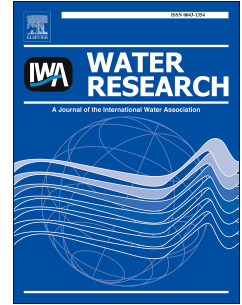


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Low pH anaerobic digestion of waste activated sludge for enhanced phosphorous release

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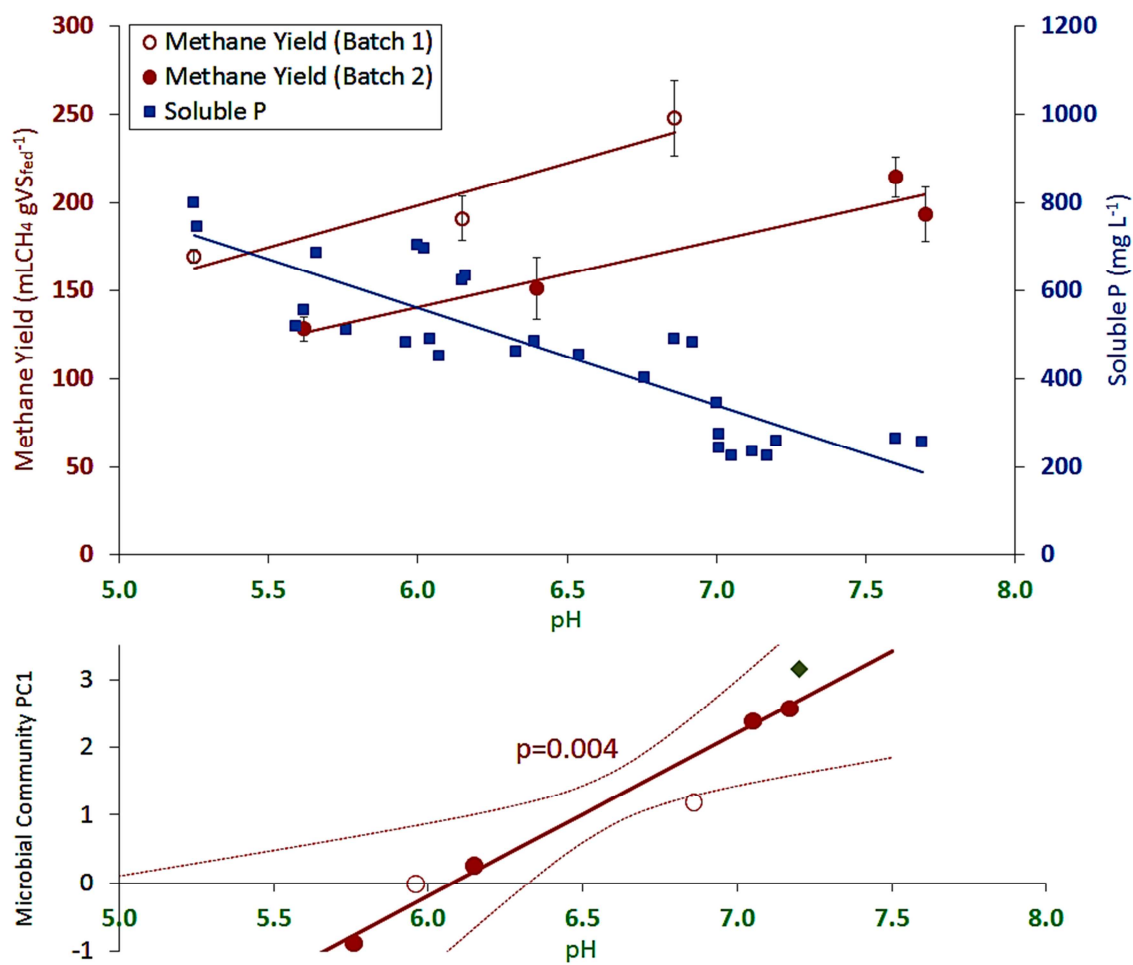
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1 **Low pH anaerobic digestion of waste activated sludge for enhanced phosphorous**
2 **release.**

3

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10

11 **Abstract**

12 This paper assesses anaerobic digestion of waste activated sludge (WAS) at low pH to
13 enhance phosphorous solubility. Batch biochemical methane potential tests were
14 conducted at a pH ranges from 5-7.2 in two separate sets (two different WAS samples
15 collected from municipal WWTP). Low pH (<5.7) caused a significant ($p=0.004$)
16 decrease in methane potential (B_0) of 33% and 3.6 times increase in phosphorus release
17 compared to high pH (>7), but with no major change in methane production rate
18 coefficient (k_{hyd}). The loss in methane yield was mainly due to decrease in hydrolytic
19 capability rather than inhibition of methanogenesis with volatile fatty acids being <300
20 mgCOD L⁻¹ and soluble COD <1300 mgCOD L⁻¹ even at low pH. While pH did not
21 influence the acetoclastic community (Methanosaeta dominated), it was the primary
22 driver for the remaining community ($p=0.004$), and caused a loss of diversity and shift
23 to Clostridia.

24

25 **Keywords:** Anaerobic digestion; Phosphorus release; Community profile; Soluble

26 COD; Batch experiments

27

28 **Nomenclature**

AD	Anaerobic Digestion
ADP	Adenosine Di-phosphate
ADS	Anaerobic Digested Sludge
Al	Aluminium
ATP	Adenosine Tri-phosphate
B_0	Methane Potential
BMP	Biochemical Methane Potential Test
Ca	Calcium
COD	Chemical Oxygen Demand
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
K	Potassium
k_{hyd}	Hydrolysis Rate Coefficient
Mg	Magnesium
N	Nitrogen
OTUs	Operational Taxonomic Units
P	Phosphorus
PCA	Principal Component Analysis
POM	Particulate Organic Matter
SCOD	Soluble Chemical Oxygen Demand
TIC	Total Inorganic Carbon
TKN	Total Kjeldahl Nitrogen
TKP	Total Kjeldahl Phosphorus
TOC	Total Organic Carbon
TP	Total Phosphorus
TPAD	Temperature Phased Anaerobic Digestion
TS	Total Solids
VFA	Volatile Fatty Acid
VS	Volatile Solids
WAS	Waste Activated Sludge
WWTP	Wastewater Treatment Plant

29

30 **1. Introduction**

31 Wastewater treatment plant (WWTP) influent contains phosphorus (P), nitrogen (N),
 32 calcium (Ca), magnesium (Mg), potassium (K) and other valuable resources. Recovery
 33 of these, particularly P has been widely studied due to rising P-fertilizer prices, limited

34 availability of natural P and environmental impact of P on natural waterways (Mehta et
35 al., 2014). P in the influent wastewater is typically present in three different forms:
36 organically bound, inorganic compounds and soluble form (free orthophosphate).
37 During anaerobic digestion, part of the organically bound P releases due to the
38 destruction of organic matter. The released P and soluble P as orthophosphate may bind
39 with cations including Mg^{2+} , Ca^{2+} , Al^{3+} and Fe^{2+} to form P-precipitants depending upon
40 system pH, temperature and, amount and type of cations present in wastewater. Typical
41 P-precipitants are struvite (magnesium/potassium ammonium phosphate), colloidal
42 aluminum and iron phosphates and a broad array of calcium phosphates (Doyle and
43 Parsons, 2002). As the amount and type of P-precipitants depend on operational
44 parameters such as pH and temperature (Szabó et al., 2008), these precipitants deposit
45 and scale within the digester and in downstream accessories (pipelines, pump heads,
46 valves and elbows). While iron and aluminum phosphates reduce bioavailability of P-
47 rich biosolids when used as agricultural additives (De-Bashan and Bashan, 2004) and all
48 precipitates make it difficult to recover P as a concentrate from digester supernatant.
49 There is therefore motivation to increase in-reactor phosphorous solubilization for both
50 operational and economic reasons.

51

52 Reduced P-precipitation during anaerobic digestion can be achieved using chemical
53 additives or by changing operational conditions such as pH and temperature. Chemical
54 additives such as ethylenediaminetetraacetic acid (EDTA) can reduce P precipitants
55 from anaerobically digested effluent (Zhang et al., 2010). EDTA is a chelating agent
56 with much higher affinity for di- and tri-valent metal ions (Ca^{2+} and Mg^{2+}). Anaerobic
57 digestion with added EDTA can reduce calcium and magnesium binding with

58 orthophosphate and thereby, reduces formation of precipitants such as struvite and
59 apatite. Commercial application of EDTA or other binding agents could be challenging
60 due to high cost of chemicals (EDTA is US \$0.95 kg⁻¹) and low phosphorus release
61 (<10 mgP g⁻¹ of EDTA added) (Zhang et al., 2010). Solubility of struvite increases with
62 temperature until approximately 30 °C, and then decreases with further increases in
63 temperature (Bhuiyan et al., 2007), while hydroxyapatite (Ca₅(PO₄)₃OH) solubility
64 increases slightly with temperature (Prakash et al., 2006). Soluble P concentrations in
65 the digested effluent were found to be similar at both mesophilic and thermophilic
66 temperatures during pilot scale operation, suggesting minimal influence of temperature
67 (Bolzonella et al., 2012).

68
69 Acidic conditions (pH <6) during anaerobic digestion can promote P solubilization.
70 This is due to lower solubility of different P-precipitants under acidic conditions, with
71 complete solubilization of P-precipitants below pH 4 (Mehta and Batstone, 2013). Wu
72 et al. (2009) observed 1.92 (48 mg-P_{soluble} L⁻¹) and 1.48 (37 mg-P_{soluble} L⁻¹) times higher
73 soluble P concentration at pH 5 and pH 6 respectively compared to pH between 7-10
74 (25 mg-P_{soluble} L⁻¹) after 9 days of batch fermentation of primary sludge. While decrease
75 in methane production was reported because of the accumulation of volatile fatty acids
76 (VFA) and soluble COD (SCOD) up to 2.35 gCOD L⁻¹ and 3.9 g L⁻¹ respectively during
77 acidic AD. Bi et al. (2012) have reported 1.26 times higher P release (213 mg-P_{soluble}
78 L⁻¹) at pH 5 compared to pH 10 (168 mg-P_{soluble} L⁻¹). They also observed 50% reduction
79 in methane production and high VFA concentration (600 mg L⁻¹) at pH 5 compared to
80 neutral pH. Acclimatization and pH tolerance have been previously observed (Kim et
81 al., 2004), which may allow digesters to operate effectively despite a low pH. However,

82 low pH conditions (pH 4.5 - 6) reduce methane production and cause accumulation of
83 SCOD, especially short-chain fatty acids (SCFAs). Low pH AD has been extensively
84 applied to multistage digestion of anaerobic sludge, with pH <6 generally applied to
85 deliberately suppress methanogens (Elefsiniotis and Oldham, 1994). However, there
86 have been limited work on activated sludge especially in a single stage AD and on low
87 pH methanogenic system, with conflicting reports of either hydrolytic (Gomec and
88 Speece, 2003), or methanogenic (Chen et al., 2007) suppression. Multiphase digestion
89 has largely focused on primary sludge, with an emphasis on solubilization in the first
90 stage (Elefsiniotis and Oldham, 1994). There is very limited work on low pH systems
91 designed to be methanogenic.

92
93 Chemicals such as EDTA are suitable for P release during anaerobic digestion without
94 compromising methane production but are expensive and may not be feasible for
95 commercial use. Previous studies showed limited or no influence of temperature on P
96 solubility but further work is justified, particularly for multistage configurations such as
97 temperature phased anaerobic digestion (TPAD) and acid-phase digestion. Acidic pH
98 conditions improve P release but could affect methane production and COD removal. It
99 should be noted that chemical free pH adjustment is possible through pressurization or
100 CO₂ recycling, and a broader range of pH levels are required to fully assess these
101 techniques. However, there is a lack of material addressing the quantitative and
102 combined outcomes of enhanced phosphorous solubility, and inhibition of either
103 methanogenesis or hydrolysis, particularly in waste activated sludge. This study is
104 designed to address these gaps, through low pH methanogenic batch testing at a range
105 of pH values.

106

107 **2. Materials and Methods**

108 Two sets of batch experiments were done, with target pH levels of 5, 5.7 and 6.5 in the
109 first batch and, pH 5, 5.7, 7, and 7.2 (no adjustment control) in a second batch. Samples
110 for batches 1 and 2 were collected in winter and summer respectively. There was no
111 motivation to collect batches in different seasons except the sampling time which was
112 due for that batch. Blank reactors were set up in both batches to account for native
113 inoculum production. Briefly, excess serum flasks were set up and sacrificially titrated
114 during adjustment events to determine the amount needed to adjust the remaining flasks
115 using 2M HCl (with triplicates remaining at the end of the batches). Batches 1 and 2
116 were first adjusted at 7d and 1.5d respectively. Microbial community analysis (pyrotag
117 sequencing) was done at 35d in both batch tests.

