New concepts in anaerobic digestion processes: recent advances and biological aspects

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Abstract

Waste treatment and the simultaneous production of energy have gained great interest in the world. In the last decades, scientific efforts have focused largely on improving and developing sustainable bioprocess solutions for energy recovery from challenging waste. Anaerobic digestion (AD) has been developed as a low-cost organic waste treatment technology with a simple set-up and relatively limited investment and operating costs. Different technologies such as, one-stage and two-stage AD have been developed. The viability and performance of these technologies have been extensively reported, showing the supremacy of two-stage AD in terms of overall energy recovery from biomass under different substrates, temperatures and pH conditions. However, a comprehensive review of the advantages and disadvantages of these technologies is still lacking. Since microbial ecology is critical to developing successful AD, many studies have shown the structure and dynamics of archaeal and bacterial communities in this type of system. However, the role of Eukarya groups remains largely unknown to date. In this review, we provide a comprehensive review of the role, abundance, dynamics and structure of archaeal, bacterial and eukaryal communities during the AD process. The information provided could help researchers to select the adequate operational parameters to obtain the best performance and biogas production results.

Keywords: anaerobic digestion; one stage vs two stage; microbiome; Archaea, Bacteria and Eukarya communities

Introduction

Energy production from renewable sources and efficient waste treatment are two of the more relevant scientific and social challenges nowadays (De Vrieze et al. 2017). In the last two decades, anaerobic digestion (AD) has been proven to be a valuable method able to solve both of these issues, combining recycling of different waste materials with the production of biogas (Oslaj et al. 2010; Tyagi and Lo 2013).Current systems based on AD aim to convert organic matter into biogas. During this process, hydrolyzing microorganisms hydrolyze organic polymers (i.e. fats and proteins) producing simple molecules (i.e. sugars, amino acids and fatty acids); acidogenic microorganisms consume free monomers generating volatile fatty acids (VFAs) and alcohols; acetogenic microorganisms transform VFA and alcohols into acetic acid, CO_2, and H_2; methanogenic archaea consume acetic acid or hydrogen to generate CH_4 (Gonzalez-Martinez et al. 2016a; Zhang et al. 2016b).

AD is a process that can be applied to almost any organic waste. Many different substrates have been discussed in the literature: agricultural waste, food waste, animal manure, feed waste, energy crops and plant residues, such as brewery wastewater (Pozo et al. 2002; Chen et al. 2008; Meulepas et al. 2010). In addition to the digestion of individual substrates, AD reactors can be loaded with mixtures of different residues. This approach, which is usually termed 'co-digestion' or 'co-fermentation', offers various technical and commercial advantages. One example is the biostimulating effect coming from the overproduction of nutrients, which can accelerate the degradation of solid waste (Beyene et al. 2018). Moreover, the application of mono or co-digestion is an efficient alternative to obtain a stabilized solid waste that can be applied as soil conditioner (Rolando et al. 2011; Gómez et al. 2006).

The aim of this review is threefold. First, we will discuss relevant features of AD: the structure of the plants (one-stage vs two-stage AD), the operational temperature (mesophilic vs thermophilic) and other technologies in biogas production. A second section will be devoted to describe the role of the microbiome (Archaea, Bacteria and Eukarya communities) involved in AD and its link to operational and performance parameters and biogas production. Finally, we will discuss future implications and prospective biotechnologies in AD.

Digester configurations: advantages and disadvantages

Since the appearance of AD, a wide variety of digester configurations has been tested such as thermophilic/mesophilic digestion, dry/wet digestion, one-phase/two-phase digestion or one-stage/two-stage digestion (Møller et al. 2009; Nizami et al. 2009; Khalid et al. 2011; Mao et al. 2015; Sun et al. 2015; Chen et al. 2016). Among these, the most relevant comparison, as well as the one most debated in the literature, is that based on the number of stages. However, independently of the digester configuration to obtain a high digestion efficiency, anaerobic bioreactors should allow a continuously high and sustainable organic load rate operating with short (Khalid et al. 2011) or long (Bergland et al. 2015) hydraulic retention time (HRT) depending on the substrate.

