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METAGENOMICS INVESTIGATION OF ANAEROBIC DEGRADATION ECOSYSTEMS

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Abstract

Biomethane has gained increasing attention in the recent years as an alternative, local energy source option. Biogas is generated during the anaerobic digestion of organic materials via a multistep process catalyzed by complex microbial communities. This review aims at providing a concise summary of recent studies on the microbial communities in various biogas reactors. The effects of acid composition, C/N ratio, mixing and the geometry of the anaerobic digester on the microbial ecosystem are discussed. The biogas microbial communities show extensive fluctuations in response to changes in temperature, substrate type, pH, type of volatile fatty acids, organic loading rate, etc. The goals to ensure efficient anaerobic degradation and to maximize the biogas production require the better understanding of these bacterial-archaeal ecosystems, since functional stability strongly correlates with the state and composition of microbial communities. The safe and controlled intensification of biogas production would be an important step to make biogas a real competitor of fossil fuels.

Keywords: Anaerobic digestion, biogas, metagenomics, methane, microbial community, syntrophic interactions

Introduction

Beside the extensive use of fossil fuels increasing attention is paid for the research and implementation of alternative methods to provide sustainable, environmentally friendly energy sources. The use of renewable energy sources can contribute to the reduction of greenhouse gas emissions thereby mitigating the proposed climate change effects.

A range of wastes such as agricultural, industrial, household and municipal wastes are available in excess and remain untapped as energy source. Furthermore, different untreated waste materials are often hazardous for the environment since these slowly degrade and the produced various gases escape into the atmosphere increasing the greenhouse gas effect. However, controlled biogas technology allows us to transform these waste materials easily and advantageously into a unique alternative energy source by anaerobic digestion. Biogas technology offers economic, health, social, and environmental benefits (Mengistu et al., 2015). This energy source is suitable to run generators to produce electricity by burning the biogas, and the generated heat also can be utilized locally. In addition, the residual digestate can replace or complete artificial fertilizers in agricultural utilization.

Anaerobic digestion/fermentative methane production

Methane-rich biogas is one of the most widely used renewable energy source, it is produced through anaerobic digestion of various organic-rich materials. Anaerobic digestion (AD) is a conversion of organic material into a mixture of mainly CH_4 and CO_2 . Coordinated interactions between the microbial consortia in the digested material are responsible of this biodegradation process (Figure 1.).