118

119 *2.1. Substrate and inoculum*

120 The substrate (WAS; waste activated sludge) and inoculum (ADS; anaerobic digested
121 sludge) were collected from a sewage treatment plant operated by Queensland Urban
122 Utilities, Brisbane. The substrate was collected from dissolved air floatation (DAF) unit
123 at 3% total solids and was stored at 4 °C. It was further thickened by removing
124 excessive water after 24 hours of storage. The inoculum was collected from mesophilic
125 anaerobic digester treating a mixture of primary and activated sludges. It was degassed
126 i.e. pre-incubated at 37 °C for one week in order to deplete the residual biodegradable
127 organic material. The physico-chemical properties of substrates and inoculums are
128 shown in Table 1.

129

130 2.2. *Biochemical Methane Potential (BMP) test*

131 A modified biochemical methane potential (BMP) test (Jensen et al., 2011) was used for
132 both batch tests. The BMP tests were conducted in 160 mL glass serum flasks with a
133 working volume of 100 mL. For batch 1, 28 mL of substrate and 72 mL of inoculum
134 while 23 mL of substrate and 77 mL of inoculum were used in batch 2 to achieve
135 inoculum to substrate ration (ISR) of 1.6 (VS basis). An ISR ratio of 1-2 is consistent
136 with normal practice (Jensen et al., 2011), and while for poorly degradable substrates
137 such as WAS, it results in a substantial methane contribution from the blank, error
138 around this is relatively low (see section 2.5). Each reactor was filled with 100 mL of
139 assay and purged with N₂ gas at a flow rate of 4-5 L min⁻¹ for 30 seconds. Each reactor
140 was immediately closed with butyl rubber after N₂ purge, sealed with an aluminum
141 crimp and placed in an incubator at 37 ± 1 °C to provide anaerobic conditions. Further
142 experimental details are given in Angelidaki et al. (2009).

143

144 Two samples of WAS were analyzed in two large experiments as briefly described at
145 the start of section 2. Initially, the reactors were incubated at pH 6.5 using 2M HCl in
146 batches 1 and 2 for 7d and 1.5d respectively to establish activity. After initial incubation
147 periods, one of the reactors was sacrificed (opened) for analysis and to estimate the
148 amount of acid required to achieve targeted pH in other reactors by titration. The pH of
149 the remaining reactors was then adjusted using this amount of acid, assuming buffering
150 of the sacrificed reactor is similar to the remaining reactors. Following this, at each pH
151 condition, one reactor was sacrificed (4 per adjustment event). The adjustment interval
152 and amount of acid added in each reactor can be found in supplementary material A and

153 was based on expected methane profile. Triplicate reactors at each pH condition
154 remained at the end of the batch.

155

156 The methane yield from sample free blanks was subtracted from the batch-containing
157 sample (using the model as in Eq. 1) and blank corrected data was fitted with the
158 relationship:

159

$$160 \quad B = B_0 \cdot (1 - e^{-k_{\text{hyd}}t}) \quad (1)$$

161

162 Where B is the methane production in $\text{mLCH}_4 \text{ gVS}_{\text{fed}}^{-1}$, B_0 is the biochemical methane
163 potential in $\text{mLCH}_4 \text{ gVS}_{\text{fed}}^{-1}$ at 25 °C, and 1 bar, k_{hyd} is the first-order degradation rate
164 coefficient (d^{-1}) and t is the batch time (days). The `lsqcurvefit()` function in
165 MATLAB was used to estimate parameter values in Eq. 1, together with parameter
166 errors calculated from linear estimate of errors based on a two-tailed t-test (95%
167 confidence interval). Only data following the first pH adjustment was used for
168 parameter estimation.

169

170 2.3. Physico-chemical analysis

171 Total solids (TS) and volatile solids (VS) were measured according to Standard
172 Methods (Clesceri et al., 1998). COD was measured by using Merck vials (range: 25-
173 1500 mg L^{-1} and 500-10000 mg L^{-1}). Diluted sample was added into vials and placed in
174 a Spectroquant TR 620 COD digester at 148 °C for 2 hours. The COD was then
175 measured using Merck Spectrophotometer, Germany (model SQ 118). The TS, VS and
176 COD measurements were performed in triplicates. Lachat Instruments USA, Quick

177 Chem 8000 flow injection analyzer (FIA) was used to measure PO_4^{3-} -P and NH_4^+ -N.
178 PerkinElmer, USA Optima 7300 DV inductivity coupled plasma-optical emission
179 spectroscopy (ICP-OES) equipped with WinLab32 for ICP software was used to
180 measure soluble and total metal ions along with the total Kjeldahl phosphorus (TKP)
181 and nitrogen (TKN). Gas chromatograph (Agilent model 78090A, USA) with flame
182 ionization detection was used to measure volatile fatty acids (VFAs). Total organic and
183 inorganic carbon were analyzed by using Analytik Jena, Germany (model Multi N/C
184 2100 S). Biogas production was recorded at regular intervals by measuring the biogas
185 pressure in serum reactors. Pressure measurements were obtained using a water-filled
186 manometer (pH acidified). Biogas volumes were converted to an equivalent volume at
187 standard room temperature and pressure (25 °C and 1 atm) using the ideal gas law. Gas
188 composition (CH_4 and CO_2) was measured using gas chromatography (GC). The GC
189 used was a Perkin Elmer, USA auto system GC equipped with a thermal conductivity
190 detector and a 2.44 m stainless steel column packed with Hayesep Q (80/100 mesh).
191 The GC was calibrated using external gas standards obtained from British Oxygen
192 Company (BOC).

193

194 *2.4. Community profiling*

195 The analysis of microbial community structure and diversity was examined using pyro
196 sequencing from samples of both batches taken at day 35 assuming that these have well
197 stabled at applied pH conditions. Total DNA was extracted from sludge samples using
198 FastDNA® Spin Kit for soil (MP Biomedicals, California, USA). Samples were
199 prepared according to the protocol provided by MP Biomedicals for DNA extraction.
200 The primers (single-stranded DNA molecules) used for pyrosequencing were universal

201 primers 962f (5'-AAACTYAA AKGAATTGACGG-3') and 1392r (5'-
202 ACGGGCGGTGTGTAC-3'). Sequencing was carried out using a Roche 454 GS FLX
203 sequencer (Roche, Switzerland). Molecular Evolutionary Genetics Analysis (MEGA)
204 version 5.2 software was used for maximum likelihood analysis of bacteria/archaea to
205 select best-fit substitutions of nucleotides (Tamura et al., 2011). Influence of batch and
206 pH was done through ANCOVA as described above, with pH as a regressor, and batch
207 number as a categorical factor. Actual pH when sampled (day 35) was used as
208 regressor, which was different only for the highest two experiments from batch 2 (7.05
209 and 7.17 at day 35 vs 7.6 and 7.7 at day 51).

210

211 2.5. Other statistical analysis

212 To test the effect of pH and batch set on B_0 and k_{hyd} , analysis of correlated variance
213 (ANCOVA) was done using the MATLAB function `anovan()`, with batch number (1
214 or 2) as a categorical variable, and pH as a regressor (continuous variable). Standard
215 linear regression has been used elsewhere for correlation analysis. p-values are provided
216 for significance testing, with a conventional 5% threshold ($p < 0.05$) applied to identify a
217 weak statistically significant relationship. Confidence intervals (CI) provided on graphs
218 and in tables are likewise based on a two-tailed t -test with a significance threshold of
219 5% (95% CI) in the mean of replicate analyses.

220

221 Error in blank methane potential was $2.9 \text{ mLCH}_4 \text{ gVS}_{\text{fed}}^{-1}$ for batch 1, and 3.6 mLCH_4
222 $\text{gVS}_{\text{fed}}^{-1}$ (95% CI) for batch 2, and hence as a small and static error (not varying within
223 experiment sets or across replicates), its contribution was not propagated through Eq. 1
224 for error in B_0 . Likewise, the error bars for methane potential curves in supplementary

225 information are based only on variation between the three experimental triplicates. The
226 effect of inoculum (and hence uncertainty in inoculum potential) was accounted for
227 implicitly in the ANOVA as outlined above.

228

229 **3. Results and Discussion**

230 *3.1. Effect of pH on biochemical methane potential*

231 The methane yield curves fit well to the first order model (see supplementary material
232 B) with minimal scatter between triplicates (see error bars). Waste activated sludge
233 degradability (B_0) was $214 \pm 12 \text{ mLCH}_4 \text{ gVS}_{\text{fed}}^{-1}$ and first order coefficient (k_{hyd}) was
234 $0.15 \pm 0.03 \text{ d}^{-1}$ at neutral conditions which is similar to previously reported (Wang et al.,
235 2014).

236

237 The influence of pH on B_0 and k_{hyd} is shown in Fig 1. Degradability was significantly
238 influenced by the batch set ($p=0.006$), with batch 2 consistently having 20% lower
239 degradability than batch 1. This could be due to seasonal factors where batch 1 samples
240 were collected in winter and batch 2 in summer. The chemical characteristics of these
241 samples are shown in Table 1. Because of different sampling times, the VS_{fed} of WAS
242 in batch 1 and 2 were 0.935 g L^{-1} and 0.774 g L^{-1} respectively. However, for both batch
243 2 and batch 1, low pH (<5.7) caused a decrease of 33% degradability compared to
244 neutral pH (>7), ($p=0.004$). Higher reductions (on the order of 64%) have been
245 observed for sludge digestion (Lay et al., 1997). There were no significant interaction
246 effects between batch number and pH on either rate or extent of degradation. Hydrolysis
247 coefficient was higher for batch 2 ($p=0.01$), but was not influenced by pH ($p=0.12$) as
248 shown in Fig. 1. The low pH conditions influenced conversion extent to methane, but

249 not rate of methane production contradicting the standard assumption in biochemical
250 process modelling, that pH will decrease rate, but not the amount of material able to be
251 degraded (Batstone et al., 2002). This was largely due to the accumulation and
252 decreased degradation of particulate organic matter (POM) at low pH (pH <5.7).
253 Identification of POM as un-degraded fraction was based on mass balancing, with only
254 a small fraction (Fig. 2) being due to increased soluble COD or VFA. Reduced methane
255 potential has been previously reported at depressed pH conditions (Lay et al., 1997),
256 and Gomec Speece (2003) particularly noted that the effect of reduced pH on WAS was
257 reduced hydrolysis, while reduced pH on primary sludge caused an accumulation of
258 organic acids and solubles. A decrease in observed methane potential at low pH has
259 been previously reported (50% loss at pH 5.25) (Chen et al., 2007).
260
261 No significant relation between hydrolysis coefficient and pH at a 5% significance
262 threshold was observed. Effect of pH on hydrolysis has been widely reported in
263 literature for various waste streams containing both primary and activated sludge,
264 between 0.1-1 d⁻¹ (Feng et al., 2009, Miron et al., 2000). This study also differs from the
265 findings of Arntz et al. (1985) who suggested that pH 6.5 can be suitable for optimal
266 hydrolysis while using beet pulp as substrate in AD. This increase in hydrolysis
267 supports multi-stage anaerobic digestion, with earlier stages focusing on low pH
268 operation (with possibly recovery of phosphorous).
269
270 VFA and SCOD concentrations remained constant for the pH range 6.2-7.6 but were
271 increased at pH below 6.2 as shown in Fig. 2. The highest SCOD concentration (1320
272 mg L⁻¹) was observed at pH 5.62 while lowest SCOD concentration (576 mg L⁻¹) was at

273 pH 7 ($p=0.006$). As shown in Fig. 2, the majority of the SCOD was VFA, with the
274 majority of this being acetate. This supports that inhibition at low pH results in residual
275 concentrations rather than a decrease in conversion rate. The increase in SCOD
276 resulting from hydrolysis and fermentation was fastest during start up after which
277 residual VFAs were reduced.

278

279 Acetic acid was found at all test conditions whereas propionic and butyric acids were
280 exclusively found at depressed pH conditions ($pH < 6.2$). The total VFA concentration
281 was 273 mg L^{-1} at pH 5.25 and 312 mg L^{-1} at pH 5.62 ($p=0.001$). Although VFAs were
282 accumulated at depressed pH conditions, the anaerobic process and methane production
283 was stable which means the fatty acids might be continuously converted into acetate
284 albeit with residual concentrations (Zoetemeyer et al., 1982).

