

1 **Biodegradation Efficiencies and Economic Feasibility of Single-stage and Two-**
2 **Stage Anaerobic Digestion of Desulfated Skim Latex Serum (SLS) by Using**
3 **Rubber Wood Ash**

4
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25 **ABSTRACT**

26 The efficiencies of single-stage anaerobic digestion (SSAD) and two-stage
27 anaerobic digestion (TSAD) of desulfated skim latex serum (DSLS) using various
28 rubber wood ash (RWA) loadings were investigated in this study. The experiments on
29 batch processes showed that DSLS gave a higher yield (6-21%) than raw SLS in both
30 SSAD and TSAD. The highest H₂ and CH₄ yields of 90.64 and 294.53 mL/g-COD_{added}
31 were achieved with DSLS using RWA loading of 5 g/L (DSLS5) and 10 g/L (DSLS10),
32 respectively in TSAD (thermophilic and mesophilic conditions, respectively). The
33 maximum 305.09 mL/g-COD_{added} CH₄ yield in SSAD was observed for DSLS10. Total
34 energy recovery in TSAD was 5% higher than that in SSAD. However, the cost
35 assessment on continuous AD using kinetics and yield from the batch experiments
36 suggests longer payback time for TSAD (4.36 years) than for SSAD (2.52 years). TSAD
37 is not economically attractive with DSLS10 due to the large total volume of digestors
38 required. This study revealed that RWA can remove sulfate from SLS to enhance biogas
39 production and reduces H₂S in the biogas, while TSAD of DSLS was not attractive
40 compared to the conventional SSAD like for some other substrates reported in the
41 literature.

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43 **Keywords:** Skim Latex Serum, Two-stage anaerobic digestion, Bio-hydrogen, Bio-
44 methane, Cost assessment

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49 **1. Introduction**

50 Thailand is the main producer and exporter of several intermediate rubber
51 products, such as concentrated latex, block rubber, and ribbed smoked sheet rubber.
52 Concentrated latex is mainly produced with annual generation capacity of around 1.1
53 million tons from concentrated rubber latex plants, which are largely located in southern
54 peninsular Thailand in 2020 (Rubber Intelligence Unit of Thailand, 2020).

55 When the fresh latex (with about 30% rubber content) is centrifuged, the main
56 product of concentrated latex (with about 60% rubber content) is collected along with
57 the skim latex by-product (with about 5% rubber content) (Danwanichakul et al., 2019).
58 In skim latex process, sulfuric acid is added to precipitate and coagulate the rubber
59 (Chaiprapat et al., 2015) and the wastewater from this process is called skim latex
60 serum (SLS). It contains a large amount of sulfate (7500 mg/L), has high chemical
61 oxygen demand (COD) (42.50 g/L), Volatile Solids (VS) (37.94), and low pH (5.51)
62 (Raketh et al., 2021). The sulfate content in SLS can be converted to hydrogen sulfide
63 under anaerobic conditions, which causes odor problems nearby the mill and has
64 become a serious issue for latex rubber factories. Therefore, treatment of this
65 wastewater before discharge to environment is crucial, otherwise it may cause water, air,
66 and even soil pollution.

67 Anaerobic digestion (AD) is one of a number of techniques for treating SLS.
68 Nonetheless, the high sulfate content in SLS could inhibit methanogens, resulting
69 uncertain AD process with possibly low biogas yield (Jariyaboon et al., 2015). In our
70 previous research work, rubber wood ash (RWA) was used to reduce sulfate in SLS with
71 42% sulfate removal efficiency, and the results indicated that RWA can reduce sulfate,
72 with adsorption possibly as the main mechanism of sulfate removal, and the desulfated

73 SLS had improved biogas production (Raketh et al., 2021)

74 There are four steps in the AD process namely hydrolysis, acidogenesis,
75 acetogenesis, and methanogenesis (Wang et al., 2020). The conventional AD process is
76 performed in a single digester and is called single-stage AD (SSAD); it focuses on
77 methane production. Actually, hydrogen is produced during acidogenesis and converted
78 to methane during methanogenesis. Two-stage AD (TSAD) has been proposed to
79 recover hydrogen in the first separated digester and the effluent is fed to the second tank
80 for further digestion. Split of TSAD to two reactor tanks allows optimizing
81 hydrolysis/acidogenesis and methanogenesis and could enhance the overall reaction
82 rate, maximize biogas yields, and create a process that is easier to control, both in meso-
83 and thermophilic states (Schievano et al., 2012).

84 Many studies have shown that TSAD gives a higher overall degradation
85 efficiency, and several authors have reported different applications of TSAD, with
86 various organic substrates and different process designs .Sani et al. (2021) reported a
87 comparative evaluation of SSAD and TSAD using palm oil mill effluent. The yield
88 $264.73 \text{ mL-CH}_4/\text{g-COD}_{\text{added}}$ was achieved in SSAD and $106.13 \text{ mL-H}_2/\text{g-COD}_{\text{added}}$ and
89 $334.56 \text{ mL-CH}_4/\text{g-COD}_{\text{added}}$ were produced in TSAD. A 38.95% higher total energy fuel
90 yield was found for TSAD compared to SSAD. Similar results were found when wheat
91 feed pellets (Massanet-Nicolau et al., 2013) and poplar wood (Akobi et al., 2016) were
92 used as substrates in a comparison study of SSAD and TSAD. Furthermore, food waste
93 was used as substrate in TSAD yielding 20 and 18% more total energy than in SSAD, in
94 the studies of De Gioannis et al. (2017) and Nathao et al. (2013), respectively.
95 Conversely, there is a case reporting no significant differences in overall energy
96 recovery between TSAD and SSAD systems using a mixture of swine manure and

97 market biowaste as substrate (Schievano et al., 2012). This result suggested the
 98 hypothesis that the TSAD systems may in some cases bring no advantage in terms of
 99 overall energy recovery yields.

100 The objective of the present study was to investigate whether TSAD of raw SLS
 101 and desulfated SLS could generate more energy than SSAD. Moreover,
 102 biodegradability, kinetics and cost assessments of both processes were also explored.

103

104 2. Materials and methods

105 2.1. Substrate and inoculum

106 Fresh raw SLS was collected from skim latex serum coagulation baths of the
 107 concentrated latex factory in Songkhla province, Thailand. The main characteristics of
 108 raw SLS and desulfated SLS (DSLSS) with the level of RWA loading at 5, 10, 15, 20, and
 109 30 g/L, are presented in Table 1. The SLS collected was stored at 4°C until use to
 110 minimize self-biodegradation and acidification (the maximum storage time was 1
 111 month).

112

113 **Table 1** Some characteristics of substrate and inoculum in AD process.

Parameters	Substrate						Inoculum	
	Raw SLS	DSLSS5	DSLSS10	DSLSS15	DSLSS20	DSLSS30	H ₂	CH ₄
RWA loading (g/L)	0	5	10	15	20	30	-	-
pH	5.51	6.46	6.83	7.04	7.49	8.26	4.81	7.88
TS (g/L)	44.74	45.31	45.99	46.67	47.36	47.70	62.64	61.19
VS (g/L)	37.94	38.70	38.85	39.00	39.15	39.23	45.23	39.43
Ash (g/L)	6.8	6.99	7.85	8.72	9.58	10.01	17.41	21.76
COD (g/L)	43.11	42.69	42.88	43.07	43.26	43.36	NA	NA
Alkalinity (mg-CaCO ₃ /L)	3,287	3,318	3,336	3,656	3,787	4,101	NA	NA

Sulfate (mg/L)	7,500	5,200	4,250	4,900	6,150	6,500	NA	NA
TOC (g/L)	14.25	NA	NA	NA	NA	NA	NA	NA
TKN (mg/L)	1,548	NA	NA	NA	NA	NA	NA	NA

NA denoted not analyzed

114

115 RWA was collected from the high-pressure steam boiler of a glove factory in
116 Songkhla province, Thailand. The main components in RWA were Ca and Si at 21.83
117 and 10.53 %w/w. K, S, Cl, Mg, and Fe were found in smallish amounts (6.43, 5.77,
118 3.15, 2.05, and 1.49 %w/w respectively) while some heavy metals were also observed
119 as presented in our previous study (Raketh et al., 2021).

