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Accepted version. *Water Research*, Volume 44, No. 12 (June 2010), DOI. © 2010 Elsevier. Used with permission.

Bioaugmentation for Improved Recovery of Anaerobic Digesters after Toxicant Exposure

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Abstract:

Bioaugmentation was investigated as a method to decrease the recovery period of anaerobic digesters exposed to a transient toxic event. Two sets of laboratory-scale digesters (SRT = 10 days, OLR = 2 g COD/L-day), started with inoculum from a digester stabilizing synthetic municipal wastewater solids (MW) and synthetic industrial wastewater (WW), respectively, were transiently exposed to the model toxicant, oxygen. Bioaugmented digesters received 1.2 g VSS/L-day of an H₂-utilizing culture

for which the archaeal community was analyzed. Soon after oxygen exposure, the bioaugmented digesters produced 25-60% more methane than non-bioaugmented controls ($p < 0.05$). One set of digesters produced lingering high propionate concentrations, and bioaugmentation resulted in significantly shorter recovery periods. The second set of digesters did not display lingering propionate, and bioaugmented digesters recovered at the same time as non-bioaugmented controls. The difference in the effect of bioaugmentation on recovery may be due to differences between microbial communities of the digester inocula originally employed. In conclusion, bioaugmentation with an H_2 -utilizing culture is a potential tool to decrease the recovery period, decrease propionate concentration, and increase biogas production of some anaerobic digesters after a toxic event. Digesters already containing rapidly adaptable microbial communities may not benefit from bioaugmentation, whereas other digesters with poorly adaptable microbial communities may benefit greatly.

1. Introduction

Bioaugmentation is the practice of adding specific microorganisms to a system to enhance a desired activity (Rittmann and Whiteman, 1994; Deflaun and Steffan, 2002). In wastewater treatment, bioaugmentation has most frequently been applied to aerobic systems to increase the population of nitrifying bacteria after upsets from uncontrolled biomass loss, fluctuations in pH, toxic events, or temperature decrease (Rittmann and Whiteman, 1994; Abeysinghe et al., 2002; Satoh et al., 2003; Head and Oleszkiewicz, 2005). Bioaugmentation has also been used for other aerobic applications, including improved flocculation and degradation of specific substrates (Van Limbergen et al., 1998), and for soil and groundwater bioremediation (Deflaun and Steffan, 2002; Singer et al., 2005).

For anaerobic processes, bioaugmentation has been investigated at laboratory scale to improve start-up of new digesters (Saravanane et al., 2001a,b), odor reduction (Duran et al., 2006; Tepe et al., 2008), and recovery after organic overload (Lynch et al., 1987). Also, anaerobic degradation rates of phenol and cresol (Charest et al., 1999; Tawfiki Hajji et al., 1999; Guiot et al., 2000; Tawfiki Hajji et al., 2000), pentachlorophenol (Tartakovsky et al., 1999; Guiot et al., 2002), 3-chlorobenzoate (Ahring et al., 1992), tetrachloroethylene (Horber et

al., 1998) and fat, oil and grease (Cirne et al., 2006) have been increased using bioaugmentation. Anaerobic bioaugmentation with cellulose degraders increased methane production rates from hemicellulose by 30% (Angelidaki and Ahring, 2000) and cattle manure by as much as 93% (Mladenovska et al., 2001; Nielsen et al., 2007); however, the significant increase in methane yield was only sustained for a limited time after inoculation. The short improvement period highlights current challenges such as washout and out-competition by indigenous organisms associated with bioaugmentation approaches (El Fantroussi and Agathos, 2005).

Bioaugmentation for rapid recovery of anaerobic digesters exposed to transient toxicants has not been reported to our knowledge. However, it may be advantageous to develop bioaugmentation as a recovery tool to address transient toxicity that can occur in full scale. Production and distribution of individual bioaugmentation cultures, each enriched to degrade a specific substrate, would be time consuming. It may be more practical to target a key, ubiquitous intermediate that accumulates during toxic events. In this regard, hydrogen (H_2) is a reasonable target intermediate since its degradation is often a rate-limiting step in methane production from many complex substrates. The H_2 concentration in anaerobic systems must be very low (<50 mM) for conversion of propionate and other intermediates to methane to be thermodynamically spontaneous (McCarty and Smith, 1986). Therefore, more rapid H_2 utilization can result in more complete conversion of propionate and other substrates to methane. In this study, bioaugmentation using an H_2 -utilizing culture was tested to determine if the approach would decrease the recovery time of anaerobic digesters exposed to a transient toxic event. A limited amount of the model toxicant, oxygen (O_2), was added to different digesters for a short period, and recovery of bioaugmented and non-bioaugmented digesters was compared.

2. Materials and methods

2.1. Anaerobic digesters

Two sets of anaerobic digesters were operated. Sets differed based upon the source of the initial inoculum employed. One set (MS systems) was inoculated with biomass from a bench-scale mesophilic anaerobic digester fed synthetic municipal sludge (Natural Choice Dog Food, NutroProducts, Inc., City of Industry, CA) with a 10-day solids retention time (SRT). The other set (WW systems) was inoculated with biomass from a bench-scale mesophilic anaerobic digester fed synthetic industrial wastewater (Instant Nonfat Dry Milk, Roundy's, Inc., Milwaukee, WI) with a 15-day SRT.

All digesters were 160-mL serum bottles containing 50 mL of active volume and operated at an SRT of 10 days and organic loading rate of 2 g COD/L-day. Five mL/day of effluent was removed and replaced with 5 mL/day of synthetic wastewater composed of nonfat dry milk (Roundy's Instant Nonfat Dry Milk) in basal nutrient medium. After one week, all digesters were exposed to the model toxicant, O_2 , by injecting 10 mL/day of air (approximately 1 atm, 20 °C) into each system for seven days. Bioaugmented digesters received 1.2 mg of volatile suspended solids (VSS) per liter of digester per day (mg VSS/L-day) of an enrichment culture described below. Control digesters received an abiotic version of the enrichment culture that had been inactivated by autoclaving.