285

286 3.2. Community profiling

287 All samples were dominated by *Methanosaeta* (see supplementary material C), with
288 40%-80% of total sequences in affiliated operational taxonomic units (OTUs) which
289 states that decrease in methane potential was mainly due to the decreased hydrolytic
290 capacity, thus, indicating that principal methanogen (*Methanosaeta*) was not affected.
291 However, this was not influenced by pH ($p=0.77$), and because it obscured other results,
292 the *Methanosaeta* OTUs were removed, and the OTU table was re-normalized and
293 analyzed. Based on principal component analysis (PCA – excluding *Methanosaeta*) of
294 samples at pH 5.25, 6.15 and 6.86 (batch 1) and 5.76, 5.96, 7.05, 7.17 (batch 2) along
295 with inoculum found that first two PCs represented the majority (64%) of variance (Fig.
296 3). Batch number did not affect PC 1 ($p=0.4$) or PC 2 ($p=0.11$) but did have an impact

297 on PC3 ($p=0.02$). Overall, pH was the primary driver of community, with an influence
298 mainly on PC1 ($p=0.03$), but not PC2 (0.06) or PC3. When inoculum was also
299 considered, the evidence of impact was even higher ($p=0.004$). Therefore, both batches
300 were affected by pH, with consistency also from inoculum. The shift in microbial
301 community was mainly due to a shift from *Clostridium sp.*, *Levilinea sp.* and
302 *Nocardioides sp.* at low pH (pH 5.25 and 5.76), *Methanoculleus sp.* was at moderate pH
303 6.15, and *Methanobrevibacter sp.*, *Candidatus Cloacamonas acidaminovorans str.*,
304 *Methanospirillum sp.* and *Methanoculleus sp.* at high pH 7.2 (inoculum). Inoculum
305 appeared to cluster with other high-pH samples with inoculum lying to the far right of
306 the pH correlated PC1. *Bacillus Aquimaris* was the next dominant OTU, and was
307 generally suppressed as pH decreased below 7. Therefore, overall pH decrease caused a
308 loss in diversity in the primary hydrolytic community with a shift towards *Clostridium*
309 from multiple bacterial hydrolytic candidates and a shift within the hydrogen utilizing
310 community towards *Methanoculleus* from *Methanobrevibacter* and *Methanospirillum*.
311 The chemical and BMP analysis identifies a reduction in hydrolytic extent as the main
312 cause of reduced potential (section 3.1). The microbial results support this, with the
313 main impact of low pH operation being related to bacterial (hydrolytic) shift rather than
314 changes in methanogenic archaea.

315

316 3.3. Effect of pH on soluble phosphorus and cations

317 Analyzing all sacrificed bottles, and end points, a strong correlation ($p<0.01$) was found
318 between pH and soluble P (Fig. 4a). Data from both batch tests is integrated to one plot,
319 with further data provided in supplementary material. At initial conditions (pH 6.8), the
320 total P concentration in each BMP bottle was the same, $1060 \pm 90 \text{ mg L}^{-1}$ and soluble P

321 concentration was 215 mg L^{-1} . During the BMP test, highest soluble phosphorus (799
322 mg L^{-1}) was observed at pH 5.25 (75% of the total P), while at neutral pH it was around
323 200 mg L^{-1} . Consequently 57% increase in soluble P was observed at pH 5.25 compared
324 to neutral conditions (pH 7.0 or above) where the soluble P concentrations remained
325 close to initial conditions. This substantially expands the range of analysis previously
326 observed (Mehta and Batstone, 2013) and indicates that almost all P can be released to
327 the soluble phase, if a suitably low pH is selected. Current findings are higher than
328 previous study by Bi et al. (2012) who observed 25% increase in P release at pH 5 (P
329 $168 \text{ mg-P}_{\text{soluble}} \text{ L}^{-1}$) compared to pH 10 ($213 \text{ mg-P}_{\text{soluble}} \text{ L}^{-1}$) in a 20d batch AD of WAS
330 where they started AD directly from pH 5 and 10.

331

332 The increased solubility of P can be related to the dissolution of Ca-P and Mg-P
333 compounds under acidic conditions (Fig. 4b). For the batch test, total calcium and
334 magnesium concentrations were 560 and 273 mg L^{-1} respectively. The soluble calcium
335 concentrations at pH 5.6 and 7.7 were 244.6 and 30 mg L^{-1} respectively. Similarly, the
336 soluble magnesium concentrations at pH 5.6 and 7.7 were 262 and 7 mg L^{-1} respectively
337 thus eliminating the possibility of struvite formation as almost whole magnesium was
338 released at this stage. However, there could be a chance of the formation of calcium
339 phosphates, as 53% of calcium was not released even at low pH (pH 5.6). Therefore, a
340 post treatment could be required to completely release calcium for increasing soluble P
341 contents in effluent. These results are comparable with the findings of (Jardin and
342 Popel, 1994) who also found a linear relationship of calcium release with P but differ
343 from the results of magnesium release with P release.

344

345 3.4. Implications of the findings

346 Struvite precipitation in anaerobic digesters causes increased maintenance costs and
347 phosphorous in biosolids is agriculturally less valuable (or has very limited value)
348 compared to mineral precipitates. This work has shown that low pH operation can
349 alleviate in-reactor precipitation to further enable downstream recovery. The reduction
350 in methane potential at low pH can be addressed through multistage AD process. In a
351 multistage process, struvite crystallization can be performed in the low-pH intermediate
352 stage followed by mesophilic AD to achieve required methane potential. Chemical costs
353 are potentially a problem for low pH AD. However, HCl can be replaced by CO₂ to
354 enable low pH operation, with potentially CO₂ recovered from biogas combustion.
355 Addition of CO₂ can reduce the reactor pH but implementation needs to be addressed in
356 a viable continuous process.

357

358 4. Conclusions

359 Phosphorous release was increased up to 3.6 times under acidic conditions with a 33%
360 reduction in methane yield compared to neutral conditions. Reduction in methane
361 potential at low pH was mainly due to reduced hydrolysis of particulate organic matters,
362 rather than an increase in soluble organics. *Methanosaeta* dominated in general, and was
363 not influenced by pH, but pH caused a shift and narrowing in bacterial diversity towards
364 *Clostridium* and within the hydrogen utilizing methanogens towards *Methanoculleus*.
365 Low pH is a suitable option for enhanced phosphorous release, but work is needed to
366 realize a chemical free option.

367

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375

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449

Table 1: Physico-chemical properties of substrate and inoculum of batch 1 and 2.

Parameter	Substrate		Inoculum		Units
	Batch 1	Batch 2	Batch 1	Batch 2	
pH	6.4	6.7	7.22	7.17	-
COD	44.3	56	26.1	26.3	(g L ⁻¹)
TS	43.6	44.8	30.3	25.6	(g L ⁻¹)
VS	33.4	33.7	20.7	16.5	(g L ⁻¹)
Total P	1060	922	568	476	(mg L ⁻¹)
Soluble P	321	340	385	398	(mg L ⁻¹)
Soluble Ca	78	72	28	32	(mg L ⁻¹)
Soluble Fe	21	20	1	0	(mg L ⁻¹)
Soluble Mg	143	121	13	22	(mg L ⁻¹)
Soluble Na	373	281	406	398	(mg L ⁻¹)
Soluble K	215	207	348	240	(mg L ⁻¹)
TKN	1700	3420	2310	2720	(mg L ⁻¹)
NH ₄ -N	34	49	1324	1140	(mg L ⁻¹)
NO _x -N	4	0	2	1	(mg L ⁻¹)
VFA	7	33	5	12	(mg L ⁻¹)
TOC	118	125	168	227	(mg L ⁻¹)
TIC	86	95	990	939	(mg L ⁻¹)

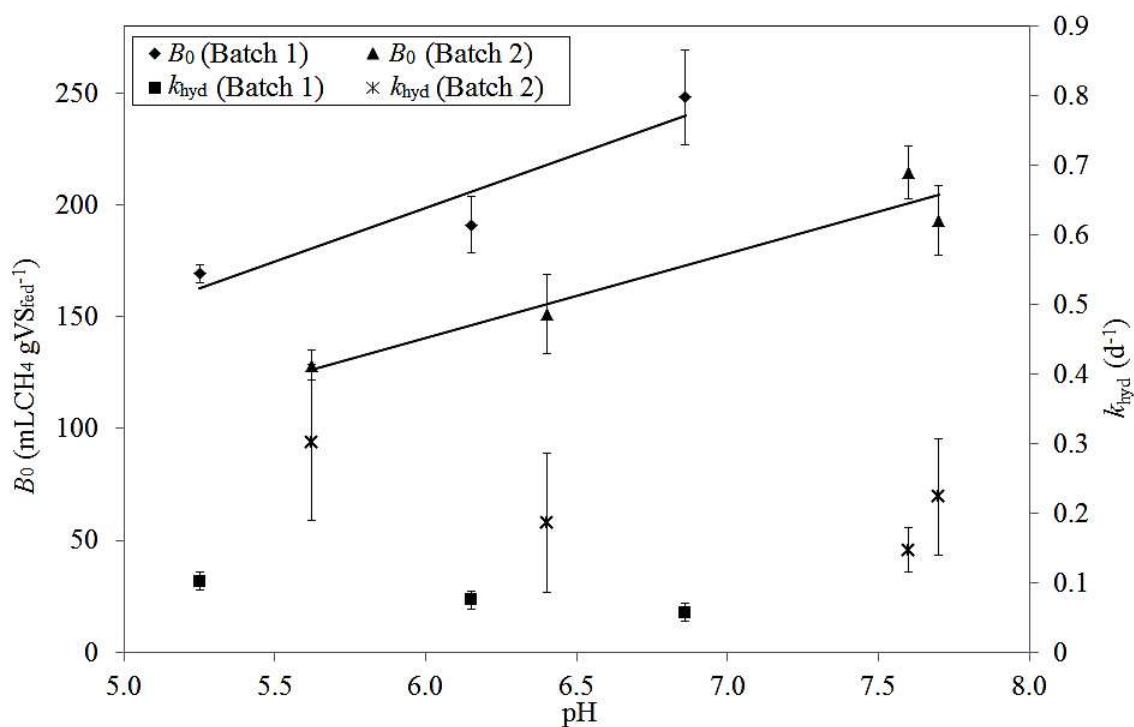


Fig. 1: Effect of pH on degradability, B_0 (mLCH₄ gVS_{fed}⁻¹) and hydrolysis rate, k_{hyd} (d⁻¹) for the batch anaerobic digestion of waste activated sludge. Linear regression lines are representing the possible trend of B_0 and k_{hyd} at each pH.

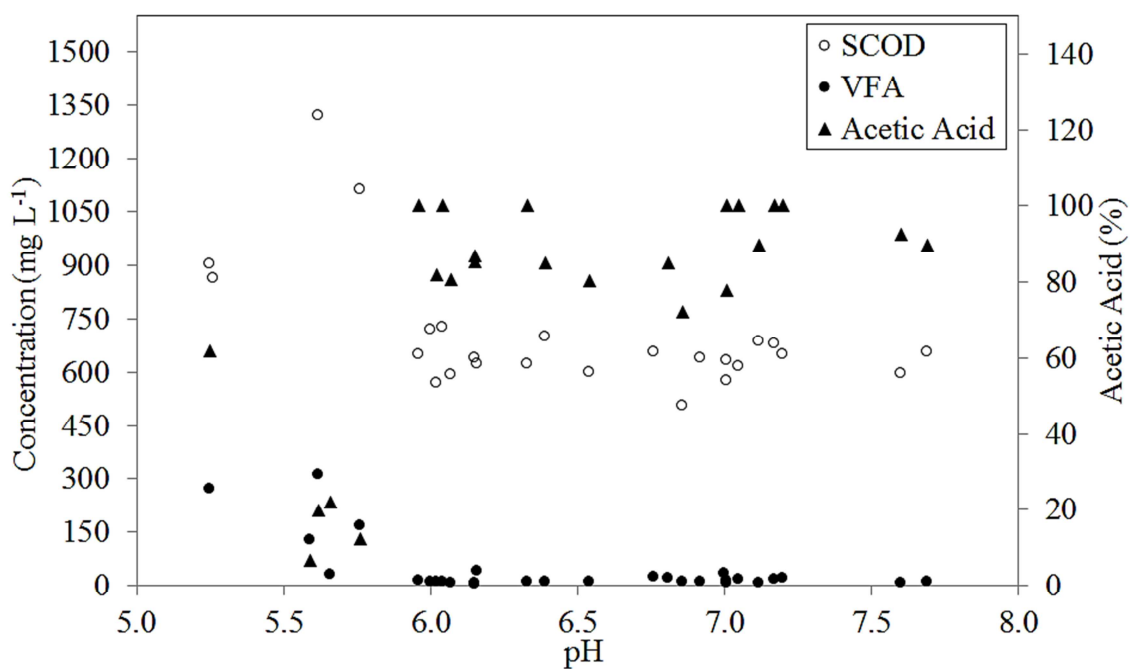


Fig. 2: Concentration of total SCOD and total VFA produced at different pH conditions. Acetic acid as a percentage of total VFA has been shown in secondary axis.