120 The mesophilic methane inoculum was obtained from the biogas plant of
121 Phasaeng Green Power Co., Ltd., Surat Thani Province, Thailand, in which palm oil
122 mill effluent was used as a substrate. The thermophilic inoculums for hydrogen
123 production were prepared by shock loading the mesophilic methane inoculum using 50
124 g/L brown sugar in 800 mL working volume serum bottle to enrich hydrogen production
125 bacteria. Then the serum bottles were closed with bottle caps and purged with nitrogen
126 gas at a flow rate of 1 L/min for 3 min to ensure anaerobic conditions before incubation
127 at 55 °C afterwards. Daily biogas volume was measured by water displacement method
128 while H₂, CO₂, and CH₄, concentrations in the biogas were analyzed by gas
129 chromatography. The sugar in the same dosage was added into the shock-loaded
130 inoculum for another acclimation. The CH₄ gas was detected only in the first day of first
131 round acclimation, after that CH₄ gas no longer appeared. The hydrogen yield of shock-
132 loaded inoculum in 3 rounds of acclimation were 63.76, 86.90, and 89.03 mLH₂/g-
133 COD, respectively, then it was further used as inoculum for hydrogen production in
134 two-stage anaerobic digestion (H₂-TSAD). Some characteristics of hydrogen and

135 methane inocula used in AD process are shown in Table 1.

136

137 2.2. Removal of sulfate from SLS

138 The rubber wood ash (RWA) levels of 5, 10, 15, 20, and 30 g/L were added to 1
139 L SLS before continuous stirring with a magnetic stirrer at a speed of 150 rpm for 10
140 minutes. After 10 minutes of mixing, the ash residue was immediately separated from
141 mixed suspensions and the desulfated SLS was then used as a substrate for anaerobic
142 digestion. The characteristics of solutions before and after adding RWA were analyzed
143 in terms of pH and sulfate.

144

145 2.3. Biohydrogen and biomethane production in batch process

146 H₂-TSAD was carried out in 120 mL serum bottle with a working volume of 60
147 mL. Raw SLS and desulfated SLS (DSLS) with various initial solid loadings of RWA
148 (5, 10, 15, 20, and 30 g/L) were used as substrate at initial loading of 28 g-COD/L and
149 70:30% v/v of substrate to inoculum ratio was added into each serum bottle (Angelidaki
150 and Sanders, 2004; Kongjan et al., 2018) and adjusted to pH 7.0 with NaHCO₃. After
151 that, the serum bottles were closed with bottle caps and purged with nitrogen gas at a
152 flow rate of 1 L/min for 3 min to ensure anaerobic conditions before incubation at 55
153 °C. The pH, sulfate, and volatile free fatty acids (VFAs) of effluent were investigated
154 afterwards.

155 The effluents from the first stage of H₂-TSAD were used to determine methane
156 production of two-stage anaerobic digestion (CH₄-TSAD), while Raw SLS and DSLS
157 corresponding to 8.5 g-COD/L initial loading were used to determine methane
158 production of single-stage anaerobic digestion (CH₄-SSAD). The procedure of CH₄-

159 TSAD and CH₄-SSAD experiments was as follows. First 60 mL of substrate was mixed
 160 with a 140 mL methane inoculum in 500 mL serum bottle, leaving a 300 mL headspace.
 161 The 70% by volume inoculum in the methane production stage was chosen to ensure
 162 sufficient microorganisms for biomethane production potential (BMP) protocol
 163 (Angelidaki and Sanders, 2004). A portion of wastewater was replaced by DI water for
 164 blank control. The serum bottle was then closed with a bottle cap and purged with
 165 nitrogen gas at a flow rate of 1 L/min for 3 min to ensure anaerobic conditions before
 166 incubation at 35 °C. The pH and sulfate of effluent were investigated afterwards.

167 All experiments were carried out in triplicate. Daily biogas volume was
 168 measured by water displacement method while CH₄, H₂ CO₂, and H₂S concentrations in
 169 the biogas were analyzed by gas chromatography. The fermentation was stopped when
 170 no significant gas production was observed. The initial characteristics in H₂-TSAD and
 171 CH₄-SSAD assay are shown in Table 2.

172

173 **Table 2** Conditions and initial main characteristics of H₂-TSAD and CH₄-SSAD

Parameter	H₂-TSAD	CH₄-SSAD
Working volume (mL)	60	200
TOC (g/L)	9.50	2.85
TKN (g/L)	1032	309
Alkalinity (g/L)	2191	657
Ash (g/L)	4.53	1.36
TS (g/L)	29.38	8.95
VS (g/L)	25.29	7.59
COD (g/L)	28.33	8.50

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176 2.4. Analytical methods

177 The biogas production volume was measured through water displacement
178 method. Hydrogen content in biogas was measured using gas chromatography (GC)
179 (Shimadzu GC 14A, Shimadzu Corp., Kyoto, Japan), equipped with a stainless-steel
180 column (2 m) packed with molecular sieve 58 (80/100 mesh) and equipped with a
181 thermal conductivity detector (TCD). Each sample of biogas (0.5 mL) was injected into
182 the GC with argon as the carrier gas at a flow rate of 35 mL/min. The GC column,
183 injection port, oven, and detector were all set at 100 °C temperature. Methane, carbon
184 dioxide, and hydrogen sulfite contents in biogas were also measured using gas
185 chromatography (Shimadzu GC 14A equipped with a thermal conductivity detector),
186 but with a 2.5 m Porapak S column with Hayesep Q (80/100). Helium was used as the
187 carrier gas at a flow rate of 30 mL/min. The injection port, oven, and detector were set
188 at 100, 60, and 110°C respective temperatures. An 0.5 mL sample of the gas was
189 injected in triplicate. VFAs were analyzed by gas chromatograph equipped with a flame
190 ionization detector (GC-FID) (Shimadzu GC 8A). The column capillary was packed
191 with fused silica and had 30 m length (Stabiwax® column). The GC-FID was equipped
192 with an autosampler and an integrator. The chromatography used this program
193 sequence: 60 °C for 35 min, 2 °C/min to 110 °C, 10 °C/min to 200 °C, and hold 1 min.
194 The inlet and detector temperatures were set at 230° and 250°C, respectively.

195 The procedures described in the standard methods (APHA, 2012) were applied
196 to determine pH, COD, TKN, total alkalinity, TS, VS, ash, and sulfate. TOC was
197 investigated with TOC-Liquid: multi N/C 3100 TOC analyzer (Analytik Jena).

198 Statistically significant differences in the results were determined using one-way
199 analysis of variance (ANOVA) in SPSS v26.0 (IBM, USA).

200

201 2.5 Kinetic model analysis for TSAD and SSAD processes

202 Models for explaining the complex metabolic processes and predicting the
203 kinetics in the AD comprise a first-order kinetic model and a modified Gompertz model,
204 presented in equations (1) and (2) (Nguyen et al., 2019; Siripatana et al., 2016). Then
205 ultimate hydrogen or methane production and the hydrolysis constants are obtained
206 from the experimental data plot.

207

208 First-order kinetic model : $M(t) = M_m \times (1 - e^{-k \times t})$ (1)

209

210 Here $M(t)$ is the cumulative production of hydrogen or methane at a given time (mL/g-
211 COD), M_m is the maximum cumulative production of hydrogen or methane (mL/g-
212 COD), k is the rate constant (1/h or 1/d), and t is the time (h or d).

213

214 Modified Gompertz model: $M(t) = M_m \times \exp \left\{ -\exp \left[\frac{R_{max} \times e}{M_m} (\lambda - t) + 1 \right] \right\}$ (2)

215

216 where R_{max} is the maximum specific of hydrogen or methane production rates (mL/g-
217 COD·h or mL/g-COD·d), λ is lag phase period or minimum time to produce biogas (h
218 or d), t is cumulative time for biogas production (h or d), and e is 2.718282, exp (1).

219 Microsoft Excel TM2010, with Solver add-in program (Microsoft, USA) was used to
220 determine the kinetic parameters .The correlation coefficients (R^2) were also obtained.