2.2. Culture used for bioaugmentation

The methanogenic culture used for bioaugmentation was developed using biomass from a mesophilic municipal anaerobic digester (South Shore Wastewater Treatment Plant, Milwaukee, WI) treating primary sludge and was enriched by feeding H_2 , carbon dioxide (CO_2), and glucose in the basal medium as well as limited O_2 over three months. Two liters of biomass were maintained in a 2.5-L glass reactor continuously stirred with a magnetic stir bar and in a temperature-controlled room (35 ± 2 °C). The culture was sparged daily with gas (1:1 v/v $H_2:CO_2$) for approximately 20 s and sealed with a rubber stopper. The CO_2 content was greater than that of the

stoichiometric amount (i.e., 4:1 v/v H₂:CO₂) so as to maintain the culture pH near 7. A glass tube was inserted through the stopper and connected to a 5-L Tedlar bag that was emptied daily and refilled with the H₂:CO₂ mixture. The liquid effluent (133 mL) was removed once per day to maintain an SRT and hydraulic retention time (HRT) of 15 days. Effluent was replaced with 133 mL of basal medium containing 84 mg glucose. Approximately 80 mg O₂/day was added by injecting 280 mL of ambient air (approximately 1 atm, 20 °C) directly into the vessel headspace with a plastic syringe. This mass of O₂ satisfied very little (i.e., 6%) of the H₂ oxygen demand. Therefore, the culture dissolved oxygen concentration was expected to be zero, and the presence of strict anaerobes (e.g., methanogens) was anticipated.

2.3. Basal nutrient medium

Basal nutrient medium contained the following [mg/L]: NH₄Cl [400]; MgSO₄·6H₂O [250]; KCl [400]; CaCl₂·2H₂O [120]; (NH₄)₂HPO₄ [80]; FeCl₃·6H₂O [55]; CoCl₂·6H₂O [10]; KI [10]; the trace metal salts (MnCl₂·4H₂O, NH₄VO₃, CuCl₂·2H₂O, Zn(C₂H₃O₂)₂·2H₂O, AlCl₃·6H₂O, NaMoO₄·2H₂O, H₃BO₃, NiCl₂·6H₂O, NaWO₄·2H₂O, and Na₂SeO₃) [each at 0.5]; yeast extract [100]; NaHCO₃ [5000]; and resazurin [1].

2.4. Specific methanogenic activity (SMA) assays

Methanogenic activity assays were conducted in triplicate at 35 °C, 250 rpm using an incubator shaker (model C25KC, New Brunswick Scientific, Edison, NJ). Published protocols for the substrates calcium acetate (Angelidaki et al., 2007) as well as H₂:CO₂ (Coates et al., 1996) were used. All assays were performed under anaerobic conditions in 160 mL serum bottles with 25 mL of enrichment culture having 100-400 mg/L VSS. Culture samples were collected from reactors on three consecutive days and composited for testing. The VSS concentration was determined at the beginning and end of activity tests and the average of the two values was employed for specific activity calculations.

For acetate activity assays, the substrate was added to serum bottle contents to achieve 10 g/L of calcium acetate. Bottles were then sparged with O₂-free gas (7:3 v/v N₂:CO₂), closed with red butyl

rubber septa and incubated. For hydrogenotrophic activity assays, serum bottles were sparged with gas (4:1 v/v H₂:CO₂) and closed with solid Balch-type butyl rubber stoppers (Geo-Microbial Technologies, Inc., Ochelata, OK) and aluminum-crimped seals. Immediately thereafter, 100 mL of the H₂:CO₂ gas blend at ambient pressure and temperature was injected through the stopper using a syringe and 23-gauge needle; then the bottles were incubated. Blanks were prepared similarly, but no substrate was added. These bottles were sparged with H₂-free gas (7:3 v/v N₂:CO₂) and sealed. Bottle headspace volume was measured at ambient pressure (approximately 1 atm) for 1-5 days. Volume was measured by inserting the needle of a glass syringe with wetted barrel. Syringe content was re-injected into the serum bottle after volume measurement. Headspace CH₄ content was measured using gas chromatography (GC).

For activity, maximum methane production rate (mL CH₄/h) was determined by linear regression of the initial, linear portion of a plot of cumulative methane production versus time. SMA values (mL CH₄/g VSS-h) were calculated by dividing maximum methane production rate values by average VSS mass. For hydrogenotrophic activity, maximum methane production rate (mL CH₄/h) was determined as described by others (Coates et al., 1996). Briefly, the decrease in headspace volume observed at any time was corrected by adding the volume of additional gas measured in blanks. The sum was divided by four based upon the stoichiometry of hydrogenotrophic methanogenesis (i.e., for every 4 mol of H₂ and 1 mol of CO₂ consumed, 1 mol of CH₄ is produced) to yield the cumulative volume of methane produced. Maximum methane production rates and SMA values against H₂:CO₂ were then determined by linear regression as described for acetate activity.

2.5. Analytical methods

Samples for propionate and soluble COD(SCOD) concentration analysis were centrifuged at 14,000 for 10 min (Galaxy 14D centrifuge, VWR International, West Chester, PA) and filtered using a 0.45 µm filter (Whatman International Ltd.,Maidstone, England). Propionate was measured in filtrate using a GC (Series 600, GOW-MAC Instrument Co., Bethlehem, MA) equipped with a flame ionized

detector (FID) and a packed stainless steel column 6' 1/4" (Alltech Associates, Inc., Deerfield, IL). Helium was used as the carrier gas at a flow of 50 ± 1 mL/min. The temperature of the injector and detector was 200 °C and the oven temperature was 150 °C. The supernatant was stored in 4-mL vials with a minimal amount of phosphoric acid to acidify the sample to a pH of <2. SCOD was measured in filtrate by standard methods (APHA et al., 1998). The biogas quantity produced in digesters was measured daily using a glass syringe with a wetted glass barrel. The headspace gas composition (CH₄, CO₂, and N₂ concentrations) was determined using a GC equipped with a thermal conductivity detector (TCD) and a packed column (CTR I column, Alltech Associates, Inc., Deerfield, IL). Helium was used as the carrier gas at a flow of 30 ± 2 mL/min with injector and detector temperatures of 120 °C and oven temperature of 38 °C. The pH was measured using a bench-top pH meter (Orion Model 720A, Thermo Fisher Scientific, Inc., Waltham, MA) and a general-purpose pH electrode (Orion, Thermo Fisher Scientific, Inc., Waltham, MA).

