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1 **Enhancing anaerobic digestion of food waste through Biochemical Methane Potential**
2 **Assays at different substrate: inoculum ratios**

3
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14 **Point of Paper:** This paper quantifies how different ratios of food waste: anaerobic
15 inoculum affect AD performance, especially methane production.
16

17 **Keywords:** Anaerobic digestion, Food waste, Municipal sludge, Hydrolysis, Organic waste,
18 Biochemical Methane Potential
19

20 **Targeted Journal:** *Journal of Industrial Microbiology & Biotechnology*
21

22 **Abstract**

23 Food waste has a high energy potential that can be converted into useful energy in the form
24 of methane via anaerobic digestion. Biochemical Methane Potential assays (BMPs) were
25 conducted to quantify the impacts on methane production of different food waste
26 compositions. Anaerobic digester sludge (ADS) was used as the inoculum, and BMPs were
27 performed at food waste: inoculum ratios of 0.42, 1.42, and 3.0 g chemical oxygen demand/
28 g volatile solids (VS). The 1.42 ratio had the highest CH₄-COD recovery: 90% of the initial
29 total chemical oxygen demand (TCOD) was from food waste, followed by ratios 0.42 and
30 3.0 at 69% and 57%, respectively. Addition of food waste above 0.42 caused a lag time for
31 CH₄ production that increased with higher ratios, which highlighted the negative impacts of
32 overloading with food waste. The Gompertz equation was able to represent the results
33 well, and it gave lag times of 0, 3.6 and 30 days and maximum methane productions of 370,
34 910, and 1950 mL for ratios 0.42, 1.42 and 3.0, respectively. While ratio 3.0 endured a long

35 lag phase and low VSS destruction, ratio 1.42 achieved satisfactory results for all
36 performance criteria. These results provide practical guidance on food-waste-to-inoculum
37 ratios that can lead to optimizing methanogenic yield.

38 **Introduction**

39 Food waste is the largest contributor to municipal solid waste, comprising 21% of waste in
40 landfills in the U.S. in 2012 (U.S. EPA, 2014a). Landfilling food waste may result in
41 significant greenhouse gas emissions from landfills, since food waste accounts for 13% of
42 methane emissions in landfills (EPA, 2015). The emission of greenhouse gases from food
43 waste has led some states, such as Massachusetts, to set limits on the amount of food waste
44 that can go to landfills (RecyclingWorks Massachusetts, 2014). A corollary drawback of
45 landfilling food waste is that its energy value is lost in proportion to the fugitive emissions
46 that contribute to greenhouse gases.

47 An alternative is to anaerobically digest the food wastes and collect the produced
48 methane. Traditionally, anaerobic digestion (AD) facilities handle organic solids from
49 municipal wastewater treatment plants and farms, and more than 180 anaerobic digester
50 facilities currently operate in the U.S. (EREF, 2015). Some of these facilities recently began
51 adding food waste to the AD input. Food waste can be an excellent candidate for AD due to
52 its high energy and moisture contents (Cirne et al., 2007; Levis & Barlaz, 2011; Moriarty,
53 2013). The carbohydrate, protein, and lipid fractions of food waste can be fermented to
54 long-chain fatty acids (LCFAs) and volatile fatty acids (VFAs) that are then converted into
55 acetate and hydrogen gas, the substrates needed by methanogens.

56 Digesting food waste alone can inhibit methanogenesis. A high risk is that LCFAs
57 and VFAs are produced faster than they can be consumed. Unless the alkalinity is high, this
58 acid accumulation will cause a drop in pH that inactivates methanogens, which function
59 well only within a near-neutral pH range (Buyukkamaci & Filibeli, 2004). The result is a

60 “pickled” digester that accumulates VFAs and H₂, but has minimal chemical oxygen demand
61 (COD) stabilization to CH₄.

62 A promising strategy is to co-digest food waste with municipal sludge (Elbeshbishy
63 et al., 2012; Liu et al., 2009; Neves et al., 2004). The key to success is a good ratio of food
64 waste to methanogenic biomass. Elbeshbishy et al. (2012) investigated the impacts of the
65 ratio of food waste to inoculum volatile solid (VS) in batch tests. With the pH held constant
66 at 7, CH₄ production increased as the ratio of food waste to methanogenic inoculum
67 increased. However, artificially maintaining a constant pH may not be realistic, and no
68 studies have evaluated co-digestion of food waste without externally controlled pH. The
69 ratio of food waste to inoculum will affect the potential to accumulate VFAs, and it also will
70 affect the pH-buffering capacity.

71 The objective of this study was to assess methane production for a range of relevant
72 ratios of food waste to methanogenic biomass. We utilized batch Biochemical Methane
73 Potential (BMP) assays and tested three ratios of food-waste COD to VS of an inoculum of
74 anaerobic digester sludge (ADS). To provide proof of concept and identify food-waste-to-
75 ADS-VS ratios that are promising for further analysis, we measured TCOD, SSCOD, TS, VS,
76 and pH at the start and end of BMP assays. Other parameters important to AD and
77 methane production were estimated via bicarbonate alkalinity calculations and the
78 Gompertz equation (Lay et al., 1996) for estimating lag times and maximum methane
79 production. Our results provide guidance on ratios needed to sustain good performance by
80 overcoming low-pH inhibition while maintaining good methanogenic yield.
81

82 **Materials and Methods**

83 ***Food waste recipe and anaerobic digested sludge***

84 The food waste recipe was developed based on weekly food scrap collections at the
85 University of Missouri campus dining operations, as outlined in Costello et al. (2015). The
86 ingredients for the food waste recipe were purchased from a local Wal-Mart food center.
87 The food waste was prepared by mixing the whole food scraps first by hand, followed by
88 grinding food scraps with 100 mL of water in a food processor (Black and Decker model
89 FP1140BD, USA; 450-Watts) for 10 minutes on setting 2, which resulted in a paste. The
90 food waste paste was blended (model Black and Decker BL1120SG, USA; 550-Watts) with
91 200 mL of water for 10 minutes on setting 4 to create a food waste slurry concentration of
92 110 g of food waste/L. The AD inoculum for the BMP test was obtained from Mesa
93 Northwest Water Reclamation Plant in Mesa, Arizona.