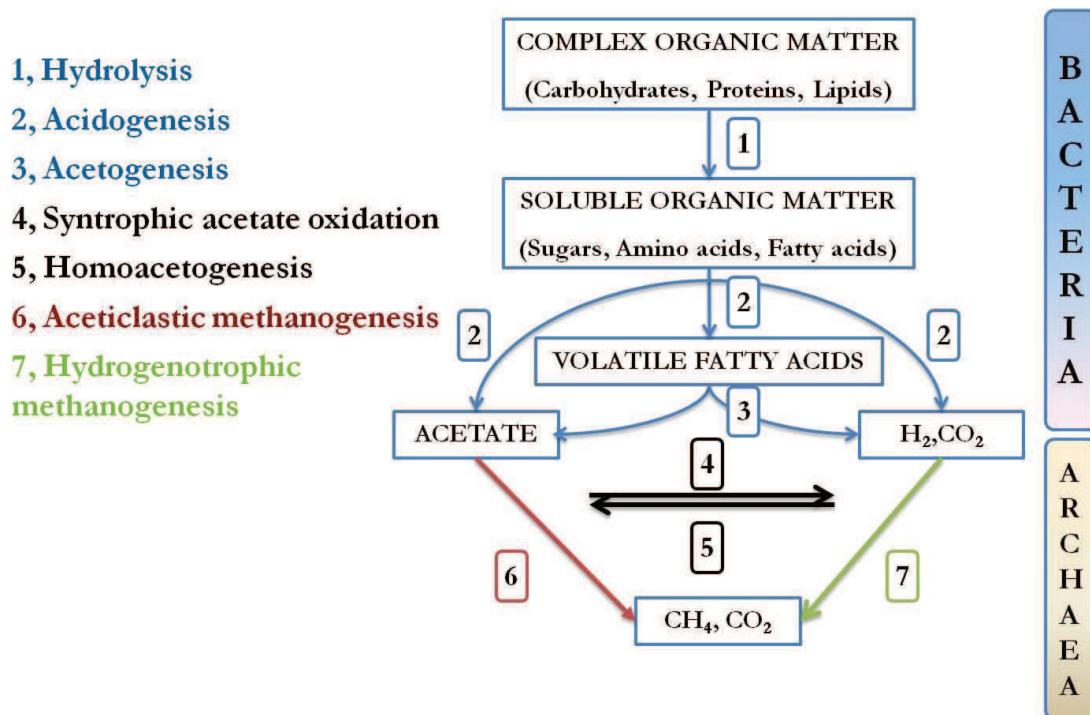


Figure 1. Generalized pathway of the anaerobic digestion process.

There are four key steps in the anaerobic digestion process: hydrolysis, acidogenesis, acetogenesis and methanogenesis. In the first step of anaerobic digestion the complex organic matter is transformed into soluble organic material, mainly carbohydrates, proteins and lipids are broken down by hydrolytic bacteria. The generated sugars, amino acids, fatty acids are appropriate substrates for fermentative bacteria involved in the acidogenesis step. Thereafter the previously formed organic acids and alcohols are transformed into acetate and hydrogen via acetogenesis. In the last step the methane production takes place via aceticlastic or hydrogenotrophic methanogenesis depending on the operating parameters and substrates (Ali Shah et al., 2014). The hydrogenotrophic methanogenesis is usually connected to syntrophic bacteria which are able to convert the previously formed short-chain fatty acids into H_2 and CO_2 through endergonic reactions. The maintenance of the equilibrium between acetogenesis and methanogenesis is a crucial factor for continuous methane production. Disturbances can initiate restricted VFA utilization leading to pH decrease which can have a strong negative effect on the methanogenesis, since the pH optimum of the methanogenic Archaea is between 6.5-8.0 (somewhat higher than the optimum for acetogen bacteria). Furthermore, too high levels of hydrogen and acetate (end products of acetogenesis) inhibit the consumption of hydrogen and acetate. A number of studies already reviewed the microbial participants of biogas generation processes. Heyer et al. discussed studies of proteomics results from full-

scale biogas plants (Heyer et al., 2015). Other studies examined the methanogenic cellulolytic communities in bioreactors using various substrates (Tsavkelova and Netrusov, 2012) or operating temperatures (Lv et al., 2010), or observed the microbial structure influenced by other operation conditions (Amani et al., 2010). Syntrophic associations in anaerobic digestion were also discussed by Kouzuma et al. in a review (Kouzuma et al., 2015). Close interactions are needed between the syntrophs and methanogens for hydrogen-transfer which is frequently strengthened by co-aggregation or biofilm production especially in the presence of poor, energetically unfavorable substrates (Kouzuma et al., 2015). In an earlier work the dynamics of the continuously stirred tank reactors (CSTRs) was examined. Microbial communities of acetate, propionate, butyrate, long-chain fatty acids, glycerol, protein, glucose and starch substrate-degrading biogas reactors were summarized (Tang et al., 2015).

