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Relating Anaerobic Digestion Microbial Community and Process Function

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Supplementary Issue: Water Microbiology

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ABSTRACT: Anaerobic digestion (AD) involves a consortium of microorganisms that convert substrates into biogas containing methane for renewable energy. The technology has suffered from the perception of being periodically unstable due to limited understanding of the relationship between microbial community structure and function. The emphasis of this review is to describe microbial communities in digesters and quantitative and qualitative relationships between community structure and digester function. Progress has been made in the past few decades to identify key microorganisms influencing AD. Yet, more work is required to realize robust, quantitative relationships between microbial community structure and functions such as methane production rate and resilience after perturbations. Other promising areas of research for improved AD may include methods to increase/control (1) hydrolysis rate, (2) direct interspecies electron transfer to methanogens, (3) community structure–function relationships of methanogens, (4) methanogenesis via acetate oxidation, and (5) bioaugmentation to study community–activity relationships or improve engineered bioprocesses.

KEYWORDS: anaerobic digestion, bioaugmentation, methanogenesis, microbial community

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Introduction

Anaerobic digestion (AD) is a microbial process that converts organic matter to biogas containing CH_4 and CO_2 in an anaerobic environment.[1] Although the technology has been employed for decades, it has received renewed attention as it provides a more sustainable alternative to waste treatment over energy-intensive methods of the past.[2,3] Compared with traditional aerobic treatment, AD has several potential advantages such as lower operational costs from lack of aeration requirements, energy production from biomethane, significantly less biomass production, which reduces handling and disposal costs, and the ability to degrade certain pollutants, which cannot be aerobically removed.[4]

Anaerobic conversion of organics to biogas involves a multistep process involving interactions among many different bacterial and archaeal species. With the increasing application of AD, there is a steady effort by both engineers and microbiologists working in this field to increase the existing knowledge of the complex, interacting microbial community that governs the overall AD process. New knowledge is crucial in order to develop better models and design improved AD systems. One key area requiring new knowledge involves the quantitative relationship between microbial community structure and AD process function. The aim of this review is to provide insight into the microbiology of anaerobic digesters and recent studies describing both qualitative and quantitative relationships between microbial community and digester function. The gaps in current knowledge and suggestions for future research are also described.

Phases in AD Process

Conceptually, the microbial processes of AD can be described by the sequential steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis.[5] Each of these steps is accomplished by a guild of microorganisms, and it is critical to maintain a *balanced* reaction rate among the steps or guilds to ensure rapid and stable digestion. As described above, balanced essentially means that acid- and H_2-consuming reactions are fast or potentially faster than acid- and H_2-producing steps. Buildup of H_2 partial pressure to more than 10^{-4} atm inhibits the destruction of propionate and butyrate intermediates.[6] Accumulation of these volatile fatty acid (VFA) intermediates can drop the pH of the digester and slow or stop methanogenesis. In addition, the rate of one of these steps limits the overall rate of methanogenesis, and the identity of the rate-limiting step can differ among systems based on substrate chemical structure and other parameters. Most importantly,

increasing the rate of the rate-limiting step will increase methane production rates, whereas improving other steps will have a little impact.

Hydrolysis. Hydrolysis involves the breakdown of polymeric substrates, such as polysaccharides, lipids, and proteins, to their respective monomers or oligomers using extracellular enzymes. These enzymes generally include amylase, cellulase, lipase, pectinase, and protease.[7] Hydrolytic bacteria are phylogenetically diverse; however, two phyla, namely, *Bacteroidetes* and *Firmicutes*, include most of the known species. Compared with methanogens, hydrolytic bacteria grow rapidly and have lower sensitivity to changes in environmental factors, such as pH and temperature. For relatively recalcitrant substrates, such as lignin, hydrolysis is often the rate-limiting step for CH_4 production. In addition to substrate chemical structure, hydrolysis rate depends on factors such as particle size, pH, enzyme production, diffusion, and adsorption of enzymes on the substrate particles.[8–10] Methods to increase hydrolysis rate using mechanical, chemical, and biological processes have been developed ,[11] but a thorough review is outside the scope of this document.

