

1 The influence of different solid- 2 liquid ratios on the thermophilic 3 anaerobic digestion performance 4 of palm oil mill effluent (POME) 5

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16

17 Abstract

18

19 An alternative method was proposed to optimize the treatment process of palm oil mill
20 effluent (POME) in an effort to address the poor removal efficiencies in terms of the
21 chemical and biological oxygen demand (COD and BOD), total suspended solids (TSS) as
22 well as oil and grease (O&G) content in treated POME along with many environmental issues
23 associated with the existing POME treatment process. The elimination of the cooling ponds
24 and the insertion of a dewatering device in the treatment process were recommended. The
25 dewatering device should enhance the anaerobic digestion process by conferring a means of
26 control on the digesters' load. The objective of this study is to identify the optimum solid:
27 liquid ratio (total solids (TS) content) that would generate the maximum amount of biogas
28 with better methane purity consistently throughout the anaerobic digestion of POME, all
29 while improving the treated effluent quality. It was established that a 40S:60L (4.02% TS)
30 was the best performing solid loading in terms of biogas production and methane yield as
31 well as COD, BOD, TSS, and O&G removal efficiencies. Meanwhile, at higher solid

32loadings, the biogas production is inhibited due to poor transport and mass transfer. It is also
33speculated that sulfate-reducing bacteria tended to inhibit the biogas production based on the
34significantly elevated H₂S concentration recorded for the 75S:25L and the 100S loadings.

35**Keywords:** Palm oil mill effluent, Solid loadings, Thermophilic anaerobic digestion, Biogas
36production, Methane yield

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| 95 | 1. Introduction | |
| 96 | | |

97 Being the second biggest palm oil producer worldwide, Malaysia faces a severe concern
98 when it comes to the disposal and management issues of the waste associated with the

99production of palm oil, Palm Oil Mill Effluent (POME). POME is a thick brownish liquid of
100pH around 4.0-5.0, with a discharge temperature of 80-90°C. POME consists of organic
101materials such as lignin, carotene, phenolic compounds, and pectin, which are at
102concentrations 4700 ppm, 8 ppm, 5800 ppm, and 3400 ppm, respectively (Choong et al.,
1032018; Iskandar et al., 2018; Khadaroo et al., 2019b). The factors making POME detrimental
104to the environment are its high Chemical Oxygen Demand (COD) (15,000-100,000mg/L),
105Biological Oxygen Demand (BOD) (10,250-43,750 mg/L), and suspended solids (5,000-
10654,000mg/L) content which causes water pollution when discharged into watercourses. The
107existing treatment process has deficiencies due to ineffective residual oil removal from the
108waste stream and a large amount of greenhouse gas emission owing to the ponding system
109(Mansor et al., 2017). After the oil removal pit, otherwise known as the cooling ponds,
110POME is allowed to cool down to a suitable mesophilic temperature, after which it is sent
111directly to anaerobic digestion. However, often, the treated effluent quality is poor and does
112not conform to the stringent environmental standards (Chin et al., 2013; Ahmed et al., 2015).
113Owing to excessive greenhouse gas emission, the Malaysian government introduced the
114National Key Economic Areas (NKEA) to enhance oil extraction efficiency and as an
115incentive for the mills to install biogas facilities to capture the biogas generated (Ministry of
116agriculture and agro-based industry, 2019). However, for mills to set up the biogas capture
117facilities as per the NKEA, the system has to be beneficial to the plants in terms of power
118generation and provide other potential uses such as use in package and/or high-pressure
119boiler as an alternative for diesel. Therefore, the amount of biogas produced during anaerobic
120digestion and the methane yield must be significantly enhanced to enable a stable generation
121of electricity downstream. Based on previous literature (Chin et al., 2013a; Lam Man & Lee,
1222010; Yacob et al., 2006), it has been reported that the percentage of methane present in the
123biogas produced from the anaerobic digestion of raw POME ranges from 35% to 54.5%

124which is too low for the required applications. Another disadvantage is the fluctuation in the
125physicochemical properties of POME from low crop season to high crop season (Poh et al.,
1262010).

127Literature on other types of substrates, predicts that more biogas would be produced if a
128lower solid content was fed to the digester. The study by Angelonidi and Smith (2015) is in
129agreement with the statement; they observed that wet anaerobic digestion of municipal solid
130waste and food waste operating at low total solids (TS) (< 20% TS) proved more cost-
131effective with higher biogas productivity. On the other hand, dry anaerobic digestion
132operating at high total solids (> 40%TS) had shorter retention time, reduced the usage of
133water, and gave a more manageable end-product for which the primary use is agricultural
134fertilizers. Both dry and wet anaerobic digestion can be advantageous depending on the
135conditions that needed to be satisfied.