Approaches for the analysis of microbial communities

A number of cultivation-independent techniques have been developed in the past, for instance fluorescence *in situ* hybridization (FISH), real-time PCR, terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE) and clone library approaches were demonstrated and shown to be suitable for the identification of complex microbial communities or at least certain groups of them (Koch et al., 2014, Su et al., 2012). However, according to a study revealed that DGGE and clone library approaches missed important taxonomic groups (Tuan et al., 2014). Thus, new techniques permitting more precise and representative community data were investigated. Nowadays, the high-throughput genomic technologies are becoming a benchmark approach for the detailed analysis of microbial communities. Accordingly, high-throughput metagenomics, metatranscriptomics, metaproteomics (Heyer et al., 2015) and metabolomics approaches supplemented with advanced visualization and isotope labeling techniques provide realistic opportunities for process engineering of anaerobic digestion through the understanding of the taxonomic and metabolic complexities (Vanwonterghem et al., 2014). Metagenomics techniques such as 454-pyrosequencing, SOLiD™ short-read DNA sequencing, Ion Torrent Personal Genome Machine™ sequencing have been successfully used to describe microbial communities of AD (De Francisci et al., 2015, Eikmeyer et al., 2013, Jang et al., 2014, Kougias et al., 2014, Kovács et al., 2015, Kovács et al., 2013, Pap et al., 2015, Solli et al., 2014, Tukacs-Hájos et al., 2014, Wirth et al., 2012).

Dominant bacterial and archaeal members of the biogas microbial communities

The *Bacteroidetes* and *Firmicutes* phyla were observed as highly stable and dominant bacterial groups in laboratory scale biogas reactors fed with various substrates (Bengelsdorf et al., 2013, Kampmann et al., 2012). Other studies also confirmed the highest abundance of the *Firmicutes* phylum in co-digesting reactors, however, under thermophilic conditions the *Thermotogae* phylum was also highly dominant (Sundberg et al., 2013). Regueiro et al. identified *Firmicutes*, *Bacteroidetes* and *Proteobacteria* as the most dominant phyla in six full-scale and one laboratory-scale co-digesters (Regueiro et al., 2012). The predominance of the *Firmicutes* phylum is mostly explained by the capability of these bacteria to produce diverse enzymes performing hydrolysis, acidogenesis and acetogenesis. Concerning the methanogenic Archaea, the predominance of the aceticlastic *Methanosaeta* genus was described principally at low acetate level in stable anaerobic digesters (Demirel and Scherer, 2008, Walter et al., 2012). Information is accumulating on the possible distinct roles of acetotrophic and hydrogenotrophic methanogens. It is to note that in numerous cases dramatic decrease in the relative abundance of the *Methanosaeta* genus was described in AD

under perturbed conditions which may indicate the high sensitivity of the members of this acetoclastic methanogenic genus. Under various disturbed circumstances (elevated temperature, decreased pH, high VFA level, high ammonia level) a clear transition was observed from acetoclastic to hydrogenotrophic methanogenesis (Fotidis et al., 2014, Hao et al., 2012, Huang et al., 2015, Karakashev et al., 2005, Kim et al., 2014, Pap et al., 2015, Sasaki et al., 2011, Song et al., 2010, Tukacs-Hájos et al., 2014). This takeover of hydrogenotrophic methanogens is mostly linked to the increased relative abundance of syntrophic acetate oxidizing bacteria (SAOB) in the biogas fermentors. Furthermore Lerm et al. revealed that the high organic loading rate (OLR) also had similar effect on methanogenesis, the enrichment of hydrogenotrophic methanogens was observed, more specifically two hydrogenotrophic species, *Methanospirillum hungatei* and *Methanoculleus receptaculi* was shown to be present with strongly increased relative abundance (Lerm et al., 2012). Further studies also revealed the *Methanoculleus* dominance over other hydrogenotrophic methanogenic genera, this phenomenon is ascribed to the possible high general stress tolerance of the members of this genus (Goberna et al., 2009, Wirth et al., 2012). It was specifically shown that certain *Methanoculleus* strains showed increased tolerance to high salt concentration, to elevated ammonium level and even against aeration in the bioreactors (Goberna et al., 2009, Liu et al., 2008, Wirth et al., 2012). The large differences of the various anaerobic digestion systems is depicted by the results of Li et al., their observations might sound to be in contradiction with the general above-mentioned picture since in this system the *Methanosaeta* genus dominated the reactors in different examined reactor states regardless of the stability level of the digestion process (Li et al., 2015). A similar observation was explained in another study where the robustness and high stability of the *Methanosaeta* genus was observed in the reactor even in the presence of high acetate level (Chen and He, 2015).