Acidogenesis and syntrophic acetogenesis. In acidogenesis, products of hydrolysis are converted primarily to VFAs, which include acetate, propionate, isobutyrate, butyrate, valerate, and isovalerate. Besides VFAs, other products of acidogenesis include alcohols, lactate, formate, CO_2, and H_2. *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, and *Proteobacteria* are phyla that contain most identified species of acidogenic bacteria.[12–15] Acidogenesis is generally rapid, which can lead to accumulation of VFAs and a drop in pH when acid utilization is inhibited or too slow due to organic overload, toxicants, or rapid temperature change. The pH drop can inhibit or stop methanogenesis completely.

Although methanogens can directly use acetate, formate, H_2/CO_2, and methyl compounds, other intermediates formed via acidogenesis, such as propionate, butyrate, isobutyrate, valerate, isovalerate, and ethanol, have to be further biodegraded by other microorganisms before methanogens can utilize them to produce methane. Syntrophic acetogenesis is the process in which these intermediates are further biotransformed to form acetate, H_2, and CO_2. Fermentation of propionate via syntrophic acetogenesis is critical because approximately 30% of complex substrates in municipal wastewater solids and other wastes can be shuttled through propionate to CH_4 during AD under typical conditions.[16] Most of the medium- to long-chain fatty acids resulting from hydrolysis of lipids and lignocellulosic compounds are also biotransformed to acetate, H_2, and CO_2 through this process.

Under standard conditions, syntrophic acetogenesis is thermodynamically unfavorable and only proceeds if the partial pressure of H_2 is lower than 10^{-4} atm.[6,17] Hydrogenotrophic methanogens and/or other H_2 utilizers live in syntrophy with acetogens, consuming H_2 released from the latter.[18] The syntrophic relationship makes acetogenesis thermodynamically feasible. Formic acid (HCOOH) and formate are similar to H_2 since they are essentially H_2 associated with CO_2 (ie, $H_2 + CO_2 = HCOOH$). Therefore, interspecies formate transfer has also been observed to play a critical syntrophic role. In addition, acetogenesis from other higher molecular weight organic acids also relies on syntrophy with H_2 or formate utilizers. This syntrophy is based on H_2/formate transfer from H_2-producing to H_2-consuming microorganisms, which is commonly referred to as interspecies H_2 transfer.[19] The H_2 also can be thought of as protons (H^+) with associated electrons, and interspecies hydrogen/formate transfer is also interspecies electron transfer. Interestingly, a recent study has shown that some microorganisms perform direct interspecies electron transfer using electrically conductive pili and electrons can be shuttled in this way from, for example, *Geobacter* to *Methanosaeta*.[20–23] This interspecies electron transfer is rapid and may prove to be an important mechanism for stable AD in the future; more research is warranted to more fully understand direct interspecies electron transfer mechanisms and how it can be encouraged in engineered systems.

Most commonly observed syntrophic acetogens in anaerobic digesters involved in propionate degradation belong to the genera such as *Pelotomaculum*, *Smithllela*, and *Syntrophobacter*.[24–26] Oxidation of butyrate and other fatty acids are performed by acetogens belonging to the genera *Syntrophus* and *Syntrophomonas*.[26–28] Syntrophic acetogenesis is a critical and, often, rate-limiting step to maintain rapid, stable AD operation because some of the VFAs, particularly propionate, inhibit methanogenesis at high concentrations even at neutral pH.[1,29–31] Syntrophic acetogens play a critical role in the overall AD process, but have not been thoroughly studied, in part, due to the difficulty in maintaining pure cultures and lack of primers to identify them in mixed cultures using molecular techniques.[32]

