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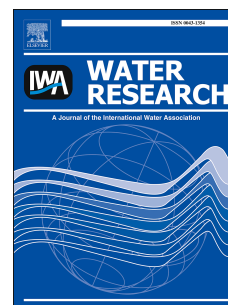
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# 1 Anaerobic digester bioaugmentation influences quasi steady state 2 performance and microbial community

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## 9 **Abstract**

10 Nine anaerobic digesters, each seeded with biomass from a different source, were  
11 operated identically and their quasi steady state function was compared. Subsequently,  
12 digesters were bioaugmented with a methanogenic culture previously shown to increase  
13 specific methanogenic activity. Before bioaugmentation, different seed biomass resulted  
14 in different quasi steady state function, with digesters clustering into three groups  
15 distinguished by methane (CH<sub>4</sub>) production. Digesters with similar functional  
16 performance contained similar archaeal communities based on clustering of Illumina  
17 sequence data of the V4-V5 region of the 16S rRNA gene. High CH<sub>4</sub> production  
18 correlated with neutral pH and high *Methanosarcina* abundance, whereas low CH<sub>4</sub>  
19 production correlated to low pH as well as high *Methanobacterium* and *DHVEG 6* family  
20 abundance. After bioaugmentation, CH<sub>4</sub> production from the high CH<sub>4</sub>-producing  
21 digesters transiently increased by 11±3% relative to non-bioaugmented controls (p <0.05,  
22 n=3), whereas no functional changes were observed for medium and low CH<sub>4</sub> producing  
23 digesters that all had pH higher than 6.7. The CH<sub>4</sub> production increase after  
24 bioaugmentation was correlated to increased relative abundance of *Methanosaeta* and

*Methaospirillum* originating from the bioaugment culture. In conclusion, different anaerobic digester seed biomass can result in different quasi steady state CH<sub>4</sub> production, SCOD removal, pH and effluent VFA concentration in the timeframe studied. The bioaugmentation employed can result in a period of increased methane production. Future research should address extending the period of increased CH<sub>4</sub> production by employing pH and VFA control concomitant with bioaugmentation, developing improved bioaugments, or employing a membrane bioreactor to retain the bioaugment.

### **Keywords**

Digester efficiency; *Methanobacterium*, *Methanosaeta*, *Methanosarcina*, *Methanospirillum*; Next generation sequencing

## 1. Introduction

Adding beneficial microorganisms to anaerobic digesters (i.e. bioaugmentation) has been shown to increase degradation rates of specific organics and reduce upset digester recovery time (Guiot et al. 2000; Hajji et al. 2000; Guiot et al. 2002; Cirne et al. 2006; Schauer-Gimenez et al. 2010; Tale et al. 2011). Anaerobic digester bioaugmentation may be more widely applicable if a culture was enriched to target a key, ubiquitous intermediate in existing anaerobic processes. The existing anaerobic processes typically treat readily degradable substrates, such as food production and dairy wastewater. When treating readily degradable substrates, one ubiquitous and potentially problematic intermediate is propionate (Schauer-Gimenez et al. 2010; Tale et al. 2015). Propionate accumulation is often an indicator of process imbalance in anaerobic digesters which can be caused by organic overload, nutrient deficiency, toxicant exposure or other factors (McCarty & Smith 1986; Speece et al. 2006; Ma et al. 2009). The subsequent recovery time of upset digesters depends on the abundance of microorganisms that can biotransform an intermediate (i.e. propionate) or inhibitory compound into less harmful products (Herrero & Stuckey 2014).

Intermediates such as propionate can be biotransformed by a specific consortium of synergistic microorganisms (McCarty & Smith 1986; Speece et al. 2006). Bioconversion of propionate to acetate and hydrogen ( $H_2$ ) is thermodynamically favorable only when the partial pressure of the generated  $H_2$  remains below  $10^{-4}$  atm. Thus, degradation of propionate requires a synergistic relationship between  $H_2$  producing and  $H_2$  consuming microorganisms to maintain low  $H_2$  concentrations (McCarty & Smith 1986). It was shown previous that adding cultures enriched to consume  $H_2$  or propionate to anaerobic

58 digesters can reduce recovery time after organic overload or toxicant exposure (Schauer-  
59 Gimenez et al. 2010; Tale et al. 2011). Tale et al., (2015) employed aerotolerant  
60 propionate consuming, methanogenic cultures for bioaugmentation. The aerotolerant  
61 culture may be commercially beneficial since it can be easily handled and dried in  
62 ambient air (Zitomer 2013). In addition, micro-aerated cultures outperformed a strictly  
63 anaerobic culture when used for bioaugmentation, resulting in higher specific  
64 methanogenic activity (SMA) against propionate and shorter recovery time after organic  
65 overload (Tale et al. 2015).

66 Despite some success, anaerobic digester bioaugmentation is still at a nascent stage. A  
67 comprehensive review published by Herrero & Stuckey (2014) reported either transient  
68 improvement in performance or a complete failure of bioaugmentation to improve  
69 anaerobic digestion, but no instances of long-term improvement. Therefore, it is still  
70 questionable whether or not adding a limited quantity of externally cultured  
71 microorganisms can increase long-term methane production (Herrero & Stuckey 2014).  
72 Microbial community analysis has often been employed to understand the relationship  
73 between microorganisms and digester function (Venkiteshwaran et al. 2016). However  
74 changes in digester microbial communities after bioaugmentation have not been  
75 extensively studied.

76 In this work, bioaugmentation using a methanogenic, aerotolerant propionate enrichment  
77 culture was investigated as a possible method to improve methane production after quasi-  
78 steady operation for anaerobic digesters fed a readily degradable waste. Nine groups of  
79 anaerobic digesters were seeded with different starting biomass to obtain different

microbial communities and digesters were then bioaugmented and monitored for changes in function and microbial community using high throughput Illumina sequencing.

## **2. Material and Methods**

### **2.1 Anaerobic digesters**

Biomass samples were obtained from nine full-scale municipal anaerobic digesters in different US states; Delaware (Set-D), Florida (Set-B), Michigan (Set-I), Mississippi (Set-F), New Jersey (Set-H), Ohio (Set-E), South Dakota (Set-G), West Virginia (Set-C) and Wyoming (Set-A) to obtain a variety of microbial communities. With the exception of a thermophilic digester in Michigan, all other biomass samples were from mesophilic digesters. All digesters were continuous stirred-tank reactors stabilizing municipal wastewater sludge with solids retention times between 15 and 30 days. The Florida digester was also fed food waste as a co-digestate.

Each biomass sample was used to seed two sub-sets (bioaugmented and non-bioaugmented) of triplicate, 160-mL lab-scale digesters with 50-mL working volume and biomass concentration of 8g volatile solids (VS)/L. Digesters were operated at a 10-day HRT and fed synthetic wastewater (non fat-dry milk) and basal nutrient media at an organic loading rate (OLR) of 3 g COD/L-day. The digesters were operated for 60 days until they attained quasi-steady state operation during which the digester daily biogas production coefficient of variation was less than 20%. The bioaugmented digesters received a daily dose of the enrichment culture from day 60 to 70. The daily dose was equivalent to 1 % of the digester biomass total adenosine triphosphate (tATP) mass (this was equivalent to 1.5-2 % of the digester VS mass). Simultaneously, the non-bioaugmented digesters received a COD equivalent dose of inactivated (autoclaved)

enrichment culture. Functional parameters including effluent soluble COD (SCOD) and volatile fatty acids (VFA) concentrations as well as biogas  $\text{CH}_4$  concentration were monitored between days 60 and 80. Digester biomass samples were collected on day 71 for amplicon sequencing.

Cumulative methane volume produced was calculated by summing the daily methane production volumes ( $\text{ml CH}_4/\text{day}$ ) from days 60 to 80. Biomass production rate was calculated as the product of VSS concentration ( $\text{mg VSS/L}$ ) and effluent flow ( $\text{L/day}$ ). Observed biomass yield was calculated as the quotient of biomass production rate and COD added to the digester per day ( $\text{mg COD/day}$ ).

## 2.2 Enrichment culture for bioaugmentation

A moderately aerated, propionate-utilizing, mixed methanogenic enrichment culture developed by Tale et al. (2011) was employed for bioaugmentation. The original seed biomass for the enrichment culture was from an upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater that exhibited a high methanogenic activity (Tale et al. 2011). When previously used for bioaugmentation, this aerotolerant mixed microbial culture reduced the recovery time of transiently organically overloaded digesters (Tale et al. 2015). The enrichment was maintained in two completely mixed vessels with a volume of four liters at  $35^\circ\text{C}$  at a 15 day HRT and fed  $0.17\text{ g propionate/L-day}$  as calcium propionate with basal nutrient media. Immediately after feeding, ambient air was added directly into the headspace of the vessel at a volume equivalent to  $25\text{ mg O}_2/\text{L-day}$  or 10% of the OLR to provide a micro-aerated environment.

