Strategies for recovery of imbalanced full-scale biogas reactor feeding with palm oil mill effluent

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Background: Full-scale biogas production from palm oil mill effluent (POME) was inhibited by low pH and highly volatile fatty acid (VFA) accumulation. Three strategies were investigated for recovering the anaerobic digestion (AD) imbalance on biogas production, namely the dilution method (tap water vs. biogas effluent), pH adjustment method (NaOH, NaHCO$_3$, Ca(OH)$_2$, oil palm ash), and bioaugmentation (active methane-producing sludge) method, and a highly economical and feasible method would be selected and validated in a full-scale application.

Results: The inhibited sludge from a full-scale biogas reactor could be recovered within 30-36 days by employing various strategies. Dilution of the inhibited sludge with biogas effluent at a ratio of 8:2, pH adjustment with 0.14% w/v NaOH, and 8.00% w/v oil palm ash were considered to be more economically feasible than other strategies tested (dilution with tap water, or pH adjustment with 0.50% w/v Ca(OH)$_2$, or 1.25% NaHCO$_3$ and bioaugmentation) with a recovery time of 30-36 days. The recovered biogas reactor exhibited a 35-83% higher methane yield than self-recovery, with a significantly increased hydrolysis constant (k_H) and specific methanogenic activity (SMA). The population of *Clostridiales* sp., *Bacilli* sp., and *Methanosarcina* sp. increased in the recovered sludge. Recovery from the imbalanced full-scale hybrid cover lagoon reactor was within 15 days by dilution with biogas effluent at a ratio of 8:2 and a better result than the lab-scale test (36.4 days).

Conclusion: Dilution of the inhibited sludge with biogas effluent could recover the imbalance of the full-scale POME-biogas reactor, with economically feasible and high biogas production performance.

1 **Strategies for recovery of imbalanced full-scale biogas reactor feeding with**

2 **palm oil mill effluent**

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23 **Abstract**

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25 low pH and highly volatile fatty acid (VFA) accumulation. Three strategies were investigated for

26 recovering the anaerobic digestion (AD) imbalance on biogas production, namely the dilution

27 method (tap water vs. biogas effluent), pH adjustment method (NaOH, NaHCO$_3$, Ca(OH)$_2$, oil

28 palm ash), and bioaugmentation (active methane-producing sludge) method, and a highly

29 economical and feasible method would be selected and validated in a full-scale application.

30 **Results:** The inhibited sludge from a full-scale biogas reactor could be recovered within 30-36

31 days by employing various strategies. Dilution of the inhibited sludge with biogas effluent at a

32 ratio of 8:2, pH adjustment with 0.14% w/v NaOH, and 8.00% w/v oil palm ash were considered

33 to be more economically feasible than other strategies tested (dilution with tap water, or pH

34 adjustment with 0.50% w/v Ca(OH)$_2$, or 1.25% NaHCO$_3$ and bioaugmentation) with a recovery

35 time of 30-36 days. The recovered biogas reactor exhibited a 35-83% higher methane yield than

36 self-recovery, with a significantly increased hydrolysis constant (k_H) and specific methanogenic

37 activity (SMA). The population of *Clostridiales* sp., *Bacilli* sp., and *Methanosarcina* sp.

38 increased in the recovered sludge. Recovery from the imbalanced full-scale hybrid cover lagoon

39 reactor was within 15 days by dilution with biogas effluent at a ratio of 8:2 and a better result

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43 performance.

44 **_Keywords_:** palm oil mill effluent, full-scale biogas plant, organic overloading, acidified

45 inhibition, recovery strategy, microbial community

Introduction

Palm oil mill effluent (POME) is the main wastewater generated from the palm oil extraction plant which is mostly treated through an anaerobic process with energy production in terms of biogas *(Wu et al., 2010)*. Biogas has been identified as one of the most profitable renewable technologies based on socio-economic analysis. The application of biogas production technology to treat POME has expanded in response to demand, but unforeseen process-related accidents occur regularly in biogas plants, where the process is inhibited and biogas production is reduced. The long-term operation of commercial biogas reactor feeding with POME was confronted with process imbalance by volatile fatty acid (VFA) inhibition, long-chain fatty acids (LCFAs) inhibition, low pH inhibition, and foaming *(Wongfaed et al., 2015; Wongfaed et al., 2020)*. The imbalanced biogas reactor resulted in a reduction of biogas production, reduction of chemical oxygen demand (COD) removal efficiency, and failure of the anaerobic digestion (AD) process *(Joo-Hwa & Xiyue, 2000; Menardo et al., 2011)*. The imbalanced biogas reactor feeding with POME was mainly caused by fluctuations in the composition and volume of POME that varied depending on the quality of palm fruit, season, harvesting period, and extraction process. The feedstock composition and organic loading rate (OLR) were affected in both bacterial and archaeal communities in the AD process *(Supaphol et al., 2011; Xia et al., 2012)*. In addition, their fluctuations always caused process imbalance of high strength feedstock resulting in unstable biogas production performance *(Joo-Hwa & Xiyue, 2000)*. Moreover, the overloading of the substrate could inhibit the AD process, resulting in losses of methane yield of up to 30% *(Fotidis et al., 2014)*. Reactor acidification by organic overload is one of the most common reasons for this AD process imbalance *(Akuzawa et al., 2011)* due to the rapid accumulation of VFA from uncoupling between the acid producers and consumers. The consequences of the AD process imbalance are financial losses due to reduced biogas yield as well as an increased deployment of staff and cost of the chemical. Therefore, it is necessary to solve these problems in a timely manner.

73   The typical recovery strategy for the AD process imbalance is stop feeding to restore the

74   ecological function of microorganisms in the AD system via self-recovery. However, this

75   strategy requires a long period of time and is not economically feasible. The stop feeding

76   strategy, combined with the addition of trace elements, could accelerate the recovery process of

77   the inhibited AD reactor, but still requires a long stop feeding period *(Voelklein et al., 2017).* The

78   recovery of VFA and low pH inhibition in real-time without stop feeding is still a significant

79   challenge. Adjusting pH of the inhibited AD reactor to near-neutral was often applied to enhance

80   the buffering capacity of the AD system against VFA disturbance, with low cost and smooth

81   operation. The adjusted pH in the AD reactor could recover the AD process from low pH

82   inhibition with a stable operation *(Zhang et al., 2013).* Alkaline addition to the AD reactor

83   improved the buffering capacity to meet the requirements of the microbial populations *(Zhang et*

84   *al., 2016)* and enhance activities of the acidogenic bacteria and methanogenic archaea *(Zhang et*

85   *al., 2012).* Alkaline substances, such as Na_2CO_3 and $NaHCO_3$, exhibited more pronounced

86   effects on the stability of the AD process than NaOH due to CO_3^{2-} and HCO_3^- having a higher

87   buffering capacity than OH^- *(Jun et al., 2009).* Additionally, and instead of alkaline chemicals,

88   wood ash was used to adjust the pH of the AD process as a cheap material alternative

89   *(Saritpongteeraka & Chaiprapat, 2008).* Oil palm ash was used to adjust the pH of POME with

90   high biogas production, rather than raw POME, due to the releasing buffer capacity and

91   micronutrient *(Gómez et al., 2006).* However, the pH adjustment strategy could not recover the

92   imbalanced AD reactor, but only delayed the AD process failure *(Gómez et al., 2006).*

93   Nevertheless, the addition of fresh and active methane-producing sludge, with the addition of

94   micronutrient, has been used to recover the imbalanced AD reactor *(Lee & Shoda, 2008; Qiang*

95   *et al., 2013).* The re-inoculation *(Wu et al., 2015)* or bioaugmentation (*Li et al., 2018*) of high-

96   activity anaerobic microorganism was used to restart the out-of-order AD reactor, and this was

97   significantly effective in the short-term, although expensive. The combination of the pH

98   adjustment with trace elements and re-inoculation was always useful, but costly *(Zhang et al.,*

99   *2018).* All strategies, described above, have proved to be effective methods to recover the

100   imbalanced AD process. However, a systematic and comprehensive evaluation of the recovery

101   strategies from the imbalanced AD reactor feed with POME has yet to be reported.