2.6. Statistical analyses

All statistical analyses were completed using the "t-test: two-sample assuming unequal variances" function in Microsoft Excel (Excel, 2004 for Mac, Version 11.5.5). Each set of replicates was analyzed in order to obtain mean and variance values for the t-test function.

2.7. Archaeal community analysis

2.7.1. DNA extraction

DNA was extracted from the bioaugmentation culture by removing and centrifuging (IEC Centra-4B, International Equipment Company) a 50-mL volume for 10 min at 2500 . The supernatant was then decanted and a 0.75-mL thickened biomass sample was used for DNA extraction (PowerSoil™ DNA Isolation Sample Kit, MoBio Laboratories, Carlsbad, CA). The standard protocol provided in the kit was followed with one exception; the Alternative Lysis Method was used (a 1 min vortex followed by a 10 min incubation at 70 °C, after adding Solution C1). The presence of DNA was confirmed and estimates of its concentration were made using gel electrophoresis (1% agarose)

stained with ethidium bromide (0.8 $\mu\text{L}/\text{mL}$) (Sambrook and Russell, 2001). A DNA ladder containing 40-ng/ μL Lambda DNA, HindIII cut and in some cases 30-ng/ μL phi X174, HaeIII cut was used as a marker.

2.7.2. Polymerase chain reaction

A fragment of the 16S rRNA genes was amplified with ArchF (5'-TTCCGGTTGATCCYGCCGGA-3') and ArchR (5'-YCCGGCGTTGAMTCCAATT-3') (DeLong, 1992) using either a Biometra Tpersonal (Biometra, Goettingen, Germany) or Bio-Rad PTC-200 DNA Engine Cycler thermal cycler (Bio-Rad, Hercules, CA). All PCR amplification mixtures (100 μL) contained EconoTaq[®] PLUS 2X Master Mix (Lucigen Corporation, Middleton, WI) and 0.1 μM of each primers. The amplification method parameters were as follows: 94 °C for 2 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min for 34 cycles and 72 °C for 13min.

2.7.3. Cloning

The 16S rRNA gene amplified products were cloned into One Shot[®] Mach1TM-T1R Chemically Competent *Escherichia coli* cells using the TOPO TA Cloning[®] Kit for Sequencing according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Bacteria were inoculated to S-GalTM/Kanamycin/Luria-Bertani Broth Agar Blend (Sigma-Aldrich, St. Louis, MO) plates containing 50 mg/mL ampicillin and incubated at 37 °C for one day. Colonies were picked using light/dark screening and directly amplified with PCR using PucF (5'-GGAATTGTGAGCGGATA ACA-3') and PucR (5'-GGCGATTAAGTTGGGTAACG-3') primers. The PCR thermal cycling parameters were as follows: 94 °C for 2 min, 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min for 30 cycles, and 72 °C for 9 min. The presence of amplified PCR products was confirmed using agarose gel electrophoresis as previously described.

2.7.4. PCR purification, sequencing and phylogenetic analysis

Samples were cleaned using the UltraClean™ PCR Clean-up™ kit according to manufacturers' instructions (MoBio Laboratories, Carlsbad, CA).

PCR products of 50 archaeal clones from the bioaugmentation culture were sequenced (University of Chicago Cancer Research Center DNA Sequencing Facility, Chicago, IL). The forward and reverse sequences were analyzed using FinchTV (Geospira Inc., Seattle, WA) and Vector NTI (Invitrogen, Carlsbad, CA) and consensus sequences were assembled. Vector sequences were removed using the Basic Local Alignment Search Tool (BLAST) to match vector sequences in the UniVec Database with the sample sequences in a manner identical to VecScreen (Altschul et al., 1997). Chimera detection analysis was performed using Chimera Check, version 2.7 (Cole et al., 2005) of the Ribosomal Database Project (RDP) and sequences determined to be chimeras were removed from further analysis.

The complete consensus sequences were submitted to BLAST to identify similar 16S rRNA gene sequences (Altschul et al., 1997). The consensus and select reference sequences were aligned using the RDP (Cole et al., 2007). A distance matrix based on the Kimura 2-parameter algorithm was constructed using the Phylogeny Inference Package (PHYLIP) dnadist program (Felsenstein, 2005). Bootstrap analyses were performed to generate 100 bootstrap samples. Neighbor-joining, maximum parsimony and maximum likelihood trees were created using PHYLIP. Consensus trees were generated with PHYLIP's consense program, using the extended majority rule. Resulting trees were visualized using FigTree v1.1.2 (Rambaut, 2008), compared and were similar. The neighbor-joining tree was presented in the text. The Fitch-Margoliash algorithm was used to add the distances to the bootstrapped trees. The SeqMatch program on the RDP website was used (Cole et al., 2007) to identify the taxonomic classifications if the 16S rRNA gene sequence similarity to known microorganisms was less than 95%.

To examine the richness of the archaeal community in the bioaugmentation culture, rarefaction analysis, Chao1 richness estimates and Shannon indices were constructed using DOTUR

(Schloss and Handelsman, 2005). Operational taxonomic units (OTUs) were defined as sequence groups in which sequences differed by 2% or less.