221

222 3. Results and discussion

223 3.1 Effect of RWA loading on biohydrogen and biomethane production

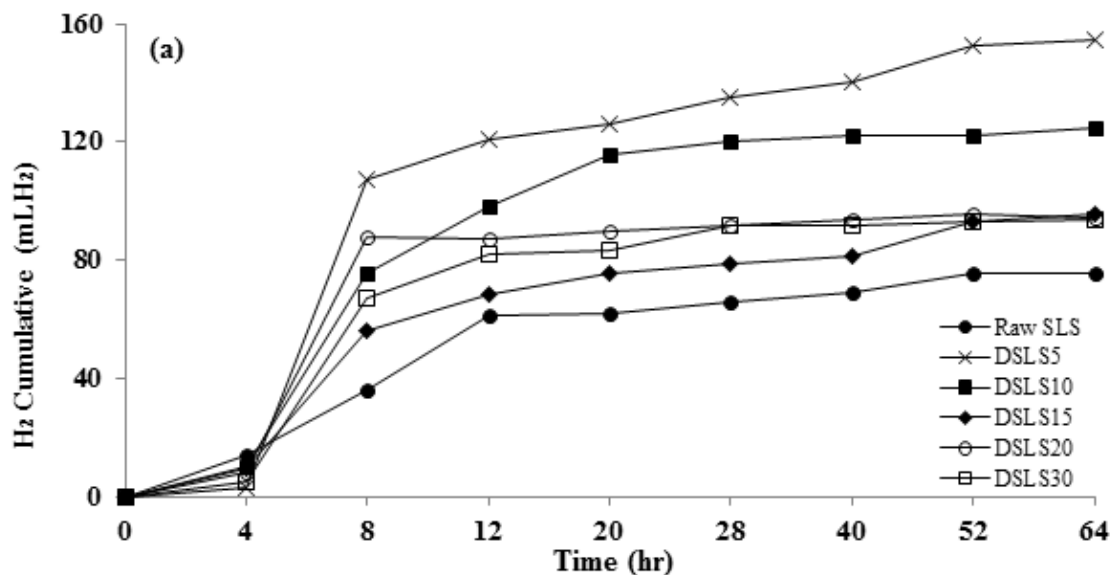
224 The cumulative H₂-TSAD of raw SLS and DSLS at various RWA loadings are

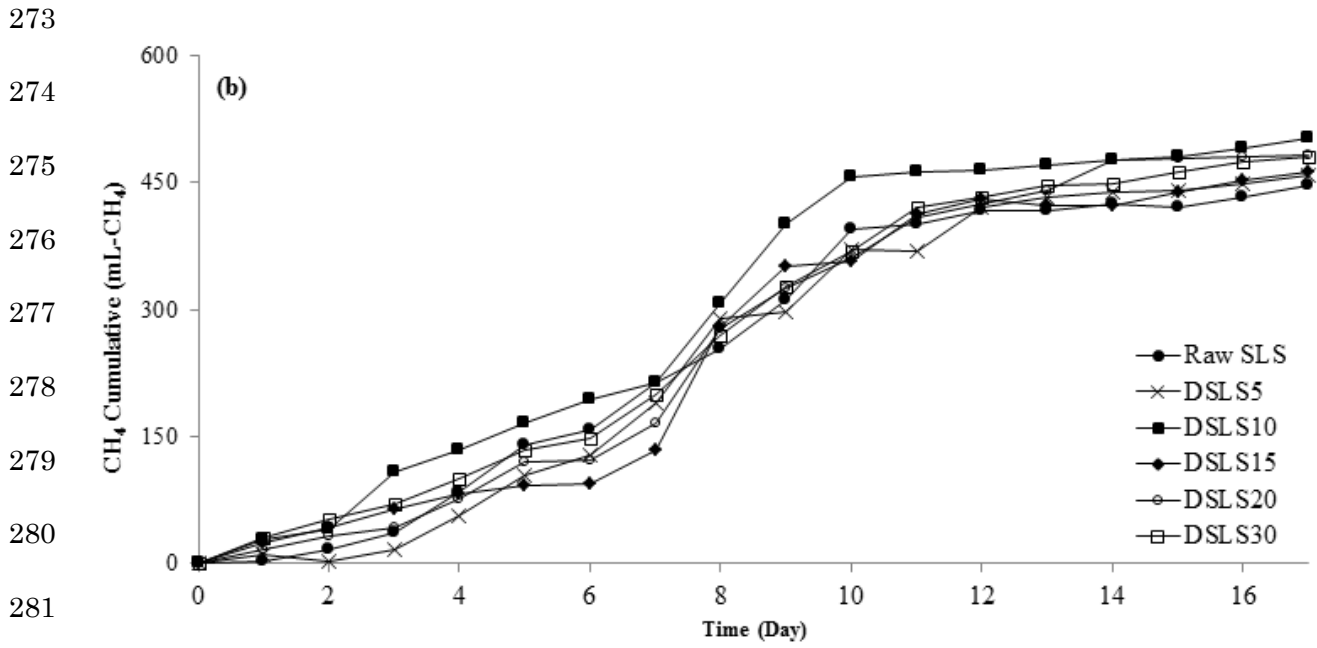
225 reported in Fig. 1a. No methane was detected during the hydrogen production in the
226 first-stage AD. All DSLS gave higher hydrogen production than raw SLS. The highest
227 154.48 mL-H₂ cumulative hydrogen in the hydrogenic stage during 64 h of operation
228 was achieved from DSLS5 (Fig. 1a), which corresponds to hydrogen yield of 90.64 mL-
229 H₂/g-COD_{added}. A lower by a half cumulation of hydrogen at 75.48 mL-H₂ was obtained
230 from raw SLS.

231 This obviously shows that, by using RWA, the sulfate in SLS could be reduced
232 (data shown in Table 3) and this decreased the competition between hydrogen
233 producing bacteria and sulfate reducing bacteria (SRB) for using the same organic
234 matters as electron donors to reduce either hydrogen or hydrogen sulfide (Kongjan et
235 al., 2014). Considering the RWA loading in SLS, 10 g/L RWA achieved the highest
236 sulfate removal efficiency. The mechanism of RWA in sulfate removal from SLS is
237 described in our previous work (Raketh et al., 2021). However, among the DSLS cases,
238 DSLS5 provided the highest H₂ production, while sulfate content was higher than in
239 DSLS10. Some metal ions can be leached from RWA and solubilized into SLS, as
240 shown in Table 4 which were investigated by inductively coupled plasma optical
241 emission spectrometry (ICP-OES) technique. The lower RWA loading should provide
242 less metal ions, so that when the RWA loading increased the metal ion concentrations
243 increased. Ni, Cu, and Mn slightly increase in DSLS while Mg was two times higher
244 than in raw SLS. K was also increased in DSLS10 and higher than in raw SLS,
245 exceeding the recommended level (2.5 mg/L) (Reungsang et al., 2019) and inhibiting
246 the anaerobic system. Possibly in these concentration ranges the metal ion effects
247 dominated over effects of the sulfate content.

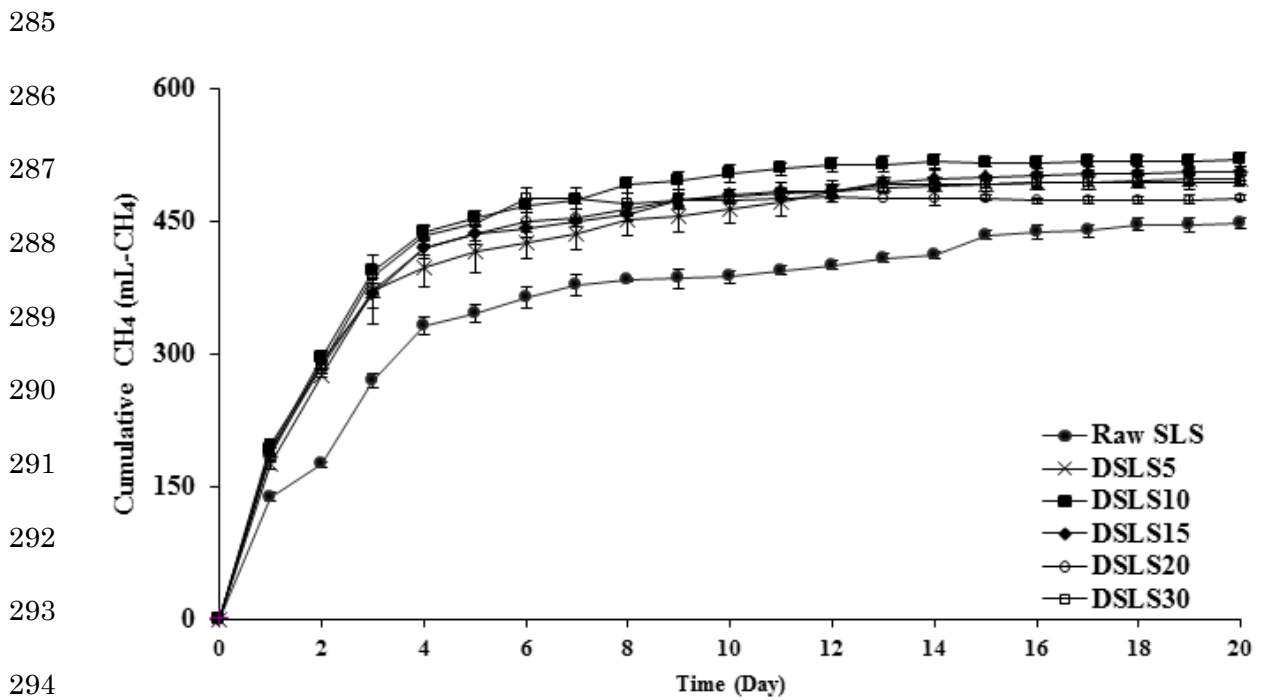
248 DSLS is also a more promising substrate than raw SLS for methane production

249 both in CH₄-TSAD and CH₄-SSAD (Fig. 1b and Fig. 2). 50.02-75.95% methane
 250 contents in the biogas were observed, demonstrating favorable metabolic pathway
 251 (Reungsang et al., 2019; Raketh et al., 2021). Using DSLS gave 13-16% methane
 252 production increases from that of raw SLS. The highest methane productions of 502
 253 mL-CH₄ and 520 mL-CH₄ were from DSLS10 in the CH₄-TSAD and the CH₄-SSAD
 254 processes, respectively. According to biomethane production protocols, the substrate in
 255 CH₄-TSAD and CH₄-SSAD assays was about 3-fold diluted by the inoculum. Thus, the
 256 concentrations of organic and inorganic compounds in SLS substrate were also reduced.
 257 This reduces the effects of RWA loading on CH₄-TSAD and CH₄-SSAD, compared to
 258 H₂-TSAD. DSLS10 achieved the highest methane productions in both CH₄-TSAD and
 259 CH₄-SSAD processes. This clearly demonstrates that at lower metal ion concentrations
 260 the sulfate content in DSLS significantly affected methane production.