102   This work aims to recover the imbalanced AD reactor feed with POME by pH adjustment

103   with an alkaline substance, the dilution of the toxic compounds with tap water and biogas

104   effluent, and re-inoculation by addition of active methane-producing sludge.The microbial

105   community responsible for each recovery strategy was investigated, and the knowledge from our

106   research can provide economically feasible and rapid recovery methods for imbalanced

107   commercial biogas reactors.

108

109   **Materials & Methods**

110   **Characteristics of inhibited AD sludge, POME, biogas effluent, and active methane-**

111   **producing sludge**

112   An inhibited sludge sample was collected from the mesophilic biogas plant ($40\pm2°C$) of a

113   palm oil mill, *Prasang Green Power Co., Ltd*., Surat Thani Province, Thailand. The biogas

114   reactor was operated in continuous mode, feeding with POME at a high OLR (4.5 gCOD/L/d),

115   resulting in acidification of the AD reactor. Biogas effluent, POME, and active methane-

116   producing sludge were collected from the biogas plant at *Pitak Palm Oil Co., Ltd*., Trang

117   Province, Thailand, and analyzed for their characteristics, according to the procedure described

118   in *APHA (2012)*. The inhibited sludge had pH, total volatile fatty acids (tVFA), suspended solids

119   (SS), and volatile suspended solids (VSS) content of 3.9, 4.8 g/L, 17.0, and 14.5 g/L,

120   respectively. The active methane-producing sludge had neutral pH (7.5), very low VFA (0.92

121   g/L), but a high SS and VSS content of 59.8 g/L and 52.2 g/L, respectively.

122

123   **Recovery of AD process imbalance**

124   With the experiments carried out in a batch reactor, an AD process imbalance, indicated

125   by inhibited sludge, was recovered using the following three strategies. Firstly, the inhibited

126   sludge sample was diluted with tap water (TW) and biogas effluent (BE) at a ratio of 9:1, 8:2,

127   7:3, 6:4, and 5:5, respectively, as a dilution strategy. Secondly, the inhibited sludge was adjusted

128   using 0.85-1.50% w/v sodium hydrogen carbonate ($NaHCO_3$), 0.10-0.14% w/v sodium

129   hydroxide (NaOH), 0.10-0.50% w/v calcium hydroxide ($Ca(OH)_2$) and 6.0-10.0% w/v oil palm

130   ash as pH adjustment strategy. Thirdly, the inhibited sludge was recovered by the addition of

131   active methane-producing sludge at 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 %v/v, as a re-

132   inoculation or bioaugmentation strategy. All strategies were combined with 20% v/v POME

133   addition as low flow rate feeding (40 m^3-POME/d), instead of stop feeding, and self-recovery

134   was used as a control. All experiments were flushed with N_2: CO_2 mixed at 80:20 ratios to create

135   the anaerobic condition, and secured tightly with a butyl-rubber septum and aluminum cap. The

136   experiment was carried out in triplicate and at a temperature of (40 ± 3^oC) for 45 days. The biogas

137   production in the headspace was measured via the water displacement method, and the biogas

138   content was analyzed by a gas chromatograph equipped with thermal conductivity detectors

139   (GC-TCD). Microbial sludge from each treatment was analyzed for the microbial community

140   structure using polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE)

141   techniques. The specific methanogenic activity (SMA) of the inhibited sludge and recovery

142   sludge was also determined *(Hussain & Dubey, 2017)*.

143

144   **Validity of lab-scale results to the full-scale recovery of AD process imbalance**

145   The selected strategy was applied to the full-scale hybrid cover lagoon reactor (6,000 m^3)

146   for recovery of the AD process imbalance, and validated the lab-scale results. The biogas reactor

147   was operated with hydraulic retention times (HRT) of 30 days and OLR of 1.25 kg COD/m^3/d.

148   The 50 m^3 of inhibited sludge and 10 m^3 of POME were diluted with biogas effluent at a ratio of

149   8:2 every day for two weeks, until the pH increased to 7.5, before adding to the full-scale biogas

150   reactor. The pH, methane production rate, and SMA of full-scale biogas sludge were monitored

151   daily, and the SMA was analyzed every 3 days using acetic acid as a substrate.

152   **Microbial activity (SMA) and community analysis (PCR-DGGE)**

153   The methanogenic activities of the inhibited and recovered sludges were evaluated by the

154   SMA test using acetic acid, glucose, crystalline cellulose, and gelatin as substrate. The SMA

155   value was calculated by the slope of methane production (based on g COD of CH_4) against

156   incubation time, and divided with VSS added of sludge sample *(Hussain & Dubey, 2017)*. The

157   microbial community structure was analyzed by PCR-DGGE techniques, according to

158   *Prasertsan et al. (2009)*. 0.2 g of the sludge sample was extracted for genomic DNA using the

159   Ultraclean Soil DNA Kit *(MoBio Laboratory Inc.*, USA). The 16S rDNA gene of bacteria was

160   amplified by the first PCR with universal primer 27f (GAGTTTGATCCTTGGCTCAG) and

161   1525r (AAGGAGGTGWTCCARCC). 16S rDNA gene for archaea was amplified using Arch21f

162   primers (TTCCGGGTTGATCCYGCCGGA) and Arch958r (YCCGGCGTTGAMTCCAATT).

163   The V_3 region of bacteria was amplified in a second PCR by primer 357f

164 (CTCCTACGGGAGGCAGCAG) with CG clamp and 518r (GTATTACCGCGGCTGCTGG),

165 using the first bacteria PCR as a template. The V_3 region of archaea was amplified in a second

166 PCR by primer 340f (CCTACGGG-GYGCASCAG) with CG clamp and 519r

167 (TTACCGCGGCKGCTG), using the product of first archaea PCR as a template. Second PCR

168 products performed the DGGE analysis using electrophoresis with 6% polyacrylamide gel for

169 bacteria and 8% polyacrylamide gel for archaea containing a linear of urea/formamide gradient

170 with denaturant ranging from 40% to 70% in 0.5 TAE buffer at 20 volts for 20 minutes, and 70

171 volts for 15 hours, at a constant temperature of 60°C. Sybr-Gold was stained in the DGGE gels

172 for 60 minutes and photographed on the Gel Doc XR system (*Bio-Rad Laboratories*).

173 Predominant DGGE bands were excised with a sterile tip and suspended in 30 μL sterilized

174 Milli-Q water. Excised DGGE band was incubated at 4°C overnight and re-amplified by PCR

175 using the same primers without the GC clamp. PCR products were purified and sequenced by

176 *Macrogen Inc*. (Seoul, South Korea). Closest matches for partial 16S rRNA gene sequences were

177 identified by database searches in Gene Bank using *BLAST (Tatusova et al., 2016)*.

178 **Analytical methods and calculation**

179 The biogas composition (H_2, N_2, CH_4, and CO_2) was determined by gas chromatograph

180 GC-8A (Shimadzu, Kyoto, Japan) with a 1-meter stainless steel column packed with Shin

181 Carbon (60/80 mesh) equipped with thermal conductivity detectors (TCD). The argon at a flow

182 rate of 14 mL/min was used as the carrier gas. The temperatures of the oven, detector, and

183 injection port were at 40°C, 100°C, and 120°C, respectively. The 0.5 mL of the biogas sample

184 was injected in duplicate for each reactor. The daily biogas production for each reactor was

185 counted using the water displacement method *(Yan et al., 2015)*. The chemical and physical

186 composition of POME, biogas effluent, active methane-producing sludge, and inhibited sludge

187 were determined for pH, lipid content, total solids (TS), volatile solids (VS), volatile suspended

188 solids (VSS), total nitrogen (TN), total volatile fatty acid (tVFA), and alkalinity according to

189 Standard Methods for the Examination of Water and Wastewater *(APHA, 2012)*. Determination

190 of TS was performed at a temperature of 90°C instead of 105°C, till constant weight to avoid

191 decreasing of VFAs *(Angelidaki et al., 2009)*. The VFAs composition was determined through a

