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Relating methanogen community structure and anaerobic digester function

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ABSTRACT

Much remains unknown about the relationships between microbial community structure and anaerobic digester function. However, knowledge of links between community structure and function, such as specific methanogenic activity (SMA) and COD removal rate, are valuable to improve anaerobic bioprocesses. In this work, quantitative structure–activity relationships (QSARs) were developed using multiple linear regression (MLR) to predict SMA using methanogen community structure descriptors for 49 cultures. Community descriptors were DGGE demeaned standardized band intensities for amplicons of a methanogen functional gene (*mcrA*). First, predictive accuracy of MLR QSARs was assessed using cross validation with training (n = 30) and test sets (n = 19) for glucose and propionate SMA data. MLR equations correlating band intensities and SMA demonstrated good predictability for glucose ($q^2 = 0.54$) and propionate ($q^2 = 0.53$). Subsequently, data from all 49 cultures were used to develop QSARs to predict SMA values. Higher intensities of two bands were correlated with higher SMA values; high abundance of methanogens associated with these two bands should be encouraged to attain high SMA values. QSARs are helpful tools to identify key microorganisms or to study and improve many bioprocesses. Development of new, more robust QSARs is encouraged for anaerobic digestion or other bioprocesses, including nitrification, nitritation, denitrification, anaerobic ammonium oxidation, and enhanced biological phosphorus removal.

1. Introduction

New sustainable waste management approaches including energy and resource recovery are now favored over energy-intensive treatment methods of the past (Angenent et al., 2004; Lettinga, 2010). Within more sustainable approaches, anaerobic biotechnology plays a central role for low-energy oxygen demand removal, lower biosolids production than aerobic systems, and renewable energy production from biomethane (Speece, 2008; Holm-Nielsen et al., 2009; Novotny et al., 2010). This, combined with recent advances that include the ability of anaerobic membrane bioreactors treating dilute, municipal wastewater to achieve effluent five-day biochemical

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oxygen demand (BOD_5) concentrations of <10 mg/L at approximately 10 °C, is making anaerobic biotechnology increasingly attractive (Shin et al., 2014), thereby encouraging the development of more robust anaerobic systems.

One challenge to anaerobic bioprocess improvement is that much remains unknown about the relationships between microbial community structure and digester function, such as biogas production and chemical oxygen demand (COD) removal rates. Although it is known that complex, interacting microbial populations accomplish the overall anaerobic degradation process, the microbial community is almost always unaccounted for in standardized testing, operation and design (Batstone et al., 2002; Curtis et al., 2003).

1.1. *Influence of environmental parameters on digester microbial community*

Numerous reports describe the influence of environmental parameters on methanogenic microbial community structure, but reverse approaches describing the influence of microbial community structure on digester functional stability or methanogenic activity are less numerous. Influences of various environmental parameters on community structure include the following: (1) higher influent SO_4^{-2} concentration leads to higher sulfate-reducing bacteria levels (Raskin et al., 1996; Pender et al., 2004), (2) higher digester acetate concentration leads to higher *Methanosarcina* and lower *Methanosaeta* abundance (Griffin et al., 1997; McMahon et al., 2001), (3) NH_3–N concentrations greater than approximately 3 g/L leads to lower *Methanosarcina* levels, higher *Methanomicrobiales* levels (Angenent et al., 2002) and a shift from acetoclastic methanogenesis to syntrophic acetate oxidation with hydrogenotrophic methanogenesis (Fotidis et al., 2013), (4) lower temperature leads to higher diversity at 37 °C versus 55 °C (Karakashev et al., 2005) and sometimes leads to a shift from acetoclastic methanogenesis to hydrogenotrophic methanogenesis at psychrophilic temperatures (Enright et al., 2009; Zhang et al., 2012), (5) different substrates lead to different community structures, including manure versus wastewater sludge (Karakashev et al., 2005) and glucose versus whey permeate and sewage sludge (Lee et al., 2009), and (6) trace nutrient deprivation causes a shift in community structure, with low cobalt or nickel concentrations causing decreased *Methanosarcina* abundance and decreased COD removal rate in methanol-fed bioreactors (Fermoso et al., 2008a, 2008b).

1.2. *Influence of community structure on digester functional stability*

Approaches describing the influence of microbial community structure on anaerobic functional stability during and after perturbation have been reviewed (Briones and Raskin, 2003; Allison and Martiny, 2008). These approaches include the work of Hashshan et al. (2000) who concluded that anaerobic digester communities with multiple microorganisms within the same trophic group (i.e., more parallel processing) exhibited greater functional stability after organic overload. The functional stability ostensibly resulted because one or more microorganisms were present and able to function in each critical group during and after upset. In addition, less stable community structure (i.e., greater community flexibility) may increase functional stability upon perturbation since less stable communities are more able to adapt to stress (Fernandez et al., 2000). Communities with higher evenness have been found to be more functionally resistant to selective stress than uneven communities. In this regard, Wittebolle et al. (2009) reported that denitrifying communities with higher evenness exhibited higher denitrification rates when exposed to salt toxicity compared to communities with low evenness. Although methanogenic systems were not their focus, the results may be applicable to anaerobic digesters; the theory being that in highly even communities there is a higher probability that one or more organisms resistant to the stress is present in significant enough numbers to proliferate and maintain functionality.

1.3. *Community structure, methanogenic activity and linear relationships*

The influence of microbial community structure on methanogenic activity during non-perturbed operation has been investigated. In a multi-year survey of nine full-scale digesters treating brewery wastewater, communities with greater evenness and redundancy exhibited higher specific methanogenic activity (SMA) values and higher COD removal (Werner et al., 2011). In addition, higher *Bacteroidetes* and *Archaea* abundances have been shown to correlate to higher hydrolytic and methanogenic specific activities, respectively (Regueiro et al., 2012).

In our laboratory, Tale et al. (2011) measured SMA against propionate for 14 different biomass samples from full-scale anaerobic digesters and the microbial communities were also compared. Principal components analysis (PCA) depicted a linear relationship between SMA with propionate and microbial community structure defined by denaturing gradient gel electrophoresis (DGGE) banding pattern of methyl coenzyme M reductase (*mcrA*), a gene ubiquitous in methanogens. Biomass with high SMA values clustered together on a PCA plot developed using only DGGE banding patterns, whereas biomass with low SMA values clustered in a different location. In addition, the presence of hydrogenotrophic methanogens closely related to *Methanospirillum hungatei* and *Methanobacterium beijingense* was associated with high propionate SMA values. Building on this work and the work of Freitag and Prosser (2009), Morris et al. (2014) found that *mcrA* gene copy numbers in a group of four different enrichment cultures were linearly correlated to SMA with H_2/CO_2 ($r^2 = 0.98$).

Multiple linear regression (MLR) has been used to develop quantitative structure activity relationships (QSARs) between chemical structure descriptors and biological or physiochemical activities, such as toxicity or Henry's Constant values (Nirmalakhandan and Speece, 1988). When Tale (2010) applied MLR to activity data, SMA values were linearly related to community structure as defined by standardized, demeaned DGGE band intensities of *mcrA* amplicons. However, the resulting MLR equation was overfitted and not predictive because too many independent variables (i.e., 10 bands) were used to predict the SMA values of too few samples (i.e., 14 biomass samples). Therefore, an insufficient number of different biomass samples was employed, preventing development of a predictive relationship (Tale, 2010).