136For dry anaerobic digestion systems, the high solids content has a significant impact on the
137rheological behavior of the digestates. High solids content suspensions are visco-elastic-
138plastic materials characterized by high yield stress. The compressibility of such materials is
139dependent upon the extent of dewatering and can be quantified by the compressive yield
140stress. The latter dictates the TS concentration, ϕ , to which a suspension will be dewatered at
141a known applied pressure (Khadaroo et al., 2019a). The yield stress was noted to increase
142significantly as the TS content increases (Abbassi-Guendouz et al., 2012). In a study using
143POME, Khadaroo *et al.*, (2019) concurred with this by establishing that at a TS content
144higher than a gel point, the compressive yield stress exponentially increases as the TS content
145increases. However, at TS concentration below the gel point, namely as solids volume
146fraction at which a network starts to form, the compressive yield stress is zero (De Kretser et
147al., 2001; Harbour et al., 2001). Controlling the solids fraction of a digestate thereby impacts
148on the rheology.

149In this work, we proposed a substitute treatment process for POME, which consists of
150eliminating the conventional cooling pond and introducing a dewatering device, for example,
151a thickener (Usher et al., 2009). The dewatering device will contribute to the solid-liquid
152separation, removal of microbes, and other impurities from the wastewater. The dewatering
153device will aid in making the anaerobic digesters used for the treatment of POME more
154effective by inferring a means of control on the digesters' load (Khadaroo et al., 2019a).
155POME will be sent from the dewatering device to the anaerobic digesters at a thermophilic
156temperature (55°C) to avoid heat loss. Nevertheless, to know at which solid loading to
157operate the digesters for optimal anaerobic digestion performance, it is fundamental to
158understand methodically how the solid loading affects the latter. However, there is no
159literature to the best of our knowledge on the study of the effect of different solid loadings on
160the anaerobic digestion of POME. This paper aims to find the optimum solid: liquid ratio (TS
161content) that would produce the highest amount of biogas at a consistent rate during the
162anaerobic digestion of POME as well as to provide a better insight into how the solid content
163of POME affects anaerobic digestion in terms of the stability of the reaction and methane
164yield.

165 2. Materials and Method

166 2.1 Materials

167

168POME was sampled during low crop season at Sime Darby East Oil Mill, Malaysia. The
169sample site lies at coordinates 2.8843° N, 101.4369° E. The temperature of POME at the
170collection location was measured to be 65°C. From batch settling experiments, it was
171inferred that raw POME has varying solid concentrations in terms of suspension phase
172ranging from 55-85% by volume (Khadaroo et al., 2019a). Anaerobic seed sludge which was
173subsequently used as inoculum in the experiments was also collected at the Sime Darby East
174Oil Mill.

175 2.2 Experimental Set up

176

177The chosen mode of anaerobic digestion was thermophilic batch anaerobic digestion. The
178temperature selected for thermophilic anaerobic digestion in this study was 55°C.

179To separate the solid and the liquid phase, 5L of POME was placed in a settling column of
180height 0.7 m with multiple sampling points to enable the removal of each phase. The solid
181flocs in POME were allowed to settle for approximately 24 hours at room temperature.
182Samples of settled suspension and clear liquid (hereafter designated "solid" and "liquid")
183obtained are shown in Figures 1a and 1b.



