure was managed in this case. From microbial analysis, the abundant presence of phylum *Firmicutes* and *Spirochaetes* was observed with 47.2 and 13.2%, respectively. The former contained *Clostridia* class and most of its OTUs belonged to *Clostridiaceae* family, a striking source of acetogens, and the latter contained *Treponema* genus but not *T. primitia* species. More species related to homoacetogens were found, including *M. glycerini* and *Sporobacter termitidis*, but just comprised the 0.5 and 1% of total OTUs abundance.

Due to the prevalence of acetogens in *Clostridia* class, a common taxonomic classification for microorganisms participating in hydrolysis and acidogenesis phase, it is complicated to ensure which of them are present in biodigesters. Furthermore, direct production of acetate by fermentation can produce more confusion. Therefore, combination of metagenomic studies with the analysis of specific genes present in Wood-Ljungdahl pathway can be a useful method to present a clearer image of microbial species involved in this phase of the process. In addition, metatranscriptomic and metaproteomic studies can enhance this purpose.

Species belonging to SAOB are *Clostridium ultunense*, *Thermacetogenium phaeum*, and *Thermotoga lettingae*, the only mesophilic microorganism of the group is *C. ultunense*, and the rest are thermophilic. In addition, it has demonstrated ammonium resistance. Furthermore, the first two microorganisms have shown the ability to produce acetate with H_2 and CO_2 as substrates (Hattori 2008). Therefore, SAOB strains can belong to acetogenic bacteria. More examples of these microorganisms are given in Table 6.

The predominance of SAO for CH_4 production in biodigesters requires to overcome acetogenic bacteria and aceticlastic methanogens; this can be reached by their inhibition. It is known that both groups are susceptible to high ammonia

concentrations (Chen et al. 2014); on the other hand, SAOB and hydrogenotrophic methanogens are more resistant to this compound (Ruiz-Sánchez et al. 2018). Therefore, it is feasible that in anaerobic digestion of nitrogen-rich compounds, which present constant release of large ammonia-nitrogen amounts, such as animal manures, slaughterhouse, and food wastes, supremacy of CH_4 production by SAO will be observed (Chen et al. 2014; Ruiz-Sánchez et al. 2018). Hence, according to ammonia content, microbial populations in biodigesters will differ (Ruiz-Sánchez et al. 2018).

A scenario that favors SAO occurs in thermophilic digesters, which are present in high metabolic rates, large OLR management, greater CH_4 production, and lower HRT (Zinder 1990) compared with mesophilic digesters. In a thermophilic biogas plant, *Tissierella* class (*Firmicutes* phylum) and a species confirmed as SAOB, *Tepidanaerobacter* and *Syntrophaceticus* (*Thermoanaerobacteraceae* family), were found. As it was expected, hydrogenotrophic methanogens (*Methanomicrobiales* and *Methanobacteriales* orders) dominate the digester (Maus et al. 2016). Due to that the identified SAOB species are very scarce, this grants the possibility that unknown taxonomic groups belong to them. In the mentioned biogas plant, *Defluviitoga tunisiensis* abundance overpassed by far other microorganisms. Albeit, this genus is not identified as a SAOB; it probably participates in syntrophy with hydrogenotrophic methanogens in the digestion of biomass (Maus et al. 2016). Stolze et al. (2016) suggested the same idea and confirmed strain's ability to produce H_2 in a thermophilic digester dominated by hydrogenotrophic methanogens.

Another investigation that strengths the taxonomic diversity of SAOB was the one done by Ruiz-Sánchez et al. (2018). Their investigation of microbial diversity in mesophilic full-scale CSTR fed with pig manure and agricultural wastes at high ammonia levels (6–7 g TAN/L) did not find known SAOB species. Instead, genera *Longilinea* and *Alloprevotella* from *Chloroflexi* and *Bacteroidetes* phylum, respectively, predominated along with hydrogenotrophic methanogens (*Methanoculleus* and *Methanomassiliicoccus*). Furthermore, reactors developed well, CH_4 content in biogas ranged between 66 and 74%, and it was positively correlated with TAN concentration (Ruiz-Sánchez et al. 2018).