2.7.5. Nucleotide sequence accession numbers

Sequences from the clone library described above were deposited in the GenBank database under accession numbers GU196151-GU196192.

3. Results

3.1. Bioaugmentation culture archaeal community analysis

An archaeal clone library was constructed for the bioaugmentation culture. Of the 50 sequences, 8 were chimeric and removed from further analysis. The rare fraction curve (see Fig. 1) indicated that the archaeal coverage was 83% (Chao1 richness estimates). Also, the Shannon indices showed that the archaeal clone library was not very heterogeneous (1.25).

Phylogenetic analysis of Archaea in the bioaugmentation culture (see Fig. 2) showed sequences that were related to three methanogenic genera (*Methanosaeta*, *Methanoculleus*, and *Methanospirillum*). Nine sequences grouped in the genus *Methanosaeta* (96% sequence similarity) and accounted for 21% of the Archaeal sequences sampled. According to the SMA assay results, the activity against acetate was relatively low (SMA = 0.25 ± 0.07 mL CH₄/g VSS-h) even though *Methanosaeta* was found in high relative abundance.

Because H₂ and CO₂ were fed to the bioaugmentation culture, it was anticipated that most of the methanogen 16S rRNA gene sequences would relate to H₂ utilizers. But only one sequence (C4-46A) was closely related to the H₂-utilizer *Methanospirillum hungatei* (98% sequence similarity, GenBank accession number: M60880) and only one sequence (C4-10A) was closely related to the H₂-utilizer *Methanoculleus* sp. *dm2* (97% sequence similarity, GenBank accession

number: AJ550158). Although these sequences accounted for only 5% of the clone library, the average SMA value against H₂ was relatively high (47 ± 30 mL CH₄/g VSS-h). It is possible that the unknown Archaeal sequences discussed below that made up 74% of the community were related to H₂ utilizers.

A number of unknown Archaeal sequences were found. For example, 14 sequences could only be classified as Euryarchaeota (33% of the community) and were most similar to an uncultured clone sequenced from a municipal mesophilic anaerobic digester (99% similarity) (Chouari et al., 2005). In addition, 17 sequences could only be placed in the phylum Crenarchaeota, class Thermoprotei (41% of the community) (see Fig. 2). These sequences were most similar to an uncultured clone sequence from leachate in a municipal solid waste landfill (98% similarity) (Huang et al., 2003).

3.2. Bioaugmented digester performance

3.2.1. Average CH₄ production rate and pH

Average CH₄ production rates for bioaugmented and control digesters were similar before and during air addition (see Figs. 3a and 4a). After air addition, however, the bioaugmented digesters produced significantly more CH₄ than the respective controls ($p < 0.05$). The higher CH₄ production rate for bioaugmented digesters was evident on Days 25-170 for MS systems (see Fig. 3a) and Days 45-70 for WW systems (see Fig. 4a). For MS systems, the average bioaugmented CH₄ production rate was approximately 60% greater than that of the non-bioaugmented controls during this period. For WW systems, the bioaugmented CH₄ production rate was approximately 25% greater. It should be noted that the apparent decrease in CH₄ production rate on Day 148 for the MS systems was due to leaking septa. These septa were replaced and the measured average CH₄ production increased in both bioaugmented and control digesters from Day 155 to 161 (27 ± 3 mL CH₄/day in the control and 28 ± 2 mL CH₄/day in the bioaugmented digesters).

For both MS and WW systems, the average pH of all digesters was approximately 7.20 before air addition. The pH then decreased

during and immediately after air addition with the lowest average pH values of 6.60 observed between Days 16 and 18 and between Days 39 and 49 for MS and WW systems, respectively. Subsequently, digester pH values increased to 7 or greater.

3.2.2. Effluent SCOD

The average effluent SCOD from all digesters significantly increased after air addition, peaking at approximately 5000 mg/L (see Figs. 3b and 4b). Subsequently, the average SCOD decreased below 2000 mg/L, with the decrease more rapid in bioaugmented systems as compared to controls (see Figs. 3b and 4b).

For MS systems, the average SCOD decreased in the bioaugmented digesters from 4000 mg/L (Day 30) to 600 mg/L during a 2.5-month period, and remained relatively low and constant for the remainder of the investigation (see Fig. 3b). In contrast, the average effluent SCOD of the non-bioaugmented digesters remained greater than 2000 mg/L for over five months, as can be seen in Fig. 3b. In addition, effluent SCOD from the non-bioaugmented digesters was significantly higher than that of the bioaugmented digesters at the conclusion of the investigation (1540 ± 290 mg/L versus 780 ± 210 mg/L, respectively).

For WW systems, the high SCOD concentrations persisted during a recovery period of only three months in contrast to the five-month recovery for MS systems (see Fig.4b). After Day 120, both bioaugmented and control digesters exhibited low average SCOD concentrations of 200 mg/L in WW systems (see Fig.4b).

3.2.3. Effluent propionate

Effluent propionate concentrations from all digesters increased from less than 600 to greater than 1000 mg/L during the four-week period after air was added, then began to decrease (see Figs. 3c and 4c). For MS systems, propionic acid in bioaugmented digesters eventually decreased to 60 ± 40 mg/L (Day 97), but lingered in control digesters and did not decrease until Day 124 (see Fig.3c). For WW systems, on the other hand, bioaugmented and control digesters

exhibited similar effluent propionate concentrations over time (see Fig.4c).

3.2.4. Recovery periods

For comparison, recovery periods were defined as the time required after air addition for the average effluent concentration of SCOD or propionate to decrease below specified threshold concentrations. After the recovery period, no subsequent increase above the threshold concentration was observed. For SCOD, threshold concentrations of 1 g/L and 2 g/L were chosen. For propionate, a threshold concentration of 200 mg/L was employed.