94 ***Biochemical Methane Potential Tests/ Experimental Design***

95 BMP tests were performed to determine the amount of CH₄ and H₂ produced from
96 three different COD-to-VS ratios that were based on previous studies with ADS (Angelidaki
97 et al., 2009; Elbeshbishy et al., 2012; Lisboa & Lansing, 2013; Owen et al., 1979): 0.42, 1.42,
98 and 3.0 g COD food waste/ g VS ADS. Negative controls (i.e., ADS in basal media without
99 electron donor) were prepared for each ratio, and the methane produced by the controls
100 was subtracted from the total CH₄ on a proportional basis to compute the methane
101 formation from the food waste alone at the end of the BMP assays. The negative controls
102 did not have any inhibition by low pH, but the food waste BMPs lowered pH and led to pH
103 inhibition at different stages during the BMP test. Thus, we could not do a control
104 subtraction until pH inhibition had been relieved, which occurred by the end of BMP tests

105 in all cases. Therefore, we eliminated the impacts of differential pH inhibition by
106 performing one-time subtraction of the gas production by the negative controls only at the
107 end of the test (day 70). Duplicate positive controls (i.e., ADS with 30 mM acetate as a
108 readily biodegradable electron donor) were set up to ensure that the inoculum was active
109 in methanogenesis and verify the COD conversion to CH₄.

110 For each ratio of COD food waste to VS ADS, 120 mL of food waste plus ADS mixture
111 was added to 200-mL serum bottles along with 60 mL of DI water. All ratio bottles were
112 prepared in triplicate. Table 1 shows the volumes of each component used for each
113 experiment. All bottles were sparged with ultra-high-purity N₂ for 10 minutes to ensure
114 anaerobic conditions. Each serum bottle was sealed with a butyl rubber septum and
115 crimped aluminum caps and placed in an incubated shaker table operated at 180 rpm and a
116 temperature of 37 ± 1°C. Experiments continued until the daily gas production was < 1%
117 of the cumulative gas production except for the 3 g COD FW/g VS condition, which is
118 discussed further in results (Koch, Plabst, et al., 2015; "VDI 4630:," 2006).

119 **Table 1: Volumes and mass of acetate used for the experiments with different ratios**
 120 **of g COD Food Waste (FW)/ g Volatile Solid (VS) Anaerobic Digested Sludge (ADS)**

Ratios	FW (L)	ADS (L)	Water (L)	Acetate (g)
0.42 g COD FW/ g VS ADS	0.014	0.106	0.06	0
1.42 g COD FW/ g VS ADS	0.037	0.083	0.06	0
3.0 g COD FW/ g VS ADS	0.06	0.06	0.06	0
Negative Control 0.42 g COD FW/ g VS ADS	0	0.106	0.074	0
Negative Control 1.42 g COD FW/ g VS ADS	0	0.083	0.097	0
Negative Control 3.0 g COD FW/ g VS ADS	0	0.06	0.12	0
Positive Control	0	0.080	0.1	0.75

121

122 *Chemical analyses*

123 All analytical tests were performed in triplicate. Chemical oxygen demand (COD)
 124 and solids analyses were performed on the food waste, ADS, and initial and final mixtures
 125 for all BMP ratios. Total chemical oxygen demand (TCOD) and semi-soluble chemical
 126 oxygen demand (SSCOD, samples filtered through 1.2- μ m glass microfiber filters (Whatman
 127 1822-047 GF/C)) were assayed using HACH HR COD kits (TNT 821, 20-1500 mg/L). Total
 128 solids (TS) and VS were determined according to *Standard Methods* (APHA, 2012).

129 pH values were measured using a Cole Parmer pH meter (Vernon Hills, USA).
 130 Ammonia nitrogen (NH₃-N) was assayed with HACH kits (TNT832), which had a detection
 131 range 2-47 mgNH₃-N/L. Total alkalinity was assayed with HACH kits (TNT870), which had
 132 a detection range of 25-400 mgCaCO₃/L. Colorimetric results from all HACH kits were
 133 measured using a HACH 2800 spectrophotometer.

134

135 ***Methane and hydrogen in the biogas***

136 Over a 70-day period, biogas production, i.e., changes in headspace volume at one
137 atmosphere, was measured with a gas-tight glass frictionless syringe (Perfektum, NY). CH₄
138 and H₂ contents were analyzed using a GC-2010 gas chromatograph (Shimadzu, Japan)
139 having a thermal conductivity detector (TCD) and Carboxen-1010 PLOT capillary column
140 (30 m, Sigma-Aldrich). The TCD was operated with an inlet temperature of 150°C, a
141 detector temperature of 220°C, and a current of 41 mA, and argon as carrier gas. Gas-
142 composition analysis involved a temperature program that began at 80°C for 3 minutes and
143 was followed by an increase in temperature of 50°C every minute until 155°C is reached,
144 giving a total run time of 4.50 minutes. Methane and hydrogen gas volumes were
145 calculated by multiplying the measured gas composition by the total biogas volume.
146 Electron-equivalent energy recovery (as equivalent COD) was calculated for CH₄ and H₂
147 according to:

148

149 (1)