The simplest possible configuration is the one-stage AD batch reactor, in which the tank is filled with the feedstock and let stand for a period after which it is emptied (Khalid et al. 2011). Although this kind of system has very low operational cost, it exhibits some limitations such as high fluctuations in gas production, biogas losses during emptying the bioreactors and restricted bioreactor heights (Khalid et al. 2011; Zhang et al. 2015; Sunyoto et al. 2016). A more widely used type of one-stage AD bioreactor is commonly defined 'one-stage continuously fed systems'(Khalid et al. 2011). In one-stage AD system, hydrolysis, acidogenesis, acetogenesis

and methanogenesis take place in the same tank. This implies that acidogenic and methanogenic microbiota have to cohabit despite the existence of marked differences regarding growth factors and kinetics, nutritional needs and environmental conditions such as pH and temperature (Gonzalez-Martinez et al. 2016b; De Gioannis et al. 2017). In this context, although the ideal pH range for AD has been reported to be between 6.8–7.4, it is known that in one-stage AD bioreactor the operational pH sometimes can affect the digestive progress and products directly. However, two-stage AD process separating the hydrolysis/acidification and acetogenesis/methanogenesis processes, provides optimal conditions for each of the microbiota, since the optimal pH levels for acidogenic (5.5–6.5) and methanogenic (7.0) microorganisms can be controlled to increase the efficiency of the process (Mao et al. 2015). Consequently, in these kinds of systems, the different sub-processes of AD take place in separate sequential reactors. The most common configuration is the two-stage continuously fed system, although three-stage systems have been proposed (Angelidaki et al. 2003). Two-stage AD were originally conceived by Pohland and Ghosh (1971), and soon gained popularity, particularly for laboratory applications (Nizami et al. 2009). Although overall performance supremacy of two-stage AD has been variously reported in the literature, one-stage AD are far from being replaced (Møller et al. 2009). According to Rapport et al. (2012), 90% of the total capacity of the full-scale AD plants installed in Europe at that time was covered by one-stage systems. The main reasons behind this are probably the simpler structural features and lower operating costs. On the other hand, two-stage AD provides higher substrate conversion and better energy recovery, as well as better process stability, resilience and reliability (Salvador et al. 2013; De Gioannis et al. 2017; Shen et al. 2017).

Multiple-stage reactors have been developed to improve process stability and efficiency (Achinas et al. 2017). In this sense, Kim et al. (2011) demonstrated significantly higher digestion efficiency of a four-stage AD system using activated sludge than a single-stage system. Likewise, a novel alternative technique based on a high working pressure (up to 100 bar), permits the production of biogas with more than 95% methane content. This technique integrate in a single process both biogas production and *in situ* increased-pressure purification, generating a clean biogas (99% methane) that can be fed directly into the natural gas networks. However, the effect of the working pressure on microbiome structure is still unknown (Lindeboom et al. 2011). The complexity and high cost of this novel technologies are barriers to commercial use and until date, few multiple-stage AD units operate on a commercial scale.

Thermophilic and mesophilic conditions

A further relevant way to classify AD systems is to consider their operating temperature. Although the biogas process can proceed at different temperatures, mesophilic (30–40°C) and thermophilic (50–60 °C) conditions are commonly used (Møller et al. 2009; Wang et al. 2018). Temperature is, indeed, one of the main environmental factors affecting physical parameters such as viscosity, surface tension and mass transfer properties. Moreover, small changes in the temperature can result in a reduction in process efficiency, so its stability is also important (Angelidaki et al. 2003).Above all, temperature must be considered in relation to microbial growth and reactions (Amani et al. 2010; Gonzalez-Martinez et al. 2017) and changes in the structure and dynamics of prokaryotic and eukaryotic groups (see Section 2). The groups of microbes that have been identified for AD are mesophilic and thermophilic strains. While great diversity exists between mesophilic and thermophilic bacteria, with the latter

showing both higher specific growth and decay rates, methanogen growth is mostly favoured by both mesophilic and thermophilic temperatures (Li et al. 2015; Kundu et al. 2017).