The recovery periods were significantly shorter for bioaugmented digesters in comparison to controls for MS systems ($p < 0.05$), but were not shorter for WW systems (see Fig.5). MS bioaugmented digesters achieved an SCOD concentration below 2 g/L over 80 days (i.e., 8 SRTs) before the controls (see Fig.5); in addition, the propionic acid concentrations in bioaugmented digesters declined below 200 mg/L approximately 70 days before that of the controls (Day 90 ± 0 versus Day 157 ± 18). In contrast, the WW bioaugmented and non- bioaugmented digesters both recovered approximately 2 months after oxygen exposure.

4. Discussion

Sequences most similar to those of H₂-utilizing methanogens (*Methanosaeta*, *Methanoculleus*, and *Methanospirillum*) were found in the bioaugmentation culture, as expected. Sequences grouped in the genus *Methanosaeta* were also found, and accounted for 21% of the Archaeal sequences sampled. These acetoclastic methanogens have also been found in other anaerobic treatment systems (Macario and de Macario, 1988; Grotenhuis et al., 1991; Raskin et al., 1995; Griffin et al., 1998; Sekiguchi et al., 1998; McHugh et al., 2003; Leclerc et al., 2004). Glucose in the feed to the bioaugmentation culture ostensibly resulted in the presence of acetoclastic methanogens in the H₂-rich environment. Fernandez et al. (2000) found that *Methanosaeta* accounted for approximately 20% of the total methanogen community in some anaerobic digesters fed glucose as the sole carbon source.

A large majority of the clones (i.e., 74%) were unknown Archaeal sequences. For example, 33% of the sequences could only be classified as Euryarchaeota and were most similar to an uncultured clone sequenced from a municipal mesophilic anaerobic digester (99% similarity) (Chouari et al., 2005). In addition, 41% of the clones could only be placed in the phylum Crenarchaeota, class Thermoprotei. These sequences were most similar to an uncultured clone sequence from leachate in a municipal solid waste landfill (98% similarity) (Huang et al., 2003). The unknown Archaeal sequences could not be further classified taxonomically and it is difficult to suggest what role they play in the microbial community. However, the Euryarchaeota could be methanogens.

Bioaugmentation of an aerobic processes has been reported to improve degradation of specific organics, start-up of new digesters, odor reduction, and recovery of organically over-loaded systems at laboratory scale. When we perturbed two different anaerobic digester sets (MS and WW) by exposing them to air, bioaugmentation with a methanogenic, H₂-utilizing culture resulted in higher methane production rates. In addition, SCOD concentrations decreased more rapidly in bioaugmented versus non-bioaugmented digesters. O'Flaherty et al. (1999) and O'Flaherty and Colleran (1999) also found that bioaugmentation increased the COD removal rate in anaerobic systems when toxicity was exerted; adding sulfate-acclimated biomass resulted in a 58% increase in steady-state COD removal for a laboratory anaerobic hybrid reactor treating high-sulfate wastewater (concentration of 4 g/L; COD/sulfate ratio of 3:1).

When effluent propionate concentrations and recovery periods were compared, bioaugmentation outcomes varied. Bioaugmentation resulted in significantly shorter recovery periods for MS systems, for which non-bioaugmented controls continued to produce chronically high, lingering propionate concentrations after the toxic event. In contrast, non-bioaugmented WW systems did not accumulate lingering high propionate concentrations, and bioaugmentation did not result in significantly shorter recovery. The addition of H₂ utilizers ostensibly reduced digester H₂ concentration, resulting in more complete propionate degradation in MS systems. The contradiction in MS and

WW results may be due to differences in microbial communities for the two digester inocula (MS and WW) employed. Others reported that recovery periods of anaerobic digesters subjected to organic overload differed based upon the microbial communities initially present (Hashsham et al., 2000; Fernandez et al., 2000). Microbial communities in which the number of individual members is approximately the same (i.e., high evenness) have been shown to accomplish higher substrate conversion when exposed to high-salts stress as compared to communities in which a few organisms dominate the population (i.e., low evenness) (Wittebolle et al., 2009). It is possible that the MS system microbial community could rapidly adapt after the toxic event to degrade propionate, whereas the WW system could not.

Therefore, the microbial community structure (i.e., richness, evenness, etc.) within an existing biological system is just as important as the community structure of any culture added when bioaugmentation is practiced. Digester microbial communities before bioaugmentation were not analyzed herein. In one review of laboratory and full-scale aerobic literature, 30% of the reports showed no benefit when bioaugmentation was practiced (Stephenson and Stephenson, 1992). One reason for the unsuccessful applications may be because biological systems with rapidly adaptable microbial communities will not benefit from bioaugmentation, whereas systems with low microbial diversity or evenness may benefit greatly.

5. Conclusions

Bioaugmentation with an H₂-utilizing culture is a potential tool to decrease the recovery period and increase biogas production of some anaerobic digesters after a toxic event. Bioaugmentation resulted in decreased recovery times for digesters that tended to produce chronically high, lingering propionate concentrations after air exposure. In contrast, recovery time was not significantly shortened for digesters that did not produce lingering propionate. This contradiction may be due to differences between microbial communities of the two digester inocula employed. Digesters already containing rapidly adaptable microbial communities may not benefit from bioaugmentation, whereas other digesters with poorly adaptable

microbial communities may benefit greatly. Analysis of microbial communities in both bioaugmentation cultures and digester biomass is suggested to develop bioaugmentation applications. In the future, the community structures of various digesters as well as bioaugmentation cultures should be determined and their response to bioaugmentation during toxicity events or periods of elevated propionate should be compared.

Notes

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Acknowledgements

- This research was supported by funding from the Naulin Foundation and Marquette University.

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Appendix

Figure 1 Rarefaction curve of archaeal clone library of the bioaugmentation culture.