192 gas chromatograph GC-17A (Shimadzu, Kyoto, Japan) with a Stabilwax®-DA fused silica

193 column (30 m of length, 0.53 mm of diameter, 85°C) connected to a flame ionization detector

194 (FID) at 240°C. The helium at 30 mL/min was used as the carrier gas. The VFA samples were

195 collected by syringe (1 mL) and filtered through a nylon membrane (0.2 µm). The filtrated

196 samples were acidified to pH 3.0–3.2 with 30% (v/v) phosphoric acid for VFAs analysis *(Raposo*

197 *et al., 2015)*. Buswell's equation was used to calculate theoretical methane yield, assuming the

198 total stoichiometry conversion of the organic matter to methane and carbon dioxide *(Buswell &*

199 *Mueller, 1952)*. The hydrolysis constants (k_H) were determined by using the first-order kinetic

200 model in equation (1) according to the protocol of *Raposo et al. (2006)*. Where B(t) is the

201 cumulative methane yield (mL-CH_4/g-VS $_{added}$) at time t, and B_∞ is the maximum cumulative

202 methane yield (mL-CH_4/g-VS $_{added}$). k_H is the hydrolysis constant (d^{-1}); t is the fermentation

203 time; λ is the lag phase (day).

204 $$B(t) = B_\infty \left[1 - \exp\left(- k_H (t - \lambda) \right) \right] \qquad (1)$$

205 The modified Gompertz model was used to predict the methane production, methane production

206 rate, and lag phase *(Nopharatana et al., 2007)* as follows in equation (2).

207 $$M = P \cdot \exp\left\{ - \exp\left[\frac{Rm \cdot e}{P} (\lambda - t) + 1 \right] \right\} \qquad (2)$$

208 Where M is the cumulative methane yield at the time t (mL-CH_4/g-VS_{added}); P is the

209 maximum cumulative methane yield (mL-CH_4/g-VS $_{added}$); R_m is the maximum methane

210 production rate (mL-CH_4/g-VS/d); λ is the lag phase (d); t is the fermentation time, and e is the

211 Euler constant (2.718282).

212 **Results**

213 **Recovery of AD process imbalance**

214 The AD process imbalance was caused by a low pH and high VFA accumulation due to

215 organic overload and low degradation efficiency. Inhibited sludge had low pH (3.9) and a high

216 total VFA (4.8 g/L) with butyric acid (2.5 g/L) and acetic acid (1.8 g/L) as the main VFA (Table

217 1). It also had a low SS and VSS of 14.5 g/L and 11.0 g/L, respectively, indicating a low number

218 of active methane-producing microorganisms in the systems. The specific methanogenic activity

219 (SMA) of the inhibited sludge with glucose, acetic acid, cellulose, and gelatin was 0.208, 0.344,

220 0.401, and 0.065 gCH_4-COD/gVSS/d, respectively (Fig. 1). The pH of inhibited sludge was

221 gradually increased from 5.7 to 7.8. Data files regarding SMA analysis of inhibited microbial

222 sludge, self-recovery sludge, and all recovered strategy were showed in Data S1. The acidic pH

223 directly affected microbial activity, leading to a low methane production rate (40.8 mL-CH$_4$/d)

224 and extended recovery time (49.4 days). The self-recovery had a low hydrolysis constant (k_H)

225 (0.005 d^{-1}) with a long time lag phase (21.2 days), and low methane yield (209 mL-CH$_4$/g-

226 VS$_{added}$) was observed (Table 2). The specific methanogenic activity of self-recovery was lower

227 than that of the inhibited sludge for cellulose and glucose as substrate (0.112 and 0.106 gCH$_4$-

228 COD/gVSS/d, respectively) but higher for acetic acids and gelatin substrate (0.481 and 0.122

229 gCH$_4$-COD/gVSS/d, respectively) (Fig. 1). Methane production was achieved from self-

230 recovery, but the low activity of specific methanogenic activity indicated low active methane-

231 producing microorganisms in the self-recovery strategy. A more detailed biogas production data

232 of each recovery strategy and self-recovery will be provided in Data S2.

233   The recovery by dilution strategy with biogas effluent (BE) gave a higher methane yield

234 (214-282 mL-CH$_4$/g-VS$_{added}$) and methane production rate (53.4-108.0 mL-CH$_4$/d) than the

235 recovery by dilution with tap water (TW) (177-190 mL-CH$_4$/g-VS$_{added}$ and 40.7-62.5 mL-CH$_4$/d,

236 respectively) and the self-recovery (Table 2). The time lag phases of dilution with BE (7.1-12.3

237 days) and dilution with TW (9.9-15.1 days) were significantly shorter than those of self-recovery

238 (21.2 days). The recovery time of dilution with TW and BE was in the same range (33.2-41.7

239 days and 32.8-42.0 days, respectively). The dilution with TW could reduce toxicity in the

240 inhibited sludge so that time lag phase and recovery time could be decreased, but not enhanced

241 methane-producing microorganisms resulting in low methane yield and methane production rate.

242 The recovery by dilution with BE at a ratio of 8:2 to 5:5 had a 20-32% shorter lag phase and

243 recovery time than the self-recovery. The recovery by dilution with BE could enhance active

244 methane-producing microorganisms in sludge and resulted in a 2.2 fold increase of the methane

245 production rate (91.4-92.8 mL-CH$_4$/d compared to 40.8 mL-CH$_4$/d, respectively). The recovery

246 by dilution with BE had specific methanogenic activity higher than self-recovery and its specific

247 methanogenic activity of sludge with glucose, acetic acid, cellulose, and gelatin was 0.234,

248 0.684, 0.528, and 0.134 gCH$_4$-COD/gVSS/d, respectively. The methane yield of dilution with

249 BE strategy was 34.9% higher than self-recovery and showed better growth of methanogens

250 under suitable pH (6.5-7.3). Therefore, the dilution of the inhibited sludge with BE at a ratio of

251 8:2 was a suitable strategy to accelerate the recovery process of the sludge from the inhibited

252 state.

253   The recovery by adjusting pH strategy with all concentrations tested of (0.10-0.5 % w/v)

254 Ca(OH)$_2$, (0.10-0.14 % w/v) NaOH, (6.00-10.00% w/v) oil palm ash, except (0.85-1.25% w/v

255 NaHCO$_3$) exhibited higher methane yield and methane production rate than self-recovery (Table

256 2). Among them, the highest methane yield (383 mL-CH$_4$/g-VS$_{added}$) was achieved from the

257 recovery by adjusting pH with 0.14% w/v NaOH with the methane production rate of 111.7 mL-

258 CH$_4$/d. Conversely, the highest methane production rate (226.3 mL-CH$_4$/d) was achieved from

259 the recovery by adjusting pH with 8.0% w/v oil palm ash with the hydrolysis constant (k_H) of

260 0.007 d^{-1} and time lag phase of 8.2 days and a recovery time of 32.4 days. In terms of the specific

261 SMA of recovery by three sources of alkaline for adjusting pH, Ca(OH)$_2$ showed the highest

262 SMA on acetic acid and cellulose, as substrates were 0.767 and 0.821 gCH$_4$-COD/gVSS/d,

263 respectively, while the highest SMA on glucose and gelatin (0.591, and 0.096 gCH$_4$-

264 COD/gVSS/d, respectively, were obtained from recovery by adjusting pH with 8.00% w/v oil

265 palm ash. Thus, the pH adjustment strategy, using 0.14% w/v NaOH and 0.40% w/v Ca(OH)$_2$,

266 gave the highest methane yields of 383 and 373 mL-CH$_4$/g.VS$_{added}$, respectively, while using

267 8.00% w/v oil palm ash and 0.40% w/v Ca(OH)$_2$ produced the highest methane production rates

268 of 226.3 and 151.4 mL-CH$_4$/d, respectively. 0.40% w/v Ca(OH)$_2$ was demonstrated as effective

269 in accelerating recovery of the inhibited sludge by saving 31.6% recovery time (from 49.4 to

270 33.8 days) and enhancing the methane yield by 78.5% (from 209 to 373 mL-CH$_4$/g.VS$_{added}$)

271 when compared with self-recovery. Nevertheless, the pH adjustment strategy from the best

272 results of each source of alkaline (0.14% w/v NaOH, 0.40% w/v Ca(OH)$_2$, 1.25% w/v NaHCO$_3$

273 and 8.00% w/v oil palm ash) was selected for economic evaluation compared to the dilution

274 strategy (dilution with TW at a 9:1 ratio and with BE at an 8:2 ratio).