Neither of the two conditions (i.e. mesophilic or thermophilic) is absolutely preferable. Although mesophilic digestion has some disadvantages (i.e. lower metabolic rate, lower rate and efficiency of particulate matter hydrolysis, smaller degree of pathogen deactivation and lower biogas production yields) (Liu et al. 2017), it has important advantages, such as a lower VFA concentration in the final effluents, maintenance of a higher organic loading rate (OLR) (Bayr et al. 2012) and a more stable performance (Guo et al. 2014), compared to thermophilic digestion (Appels et al. 2008; Wang et al. 2017). On the other hand, thermophilic temperatures can produce large quantities of dissolved solids in the digester supernatant and more odours, and have acidification potential and higher energy requirements. For these reasons, two-stage AD offers the opportunity to operate thermophilic hydrolysis/acidogenesis and mesophilic methanogenesis, as a good compromise. Of note, a different approach not requiring an extra heat supply, named '*ambient/seasonal temperature AD*', has also been used for organic waste. However, the changes in temperature induce less stability and lower methane production compared with the mesophilic process (Mao et al. 2015).

Biogas production

Currently, AD is implemented in various ways worldwide. In the Western world there are, to date, about ten thousands of operational AD plants (Yousuf et al. 2016; Vasco-Correa et al. 2018). A comparable amount can be found in Asia, where rural communities use small-scale household digesters for domestic necessities (Surendra et al. 2014). Similar small-scale digesters have also been installed in rural regions of Latin America and Africa during the last few years (REN21, 2016). Laws on the subject of environmental protection and waste treatment, as well as new emerging candidate substrates and innovative technologies, will surely guide the evolution of AD.

Different compositions of mixed substrates have been reported to increase the production of biogas, such as mixing municipal solid waste with industrial sludge (Ağdağ and Sponza 2007) or olive mill wastewater with olive mill solid waste (Fezzani and Cheikh 2010). In addition, co-digestion has been proved to stabilize reactor performance (Lo et al. 2010; Beyene et al. 2018). Interestingly, the use of this approach with substrates rich in carbon has been proposed as a solution to reduce ammonia and other toxic substances (Rajagopal et al. 2013; Fitamo et al. 2017). Moreover, co-digestion is an efficient strategy to degrade those kinds of waste that are difficult to process as a unique substrate. Recently, Park et al. (2016) tested different mixtures in order to optimize the processing of sewage sludge, obtaining optimal results in combination with food waste. As a further solution, Shen et al. (2017) proved that the combination of sewage sludge and pyro-biochar can improve biomethane production, compared with the digestion of sewage sludge alone.

As an example, the Korean government recently solicited the use as an AD substrate of organic waste from ocean dumping or landfill, with the aim to produce renewable energy; this raises the issue of efficiently degrading septage and sewage sludge, and the consequent investigation of different mixtures for co-digestion approaches (Park et al. 2016). Otherwise, good availability of a specific kind of waste can turn it into a candidate substrate. In Colombia, for example, the massive production of coffee generates a large amount of coffee mucilage, a crop residue rich in carbohydrates. This organic matter has been successfully used in co-digestion with pig manure to produce biohydrogen, taking advantage of two types of organic waste readily available in the

same geographical region (Hernández et al. 2014). Finally, technical innovations will help the scale-up of currently experimental systems.

Biohythane is a promising sustainable alternative to hythane. It is more environmentally friendly, requires a shorter fermentation time and offers better energy recovery than traditional biogas. Despite research interest in the production of this gas, numerous challenges have still to be addressed in order to allow large-scale production of biohythane by means of AD (Liu et al. 2018). Similarly, technical improvements are needed for the realization of full-scale three-stage AD plants. Hitherto, an in-lab preliminary study has proved that this approach could considerably improve the production of methane (Zhang et al. 2017). A further promising strategy to increase biogas yield and system performance is the application of selected microbial consortia, often taken from another operating plant. However, more accurate knowledge concerning adaptation of the inoculum is required in order to maximize the potential advantages of this approach (Wojcieszak et al. 2017).

Archaea, Bacteria and Eukarya communities in anaerobic digestion processes

Integration of microbial aspects within the framework of AD is critical to achieve the desired performance and biogas production. The microbiome as an entity does not work as a randomized mix, and scientific efforts focus largely on linking operational and performance parameters with the structure of microbial communities. Here, we highlight engineering of the microbiome, focusing on the most crucial Archaea, Bacteria and Eukarya groups.