Syntrophic associations in AD are gaining more and more attention, the acetate-, propionate- or butyrate-oxidizing bacteria interact with H₂-scavenging methanogens. Syntrophic acetate oxidation (SAO) was described in stressed (particularly high ammonia-level) biogas reactors (Karakashev et al., 2006, Schnurer and Nordberg, 2008). Westerholm et al. reported a study about quantification of syntrophic acetate-oxidizing bacteria (SAOB), the putative roles of *Thermacetogenium phaeum*, *Clostridium ultunense*, *Syntrophaceticus schinkii* and *Tepidanaerobacter acetatoxydans* were discussed (Westerholm et al., 2011). The presence of syntrophic fatty acid degrading bacteria (SFAB) in biogas reactors was also shown, members of the *Syntrophobacter*, *Smithella*, *Pelotomaculum* and *Syntrophomonas* bacterial genera were identified, their relative abundances were observed to vary according to reactor configuration and substrate characteristics (Mathai et al., 2015).

Effects of temperature on the microbial community structure

Temperature is one of the crucial factors in shaping the microbial community structure during the anaerobic digestion (beside substrate type, OLR (organic loading rate), VFA (volatile fatty acids) composition, ammonium concentration, pH of the digested sludge, alkalinity, mixing and the geometry of the anaerobic digester) (Levén et al., 2007, Tukacs-Hájos et al., 2014). Elevated operation temperature enhances the efficacy of the enzymatic processes and initiates faster growth rate of the methanogens, thereby ensure that besides lower hydraulic retention time (HRT) microbes remain in optimal concentration within the fermentor (Weiland, 2010, Yadavika et al., 2004). Moreover, thermophilic anaerobic fermentation minimizes the numbers of pathogens, viruses, fungi, and parasites, which is an important requirement for the agricultural utilization of the residual digested sludge as fertilizer (Sahlström, 2003, Weiland,

2010). Community analyses revealed the fundamental effect of the operation temperature on the microbial diversity. In general the mesophilic bioreactors maintain higher general biodiversity than the thermophilic reactors (Guo et al., 2014, Hollister et al., 2012, Levén et al., 2007). Under mesophilic conditions the major bacterial components of the fermentation ecosystem are the *Bacteroidetes* (in some cases up to 50% of the bacterial community), the *Firmicutes* and the *Proteobacteria* phyla according to various studies (Pap et al., 2015, Tukacs-Hájos et al., 2014). However, in the thermophilic anaerobic digestion process *Firmicutes* phylum was identified as the most abundant bacterial group in the ecosystem, while the relative abundance of the *Bacteroidetes* and *Proteobacteria* phyla were very low indicating that these organisms play only marginal role in the decomposition of the organic materials at elevated temperature (Pap et al., 2015, Rademacher et al., 2012)(Figure 2A).