Sun et al. (2014) investigated specifically the presence of SAOB and the dominant methanogenic pathway in 13 well-functioning biogas plants and three thermophilic, and the rest were mesophilic. All thermophilic and seven mesophilic SAO coupled with hydrogenotrophic methanogens prevailed. In contrast, the rest three mesophilic digesters were dominated by aceticlastic methanogenesis. Interestingly, SAO process was observed in co-digestion plants, while one substrate biogas plant was managed by aceticlastic methanogenesis. In addition, higher free ammonia levels were present in co-digestion plants compared with single substrate plants; the former ranged values between 0.16 and 0.82 g/L and the latter 0.03–0.09 g/L of free ammonia. The results from metagenomic reads demonstrated that in all digesters, *Syntrophaceticus schinkii* was present; clearly, in digesters dominated by SAO, their abundance was higher. Another representative microorganism was *Tepidanobacter acetotoxidans*; however, it wasn't found where aceticlastic methanogenesis predominated. The mesophilic *Clostridium ultunense* was limited

to the digesters with high ammonia content, and *Thermacetogenium phaeum* showed the same behavior but in thermophilic conditions. Dominant methanogenic orders that accompanied SAOB were *Methanomicrobiales* and *Methanobacteriales* with more than 80% of methanogenic reads, overpassing the percent reached by aceticlastic methanogens in their digesters. The first order demonstrated high abundance in all digesters, and the second was preferentially found in thermophilic digesters (Sun et al. 2014).

It is generally thought that hydrogenotrophic methanogens contributes for a little fraction of the total methane produced. However, in digesters dominated by SAO, these methanogens dominate and can work under stressful conditions in a stable way. Therefore, the development of knowledge about the network involving this syntrophic relationship is an outstanding topic for a better understanding in process efficiency. Finally, Table 7 shows the microorganisms that have been identified in anaerobic full-scale reactors involved in acetate oxidation.

3.4 Methanogens

Methanogenesis is an antique process carried by methanogenic archaea which belongs to the phylum *Euryarchaeota*. These microorganisms are distributed around the planet, and they are the main source of methane emissions to the atmosphere. Up this date, seven taxonomic orders are acknowledged, each one grouping members with unique features. The methanogens' division included the orders *Methanobacteriales*, *Methanomicrobiales*, *Methanocellales*, *Methanopyrales*, *Methanococcales*, *Methanosarcinales*, and *Methanoplasmatales* (Alvarado et al. 2014). Albeit, in anaerobic biogas digesters, only *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales* group members were recognized (Alvarado et al. 2014). In biogas reactors, the amount of profitable methanogenesis is perhaps the most prominent indicator of a good performance and efficient.

It is well documented that 70% of methane production in biodigesters is carried out by the acetoclastic pathway, meanwhile the other 30% corresponding to the CO_2-reduction pathway. Members of *Methanobacteriales* and *Methanomicrobiales* utilize the hydrogenotrophic pathway. Hydrogen is commonly used as electron donor in this case, but some species also use formate and alcohols. On the other hand, *Methanosarcinales* are the most diverse in terms of metabolism. Acetate, hydrogen, format, ethanol, isopropanol, and methylated compounds can be metabolized by members from this order (Kendall and Boone 2006). Microbial community structure of archaeal communities has been evaluated in recent publications on full-scale biodigesters (Cheon et al. 2008; Werner et al. 2011; Regueiro et al. 2012; Sundberg et al. 2013; Li et al. 2015; Abendroth et al. 2015). Studies that describe the archaeal population in full-scale mesophilic biodigesters feed with dairy manure indicates a major prevalence of *Methanosarcina thermophila* with an abundance of 98.5% (St-Pierre and Wright 2013). In addition, Li et al. (2014) evaluated a mixed plug-flow loop reactor; the results indicate a high proportion (86%) assigned to the

Table 7 Presence of possible acetogenic and syntrophic acetate-oxidizing bacteria in anaerobic full-scale reactors