275   The recovery by bioaugmentation or re-inoculation of active methane-producing sludge

276 at 5-50% into the inhibited sludge was able to accelerate the recovery process in terms of

277 methane yield (212-237 mL-CH$_4$/g-VS$_{added}$), methane production rate (40.4-83.5 mL-CH$_4$/d), and

278 time lag phase (15.2-11.0 days), but did not reduce the recovery time (43.6-47.2 days) (Table 3).

279 Its k_H value was 0.006-0.008 d^{-1}. The addition of active methane-producing sludge at 15-50%

280 had a methane production rate higher than the self-recovery as it can improve the amount of

281 active biomass. The recovery of this strategy gave a small improvement with a long recovery

282   time of 45.8 days not too different from that of self- recovery (49.20 days). In particular, k_H of

283   the addition of active methane-producing sludge strategy showed a small increased when

284   compared with self-recovery. The amount of active methane-producing sludge at 30%-50% was

285   suitable for recovery of the inhibited sludge with a shorter time lag phase and increased tolerance

286   of microorganism to low pH and high VFA. However, the addition of the active methane-

287   producing sludge strategy had a lower recovery efficiency than alkali addition due to lack of

288   buffering capacity.

289   **Economic evaluation**

290   The energy and economic evaluation of each recovery strategy was provided in this

291   study. The price of reagents, energy consumption, energy loss, and human resources was

292   achieved according to the current market price, and the average price of industrial water in

293   Thailand. According to Table 4, there were no extra economic benefits from either of the

294   recovery strategies. The recovery by NaOH addition, dilution with biogas effluent, and oil palm

295   ash addition could save the cost of recovery of the inhibited AD systems more than other

296   strategies. The net profit of recovery of the inhibited sludge is in the following order

297   (USD/m^3/d): NaOH addition (-1.76), dilution with biogas effluent (-1.77), oil palm ash addition

298   (-1.79) Ca(OH)$_2$ addition (-2.49), dilution with tap water (-3.33), and NaHCO$_3$ addition (-7.2).

299   The recovery by 0.14 % w/v NaOH addition corresponding to NaOH of 1.40 kg/m^3-inhibited

300   sludge had the cost of chemicals at 0.49 USD/m^3/d. The recovery by dilution with biogas

301   effluent at a ratio of 8:2 corresponding to 0.2 m^3/m^3-inhibited had no cost for biogas effluent.

302   The recovery by 8.00 % w/v of oil palm ash addition corresponding oil palm ash of 80 kg/m^3-

303   inhibited sludge had no cost for oil palm ash. Oil palm ash is a by-product obtained by burning

304   fibers, shells, and empty fruit bunches as fuel in palm oil mill boilers while biogas effluent is that

305   from POME AD digester, and free of charge. Thus, we suggest that NaOH addition, dilution

306   with biogas effluent, and oil palm ash addition could be economically feasible strategies to

307   recover the inhibited AD system.

308   **Validity of lab-scale results to the full-scale recovery of AD process imbalance**

309   Full-scale recovery was conducted by diluting 50 m^3 of inhibited sludge with biogas

310   effluent at a ratio of 8:2, which was added to 6,000 m^3 biogas reactor every day. The AD

311 imbalance reactor was maintained by a low feeding rate of 10 m^3-POME/d. After dilution, the
312 pH increased from 5.6 to 6.8 in the first week and to 7.8 in the second week. After that, the
313 reactor was operated normally with a feeding rate of 200 m^3-POME/d. The methane production
314 rates of the first, second, and third weeks of dilution with biogas effluent were 0.8, 2.0, and 2.86
315 m^3-CH_4/m^3-reactor /d, respectively, with the pH values of 6.8, 7.8 and 7.8, respectively (Fig. 2).
316 The SMA values also increased to 0.44, 0.70, and 0.71 gCH_4-COD/gVSS/d, respectively. Results
317 indicated that the reactor recovered within two weeks after dilution with biogas effluent.
318 Therefore, this recovery time (15 days) gave a better result than lab-scale reactor (36.4 days).

319 **Microbial community responsible for high potential recovery strategies**

320   The microbial community from the four high potential recovery strategies (dilution with
321 BE at a ratio of 8:2, 0.14% w/v NaOH addition, 0.50% w/v Ca(OH)$_2$addition, and 8.00% w/v oil
322 palm ash addition) indicated by short time lag phase, short recovery time, high SMA activity,
323 and high methane production was analyzed. The heat map of the bacterial and archaeal
324 communities of these four recovered sludges were higher than those of self-recovery (Fig. 3).
325 The bacterial community of self-recovery strategy was dominated by *Desulfotomaculum* sp.,
326 *Bacteroidetes* sp., and *Lactobacillus* sp. (Fig. 3A). The number of *Clostridium* sp., *Anaerostipes*
327 sp., *Lynsinibacillus* sp. increased after 12 days of self-recovery. For the archaea community, self-
328 recovery was dominated by *Methanosaeta* sp. (Fig. 3B). The numbers of *Methanosaeta* sp.,
329 *Methanosarcina* sp. increased after 12 days of self-recovery, while the bacteria in recovery by
330 dilution with BE, at a ratio of 8:2, was dominated by *Desulfotomaculum* sp., *Lactobacillus* sp.,
331 *Bacteroidetes* sp., *Clostridium* sp., *Staphylococcus* sp., *Selenomonas* sp., and *Lynsinibacillus* sp.,
332 in which the last four species increased after 5 days of recovery. The archaea community was
333 dominated by *Methanosaeta* sp., and *Methanosarcina* sp. in which the latter species decreased
334 after 5 days of recovery. The recovery by 0.14% w/v NaOH addition was dominated by
335 *Desulfotomaculum* sp., *Blautia* sp., *Lactobacillus* sp., *Bacteroidetes* sp., and *Selenomonas* sp.,
336 with the number of *Blautia* sp., *Clostridium* sp., *Anaerostipes* sp., *Lynsinibacillus* sp.,and
337 *Staphylococcus* sp. increasing after 5 days of recovery. Furthermore, the archaea community was
338 dominated by *Methanosaeta* sp., *Methanosarcina* sp., and *Methanococcoides* sp. after 5 days of
339 recovery. The recovery by 0.50% w/v Ca(OH)$_2$ addition was dominated by *Desulfotomaculum*
340 sp., *Blautia* sp*., Clostridium* sp., *Anaerostipes* sp*., Kurthia sp., Exiguobacterium* sp*.,*

341 *Staphylococcus* sp., *Selenomonas* sp., *Bacillus* sp*., Lynsinibacillus* sp., *Lactobacillus* sp., and

342 *Bacteroidetes* sp., in which the last two species did not appear at 5 days of recovery. The archaea

343 community was dominated by *Methanosaeta* sp., with additional species of *Methanosarcina* sp.,

344 and *Methanococcoides* sp. at 5 days of recovery. The recovery by 8.0% w/v oil palm ash

345 addition was dominated by *Desulfotomaculum* sp., *Blautia* sp*., Lactobacillus* sp., *Bacteroidetes*

346 sp*., Clostridium* sp., *Anaerostipes* sp., *Kurthia sp., Exiguobacterium* sp*., Staphylococcus* sp.,

347 *Selenomonas* sp., *Bacillus* sp*.,* and *Lynsinibacillus* sp., where the first four species did not appear

348 at 5 days of recovery, while the dominated archaea community is similar with the recovery by

349 0.50% w/v Ca(OH)$_2$ .The orders *Clostridiales* and *Bacilli* were observed as main bacteria in all

350 recovered sludges. *Desulfotomaculum* sp.was found in all recovered strategies while *Blautia* sp.,