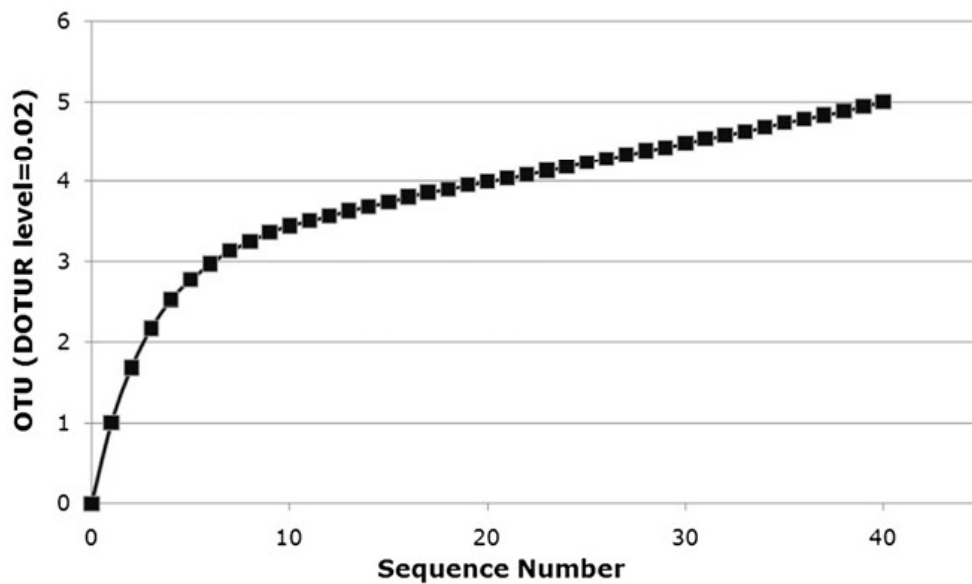
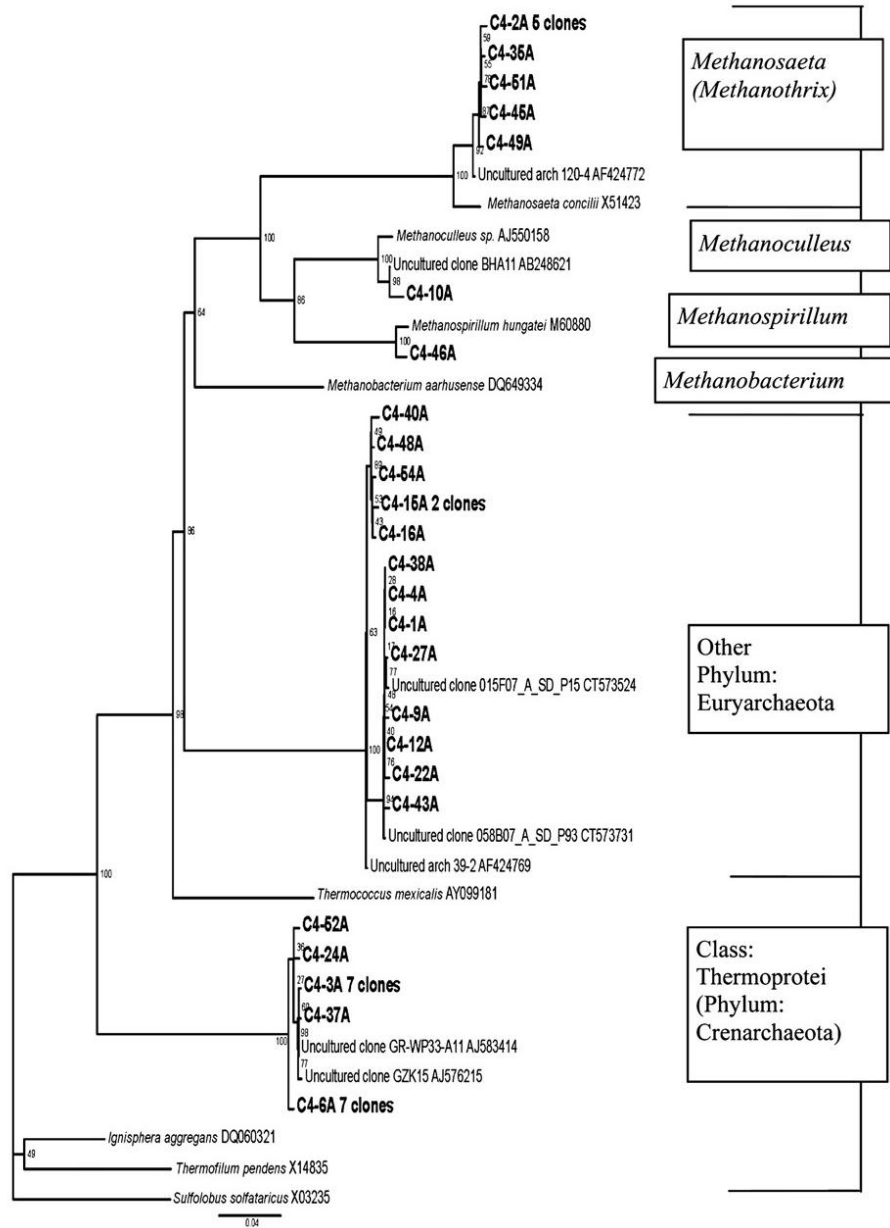
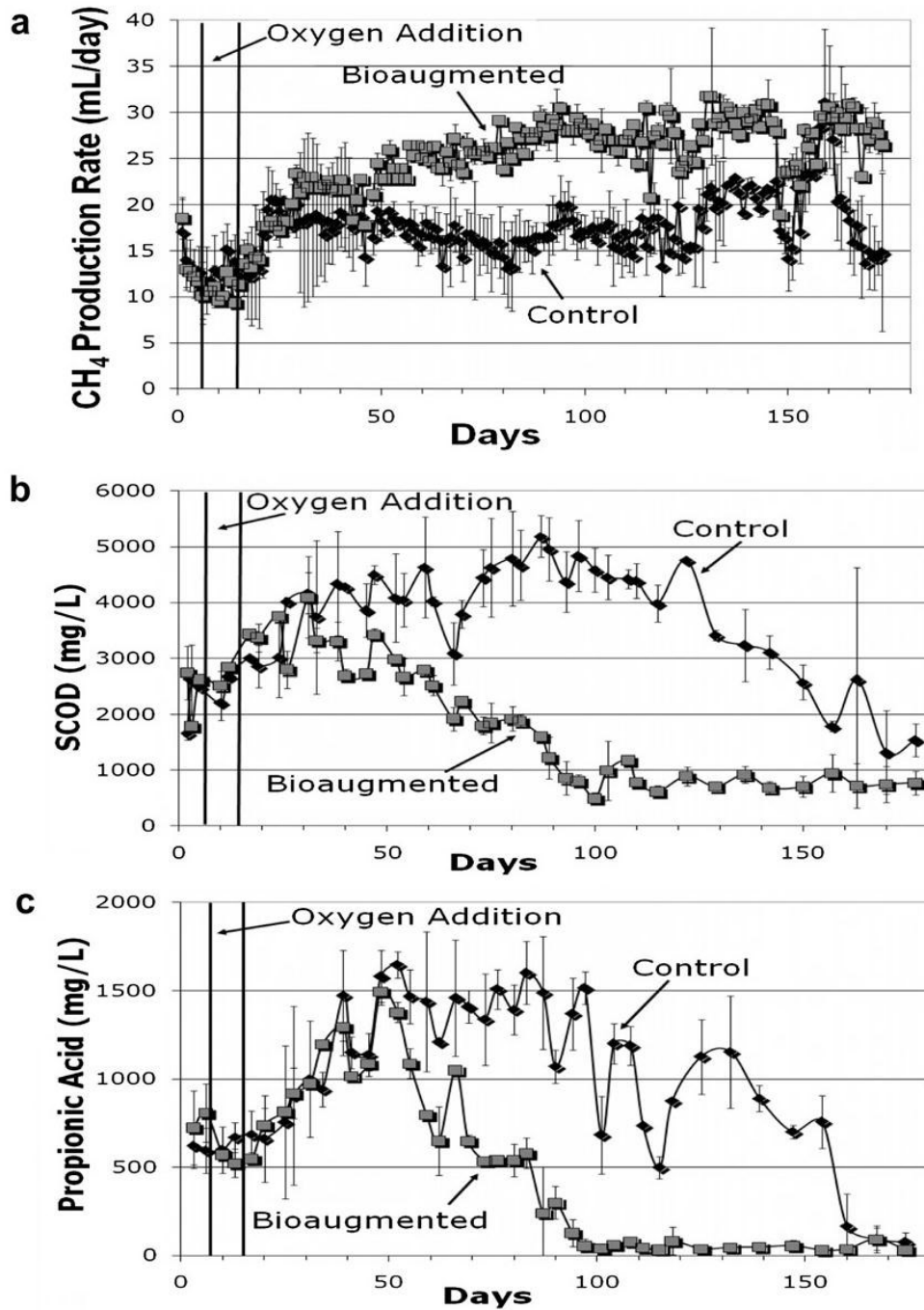