Methanogenesis. The final step in biomethane production, methanogenesis, is performed by a specialized group of microorganisms belonging to the domain *archaea*, called methanogens. There are three known types of methanogens: acetoclastic, hydrogenotrophic, and methylotrophic. Acetoclastic methanogens convert acetate to CH_4 and CO_2, hydrogenotrophic methanogens use H_2 or formate to reduce CO_2 to CH_4, and methylotrophic methanogens produce CH_4 from methyl compounds, such as methanol, methylamines, and methylsulfides.[33] In typical municipal anaerobic digesters, about 70% of the CH_4 is produced from acetate, and the rest from H_2 and CO_2. Only a minimal amount of CH_4 is produced via methylotrophic methanogenesis.[34]

Hydrogenotrophic methanogens are critical for AD process owing to their ability to scavenge H_2 and maintain the partial pressure low. *Methanobacterium*, *Methanobrevibacter*, *Methanoculleus*, *Methanospirillum*, and *Methanothermobacter* are the most commonly observed hydrogenotrophic methanogens in anaerobic digesters.[35–38]

Acetoclastic methanogens belong to two genera: *Methanosaeta* and *Methanosarcina*. *Methanosaeta* are obligate acetoclastic methanogens and are only known to use acetate or direct electron transport as the substrate or electron donor. *Methanosaeta* have a relatively slow growth rate but possess a high affinity for acetate and hence dominate at low acetate concentration.[33] *Methanosarcina* are facultative acetoclastic methanogens. Most *Methanosarcina* species can use H_2/CO_2, and C-1 compounds in addition to acetate.[33,39,40] In addition to its wider range of substrates, *Methanosarcina* has a higher growth rate and lower affinity for acetate so it can dominate over *Methanosaeta* in digesters, where the concentration of acetate is high.[38–40] The filamentous morphology of *Methanosaeta* can play an important role in granule formation since the filaments act as binders to help hold the granule together. Many AD configurations, such as the upflow anaerobic sludge blanket, rely on the formation of these microbial agglomerations, called granules that are 1–5 mm particles, containing microbes that settle rapidly. When granulation does not occur in these bioreactors, the process is difficult to maintain and can fail.

Syntrophic acetate-oxidizing bacteria. Under certain conditions, an alternative pathway for CH_4 production from acetate has been observed in some anaerobic digesters. This pathway combines the conversion of acetate to H_2 and CO_2 by acetate-oxidizing bacteria that are subsequently converted to CH_4 by hydrogenotrophic methanogens.[41] Only few species of microorganisms have been identified that perform syntrophic acetate oxidization in conjunction with H_2-consuming methanogens, which include strain AOR (ie, Reversibacter), *Clostridium ultunense*, *Thermacetogenium phaeum*, *Tepidanaerobacter acetatoxydans*, *Thermotoga lettingae*, and *Syntrophaceticus schinkii*.[42–45] This pathway is not believed to be a typical AD pathway for CH_4 production because acetoclastic methanogens outcompete syntrophic acetate-oxidizing bacteria in most digesters; however, more work is required to understand the importance of the process in AD systems.[46] Under conditions that might be inhibitory to acetoclastic methanogens, such as high ammonia (>3 g/L NH_3-N) or sulfate concentration and/or high temperature, this pathway can be critical for biogas production.[42,46–51] Studies have also shown that a long hydraulic retention time along with a low abundance of *Methanosaeta* can promote a shift toward syntrophic acetate-oxidizing pathway from acetoclastic methanogenesis.[52,53]

Environmental Parameters Affecting Digester Microbial Community

Many studies have reported the influence of environmental parameters on the microbial community structure of a digester, primarily focusing on the methanogenesis pathway since it plays a direct role in reducing the pollutant load and producing CH_4 as a renewable energy source.[54] Compared with bacteria, methanogens have a lower growth rate and are sensitive to environmental disturbances, such as pH decline, high VFA, and ammonia concentrations.[55,56] Environmental parameters such as pH, temperature, substrate concentration, substrate composition, and presence of toxic or inhibitory compounds can cause a shift in the methanogenic community structure and affect the overall digestion process.[55]

