Predicting bioaugmentation outcome based on SMA screening and methanogen community structure

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Abstract
A novel approach to improve anaerobic digester performance by bioaugmenting with cultures enriched for propionate and H_2/CO_2 degradation was investigated. First, biomass samples from nine different full-scale anaerobic digesters were screened for bioaugmentation outcomes by comparing SMA values against propionate with bioaugmentation (bSMA) and conventional SMA values without bioaugmentation (cSMA). The bSMA values were determined using a 4:1 mixture (based on ATP concentration) of each biomass with the enrichment (augment) culture, whereas the cSMA values were determined for each biomass alone. DGGE analysis using *mcrA* primers was also performed to relate methanogen community structure to digester function. Six of the nine biomass samples showed increased SMA upon bioaugmentation (bSMA > cSMA). Furthermore, biomass with methanogenic community structure less similar to that of the augment culture exhibited a greater difference between bSMA and cSMA. Two biomass samples, one that demonstrated increased SMA due to bioaugmentation (Biomass G) and another that did not (Biomass C), were used to seed separate laboratory anaerobic digesters. After quasi steady state was achieved, digesters seeded with Biomass G exhibited increased steady-state methane production and lower propionate concentration when bioaugmented, whereas digesters seeded with Biomass C did not. We anticipate concluding the steady state analysis of the seven other biomass samples by April 2013. We hypothesize that the degree of functional improvement from bioaugmentation for a given biomass during steady state operation correlates to ΔSMA, where ΔSMA = (bSMA – cSMA)/cSMA. In addition, a greater difference between existing digester and augment culture methanogen community structures correlates to higher steady-state methane production in bioaugmented digesters.

Keywords
Anaerobic digestion; bioaugmentation; propionate; specific methanogenic activity; steady state

INTRODUCTION

In anaerobic digestion (AD), the bioconversion of complex substrates to biogas relies upon healthy relationships between different groups of microorganisms. An imbalance in these relationships results in high volatile acid concentrations and subsequently in poor AD function and possible failure (Hatamoto et al., 2007). This susceptibility to process upset has limited AD technology from operating at maximum efficiency. Bioaugmentation offers great potential for countering these process upsets caused by an imbalance in healthy relationships. Indeed, mixed cultures enriched for propionate degradation have been shown to reduce transient high concentrations of volatile acids in stressed digesters after toxicant exposure and organic overload (Schauer-Gimenez et al., 2010; Tale et al., 2011). Bioaugmentation with propionate-degrading cultures should be investigated to improve steady-state functionality, particularly since it is estimated that up to 30% of the COD from complex substrates flows through propionate as an intermediate (Speece, 2008). Additionally, it is pertinent to examine the microbial community characteristics since prior studies have stated the importance it plays in digester function (Fernandez et al., 2000; Hashsham et al., 2000). In this research, SMA testing was employed to screen biomass samples for potential improvement due to bioaugmentation. Different biomass samples were used to seed anaerobic digesters and then bioaugmented with a propionate-degrading enrichment (augment) culture to determine if the rate of propionate-to-methane conversion and steady-state methane production can be increased.

MATERIALS AND METHODS

Cultures used for bioaugmentation. The added augment culture was a 1:1 (VSS mass-ratio) mix of two cultures. One was enriched using H_2/CO_2 (Schauer-Gimenez et al., 2010), since low H_2 parital pressure ($< 10^{-4}$ atm) is essential for propionate degradation (McCarty and Smith, 1986), and the other was enriched using propionate (Tale et al., 2011). These cultures previously have been used to bioaugment digesters after transient organic overload and toxicity events, resulting in increased methane production and COD reduction during non-steady-state periods. Aliquots of the mixture were also autoclaved to produce inactive material for controls.

Specific methanogenic activity (SMA). SMA analysis was performed against calcium propionate to estimate the maximum rate of propionate-to-methane conversion for nine biomass samples from different full-scale anaerobic digesters (labelled A through I in Figure 1). SMA analysis for each biomass was performed under both bioaugmented (bSMA) and non-bioaugmented, conventional conditions (cSMA). For bSMA, the biomass sample was mixed with the augment culture at a ratio of 1:4 (augment:biomass) based on intracellular adenosine triphosphate (iATP) concentration . The iATP was measured using an ATP test kit by the manufacturer protocol (QuenchGone21TM, LuminUltra, Fredericton, New Brunswick, Canada). The cSMA was measured using the full-sale digester biomass samples alone.

Steady-state digesters. Each biomass sample was used to seeds different groups of anaerobic digesters containing 3 sets, (1) triplicate digesters bioaugmented with live culture (Active), (2) triplicate digesters augmented with autoclaved culture (Inactive), and (3) triplicate digesters that were not augmented (Control). The digesters were 160-mL serum bottles with a 50-mL working volume and had a 10-day hydraulic residence time (HRT). All digesters were fed synthetic industrial wastewater (nonfat-dry milk, OLR of 4 g COD/L_R-day) and basal nutrients for methanogenic cultures (Speece, 2008). From day 1, the Active and Inactive digesters receive a daily augment dose of approximately 1% of digester VSS mass as active and autoclaved augment, respectively.