## 2.3 Basal nutrient media



Basal nutrient media, as described by Speece (2008), contained the following [mg/L]:  
 $\text{NH}_4\text{Cl}$  [400];  $\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$  [250];  $\text{KCl}$  [400];  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  [120];  $(\text{NH}_4)_2\text{HPO}_4$  [80];  
 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  [55];  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  [10];  $\text{KI}$  [10]; the trace metal salts  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{NH}_4\text{VO}_3$ ,  
 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ,  $\text{H}_3\text{BO}_3$ ,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ,  
 $\text{NaWO}_4 \cdot 2\text{H}_2\text{O}$ , and  $\text{Na}_2\text{SeO}_3$  [each at 0.5]; yeast extract [100];  $\text{NaHCO}_3$  [6000]; and  
 resazurin [1].

#### 2.4 Analytical methods

tATP concentration was analyzed using a commercial kit following the manufacturer  
 instructions (BacTiter-Glo, Promega, Madison WI, USA). The inactive culture added to  
 non-bioaugmented digesters was placed in an autoclave (Model 3870E, Tuttnauer Co.,  
 Hauppauge, NY, USA) at 15 Psi and 121°C for 30 minutes prior to augmentation  
 experiments. Daily biogas volume produced was measured by inserting a needle with a  
 wetted glass barrel syringe through serum bottle septa. SCOD was measured by filtering  
 the sample through a 0.45  $\mu\text{m}$  pore size membrane syringe filter and determining the  
 filtrate COD by standard methods (APHA et al., 1998). Biogas methane concentration  
 was measured by gas chromatography (GC System 7890A, Agilent Technologies, Irving,  
 TX, USA) using a thermal conductivity detector. VFA concentrations were measured by  
 gas chromatography (GC System 7890A, Agilent Technologies, Irving, TX, USA) using  
 a flame ionization detector. The VS, TSS and VSS analyses were performed by standard  
 methods (APHA et al., 1998). Statistical analysis such as two-sample Student's t-test  
 with unequal variance and Pearson's coefficient were calculated on Microsoft Excel 2010  
 (Version 14.3.2) using built in functions.

#### 2.5 Microbial community analysis

148 DNA was extracted from all bioaugmented and non-bioaugmented digesters (including  
149 all replicates, n=54) on Day 71 using the PowerSoil™ DNA Isolation Sample Kit  
150 (MoBio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's  
151 instructions. The biomass samples were subjected to bead beating on a vortex (Model  
152 58816-121, VWR International, Radnor, PA, USA) for 10 minutes. Primer pair 515-532U  
153 and 909-928U was used (Wang & Qian 2009) including their respective linkers, to  
154 amplify the V4-V5 region of the 16S rRNA gene over 30 amplification cycles at an  
155 annealing temperature of 65 °C. The primer pairs target both archaeal and bacterial 16S  
156 rRNA genes. An index sequence was added in the second PCR of 12 cycles, and the  
157 resulting products were purified and loaded onto the Illumina MiSeq cartridge for  
158 sequencing of paired 300 bp reads following manufacturer's instructions (v3 chemistry).  
159 Sequencing and library preparation were performed at the Genotoul Lifescience Network  
160 Genome and Transcriptome Core Facility in Toulouse, France (get.genotoul.fr). A  
161 modified version of the standard operation procedure for MiSeq data (Kozich et al. 2013)  
162 in Mothur version 1.35.0 (Schloss et al. 2009) was used to assemble forward and reverse  
163 sequences and preclustering at 4 differences in nucleotides over the length of the  
164 amplicon. Uchime was used for chimera checking (Edgar et al. 2011). Sequences that  
165 appear less than three times in the entire data set were removed. Alignment of the 16S  
166 rRNA sequences was done using SILVA SSURef NR99, release 119, as provided by  
167 Mothur (Schloss et al. 2009). The same database was used in Mothur's classify.seqs()  
168 command to assign taxonomic affiliation using a cutoff value of 80%. Custom R scripts  
169 were used to perform dual hierarchical clustering (using R command hclust and heatmap)  
170 and nonmetric multidimensional scaling (nMDS) (using the default Bray-Curtis index),

of anaerobic community sequence data gathered from Illumina sequencing (Carey et al. 2016; McNamara & Krzmarzick 2013).

### **3. Results and Discussion**

All nine digester sets reach quasi-steady state based on less than 20% coefficient of variation in daily biogas production by day 60 when bioaugmentation was initiated (Figure 1 and S1). Effluent VFA concentrations for all digesters were higher than 2 g/L and methane production was below 70% of the theoretical value assuming all COD was converted to methane. Therefore, residual COD was available and could possibly be removed if system changes occurred. This challenged condition was desired so that bioaugmentation effects could be observed. During the dosage period, autoclaved propionate enrichment culture was added to the non-bioaugmented digesters, whereas live propionate enrichment culture was added to the bioaugmented digesters. Adding inactivated enrichment culture did not result in a statistical change in biogas production rate (Figure 1 and S1). This was expected since the daily COD fed to the digesters from the augments was low and was less than 8% of the total synthetic wastewater COD fed.

#### **3.1 Non-bioaugmented digester function**

Although operated identically, the digester sets did not achieve identical operational values. For example, the quasi steady state methane production rates ranged from 0.3 to 0.8 L CH<sub>4</sub> per L of digester per day (L CH<sub>4</sub>/L<sub>R</sub>-day) (Table 1). Each non-bioaugmented digester set was classified into one of three distinct groups based on statistically similar methane production rate, SCOD removal, pH and effluent acetate concentration (p value <0.05, n=3) (Table 1). Group 1 (G1) (sets A, B and C) contained the best performing digesters with the highest methane production rate, highest SCOD removal rate, highest

pH and lowest effluent acetate concentration; Group 2 (G2) (sets D, E, F and G) showed intermediary performance and Group 3 (G3) (sets H and I) contained the poorest performing digesters with the lowest methane production rate, lowest SCOD removal rate, lowest pH and highest effluent acetate concentration (Figure 2). The functional variation among digester sets can be attributed to the differences in the microbial communities the seed biomass used for each set. Future research is warranted to elucidate quantitative relationships between microbial community descriptors and digester function so that the suitability of various seed biomass samples can be estimated. This would be helpful to identify the most suitable biomass for a given process startup or re-seeding application.

### 3.2 Non-bioaugmented digester archaeal community

A total of 32 archaeal OTUs, based on 97% similarity, were identified among all the digester samples analyzed. The relative abundance of archaeal sequences varied from 1 to 4% for G1, G2 and 0.1 to 1% for G3 digesters, respectively. Eight archaeal OTUs represented more than 99% of the archaeal abundance in all non-bioaugmented digesters (Figure S2). These eight OTUs were most similar to the genera *Methanofollis*, *Methanosarcina*, *Methanospirillum*, *Methanosaeta*, *Methanobacterium*, *Candidatus Methanomethylophilus* and two unclassified genera in the order *WCHA1-57* and the family *Deep Sea Hydrothermal Vent Grp 6 (DHVEG 6)*, based on the SILVA SSURef NR99 v119 reference database from Mothur (Schloss et al. 2009) (Figure S2).

The nine non-bioaugmented digester sets clustered in the same three groups that were identified by functional data (95% confidence interval) (Figure 3). Digesters with similar functional performance contained similar archaeal communities. Non-bioaugmented G1

digester communities were distinguished from G2 and G3 communities by high relative abundance of *Methanosarcina*, which ranged from 60 to 95% of archaea in G1 digesters (Figure S2). *Methanobacterium* dominated non-bioaugmented G2 digesters, with a relative abundance that ranged from 80 to 99% of the archaeal diversity. The G3 digesters were distinguished by high relative abundance of the *DHVEG 6* family, with *Methanobacterium* also observed in high relative abundance (Figure S2). *Methanosarcina* and *Methanosaeta* are the only two methanogenic genera known to consume acetate (Liu & Whitman 2008). High relative abundance of *Methanosarcina* in the high-performing G1 digesters correlated to higher methane production and lower effluent acetate concentration. This is ostensibly because the *Methanosarcina* maximum specific substrate utilization rate is higher than that of *Methanosaeta* (Liu & Whitman 2008). Therefore, the presence of *Methanosarcina* in digesters with moderate to high VFA concentrations, such as the ones of this study, may be beneficial to maintain more rapid bioconversion of acetate to methane. Compared to G1 digesters, *Methanosarcina* relative abundance in the lesser performing, non-bioaugmented G2 digesters was lower, ranging from 0.5 to 18%. In addition, *Methanosarcina* was undetectable in the poorest performing, non-bioaugmented G3 digesters which had the highest acid concentrations and lowest pH. The poor performing G3 digesters were distinguished from the better performing G1 and G2 digesters by the low archaeal sequence abundance ( $\leq 1\%$  of the total sequence) and further by high relative abundance of *DHVEG 6*, which ranged from 60-90% in G3 digesters (Figure S2). *DHVEG 6* have been observed in acidic environments, marine environments, terrestrial soils, hydrothermal sediments, deep sea methane seep

sediments, rice paddy soil and saline lakes (Casamayor et al. 2013; Nunoura et al. 2010; Nunoura et al. 2011; Hugoni et al. 2013; Grosskopf et al. 1998). Given that *DHVEG6* microorganisms have been observed in extreme environmental conditions that typically are not present in a healthy functioning digester, high abundance of *DHVEG 6* in anaerobic digesters ostensibly indicates an upset digester with low pH and low biogas production such as the G3 digesters.