Substrate	Reactor type	HRT (D)	T (°C)	CH_4 yield (m^3/d)	kWh (month)	Phylum	%	Taxonomic classification	%	References
Acetogens										
Activated sludge	WWTP	5	35	1500	N/A	*Firmicutes*	12.5	*Clostridia*^{C} *Negativicutes*^{C}	72.5 3.32	(Guo et al. 2015)
Cattle manure, whey silage	Plug-flow digester	21	37.8	N/A	$1.29x10^5$	*Firmicutes*	52	*Clostridia*^{C} *Negativicutes*^{C}	44.4 0.1	(St-Pierre and Wright 2014)
Cattle manure, waste of ice cream factory	Plug-flow digester	25-27	38.3	N/A	$9.49x10^4$	*Firmicutes*	34.1	*Clostridia*^{C} *Negativicutes*^{C}	25.7 0.1	(St-Pierre and Wright 2014)
Cattle manure, oil waste	Plug-flow digester	30	36.1	N/A	$1.42x10^5$	*Firmicutes*	49.5	*Clostridia*^{C} *Negativicutes*^{C}	25.7 0.1	(St-Pierre and Wright 2014)
Primary sludge, waste, and activated sludge	WWTP	30-35	35-38	N/A	$6.39x10^5$	*Spirochaetes*	13.8	*Treponema primitia*^{S}	11.5	(Rittmann et al. 2008; Zhang et al. 2009)
Poultry manure	Pilot-scale CSTR	29.5	56.7	7.088	N/A	*Firmicutes*	76	*Clostridia*^{C} *Clostridiales*^{O}*Thermoanaerobacterales*^{O}	52 64 11	(Bombardiere et al. 2007; Smith et al. 2014.)
Pig manure	Pilot-scale digester	N/A	N/A	150	N/A	*Firmicutes* *Spirochaetes*	47.2 13.2	*Clostridia*^{C} *Moorella glycerini*^{S}	45.5 0.5	(Liu et al. 2009)

(continued)

Table 7 (continued)

Substrate	Reactor type	HRT (D)	T (°C)	CH_4 yield (m^3/d)	kWh (month)	Phylum	%	Taxonomic classification	%	References
								Sporobacter termitidis^S *Treponema*^{G}	1 4.2	
Syntrophic acetate oxidizers										
Maize and barley silage, cattle and pig manure	Thermophilic biogas plant (three digesters)	19.8	54	394.2	$5.13x10^3$	*Firmicutes* *Thermotogae*	36.5 7.1	*Tepidoanaerobacter*^{G} *Tissierella*^{G} *Defluviitoga*^{G}	0.41 0.02 5.53	(Maus et al. 2016)
Poultry manure	Pilot-scale CSTR	29.5	56.7	7.088	N/A	*Thermotoga*	N/A	*Defluviitoga teniensis*^{C}	3	(Bombardiere et al. 2007; Smith et al. 2014)

HRT hydraulic retention time, *WWTP* wastewater treatment plant; *N/A* not available; ^{C}Class; ^{O}Order; ^{G}Genus; ^{S}Species system. Methanogenic communities possess a specialized metabolism, features that make them more likely to be inhibited

genus *Methanosaeta* sp. Apart from that, a lagoon-type biodigester feed with pig waste showed a relative abundance of 52% and 42% of hydrogenotrophic *Methanospirillum* and acetoclastic *Methanosaeta*, respectively (Pampillón-González et al. 2017). Sundeberg et al. (2013) carried out a study in which 21 full-scale biogas digesters were evaluated. The microbial diversity indicates a prevalence of acetoclastic *Methanosaeta* sp. across all sewage sludge digesters. Meanwhile co-digestion at mesophilic conditions reactors was dominated by hydrogenotrophic *Methanoculleus* sp. and *Methanobrevibacter* sp. In addition, reactors operated under thermophilic conditions were dominated by *Methanobacterium* sp. Kirkegaard et al. (2017) evaluated 32 full-scale anaerobic digester systems fed with activated sludge. The report found that *Methanosaeta* sp. genus dominates the mesophilic reactors, and genus *Methanothermobacter* sp. was more abundant in thermophilic conditions over the 6-year period of the study.

4 Conclusions

The complexity of microbial diversity, their functional role, and its community interactions in a specialized environment such as biogas digester denote how particular is the phenomena behind AD process. Several microbial groups belonged to the phylum of *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* indicate that AD have stronger relationship between community structure and its function rather than its environment. On the other hand, methanogens seem to have more heterogeneity across full-scale biogas digesters and might substrate and operational conditions be the main factors that affect methanogen populations. In this regard, hydrogenotrophic methanogens show a high relative abundance in more biodigesters.

The multifunctionally of this process and the recent advances in next-generation sequencing technology will allow a best understanding of the microbial populations and their responses to environmental gradients during the digestion course. Furthermore, a comprehensive analysis of the microbial populations in full-scale anaerobic digesters allows to create economical strategies to improve bioenergy production in form of methane.

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