150
$$1\text{ mL CH}_4 \text{ gas} = \frac{\text{L}}{10^3 \text{ ml}} \cdot \frac{1 \text{ mol CH}_4}{22.4 \text{ L}} \cdot \frac{273 \text{ K}}{313 \text{ K}} \cdot \frac{8 \text{ e}^- \text{ eq}}{\text{mol CH}_4} \cdot \frac{8 \text{ g COD}}{\text{e}^- \text{ eq}} \cdot \frac{10^3 \text{ mg}}{\text{g}} = 2.52 \text{ mg COD}$$

151

152 (2)

153
$$1\text{ mL H}_2 \text{ gas} = \frac{\text{L}}{10^3 \text{ ml}} \cdot \frac{1 \text{ mol CH}_4}{22.4 \text{ L}} \cdot \frac{273 \text{ K}}{313 \text{ K}} \cdot \frac{2 \text{ e}^- \text{ eq}}{\text{mol H}_2} \cdot \frac{8 \text{ g COD}}{\text{e}^- \text{ eq}} \cdot \frac{10^3 \text{ mg}}{\text{g}} = 0.62 \text{ mg COD}$$

154 ***Bicarbonate alkalinity estimation and total alkalinity measurement***

155 The concentration of bicarbonate alkalinity was computed from the final pH and the
156 final CO₂ content in the headspace for each BMP bottle. Equation 3 was used to estimate
157 the bicarbonate alkalinity:

158 **(3)**

159
$$\text{pH} = \text{p}K_{a,1} + \log \left(\frac{\text{Alkalinity (bicarbonate)}}{50,000 \frac{\text{CO}_2(\text{g})}{K_H}} \right)$$

160 where $\text{p}K_{a,1} = 6.33$ for the bicarbonate system at 35°C and 1 atm, *Alkalinity (bicarbonate)* =
161 bicarbonate alkalinity in the anaerobic reactor (mg/L as CaCO₃), $\text{CO}_2(\text{g})$ = gas phase carbon
162 dioxide concentration in anaerobic digester headspace (atm), and K_H = Henry's law
163 constant for carbon dioxide at 35°C and 1 atm, which is 38 atm.

164 The CO₂ concentration at the end of the batch BMP tests were obtained from the GC-
165 TCD data and substituted into the equation along with pH values. Since CO₂ was being
166 generated and out-gassed from solution, the computation may slightly under-estimate the
167 actual bicarbonate concentration.

168

169 ***COD-CH₄ Normalization and Calculating Volatile Solid Destruction***

170 COD-CH₄ normalization was used to show the conversion efficiencies of the volume
171 of methane produced at a given day. The COD-CH₄ normalization can be calculated each day
172 using equation 4:

173

174 (4)

175
$$E = \frac{V_{\text{methane}} \cdot \text{COD}_{\text{methane}}}{\text{COD}_{\text{FW}} \cdot V_{\text{FW}}}$$

176 where E = conversion efficiency of COD-CH₄, V_{methane} = volume of methane production,
177 $\text{COD}_{\text{methane}}$ = 2.52mg COD/mL CH₄ at 35° C at 1atm, COD_{FW} = measured chemical oxygen
178 demand of food waste and V_{FW} = volume of food waste in the BMP test.

179 Volatile solid destruction (VSD) was used to measure the amount of volatile solids
180 removed during the BMP test. The VSD was calculated by using equation 5:

181

182 (5)

183
$$\% \text{VSD} = \frac{(\text{VS}_{\text{FWinitial}} + \text{VS}_{\text{Sinitial}} - \text{VS}_{\text{final}} - \text{VS}_{\text{negfinal}})}{\text{VS}_{\text{FWinitial}} + \text{S}_{\text{initial}}} \cdot 100$$

184 where VSD = volatile solid destruction at day 70 (%), $\text{VS}_{\text{FWinitial}}$ = VS of food waste at day 1
185 (g/L), $\text{VS}_{\text{Sinitial}}$ = VS of ADS at day 1, VS_{final} = VS of ratio on day 70 of the BMP assay test (g/L)
186 and $\text{VS}_{\text{negfinal}}$ = VS of negative control on day 70.

187

188 ***Gompertz-equation fit to the batch BMP data***

189 The Gompertz equation (Lay et al., 1996) often is used to fit batch methanogenic
190 data:

191 (6)

193
$$M_p = \exp\left[-\exp\left\{\frac{R_M}{P_M(x_0 - x)e} + 1\right\}\right]$$

192

194 where M_p = observed cumulative methane production (mL), P_M = ultimate methane
195 production (mL), R_M = observed methane production rate (mL/day), x_0 = lag phase time

197 (days), & x = time of observation (days), and e = exponential (2.718). All the parameters in
 198 the Gompertz equation were evaluated using the techniques of Parameswaran & Rittmann
 199 (2012), implemented in Microsoft Excel Solver, to determine the set of P_M , R_M , and x_0
 200 parameters giving the lowest sum of squares of error between the model and experimental
 201 values based on the observed experimental limitations that P_M is less than 2000 mL, R_M is
 202 less than 100 mL/day, and x_0 is less than 30 days.

203

204 **Results and discussion**

205 ***Chemical characteristics of the feed and final results for the BMP ratios***

206 Table 2 presents the characteristics of the food waste and the starting mixtures for
 207 each experimental ratio. Although the initial pH of the food waste alone was lower than 5,
 208 the pH values for all combined mixtures exceeded 6.3, while the 0.42 and 3.0 mixtures were
 209 in the ideal range for anaerobic digestion (Parkin & Owen, 1986), 6.8 – 7.2. SSCOD
 210 increased as the amount of food waste increased, suggesting that the organic material
 211 would be more readily bioavailable for conversion to CH₄.