### 3.3 Non-bioaugmented digester bacterial community

Approximately 1300 bacterial OTUs were identified based on 97% similarity among all the biomass samples analyzed in this study. The 29 OTUs having the highest relative abundance among all the digesters and the enrichment culture were considered for bacterial community analysis. These 29 OTUs contributed 70-85% of the total bacterial sequences in the non-bioaugmented digesters.

The nine digesters bacterial communities formed two clusters, with G1 and G2 non-bioaugmented digesters forming one bacterial cluster and G3 non-bioaugmented digesters in the second bacterial cluster (Figure 4). Bacterial communities in all nine digester sets were dominated by OTUs most similar to fermenters belonging to the phyla *Firmicutes*, *Bacteroidetes* and *Synergistetes* (Figure S3). The most common bacterial OTUs that were observed in the digesters were the genera *Bacteroides*, *Peptostreptococcus*, *Pyramidobacter*, *Aminobacterium*, *Atopobium* and *RC9 Gutgroup*. Non-bioaugmented G1 and G2 digesters were distinguished from G3 digesters by the higher abundance of the genera *Porphyromonas*, *Petrimonas* and unclassified *FamilyXI*, whereas non-bioaugmented G3 digesters were dominated by OTUs most similar to RC9 Gut Group

microorganisms which contributed more than 60% of the total bacterial relative abundance (Figure S3).

### 3.4 Enrichment culture

The enrichment culture functional parameters were stable between days 60 to 80, with 99% SCOD removal, no detectable VFAs, pH of  $7.5 \pm 0.2$ ,  $60 \pm 3\%$  biogas methane content, methane yield of  $330 \pm 16$  mLCH<sub>4</sub>/g-COD removed and observed biomass yield of  $0.08 \pm 0.01$  gVSS/gCOD.

More than 700 bacterial OTUs were identified in the enrichment culture based on 97% similarity. The 25 bacterial OTUs with the highest relative abundance represented approximately 80% of total bacterial sequences and are shown in Figure S4. The two most abundant bacterial taxa were most similar to an unclassified genus within *Spirochaetaceae* (30% of the total bacterial relative abundance) and *Thermovirga* within *Synergistaceae* (12% of the total bacterial relative abundance) (Figure S4). *Thermovirga* is currently represented by a single member species *Thermovirgalienii*, which is a moderately thermophilic, amino acid degrading fermentative bacterium (Dahle 2006). Some members of the *Spirochaetaceae* family such as *Treponema* species, are reported to be abundant in iron-reducing consortia that were used by othersto bioaugmentanaerobic digesters (Baek et al. 2015). Bacteria related to *Spirochaetes* may be beneficial for the overall process since some of them have been suggested as syntrophic acetate-oxidizing bacteria (Hattori 2008; Lee et al. 2013; Lee et al. 2015). Iron-reducing bacteria (IRB) are commonly observed in anaerobic systems and can utilize acetate, H<sub>2</sub>, ethanol and other complex substrates and ferric iron as an electron acceptor (Kim et al. 2014). They are also known to form syntrophic associations and, via interspecies electron transfer,



285 transfer electron directly to their methanogenic partner, which can facilitate CO<sub>2</sub> reduction  
 286 to CH<sub>4</sub> (Stams & Plugge 2009; Rotaru et al. 2014). Addition of an IRB consortium has  
 287 been shown to increase the methane production rate in anaerobic digesters (Baek et al.  
 288 2015).

289 Given that the enrichment culture was fed calcium propionate, it was expected that  
 290 bacteria associated with syntrophic propionate degradation would be abundant. Of the  
 291 known bacterial genera with members capable of degrading propionate, *Desulfobulbus*,  
 292 *Smithella*, and *Syntrophobacter* were observed with a combined relative abundance of  
 293 9% (De Bok et al. 2001; De Bok et al. 2004; Stams & Plugge 2009), with *Desulfobulbus*  
 294 contributing 7% (Figure S4).

295 15 archaeal OTUs were detected in the enrichment culture based on 97% similarity, of  
 296 which 6 OTUs contributed more than 99% of the total archaeal sequences (Figure S5).  
 297 Archaeal sequences constituted approximately 5-6% of the total sequences detected in the  
 298 enrichment culture. The archaeal community was dominated by sequences most similar  
 299 to *Methanosaeta*, constituting 65% of the total archaeal sequences (Figure S5).  
 300 *Methanosarcina* constituted only 1.2% of the total archaeal sequences in the enrichment  
 301 culture (Figure S5). Unlike *Methanosarcina*, *Methanosaeta* have a high substrate affinity  
 302 and a lower maximum specific substrate utilization rate. Hence, *Methanosaeta* usually  
 303 dominate over *Methanosarcina* in cultures such as the enrichment culture in this study  
 304 having acetate concentrations lower than 500 mg/L (Liu & Whitman 2008).

305 Apart from acetoclastic methanogens, the enrichment culture archaeal composition  
 306 consisted of OTUs most similar to known hydrogenotrophic methanogens including  
 307 *Methanospirillum*, *Methanobacterium*, *Methanolinea* and an unclassified genus in the



order WCHA1-57 (Figure S5) (Liu & Whitman 2008). Conversion of propionate to methane only becomes thermodynamically favorable through H<sub>2</sub> utilization. Therefore, the significant presence of hydrogenotrophic methanogens contributing 30-35% of the total archaeal sequences could have positive functional results. The presence of microorganisms classified in the genera *Methanospirillum*, *Methanobacterium*, *Methanolinea* has previously been reported to play an important role in propionate utilization during digester recovery after organic overload (Tale et al. 2011; Tale et al. 2015; Schauer-Gimenez et al. 2010).

The archaeal order WCHA1-57 was observed at a significant relative abundance (12%) in the enrichment culture. Although many WCHA1-57-related 16S rRNA gene sequences have been identified in anaerobic digesters (Chouari et al. 2005; Rivière et al. 2009; Schauer-Gimenez et al. 2010), no reports were found regarding their role in propionate oxidation or methane production. In some anaerobic digesters treating municipal sewage sludge, the WCHA1-57 phylotype population represented one of the predominant archaeal components, with relative abundance >70% in archaeal clone libraries (Chouari et al. 2005; Rivière et al. 2009). These observations indicate that WCHA1-57 archaea represent a potentially important group in anaerobic digesters. Chouari et al. (2005) reported the enrichment of WCHA1-57 phylotypes in cultures fed formate or H<sub>2</sub>/CO<sub>2</sub>. This indicates that WCHA1-57 plays a role in reducing hydrogen concentration and, therefore, aiding in conversion of propionate to methane.

Both bacterial and archaeal enrichment culture communities were distinct from those of the nine digester sets. The nMDS scaling plots based on the top eight archaeal (Figure 3) and 29 bacterial (Figure 4) OTUs, selected based on their relative abundance and

prevalence among all the biomass samples, shows distinct clustering of the enrichment culture separate from the G1, G2 and G3 non-bioaugmented and bioaugmented digesters.

### 3.5 Bioaugmentation, digester function and microbial community changes

Cumulative methane produced by both non-bioaugmented and bioaugmented digesters between days 60 and 80 were calculated and compared to observe any difference in performance (Figure 5). Only the three G1 digester sets A, B and C showed a statistically significant increase ( $P < 0.05$ ,  $n = 3$ ) in average methane production of  $11 \pm 3\%$  after bioaugmentation, with increases of  $9 \pm 1\%$ ,  $12 \pm 2\%$  and  $13 \pm 2\%$ , respectively, compared to non-bioaugmented controls (Figure 5).

The increased methane production in G1 digesters after bioaugmentation was not sustained. The period of increased methane production averaged 9 days, and was 7, 11 and 9 days for sets A, B and C, respectively (Figure 1, S1A and S1B). Also, the increased methane production did not occur immediately after bioaugmentation was initiated. The lag between the dosage period start (Day 60) and the first day of increased methane production for set A, B and C bioaugmented digesters averaged 10 days, and was 12, 8 and 9 days, respectively (Figure 1, S1A and S1B).