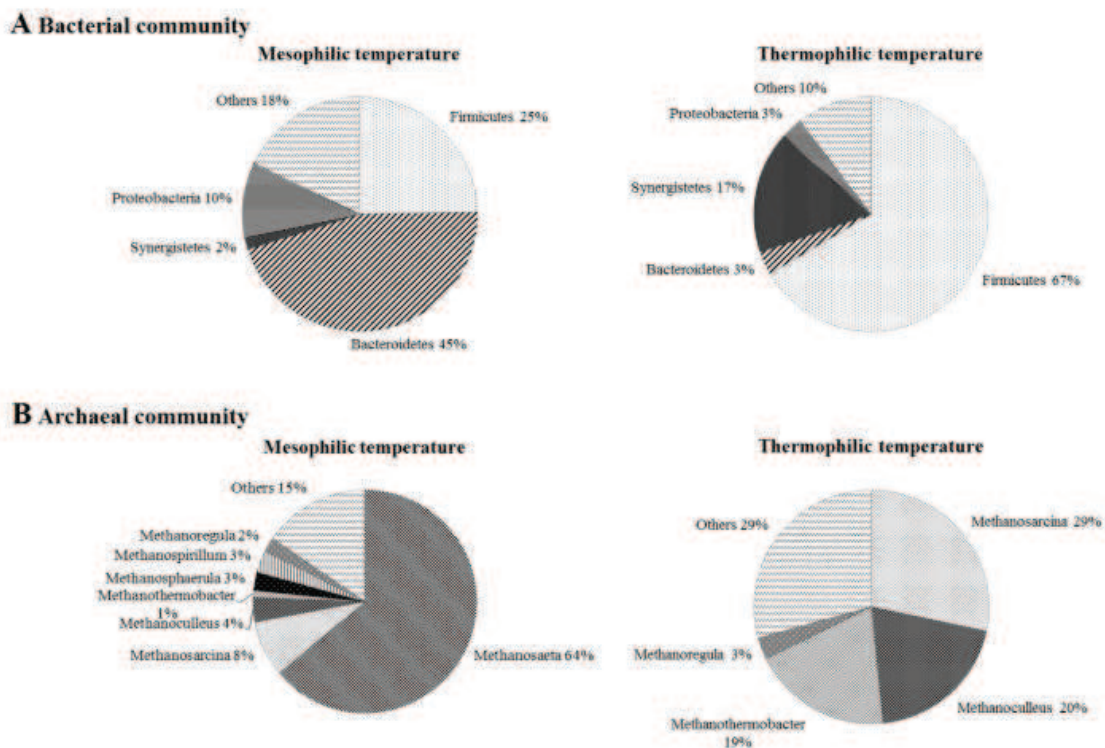


Figure 2. An example for the microbial diversity in biogas reactors. (A) Phylogenetic distribution of the bacterial communities (at phylum level) at mesophilic and thermophilic temperatures in a reactor fed with maize silage. (B) Structure of the archaeal communities (at genus level) at mesophilic and thermophilic temperatures in a reactor run on maize silage.

In sugar beet pressed pulp-degrading biogas reactors the *Thermotogae* phylum was also enriched during an adaptation from mesophilic to thermophilic operation, *Petrotoga mobilis* became the most abundant species in the system (Tukacs-Hájos et al., 2014). In another temperature adaptation study using maize-silage as substrate the most striking expansions were detected for the hydrogen-producing *Clostridium* and *Caldicellulosiruptor* genera within the *Firmicutes* phylum (Goberna et al., 2009, Pap et al., 2015). Hollister et al. compared esophilic and thermophilic microbial communities in bioreactors fed by lignocellulosic feedstock (Hollister et al., 2012). The mesophilic anaerobic digester was dominated by

Firmicutes, *Proteobacteria*, *Actinobacteria* phyla and *Bacteroidia* class within *Bacteroidetes* phylum, while under thermophilic operation two classes of *Firmicutes* phylum *Clostridia* and *Bacilli* and *Thermoanaerobacterium* genus within *Clostridia* class were identified as the most abundant taxons. In the mesophilic reactor the majority of the genes could be assigned to biodegradation of hemicellulose derivatives (especially for the five-carbon sugar arabinose degradation). The ability for degradation of arabinose and other hemicellulose derivatives by *Bacteroidetes* phylum and arabinose transformation into propionate by members of the *Bacteroides* genus were shown (Caldwell and Newman, 1986). Thus, the elevated propionate concentration is likely the result of the dominant *Bacteroidetes* phylum metabolism. The presence of higher concentration of propionate under mesophilic conditions (compared to the thermophilic reactor) confirmed this observation. However, the thermophilic reactor was rather enriched in genes related to cellobiose uptake. *Clostridia* and *Bacilli* classes were identified also by Ritari et al. as the most prevalent groups in thermophilic reactors, moreover members of the *Thermotogae* phylum could be exclusively identified in the thermophilic reactor confirming the results of other studies (Ritari et al., 2012). Thus, in general *Firmicutes*, *Bacteroidetes* and *Thermotogae* are the most abundant bacterial phyla under thermophilic operation of the anaerobic bioreactor. In response to temperature drop (switch from mesophilic to psychrophilic operation) an increase was observed in the *Bacteroides* genus and significantly decreased relative abundances were found for *Syntrophomonas* and *Clostridium* genera (Regueiro et al., 2014). These observations indicate a generally increasing importance of *Clostridia* by elevating the temperature and vice versa. At the same time the members of phyla *Proteobacteria* and *Bacteroidetes* are more characteristic for mesophilic systems.