283 **Fig.1** The experimental results from TSAD. (a) Cumulative hydrogen production, and
 284 (b) cumulative methane production.



295 **Fig.2** Cumulative methane production from SSAD

296 **Table 3** pH and sulfate concentration during H₂-TSAD, CH₄-TSAD, and CH₄-SSAD runs.

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Substrate	Two-stage AD									Single-stage AD						
	H ₂ -TSAD			CH ₄ -TSAD			Sulfate mass distribution		Overall sulfate removal (%)	CH ₄ -SSAD				Sulfate mass distribution		Overall sulfate removal (%)
	Initial pH ^a	Final pH	Initial Sulfate (mg/L)	Initial pH	Final pH	Final Sulfate (mg/L)	Initial ^b (mg)	Final (mg)		Initial pH	Final pH	Initial Sulfate (mg/L)	Final Sulfate (mg/L)	Initial ^c (mg)	Final (mg)	
Raw SLS	7.00	6.93	5417	7.56	7.64	261	325	52	83.94	7.31	7.34	1625	250	325	50	84.62
DSLS5	7.03	6.94	3467	7.6	7.65	155	208	31	85.10	7.49	7.33	1040	145	208	29	86.11
DSLS10	6.98	6.86	2833	7.55	7.63	111	170	22	86.94	7.58	7.35	850	102	170	20	88.00
DSLS15	7.00	6.93	3267	7.61	7.65	149	196	30	84.80	7.6	7.34	980	145	196	29	85.20
DSLS20	7.06	6.95	4100	7.62	7.66	152	246	30	87.64	7.65	7.37	1230	149	246	30	87.89
DSLS30	7.12	6.98	4333	7.63	7.66	174	260	35	86.62	7.68	7.4	1300	170	260	34	86.92

^a pH adjusted with NaHCO₃, ^b Working volume of H₂-TSAD was 60 mL, and ^c Working volume of CH₄-SSAD was 120 mL

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305 **Table 4** Dissolved elements in raw SLS and DSLS10 from ICP-OES technique.

Dissolved element	Concentration (mg/L)	
	Raw SLS	DSLS10
Cobalt (Co)	< 0.003	< 0.003
Nickel (Ni)	< 0.005	0.06±0.01
Copper (Cu)	< 0.016	0.18 ± 0.02
Manganese (Mn)	0.14 ± 0.00	2.69 ± 0.00
Iron (Fe)	0.60 ± 0.02	1.34 ± 0.02
Sodium (Na)	10.62 ± 0.02	12.22 ± 0.09
Calcium (Ca)	12.91 ± 0.05	302.40 ± 6.56
Magnesium (Mg)	32.41 ± 0.06	66.41 ± 0.10
Phosphorus (P)	223.90 ± 2.64	209.20 ± 1.56
Zinc (Zn)	295.70 ± 3.93	240.80 ± 2.88
Potassium (K)	3,728.00 ± 47.85	4,015.00 ± 59.26

306

307 Under anaerobic conditions, sulfate reducing bacteria (SRB) use sulfate as a terminal
308 electron acceptor to suppress organic compounds (Mu et al., 2019). The SRB is not only
309 competing with methane-producing bacteria (MPB) to use organic substances resulting
310 in lower methane production yield (Gustavsson et al., 2013), but SRB also could
311 produce a significant amount of H₂S from sulfate in SLS, which inhibits MPB, causing
312 slowing or stop of the methane production. Fig. 3 shows the cumulative H₂S from
313 TSAD and SSAD processes. As expected, the more sulfate was in the substrate, the
314 more H₂S was produced. In both TSAD and SSAD processes, the raw SLS digestion
315 produced the most H₂S by cumulative volume and its concentration in biogas ranged
316 within 0.21-3.57%. The lowest cumulative H₂S was given by DSLS10 digestion and the
317 concentration in biogas ranged within 0.22-1.56%. The TSAD started to generate H₂S
318 on the 2nd day of fermentation (Fig. 3a), whereas H₂S was detected after the 3rd day
319 from SSAD, as illustrated in Fig. 3b. This is because sulfates are consumed in the first

320 stage and converted to hydrogen sulfide. Organic substrates in H₂-TSAD are also
 321 utilized by SRB and produce HS⁻. Thus, the effluent of H₂-TSAD, which was the feed to
 322 CH₄-TSAD, was rich in SRB and HS⁻ causing H₂S detection early on from this stage. In
 323 contrast, SRB in SSAD took the time for favorable growth in the methane production
 324 processes, hence H₂S was generated later than by TSAD. This investigation found that
 325 the TSAD produced 6% less cumulation of H₂S than the SSAD process.

326 RWA does not reduce the sulfate to lower than the inhibition level (500 mg/L)
 327 due to the equilibrium limit and because sulfate contained in RWA was released into
 328 SLS (Raketh et al., 2021). However, RWA clearly enhanced the biogas production from
 329 DLSLs which was higher than from raw SLS both in TSAD and SSAD processes.
 330 Sulfate concentrations and sulfate mass distribution during H₂-TSAD, CH₄-TSAD, and
 331 CH₄-SSAD runs were also observed and are shown in Table 3. It was found that the
 332 overall sulfate removal efficiencies (83.94-88.00%) achieved were similar for TSAD
 333 and SSAD processes in SLS digestion.

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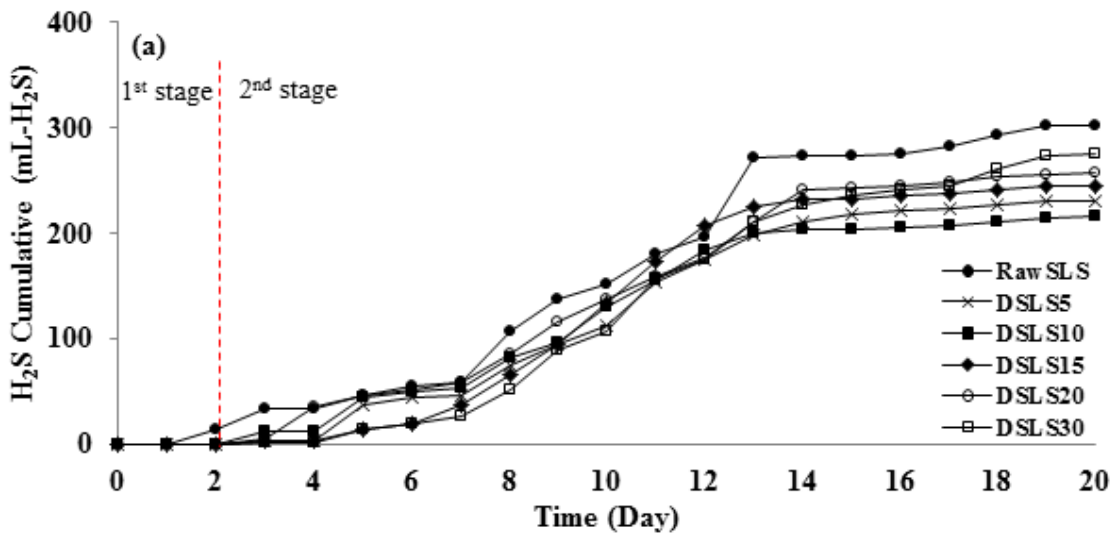
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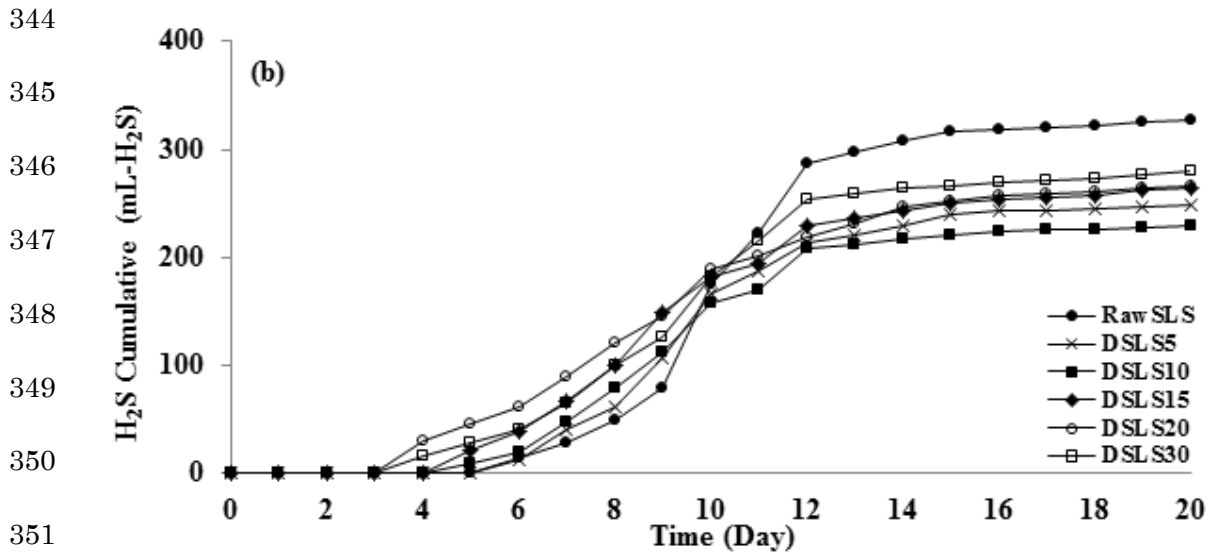