The archaeal communities in the bioaugmented digesters were grouped into three distinct clusters based on archaeal sequences (Figure 3). The archaeal community of the bioaugmented digesters belonging to functional groups G2a and G3a, which did not improve after bioaugmentation, did not significantly change after bioaugmentation (Figure 3). In contrast, however, the G1 bioaugmented digesters showed a statistical improvement in methane production and the archaeal community changed significantly

after bioaugmentation (Figure 3). After bioaugmentation, the archaeal community of G1 digesters became more similar to that of the enrichment culture (G4). The community structure shift in G1 digesters after bioaugmentation was primarily caused by the increased abundance of two archaeal genera: *Methanosaeta* and *Methanospirillum* (Figure 6), which are in high abundance in the enrichment culture (Figure S5). In contrast, the relative abundance values of *Methanosaeta* and *Methanospirillum* were very low (<1%) in G2 and below detection in the G3 bioaugmented digesters, respectively. The bacterial community compositions of the bioaugmented digesters did not show any significant shift after bioaugmentation (Figure 4). Sequences related to the two most abundant bacterial genera observed in the enrichment culture, unclassified *Spirochaeta* and *Thermovirga*, which were not detected in the non-bioaugmented digesters, were detected in all the bioaugmented digesters, but their relative abundance remained below 1% after bioaugmentation. The resulting increase in methane production observed in G1 digesters from bioaugmentation was associated with a shift in the archaeal community structure. Increased relative abundance of the genera *Methanosaeta* and *Methanospirillum* was observed in digesters with improvement in the methane production rate. The relative abundance of *Methanosaeta* and *Methanospirillum* increased from below detection in the non-bioaugmented digesters to 10-40% and 10-30%, respectively, in the bioaugmented G1 digesters (Figure 6). However, it is important to note that the methane production rate increase lasted only 7 to 11 days in the bioaugmented G1 digesters. This could be due to washout of *Methanosaeta* and *Methanospirillum* once bioaugmentation ceased. It may be possible to improve the methane production further by increasing the dose of the

enrichment biomass or extending the duration of the dosage period. In addition microbial carriers, such as alginate beads or granular activated carbon, or membrane bioreactors could be used to retain the bioaugment in the bioreactor.

The enrichment culture used in this study was produced at a pH of 7.5 with no detectable VFAs present. The most abundant methanogens in the enrichment culture, *Methanosaeta* and *Methanospirillum* (i.e., *M. hungatii*), are sensitive to low pH and high acid or propionate concentrations (Liu & Whitman 2008; Barredo & Evison 1991). It is likely that the methane production increase in G1 digesters after bioaugmentation was due to the relatively low VFA concentration and neutral pH, which was conducive for the activity of *Methanosaeta* and *Methanospirillum* added via the bioaugment. In contrast, the low pH, high VFA concentration environment in G2 and G3 digesters may have inhibited the enrichment culture microorganisms. Therefore, the environment the enrichment culture is being added into must be carefully considered and additional steps such as acclimating the augment culture or increasing the digester pH before bioaugmentation may be required to increase methane production and COD removal by bioaugmentation.

#### **4. Conclusion**

Different anaerobic digester seed biomass can result in significantly different quasi steady state functional parameters, including methane production rate, SCOD removal, pH and effluent VFA concentration. Therefore, care should be taken to select seed biomass with high activity for digester startup or re-seeding.

Identically operated digesters that contain different archaeal communities can exhibit different functional characteristics during quasi steady state operation. When operating

under challenging conditions, digesters with high methane production rates can be distinguished by high *Methanosarcina* relative abundance. The presence of *Methanosarcina* in digesters with moderate to high VFA concentrations is beneficial to maintain more rapid bioconversion of acetate to methane. In contrast, digesters with low methane production can be distinguished by high abundance of *Methanobacterium* and *DHVEG 6* family organisms. Since *DHVEG6* microorganisms have been found in extreme environments, including deep-sea hydrothermal vents, their high abundance in anaerobic digesters may indicate past or current digester upset (i.e., high VFA concentration and low methane production).

Bioaugmentation with a methanogenic, propionate degrading enrichment culture resulted in a significant increase in methane production when digester pH was neutral or greater. However, methane production did not change after bioaugmenting digesters that had pH values less than neutral. Therefore, when predicting bioaugmentation outcomes, the environment into which an augment culture is added must be carefully considered as well as the composition of the bioaugment itself. Steps such as increasing low digester pH before bioaugmentation may be necessary to improve digester function.

The methane production increase after bioaugmentation was correlated with increased relative abundance of *Methanosaeta* and *Methaospirillum* that were in the bioaugment culture employed. However, the methane production rate increase was only temporary. More research is warranted to develop sustained, steady state improvements via bioaugmentation or bioaugmentation combined with pH adjustment for challenged digesters.

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## **References**

- American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), et al., 1998. Standard Methods for the Examination of Water and Wastewater, twentieth ed.
- Baek, G., Kim, J., Shin, S. G., & Lee, C. (2016). Bioaugmentation of anaerobic sludge digestion with iron-reducing bacteria: process and microbial responses to variations in hydraulic retention time. *Applied Microbiology and Biotechnology*, 100(2), 927–37. doi:10.1007/s00253-015-7018-y.
- Barredo, M. S., & Evison, L. M. (1991). Effect of propionate toxicity on methanogen-enriched sludge, *Methanobrevibacter smithii*, and *Methanospirillum hungatii* at different pH values. *Applied and Environmental Microbiology*, 57(6), 1764–9.
- Carey, D. E., Zitomer, D. H., Hristova, K. R., Kappell, A. D., & McNamara, P. J. (2016). Triclocarban Influences Antibiotic Resistance and Alters Anaerobic Digester Microbial Community Structure. *Environmental Science & Technology*, 50(1), 126–34. doi:10.1021/acs.est.5b03080.
- Casamayor, E. O., Triadó-Margarit, X., & Castañeda, C. (2013). Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain. *FEMS Microbiology Ecology*, 85(3), 503–18. doi:10.1111/1574-6941.12139.
- Chouari, R., Le Paslier, D., Daegelen, P., Ginestet, P., Weissenbach, J., & Sghir, A. (2005). Novel predominant archaeal and bacterial groups revealed by molecular

- analysis of an anaerobic sludge digester. *Environmental Microbiology*, 7(8), 1104–15. doi:10.1111/j.1462-2920.2005.00795.x.
- Cirne, D. G., Björnsson, L., Alves, M. M., & Mattiasson, B. (2006). Effects of bioaugmentation by an anaerobic lipolytic bacterium on anaerobic digestion of lipid-rich waste. 81(11), 1745-52. doi: 10.1002/jctb.1597.
- Dahle, H. (2006). *Thermovirga lienii* gen. nov., sp. nov., a novel moderately thermophilic, anaerobic, amino-acid-degrading bacterium isolated from a North Sea oil well. *International journal of systematic and evolutionary microbiology*, 56(7), 1539–45. doi:10.1099/ijs.0.63894-0.
- De Bok, F. A., Plugge, C. M., & Stams, A. J. M. (2004). Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Research*, 38(6), 1368–75. doi:10.1128/AEM.67.4.1800-1804.2001.
- De Bok, F. A., Stams, A. J. M., Dijkema, C., & Boone, D. R. (2001). Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. *Applied and Environmental Microbiology*, 67(4), 1800–4. doi: 10.1128/AEM.67.4.1800-1804.2001
- Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* (Oxford, England), 27(16), 2194–200. doi:10.1093/bioinformatics/btr381.
- Grosskopf, R., Janssen, P. H., & Liesack, W. (1998). Diversity and structure of the methanogenic community in anoxic rice paddy soil microcosms as examined by cultivation and direct 16S rRNA gene sequence retrieval. *Applied and Environmental Microbiology*, 64(3), 960–9.