Abundance, structure and dynamics of the microbiome in anaerobic digestion processes

Microbial ecologists and engineers have shown increasing interest concerning insight into the microbiome in anaerobic digesters. So far, the most crucial microorganisms have been identified although few authors have linked operational and performance parameters and microbiome response at laboratory or full-scale conditions (Carballa et al. 2011; Werner et al. 2011; Carballa et al. 2015; Gonzalez-Martinez et al. 2016b; De Vrieze et al. 2017; Kundu et al. 2017; Wang et al. 2018). Since a strong syntrophic relationship exists between acetogenic and methanogenic organisms involved in AD, biomonitoring of the system could be an important feature for engineers to obtain a highly efficient microbiome and to predict and prevent system failure (Amani et al. 2010). For example, Kundu et al. (2013) showed that a high degree of microbial diversity could be indicative of stable AD performance. Recently, a methodological approach to link microbial and operational data has also been described (de Los Reyes III et al. 2015).

The development of next-generation sequencing technologies has offered an opportunity to describe the microorganisms present (DNA) or active (RNA) in engineered ecosystems as well as their abundance (Muñoz-Palazon et al. 2018). Nevertheless, a combined DNA–RNA approach would result in a more accurate methodology to link the microbial community's structure and its metabolic ability requirements (Kaever et al. 2014; Maus et al. 2016). Identification of the critical representative species by means of these techniques can help to increase the efficiency and stability of AD (Venkiteshwaran et al. 2015; Dang et al. 2017). In this sense, the presence of sulphate-reducing bacteria in AD can decrease methane production because of substrate competition and sulphide inhibition of the methanogenic community (Chen et al. 2008; Sasaki et al. 2011). Thus, biomonitoring tools can help to prevent inefficiencies in AD.

The AD process comprises four interdependent steps in which microorganisms responsible for a specific stage provide the intermediates for the next. Microbial community structure and dynamics are important to sustain functional redundancy and to maintain a well-balanced process (Allison and Martiny 2008; Ziganshin et al. 2013). Archaea, Bacteria and Eukarya communities form the microbiome of the anaerobic digester and change during the stages of the AD process (Matsubayashi et al. 2017).

Archaea play a central role during methanogenic processes of AD, and it has been reported that these microorganisms can be related to different operational parameters (Zhang et al. 2012; Smith et al. 2014; Hao et al. 2016). Synthesis of CH_4 is carried out both by acetoclastic (e.g. *Methanosaeta*, *Methanosarcina* and *Methanothrix*) and hydrogenotrophic methanogens (e.g. *Methanobacterium*, *Methanomicrobium*, *Methanococcus*, *Methanobrevibacter*, *Methanomassilii* and *Methanospirillum*) using acetic acid, or by using H_2 and CO_2 or methyl compounds to synthesize CH_4 (Calderón et al. 2013; Gonzalez-Martinez et al. 2016b). The characteristics and properties of the main methanogens involved in an AD as well as their substrates and products have been reported (McHugh et al. 2003; Amani et al. 2010; Goswani et al. 2016; Kundu et al. 2017). In most of the studies in the literature, Archaea diversity decreases with temperature elevation (Kundu et al. 2012; Guo et al. 2014), an effect more remarkable than changes in OLR which abrupt increase (from 1 to 8 g VS L^{-1} d^{-1}) seemed to have little influence on the microbial community (Gou et al. 2014). Hao et al. (2016) compared the effect of total solid (TS) concentrations on archaeal diversity in sludge-fed digesters. Under high TS conditions (TS > 44 g/L), the relative abundance of *Methanosarcinaceae* and *Methanobacteriaceae* families increased whereas when digesters operated at lower-TS (TS $\leq$ 44 g/L) only *Methanosaetaceae* family was favoured. Under the use of continuous lab and full-scale reactors and food waste substrate the genus *Methanosarcina* is dominant under thermophilic conditions, with abundance higher than 80%, although *Methanothermobacter* and *Methanoculleus* are also favoured (Cho et al. 2013; Wang et al. 2018), whereas *Methanosaeta* is dominant under mesophilic conditions (accounting for>25% of relative abundance) (Gonzalez-Martinez et al. 2016b). On the other hand, *Methanosaeta* instead of *Methanosarcina* is favoured under low acid concentrations. Since VFA accumulation results in lower values for pH, Guo et al. (2014) showed a decrease in archaeal diversity when VFAs produced in the hydrolytic step are not consumed by methanogens. In fact, acetoclastic methanoarchaea have a positive correlation with VFAs and NH_4^+ (Lin et al. 2012). Methanogen diversity is also sensitive to a pH value lower than 6.5, particularly during acid and acetate accumulation (Bräuer et al. 2006). In general, lower hydraulic retention time values decrease archaeal diversity by selecting organisms with a high growth rate and poor substrate affinity. In this sense, *Methanosaetaceae* (slower growth rate) predominate when HRT> 5 days, while *Methanosarcinaceae*, *Methanobacteriales* and *Methanomicrobiales* (faster growth rate) become dominant at HRT< 2 days (Padmasiri et al. 2007; Chelliapan et al. 2011). Regueiro et al.(2014) reported that *Methanosaeta* is crucial for reaching stable reactor performance although the archaeal community structure is affected by substrate type. Moreover, taking into account operational performance parameters, Kundu et al. (2017) indicated *Methanosaetaceae* as the best candidate for biomonitoring based on its sensitivity to fluctuations in the AD process.