Compared with thermophilic temperature (55°C), the methanogenic community exhibits higher diversity at mesophilic temperature (37°C).[33] Lowering the temperature further to psychrophilic values may shift the community from acetoclastic to hydrogenotrophic methanogens, but the relationship is currently unclear and requires additional research.[57,58] Substrate disturbances, which include changes in the substrate concentration and composition, can affect the methanogenic community and its activity.[59] Different substrates can lead to development of different methanogenic communities, for example, manure versus wastewater sludge[56] and glucose versus whey permeate and sewage sludge.[60] Higher acetate concentration can lead to *Methanosarcina* being selected as a dominant acetoclastic methanogen over *Methanosaeta*.[61,62]

In most large-scale industrial or municipal anaerobic digesters, changes in substrate concentration or substrate overload due to the variability in wastewater streams are the most common causes of digester instability. Of the four trophic phases, hydrolysis and acidogenesis can proceed at a faster rate than acetogenesis and methanogenesis.[63] During substrate overload, the rate of formation of VFA intermediates is higher than that of their conversion to methane. Therefore, the VFAs accumulate to high concentrations in the digester, causing a pH decrease from the typical optimal values of pH 7–8 for efficient methanogenic activity.[63] Apart from lowering the pH, VFAs can inhibit methanogenesis at high concentrations, and the inhibition is much stronger at lower pH values.[64] The pH influences the ratio of undissociated to dissociated forms of VFAs, and the former is more toxic to microorganisms as the undissociated form can diffuse through cell membrane and cause damage by decreasing the intracellular pH.[65]

Many studies have investigated a wide range of environmental and nutrient factors that might severely inhibit the methanogenic process. Comprehensive reviews published by Blum and Speece[66] and Chen et al[55] provide detailed summaries of factors that might cause inhibition of anaerobic processes, which include specific organic chemicals, ammonia, sulfate/sulfite, and toxicity due to light metal ions (Na, K, Mg, Ca, and Al) and heavy metal ions (Fe, Zn, Ni, Co, Mo, Cu, etc.). However, it is important to note that metal ions are also essential trace elements for methanogenesis and are required at adequate concentrations, below inhibitory levels, for sustained methanogenesis.[67]

Relating Microbial Community Structure to Digester Stability and Function

Despite numerous reports describing the effect of environmental parameters on the microbial community structure, the reverse approach describing the influence of microbial community structure on digester function and its stability

has been studied less. Researchers have just begun to utilize the information pertaining to microbial community structure to understand or predict its underlying influence on digester performance.

Qualitative structure–function relationships. Microbial diversity, specifically quantified as species richness (number of species) and evenness (relative abundance of species), has been shown to play an important role in both natural and engineered ecosystem function.[68–73] Ecosystems containing more than one organism capable of performing a specific function (high richness) with a relatively equal abundance (high evenness) have a higher probability of functional redundancy or functional stability. It is a form of functional *insurance* for an ecosystem to have high richness and evenness based on compensatory growth. If the population of one species within a functional group is reduced or lost due to system perturbation, then another species from the same functional group, but higher resistance to the perturbation, may rapidly take its place if originally present in enough numbers.[68–70,74]

Engineered biological systems, such as anaerobic digesters, are often prone to and criticized for functional instability; therefore, studies involving functional resistance and resilience of biological treatment systems after perturbation have focused on relating species richness and evenness to overall functional stability. Although not a methanogenic system, Wittebolle et al[72] working on denitrifying bacteria reported that communities with higher evenness exhibited higher rates of denitrification when exposed to salt toxicity compared with communities with low evenness. In parallel papers, Fernandez et al[68] and Hashsham et al[69] studying perturbation of methanogenic digesters using glucose overload concluded that greater functional stability was observed in communities exhibiting multiple microorganisms within the same functional group.