212

213 **Table 2. Characteristics of food waste and the starting mixtures for the three ratios**
 214 **of food waste to inoculum**

Initial Characteristics	0.42 g COD/g VS	1.42 g COD/g VS	3.0 g COD/g VS
TCOD (g/L)	21.1 ± 3.75	23.4 ± 7.11	28.8 ± 3.9
SSCOD (g/L)	2.87 ± 0.55	4.13 ± 0.25	6.34 ± 0.4
SSCOD/TCOD (%)	13.6	17.6	22.0
TS (g/L)	33.4	34.1	34.6
VS (g/L)	23.2	24.8	25.0
VS:TS	0.69	0.73	0.72
pH	7.32	6.8	6.3

215
220

221 Table 3 tabulates the characteristics of the mixtures at the end of the 70-day BMP
 222 tests. Final SSCOD was lower for 0.42 and 1.42 ratios, 0.41 g/L and 0.49 g/L, respectively,
 223 compared to 1.29 g/L SSCOD for the 3.0 g COD/g VS ratio. The high final SSCOD for the 3.0
 224 ratio implies that stabilization was incomplete at 70 days, but the 3.0 run was stopped
 225 along with ratios 0.42 and 1.42 to enable comparison within the same timeframe and, more
 226 importantly, within realistic operational timeframe for commercial AD systems (Rapport et
 227 al., 2008). Early and transient hydrogen production for ratio 3.0 indicates that inhibition
 228 based on VFA accumulation and low pH likely occurred during the first 10-15 days, after
 229 which the inhibition was overcome. The lag led to postponed methane production and,
 230 consequently, complete stabilization (shown in Figure S6). Correspondingly, the VS:TS
 231 ratio at the end of the batch BMP assays was the highest for the 3.0 ratio, which is another
 232 sign of less complete stabilization compared to ratio 0.42 and 1.42. This kind of inhibition
 233 has been seen previously when digesting food wastes: LCFA and VFAs are produced faster
 234 than they can be consumed, and the acid accumulation causes a drop in pH that inactivates
 235 methanogens, resulting in minimal chemical oxygen demand (COD) stabilization to CH₄
 236 (Buyukkamaci & Filibeli, 2004).

237 **Table 3. Characteristics of the mixtures at the end of the 70-day BMP assays**

Final Characteristics	0.42 g COD/g VS	1.42 g COD/g VS	3.0 g COD/g VS
TCOD (g/L)	9.37 ± 0.13	9.41 ± 2.19	10.49 ± 2.10
SSCOD (g/L)	0.41 ± 0.14	0.49 ± 0.15	1.29 ± 0.37
TS (g/L)	9.88 ± 1.61	8.76 ± 0.59	7.54 ± 1.62
VS (g/L)	7.84 ± 1.18	7.12 ± 0.44	6.71 ± 1.32
VS:TS	0.79	0.81	0.89
pH	7.09	7.02 ± 0.01	7.36 ± 0.14

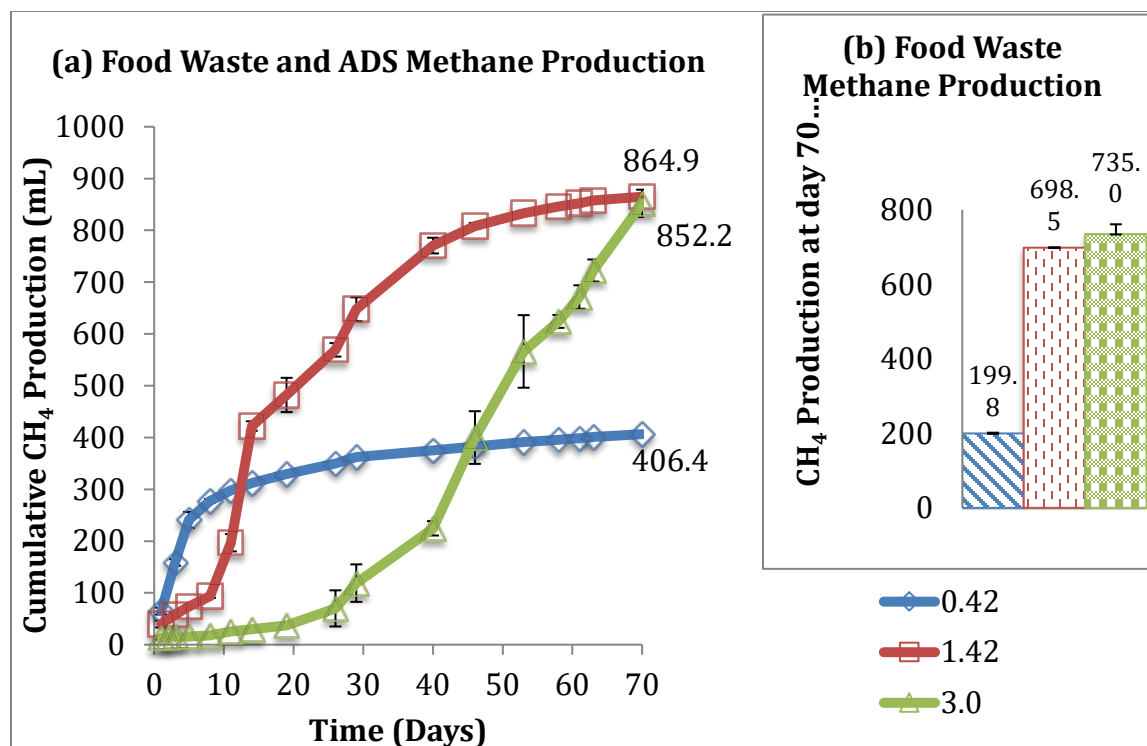
238

239 ***Methane generation during BMP tests***

240 Figure 1a illustrates cumulative CH₄ production for the three ratios of food waste
241 and ADS. The BMP results for the 0.42 ratio had a minimal lag time, with rapid and highest
242 rate of methane production within the first 10 days, after which gas production slowed
243 significantly. The lack of a lag likely was due to the high amount of AD inoculum, which
244 provided relatively large concentrations of hydrolytic enzymes, fermenting bacteria, and
245 acetoclastic methanogens. The activity of acetoclastic methanogens in the inoculum was
246 confirmed by the immediate gas production in the positive control (Figure S2, Supporting
247 Information).