The presence of bacterial genera such as *Desulfotomaculum*, *Desulfovibrio*, *Syntrophobacter*, *Syntrophomonas*, *Syntrophospora*, *Clostridium*, *Bacteroides*, *Bifidobacterium*, *Butyrivibrio*, *Pseudomonas*, *Bacillus*, *Streptococcus* and *Eubacterium* has been related to acid formation and hydrogen release (Yamada et al. 2006; Gonzalez-Martinez et al. 2016a), and synergistic cooperation with methanogenic archaeal groups in

methanogenesis bioreactors has also been considered (Demirel and Scherer 2008). González-Martinez et al. (2016b) studied archaeal and bacterial community dynamics of a bench-scale two-stage anaerobic digester. An overview of the response of key archaeal and bacterial phylotypes to changes in performance parameters is presented in Fig. 1a and 1b, respectively.

In the acidogenic phase, organic matter is biodegraded to VFAs by bacterial communities. During this phase, *Bacteroidetes*, *Chloroflexi*, *Cloacimonetes*, *Firmicutes* and *Proteobacteria* are the predominant phyla. Moreover, *Microthrix* spp. are usually associated with operational dysfunction while *Firmicutes* species in the digesters are important acetogens utilizing simple and complex carbohydrates (Tracy et al. 2012). *Synergistetes* spp. can utilize amino acids as an energy source to produce VFAs for methanogens (Vartoukian et al. 2007), whereas *Proteobacteria* have been recognized as one of the main consumers of VFAs (Ariesyady et al. 2007). Moreover, *Syntrophomonas* strains are present during this phase and are able to syntrophically degrades straight-chain fatty acids (4–8 carbon atoms) into propionate, acetate and methane in co-culture with methanogens (Zhang et al. 2005).

Changes in operational and performance parameters influence bacterial diversity. Hao et al. (2016) found that under high TS conditions, the relative abundance of *Thermoanaerobacteraceae, Syntrophomonadaceae, Rhodobacteraceae, Comamonadaceae* and *Xanthomonadaceae* families were enriched. In contrast, digesters at lower-TS favoured *Syntrophaceae, Syntrophobacteraceae, Anaerolineaceae, Rikenellaceae* and *WCHB01-69* and *Candidatus Cloacamonas* families. Under thermophilic and mesophilic conditions, Guo et al. (2014) found that *Firmicutes* was the common phylum appearing at both temperatures, accounting for 10–20% of relative abundance. *Thermotogae* (60–80% of relative abundance) and *Bacteroidetes* (5–45% of relative abundance) were the dominant taxa under both conditions, respectively. *Proteobacteria* were present in limited amounts and only in thermophilic AD whereas *Synergistetes* appeared in both reactors. Although the relative abundance of *Chloroflexi*, *Actinobacteria* and *Spirochaetes* was higher than that in thermophilic AD, they were poorly represented, accounting for <3% of relative abundance. Finally, *Gelria*, uncultured *Lachnospiraceae, Ruminococcaceae Incertae Sedis, Sporanaerobacter, Tepidanaerobacter, Petrobacter* and *Anaerobaculum* were related to performance variations with OLR elevation.