Fig. 3 Cumulative hydrogen sulfide production from (a) TSAD, and (b) SSAD.

3.2 Kinetics study

H₂ and CH₄ production rates are also important for further process design, so it is necessary to simplify and accurately explain the mechanisms and metabolic pathways related to AD of the substrates under varied operating conditions, for predicting the performance of an individual digester (Nguyen et al., 2019). The kinetic parameters of the models used to explain the rates of substrate degradation and H₂-TSAD, CH₄-TSAD, and CH₄-SSAD were determined by model fits to the experimental data. Table 5 summarizes the results of kinetic study of TSAD and SSAD processes using the first-order kinetic model and the modified Gompertz model.

Using the first-order kinetic model, H₂-TSAD ($R^2 > 0.91$), and CH₄-SSAD ($R^2 > 0.96$) data were fitted better than the CH₄-TSAD data ($R^2 > 0.90$). This is probably due to high VFAs in substrate loaded into CH₄-TSAD, which could slow down the methanogen activity. Moreover, the first-order kinetic model provided higher R^2 than 0.90 and was appropriate in all these cases. This is reconfirmed by plots of measured

368 and model predicted H₂-TSAD, CH₄-TSAD, and CH₄-SSAD in Fig. 4. The predicted
369 H₂-TSAD by the model was slightly below the experimental results. For CH₄-TSAD,
370 both of models slightly overestimated the experiments, in contrast to the CH₄-SSAD for
371 which model predictions were below the values from experiments. The rate constants
372 (*k*) of first-order models varied in the range 0.12-0.31 (1/h) for H₂-TSAD and 0.11-0.51
373 (1/d) for CH₄-TSAD and CH₄-SSAD.

374 Theoretically, methane production rate in CH₄-TSAD should faster than in CH₄-
375 SSAD because some organic matter, especially carbohydrates, is degraded during the
376 first stage before feeding to the second. It can be observed that most studies reporting
377 faster methane production rate in the CH₄-TSAD used difficult to digest substrates. Sani
378 et al. (2021) report on biohydrogen and biomethane potential of palm oil mill effluent
379 (POME). The POME is a poorly digestible substrate, and the *k* for CH₄-TSAD (0.12-
380 0.39) is higher than for CH₄-SSAD (0.08-0.27) (Sani et al., 2021). On the other hand, in
381 this study the *k* of first-order kinetic model for CH₄-SSAD (0.24-0.51) was higher than
382 for CH₄-TSAD (0.11-0.14) as shown in Table 5.

383 For the modified Gompertz model, different interpretable parameters of M_m ,
384 R_{max} , and λ were obtained by fitting with cumulative production of hydrogen and
385 methane (Fig.4). The R² values were in range 0.92-0.99 of H₂-TSAD, 0.96-0.99 of CH₄-
386 TSAD, and 0.94-0.9789 of CH₄-SSAD. The R² of the modified Gompertz model fits
387 was excellent indicating good suitability of this model type, and good correlation to
388 experimental data to explain cumulative production of hydrogen and methane from AD
389 process. λ of H₂-TSAD and CH₄-SSAD were shorter than for CH₄-TSAD, indicating
390 microorganisms in an inoculum used are rapidly starting AD of the substrate. The lag
391 time of raw SLS of H₂-TSAD was shorter than of DSLS, while in contrast DSLS10 of

392 both CH₄-TSAD and CH₄-SSAD achieved a shorter lag time than others. Therefore, the
393 RWA loading affects lag time. Nonetheless, the modified Gompertz model is not
394 directly related to biogas or other kinds of accumulated product data in a context of
395 enzymatic kinetics (Siripatana et al., 2016), hence using kinetic constant obtained from
396 the first-order kinetic model in combination with those interpretable parameters attained
397 from the modified Gompertz model could be further used, possibly for optimizing the
398 design, start-up, and functioning of a continuous anaerobic digester.

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416 **Table 5** Kinetic parameters according to model fits for TSAD and SSAD processes.

	H₂-TSAD						CH₄-TSAD						CH₄-SSAD					
	rawSLS	DSLS5	DSLS10	DSLS15	DSLS20	DSLS30	rawSLS	DSLS5	DSLS10	DSLS15	DSLS20	DSLS30	rawSLS	DSLS5	DSLS10	DSLS15	DSLS20	DSLS30
Experimental <i>M_m</i> (mL/g-COD)	44.29	90.64	73.04	56.02	56.16	55.11	261.68	269.35	294.53	271.48	283.38	281.62	263.28	292.77	305.10	297.17	279.86	290.42
First order model																		
<i>M_m</i> (mL/g-COD)	41.54	79.44	67.69	46.42	52.26	48.22	281.24	270.06	305.80	293.96	293.45	296.86	263.74	290.93	301.34	293.57	278.63	288.06
<i>k</i> *	0.12	0.19	0.17	0.21	0.26	0.31	0.12	0.13	0.14	0.11	0.12	0.12	0.24	0.35	0.45	0.39	0.51	0.49
R-Squared	0.9514	0.9343	0.9927	0.9442	0.9169	0.9887	0.9571	0.9352	0.9373	0.9002	0.9296	0.9402	0.9626	0.9793	0.9913	0.9855	0.9956	0.9892
Modified Gompertz model																		
<i>M_m</i> (mL/g-COD)	44.13	88.00	73.00	50.09	54.64	53.25	271.66	263.32	316.97	279.61	307.83	309.32	243.04	278.12	296.49	283.83	274.92	283.32
Lag time**	1.01	3.98	2.50	1.95	2.89	2.85	2.60	2.69	1.93	3.15	3.20	2.25	0.63	0.43	0.15	0.39	0.51	0.55
<i>R_{max}</i> ***	3.28	15.02	8.39	4.93	11.21	7.63	31.01	33.01	32.34	35.92	32.39	28.44	66.37	97.86	93.77	101.36	114.56	119.80
R-Squared	0.9248	0.9591	0.9908	0.9372	0.9818	0.9766	0.9904	0.9908	0.9784	0.9616	0.9902	0.9891	0.9404	0.9470	0.9768	0.9556	0.9629	0.9602

* *k* in (1/h) for H₂-TSAD; and *k* in (1/d) for CH₄-TSAD and CH₄-SSAD.