(a) Solids Sample

(b) Liquid Sample

184

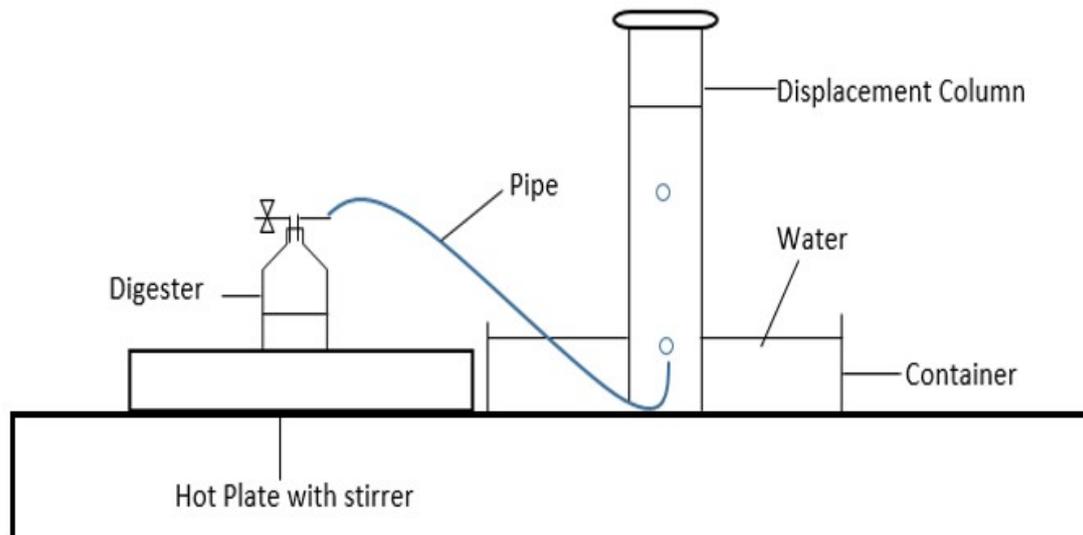
185 Figure 1a and 1b: Dewatered Solids and Liquid samples used in this study

186

187 Having settled raw POME into solid and liquid phases, they were recombined to achieve the
188 desired ratios by volume for this study. The solid: liquid ratios studied were 20:80, 40:60,
189 50:50, 75:25 and 100:0. These ratios were chosen to allow the study over the full range of
190 solids ratios. The 0:100 ratio consisting of 100% liquid was not investigated as the COD of
191 the latter was found to be 1500 mg/L while the average COD of raw POME is 50,000mg/L
192 indicating that the solid portion of POME is primarily where the organic matter is found for
193 anaerobes to thrive.

194 The selected solid: liquid ratio of POME was placed in a 250ml Schott bottle with two
195 outlets. The working volume of the system was taken as 100mL. An inoculum (anaerobic
196 seed sludge) volume of 20% of the working volume was used and maintained throughout the
197 experiments. The inoculum was cultivated and acclimatized in a reactor heated at 55°C under
198 anaerobic conditions to allow the bacteria to become accustomed to the conditions under
199 which the anaerobic digestions experiments were undertaken. The digesters were placed on a
200 heated magnetic stirrer for proper homogenization of the medium, as sketched in Figure 2.
201 Each digester was connected to a water displacement column via a pipe to allow the
202 measurement of the volume of biogas produced. The pH of the water in the displacement
203 column was adjusted to 2.0 to ensure that carbon dioxide does not readily dissolve in the
204 latter, which is in concurrence with ASTM D5511 (Müller et al., 2004). Since the pH of
205 water in the displacement column is low, the solubility of CO₂ decreases, which makes
206 measuring the total biogas volume with the water displacement method more accurate.

207 The pH of the digester system, however, was kept between 6.8 to 8.0 by dosing with 1M
208 NaHCO₃ to ensure optimum conditions for the methanogenic bacteria to produce methane
209 (Choong et al., 2018; Lindner et al., 2015). The composition of biogas was measured using
210 the COMBIMASS Gas Analyzer.



211

212 Figure 2: Thermophilic anaerobic digestion experimental set up
213

214 2.3 Physico-Chemical Analysis 215

216 Characterization tests such as the COD, BOD, TSS, and O&G experiments were conducted
217 before and after anaerobic digestion to determine the quality of the discharged effluent from
218 the anaerobic digesters. The COD, BOD, and TSS were undertaken as per the HACH
219 Standard Methods 8000, 8043, and 8006, respectively, while the O&G test was done as per
220 the ASTM method D7066-04. The most probable number (MPN), i.e., total anaerobes count,
221 was performed as per ASTM STP695. Total solids (TS) test was undertaken as per the
222 standard methods for the examination of water and wastewater (APHA, 1998). The methane
223 yield was calculated based on the volume of methane produced and the mass of COD
224 removed (Jingura and Kamusoko, 2017). For each condition (20S:80L, 40S:60L, 50S:50L,
225 75S:25L, and 100S) three runs were conducted. Each run consisted of 3 batch anaerobic
226 digesters and triplicates were used for the physico-chemical analyses carried out. This set-up
227 produced a total of 9 sets of data for each condition providing a good range of data to gain a
228 better insight into how each tested ratio performed. The error bars in Figure 3 and 4 were
229 calculated based on the standard deviations from the 9 sets of data obtained.