Roles of ammonia, VFA level and pH in shaping the microbial ecosystem

The free ammonia diffusing across cell membranes is considered the main form of ammonium toxicity (Kadam and Boone, 1996, Sprott and Patel, 1986, Bittsánszky et al., 2015). Methanogens in biogas reactors exhibit different reactions to ammonia stress. In general, the acetoclastic methanogens are more sensitive than the hydrogenotrophic ones (Schnürer et al., 1994, Sprott and Patel, 1986). It has been shown that the relative abundance of the acetoclastic groups declined whereas that of the hydrogenotrophic methanogens increased when total ammonium concentration reached 3 g L⁻¹ in anaerobic digesters (Angenent et al., 2002, Westerholm et al., 2011). For example, the extreme reduction of the *Methanosaeta* genus and a clear shift from acetate to hydrogen utilization was observed in the anaerobic digesters at increased ammonium concentration and VFA level (Karakashev et al., 2005, Williams et al., 2013). Also, the increasing ammonium level favor the development of syntrophic acetate oxidation (SAO) (Schnürer et al., 1999). Certain syntrophic acetate oxidizers were found to be tolerant up to 8 g L⁻¹ ammonium level at neutral pH (Schnürer et al., 1996, Westerholm et al., 2011). Under high ammonium conditions CH₄ production from acetate is probably shifted from acetoclastic methanogenesis to SAO combined with hydrogenotrophic methanogenesis (Schnürer et al., 1994, Westerholm et al., 2011, Westerholm et al., 2012). However, the sensitivity of individual methanogens can be highly different and is influenced by further reactor parameters like pH and temperature. *Methanosarcina* spp. were found to be either sensitive (Angenent et al., 2002, Westerholm et al., 2011) or tolerant to ammonia stress (Fotidis et al., 2013) in different systems. Hydrogenotrophic methanogens also showed differential responses in different setups (Fotidis et al., 2013, Westerholm et al., 2012, Zeeman et al., 1985). Interestingly, the species *Methanoculleus bourgensis* was identified to play a significant role in different biogas reactor systems especially under high ammonium concentration. Comparative genome analysis of *M. bourgensis* MS2 and *Methanoculleus*

marisnigri JR1 revealed significant similarities and differences between the two *Methanoculleus* species. The absence of genes for a putative ammonium uptake system in *M. bourgensis* MS2 may indicate that this species is specifically adapted to environments with high levels of ammonium/ammonia (Maus et al., 2015). Although the dominance of the acetoclastic *Methanosaeta* spp. was generally observed in anaerobic digesters operating under low ammonia/ammonium level (Fotidis et al., 2014), some exceptions can be found in the literature again (Chen and He, 2015, Li et al., 2015).

The accumulation of volatile fatty acids (VFA) also represents stress for the microbial communities, especially for the methanogenic Archaea fraction. *Methanosarcina* the *Methanoculleus* genera showed the most robust tolerance to rapid VFA increase in biogas reactors, where VFA increase was induced by gradual temperature elevation causing a perturbation in the stable mesophilic community. The relative abundance of *Methanosarcina* the *Methanoculleus* genera significantly increased in response to temperature adaptation and replaced the members of the *Methanosaeta* genus being dominant in the mesophilic reactor with low VFA level (Pap et al., 2015) (Figure 2B). The pH and alkalinity are also among the most important influencing parameters since sufficient methanogenesis requires pH value between 6.5-8.0, the methanogens are especially sensitive for the acidic environment. As acidogenic bacteria are capable to lower the pH below the optimal range it is essential to continuously monitor the anaerobic digestion process to maintain constant and safe methane production.