- 468 <http://aem.asm.org/content/64/3/960.full.pdf+html>
- 469 Guiot, S. R., Tartakovsky, B., Lanthier, M., Lévesque, M. J., Manuel, M. F., Beaudet, R.,
- 470 Greer C. W., & Villemur, R. (2002). Strategies for augmenting the
- 471 pentachlorophenol degradation potential of UASB anaerobic granules. *Water*
- 472 *Science and Technology : A Journal of the International Association on Water*
- 473 *Pollution Research*, 45(10), 35–41. <http://www.ncbi.nlm.nih.gov/pubmed/12188570>
- 474 Guiot, S. R., Tawfiki Hájji, K., & Lépine, F. (2000). Immobilization strategies for
- 475 bioaugmentation of anaerobic reactors treating phenolic compounds. *Water Science*
- 476 *and Technology*, 42(5), 245–50. [http://wst.iwaponline.com/content/ppiwawst/42/5-](http://wst.iwaponline.com/content/ppiwawst/42/5-6/245.full.pdf)
- 477 [6/245.full.pdf](http://wst.iwaponline.com/content/ppiwawst/42/5-6/245.full.pdf).
- 478 Hajji, K. T., Lépine, F., Bisaillon, J. G., Beaudet, R., Hawari, J., & Guiot, S. R. (2000).
- 479 Effects of bioaugmentation strategies in UASB reactors with a methanogenic
- 480 consortium for removal of phenolic compounds. *Biotechnology and Bioengineering*,
- 481 67(4), 417–23. doi:10.1002/(SICI)1097-0290(20000220)67:4<417::AID-
- 482 BIT5>3.0.CO;2-#.
- 483 Hattori, S. (2008). Syntrophic acetate-oxidizing microbes in methanogenic environments.
- 484 *Microbes and Environments / JSME*, 23(2), 118–27.
- 485 <http://www.ncbi.nlm.nih.gov/pubmed/21558697>.
- 486 Herrero, M., & Stuckey, D. C. (2014). Bioaugmentation and its application in wastewater
- 487 treatment: A review. *Chemosphere*, 44. doi:10.1016/j.chemosphere.2014.10.033.
- 488 Hugoni, M., Taib, N., Debroas, D., Domaizon, I., Jouan Dufournel, I., Bronner, G., Salter
- 489 I., Agogué H., Mary I., Galand, P. E. (2013). Structure of the rare archaeal biosphere
- 490 and seasonal dynamics of active ecotypes in surface coastal waters. *Proceedings of*



- 491 the National Academy of Sciences of the United States of America, 110(15), 6004–  
 492 9. doi:10.1073/pnas.1216863110.
- 493 Jumas Bilak, E., Roudière, L., & Marchandin, H. (2009). Description of “Synergistetes”  
 494 phyl. nov. and emended description of the phylum “Deferribacteres” and of the  
 495 family Syntrophomonadaceae, phylum “Firmicutes”. International Journal of  
 496 Systematic and Evolutionary Microbiology, 59(Pt 5), 1028–35.  
 497 doi:10.1099/ijs.0.006718-0.
- 498 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013).  
 499 Development of a dual-index sequencing strategy and curation pipeline for  
 500 analyzing amplicon sequence data on the MiSeq Illumina sequencing platform.  
 501 Applied and Environmental Microbiology, 79(17), 5112–20.  
 502 doi:10.1128/AEM.01043-13.
- 503 Lee, S. H., Park, J. H., Kang, H. J., Lee, Y. H., Lee, T. J., & Park, H. D. (2013).  
 504 Distribution and abundance of Spirochaetes in full-scale anaerobic digesters.  
 505 Bioresource Technology, 145, 25–32. doi:10.1016/j.biortech.2013.02.070.
- 506 Lee, S. H., Park, J. H., Kim, S. H., Yu, B. J., Yoon, J. J., & Park, H. D. (2015). Evidence  
 507 of syntrophic acetate oxidation by Spirochaetes during anaerobic methane  
 508 production. Bioresource Technology, 190, 543–549.  
 509 doi:10.1016/j.biortech.2015.02.066.
- 510 Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of  
 511 the methanogenic archaea. Annals of the New York Academy of Sciences, 1125,  
 512 171–89. doi:10.1196/annals.1419.019.
- 513 Ma, J., Carballa, M., Van De Caveye, P., & Verstraete, W. (2009). Enhanced propionic

- 514 acid degradation (EPAD) system: proof of principle and feasibility. *Water Research*,  
 515 43(13), 3239–48. doi:10.1016/j.watres.2009.04.046.
- 516 McCarty, P. L., & Smith, D. P. (1986). Anaerobic wastewater treatment. *Environmental*  
 517 *Science & Technology*, 20(12), 1200–6. doi:10.1021/es00154a002.
- 518 McNamara, P. J., & Krzmarzick, M. J. (2013). Triclosan enriches for Dehalococcoides-  
 519 like Chloroflexi in anaerobic soil at environmentally relevant concentrations. *FEMS*  
 520 *Microbiology Letters*, 344(1), 48–52. doi:10.1111/1574-6968.12153.
- 521 Nunoura, T., Takaki, Y., Kazama H., Hirai M., Ashi J., Imachi H., & Kazama, H. (2012).  
 522 Microbial diversity in deep-sea methane seep sediments presented by SSU rRNA  
 523 gene tag sequencing. *Microbes and Environments*, 27(4), 382 – 390.  
 524 <http://europepmc.org/articles/pmc4103545>.
- 525 Nunoura, T., Oida, H., Nakaseama, M., Kosaka, A., Ohkubo, S. B., Kikuchi, T., Kazama  
 526 H., Hosoi Tanabe S., Nakamura K., Kinoshita M., Hirayama H., Inagaki  
 527 F., Tsunogai U., Ishibashi J., Takai, K. (2010). Archaeal diversity and distribution  
 528 along thermal and geochemical gradients in hydrothermal sediments at the Yonaguni  
 529 Knoll IV hydrothermal field in the Southern Okinawa trough. *Applied and*  
 530 *Environmental Microbiology*, 76(4), 1198–211. doi:10.1128/AEM.00924-09.
- 531 Rivière, D., Desvignes, V., Pelletier, E., Chaussonnerie, S., Guermazi, S., Weissenbach  
 532 J., Li T., Camacho P., & Sghir A. (2009). Towards the definition of a core of  
 533 microorganisms involved in anaerobic digestion of sludge. *The ISME Journal*, 3(6),  
 534 700–14. doi:10.1038/ismej.2009.2.
- 535 Rotaru, A. E., Shrestha, P. M., Liu, F., Shrestha, M., Shrestha, D., Embree, M., Zengler  
 536 K., Wardman C., Nevin K. P., & Lovley D. R. (2014). A new model for electron

- 537 flow during anaerobic digestion: direct interspecies electron transfer to  
 538 Methanosaeta for the reduction of carbon dioxide to methane. Energy &  
 539 Environmental Science, 7(1), 408-15. doi:10.1039/c3ee42189a.
- 540 Schauer Gimenez, A. E., Zitomer, D. H., Maki, J. S., & Struble, C. A. (2010).  
 541 Bioaugmentation for improved recovery of anaerobic digesters after toxicant  
 542 exposure. Water Research, 44(12), 3555–3564. doi:10.1016/j.watres.2010.03.037.
- 543 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,  
 544 Lesniewski R. A., Oakley B. B., Parks D. H., Robinson C. J., Sahl J. W., Stres B.,  
 545 Thallinger G. G., Van Horn D. J., & Weber C. F. (2009). Introducing mothur: open-  
 546 source, platform-independent, community-supported software for describing and  
 547 comparing microbial communities. Applied and Environmental Microbiology,  
 548 75(23), 7537–41. doi:10.1128/AEM.01541-09.
- 549 Speece, R E, Saroch Boonyakitsombut, Moonil Kim, Nuri Azbar, and Pepi Ursillo. 2006.  
 550 “Overview of Anaerobic Treatment: Thermophilic and Propionate Implications.”  
 551 Water Environment Research : A Research Publication of the Water Environment  
 552 Federation 78 (5): 460–73. <http://www.ncbi.nlm.nih.gov/pubmed/16752608>.
- 553 Speece, R. E. (2008). Anaerobic Biotechnology and Odor/corrosion Control for  
 554 Municipalities and Industries. Fields Publishing, Inc., Nashville, TN. ISBN: 1-  
 555 57843-052-9
- 556 Stams, A. J. M., & Plugge, C. M. (2009). Electron transfer in syntrophic communities of  
 557 anaerobic bacteria and archaea. Nature Reviews Microbiology, 7(8), 568–77.  
 558 doi:10.1038/nrmicro2166.
- 559 Tale, V. P., Maki, J. S., Struble, C. A., & Zitomer, D. H. (2011). Methanogen community