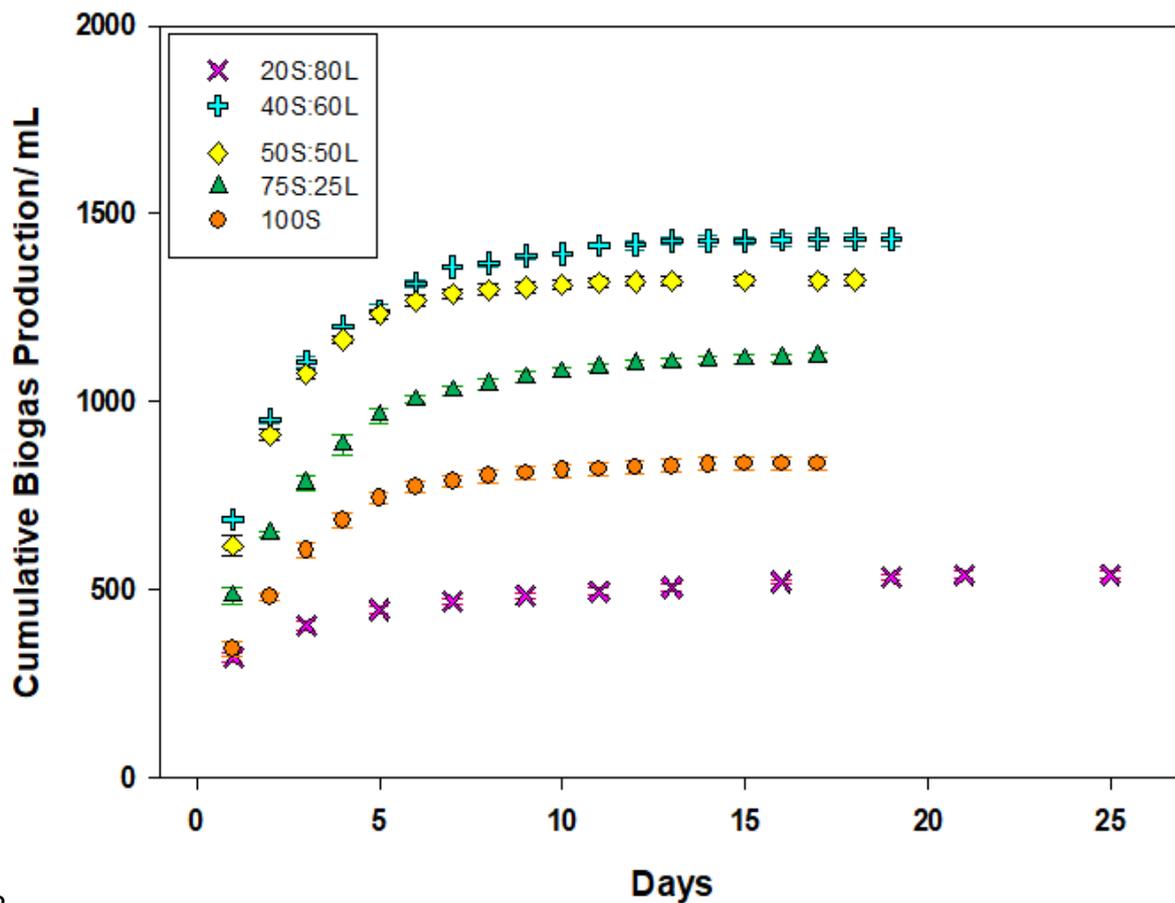
230 3. Results and Discussion

231

232 3.1 The effect of solids content on the anaerobic digestion of 233 POME

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235 This section describes the anaerobic digestion performance results obtained with varying
236 solid loadings of POME. The effect of varying solids loading turned out quite significant. The
237 findings for each ratio have been thoroughly discussed below.