351 *Clostridium* sp., and *Anaerostipes* sp. were remarkably abundant during 12-20 days in pH

352 adjustment with 0.50% w/v of Ca(OH)$_2$ and 8.0 % w/v of oil palm ash addition. During the third

353 week, *Bacillus* sp., *Lactobacillus sp.*, *Staphylococcus sp., Kurthia sp.*, *Lysinibacillus* sp.,

354 *Exiguobacterium* sp., and *Bacillus* sp. were observed in all strategies. The recovery by 0.50 %

355 w/v Ca(OH$_2$) and 8.0 % w/v oil palm ash addition was predominant with bacteria belonging to

356 *Kurthia sp.*, *Exiguobacterium* sp., and *Bacillus* sp. These bacteria can produce VFA from

357 monomers after hydrolysis, especially *Exiguobacterium* sp., which was a high number in the

358 third week. The member of *Bacteroides* sp., and *Selenomonas* sp. had a low number in self-

359 recovery but abundant in the recovered sludge with 0.14% of NaOH, 0.50% of Ca(OH)$_2$, and

360 8.00% of oil palm ash addition. The distribution of the exclusive bacteria in different groups

361 clearly showed that the recovery strategy significantly influences the bacterial community

362 structure and selectively enriches specific acidogenic bacteria during the recovery process. The

363 acetoclastic methanogen (*Methanosaeta* sp.) was more abundant than hydrogenotrophic

364 methanogens in the recovered sludge. *Methanosaeta* sp. and *Methanosarcina* sp. were

365 predominant in the recovered sludge with 0.50%w/v Ca(OH)$_2$ and 8.00 %w/v oil palm ash

366 addition. Molecular identification of bacteria and archaea from recovery strategy by dilution with

367 BE 8:2, addition with 0.14% w/v NaOH, 0.50% w/v Ca(OH)$_2$, 8.0% w/v oil palm ash and self-

368 recovery by denaturing gradient gel will be offered in Table S1 and Table S2, respectively).

369 **Discussion**

370   The inhibited AD sludge was caused by low pH and high VFA accumulation resulting in
371 extreme consumption of alkalinity from the AD reactor. This indicated that the pH directly
372 affected the microbial activity leading to low biodegradability and methane production. The long
373 time lag phase of self-recovery indicated that adaptation and initiating bacterial multiplication
374 are required due to the loss of a dynamic balance between acidogens and methanogens. Methane
375 production was observed in self-recovery under low initial pH of 5.7. Several hydrogenotrophic
376 methanogens are capable of growth and metabolism at acidic pH (often less than 6.0)
377 *(Charalambous et al., 2020)*. The prevalence of hydrogenotrophic methanogens over acetoclastic
378 methanogens was also reported by *Kim et al. (2004)* during the operation of the bioreactor for
379 hydrogen production at pH below 5.0. The dilution with tap water was not significant to
380 accelerate the recovery of the inhibited sludge. This strategy can not only dilute inhibitors but
381 also the active microbes and substrates, resulting in a reduced methane production rate and
382 providing long-time recovery. The dilution with water at the ratio of 5:5 achieved a shorter time
383 lag phase (9.1 days) than self-recovery (21.2 days), but did not accelerate the recovery process
384 *(Wu et al., 2015)*. The dilution with BE at a ratio of 8:2 could enhance methane yield of 34.7%
385 compared with self-recovery, with a shorter recovery time and high k_H. The high k_H indicates a
386 high conversion rate of the recovered sludge *(Sosnowski et al., 2008)*. The recirculation of BE to
387 adjust the pH of POME could enhance methane production by two-stage anaerobic digestion
388 with the highly flavored activity of acidogens *(O-Thong et al., 2016)*.

389   The inhibited sludge was recovered by providing alkalinity sources for methane-
390 producing bacteria *(Chen et al., 2015)*. It was reported that increased alkalinity resulted in higher
391 methane production than non-alkalinity addition reactors *(Lens et al., 2003)*. A significant
392 increase in methane production (3.03%) was observed in the AD of wet poultry with NaOH
393 addition *(Ajiboye et al., 2018)*. The adjusting pH with 0.14 % w/v NaOH addition could adjust
394 the pH of the inhibited sludge to pH 7.30. The recovered sludge was a relatively stable pH of
395 6.80-7.50 with an enhanced methane yield of 83.3% compared with self-recovery. The results
396 agreed with *Zhang et al. (2018)*, who reported that the pH adjustment of the AD system by
397 0.013% of NaOH addition could delay the time of process failure by enhancing the tolerance of
398 methanogens to the high concentration of VFA via reducing the ratio of un-dissociated VFA.

399   NaOH is one of the most popular alkaline chemicals used in the AD system due to its potential to

400   buffer the pH *(Gáspár et al., 2007)*. The recovery by Ca $(OH)_2$ addition for adjusting the pH of

401   inhibited sludge showed a high function as a buffering capacity for the AD system with a stable

402   pH in the recovered AD systems. In addition, *Li et al. (2009)* reported that the pH adjustment

403   with $Ca(OH)_2$ improved the methanogenic activity by maintaining a stable pH for methanogens.

404   The $Ca(OH)_2$ addition at a concentration of 0.6% to 1.0% w/v could maintain active methane-

405   producing microorganisms and stability in the AD process *(Zhang et al., 2014)*. However, the

406   accumulation of Ca^{2+} may lead to the precipitation of calcium salt and accumulation on the

407   reactor walls leading to a loss of nutrition and a lower buffer ability in the AD system *(Zhu et al.,*

408   *2010)*. The recovery by oil palm ash addition can improve the buffer capacity and methane

409   production rate of the inhibited sludge with a short time lag phase and recovery time. The

410   addition of ash could increase functional buffering capacity corresponding with *Bunrung et al.*

411   *(2011)*, who reported that 15% (w/v) oil palm ash addition resulted in an increase of pH from 7.5

412   to 9.1. Oil palm ash composed of silicon dioxide (58-65%), calcium oxide (6-7%), and potassium

413   oxide (7-8%) could improve buffer capacity and pH of the AD systems in the range of 8.25-9.14

414   *(Tangchirapat et al., 2009)*. The oil palm ash addition of 1.18% w/v into POME improved

415   methane yield (218.79 mL-CH_4/g-COD) and adjusted the pH in the suitable range for AD

416   systems *(Jijai et al., 2017)*. The recovery by $NaHCO_3$ addition had low recovery efficiency due

417   to Na^+at a high concentration which could inhibit the methanogens resulting in a low methane

418   production rate *(Zhang et al. 2016)*. The Na^+ slightly inhibited the methanogens in AD systems

419   at 0.31% w/v of $NaHCO_3$ addition *(Chen et al., 2008)*.

420   The recovery by the addition of active methane-producing sludge can reduce the time lag

421   phase with a small improvement of biogas production comparing to self-recovery. The results are

422   in line with previous research *(Salminen & Rintala, 2002; Cirne et al., 2007)* that an increase of

423   active methane-producing sludge proportion can reduce the recovery time from 45 to 28 days.