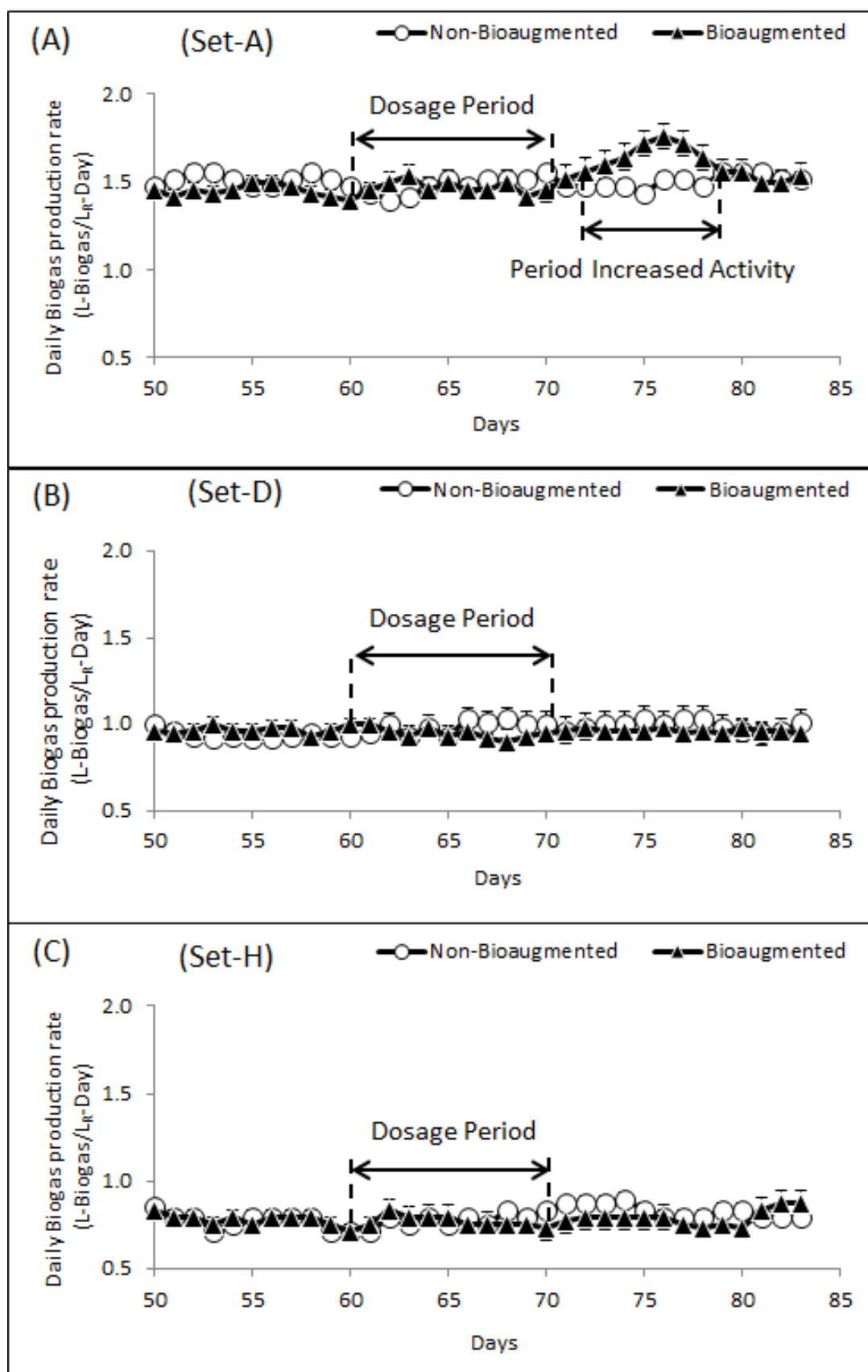
- 560 structure-activity relationship and bioaugmentation of overloaded anaerobic  
561 digesters. *Water Research*, 45(16), 5249–56. doi:10.1016/j.watres.2011.07.035.
- 562 Tale, V. P., Maki, J. S., & Zitomer, D. H. (2015). Bioaugmentation of overloaded  
563 anaerobic digesters restores function and archaeal community. *Water Research*, 70,  
564 138–147. doi:10.1016/j.watres.2014.11.037.
- 565 Venkiteshwaran, K., Bocher, B., Maki, J., & Zitomer, D. (2016). Relating Anaerobic  
566 Digestion Microbial Community and Process Function. *Microbiology Insights*, 8(2),  
567 37–44. doi:10.4137/MBI.S33593.
- 568 Wang, Y., & Qian, P.-Y. (2009). Conservative fragments in bacterial 16S rRNA genes  
569 and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PloS*  
570 *One*, 4(10), e7401. doi:10.1371/journal.pone.0007401.
- 571 Zitomer, D. (2013) Preservation of Methanogenic, Hydrogen- utilizing Microbial  
572 Cultures. U.S. Patent US 8,557,563 B2, October 15th.

**Table 1:** Performance parameters of digester groups.

Group->	G1	G2	G3
Digester Sets->	A, B and C	D, E, F and G	H and I
Methane production rate (L-CH <sub>4</sub> /L <sub>R</sub> -day at 35°C, 1 atm)	0.77±0.12	0.6±0.04	0.34±0.02
SCOD removal (%)	67±10	55±4	30±4
pH	7.2±0.06	6.6±0.05	6.3±0.0
Acetate (g/L)	2.4±0.6	5.4±1	7.3±2
Propionate (g/L)	2.1±1	4±1	4.3±3

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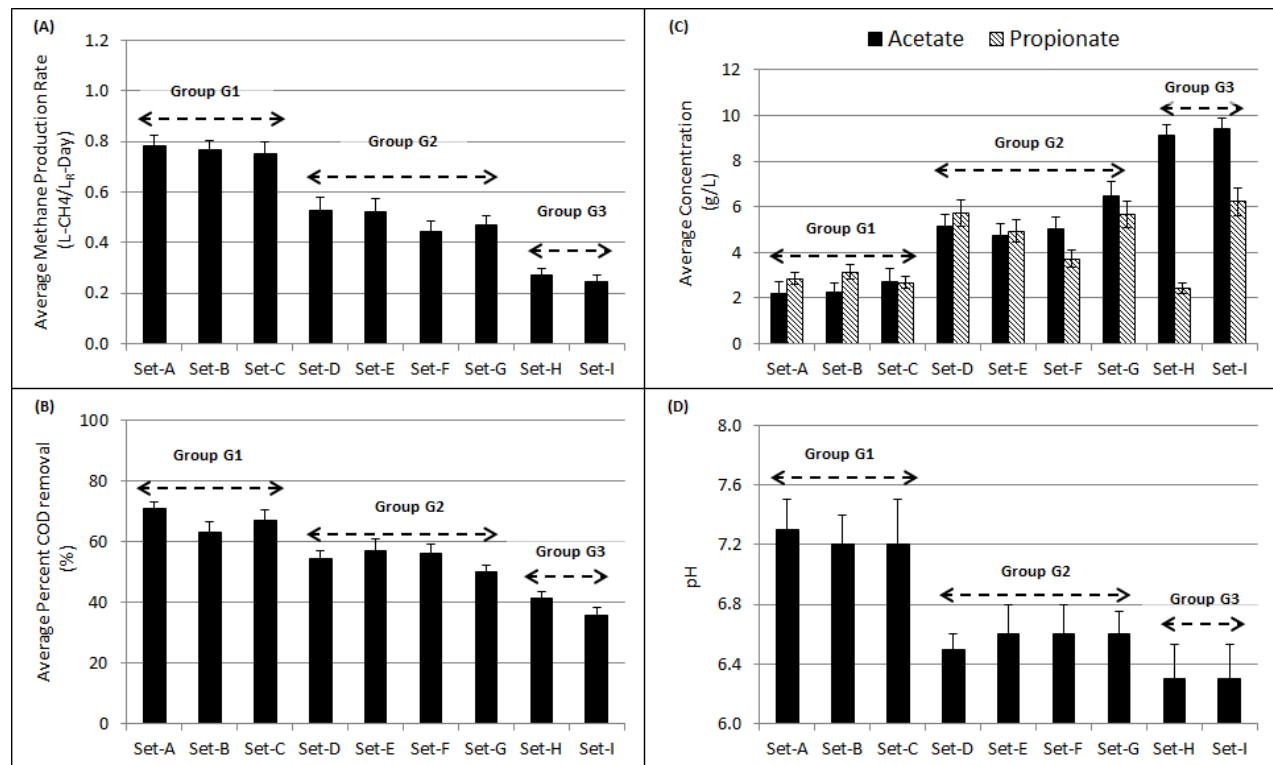
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**Figure 1:** Typical quasi steady state biogas production.

Daily biogas production rate observed from non-bioaugmented and bioaugmented digesters of (A) Set-A, (B) Set D and (C) Set H, respectively. The error bars represent standard deviation among triplicate digesters; some error bars are small and not visible. The dosage period represents the 10-day period during which inactivated and active enrichment cultures were added to non-bioaugmented and bioaugmented digesters, respectively. The Period of Increased Activity (period when bioaugmented digester methane production was statistically greater than that of non-bioaugmented digesters ( $p$  value  $<0.05$ ,  $n=3$ )). PIA was not observed for Set-D (B) and Set-H (C) digester systems.