248 After a lag of about 8 days, the BMP for ratio 1.42 began producing CH₄ gas, and the
249 production rate was greater than that achieved in the first few days for ratio 0.42. This rapid
250 increase in CH₄ production suggests that hydrolysis and fermentation had been occurring
251 over the first 10 days; thus, an increase in the activity of methanogen by day 10 allowed
252 rapid conversion of the accumulated VFAs to CH₄.

253 Although ratio 3.0 eventually yielded the most methane from food waste only,
254 (Figure 1b), it also had the most transient hydrogen production and longest lag time, as
255 discussed previously (Supplemental Material Figure S6). Although ratios 0.42 and 1.42
256 were close to reaching saturation for methane generation, ratio 3.0 clearly had not reached
257 saturation by the end of the experiment.

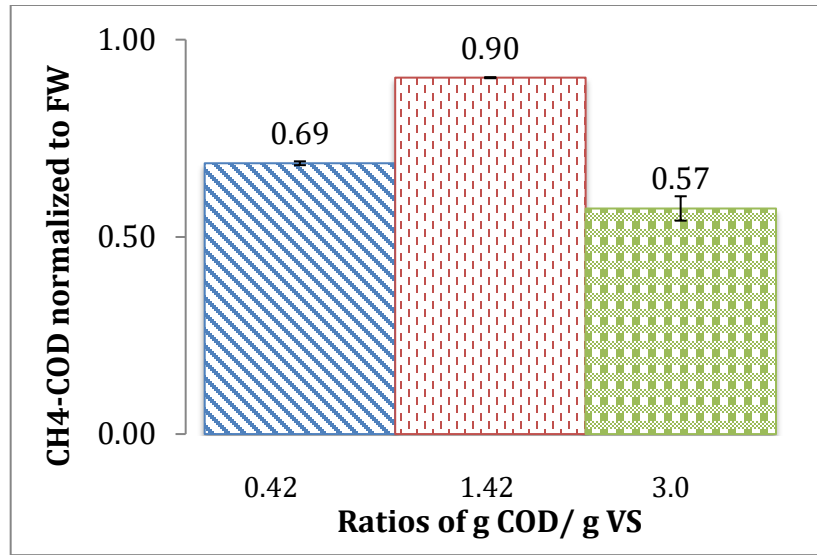


258

259 **Figure 1. (a) Methane production of food waste and ADS. (b) Methane production of**
 260 **food waste at day 70 adjusted to subtract gas produced from negative controls.**
 261 **(Panel (a) does not have subtraction of the negative controls, because pH inhibition**
 262 **was not relieved throughout the assays and thus cannot be compared directly.) Error**
 263 **bars in graph represent standard deviation.**
 264

265 Figure 2 shows that the food waste COD-to-ADS-VS ratio affected the fraction of food
 266 waste COD removed as CH₄ after the cumulative one-time subtraction of negative controls
 267 at the end (day 70). The 0.42 ratio demonstrates low CH₄ production in Figure 1 and
 268 consequently results in low COD as CH₄ at 69% in Figure 2. Ratio 1.42 had the highest CH₄-
 269 COD conversion efficiency, 90% for food waste alone. Ratio 3.0 gave the lowest adjusted
 270 methane yield (57%), and this was caused by the long lag time and clearly incomplete
 271 conversion at the end of the test (Figure 1a).

272

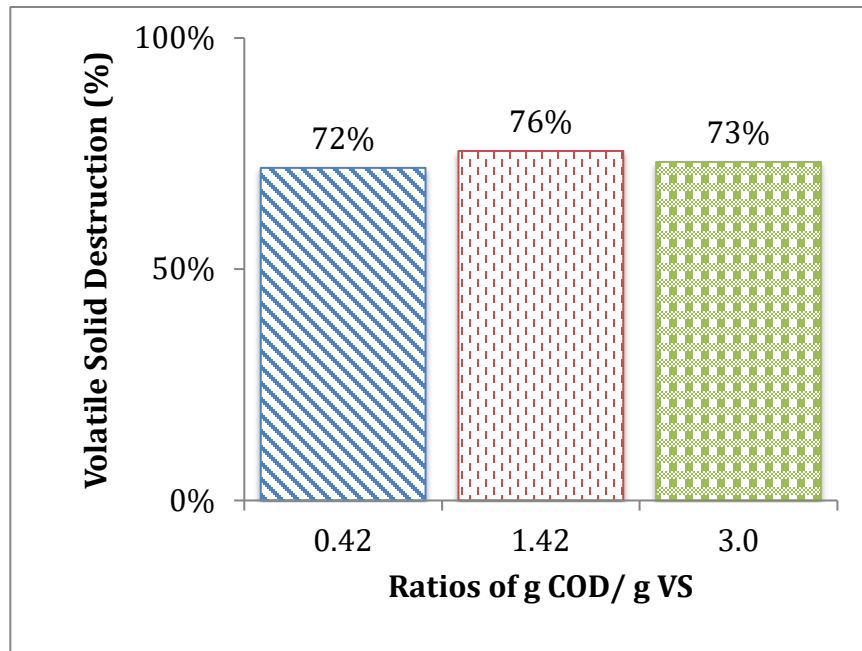


273

274 **Figure 2. CH₄-COD normalized to food waste (FW) COD at day 70. Error bars show**
 275 **the relative standard deviation.**

276

277 All three ratios showed similar values for volatile solids reduction (VSD), shown in
 278 Figure 3, indicating that the hydrolysis of the particulate fraction was not limiting
 279 methanogenesis at the end of the BMP assays; hence, the differences in methanogenic
 280 yields were likely linked with VFA production and pH inhibition of VFA conversion to
 281 methane. The VSD for ratios 0.42 and 3.0 were slightly greater than the conversion of FW
 282 COD to methane (Fig. 2), which is consistent with accumulation of hydrolysis and
 283 fermentation products, including VFAs. The highest volatile solid destruction (VSD), 76%
 284 for ratio 1.42, corresponds to the highest cumulative methane production Figure 1a.