** Lag time in (h) for H₂-TSAD; and in (d) for CH₄-TSAD and CH₄-SSAD.

*** *R_{max}* in (mL/g-COD-h) for H₂-TSAD; and in (mL/g-COD-d) for CH₄-TSAD and CH₄-SSAD.

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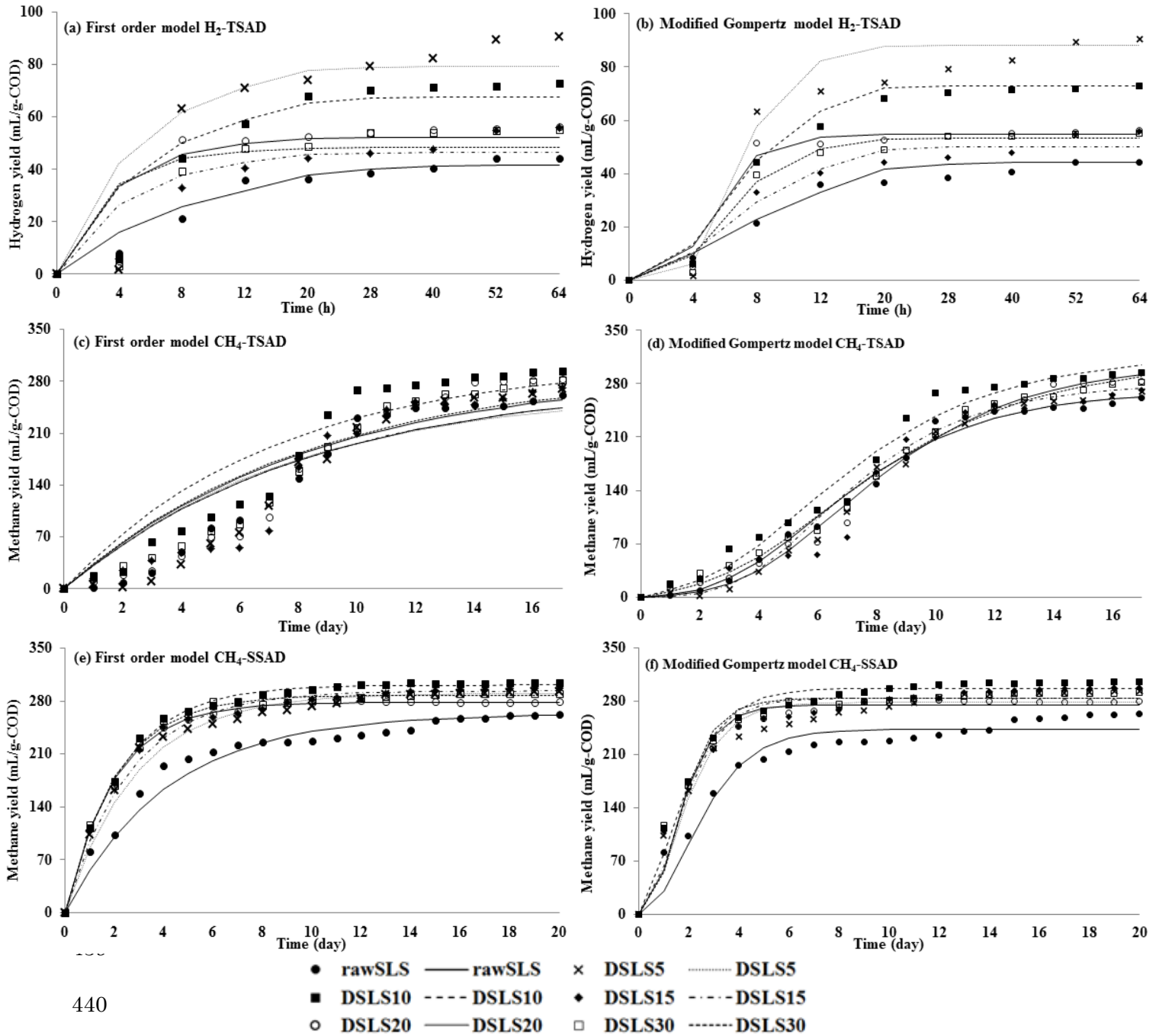
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442 **Fig. 4** Experimental data (symbols) and model fits (lines) of cumulative production

443 yield from TSAD and SSAD process: (a) First order model of H_2 -TSAD, (b) Modified

444 Gompertz model of H_2 -TSAD, (c) First order model of CH_4 -TSAD, (d) Modified

445 Gompertz model of CH_4 -TSAD, (e) First order model of CH_4 -SSAD, and (f) Modified

446 Gompertz model of CH_4 -SSAD.

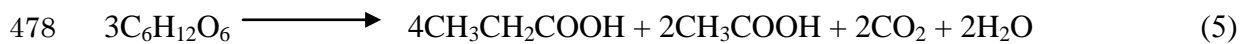
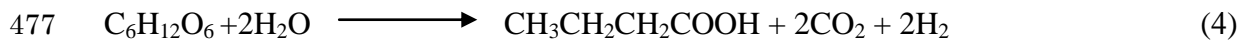
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448 Table 6 shows VFA (acetic, propionic, and butyric acid) profiles of all
449 experiments. Rich VFAs of 1.32-2.90 g/L acetic, 2.05-3.47 g/L propionic, and 6.21-7.60
450 g/L butyric acid were observed from H₂-TSAD. Wang et al. (2009) reported the
451 optimum conditions for acetic acid, propionic acid, and butyric acid at concentrations of
452 1.6, 0.3, and 1.8 g/L, respectively, as those giving the maximum cumulative methane
453 yield. Nevertheless, the inhibiting levels of VFAs are 2.0 g/L of acetic acid (Demirel
454 and Yenigün, 2002), 0.9 g/L of propionic acid (Wang et al., 2009) and, 4.5 g/L of butyric
455 acid (Aguilar et al., 1995). SLS has a high carbohydrate content in the form of
456 fermentable sugar. Danwanichakul et al. (2019) reported that SLS contained 1.2 g/L L-
457 Quebrachitol. Thus, the sugar can be mostly consumed during acidogenesis step and this
458 produces high VFAs. It can be seen from Table 6 that initially CH₄-TSAD contained
459 propionic acid at a concentration near the inhibition level, and contained VFAs at a
460 higher level than CH₄-SSAD. This made CH₄-TSAD give a lesser CH₄ production in the
461 first period. Even though the microorganisms in CH₄-TSAD needed more time for
462 acclimation, the final methane yield achieved was similar to CH₄-SSAD.

463 Feng et al., 2017 and Lyberatos and Pullammanappallil, 2010 present reactions
464 that can take place in hydrogen production. The pathways of acetic and butyric
465 production shown in equations (3) and (4) are the main pathways of hydrogen
466 production during acidogenesis stage. The stoichiometrically calculated H₂ production
467 yields based on equations (3) and (4) are compared to the experimental yields in Table
468 7. It was found that the experimental H₂ production yields are lower than the calculated
469 theoretical H₂ production yields in all AD process cases. This can be explained by other
470 pathways generating acetate, propionate, and butyrate but without hydrogen production,
471 as shown in equations (5) and (6). Moreover, propionate producing during AD process

472 is probably generated via the pathway showing in equations (7) which the produced H₂
473 and sugar are consumed. (Feng et al., 2017; Lyberatos and Pullammanappallil, 2010;
474 Sani et al., 2021).

475



481

482 The optimum pH in acidogenesis and methanogenesis stages is 5-6.7 and 7-8,
483 respectively (Kongjan et al., 2018). In H₂-TSAD, pH slightly dropped due to the
484 addition of NaHCO₃ to maintain the pH and buffer capacity of the system at their initial
485 levels. All of the cases H₂-TSAD, CH₄-TSAD and CH₄-SSAD had a suitable initial pH
486 in the ranges 6.98-7.12 and 7.31-7.68, respectively, as presented in Table 3.

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495 **Table 6** Volatile fatty acids on TSAD and SSAD.

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Sample	H ₂ -TSAD						CH ₄ -TSAD			CH ₄ -SSAD		
	Initial (g/L)			Final (g/L)			Initial (g/L)			Initial (g/L)		
	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
Raw SLS	0.88	0.81	0.62	1.32	2.05	6.94	0.40	0.62	2.08	0.26	0.24	0.19
DSLS5	0.91	0.83	0.64	2.90	3.47	7.25	0.87	1.04	2.18	0.27	0.25	0.19
DSLS10	0.92	0.81	0.66	2.34	2.79	7.60	0.70	0.84	2.28	0.28	0.24	0.20
DSLS15	0.96	0.85	0.69	1.79	2.38	6.72	0.54	0.71	2.02	0.29	0.26	0.21
DSLS20	1.01	0.89	0.73	1.77	2.35	6.21	0.53	0.71	1.86	0.30	0.27	0.22
DSLS30	1.06	0.94	0.76	1.76	2.34	6.17	0.53	0.70	1.85	0.32	0.28	0.23

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505 **Table 7** Stoichiometric calculation of H₂ generation yield from produced VFAs.