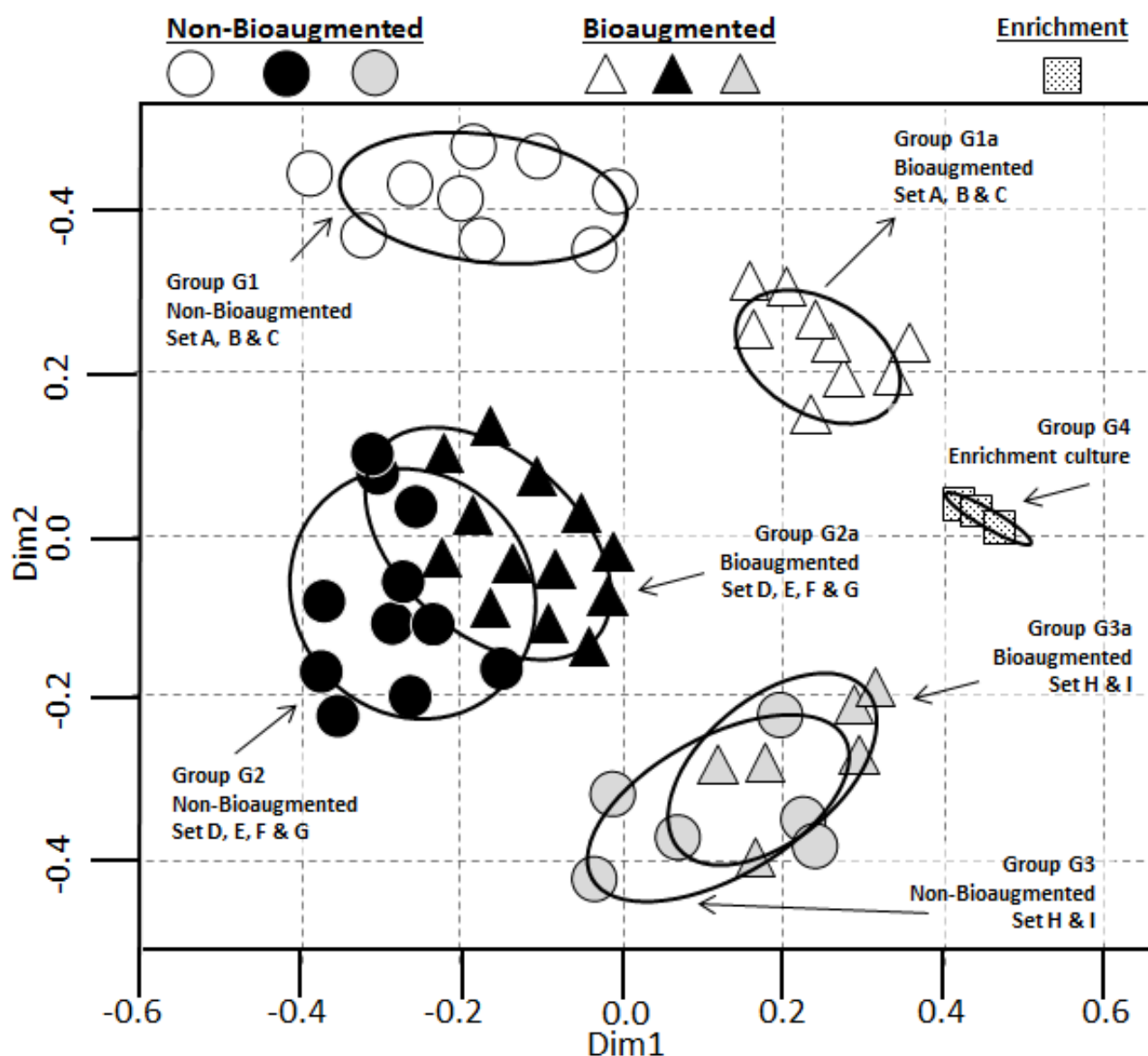
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**Figure 2:** Digester group functional parameters.

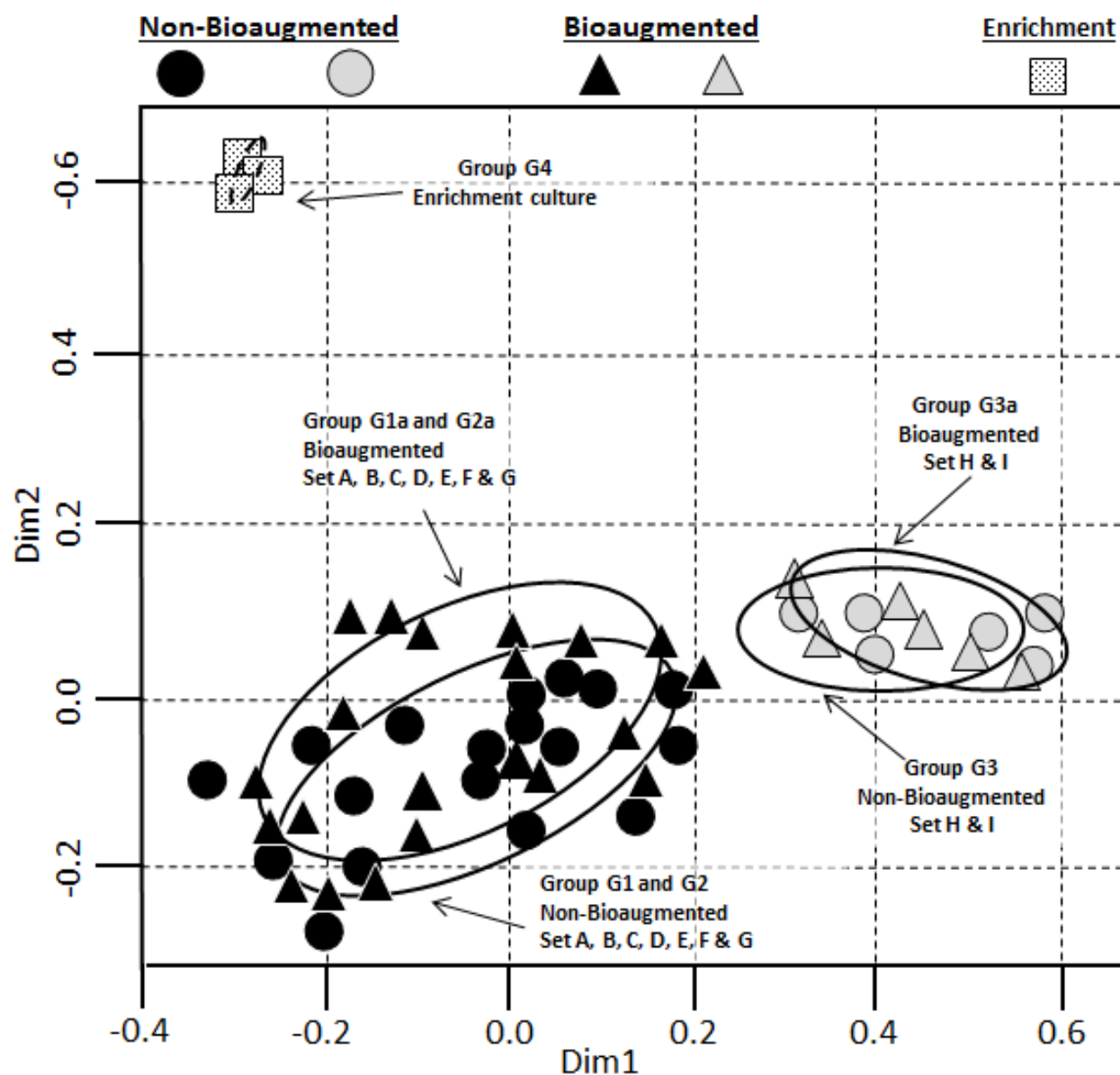
(A) Average methane production rate (L-CH<sub>4</sub>/L<sub>R</sub>-day), (B) average percent COD removal, (C) average effluent acetate and propionate concentration, (D) and average effluent pH. Error bars represent standard deviation among triplicates. Based on average functional performance, the nine digester sets were divided into three statistically distinct groups ( $p < 0.05$ ,  $n = 3$ ): digester groups G1 (Sets A, B and C), G2 (Sets D, E, F and G) and G3 (Sets H and I).