The substrate dependence of the AD microbial communities

It is evident, that different substrates are metabolized through different pathways either by single microbes or by complex microbial communities as well. A large number of studies examined the microbial composition dynamics in response to changes in feedstock composition and feeding rates. For example, propionic acid accumulates in reactors overloaded with carbohydrates, in such cases members of the *Lactobacillus* genus were found to increase significantly. Acetate accumulation was observed in reactors supplemented with lipids, which was accompanied by the occurrence of *Dialister* and *Kyrpidia* genera. *Desulfotomaculum* was the only bacterial genus exhibiting significant increase in response to addition of proteins, it can be justified by the appearing need for H₂S elimination as a consequence of protein degradation (De Francisci et al., 2015). According to many scientists the efficient biodegradation of the excessively available cellulosic materials could be a competitive solution for biomass-based energy generation. However, the anaerobic degradation of cellulosic biomass is not a straightforward approach. In most cases the hydrolysis of such substrates is a major rate-limiting factor in AD, consequently pretreatment strategies are required (Lynd et al., 2002). During anaerobic digestion of grass silage which consists mainly of polymers of cellulose, hemicellulose and lignin mostly various members of *Firmicutes* (dominated by *Clostridia*) and *Bacteroidetes* bacterial phyla were identified by clone library analysis, while the methanogenic Archaea were represented by the hydrogenotrophic *Methanobacterium* genus as the most dominant group (Wang et al., 2010). Maize-silage is a common substrate for biogas generation, similar community structures were observed in different mesophilic biogas digesters with the clear dominance of the *Firmicutes* and *Bacteroidetes* phyla (Kröber et al., 2009, Pap et al., 2015). Within the thermophilic cellulolytic community fermenting microcrystalline cellulose as substrate and glucose as co-substrate the *Anaerolineales*, *Clostridiales*, *Bacteroidales* and *Thermotogales* orders belonging to *Chloroflexi*, *Firmicutes*, *Bacteroidetes* and *Thermotogae* phyla, respectively, were dominant. The dominance of *Methanobacteriales* and *Methanosarcinales* archaeal orders were observed in

this system. Interestingly, metatranscriptomic analysis showed stronger transcriptional activities of genes and pathways involved in acetoclastic methanogenesis of *Methanosarcinales* compared to the hydrogenotrophic pathways of *Methanobacteriales* suggesting that acetoclastic methanogens were more active than the hydrogenotrophic methanogens (Xia et al., 2014). This finding also highlights the importance of the combination of various approaches to describe the microbial communities.

Application of algal biomass for biogas production is another promising possibility due to its low lignin content, certain algal species are described to have advantageous cell wall characteristics with easily degradable materials. A single physical pretreatment of the algal biomass might be sufficient to use it as substrate (Montingelli et al., 2015). During co-fermentation of mixed algal-bacterial biomass with maize silage a microbial community dominated by the *Proteobacteria* phylum was developed, which showed clear differences compared to the anaerobic digestion based on maize silage alone where *Firmicutes* and *Bacteroidetes* were the most abundant phyla (Wirth et al., 2015).

Byproducts of various food processing industries, predominantly dairy and meat industries are often used as substrates for biogas generation. The anaerobic digestion of such protein-rich waste materials containing high concentration of nitrogen (resulting in elevated ammonia/ammonium level) has an intrinsic risk for inhibition issues in the biogas fermentors. However, these materials are valuable and suitable for biogas production under well-controlled conditions and with careful operation, the bacterial and archaeal communities can be successfully adapted to the protein-rich substrate (Chen et al., 2008, Kovács et al., 2015). *Firmicutes* was shown to be the most abundant phylum in the biogas reactor fed by protein-rich substrates and within the *Firmicutes* phylum a decreasing relative abundance of the *Bacilli* class was observed over time (Kovács et al., 2015, Kovács et al., 2013a). However, the *Thermotogae* phylum also showed constant increase due to its adaptability for protein-rich substrates (Kovács et al., 2015, Kovács et al., 2013a). The highest methane yield was described in protein-degrading batch reactors compared to batch systems running on cellulose-rich material or using high lipid content. The most explicit dominance of the *Methanoculleus* genus was described in the batch reactors fed with protein-rich substrate suggesting that this group was responsible for the surplus methane production (Wagner et al., 2013). The archaeal community was examined by terminal restriction fragment length polymorphism (T-RFLP) method when the biogas fermentor was fed with protein-rich substrate and the overwhelming dominance of the hydrogenotrophic *Methanoculleus* genus was observed (Ács et al., 2013).