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Substrate	VFAs appeared in H ₂ -TSAD				Stoichiometrically calculated H ₂ production					Experimental H ₂ production
	Acetic		Butyric		H ₂ from acetic		H ₂ from butyric		Total H ₂	Total H ₂
	(g/L)	(mmol) ^a	(g/L)	(mmol) ^a	(mmol)	mL(STP)	(mmol)	mL(STP)	mL (at 55°C)	mL (at 55°C)
Raw SLS	0.44	0.44	6.32	4.31	0.88	19.71	8.62	193.05	255.62	75.48
DSLS5	1.99	1.99	6.61	4.51	3.99	89.30	9.01	201.91	349.87	154.48
DSLS10	1.42	1.42	6.94	4.73	2.84	63.62	9.46	211.99	331.13	124.48
DSLS15	0.83	0.83	6.03	4.11	1.66	37.18	8.22	184.09	265.85	95.48
DSLS20	0.76	0.76	5.48	3.74	1.53	34.20	7.48	167.49	242.32	95.77
DSLS30	0.70	0.70	5.41	3.69	1.40	31.36	7.38	165.25	236.22	93.93

^a using working volume = 0.06 L for H₂-TSAD

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513 3.3 Anaerobic biodegradability

514 After 14 days of the CH₄-TSAD and 12 days of the CH₄-SSAD runs, a nearly
 515 steady methane production rate was observed. The overall COD distributions for the
 516 TSAD and SSAD processes are presented in Table 8. It was found that both systems
 517 gave an overall COD removal efficiency exceeding 65 % and the difference between the
 518 two systems was not significant, although % COD removal efficiency of the TSAD was
 519 slightly higher.

520 In TSAD, the highest COD removal efficiency of 78.02 % was achieved with
 521 DSLS10 while raw SLS gave the lowest COD removal efficiency of 68.10%. In the
 522 SSAD process, more than 70 % of COD removal was attained in DSLS digestion.
 523 DSLS10 gave the maximum COD removal (76.37%), whereas 65.90 % COD removal
 524 was attained with raw SLS.

525 An enhancement in the COD removal efficiency from using DSLS demonstrates
 526 that the substrate was more suited to the microorganisms metabolizing it than raw SLS
 527 was. DSLS had a lower initial sulfate concentration than raw SLS, resulting in less
 528 competition of SRB and MPB, and MPB can digest the organic compounds in DSLS
 529 better than in raw SLS, in the lower H₂S environment.

530

531 **Table 8** COD balance for the TSAD and the SSAD processes.

Sample	COD converted to gaseous compound (g)		COD in liquid phase (g)		Overall COD removal (%)
	H ₂ production	CH ₄ production	Initial ^a	Final ^b	
Two stages AD					
Raw SLS	0.04	1.13	1.72	0.55	68.10
DSLS5	0.09	1.16	1.72	0.47	72.75
DSLS10	0.07	1.27	1.72	0.38	78.02

DSLS15	0.06	1.17	1.72	0.50	71.25
DSLS20	0.06	1.22	1.72	0.45	74.19
DSLS30	0.06	1.22	1.72	0.45	73.73
Single stage AD					
Raw					
SLS	-	1.14	1.72	0.59	65.90
DSLS5	-	1.26	1.72	0.46	73.28
DSLS10	-	1.32	1.72	0.41	76.37
DSLS15	-	1.28	1.72	0.44	74.38
DSLS20	-	1.21	1.72	0.52	70.05
DSLS30	-	1.25	1.72	0.47	72.70

^a COD in substrate.

^b Remaining COD in fermentation broth was calculated from COD balance.

532

533 3.4 Hydrogen and methane energy yields

534 Hydrogen and methane production yields together with overall energy

535 production yields from each substrate in TSAD and SSAD processes were calculated

536 and are shown in Table 9. For the TSAD, energy production in H₂-TSAD ranged in

537 0.47-0.97 kJ/g-COD_{added} and the DSLS5 fermentation gave the highest 90.64 mL-H₂/g-

538 COD_{added} yield (0.97 kJ/g-COD_{added}), which is 16.15% of the theoretical yield (467 mL-

539 H₂/g-COD_{added}).

540 A low substrate conversion was obtained in the first stage because only short

541 chain carbohydrates were digested (Liu et al., 2013). The comparatively low hydrogen

542 yields of raw SLS relative to DSLS were due to the higher sulfate content in raw SLS.

543 The CH₄-TSAD generated energy in the range 8.30-9.35 kJ/g-COD_{added}. The

544 maximum yield was from DSLS10 at 294.53 mL-CH₄/g-COD_{added} (9.35 kJ/g-COD_{added})

545 which is 74.59% of the theoretical yield (350 mL-CH₄/g-COD_{added}). The SSAD process

546 produced more methane corresponding to energy range 8.35-9.68 kJ/g-COD_{added}, and

547 the DSLS10 gave the highest yield of 305.09 mL-CH₄/g-COD_{added} (9.68 kJ/g-COD_{added}),

548 which is 77.26 % of the theoretical yield. The lower yield of methane from TSAD was

549 because some COD was converted to H₂ in the first stage of AD. However, when the
 550 overall energy production was assessed, it was 5% higher for TSAD than for SSAD
 551 process.

552

553 **Table 9** Summary of hydrogen and methane yields from TSAD and SSAD processes.

		Hydrogen production		Methane production		Overall energy production yield (kJ/g-COD _{added})		
		Yield (mL/g-COD _{added})	% of theoretical Yield*	Yield (mL/g-COD _{added})	% of theoretical Yield*	H ₂	CH ₄	Total
Two-stage AD	Raw SLS	44.29	7.89	261.68	66.27	0.47	8.30	8.78
	DSLS5	90.64	16.15	269.35	68.21	0.97	8.55	9.51
	DSLS10	73.03	13.02	294.53	74.59	0.78	9.35	10.12
	DSLS15	56.02	9.98	271.48	68.75	0.60	8.61	9.21
	DSLS20	55.43	9.88	283.38	71.77	0.59	8.99	9.58
	DSLS30	55.11	9.82	281.62	71.32	0.59	8.94	9.52
Single-stage AD	Raw SLS	-	-	263.28	66.68	-	8.35	8.35
	DSLS5	-	-	292.77	74.14	-	9.29	9.29
	DSLS10	-	-	305.09	77.26	-	9.68	9.68
	DSLS15	-	-	297.17	75.26	-	9.43	9.43
	DSLS20	-	-	279.86	70.87	-	8.88	8.88
	DSLS30	-	-	290.42	73.55	-	9.22	9.22

* H₂ and CH₄ theoretical yields of 467 mL/g-COD glucose at STP and 350 mL/g-COD at STP.

554

555 In most cases the TSAD was more productive than the SSAD process, because
 556 TSAD offers several advantages. The TSAD is split in stages that allow separately
 557 optimizing hydrolysis/acidogenesis and methanogenesis, to improve the overall reaction
 558 rate, maximize biogas yields, and make the process easier to control, both in meso- and
 559 thermophilic states. In addition, TSAD is enriching different microorganisms in each
 560 anaerobic digester (Schievano et al., 2012). Similarly, better process conversion
 561 efficiency and maximum overall energy yield were reported previously for TSAD, it

562 being more productive than SSAD.

563 Table 10 has a comparison of TSAD and SSAD process performances on
564 using several substrates, in the form of total energy recovery. Sani et al. (2021) reported
565 a comparative evaluation of SSAD and TSAD using palm oil mill effluent. The results
566 shows a 38.95% higher total energy production for TSAD compared to SSAD. Similar
567 results were reported by Massanet-Nicolau et al. (2013) and Akobi et al. (2016), who
568 observed over 30% increases in overall energy recovery for TSAD compared to SSAD,
569 with wheat pellet and poplar wood substrates, respectively. Arreola-Vargas et al. (2016)
570 reported similarly a 22% increase when bagasse was used as the substrate. Moreover,
571 food waste was also used as the substrate in an investigation of overall energy recovery,
572 and De Gioannis et al. (2017) and Nathao et al. (2013) reported 20 and 18 % higher
573 energy recoveries for TSAD.

574 Aside from that, there is a case that reported no significant differences in overall
575 energy recovery for TSAD and SSAD systems using a mixture of swine manure and
576 market biowaste as the substrate (Schievano et al., 2012). This suggests the hypothesis
577 that TSAD may in some cases have no advantage in terms of the overall energy
578 recovery. This is somewhat conflicting with various prior studies. The listing in Table
579 10 was arranged starting from more difficult to digest substrates. Therefore, it is in a
580 good agreement with our study, in which DSLS is easier to biodegrade compared to the
581 other substrates, and only a 5% increase in overall energy recovery was obtained. Thus,
582 the total energy recovery depends strongly on both substrate and operating conditions.
583 The various substrates gave different total energy recoveries, as mentioned above, and
584 different operating conditions also affected energy recovery.

585

586 **Table 10** Total energy recovery in forms of hydrogen and methane production by TSAD
 587 and by SSAD processes.