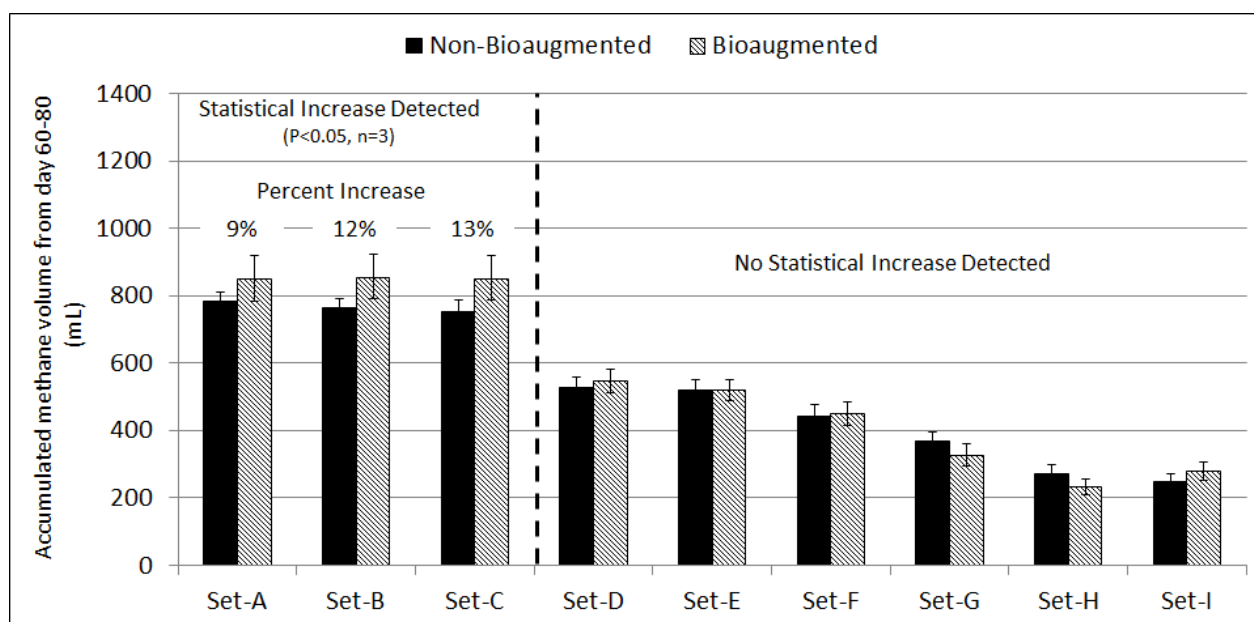
**Figure 3:** Archaeal sequence nMDS plot.

Non-bioaugmented digesters cluster 1, G1 (○); non-bioaugmented digesters cluster 2, G2 (●); non-bioaugmented digesters cluster 3, G3 (◐); bioaugmented digesters cluster 1, G1a (△); bioaugmented digesters cluster 2, G2a (▲); bioaugmented digesters cluster 3, G3a (◑); and enrichment culture G4 (▨). The ellipses represent 95% confidence interval for each cluster. Eight archaeal OTU's, identified based on 97% similarity and representing >99% of the total archaeal sequences in all digesters, including the enrichment culture, were employed for nMDS analysis.

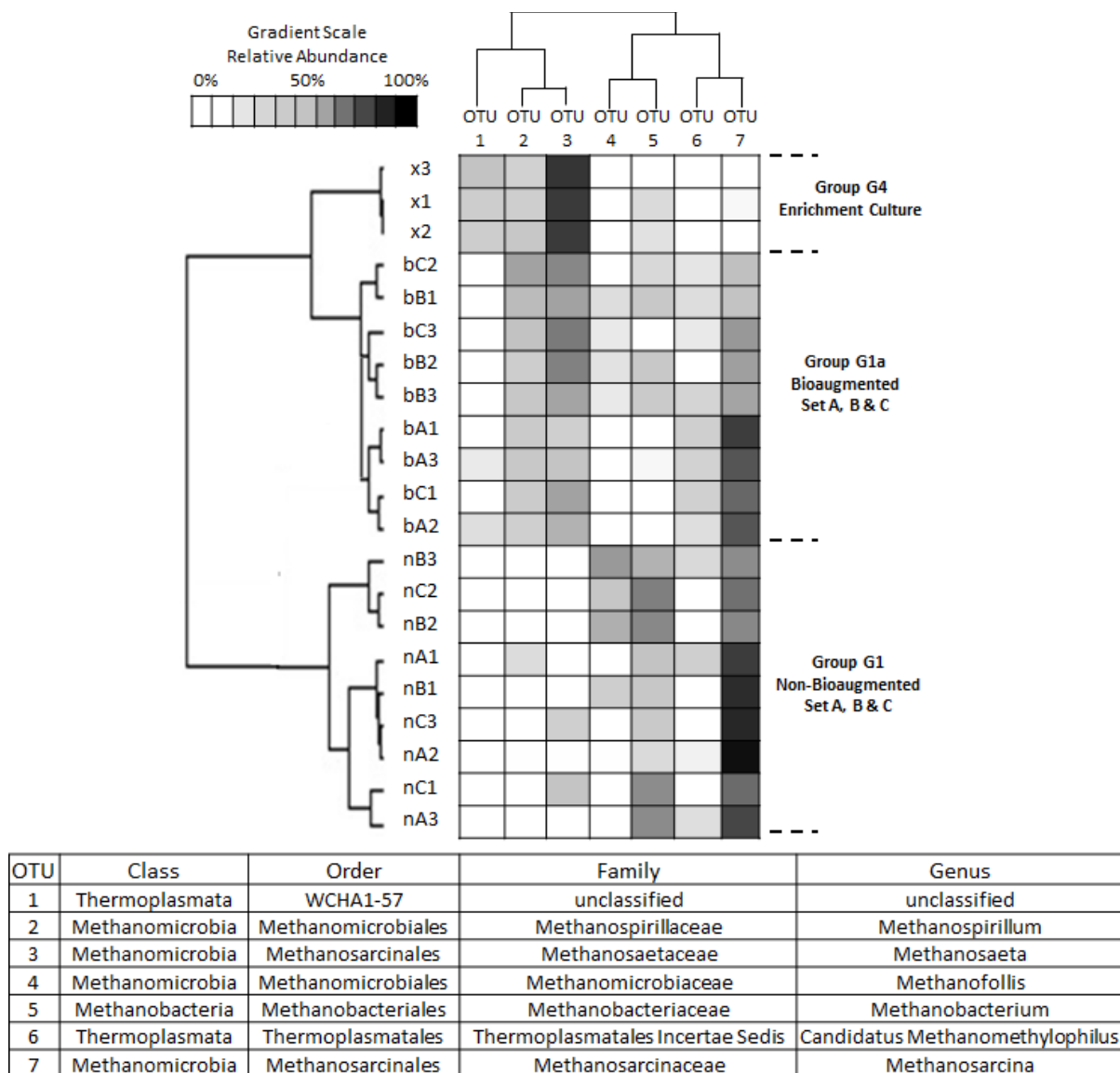


**Figure 4:** Bacterial sequence nMDS plot.

Non-bioaugmented digesters cluster 1, G1 and G2 (●); non-bioaugmented digesters cluster 2, G3 (○); enrichment culture G4 (▣); bioaugmented digesters cluster 1, G1a and G2a (▲); and bioaugmented digester cluster 2, G3a (△). The ellipses represent 95% confidence interval for each cluster. The 29 OTUs detected in all digesters with the highest relative abundance, including the enrichment culture, were employed for nMDS analysis.



**Figure 5:** Cumulative  $\text{CH}_4$  produced between day 60-80. Error bars represent standard deviation among triplicates. Bioaugmented digesters of Sets A, B and C showed statistically higher cumulative  $\text{CH}_4$  production ( $p < 0.05$ ,  $n=3$ ).



**Figure 6:** Dual hierarchical clustering of the top seven archaeal OTUs observed in the enrichment culture and G1 digesters.

These seven OTU's, based on 97% similarity, represent >99% of the total archaeal abundance in the enrichment culture and G1 digesters. The gradient scale ranges from 0 to 100% relative abundance. Sample names x1, x2 and x3 represent the enrichment culture. The sample names for digesters are denoted as follows: for example "nB1" and bB1 – the prefix "n" and "b" represents "non-bioaugmented" and "bioaugmented", respectively, the middle letter "B" represents Set-B and the suffix "1" represent the replicate number. The enrichment culture is dominated by *Methanosaeta* (OTU 3), *Methanospirillum* (OTU 2) and *WCHA1-57* (OTU 1). The non-bioaugmented digesters are dominated by *Methanosarcina* (OTU 7), followed by *Methanobacterium* (OTU 5). The bioaugmented digester of Set-A, B and C showed a significant abundance of *Methanosaeta* (OTU 3) and *Methanospirillum* (OTU 2) as compared to the non-bioaugmented digesters.

## Highlights

- Digesters with similar functional performance have similar methanogenic community structure and vice versa
- Bioaugmentation can improve methane production by shifting the digester's methanogenic community structure
- Washout of microorganisms, low pH and high acid concentrations may decrease the effectiveness of bioaugmentation

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