Apart from qualitatively linking species richness and evenness to digester stability during perturbation, studies have shown the relationship between microbial community structure and digester activity under nonperturbed conditions. Clustering analysis performed by Carballa et al[75] using two molecular techniques, denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP), showed similar results, with a clear separation between the mesophilic and thermophilic communities. The bacterial and mesophilic communities were more diverse and even than the archaeal and thermophilic communities. The study also concluded that a digester with a higher evenness and diversity in its bacterial community resulted in a higher biogas/methane production.[75] Tale et al[76] measured specific methanogenic activity (SMA) against propionate for 14 different biomass samples from full-scale anaerobic digesters. Microbial community analysis was performed to elucidate only the methanogenic community structure defined by DGGE banding pattern of a gene ubiquitous to methanogens, the methyl coenzyme M reductase (*mcrA*) gene. Using the band intensities as a quantitative measure and employing principal component analysis, Tale et al[76] showed that the digesters with high SMA values clustered together on the principal component analysis plot and correlated linearly with the DGGE banding patterns. Upon excising and sequencing the gel bands, the presence of hydrogenotrophic methanogens, *Methanospirillum hungatei* and *Methanobacterium beijingense*, was prominent in digesters with high propionate SMA. In another multiyear study, Werner et al[73] looked at the bacterial communities of nine full-scale digesters treating brewery wastewater by employing 454 pyrosequencing to sequence the 16S rRNA gene. Using principal coordination analysis, they observed that digesters with higher SMA against acetate correlated with high community evenness.

Quantitative structure–function relation. Though the general understanding of the relationship between digester function and microbial community structure is increasing rapidly, the relationship is still difficult to quantitatively model. Current AD mathematical models consider biomass to be one independent population that is viewed as a *black box*[77] or, as in the case of models such as AD Model 1 (ADM1) and others,[78] as trophic groups containing one member. The lack of microbial community descriptors that may quantify, for example, functional redundancy in models is an obvious hurdle to improving design and operation of anaerobic digesters. The very important, but underappreciated, work of Ramirez et al[79] began to tackle this issue by including microbial diversity concepts into an extended ADM1 model. However, more work is required to improve AD models using microbial community descriptors.

A few studies have reported a direct correlation between methanogenic activity and microbial community descriptors. Although not an anaerobic digester, Freitag and Prosser[80] observed a linear correlation between the methanogenic activities of peat soil samples and *mcrA* gene copy numbers quantified using qPCR. The *mcrA* gene copy number has also been shown to linearly correlate with SMA values against H_2/CO_2 in four biomass samples.[81] Regueiro et al[82] reported that higher hydrolytic and methanogenic activities were correlated with higher *Bacteroidetes* and *Archaea* abundances. The linear correlation observed in these studies is encouraging; however, multiple linear regression (MLR) models, when performed using a small sample size and a high number of independent variables (10 DGGE bands in the case for Tale et al),[76] are overfitted and not predictive.[83] Therefore, a large number (ie, >30) of different microbial communities must be analyzed to develop statistically relevant empirical correlations, which is one issue that has limited the development of structure–function relationships.

Based on the studies by Tale et al[76] and Morris et al,[81] a study by Bocher et al[84] utilized MLR modeling and addressed the issue of overfitting by increasing the sample size (49 samples) and reducing the number of independent variables

(5 DGGE bands) to develop the MLR equations relating community and functional descriptors. Methanogenic microbial communities were assayed for methanogenic activity against glucose and propionate and the methanogenic community structure was quantified using *mcrA* gene DGGE band intensities. Of the 49 microbial samples, 30 were randomly selected and used as a training set to develop MLR equations relating propionate and glucose SMA values to band intensities [equations (1) and (2)]. The maximum correlation coefficient (R^2) value was observed using a minimum of five bands. The MLR equations derived were then used to predict the activity of remaining 19 samples (the test set). In conclusion, the MLR equation described a regression with good quantitative predictability with the validation parameter (q^2) value higher than the threshold value of 0.5 for glucose ($q^2 = 0.53$) and propionate ($q^2 = 0.52$) relationships.