Adaptation of bacterial communities during the start-up stage of thermophilic and mesophilic AD was explored by Wu et al. (2016) and González-Martínez et al. (2016b), respectively. Under thermophilic conditions, the relative abundance of *Firmicutes* increased gradually; on the contrary, *Proteobacteria* and *Thermotogae* decreased. Under mesophilic conditions, the more abundant microorganisms were related to *Clostridiaceae* (21.49%), *Treponema* (5.10%), *Synergistetes* (4.11%) and *Paenibacillaceae* (3.25%) whereas *Cloacamonas* and *Comamonas* were present at >3% abundance only at the beginning of AD, decreasing after that. Zhang et al. (2016a) analysed the microbial community in the anaerobic co-digestion of food waste and sewage sludge. *Firmicutes*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* were found as the predominant phyla in the bacterial community. *Firmicutes* increased significantly on day 5 at acidification phase corresponding to VFAs accumulation. After that, the abundance of *Firmicutes* and *Bacteroidetes* increased much more from day 12 at the active methane production phase. *Proteobacteria* and *Actinobacteria* decreased significantly during the experimental period. The greatest changes in these four dominant phyla all appeared on day 5, which could be an indicator of the acidification phase corresponding to VFA accumulation. Hydrolytic bacteria are known to have a lower sensitivity to changes in environmental factors, such as pH and temperature, than methanogens.

Although the role of eukaryotes in performance, predation on bacteria and excess sludge production has been reported during aerobic treatment processes (Ntougias et al. 2011), it is also important to investigate the diversity, roles and functions of eukaryotes in AD. Few authors have reported on diversity and roles/functions in AD (Luo et al. 2005; Matsubayashi et al. 2017). Under mesophilic AD, *Rotifera* and *Phragmoplastophyta* are the most representative phyla and the majority of eukaryal phylotypes belong to Fungi (42.2%), followed by Animalia (28.8%), Protista (13.3%) and finally Plantae (8.9%). In addition, Luo et al.(2005) described the microeukaryotic community in anaerobic sulphide- and sulphur-rich springs, whereas Matsubayashi et al. (2017) constructed clone libraries by sequencing the 18S rRNA gene in anaerobic sludge digesters (Table 1). The latter study suggested that prokaryotic and eukaryotic community structures do not work independently, and that the functional features of both communities are closely related.

Very limited information on the physiology of anaerobic or facultative anaerobic eukaryotic organisms is available to date. Some of the Fungi found in AD contribute to the degradation of some organic matter in anaerobic environments and they could be implicated in the hydrolysis of organic matter in anaerobic sludge digestion processes. Previous studies have demonstrated that phylotypes in Plantae, Animalia and Fungi can produce CH_4 (Liu et al. 2015; Gorrasi et al. 2014).

Regarding the dynamics of the microbiome during AD, contrasting results have been obtained, showing large changes (>25%) from bench-scale mesophilic anaerobic digesters inoculated with sludge from wastewater treatment plants (Schauer-Gimenez et al. 2010; De Vrieze et al. 2013) or high consistency from reactors with an upflow configuration with anaerobic granular biomass (Werner et al. 2011). Given the presence of a wide variety of microorganisms in the influent of AD, dynamic changes in community diversity are likely the result of proliferation of organisms that are adapted to the selective pressures in each bioreactor. However, a core microbiome dominates the reactors, showing the strong selective pressures present in this type of environment (Town et al. 2014; Gonzalez-Martinez et al. 2016b). Maspolim et al. (2015) compared the microbial community dynamics in single-stage and 2-phase anaerobic AD systems treating municipal sludge and the analysis revealed that microbial adaptation occurred as the sludge formed a different community in each reactor at 30 d HRT but no significant microbial changes occurred at lower HRTs. Engineering of the microbiome by adjusting operational parameters leads to a stable microbial structure (Vanwonterghem et al. 2014; De Vrieze et al. 2016). Accurate monitoring of the microbial community requires that the metabolic potential and mode of interaction between the different microorganisms are distinguished from sudden unwanted changes related to unfavourable operational conditions. While generalist microorganisms are able to occupy a broad range of niches based on their greater phenotypic plasticity (van Tienderen 1997), specialists occupy only a limited number of niches and show high levels of specificity. The former can be considered as keepers of process stability (Matias et al. 2013) whereas the latter may drive evolution towards new traits in the microbial community and could be of direct interest in the search for new potential.