424   Previous reports also showed that a re-inoculum size of 80% could recover the inhibition of

425   mesophilic anaerobic sludge treating the de-oiled grease trap waste *(Wu et al., 2015)*. The high

426   recovery efficiency strategies (0.50 %w/v $Ca(OH_2$ addition), dilution with biogas effluent at a

427   ratio of 8:2, and 8.00 % w/v oil palm ash) were dominated by *Clostridium* sp.*, Kurthia* sp.,

428   *Exiguobacterium* sp., *Bacteroides* sp., and *Bacillus* sp. which had been identified as being

429   involved in biogas production, especially in hydrolysis and acidogenesis stages *(Wirth et al.,*

430   *2012). Exiguobacterium* sp. has been confirmed as amylase and protease producing bacterium

431   *(Kumar et al., 2014)* and produces highly effective proteolytic enzymes *(Oh et al., 2018)*. A

432   member of *Bacteroides* sp. has been shown as a main microbe in anaerobic reactors with

433   polysaccharide degradation *(Levén et al., 2007; Trzcinski et al., 2010). Desulfotomaculum* sp.,

434   *Blautia* sp., and *Clostridium* sp. were fermentative bacteria and acetogenic bacteria that could

435   convert soluble organics to VFAs. The *Clostridiales* was generally found in the stable AD

436   digester *(Li et al., 2015).*

437   The high microbial diversity in recovered sludge results in higher abundant functions that

438   are able to operate under stable conditions resulting in good AD performances *(Carballa et al.,*

439   *2015*). The acetoclastic methanogens were more abundant than hydrogenotrophic methanogens

440   in the recovered AD system. The inhibited sludge commonly induced hydrogen production and

441   consequently facilitated the growth of hydrogenotrophic methanogens *(Liu et al., 2016)* which

442   decreased in the recovered AD system. The recovery by 0.50% w/v $Ca(OH)_2$ and 8.00 % w/v oil

443   palm ash addition enhanced acetoclastic methanogens resulted in the highest methane yield and

444   methane production rate. The dominant *Methanosarcina* sp. was most important in the recovered

445   AD system, which utilizated acetate to produce CH_4. Maintenaning the number of

446   *Methanosarcina* sp. during the AD process is critical for performance stability *(Yang et al.,*

447   *2016)*.

448   **Conclusions**

449   Recovery of the inhibited sludge by the addition of 0.14% w/v NaOH, 0.50% w/v

450   $Ca(OH)_2$, 8.00% w/v oil palm ash, and dilution with biogas effluent at a ratio of 8:2 had a short

451   time lag phase, with a short recovery time of 30-36 days. The dilution with biogas effluent at a

452   ratio of 8:2, 0.14% w/v NaOH addition and 8.00% w/v oil palm ash addition was considered a

453   more economical strategy with a recovery time of 30-36 days. The recovered AD system can

454   increase methane yield by 35-83% and significantly higher kinetics, SMA activity, and short

455   time lag phase compared to self-recovery. The *Clostridiales* sp., *Bacilli* sp., and *Methanosarcina*

456   sp. were dominant in the recovered AD system. The imbalanced full-scale hybrid cover lagoon

457   reactor (6,000 m^3) was recovered within 15 days by dilution with biogas effluent at a ratio of 8:2

458   with a better result than the lab-scale reactor (36.4 days).

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643   **This work is no conflict of interest.**

Figure 1

Specific methanogenic activity (SMA) of the inhibited sludge and recovered sludge by different recovery strategies.

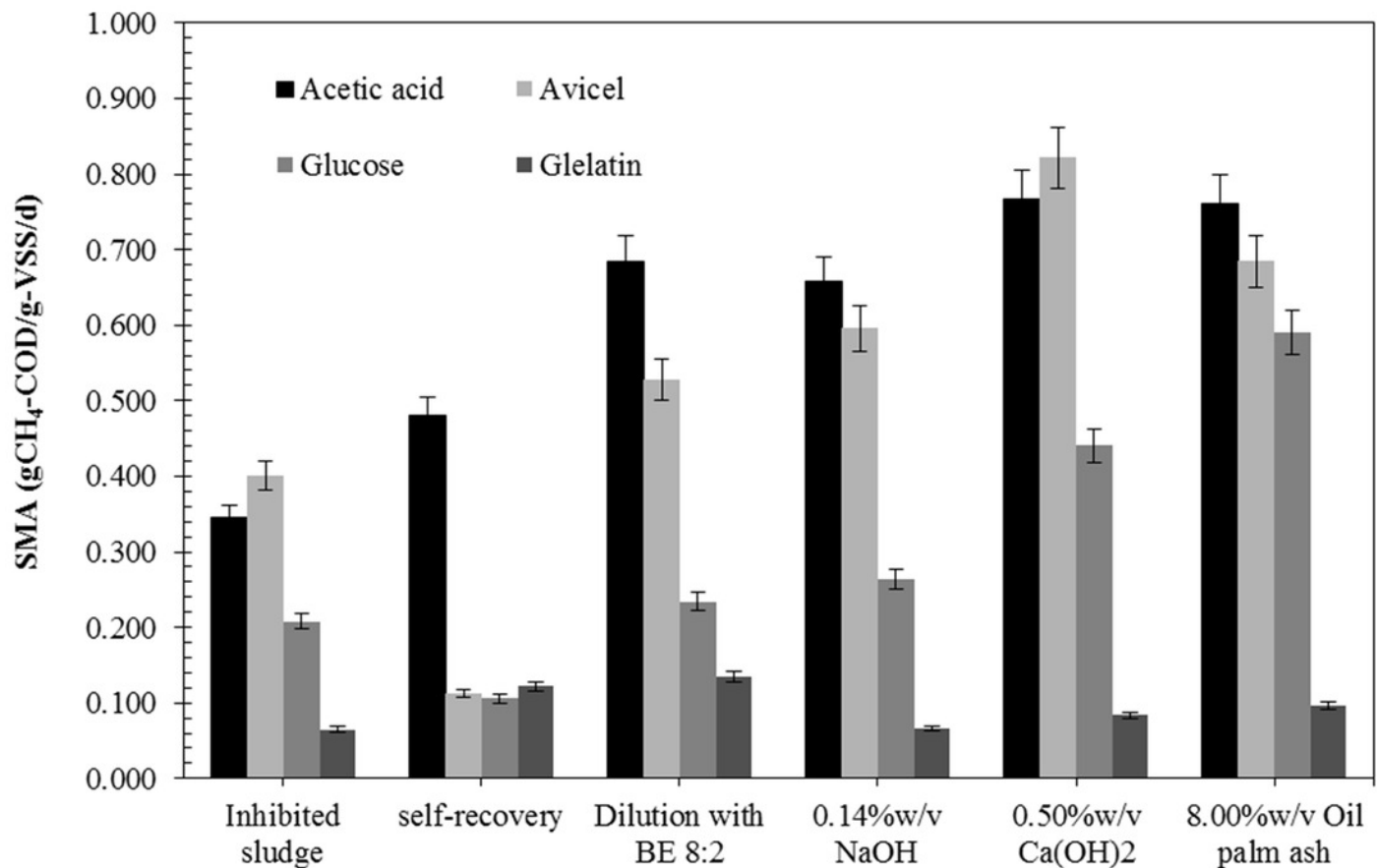



Figure 2

Full-scale application for recovery inhibited sludge by dilution with biogas effluent at a ratio of 8:2.