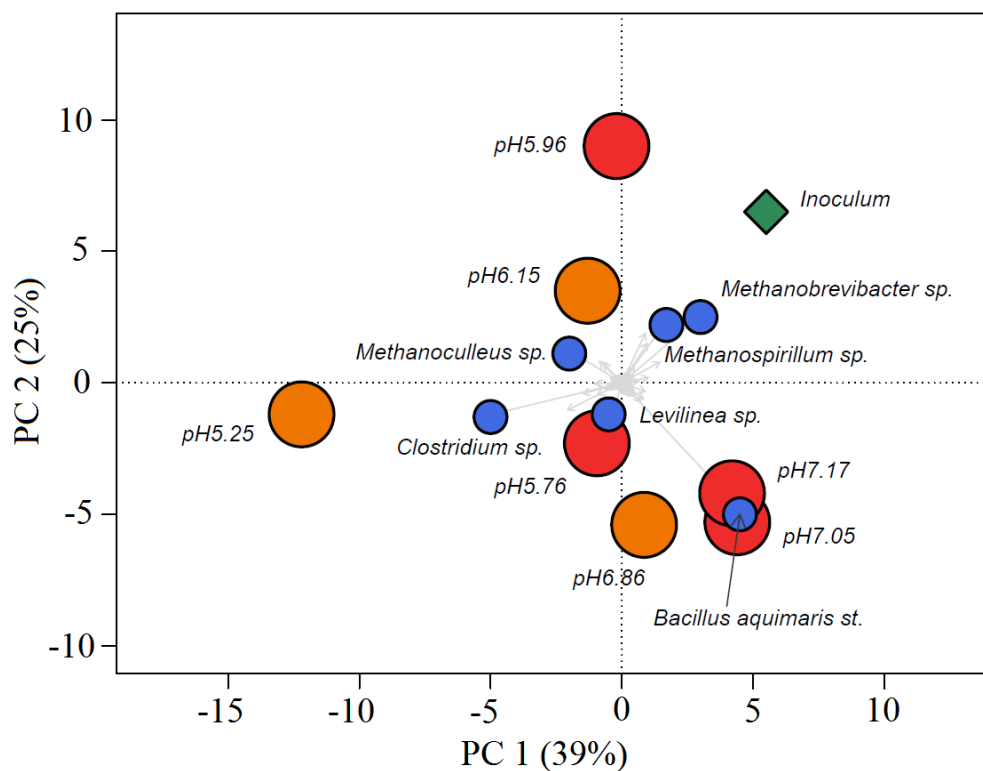


Fig. 3: Principal component analysis biplot at each pH at 35 days of batch 1 and 2. Orange and red circles represent the pH of sludge samples for batch 1 and 2 respectively. Blue circles are the vectors representing orientation of microorganisms with respect to pH. Green box is showing the inoculum of batch 2 only.

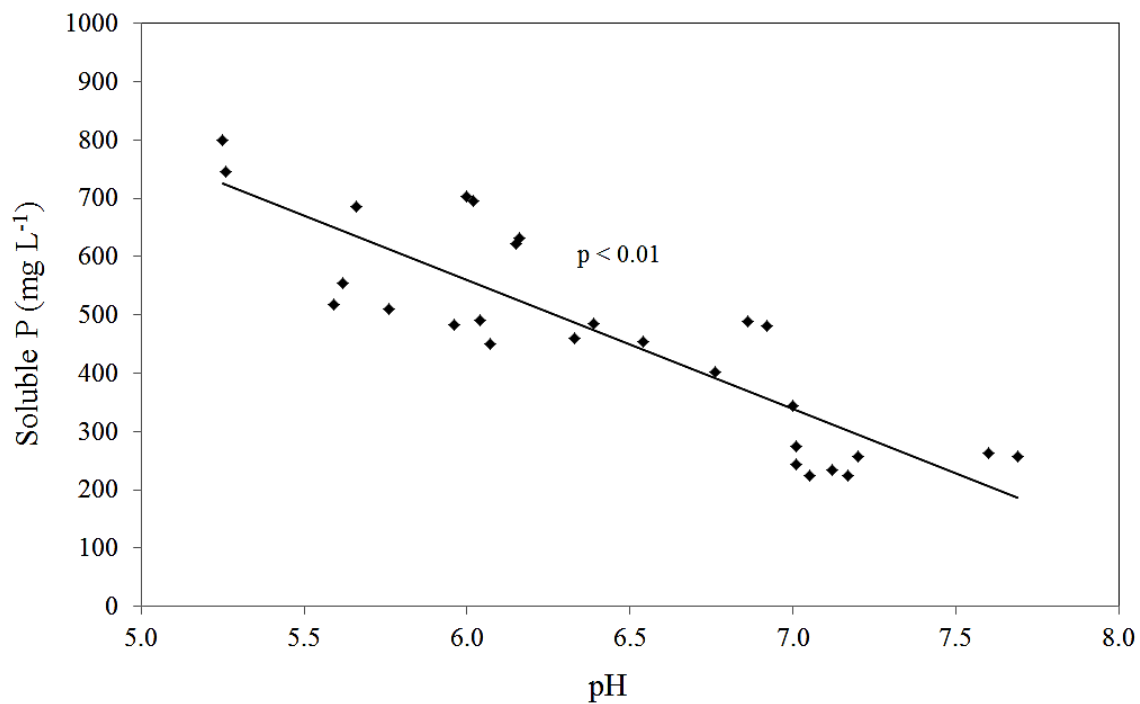


Fig. 4a: Soluble phosphorus concentration during anaerobic digestion of waste activated sludge at different pH values. Linear regression line is showing the possible trend of P release with decreasing pH.

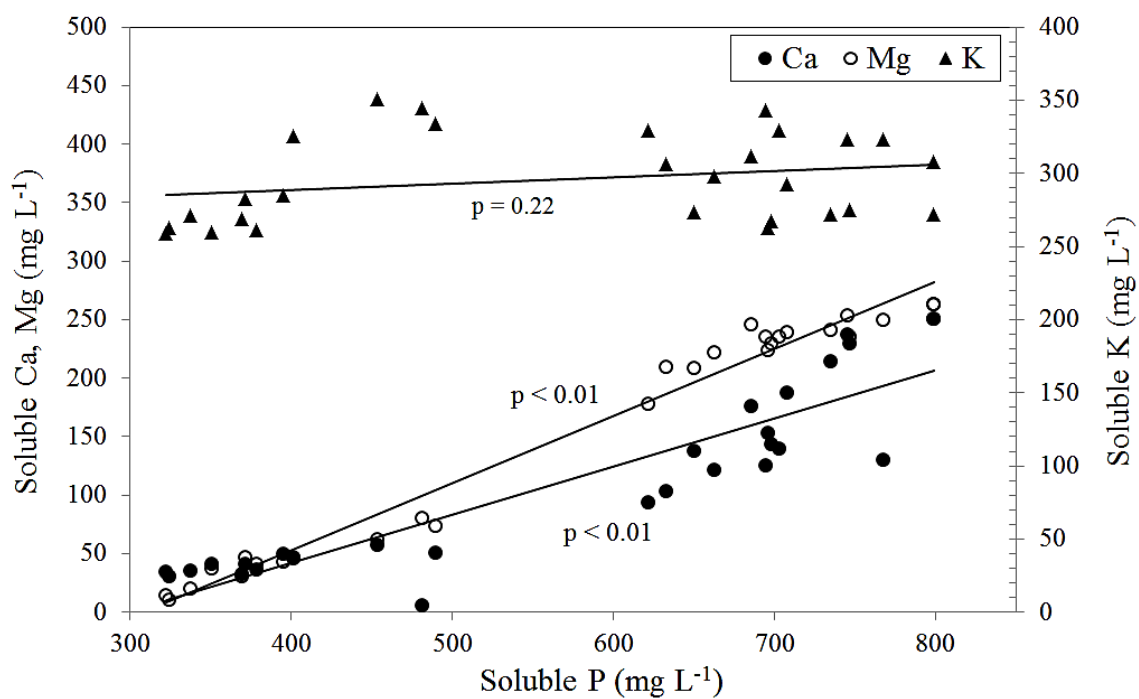


Fig. 4b: Correlation between soluble P, Ca, Mg and K. Linear regression lines are showing the possible trend of cations release with released P.

Highlights

- Preventing phosphorous binding by low pH AD can enable resource recovery.
- A decrease in CH₄ yield at low pH caused decrease in particulates solubilization.
- No change in kinetics at low pH.
- Bacterial community influenced by pH but not acetoclastic methanogens.
- Multi-stage AD with an intermediary struvite recovery could avoid methane loss.

Supplementary Material A

Table: Sacrificing schedule and amount of acid used to achieve desired pH in both experiments

Batch 1				Batch 2			
Days	Final pH	Adjusted pH	HCl (ml)	Days	Final pH	Adjusted pH	HCl ¹ (ml)
7	6.8	5.0	7.6	1.5	6.8	5.0	3.2
15	6.0	5.0	2.5	9	6.0	5.0	0.8
29	5.7	5.0	0.6	21	5.6	5.0	0.4
36	5.3	5.0	0.2	35	5.8	5.0	0.6
51	5.3			51	5.6		
7	6.8	5.7	6.1	1.5	6.8	5.7	2.4
15	6.2	5.7	1.6	9	6.3	5.7	0.6
29	6.2	5.7	0.9	21	6.1	5.7	0.5
36	6.0	5.7	0.3	35	6.0	5.7	0.3
51	6.2			51	6.4		
7	6.8	6.5	2.3	1.5	6.8	7.0	0.2 ²
15	6.5	6.5	0.4	9	7.0	7.0	0.0
29	6.8	6.5	0.9	21	7.0	7.0	0.0
36	6.9	6.5	0.3	35	7.1	7.0	0.0
51	6.9			51	7.6		

¹ as 2M HCl

² as 1M NaOH

Supplementary Material B

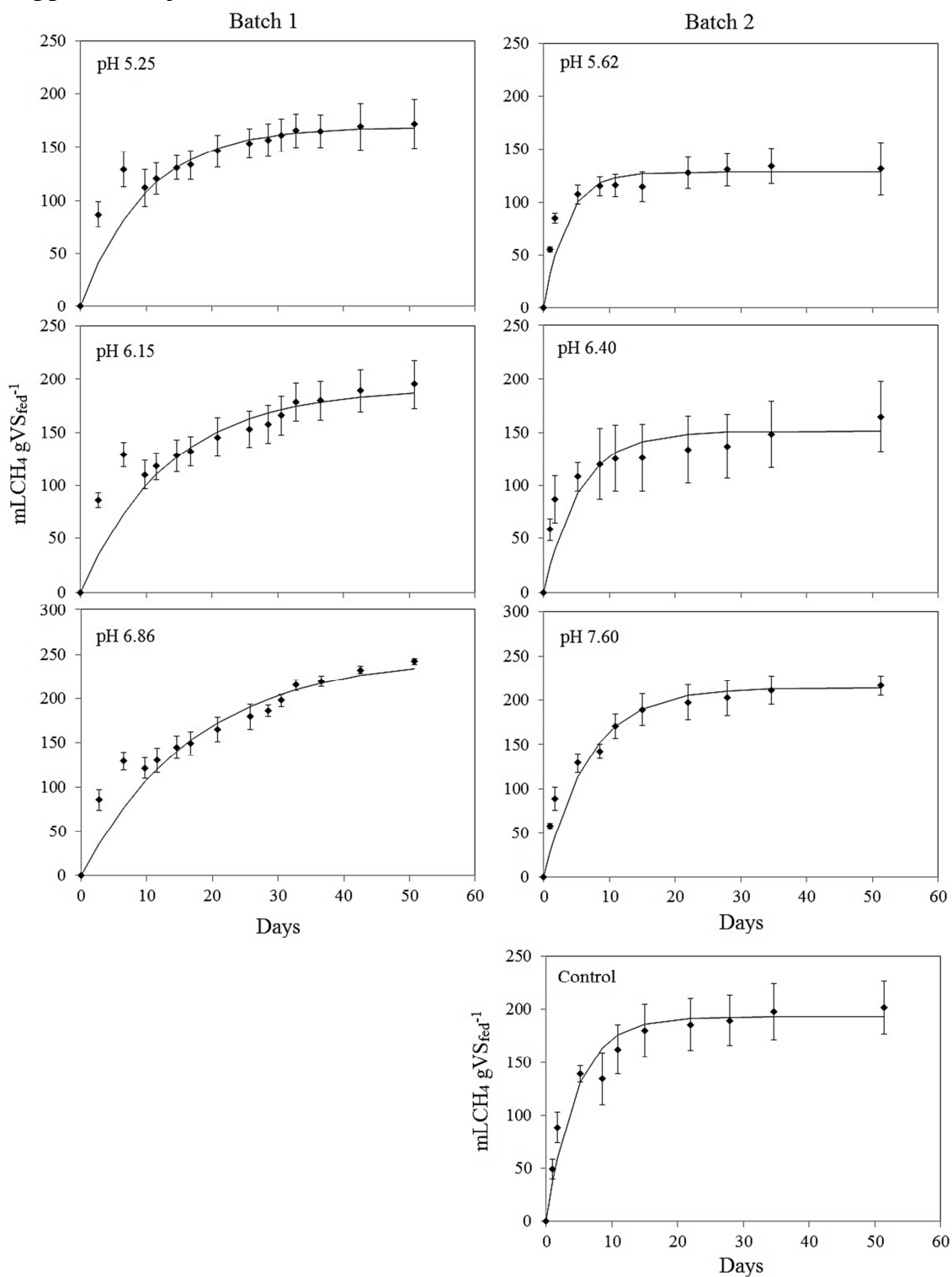


Fig.: Methane production per gVS_{fed} at different pH conditions during batch 1 and 2. The data points are the actual methane produced per gVS_{fed} and lines are fitted by first order kinetic model.