285 **Figure 3. Percent volatile solid destruction for each volumetric ratio.**

286

287 *Gompertz Equation Analysis*

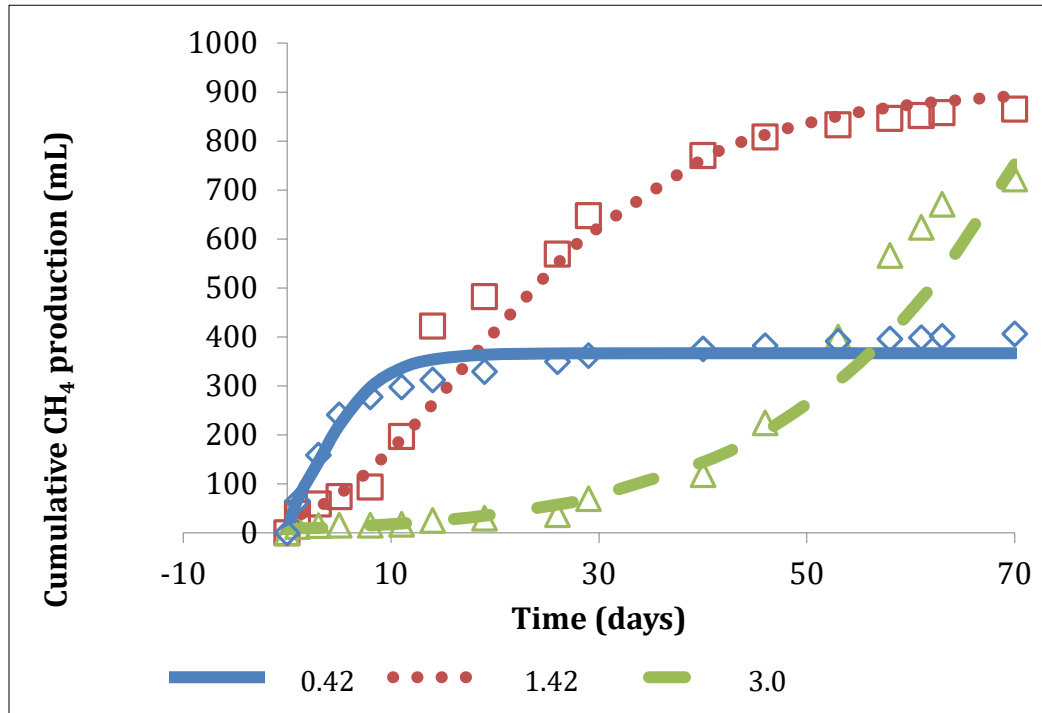
288 Previous studies have shown that the Gompertz equation fits experimental data
 289 when the BMP data follow a typical pattern with initial lag, exponential, and saturation
 290 phases (Parameswaran & Rittmann, 2012). The Gompertz equation described in the
 291 methods section fit the corresponding volumetric ratios 0.42 and 1.42 experiments very
 292 well, as shown in Figure 5, which uses the parameters in Table 4. The model fit for ratio 3.0
 293 is not as accurate, due to the ultimate methane production value being estimated from
 294 projected saturation rather than an observed value. For ratio 3.0, the model fit the data
 295 well through day 46. After the time, the experimental rate of methane production began to
 296 slow, while the modeled production rate continued to increase. Bakhov et al. (2014) also
 297 employed the Gompertz equation and were unable to provide a good representation when
 298 the food waste loading was high.

299

300 **Table 4. Estimated parameters from the fit of Gompertz equation to the BMP assays**
 301 **of corresponding ratios.**

Substrate Ratio	P_m (mL CH ₄)	Ratio Pm:initial FW (mL CH ₄ /g COD _{FW})	Ratio Pm:initial ADS (mL CH ₄ /g COD _{ADS})	R_m (mL CH ₄ /day)	Ratio of R_m :initial ADS (mL CH ₄ /g COD _{ADS-d})	x_0 (days)	Total Sum of Squares Errors between model and actual
0.42	367	874	25	40.2	2.71	0	0.11
1.42	908	639	94	25.4	2.63	3.6	0.38
3.0	1947	649	299	17.2	2.64	30	1.15

302



303

304 **Figure 5. Cumulative CH₄ production as a function of time, where symbols represent**
 305 **the experimental data and lines represents the Gompertz equation model fit.**
 306

307 Three significant trends exist for all ratios. First, the ratio of R_m to ADS inoculum
 308 had a narrow range, between 2.63-2.71 mL CH₄/g COD_{ADS-d}, for all ratios, reinforcing that
 309 the CH₄ production rate was dictated by ADS inoculum dose, which contained the bacteria

310 responsible for hydrolysis. Second, the ratio of Pm to initial FW COD loading was relatively
311 narrow, ranging from 874 mL CH₄/g COD_{FW} for the 0.42 ratio to 649 mL CH₄/g COD_{FW} for
312 the 3.0 ratio. In contrast, the ratio of Pm to initial ADS COD loading increased steadily from
313 25 to 299 mL CH₄/g COD_{AD} with increasing FW:ADS ratio. These differing trends indicate
314 that the maximum amount of CH₄ produced in the system was controlled by the added COD
315 from the FW. Third, the increasing x_0 with increasing FW: AD clearly illuminates the
316 delayed onset of methanogenesis due to pH inhibition from the high accumulation of VFAs
317 from the fermentation step.