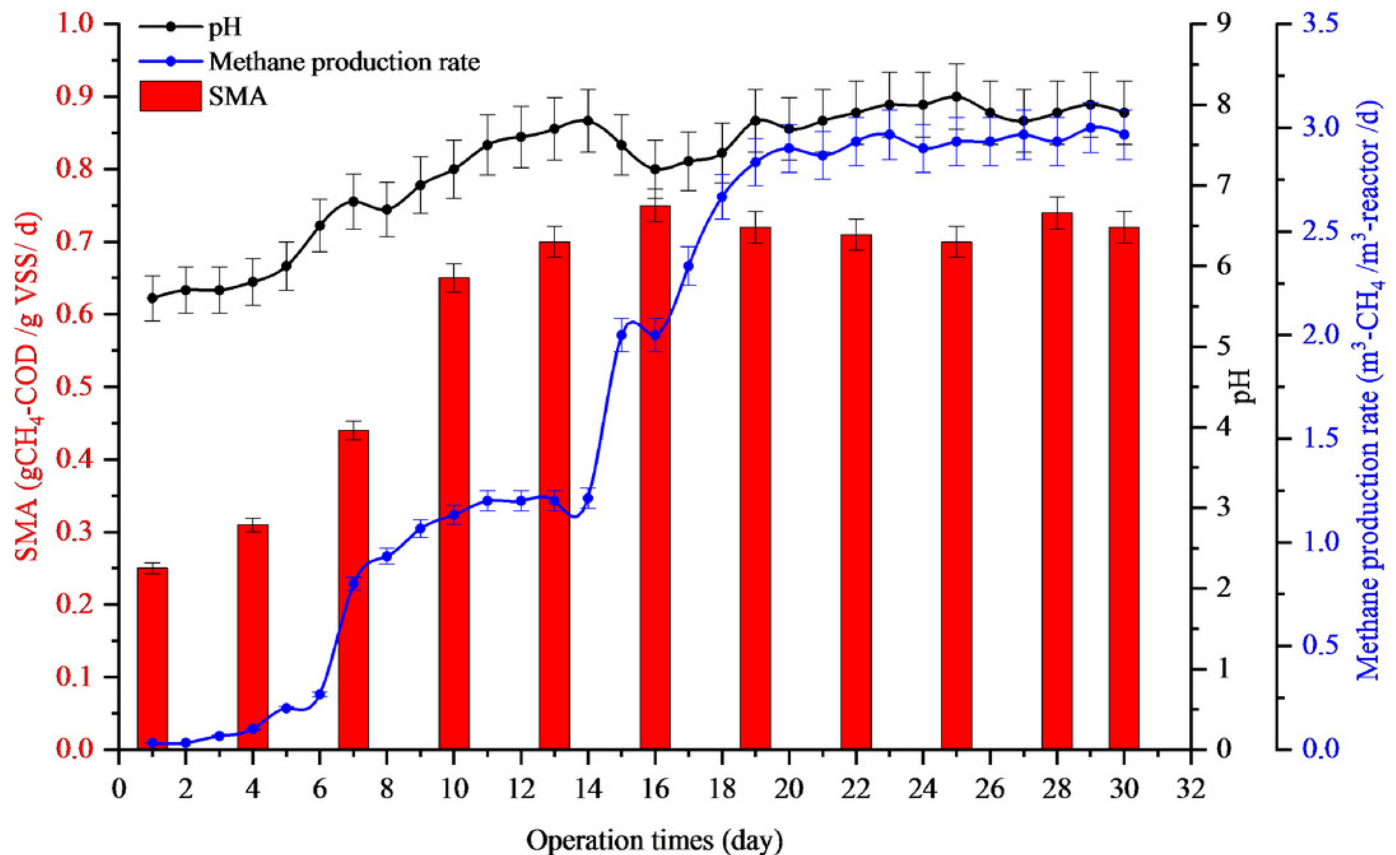
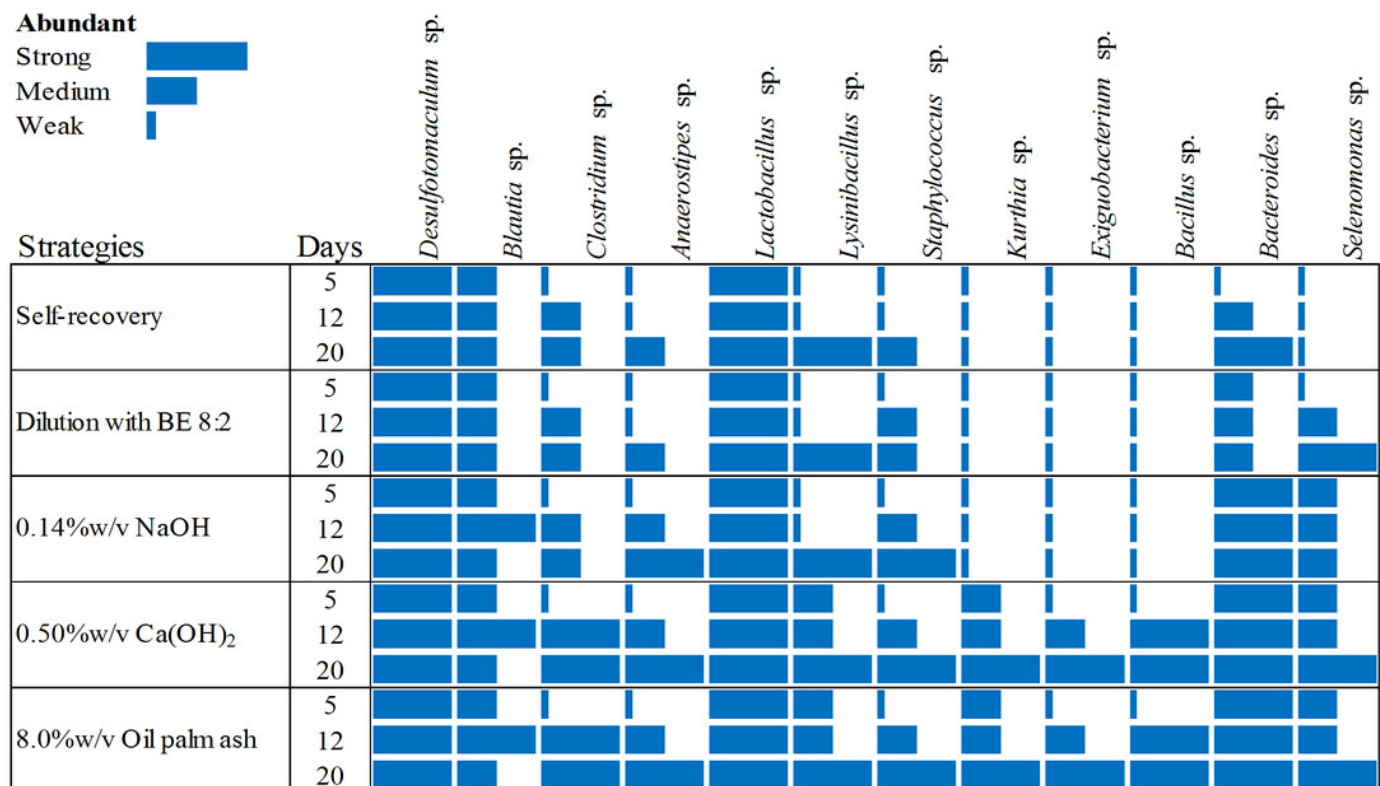
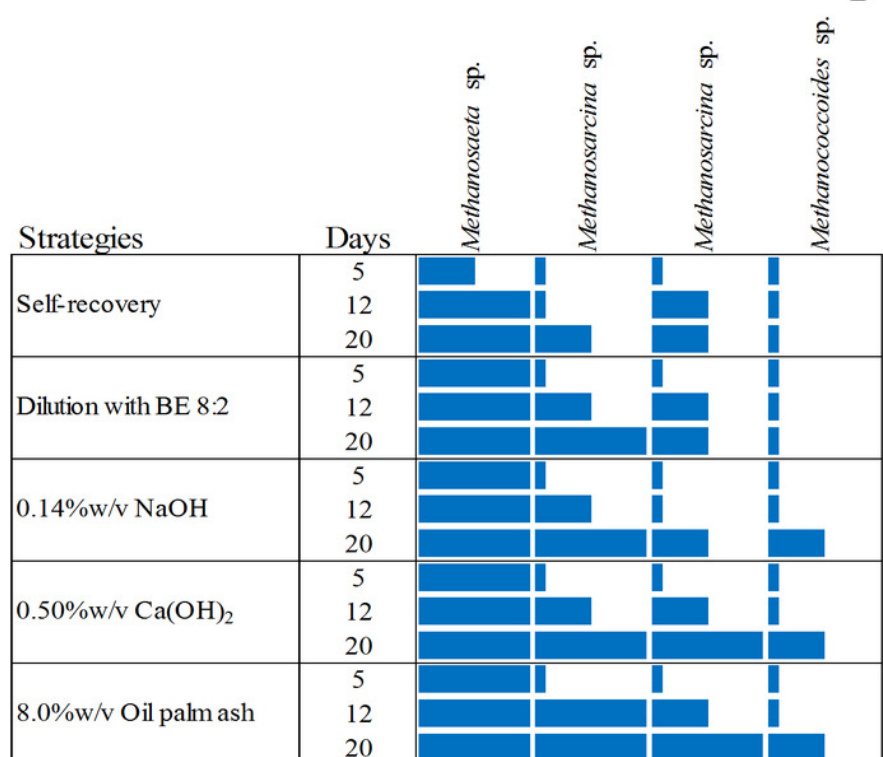



Figure 3

Dynamic diversity of bacteria (A) and archaea (B) during recovery by self-recovery, dilution with BE 8:2, 0.14% w/v NaOH addition, 0.50% w/v Ca(OH)$_2$ addition ,and 8.00% w/v oil palm ash addition.