Figure 2 Phylogenetic analysis of the bioaugmentation culture archaeal clone library.



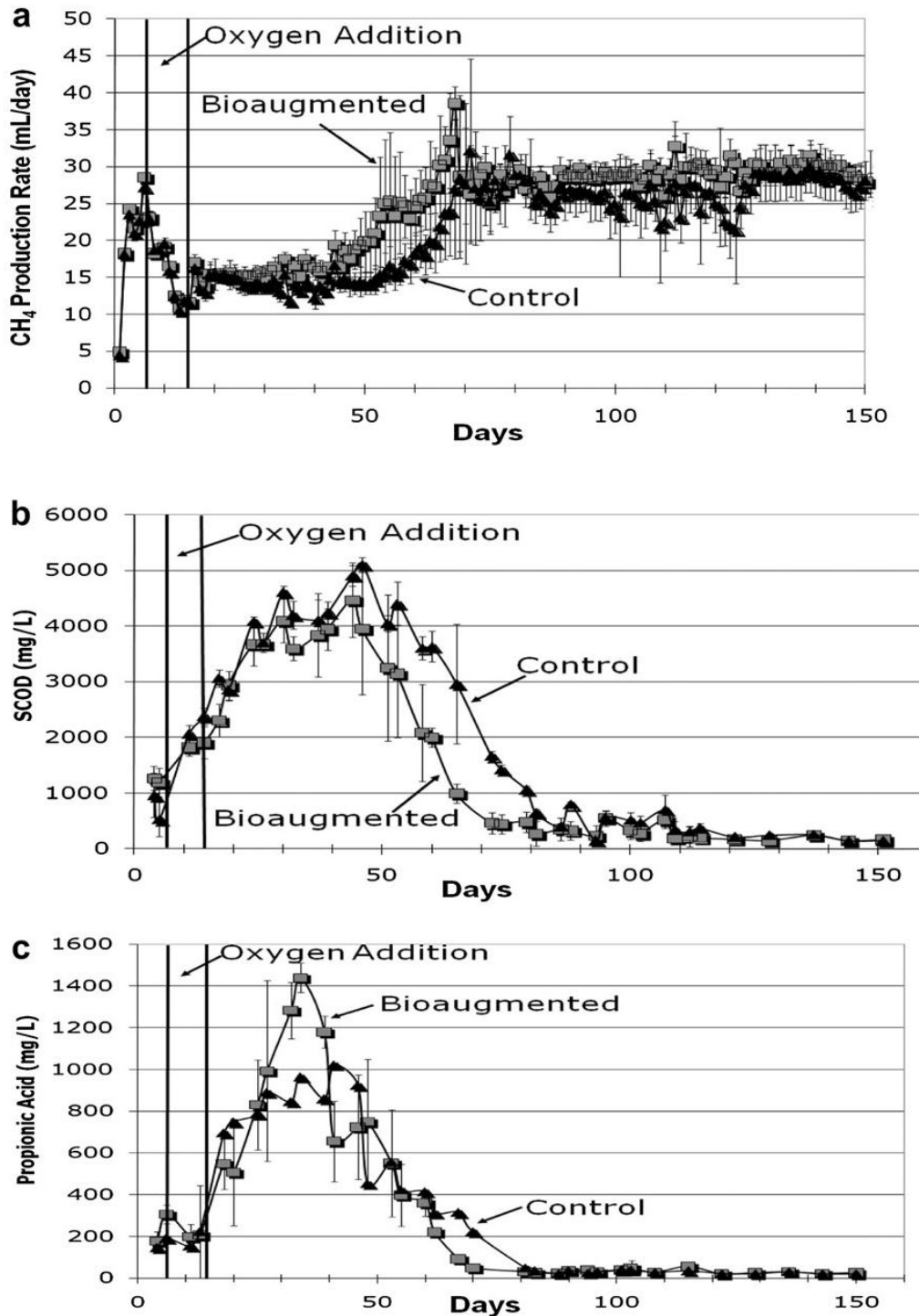
Calculations were based on the neighbor-joining algorithm (bootstrap number = 100). Numbers at node represent bootstrap values. The scale bar represents the number of nucleotide changes per sequence position. The tree was rooted to the organism *Sulfolobus solfataricus* (X03235).