Supplementary Material C

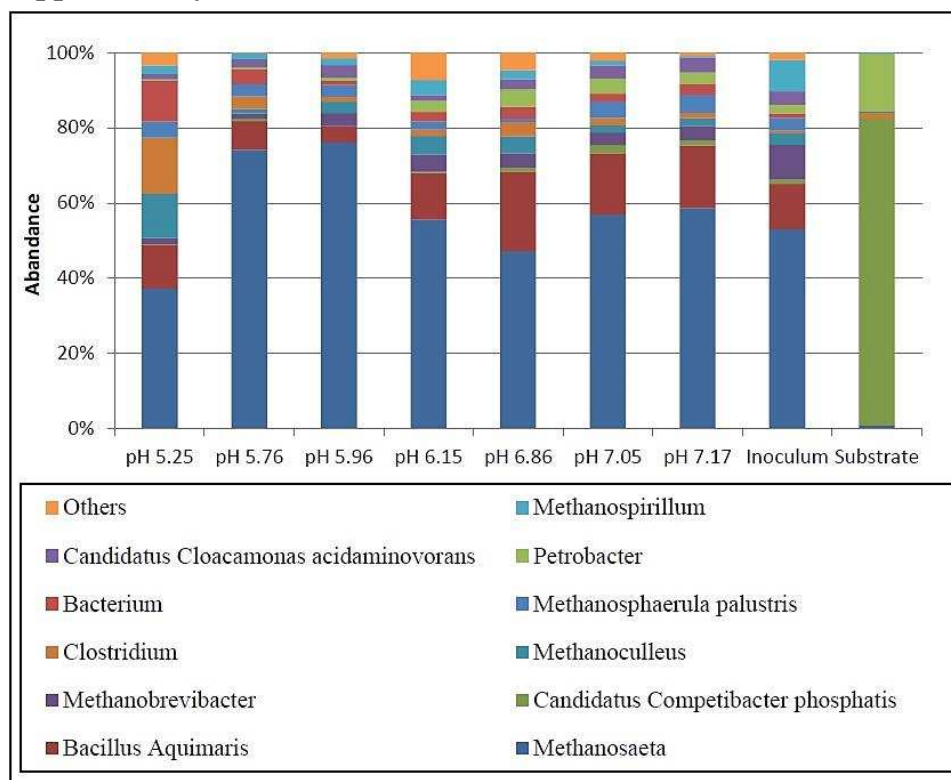


Fig.: Relative abundance of microorganisms at various pH conditions in both batch tests

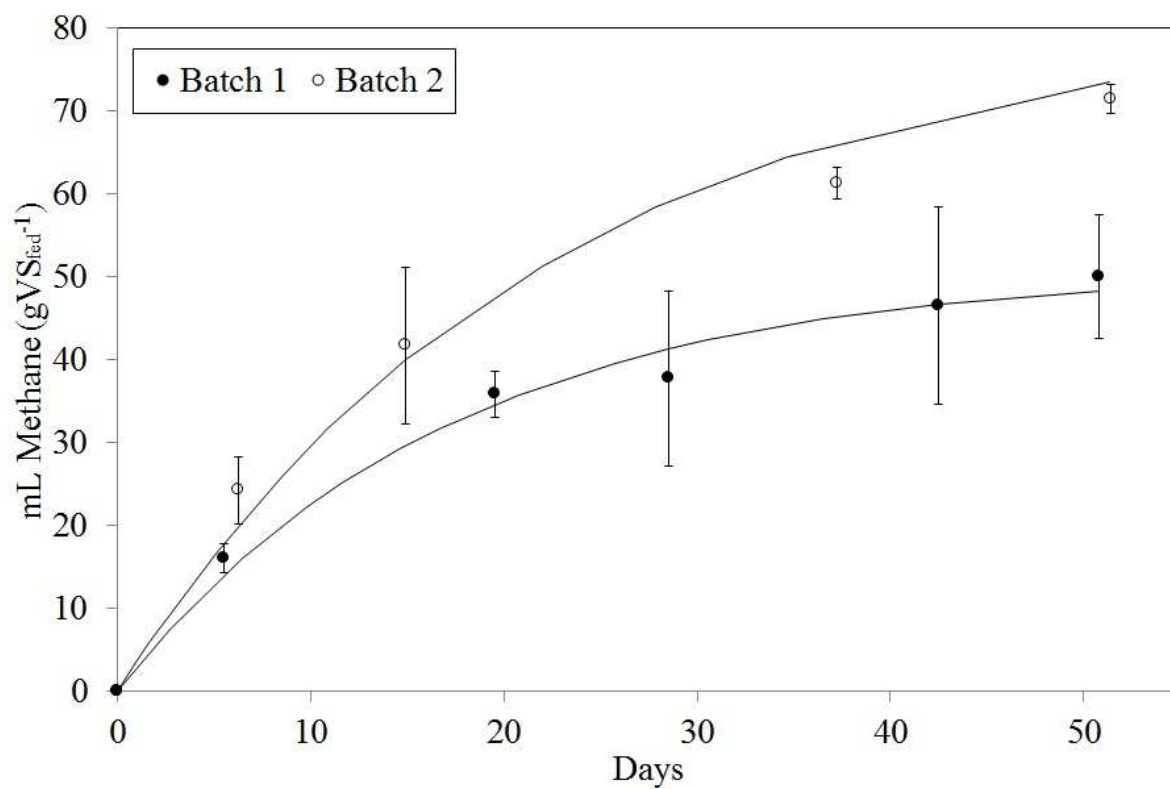


Fig.: Native methane production from inoculums (as blank) per gVS_{fed} along the experimental period and lines are fitted using first order kinetic model.

Supplementary Material E

pH	Total VFA	Acetic Acid (%)	Propionic Acid (%)	Butyric Acid (%)	Valeric Acid (%)	Hexanoic Acid (%)
5.3	273	62	13	10	15	1
5.6	312	20	53	3	23	2
5.7	30	22	18	13	13	34
5.8	168	12	64	0	24	0
6.1	7	81	11	9	0	0
6.2	5	87	0	0	13	0
6.4	9	85	7	0	8	0
6.5	9	80	11	0	8	0
6.8	23	45	27	6	13	9
6.9	9	72	6	1	7	13
7.0	7	78	11	0	11	0
7.1	6	90	0	0	10	0
7.6	7	93	1	0	7	0

Table: Individual VFAs as percentage of total VFA at major pH values of both batch tests.