$$\text{SMAp} = -220(X_4) - 82(X_8) + 340(X_{10}) - 52(X_{14}) + 180(X_{15}) + 50 \quad (1)$$

$$\text{SMAg} = -430(X_4) - 470(X_7) - 76(X_{11}) + 170(X_{15}) + 89 \quad (2)$$

SMAp and SMAg are the SMA values against propionate and glucose, respectively (mL CH_4/mg iATP-h), and X_n is the demeaned, normalized band intensity value for band n on a DGGE gel of amplified *mcrA* products.[84]

To the best of authors' knowledge, this is the only study that has reported a quantitative, predictive model between methanogenic community structure and anaerobic biomass activity. The model as described by equations (1) and (2) shows, for example, that the presence of methanogens represented by DGGE bands X_{10} and X_{15} for SMAp and X_{15} for SMAg positively correlates with higher SMA. This kind of information could be used in the future to select or design microbial communities to seed or bioaugment anaerobic digesters for more rapid methane production. Similarly, methanogens represented by bands X_4, X_8, and X_{14} for SMAp and bands X_4, X_7, and X_{11} for SMAg negatively correlate with higher SMA.

This is a first step and does not describe the many ways microbial community structure relates to digester function. In the future, however, these and other, more robust quantitative structure–activity relationships (QSARs) could be used to develop specific cultures that could increase process performance via digester seeding or bioaugmentation. The recently developed next-generation sequencing technologies may provide a breakthrough, as they allow sequencing of a large number of 16S rRNA gene PCR amplicon samples and have a rapid turnover time. At the same time, instead of analyzing for a specific functional or taxonomical group, next-generation sequencing can be used to thoroughly describe the digester microbial community, either by using a metagenomic approach, employing universal 16S rRNA gene primers, or by other approaches.[85]

Bioaugmentation as a tool to study structure–function relationships. Bioaugmentation is the practice of adding specialized or a mixed community of microorganisms to a system to obtain a desired process function.[86] A recent review published by Herrero and Stuckey[87] broadly covers the application of bioaugmentation in wastewater treatment. Bioaugmentation of anaerobic digesters has now been demonstrated in studies for reactor start-up,[88] odor reduction,[89] and degradation of organic compounds, including 3-chlorobenzoate,[90] pentachlorophenol,[91] tetrachloroethene,[92] benzene,[93] selenate and nitrate,[94] phenol and cresol,[95] fat, oil, and grease,[96] and oleate,[97] and to aid in the recovery of upset digesters.[76,98,99]

The relevance of bioaugmentation to study structure–function relationships comes from the underlying hypothesis that addition of an exogenous culture ostensibly alters the original microbial community that may, in turn, change digester function. Hence, if the microbial community structure of the augment culture, the original digester biomass, and their mixture are well characterized, then their functional activities could be used to relate function and community structure. This concept was tested in a study performed by Bocher,[100] who compared the rates of propionate conversion to CH_4 before and after bioaugmentation with a propionate degrading, methanogenic augment. Nine different biomass samples, each with a different microbial community, were collected from different full-scale anaerobic reactors. Bioaugmentation was done by mixing the augment with each digester biomass sample at an iATP ratio of 1:5 (augment:biomass). Six of the nine biomass samples assayed showed a statistically significant increase in the SMA after bioaugmentation. The bioaugmentation results were correlated with the dissimilarity (calculated as 1—Pearson's correlation coefficient) between the methanogenic community structure of the augment and original digester biomass cultures (Fig. 1). The results of bioaugmentation were measured as the percentage increase in SMA against propionate, before and after bioaugmentation. The dissimilarity between the methanogenic community structure of the digester biomass and the augment culture was quantified as the distance, calculated using 1—Pearson's

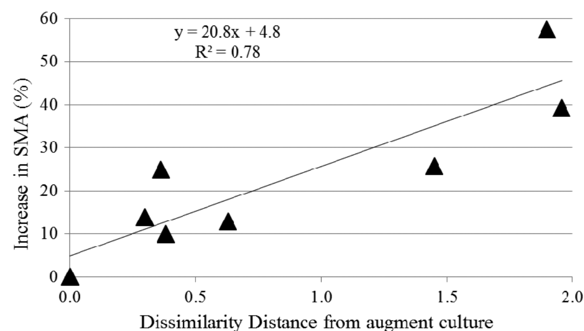

Figure 1. Difference between methanogen community structures in the augmented and biomass samples correlated with percent increase in SMA values.[100]

correlation coefficient, of the *mcrA* DGGE banding patterns of each digester biomass sample and the augment culture.