Figure 3 Results of bioaugmented and control MS digesters.



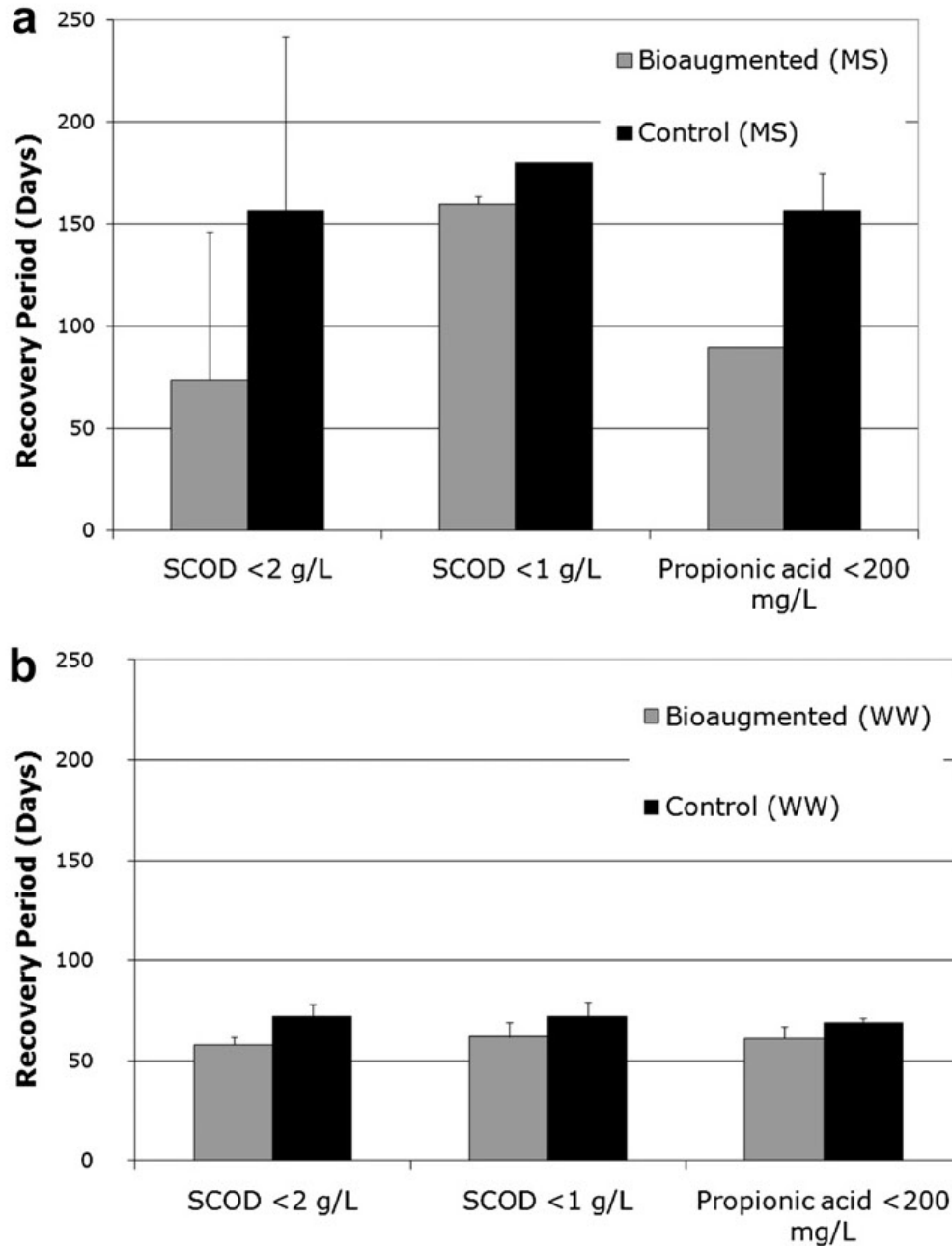
(a) Methane production; (b) SCOD; (c) propionic acid. Error bars represent standard deviation of duplicate digesters.

Figure 4 Results of bioaugmented and control WW digesters.



(a) Methane production; (b) SCOD; (c) propionic acid. Error bars represent standard deviation of four replicate digesters.

Figure 5 Comparison of bioaugmented and control system recovery periods.



Average bioaugmented and control digester recovery periods for MS systems (a); and WW systems (b). Recovery periods for MS control and bioaugmented systems were different, whereas that for WW systems were not ($p < 0.05$). Error bars represent standard deviation. Some error bars are small and not visible.