The dynamics of prokaryotic organisms have been described during the start-up stage of AD (Gonzalez-Martinez et al. 2016b) as showing substantial changes under unstable conditions. Thus, a challenge exists to develop a useful biomonitoring tool for environmental engineers. Many studies have indicated that *Methanosaeta* and *Methanosarcina* are competitive genera in the AD process. During the acidification phase, *Methanosaeta*, an acetoclastic methanogen, is the dominant genus but its abundance decreases significantly during the methane production phase. In the latter phase, the acetoclastic methanogen *Methanosarcina* increases

significantly. *Methanosarcina* is more tolerant to inhibitors than *Methanosaeta* (Cho et al. 2013). At the end of AD, *Methanoculleus*, a hydrogenotrophic methanogen, becomes dominant because of the exhaustion of acetic acid. Previous studies have reported that for continuous and fed-batch systems, bacterial community dynamics show larger changes than those for the archaeal community, but there is similar diversity, and VFA-producers show greater relative abundance. Generally considered, the hydrolysing bacterial groups *Bacteroides*, *Cloacamonas*, *Clostridiaceae* and *Rikenellaceae* are dominant at the beginning of AD and finally change to other bacterial groups such as *Clostridiaceae*, *Fervidobacterium*, *Paenibacillus* and *Spirochaetes* (Ghasimi et al. 2015;Gonzalez-Martinez et al. 2016b).

Microbial and Eukaryal groups involved in biogas production

AD for methane production has already been widely adopted (Cavinato et al. 2013; Carrere et al. 2016) using methanogenic microorganisms able to utilize simple organic substrates, such as acetate, CO_2/H_2, methanol and formate (de Bok et al. 2004). A deep insight into the main archaeal and bacterial phylotypes of AD involved in biogas production under different operational conditions can be seen in Hao et al. (2016). There are three main types of methanogen, namely acetoclastic, hydrogenotrophic and methylotrophic. Most archaea produce methane by the hydrogenotrophic route and only those belonging to the order *Methanosarcinales* produce it by the acetoclastic route. *Methanobacterium*, *Methanothermobacter*, and *Methanospirillum* are the most commonly identified hydrogenotrophic methanogens in the AD process. Acetoclastic methanogens belong to two genera: *Methanosaeta* and *Methanosarcina* (Venkiteshwaran et al. 2015; Gonzalez-Martinez et al 2016b). *Methanosaeta* can be considered a key methanogen in the AD process, given its unique morphology and physiology (De Vrieze et al. 2012; 2015), catalysing renewable energy production from organic waste streams.

Bacteria can support methane production during the process of methanogenesis by hydrolysation of organic matter. Positive correlation of *Cytophaga*, *Herbaspirillum*, *Symbiobacterium*, *Comamonas* and *Allochromatium* with biogas production has been found (Gonzalez-Martinez et al. 2016b). The genera *Cytophaga* and *Symbiobacterium* are important organic matter degraders in AD in the hydrolysis and acidogenesis processes, respectively (Panichnumsin et al. 2012; Yi et al. 2014)**.**The importance of *Herbaspirillum* sp. remains widely unclear due to its inability to carry out fermentation (Schmid et al. 2006), but its relationship to biogas production (Gonzalez-Martinez et al. 2016b) and degradation of complex organic matter has been reported (Guo et al. 2015).

The role of Eukarya in the production of methane remains largely unknown although Plantae, Animalia and Fungi eukaryal phylotypes have been reported to direct produce CH_4,even in the presence of oxygen (Liu et al. 2015; Gorrasi et al. 2014). However, the mechanisms involved in this pathway remain largely unclear and it has been proposed that CH_4 originates from organic methyl-type compounds in response to environmental stresses. Although it is estimated that plants could contribute around 3–24% to the global CH_4 budget, an estimate of CH_4 production by animals and fungi is still lacking. Consequently, Eukarya are not considered as a CH_4 source by the Intergovernmental Panel on Climate Change (IPCC), and their role in biogas production could be useful for better quantitation of the global CH_4 budget. The influence of rumen fungi for improvement of biogas production from animal manure on anaerobic digesters have gained attention as a biological pre-treatment option of various polymeric substances. These microorganisms are able to effectively digest lignocellulosic compounds, providing energy due to symbiotic associations with rumen microorganisms (Yıldırım et al. 2017). For instance, Gorrasi et

al. (2014) demonstrated the potential application of chitinolytic fungi to obtain H and Ma et al. (2015) determined that rumen microorganisms have higher hydrolytic and acidogenic activity than other microbial species using lignocellulosic biomass as substrates.