Analytical Methods. COD, VFA and biogas CH_4 concentrations were measured by standard methods (APHA et al., 1998). Digester daily biogas volume was measured using a glass syringe with wetted glass barrel, the needle of which was inserted through digester butyl rubber septa. DNA was extracted using a DNA isolation sample kit (PowerSoil™ DNA Isolation Sample Kit, MoBio Laboratories, Inc., Carlsbad, CA). Extracted DNA was amplified using primers for the *mcrA* gene which has been used previously to compare and identify taxonomically different methanogens (Luton et al., 2002). Amplicons were separated using denaturing gradient gel electrophoresis (DGGE). Densitometric data of gel bands were obtained using gel viewing software (Lab Works v. 4.6.00.0 Lablogics, Inc., Mission Viejo, CA).

RESULTS AND DISCUSSION

SMA analysis. Bioaugmentation in six out of nine biomass samples (A, E, F, G, H and I) resulted in increased average SMA values ($p < 0.05$), as shown in Figure 1. Previous studies have shown that higher community diversity can result in greater functional stability (Fernandez et al., 2000; Hashsham et al., 2000). The methanogenic community structures of the nine biomass samples and the augment culture were quantified using DGGE band intensity data. The dissimilarity between the methanogenic community structure of a biomass sample and the augment culture was quantified as the distance, calculated as one minus the Pearson's correlation coefficient, of the DGGE banding patterns. The percentage increase in the SMA observed due to bioaugmentation were plotted against the dissimilarity of the biomass from the augment culture (Figure 2).

The extent of dissimilarity between initial seed biomass and the augment culture correlated to the increase in SMA observed. In other words, digester biomass that was more similar to that of the augment culture appeared to benefit less from bioaugmentation and *vice versa*.

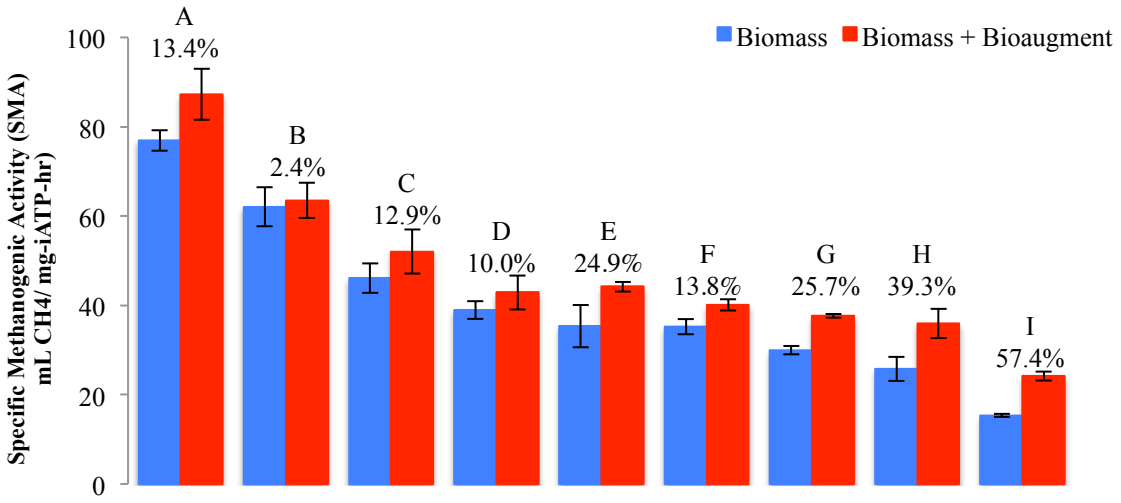

Figure 1: Comparison of the SMA against propionate for nine biomass samples (labelled A through I). The percentage value depicts the measured increase in the SMA after bioaugmentation.

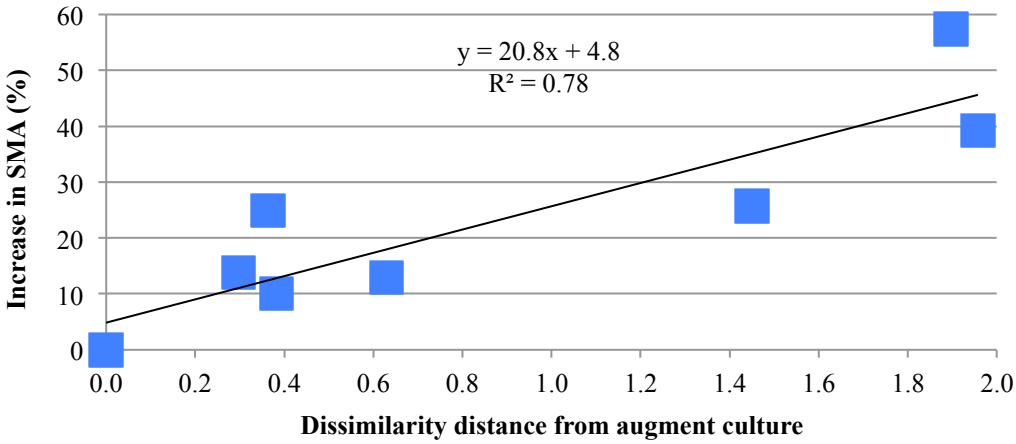

Figure 2: Difference between methanogen community structures in the augmented and biomass samples correlated with percent increase in SMA values