A linear correlation was observed and supported the argument that functional improvement (ie, increased rate of propionate degradation) after bioaugmentation is not only a function of the augment culture community structure but also the methanogenic community structure of the original biomass within a digester (ie, how much different it is from the augment culture). This correlation provides a rationale to further study bioaugmentation as a tool to analyze structure–function relationship in AD process. Bioaugmentation will not improve every existing anaerobic biomass, but may improve some, and a method to quantify potential improvement based on microbial community descriptors should exist.

Although linear models have been successful, other structure–activity models may prove to be more appropriate. This is similar to the historical development of quantitative QSARs for drug activity and other physiochemical parameter estimation over the past 80 years; these QSARs initially relied on linear relationships, but were later refined using nonlinear relationships such as artificial neural networks.[101,102] In any event, the initial success using empirical, linear relationships encouraged the development of more robust and accurate empirical and mechanistic models. It is probable that more robust models can be developed in the near future to predict the function of engineered microbial processes using microbial community descriptors as well as environmental parameter values. These new, robust models will be very helpful to improve engineered bioprocesses.

Conclusion

As a biological treatment process, both efficiency and stability of AD technology depend fundamentally on the complex microbial communities and their activities in digesters. Owing to this, over the years, scientists and engineers working in this field have focused their attention to answering the central questions: (1) which microorganisms are present, (2) how many different types of microorganisms are present, (3) which microorganisms are active and growing, (4) how do microorganisms behave under certain environmental conditions, and (5) how does the microbial community structure relate to digester function.

Considerable progress has been made in the last decade to identify the key groups of microorganisms that influence the trophic phases of AD as well as how various environmental conditions affect the microbial community structure and digester function. Yet, more work is required to realize quantitative, predictive relationships between the complex microbial community structure and the digester functional output. A robust quantitative microbial structure–function relationship would be a *holy grail* for engineers and scientists who are looking to develop new predictive models that can be used to improve the design and operation of anaerobic digesters for waste treatment and renewable energy generation. However, for a valid quantitative relationship, it is essential to analyze the microbial community structure and monitor the functional and environmental parameters for a large sample of different anaerobic digester communities, and this has limited model development. Future experimental work can be envisioned, in which a large number of different microbial communities from various, controlled anaerobic digesters are analyzed using next-generation sequencing technology. The community and functional data could then be used to determine predictive, empirical, or mechanistic relationships between community structure and digester function descriptors, including CH_4 production rate. It would be a worthwhile endeavor and an important step forward in this field.

Other promising areas of research for improved AD processes may include (1) methods to increase hydrolysis rate, (2) direct interspecies electron transfer to methanogens via conductive pili or other mechanisms, (3) community structure and function relationships of methanogenic communities, (4) methanogenesis via acetate-oxidizing bacteria, and (5) bioaugmentation to study microbial community–activity relationships or improve engineered bioprocesses.

Author Contributions

Conceived and designed the experiments: KV, BB, JM, and DZ. Analyzed the data: KV, BB, JM, and DZ. Wrote the first draft of the manuscript: KV and DZ. Contributed to the writing of the manuscript: KV, BB, JM, and DZ. Agreed with manuscript results and conclusions: KV, BB, JM, and DZ. Jointly developed the structure and arguments for the paper: KV, BB, JM, and DZ. Made critical revisions and approved the final version: KV, BB, JM, and DZ. All the authors reviewed and approved the final manuscript.

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