#FrankenQIIME 1.1.0 OTU Table
#rarefaction_1500_297.txt

	pH 5.76	pH 5.96	pH 7.05	pH 7.17	Inoculum	pH 5.25	pH 6.15	pH 6.86	Substrate	% Identity	total	Alignment	MismatchGaps	Query Star	Query End	Subject Sta	Subject End	E-Value	Hit ID	Consensus Lineage							
0	53.60	59.53	37.20	38.27	32.13	20.47	30.00	25.93	0.20	100	297.3333	250	0	0	1	250	1326	1077	3.00E-139	149173 k_Bacteria	p_Euryarchaeota	c_Methanomicrobia	o_Methanosarcinales	f_Methanosacetaceae	g_Methanoseta	s_	
1	5.53	3.40	10.67	10.87	7.33	6.40	6.73	11.67	0.00	99.6	62.6	250	1	0	1	250	1388	1139	7.00E-137	817453 k_Bacteria	p_OPB8	c_OPB8_1	o_	f_	g_	s_	
14	0.40	0.00	1.40	0.93	0.67	0.13	0.20	0.50	18.33	99.6	22.66667	250	1	0	1	250	1381	1132	7.00E-137	745322 k_Bacteria	p_Proteobacteria	c_Gammaproteobacteria	o_Xanthomonadales	f_Sinobacteraceae	g_	s_	
6	1.00	2.73	2.20	2.47	5.60	0.87	2.47	2.07	0.00	100	19.4	250	0	0	1	250	1266	1017	3.00E-139	204914 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanobacteriales	f_WSA2	g_	s_	
8	0.93	2.40	1.20	1.33	1.87	6.47	2.67	2.47	0.00	99.6	19.33333	250	1	0	1	250	1343	1094	7.00E-137	270985 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanomicrobiales	f_Methanomicrobiaceae	g_Methanoculleus	s_	
3	2.40	1.00	1.33	0.93	0.40	8.20	0.93	2.07	0.33	100	17.6	250	0	0	1	250	1347	1098	3.00E-139	348237 k_Bacteria	p_Firmicutes	c_Clostridia	o_Clostridiales	f_Peptostreptococaceae	g_	s_	
7	2.33	2.40	2.80	3.13	2.07	2.33	1.20	0.53	0.07	98.8	16.86667	250	3	0	1	250	1420	1093	4.00E-132	549891 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanomicrobiales	f_Methanoregellaceae	g_	s_	
4	2.80	0.93	1.40	1.80	0.73	5.93	1.33	1.73	0.00	99.6	16.66667	250	1	0	1	250	1359	1110	7.00E-137	330235 k_Bacteria	p_Chloroflexi	c_Anaerolineae	o_Anaerolineales	f_Anaerolineaceae	g_SHD-231	s_	
9	0.40	0.67	2.60	2.07	1.40	0.20	1.60	2.60	3.47	99.6	15	250	1	0	1	250	1388	1139	7.00E-137	822726 k_Bacteria	p_Proteobacteria	c_Betaproteobacteria	o_SC1-84	f_	g_	s_	
12	1.60	2.60	2.27	2.60	2.13	0.80	0.80	1.40	0.00	100	14.2	234	0	0	17	250	1341	1108	9.00E-130	727822 k_Bacteria	p_WPS-2	c_	o_	f_	g_	s_	
10	1.13	1.40	0.87	0.27	5.07	1.27	2.20	1.33	0.07	100	13.6	250	0	0	1	250	1323	1074	3.00E-139	145915 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanomicrobiales	f_Methanomicrobiaceae	g_Methanoculleus	s_	
2	0.13	1.20	1.40	0.60	1.20	1.87	3.93	2.60	0.00	100	12.93333	234	0	0	17	250	1425	1192	9.00E-130	540398 k_Bacteria	p_Spirochaetes	c_WWE1	o_[Cloacomonales]	f_[Cloacomonaceae]	g_W22	s_	
5	0.67	0.27	1.67	1.40	0.40	2.23	2.00	2.27	1.80	99.6	12.8	250	1	0	1	250	1381	1132	7.00E-137	3951715 k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Actinomycetales	f_	g_	s_	
11	1.53	0.80	1.60	2.40	1.47	1.40	1.07	1.13	0.07	98.8	11.46667	250	3	0	1	250	1361	1112	4.00E-132	2215671 k_Bacteria	p_Chloroflexi	c_Anaerolineae	o_Anaerolineales	f_Anaerolineaceae	g_T78	s_	
15	0.53	1.20	1.47	1.20	0.53	2.20	1.93	2.00	0.00	99.2	11.06667	250	2	0	1	250	1378	1129	2.00E-134	547268 k_Bacteria	p_Spirochaetes	c_Spirochaetes	o_Spirochaetales	f_Spirochaetaceae	g_Treponema	s_	
13	0.60	0.87	1.13	1.20	0.60	1.27	1.53	2.87	0.00	99.6	10.66667	250	1	0	1	250	1316	1067	7.00E-137	339192 k_Bacteria	p_Firmicutes	c_Clostridia	o_OPB54	f_	g_	s_	
16	0.20	0.20	0.67	0.27	0.73	1.80	1.80	1.47	1.40	98.8	8.533333	250	3	0	1	250	1360	1111	4.00E-132	104811 k_Bacteria	p_Chloroflexi	c_Anaerolineae	o_DRC31	f_	g_	s_	
20	1.67	1.33	0.33	0.73	0.73	0.67	0.47	0.07	0.13	1.13	98.6	8.533333	250	1	0	1	250	1255	1006	7.00E-137	167356 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanobacteriales	f_Methanobacteriaceae	g_Methanobrevibacter	s_
24	0.07	0.00	0.20	0.13	0.00	0.00	0.00	0.00	8.00	96.8	8.2	250	8	0	1	250	1292	1043	3.00E-120	2242064 k_Bacteria	p_Bacteroidetes	c_Sphingobacteriia	o_Sphingobacteriales	f_Saprospiraceae	g_	s_	
32	0.87	0.87	1.07	1.13	0.80	0.40	1.27	1.73	0.00	99.8	8.133333	250	1	0	1	250	1386	1137	7.00E-137	109155 k_Bacteria	p_Synergistetes	c_Synergistia	o_Synergistales	f_Thermovirgaceae	g_	s_	
28	0.80	0.13	0.20	1.07	0.47	2.73	0.73	0.93	0.00	99.7	7.066667	250	1	0	1	250	1251	1002	7.00E-137	151815 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanobacteriales	f_Methanobacteriaceae	g_Methanobacterium	s_	
17	0.20	0.60	1.20	0.73	1.20	0.13	1.80	0.87	0.07	97.2	6.8	250	7	0	1	250	1358	1109	1.00E-122	100817 k_Bacteria	p_Chloroflexi	c_Anaerolineae	o_Anaerolineales	f_Anaerolineaceae	g_WCHB1-05	s_	
38	1.80	1.73	0.07	0.00	0.00	1.40	0.20	0.93	0.00	99.6	6.133333	250	1	0	1	250	1393	1144	7.00E-137	584646 k_Bacteria	p_Spirochaetes	c_[Brachyspirae]	o_[Brachyspirales]	f_Brachyspiraceae	g_	s_	
38	0.60	0.07	0.13	0.07	0.33	0.00	0.07	5.40	0.00	99.2	6.066667	251	1	0	1	250	1375	1125	3.00E-139	241514 k_Bacteria	p_Bacteroidetes	c_Sphingobacteriia	o_Sphingobacteriales	f_Chitinophagaceae	g_	s_	
30	1.13	0.33	0.47	0.60	2.07	0.20	0.33	0.07	0.73	100	5.933333	250	0	0	1	250	1351	1102	3.00E-139	1109113 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanosarcinales	f_Methanosarcinaceae	g_Methanosarcina	s_	
29	0.67	0.67	0.40	0.47	0.40	1.53	0.87	0.87	0.00	100	5.866667	247	0	0	4	250	1384	1138	2.00E-137	335574 k_Bacteria	p_Spirochaetes	c_WWE1	o_[Cloacomonales]	f_[Cloacomonaceae]	g_W5	s_	
27	1.27	0.27	0.47	0.13	0.07	2.33	0.27	0.73	0.13	100	5.666667	250	0	0	1	250	1374	1125	3.00E-139	296795 k_Bacteria	p_Firmicutes	c_Bacilli	o_Turicibacterales	f_Turicibacteraceae	g_Turicibacter	s_	
31	0.80	0.80	0.47	0.80	0.00	1.00	0.67	0.67	0.00	99.15	5.2	234	2	0	17	250	1360	1127	6.00E-125	571020 k_Bacteria	p_WS1	c_	o_	f_	g_	s_	
34	0.40	0.13	0.53	0.80	0.40	0.47	0.40	0.33	1.67	99.6	5.133333	250	1	0	1	250	1358	1109	7.00E-137	1111460 k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Actinomycetales	f>Intrasporangiaceae	g_	s_	
22	0.20	0.33	0.33	0.27	0.13	0.33	2.20	1.33	0.00	99.54	5.133333	218	1	0	1	218	1383	1166	8.00E-118	77438 k_Bacteria	p_Thermotogae	c_Thermotogae	o_Thermotogales	f_Thermotogaceae	g_Kosmotoga	s_mrcj	
19	0.27	0.13	0.00	0.40	0.53	1.47	0.67	1.20	0.33	5	5									No blast hit							
25	0.13	0.13	0.40	0.67	0.60	0.27	1.40	1.00	0.00	99.6	4.6	250	1	0	1	250	1369	1120	7.00E-137	148129 k_Bacteria	p_Firmicutes	c_Clostridia	o_SHA_98	f_	g_	s_	
23	0.07	0.33	0.33	0.40	1.13	1.13	1.13	1.07	0.00	96	4.6	250	10	0	1	250	1391	1142	2.00E-115	651287 k_Bacteria	p_Bacteroidetes	c_Bacteroidia	o_Bacteroidales	f_	g_	s_	
21	0.07	0.13	0.73	0.73	0.53	0.40	0.87	1.00	0.00	98.8	4.666667	250	3	0	1	250	1416	1167	4.00E-132	590108 k_Bacteria	p_Proteobacteria	c_Deltaproteobacteria	o_GW-28	f_	g_	s_	
37	0.00	0.13	0.67	0.53	0.40	0.00	0.20	0.73	0.00	99.2	4.2	250	1	0	1	250	1374	1124	2.00E-134	210473 k_Bacteria	p_Proteobacteria	c_Betaproteobacteria	o_Burkholderiales	f_Comamonadaceae	g_	s_	
40	0.00	0.13	0.67	0.80	0.07	0.07	0.07	0.20	1.60	99.2	3.6	250	3	0	1	250	1372	1124	2.00E-134	210473 k_Bacteria	p_Proteobacteria	c_Betaproteobacteria	o_Burkholderiales	f_Comamonadaceae	g_	s_	
51	0.47	0.20	0.20	0.20	0.20	0.93	0.20	0.40	0.27	100	3.066667	250	0	0	1	250	1371	1122	3.00E-139	298037 k_Bacteria	p_Firmicutes	c_Clostridia	o_Clostridiales	f_Lachnospiraceae	g_	s_	
65	0.27	0.33	0.00	0.13	0.20	0.27	0.33	0.33	1.07	99.6	2.933333	250	1	0	1	250	1369	1120	7.00E-137	520213 k_Bacteria	p_Firmicutes	c_Clostridia	o_Natronasporales	f_Anaerobrancheaceae	g_	s_	
86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.87	98.8	2.866667	251	2	1	1	250	1368	1118	2.00E-130	22845 k_Bacteria	p_Chloroflexi	c_Anaerolineae	o_EnvOP52	f_	g_	s_	
33	0.27	0.27	0.67	0.33	0.40	0.07	0.53	0.27	0.00	100	2.8	234	0	0	17	250	1370	1137	9.00E-130	637843 k_Bacteria	p_Proteobacteria	c_Deltaproteobacteria	o_Syntrophobacterales	f_Syntrophorhabdaceae	g_	s_	
82	0.33	0.40	0.53	0.40	0.60	0.00	0.00	0.13	0.40	100	2.8	250	0	0	1	250	1324	1075	3.00E-139	1127026 k_Archaea	p_Euryarchaeota	c_Methanomicrobia	o_Methanomicrobiales	f_Methanospirillaceae	g_Methanospirillum	s_	
79	0.13	0.33	0.73	0.07	0.73	0.07	0.40	0.07	0.00	99.54	2.533333	219	1	0	1	219	1364	1146	2.00E-118	201151 k_Bacteria	p_Bacteroidetes	c_Bacteroidia	o_Bacteroidales	f_	g_	s_	
49	0.13	0.00	0.60	0.40	0.13	0.13	0.20	0.40	0.53	99.2	2.533333	250	1	0	1	250	1355	1106	7.00E-137	96649 k_Bacteria	p_Actinobacteria	c_Actinobacteria	o_Actinomycetales	f_Nocardioideae	g_	s_	
48	0.07	0.00	0.33	0.33	0.20	0.20	0.20	0.53	0.60	99.2	2.466667	250	2	0	1	250											