Substrate	Total Energy Recovery		% increase	Reference
	Single-stage	Two-stage		
Palm oil mill effluent	9.79 kJ/gCOD _{feedstock}	13.61 kJ/gCOD _{feedstock}	38.95	(Sani et al., 2021)
Wheat feed pellets	8.73 MJ/kg-VS	12.09 MJ/kg-VS	37	(Massanet-Nicolau et al., 2013)
Poplar wood	8.70 kJ/gCOD _{feedstock}	11.60 kJ/gCOD _{feedstock}	33	(Akobi et al., 2016)
Bagasse	12.80 kJ/L	35.80 kJ/L	22	(Arreola-Vargas et al., 2016)
Food waste	11.60 MJ/kg-VS	14.50 MJ/kg-VS	20	(De Gioannis et al., 2017)
Food waste	3.40×10^{-3} kW-h	4.00×10^{-3} kW-h	18	(Nathao et al., 2013)
Mixture of swine manure and market biowaste	14.21 kJ/kg-VS _{added}	14.13 kJ/kg-VS _{added}	No sd*	(Schievano et al., 2012)
DSLS10	9.68 kJ/g-COD _{added}	10.12 kJ/g-COD _{added}	5	This study

*No sd means “not significant difference”

588

589 3.5 Cost assessment and energy outcome from AD process

590

591 The costs assessment with DSLS10 substrate for SSAD and TSAD was based on
 592 400 m³/d wastewater production of the concentrated latex factory in Songkhla province,
 593 Thailand. Continuous stirred tank reactors (CSTR) were chosen for both SSAD and
 594 TSAD processing of DSLS10. Firstly, Organic loading rate (OLR) (g-COD/L·d) was
 595 calculated from equation (8) (Kongjan et al., 2018)

596

$$597 \quad OLR = \frac{kC_0}{y/(y_m - y)} \quad (8)$$

598

599 Here C₀ is the initial concentration of substrate equaling 8 g-COD /L, y is the
 600 required hydrogen/methane yield (mL/g-COD_{added}), and y_m is the ultimate

601 hydrogen/methane yield at the end of hydrogen/methane production assay (mL/g-
602 COD_{added}). Then the working volume of CSTR (V_R) can be calculated from equation (9)

603

$$604 \quad V_R = Q \cdot C_0 / OLR \quad (9)$$

605

606 Here, Q is influent flow rate (m³/d). The purchase cost, variable cost, and
607 revenues were calculated and are summarized in Table 11. The total purchase cost will
608 vary depending on the type of reactor and its size, and the mixing system. The results
609 show that the purchase cost of TSAD is higher than of SSAD, and these are estimated as
610 707,172 and 1,131,693 USD, respectively. TSAD needs more investment because it
611 includes 2 digesters for H₂-TSAD and CH₄-TSAD, has a lower digestion rate, and
612 operates at the higher temperature of 55°C. There are very few studies that report a
613 comparison of purchase costs between SSAD and TSAD. 46% higher purchase cost of
614 SSAD required for DSLS in this study is much higher than the 3% more expensive
615 SSAD for food waste reported by Rajendran et al. (2020).

616 The variable costs consisted of labor, operating costs (electricity cost for
617 pumping and digester, upgrading costs, and power generation costs), and maintenance
618 costs. The total variable costs of TSAD were 239,145 USD/year while for SSAD they
619 were 177,397 USD/year. The revenues were calculated from the electricity produced
620 from the purified biogas. The revenue of gas from TSAD was comparatively higher
621 than that from SSAD by 9%, due to the higher energy yield described in a previous
622 section.

623 The payback time was evaluated for purchase cost using the profit difference of
624 revenues and variable costs. Even though the revenue was higher for TSAD than for

625 SSAD, TSAD had a larger investment, so its payback time at 4.36 years was longer than
626 for SSAD at 2.52 years.

627 There is a lack of reports on payback time of electricity production from biogas
628 generated from TSAD, while some reports on SSAD are accessible. Chungchaichana
629 and Vivanpatarakij (2012) reported a 3 years payback time for 94.19 ton/day fresh-food
630 market waste treatment for biogas production (17,807 m³/day) used to produce
631 electricity (12,643 - 24,929 kWh). An AD plant for animal manure, built and operated
632 by Ilci Agricultural, Livestock & Biogas Company in Turkey, has a reasonable energy
633 based payback time of 3.7 years when using the SSAD process (Akbulut, 2012). In
634 addition, the mature technology that uses dairy cattle manure and corn insilate co-
635 digestion to produce biogas and convert it to electricity via Solid Oxide fuel cells, has
636 the reasonable 4 years payback time for large-sized plants (manure availability 103.5
637 tons/day) (Baldinelli et al., 2018).

638 As a practical assessment, the advantages of TSAD include providing optimum
639 operating conditions by stage separation, faster degradation of substrates, and higher
640 loading. On the other hand, the disadvantages include ammonia accumulation due to
641 recirculation that might be toxic to the microbes, uncertainty around reactor
642 configuration for several feedstock types, and poor economic viability (Rajendran et al.,
643 2020). Therefore, the disadvantages of the TSAD need considerable research. The
644 calculations indicate that TSAD is not economically attractive for DSLS10. Hence,
645 there is low potential for replacing SSAD with TSAD in treatment of DSLS10.
646 However, studies on continuous AD production in pilot-scale with variations of OLRs
647 are required for evaluating the real kinetic parameters needed in digester sizing.

648

Category	SSAD	TSAD	Units	References
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649 **Table 11** Cost assessment and energy outcomes for SSAD and TSAD processes.

Design parameter				
Wastewater production (8 g-COD/L)	400	400	m ³ /d	Based on Top Glove Technology factory data
OLR (Estimate from equation (3)) ^a	0.60	0.60 and 0.39	g-COD/L·d	(Kongjan et al., 2018)
HRT	13	0.56 and 21	d	(Kongjan et al., 2018)
Size of CSTR reactor (Estimate from equation (4))	5,910	245 and 9,026	m ³	(Rajendran et al., 2020)
Investment costs				
Digester				
CSTR reactor (assumed based on literature)	704,822	1,111,993	USD	(Rajendran et al., 2020; Vo et al., 2018)
Mixer pump ^b (4kW)	1,000	2,000	USD	(Alibaba., 2021)
Total facility investment				
Pump Power:2 pumps in each stage ^c (1.5 kW)	1,350	2,700	USD	(Kreng thai wattana., 2021)
Heat Exchanger	-	15,000	USD	(Han et al., 2016)
Total	707,172	1,131,693	USD	
Variable costs				
Labor wages (gross)				
2 operators in each AD process	7,091	7,091	USD/year	(Vo et al., 2018)
Operating				
Electricity cost of pump power and digester ^d	8,640	17,512	USD/year	
Upgrading cost ^e	118,732	151,300	USD/year	(Patterson et al., 2011)
Power generation cost ^f	16,770	21,370	USD/year	(Department of alternative energy development and efficiency., 2013)
Maintenance costs				
3% equipment purchase cost	21,215	33,951	USD/year	(Vo et al., 2018)
0.7% insurance	4,950	7,922	USD/year	(Vo et al., 2018)
Total	177,397	239,145	USD/year	
Revenues				
Cumulative biogas production ^g	670,802	854,801	m ³ -biogas/year	This study
Electricity production ^h	1,992,282	2,167,786	kWh/year	(Abdelsalam et al., 2019)
Revenues from gasⁱ	458,225	498,591	USD/year	(Kavitha et al., 2015)
Payback time	2.52	4.36	year	(Sritrakul, 1995)

^ak constant was chosen from first order kinetic model for calculation OLRs, ^bQJB Series, QJB4/12-620/3-480/S, ^cEBARA, DVSA Series, 80DVS 51.5, ^d0.18USD/kWh, ^e0.18 USD/m³-biogas (water scrubbing method), ^f0.025 USD/m³-biogas, ^g85% of maximum yield in this study, ^henergy conversion efficiency 45% ; energy content 6.60 kWh/m³-produced gas (SSAD); 1.15 kWh/m³-produced gas (H₂-TSAD); 6.80 kWh/m³-produced gas (CH₄-TSAD), and ⁱestimation of output from energy was obtained from 1 kWh = 0.23USD.

650

651 **4. Conclusions**

652 This study indicated that RWA can reduce sulfate content in SLS and thereby
653 improve biogas production and biogas quality from both TSAD and SSAD processes.
654 The methane yields in second stage of TSAD and SSAD were found not significantly
655 different. However, hydrogen was also collected in the first stage of TSAD, so that the
656 total energy recovery from TSAD (in forms of H₂ and CH₄) was improved by 5%
657 compared with the SSAD process. Furthermore, the cost assessment of continuous AD
658 process was based on kinetics and yields in batch experiments, and it suggested that the
659 payback time of TSAD (4.36 years) is longer than that of SSAD (2.52 years), because
660 methane production in the second stage of TSAD is slower than in SSAD so that a
661 larger digester is needed. Based on information from batch experiments, there is low
662 potential for replacing SSAD with TSAD to treat DSL10, according to this cost
663 assessment. Further work is necessary to study continuous AD production on a pilot-
664 scale, with variation of OLRs, to investigate the kinetic parameters further for digester
665 sizing.

666

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