Future implications and prospective biotechnologies

New advances in monitoring AD will require the application of control strategies to redirect the microbiome to reach a stable functionality. Until now, microbial process control actions have usually taken place by altering basic operational parameters, such as pH and temperature. For example, increases in AD efficiency were done using different ways: bioaugmentation, as a suitable alternative to increase VFA removal (Town and Dumonceaux 2016) or hydrolysis (Martin-Ryals et al. 2015); microwave (MW) pre-treatment, as an effective way of enhancing biogas production and solids removal (Coelho et al. 2011). However, to engage direct steering of the microbiome to sustain process stability, this knowledge has to be integrated into advanced monitoring and control strategies. For example, the ratio of syntrophic acetate-oxidizing bacteria or methanogenic archaea to total bacteria has been suggested as a possible microbial community monitoring strategy for AD (De Vrieze et al. 2012). This has to be based on specific genes and/or their transcripts, such as the methyl co-enzyme M reductase (*mcrA*) gene for methanogens (Wilkins et al. 2015) and the formyl tetrahydrofolate synthetase (FTHFS) gene for syntrophic acetate-oxidizing bacteria (Akuzawa et al. 2011; Hori et al. 2011).

The study of biogeochemical cycles in natural ecosystems can drive innovation in bioenergetics applications to support improvements of AD. In this sense, Izzo et al. (2014) explored the potentials offered by the structural and functional microbial biodiversity in hypertrophic lagoons characterised by rapid and huge biomass blooms and decomposition. They selected the microbial communities as inoculum and successfully tested for hydrogen production on different kinds of organic wastes.

To decrease the cost of the treatment is of vital importance in AD. This can be achieved by using raw material with lower water content and running the process with a higher dry matter content. The biogas produced can often be utilized to cover the need for process energy. Thus, the economy of a biogas plant is directly linked to the amount of biogas produced per unit of raw material treated in the plant. In short, advanced and direct monitoring of the microbiome is possible through the application of different microbial techniques. Accurate and quick decision tools have to be developed. The integration of existing physicochemical techniques and microbiome-based monitoring is necessary to increase product recovery and the overall energy efficiency of microbial processes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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715

716 **Table 1** Main eukaryal phylotypes found in anaerobic digesters. Data were taken from Matsubayashi et al. (2017).

Kingdom/ Superphylum	*Phylum*	
Alveolata	*Perkinsozoa*	*A31*
Amoebozoa	*Discosea*	*Order Dactylopodida*
	Gastrotricha	*Chaetonotus cf.*
Animalia	*Gastrotricha*	*Chaetonotus cf.*
Archaeplastida	*Chlorophyta*	*ANI-3*
	Chlorophyta	*Family Chlorellaceae*
	Chlorophyta	*Prototheca zopfi*
	Ciliophora	*Acaryophrya sp.*
	Ciliophora	*Vorticellides aquadulcis*
Fungi	*Arthropoda*	*Allonothrus sp.*
	Arthropoda	*Boletoglyphus sp.*
	Arthropoda	*Naidacarus arboricola*
	Arthropoda	*Rhizoglyphus sp.*
	Ascomycota	*Candida sp.*
	Ascomycota	*Exophiala equine*
	Ascomycota	*Family Dipodascaceae*
	Ascomycota	*Penicillium chrysogenum*
	Ascomycota	*Phoma sp.*
	Ascomycota	*Xenobotrytis sp.*
	Basidiomycota	*Lentinus sp.*
	Basidiomycota	*Trichosporum cutaneum*
	Cryptomycota	*LKM11*
	Cryptomycota	*LKM15*
Metazoa	*Platyhelminthes*	*Gieysztoria sp.*
	Rotifera	*Brachionus calyciflorus*
Rhizaria	*Cercozoa*	*Rhogostoma minus*
Stramenopiles	*Hyphochytriomycetes*	*Rhizidiomyces apophysatus*

717

718 **Figure legends**

719

720 **Fig. 1** Multivariate redundancy analyses relating performance parameters (dried sludge, volatile dried sludge,
721 pH, acid/alkalinity ratio AC/AL, O_2, CO_2 CH_4 and biogas production) with changes in diversity or abundance of
722 the most representative archaeal **(a)** and bacterial **(b)** phylotypes in anaerobic digestion. Data were taken from
723 González-Martínez et al. (2016b)