71	0.00	0.07	0.47	0.07	0.00	0.20	0.13	0.20	0.00	98	1.133333	250	5	0	1	250	1361	1112	2.00E-127	518417	k__Bacteria	p__Chloroflexi	c__Thermomicrobia	o__JG30-KF-CM45	f__	g__	s__	
58	0.13	0.07	0.00	0.27	0.00	0.33	0.27	0.00	0.00	100	1.066667	250	0	0	1	250	1346	1097	3.00E-139	107678	k__Archaea	p__Crenarchaeota	c__MCG	o__pGrC26	f__	g__	s__	
74	0.00	0.00	0.13	0.00	0.00	0.07	0.13	0.00	0.73	99.6	1.066667	250	1	0	1	250	1344	1095	7.00E-137	956624	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Erythroacteraceae	g__	s__	
78	0.27	0.33	0.00	0.07	0.20	0.13	0.07	0.00	0.00	100	1.066667	250	0	0	1	250	1373	1124	3.00E-139	744935	k__Bacteria	p__OP11	c__OP11-2	o__WCHB1-07	f__	g__	s__	
106	0.33	0.07	0.00	0.00	0.40	0.00	0.00	0.27	0.00	100	1.066667	234	0	0	17	250	1335	1102	9.00E-130	699250	k__Bacteria	p__OP8	c__	o__	f__	g__	s__	
107	0.00	0.07	0.07	0.07	0.53	0.00	0.13	0.20	0.00	100	1.066667	234	0	0	17	250	1346	1113	9.00E-130	675414	k__Bacteria	p__WS1	c__	o__	f__	g__	s__	
117	0.00	0.00	0.00	0.07	0.27	0.00	0.00	0.00	0.73	100	1.066667	250	0	0	1	250	1393	1144	3.00E-139	140163	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Rhodocyclales	f__Rhodocyclaceae	g__Methyloversatilis	s__	
260	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.07	98.01	1.066667	251	4	1	1	250	1360	1110	1.00E-125	165491	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__DRC31	f__	g__	s__
69	0.07	0.20	0.00	0.00	0.33	0.00	0.27	0.13	0.00	98.4	1	250	4	0	1	250	1396	1147	9.00E-130	277877	k__Bacteria	p__Spirochaetes	c__Spirochaetes	o__Sphaerochaetales	f__Sphaerochaetaceae	g__Sphaerochaeta	s__	
116	0.27	0.00	0.13	0.20	0.00	0.20	0.13	0.07	0.00	99.6	1	250	1	0	1	250	1428	1179	7.00E-137	105201	k__Bacteria	p__OP9	c__OP946	o__SHA-1	f__	g__	s__	
272	0.00	0.00	0.20	0.07	0.57	0.00	0.07	0.00	0.00	99.6	0.57	250	1	0	1	250	1335	1086	7.00E-137	201253	k__Bacteria	p__Deltaproteobacteria	c__Deltaproteobacteria	o__Syntrophobacteriales	f__Syntrophaceae	g__	s__	
290	0.00	0.00	0.00	0.00	0.93	0.00	0.00	0.00	0.00	99.6	0.933333	247	1	0	4	250	1388	1142	4.00E-135	1884905	k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__	g__	s__	
81	0.07	0.07	0.07	0.20	0.00	0.00	0.40	0.07	0.00	99.2	0.866667	250	2	0	1	250	1272	1023	2.00E-134	811104	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SHA-20	f__	g__	s__	
91	0.13	0.07	0.13	0.07	0.00	0.00	0.20	0.27	0.00	99.6	0.866667	250	1	0	1	250	1383	1134	7.00E-137	2338278	k__Bacteria	p__Synergistetes	c__Synergistetes	o__Synergistales	f__Thermovirgaceae	g__	s__	
108	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.80	98.8	0.866667	250	3	0	1	250	1345	1096	4.00E-132	1141775	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__Pseudonocardiales	g__Pseudonocardia	s__	
109	0.00	0.00	0.00	0.13	0.00	0.13	0.07	0.07	0.47	97.6	0.866667	250	6	0	1	250	1325	1076	6.00E-125	1116330	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__	f__	g__	s__	
133	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.80	96.81	0.866667	251	7	1	1	250	1359	1109	2.00E-118	274989	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__DRC31	f__	g__	s__	
158	0.00	0.00	0.07	0.00	0.13	0.07	0.07	0.00	0.53	99.2	0.866667	250	2	0	1	250	1389	1140	2.00E-134	1139633	k__Bacteria	p__Bacteroidetes	c__Sphingobacteria	o__Sphingobacteriales	f__	g__	s__	
68	0.07	0.07	0.13	0.07	0.07	0.13	0.20	0.07	0.00	99.6	0.8	250	1	0	1	250	1332	1083	7.00E-137	565215	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__SHD-231	s__	
101	0.07	0.07	0.07	0.07	0.07	0.20	0.20	0.07	0.00	98.8	0.8	250	3	0	1	250	1355	1106	4.00E-132	831305	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__T78	s__	
125	0.07	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.60	98.8	0.8	250	3	0	1	250	1375	1126	4.00E-132	1991830	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__Actinomycetales	f__	g__	s__	
134	0.00	0.00	0.13	0.07	0.00	0.20	0.27	0.07	0.00	98.8	0.8	250	1	1	1	250	1341	1094	2.00E-130	511430	k__Bacteria	p__Actinobacteria	c__Acidimicrobia	o__Acidimicrobiales	f__	g__	s__	
148	0.00	0.00	0.00	0.00	0.07	0.00	0.13	0.13	0.47	97.2	0.8	250	7	0	1	250	1382	1133	1.00E-122	1144467	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Chromatiaceae	g__Thiococcus	s__	
166	0.00	0.00	0.00	0.07	0.33	0.00	0.33	0.07	0.00	100	0.8	234	0	0	17	250	1368	1135	9.00E-130	534812	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Syntrophobacteriales	f__Syntrophorhabdaceae	g__	s__	
50	0.00	0.00	0.00	0.00	0.00	0.27	0.13	0.33	0.00	98.4	0.733333	250	4	0	1	250	1396	1147	9.00E-130	220428	k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__	g__	s__	
56	0.00	0.07	0.07	0.27	0.07	0.00	0.20	0.07	0.00	100	0.733333	234	0	0	17	250	1338	1105	9.00E-130	562214	k__Bacteria	p__Synergistetes	c__Synergistetes	o__Synergistales	f__Dethiosulfobrivionaceae	g__HA73	s__	
76	0.00	0.07	0.13	0.00	0.13	0.13	0.20	0.07	0.00	90.36	0.733333	249	22	2	1	248	1355	1108	1.00E-76	781763	k__Bacteria	p__NK819	c__	o__	f__	g__	s__	
87	0.07	0.00	0.20	0.13	0.00	0.07	0.07	0.00	0.20	99.2	0.733333	250	2	0	1	250	1403	1154	2.00E-134	546149	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__	f__	g__	s__	
89	0.00	0.00	0.00	0.27	0.00	0.00	0.07	0.40	0.00	100	0.733333	234	0	0	17	250	1348	1115	9.00E-130	792729	k__Bacteria	p__Bacteroidetes	c__Sphingobacteria	o__Sphingobacteriales	f__Saprospiraceae	g__	s__	
132	0.13	0.13	0.13	0.20	0.13	0.00	0.00	0.00	0.00	99.2	0.733333	250	2	0	1	250	1321	1072	2.00E-134	145231	k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanomicrobiales	f__Methanospirillaceae	g__Methanospirillum	s__	
277	0.07	0.00	0.00	0.07	0.00	0.00	0.33	0.00	0.27	99.6	0.733333	250	1	0	1	250	1349	1100	7.00E-137	89473	k__Bacteria	p__Cyanobacteria	c__AC01-2	o__MLE1-12	f__	g__	s__	
139	0.00	0.00	0.13	0.20	0.00	0.13	0.07	0.00	0.20	99.2	0.733333	250	2	0	1	250	1390	1141	2.00E-134	814427	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Burkholderiaceae	g__	s__	
99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.73	99.2	0.733333	250	2	0	1	250	1367	1118	2.00E-134	819575	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__envOPS12	f__	g__	s__	
213	0.00	0.00	0.00	0.00	0.07	0.00	0.07	0.00	0.60	92.68	0.733333	246	18	0	1	246	1325	1080	6.00E-94	1116330	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__	f__	g__	s__	
66	0.00	0.00	0.07	0.13	0.40	0.00	0.07	0.00	0.00	99.6	0.666667	250	1	0	1	250	1394	1145	7.00E-137	1059339	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Syntrophobacteriales	f__Syntrophaceae	g__Syntrophus	s__	
138	0.00	0.13	0.07	0.13	0.07	0.00	0.00	0.07	0.20	100	0.666667	234	0	0	17	250	1332	1099	9.00E-130	560432	k__Bacteria	p__Armatimonadetes	c__[Fimbrimonadetes]	o__[Fimbrimonadales]	f__[Fimbrimonadaceae]	g__	s__	
162	0.00	0.00	0.00	0.13	0.07	0.00	0.07	0.07	0.33	97.6	0.666667	250	6	0	1	250	1355	1106	6.00E-125	576076	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SHA-20	f__	g__	s__	
204	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	99.2	0.666667	251	1	1	1	250	1379	1129	1.00E-132	3461889	k__Bacteria	p__Bacteroidetes	c__Sphingobacteria	o__Sphingobacteriales	f__Saprospiraceae	g__	s__	
285	0.00	0.00	0.20	0.00	0.33	0.00	0.07	0.07	0.00	99.6	0.666667	250	1	0	1	250	1411	1162	7.00E-137	13692	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Syntrophomonadaceae	g__Syntrophomonas	s__	
77	0.00	0.07	0.07	0.00	0.00	0.00	0.53	0.00	0.00	99.6	0.666667	250	1	0	1	250	1357	1108	7.00E-137	223241	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__T78	s__	
104	0.00	0.00	0.07	0.00	0.40	0.07	0.07	0.00	0.00	1138	2.00E-134	145398	k__Bacteria	2	0	1	250	1387	1138	2.00E-134	145398	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Syntrophobacteriales	f__Syntrophaceae	g__	s__
156	0.07	0.07	0.00	0.00	0.00	0.00	0.40	0.13	0.00	97.2	0.666667	250	7	0	1	250	1360	1111	1.00E-122	104665	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__WCHB1-05	s__	
160	0.13	0.00	0.13	0.13	0.13	0.00	0.07	0.07	0.00	100	0.666667	234	0	0	17	250	1359	1126	9.00E-130	562337	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__	s__	
188	0.00	0.07	0.00	0.00	0.60	0.00	0.00	0.00	0.00	100	0.666667	234	0	0	17	250	1357	1124	9.00E-130	772955	k__Bacteria	p__Firmicutes	c__RF3	o__ML6151-28	f__	g__	s__	
131	0.00	0.00	0.13	0.00	0.07	0.00	0.00	0.00	0.40	99.2	0.6	250	2	0	1	250	1390	1141	2.00E-134	525955	k__Bacteria	p__Bacteroidetes	c__Flavobacteria	o__	f__	g__	s__	
146	0.00	0.00	0.13	0.47	0.00	0.00	0.00	0.00	0.00	99.2	0.6	250	2	0	1	250	1246	997	2.00E-134	581399	k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanomicrobiales	f__Methanospirillaceae	g__Methanospirillum	s__	
161	0.00	0.00	0.07	0.13	0.33	0.00	0.07	0.00	0.00	99.6	0.6	250	1	0	1	250	1386	1137	7.00E-137	3240442	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__Clostridium	s__	
202	0.00	0.00	0.07	0.07	0.00	0.00	0.07	0.00	0.40	99.6	0.6	250	1	0	1	250	1390	1141	7.00E-137	579023	k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__SC1-84	f__	g__	s__	
209	0.00	0.00	0.00	0.07	0.00	0.07	0.27	0.00	0.20	98	0.6	250	5	0														

481	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	94.42	0.133333	251	13	1	1	250	1391	1141	4.00E-104	538801	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__	s__
483	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	94.8	0.133333	250	13	0	1	250	1351	1102	3.00E-108	538684	k__Bacteria	p__Spirrochaetes	c__Sphingobacteria	o__Sphingobacteriales	f__	g__	s__
493	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	98.61	0.133333	216	3	0	17	232	1425	1210	8.00E-112	540398	k__Bacteria	p__Spirochaetes	c__WWE1	o__[Cloacamonales]	f__[Cloacamonaceae]	g__	W22
495	0.00	0.13	0.00	0.00	0.00	0.00	0.00	0.00	91.9	0.133333	247	20	0	1	247	1320	1074	9.00E-90	279611	k__Bacteria	p__OD1	c__SM2F11	o__	f__	g__	s__
496	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	94.02	0.133333	234	13	1	1	233	1315	1082	6.00E-94	265485	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__	WCHB1-05
509	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.07	97.2	0.133333	250	7	0	1	250	1344	1095	1.00E-122	1129233	k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanosarcinales	f__Methanosaetaceae	g__	Methanosaeta
514	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	98	0.133333	250	5	0	1	250	1312	1063	2.00E-127	2547741	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__	g__	AB4
516	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	94.78	0.133333	249	12	1	2	250	1296	1049	3.00E-105	577182	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__	s__
526	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	97.36	0.133333	227	6	0	1	227	1371	1145	3.00E-111	111350	k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__	g__	s__
533	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.07	99.2	0.133333	251	1	1	1	250	1317	1067	1.00E-132	639742	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Planctomycetiales	f__Planctomycetaceae	g__	Planctomyces
536	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	94.8	0.133333	250	12	0	1	250	1388	1139	3.00E-108	837453	k__Bacteria	p__OP8	c__OP8_1	o__	f__	g__	s__
563	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	95.2	0.133333	250	12	0	1	250	1362	1113	1.00E-110	155633	k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__	g__	s__
564	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	96.8	0.133333	250	8	0	1	250	1415	1166	3.00E-120	349535	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Deltavibrionales	f__Bacterioviraceae	g__	s__
588	0.07	0.00	0.00	0.07	0.00	0.00	0.00	0.00	96.15	0.133333	234	9	0	17	250	1308	1075	3.00E-108	572118	k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanomicrobiales	f__	g__	s__
594	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	99.15	0.133333	234	2	0	1	234	1340	1107	6.00E-125	784307	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__	T78
604	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	98.15	0.133333	250	4	0	1	250	1344	1095	9.00E-130	3880686	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Sphingomonadales	f__Sphingomonadaceae	g__	s__
611	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	98.61	0.133333	216	3	0	17	232	1425	1210	8.00E-112	540398	k__Bacteria	p__Spirochaetes	c__WWE1	o__[Cloacamonales]	f__[Cloacamonaceae]	g__	W22
614	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	97.2	0.133333	250	7	0	1	250	1348	1099	1.00E-122	358361	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	g__	s__
616	0.00	0.00	0.00	0.00	0.00	0.07	0.07	0.00	99.2	0.133333	250	2	0	1	250	1323	1074	2.00E-134	838180	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacteriales	f__Hyphomonadaceae	g__	s__
652	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	98.4	0.133333	250	4	0	1	250	1433	1184	9.00E-130	579324	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Dehalobacteriaceae	g__	Dehalobacterium
657	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.2	0.133333	250	11	0	1	250	1306	1057	5.00E-113	3230114	k__Bacteria	p__NK1819	c__TSBW08	o__	f__	g__	s__
659	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	92.95	0.133333	227	16	0	1	227	1377	1151	2.00E-87	684719	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	g__	PD-UASB-13
666	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	98.8	0.133333	250	3	0	1	250	1374	1125	4.00E-132	346233	k__Bacteria	p__Synergistetes	c__Synergistia	o__Synergistales	f__Dethiosulfobionaceae	g__	s__
675	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	94.8	0.133333	250	13	0	1	250	1245	996	3.00E-108	140685	k__Archaea	p__Euryarchaeota	c__Methanomicrobia	o__Methanomicrobiales	f__Methanoculleus	g__	s__
685	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	99.55	0.133333	224	1	0	27	250	1371	1148	2.00E-121	741310	k__Bacteria	p__OP8	c__OP8_1	o__	f__	g__	s__
686	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	92.8	0.133333	250	18	0	1	250	1323	1074	2.00E-96	3809681	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__	g__	s__
688	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	92.4	0.133333	250	19	0	1	250	1396	1147	6.00E-94	1147253	k__Bacteria	p__Proteobacteria	c__SJA-4	o__	f__	g__	s__
689	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	98	0.133333	250	5	0	1	250	1396	1147	2.00E-127	2051918	k__Bacteria	p__Acidobacteria	c__Holophagae	o__Holophagales	f__Holophagaceae	g__	Geothrix
695	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	99.6	0.133333	250	1	0	1	250	1369	1120	7.00E-137	342714	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Hallangiaceae	g__	s__
702	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	96.4	0.133333	250	9	0	1	250	1391	1142	8.00E-118	822086	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Eubacteriaceae	g__	s__
703	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	94.38	0.133333	249	13	1	1	248	1366	1118	3.00E-108	806329	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__GCAD04	f__	g__	s__
713	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	98.41	0.133333	251	3	1	1	250	1389	1139	6.00E-128	221605	k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__	g__	s__
716	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.6	0.133333	250	1	0	1	250	1397	1148	7.00E-137	1143179	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__Hallangiaceae	g__	s__
721	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	99.6	0.133333	250	1	0	1	250	1359	1110	7.00E-137	271289	k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__	Clostridium
729	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	98.4	0.133333	250	4	0	1	250	1403	1154	9.00E-130	552017	k__Bacteria	p__NK1819	c__TSBW08	o__	f__	g__	s__
730	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	93.6	0.133333	250	16	0	1	250	1376	1127	4.00E-101	1770075	k__Bacteria	p__Planctomycetes	c__Pla3	o__	f__	g__	s__
741	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	98.8	0.133333	250	3	0	1	250	1309	1060	4.00E-132	188150	k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodobacteriales	f__Rhodobacteraceae	g__	s__
743	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	99.2	0.133333	250	2	0	1	250	1310	1061	2.00E-134	1963655	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__	g__	s__
744	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	98.4	0.133333	250	4	0	1	250	1329	1080	3.00E-117	807206	k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Syntrophobacteriales	f__Syntrophaceae	g__	s__
757	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.00	99.2	0.133333	250	2	0	1	250	1393	1144	2.00E-134	344848	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__	s__
764	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	98.4	0.133333	250	4	0	1	250	1399	1150	9.00E-130	647335	k__Bacteria	p__Spirochaetes	c__Spirochaetes	o__Spirochaetales	f__Treponemaceae	g__	Treponema
766	0.00	0.00	0.00	0.07	0.00	0.07	0.00	0.00	97.2	0.133333	250	7	0	1	250	1420	1171	1.00E-122	97060	k__Bacteria	p__Actinobacteria	c__Actinobacteria	o__WCHB1-81	f__	g__	A1425_EubF1
111	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	97.2	0.133333	250	7	0	1	250	1391	1142	1.00E-122	240019	k__Bacteria	p__Planctomycetes	c__Planctomycetia	o__Pirellulales	f__Pirellulaceae	g__	s__
129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	99.6	0.133333	250	1	0	1	250	1338	1089	7.00E-137	2183844	k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Caldilineales	f__Caldilineaceae	g__	s__
143	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	99.6	0.133333	251	0	1	1	250	1414	1164	4.00E-135	202078	k__Bacteria	p__WS6	c__SC72	o__A-2AF	f__	g__	s__
150	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.6	0.133333	250	1	0	1	250	1341	1092	7.00E-137	1111079	k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__	g__	s__
153	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	96.69	0.133333	242	8	0	1	242	1375	1134	2.00E-115	823226	k__Bacteria	p__Chlamydiae	c__Chlamydia	o__Chlamydiales	f__Rhabdochlamydiaceae	g__	Candidatus Rhabdochlamydia
176	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	96.4	0.133333	250	9	0	1	250	1307	1058	8.00E-118	314077	k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__	s__
185	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	86.32	0.133333	234	31	1	1	233	1300	1067	6.00E-51	747039	k__Bacteria	p__	c__	o__	f__	g__	s__
210	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	96.4	0.133333	250	8	0	1	250	1493	1244	3.00E-120	142693	k__Bacteria	p__OP9	c__J51	o__BA021	f__	g__	s