Steady-state digesters. For the steady-state digester investigation, two sets of digesters, seeded with biomass samples C and G (refer to Figure 1), have been operated so far. An additional seven digester sets will be analysed by April 2013. Of the two sets operated, Set G showed a significant improvement in performance after bioaugmentation (i.e, higher methane production and lower effluent propionic acid concentration), whereas Set C did not show improvement (Figure 3) (Venkiteshwaran et al., 2012). Based on the conclusions obtained from the SMA experiment of the nine biomass samples, we hypothesize that the degree of functional improvement from bioaugmentation for a given biomass during steady state operation correlates to DSMA, where DSMA = (bSMA – cSMA)/cSMA. In addition, a greater difference between existing digester and enrichment culture methanogen community structures correlates to higher steady-state methane production in bioaugmented digesters. This research will help develop a tool to predict the success of bioaugmentation based on methanogen community, and will contribute to our understanding of when bioaugmentation can be implemented effectively.

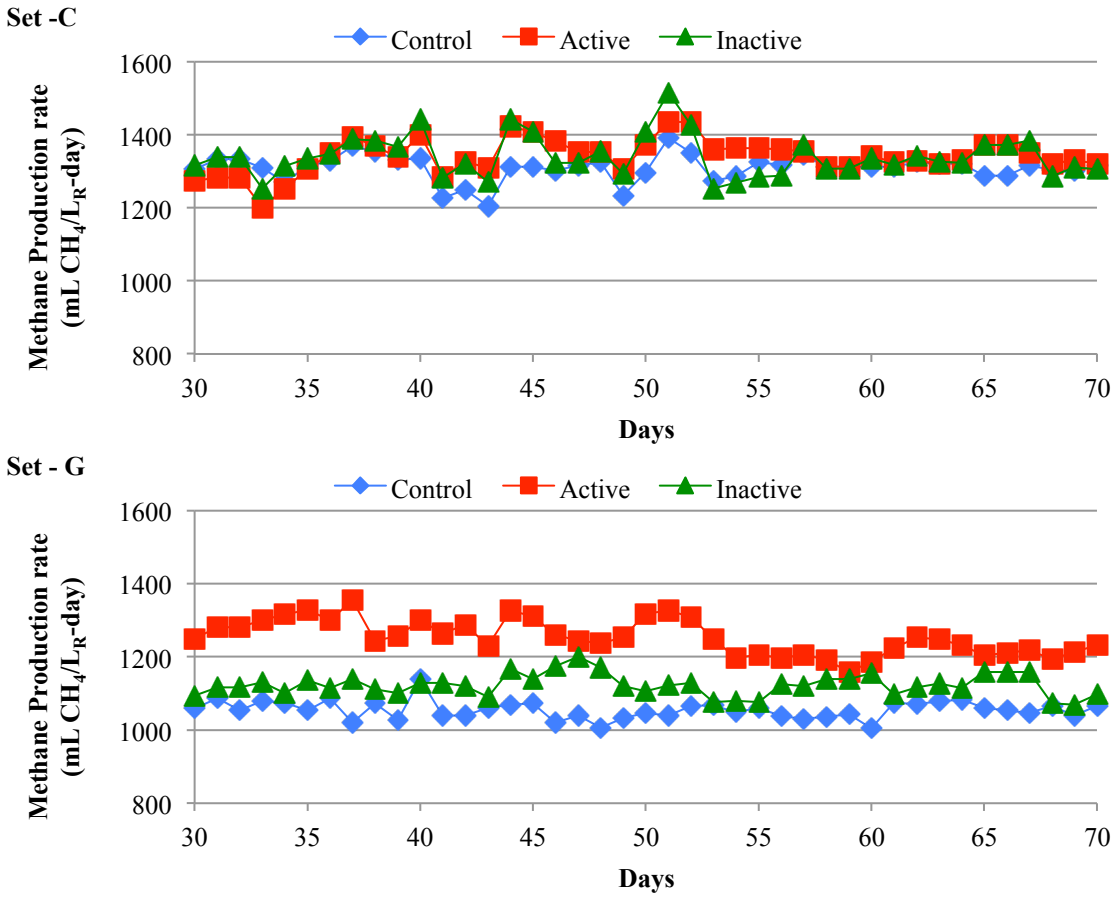

Figure 3: Average daily methane production of the two sets of steady-state digesters (Venkiteshwaran et al., 2012)

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