318

319 ***Alkalinity estimation at the end of batch BMPs***

320 Final bicarbonate-alkalinity values, summarized in Table 5, indicate that ratio 3.0
321 had a lower value than ratio 1.42, possibly indicating a greater alkalinity consumption
322 associated with the higher food waste fraction, possibly leading to a pH induced inhibition.
323 The final NH₃-N concentrations can be expressed in alkalinity equivalents by multiplying
324 the NH₃-N concentration by a factor of 50 mg as CaCO₃/14 mg N. The values of alkalinity
325 added by NH₃ release clearly point out that a major fraction of the bicarbonate alkalinity
326 originated from NH₃-N. The exponential phase of methanogenesis often coincides with
327 increases in bicarbonate alkalinity and pH, both of which occurred in the 3.0 ratio at the
328 end of 70 days. In fact, there was an abundance of NH₃-based alkalinity for the ratio 1.42,
329 which also correlates with the superior methane production performance at this ratio. On
330 the other hand, the lower final NH₃-N for ratio 3.0 may mean that the Food Waste
331 underwent less hydrolysis, atleast to the point of releasing lower NH₃-N compared to the
332 other two ratios.

333 **Table 5. Estimated Characteristics of the mixtures at the end of the 70-day BMP**
 334 **assays.**

Estimated bicarbonate Alkalinity (g/L as CaCO ₃)	3.6	2.9	4.5
Ammonia nitrogen (g/L)NH ₃ -N/L)	0.81 ±0.02	0.90 ±0.03	0.76 ± 0.08
Alkalinity originating from Ammonia-N in food waste (g/L as CaCO ₃)	1.4	2.6	2.1
% of Estimated bicarbonate alkalinity that came from food waste NH ₃	39%	90%	47%

335

336 **Conclusion**

337 The effects of the food-waste-to-inoculum ratio provide insights into the
 338 performance of co-digestion with food waste. A high ratio of food waste COD to ADS VS
 339 (3.0 g FW COD/g AD VS) eventually gave greater volumetric methane production, but VFA-
 340 induced pH inhibition caused a large lag period (about 10 days). An intermediate ratio
 341 (1.42 g FW COD/g AD VS) gave the best balance of high methanogenic yield with a short lag
 342 time. A key factor was the balance of food waste COD that could be fermented to VFAs
 343 versus alkalinity in the AD and generated by NH₃ release from food waste. Due to the
 344 relatively labile nature of food waste COD, the generation of VFAs could suppress the pH
 345 and inhibition methanogenesis. Thus, this work underscores the importance of measuring
 346 COD, alkalinity, VFAs, and N in food waste and in the mixture of food waste with AD.

347

348

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355

356 **Work Cited**

- 357 Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J. L., Guwy, A. J., . . . van Lier, J.
358 B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and
359 energy crops: a proposed protocol for batch assays. *Water Sci Technol*, 59(5), 927-
360 934. doi:10.2166/wst.2009.040
361
- 362 APHA, A. P. H. A. (2012). *Standard Methods for the Examination of Water and Wastewater*,
363 *22nd Edition* American Public Health Association, American Water Works
364 Association, Water Environment Federation.
365
- 366 Bakhov, Z. K., Korazbekova, K., & Lakhanova, K. (2014). Kinetics of Methane Production
367 from Co-digestion of Cattle Manure. *Pakistan Journal of Biological Sciences*, 17(8),
368 1023.
369
- 370 Bolzonella, D., Fatone, F., Pavan, P., & Cecchi, F. (2005). Anaerobic Fermentation of Organic
371 Municipal Solid Wastes for the Production of Soluble Organic Compounds. *Industrial*
372 *& Engineering Chemistry Research*, 44(10), 3412-3418. doi:10.1021/ie048937m
373
- 374 Buyukkamaci, N., & Filibeli, A. (2004). Volatile fatty acid formation in an anaerobic hybrid
375 reactor. *Process Biochemistry*, 39(11), 1491-1494.
376 doi:[http://dx.doi.org/10.1016/S0032-9592\(03\)00295-4](http://dx.doi.org/10.1016/S0032-9592(03)00295-4)
377
- 378 Cirne, D. G., Paloumet, X., Björnsson, L., Alves, M. M., & Mattiasson, B. (2007). Anaerobic
379 digestion of lipid-rich waste—Effects of lipid concentration. *Renewable Energy*,
380 32(6), 965-975. doi:<http://dx.doi.org/10.1016/j.renene.2006.04.003>
381
- 382 Costello, C., Birisci, E., & McGarvey, R. G. (2015). Food waste in campus dining operations:
383 Inventory of pre- and post-consumer mass by food category, and estimation of
384 embodied greenhouse gas emissions. *Renewable Agriculture and Food Systems*,
385 *FirstView*, 1-11. doi:doi:10.1017/S1742170515000071
386
- 387 Elbeshbishy, E., Nakhla, G., & Hafez, H. (2012). Biochemical methane potential (BMP) of
388 food waste and primary sludge: Influence of inoculum pre-incubation and inoculum
389 source. *Bioresource Technology*, 110, 18-25.
390 doi:<http://dx.doi.org/10.1016/j.biortech.2012.01.025>
391
- 392 EPA, U. S. (2015). Inventory of U.S. Greenhouse Gas Emission and Sinks: 1990-2013.
393 Retrieved from
394 <http://www.epa.gov/climatechange/emissions/usinventoryreport.html>
395
- 396 EREF. (2015). *Anaerobic Digestion of Municipal Solid Waste: Report on the Ste of Practice*. .
397 Retrieved from <http://www.erefdn.org>
398
- 399 Koch, K., Helmreich, B., & Drewes, J. E. (2015). Co-digestion of food waste in municipal
400 wastewater treatment plants: Effect of different mixtures on methane yield and