592	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	96.8	0.066667	250	8	0	1	250	1372	1123	3.00E-120	817930_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__	g__	s__		
593	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	98.8	0.066667	250	3	0	1	250	1346	1097	4.00E-132	782547_k__Bacteria	p__Proteobacteria	c__Alphaproteobacteria	o__Rhodospirillales	f__Acetobacteraceae	g__	s__		
595	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.6	0.066667	250	1	0	1	250	1416	1167	7.00E-137	160380_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Syntrophomonadaceae	g__Syntrophomonas	s__		
597	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	100	0.066667	234	0	0	17	250	1368	1135	9.00E-130	533248_k__Bacteria	p__NKB19	o__	f__	g__	s__	
598	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	94	0.066667	250	15	0	1	250	1266	1017	2.00E-103	204914_k__Archaea	p__Euryarchaeota	c__Methanobacteria	o__Methanobacteriales	f__WSA2	g__	s__
599	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	98.4	0.066667	250	4	0	1	250	1365	1116	9.00E-130	1125298_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Ruminococcaceae	g__Ruminococcus	s__
600	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	98	0.066667	250	5	0	1	250	1359	1110	2.00E-127	232590_k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Bdellovibrionales	f__Bacterioviracaceae	g__	s__
601	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	96.4	0.066667	250	8	1	1	250	1369	1121	2.00E-115	138611_k__Bacteria	p__Firmicutes	c__Clostridia	o__SHA-98	f__	g__	s__
605	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	95.7	0.066667	187	8	0	1	187	1382	1196	1.00E-82	1144467_k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Chromatiales	f__Chromatiaceae	g__Thiococcus	s__
606	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	99.6	0.066667	250	1	0	1	250	1371	1122	7.00E-137	147679_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__	g__	s__		
612	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	99.6	0.066667	251	1	0	0.07	1	250	1393	1144	7.00E-137	145637_k__Bacteria	p__Bacteroidetes	c__Bacteroidetes	o__Nitrosomonadales	f__Nitrosomonadaceae	g__	s__	
613	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	91.6	0.066667	251	20	1	1	250	1376	1126	2.00E-87	152262_k__Bacteria	p__Chlamydiae	c__Chlamydiae	o__Chlamydiales	f__	g__	s__		
621	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	94	0.066667	250	15	0	1	250	1351	1102	2.00E-103	1109113_k__Archaea	p__Euryarchaeota	c__Methanomicrobium	o__Methanosarcinales	f__Methanosarcinaceae	g__Methanosarcina	s__		
623	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	91.88	0.066667	234	19	0	1	234	1356	1123	2.00E-84	303832_k__Bacteria	p__Firmicutes	c__Clostridia	o__Coriobacteriales	f__	g__	s__
625	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	99.19	0.066667	246	2	0	1	246	1358	1113	4.00E-132	635410_k__Bacteria	p__Proteobacteria	c__Betaproteobacteria	o__Burkholderiales	f__Comamonadaceae	g__	s__
627	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	95.2	0.066667	250	12	0	1	250	1388	1139	1.00E-110	821341_k__Bacteria	p__Firmicutes	c__Clostridia	o__SHA-98	f__	g__	s__
628	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	97.01	0.066667	234	7	0	1	234	1381	1148	5.00E-113	629200_k__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Xanthomonadales	f__Sinobacteraceae	g__	s__
629	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	97.2	0.066667	250	7	0	1	250	1359	1110	1.00E-122	330235_k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__SHD-231	s__
631	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	93.09	0.066667	246	17	0	5	250	1383	1138	3.00E-86	792230_k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__	g__	s__		
637	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	98	0.066667	250	5	0	1	250	1312	1063	2.00E-127	809180_k__Bacteria	p__Chloroflexi	c__Dehalococcoidetes	o__FS117-238-02	f__	g__	s__
639	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	96.6	0.066667	235	8	0	1	235	1358	1124	3.00E-111	100817_k__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__WCHB1-05	s__
640	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	99.2	0.066667	250	2	0	1	250	1311	1062	5.00E-122	575820_k__Bacteria	p__	c__	o__	f__	g__	s__
642	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	94.02	0.066667	251	14	1	1	250	1369	1119	1.00E-101	520213_k__Bacteria	p__Firmicutes	c__Clostridia	o__Natranaerobiales	f__Anaerobrancaeae	g__	s__		
644	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	96.4	0.066667	250	9	0	1	250	1369	1120	8.00E-118	103256_k__Bacteria	p__Firmicutes	c__Clostridia	o__MBA08	f__	g__	s__
646	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	93.23	0.066667	251	16	1	1	250	1383	1133	6.00E-97	576477_k__Bacteria	p__Armatimonadetes	c__SIA-176	o__	f__	g__	s__
648	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	97.2	0.066667	250	7	0	1	250	1362	1113	1.00E-122	287441_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Peptostreptococcaceae	g__	s__
649	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.066667								No blast hit								
651	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	94	0.066667	250	15	0	1	250	1424	1175	2.00E-103	156269_k__Bacteria	p__TA06	c__	o__	f__	g__	s__
654	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	95.18	0.066667	249	12	0	2	250	1380	1132	5.00E-110	215003_k__Bacteria	p__Actinobacteria	c__Acidimicrobia	o__Acidimicrobiales	f__	g__	s__		
655	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	96.8	0.066667	250	8	0	1	250	1387	1138	3.00E-120	1140627_k__Bacteria	p__Bacteroidetes	c__Sphingobacteria	o__Sphingobacteriales	f__	g__	s__		
656	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	99.6	0.066667	250	1	0	1	250	1315	1067	7.00E-137	1141697_k__Bacteria	p__Bacteroidetes	c__Bacteroidia	o__Bacteroidales	f__Bacteroidaceae	g__	s__		
658	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	92.4	0.066667	250	19	0	1	250	1394	1145	6.00E-94	2174217_k__Bacteria	p__BRC1	c__NPL-UPA2	o__	f__	g__	s__		
660	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	92.27	0.066667	233	18	0	1	233	1314	1082	3.00E-86	589266_k__Bacteria	p__Chloroflexi	c__SAR202	o__	f__	g__	s__		
662	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	98.8	0.066667	250	3	0	1	250	1390	1141	4.00E-132	1105310_k__Bacteria	p__OP9	c__OPB46	o__OPB72	f__TBD11	g__	s__
663	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	89.79	0.066667	235	23	1	1	235	1389	1156	1.00E-70	2881195_k__Bacteria	p__Gemmatimonadetes	c__Gemm-5	o__	f__	g__	s__
665	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	95.55	0.066667	247	11	0	1	247	1379	1133	3.00E-111	91474_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Ruminococcaceae	g__Ethanologenes	s__harbiense
667	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	93.2	0.066667	250	16	1	1	250	1369	1121	2.00E-96	151986_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Caloramator	g__	s__
668	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	94.8	0.066667	250	13	0	1	250	1373	1124	3.00E-108	744935_k__Bacteria	p__OP11	c__OP11-2	o__WCHB1-07	f__	g__	s__		
670	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	99.6	0.066667	250	1	0	1	250	1401	1152	7.00E-137	824462_k__Bacteria	p__Actinobacteria	c__OPB41	o__	f__	g__	s__
671	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	96.8	0.066667	250	8	0	1	250	1329	1080	3.00E-120	209573_k__Bacteria	p__Bacteroidetes	c__Sphingobacteria	o__Sphingobacteriales	f__	g__	s__		
672	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	96.8	0.066667	250	8	0	1	250	1379	1130	3.00E-120	821689_k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Syntrophobacteriales	f__Syntrophaceae	g__	s__		
676	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	93.99	0.066667	233	14	0	1	233	1389	1157	6.00E-97	53679_k__Bacteria	p__OP8	c__OP8_1	o__	f__	g__	s__
679	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	93.56	0.066667	233	15	0	1	233	1351	1119	2.00E-93	832804_k__Bacteria	p__Actinobacteria	c__Actinomycetales	o__Actinomycetales	f__	g__	s__		
681	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	97	0.066667	250	10	0	1	250	1411	1162	2.00E-115	141905_k__Bacteria	p__Proteobacteria	c__Deltaproteobacteria	o__Myxococcales	f__	g__	s__		
682	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	99.2	0.066667	251	1	1	1	250	1387	1137	1.00E-132	259078_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__	s__		
687	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.066667								No blast hit								
693	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100	0.066667	214	0	0	37	250	1244	1031	8.00E-118	1767034_k__Bacteria	p__Armatimonadetes	c__SHA-37	o__	f__	g__	s__		
696	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	94.74	0.066667	247	13	0	1	247	1288	1042	2.00E-106	541232_k__Bacteria	p__Firmicutes	c__Clostridia	o__Clostridiales	f__Clostridiaceae	g__	s__
697	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	95.62	0.066667	251	10	1	1	250	1388	1138	3.00E-111	813687_k__Bacteria	p__OP8	c__OP8_1	o__	f__	g__	s__
699	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	95.6	0.066667	250														

Supplementary Material G

Table 1: Total and soluble phosphorus concentration measured at the end of batch 1.

Set-point pH	Final pH	Total P (mg L⁻¹)	Soluble P (mg L⁻¹)
5	5.25	1068	799
5.7	6.15	1133	768
6.5	6.86	1076	490

Table 2: Total and soluble phosphorus concentration measured at the end of batch 2.

Set-point pH	Final pH	Total P (mg L⁻¹)	Soluble P (mg L⁻¹)
5	5.54	977	554
5.7	6.4	1019	484
7	7.65	993	262
Control	7.7	963	224

Supplementary Material H

Table: Average biogas composition of batch 1 at different time intervals.

Biogas Composition of Batch 1									
	Set-point pH 5.0			Set-point pH 5.7			Set-point pH 6.5		
Day	pH	CH₄	CO₂	pH	CH₄	CO₂	pH	CH₄	CO₂
15	6.02	39	58	6.15	39	58	6.54	48	46
29	5.66	37	59	6.16	41	56	6.76	49	45
36	5.26	30	64	6	37	62	6.92	42	53
51	5.25	37	58	6.15	39	54	6.86	48	54

Table: Average biogas composition of batch 2 at different time intervals.

Biogas Composition of Batch 2											
	Set-point pH 5.0			Set pH-point 5.7			Set-point pH 7			Control	
Day	pH	CH₄	CO₂	pH	CH₄	CO₂	pH	CH₄	CO₂	CH₄	CO₂
9	6.04	41	27	6.33	42	27	7.01	43	29	48	20
21	5.59	46	52	6.07	47	51	7.01	62	34	62	32
35	5.76	44	53	5.96	47	50	7.05	60	33	61	33
51	5.54	43	53	6.4	52	43	7.65	60	34	61	33