The size of the rectangle respect to the dominance of microorganisms is represented from long-size to short-size for strong dominant to low dominant of microorganisms, respectively.

The characteristics of inhibited sludge, active methane-producing sludge, POME, and biogas effluent.

1 **Table 1.** The characteristics of inhibited sludge, active methane-producing sludge, POME, and biogas effluent.

Parameter	Unit	POME	Biogas effluent	Active methane-producing sludge	Inhibited sludge
pH	-	4.1±0.1	7.8±0.1	7.5±0.1	3.9±0.1
Total solids (TS)	g/L	55.4±0.2	13.4±0.3	68.4±0.3	24.0±0.2
Volatile solids (VS)	g/L	45.1±0.3	4.8±0.2	61.5±0.2	17.0±0.1
Suspended solids (SS)	g/L	34±0.3	2.4±0.3	59.8±0.1	14.5±0.2
Volatile suspended solids (VSS)	g/L	17.0±1.2	1.3±0.4	52.2±0.3	11.0±0.3
Total nitrogen (TN)	g/L	1.2±0.2	0.2±0.3	2.5±0.4	0.7±0.2
Alkalinity	g/L as CaCO$_3$	2.9±0.2	3.9±0.2	6.1±0.1	2.4±0.2
Lipid	g/L	6.5±0.3	0.1±0.02	0.5±0.1	4.4±0.1
Total chemical oxygen demand (tCOD)	g/L	59.0±0.1	7.5±0.1	N.D.	28.9±0.2
Soluble chemical oxygen demand (sCOD)	g/L	38.2±0.3	1.2±0.3	2.5±0.2	23.9±0.3
Total volatile fatty acids (TVFAs)	g/L	1.3±0.1	0.2±0.2	0.9±0.3	4.8±0.1
Acetic acid	g/L	0.4±0.04	0.02±0.01	0.3±0.01	1.8±0.2
Propionic acid	g/L	0.06±0.01	0.05±0.02	0.07±0.02	1.4±0.1
Isobutyric acid	g/L	0.03±0.01	0.03±0.01	0.05±0.01	0.3±0.1
Butyric acid	g/L	0.6±0.05	0.02±0.01	0.5±0.04	2.5±0.1

3 Note: N.D. = Not determined

Performance of self-recovery and recovered sludge by various strategies.

The colors respect to the high performances in dark red to low performance in light red.

1   **Table 2.** Performance of self-recovery and recovered sludge by various strategies with the colors respect to the high performances in
2   dark red to low performance in light red.

Strategies	Initial pH	Final pH	Methane yield (mL-CH$_4$/g-VS$_{added}$)	Methane Production rate (mL-CH$_4$/d)	k_H (d^{-1})	Lag phase (d)	Recovery time (d)
Self-recovery	5.7	7.8	209	40.8	0.005	21.2	49.4
Dilution with TW 9:1	6.2	7.3	190	42.7	0.007	15.1	41.7
Dilution with TW 8:2	6.3	7.4	187	40.6	0.006	12.4	39.2
Dilution with TW 7:3	6.3	7.4	178	46.0	0.007	14.3	38.1
Dilution with TW 6:4	6.5	7.5	173	46.4	0.007	10.9	36.5
Dilution with TW 5:5	6.5	7.5	177	62.5	0.006	9.9	33.2
Dilution with BE 9:1	6.7	7.3	214	53.4	0.008	12.3	42.0
Dilution with BE 8:2	6.8	7.4	282	91.4	0.009	7.1	36.4
Dilution with BE 7:3	6.9	7.5	253	108.0	0.008	7.7	30.8
Dilution with BE 6:4	6.5	7.6	229	100.4	0.009	7.2	31.2
Dilution with BE 5:5	6.5	7.8	230	92.8	0.008	9.9	32.8
0.10%w/v NaOH	6.5	8.6	218	85.9	0.007	9.5	30.8
0.11%w/v NaOH	6.6	8.8	238	87.3	0.007	9.5	30.0
0.12%w/v NaOH	6.8	8.8	278	87.0	0.006	8.9	35.0
0.13%w/v NaOH	7.0	8.2	263	85.9	0.006	9.1	34.1
0.14%w/v NaOH	7.3	8.5	383	111.7	0.006	9.0	35.0
0.85%w/v NaHCO$_3$	6.7	7.2	223	31.8	0.004	14.0	45.8
1.00%w/v NaHCO$_3$	6.8	7.3	224	24.2	0.004	14.0	58.8
1.25%w/v NaHCO$_3$	6.9	7.5	268	27.2	0.005	14.0	61.6
1.45%w/v NaHCO$_3$	6.9	7.6	168	20.8	0.004	16.0	58.5
1.50%w/v NaHCO$_3$	7.0	7.7	158	22.0	0.004	16.0	53.4
0.10%w/v Ca(OH)$_2$	6.7	8.1	319	103.8	0.008	7.6	36.7
0.20%w/v Ca(OH)$_2$	6.8	8.1	333	129.5	0.007	9.0	35.3
0.30%w/v Ca(OH)$_2$	6.8	8.0	368	112.0	0.007	8.3	35.3
0.40%w/v Ca(OH)$_2$	7.0	8.3	373	151.4	0.008	9.1	33.8
0.50%w/v Ca(OH)$_2$	7.1	8.6	360	137.7	0.006	9.4	33.9
6.00%w/v Oil palm ash	6.5	7.5	239	155.4	0.007	7.9	33.2
7.00%w/v Oil palm ash	6.9	7.8	265	155.2	0.006	8.6	32.1
8.00%w/v Oil palm ash	6.9	8.0	347	226.3	0.007	8.2	32.4
9.00%w/v Oil palm ash	6.9	8.3	218	147.5	0.006	9.2	33.0
10.00%w/v Oil palm ash	6.9	8.7	211	152.2	0.006	9.1	32.5

Performance of recovered sludge by addition of active methane-producing sludge.

The colors respect to the high performances in dark red to low performance in light red.

1   **Table 3.** Performance of recovered sludge by addition of active methane-producing sludge with the colors respect to the high

2   performances in dark red to low performance in light red.

Active methane-producing sludge (% v/v)	Initial pH	Final pH	Methane yield (mL-CH$_4$/g-VS$_{added}$)	Methane production rate (mL-CH$_4$/d)	k_H (d^{-1})	Lag phase (d)	Recovery time (d)
5	6.4	7.5	212	40.4	0.007	15.2	45.8
10	6.4	7.5	216	47.2	0.007	15.1	46.1
15	6.4	7.5	214	60.8	0.007	15.0	47.2
20	6.4	7.5	222	77.0	0.006	15.0	45.2
25	6.4	7.5	222	70.7	0.006	15.0	44.2
30	6.4	7.6	224	65.9	0.006	13.2	43.6
35	6.4	7.6	222	67.9	0.006	12.0	43.9
40	6.4	7.6	227	80.9	0.006	12.0	44.0
45	6.4	7.9	230	80.1	0.007	11.0	44.5
50	6.4	8.0	237	83.5	0.008	11.0	45.8

The economic evaluation and energy balance of different recovery strategies.

Table 4. The economic evaluation and energy balance of different recovery strategies.

Items	Dilution with tap water (TW)	Dilution with biogas effluent (BE)	NaOH addition	Ca(OH)$_2$ addition	NaHCO$_3$ addition	Oil palm ash addition
Chemical cost (USD/m^3/d)	-0.02	0.00	-0.49	-0.19	-1.73	0.00
Human resource (USD/m^3/d)	-0.05	-0.05	-0.15	-0.15	-0.15	-0.15
Energy required (USD/m^3/d)	-0.10	-0.10	-0.02	-0.02	-0.02	-0.02
Biogas loss (USD/m^3/d)	-4.26	-3.46	-3.20	-3.73	-5.30	-3.46
Biogas production (USD/m^3/d)	1.04	1.84	2.10	1.60	0.00	1.84
Net profit (USD/m^3/d)	-3.39	-1.77	-1.76	-2.49	-7.20	-1.79

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