401 hydrolysis rate constant. *Applied Energy*, 137, 250-255.
402 doi:<http://dx.doi.org/10.1016/j.apenergy.2014.10.025>
403

404 Koch, K., Plabst, M., Schmidt, A., Helmreich, B., & Drewes, J. E. (2015). Co-digestion of food
405 waste in a municipal wastewater treatment plant: Comparison of batch tests and
406 full-scale experiences. *Waste Management*.
407 doi:<http://dx.doi.org/10.1016/j.wasman.2015.04.022>
408

409 Lay, J.-J., Li, Y.-Y., & Noike, T. (1996). EFFECT OF MOISTURE CONTENT AND CHEMICAL
410 NATURE ON METHANE FERMENTATION CHARACTERISTICS OF MUNICIPAL SOLID
411 WASTES. *Doboku Gakkai Ronbunshu*, 1996(552), 101-108.
412 doi:10.2208/jscej.1996.552_101
413

414 Levis, J. W., & Barlaz, M. A. (2011). What Is the Most Environmentally Beneficial Way to
415 Treat Commercial Food Waste? *Environmental Science & Technology*, 45(17), 7438-
416 7444. doi:10.1021/es103556m
417

418 Lisboa, M. S., & Lansing, S. (2013). Characterizing food waste substrates for co-digestion
419 through biochemical methane potential (BMP) experiments. *Waste Management*,
420 33(12), 2664-2669. doi:<http://dx.doi.org/10.1016/j.wasman.2013.09.004>
421

422 Liu, G., Zhang, R., El-Mashad, H. M., & Dong, R. (2009). Effect of feed to inoculum ratios on
423 biogas yields of food and green wastes. *Bioresour Technol*, 100(21), 5103-
424 5108. doi:<http://dx.doi.org/10.1016/j.biortech.2009.03.081>
425

426 Moriarty, K. (2013). *Feasibility Study of Anaerobic Digestion of Food Waste in St. Bernard,*
427 *Louisiana: A study prepared in partnership with the Environmental Protection Agency*
428 *for the RE-Powering America's Land Initiative: Siting Renewable Energy on Potentially*
429 *Contaminated land and Mine Sites*. Retrieved from Golden, CO:
430 <http://www.nrel.gov/docs/fy13osti/57082.pdf>
431

432 Neves, L., Oliveira, R., & Alves, M. M. (2004). Influence of inoculum activity on the bio-
433 methanization of a kitchen waste under different waste/inoculum ratios. *Process*
434 *Biochemistry*, 39(12), 2019-2024.
435 doi:<http://dx.doi.org/10.1016/j.procbio.2003.10.002>
436

437 Obulisamy, P. K., Chakraborty, D., Selvam, A., & Wong, J. W. C. (2016). Anaerobic co-
438 digestion of food waste and chemically enhanced primary-treated sludge under
439 mesophilic and thermophilic conditions. *Environmental Technology*.
440 doi:10.1080/09593330.2016.1181112
441

442 Owen, W. F., Stuckey, D. C., Healy Jr, J. B., Young, L. Y., & McCarty, P. L. (1979). Bioassay for
443 monitoring biochemical methane potential and anaerobic toxicity. *Water Research*,
444 13(6), 485-492. doi:[http://dx.doi.org/10.1016/0043-1354\(79\)90043-5](http://dx.doi.org/10.1016/0043-1354(79)90043-5)
445

- 446 Parameswaran, P., & Rittmann, B. E. (2012). Feasibility of anaerobic co-digestion of pig
447 waste and paper sludge. *Bioresource Technology*, 124, 163-168.
448 doi:<http://dx.doi.org/10.1016/j.biortech.2012.07.116>
449
- 450 Parkin, G. F., & Owen, W. F. (1986). Fundamentals of Anaerobic Digestion of Wastewater
451 Sludges. *Journal of Environmental Engineering*, 112(5), 867-920.
452
- 453 Pavlostathis, S. G., & Giraldo - Gomez, E. (1991). Kinetic of anaerobic treatment: A critical
454 review. *Critical Reviews in Environmental Control*, 21(5-6). doi:
455 10.1080/10643389109388424
456
- 457 Rapport, J., Zhang, R., Jenkins, B. M., & Williams, R. B. (2008). *Current Anaerobic Digestion*
458 *Technologies Used for Treatment of Municipal Organic Solid Waste*. Retrieved from
459 Davis, California
- 460 RecyclingWorks Massachusetts. (2014). Options for Complying with the Commercial
461 Organics Waste Ban. Retrieved from
462 <http://www.recyclingworksma.com/commercial-organics-waste-ban/>
463
- 464 Tandukar, M., & Pavlostathis, S. G. (2015). Co-digestion of municipal sludge and external
465 organic wastes for enhanced biogas production under realistic plant constraints.
466 *Water Research*. doi:<http://dx.doi.org/10.1016/j.watres.2015.04.031>
467
- 468 U.S. EPA. (2014a). Municipal Solid Waste Generation, Recycling, and Disposal in the United
469 States: Facts and Figures for 2012. Retrieved from
470 http://www.epa.gov/osw/nonhaz/municipal/pubs/2012_msw_fs.pdf
471
- 472 VDI 4630:. (2006). *Fermentation of organic materials– characterisation of the substrate,*
473 *sampling, collection of material data, fermentation test* Verein Deutscher Ingenieure,
474 Düsseldorf.
475
476