Industrial wastewaters, sewage sludges, swine manure and biofermentors digesting different organic solids such as slaughter-house waste, animal manure and food wastes are often sources of toxic phenolic compounds which can negatively affect the microorganisms involved in anaerobic digestion. Inhibited degradation of phenol was observed at thermophilic operation temperature while these phenols and derivatives were degraded rapidly into methane in mesophilic reactors (Levén et al., 2012, Tukacs-Hájos et al., 2014). This can be explained by the different metabolic capability and enzyme sortiment (harbored by the strongly different microbial communities) under mesophilic or thermophilic operation. The members of *Syntrophorhabdaceae* (*Proteobacteria* phylum) and the *Desulfotomaculum* genus (*Firmicutes* phylum) were identified as community members with potential phenol-degrading capability (Levén et al., 2012, Levén and Schnürer, 2010).

Microbes used for bioaugmentation and pretreatment

Biological pretreatments of various substrates might provide more improvements in the anaerobic biodegradation processes than thermochemical pretreatments (Fdez-Güelfo et al., 2011). Efficiency of the lignocellulosic biomass degradation is a key factor to provide appropriate substrate for sufficient methane production via anaerobic digestion. However, lignocellulose is a barely degradable material, its breakdown into lignin, cellulose and hemicellulose might be enhanced by various biological pretreatment approaches. The controlled addition of selected hydrogen evolving bacteria (e.g. *Enterobacter cloacae* and/or *Caldicellulosiruptor saccharolyticus*) into the biogas fermentor may enhance the biogas production rate by promoting hydrogenotroph methanogenesis through mitigating the hydrogen limitation (Ács et al., 2015, Kovács et al., 2013b). Biogas production using protein-rich meat extract as substrate was improved by bioaugmentation, a mixture of three bacterial strains naturally displaying high affinity for protein degradation (*Pseudomonas fluorescens*, *Bacillus coagulans* and *B. subtilis*) was applied to enhance hydrolysis in the anaerobic digester (Kovács et al., 2015). It was suggested and demonstrated that specific fungi able to survive anoxic conditions at mesophilic and thermophilic temperature may contribute to feeding hydrolytic bacteria and methanogenic Archaea during the biogas generation process (Ritari et al., 2012). Thus, beside bacteria, some fungi are also able to help the better utilization of biomass and to speed up the anaerobic degradation of fiber-rich substrates in biogas fermentors (Kazda et al., 2014). Another study described experiments where biogas production was enhanced by 4-22% in pig slurry fermenting reactors supplemented with cellulose-rich energy crop substrate by the addition of a mixture of rumen anaerobic fungi, the *Anaeromyces*, *Piromyces* and *Orpinomyces* strains possess fibrolytic activity (Procházka et al., 2012). Similarly, improved biogas production was observed when paddy straw was pretreated by the *Thermoascus aurantiacus* MTCC 375 fungus (Phutela and Dar, 2014).

Conclusion

Biogas production can be coupled to the recycling of various agricultural, municipal, food, animal and forestry wastes. The anaerobic digestion process is relying on a well-concerted microbial network, the stability of this complex and sensitive community depends on the process parameters including substrate-type and feeding rate (C/N ratio, specific inorganic compounds), operation temperature, mixing and the geometry of the digester. A more detailed insight into the microbial community and especially the differential tolerance levels of community members to various stress factors can help us in developing and maintaining efficient and safe biogas production, thereby contribute to the competitiveness of the production and utilization of biomass-based energy sources.

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