238

239 Figure 3: Combined cumulative biogas production graphs for different solid:
240 liquid ratios

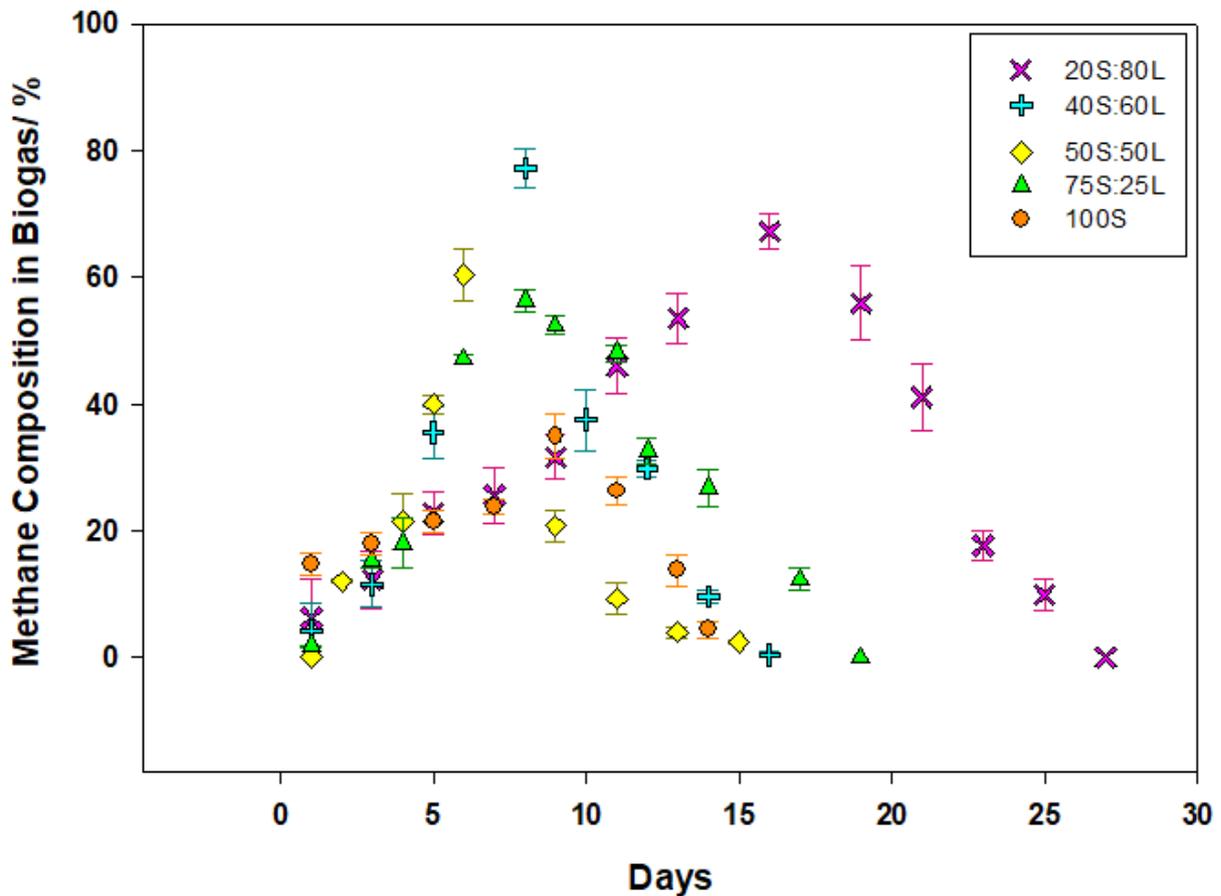
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242 Figure 3 illustrates the graph of cumulative biogas volume produced daily. As shown in the
243 latter, the duration for anaerobic digestion determined as the time taken for methane to stop
244 being produced, varies from ratio to ratio. It can be seen that as the solid content increases,

245the final cumulative biogas attains a plateau more rapidly. This observation is in agreement
246with a study conducted by Angelonidi and Smith (2015). From the daily biogas production, it
247was observed that the rate of biogas production is at its maximum during the first 8-10 days,
248after which it steadily decreases until no biogas is produced exemplified by the plateaus in
249Figure 3. A possible explanation for the shortened anaerobic digestion duration of the higher
250solids content ratios may be owing to the transport restriction within the digester making part
251of the system inaccessible for digestion (Benbelkacem et al., 2015; Markis et al., 2014). This,
252in turn, prevents the bacteria from thriving; an indication of the reduced bacterial activity is
253made known by the anaerobes count undertaken using the most probable number (MPN)
254method presented in Table 1. As that table shows, as the solid loading increased, the total
255number of anaerobes decreased, resulting in a deficit in active microorganisms present in the
256medium, thereby causing the biogas production to subside until it ceased altogether.

257It was observed that 20S:80L ratio generated the least amount of biogas compared to the
258other ratios; an explanation for this incidence is due to the restricted amount of solids
259available for the bacteria to hydrolyze the substrate effectively to produce biogas (Cappai et
260al., 2015; Mishra et al., 2019). Conversely, the 40S:60L solid loading exhibited the maximum
261biogas volume produced compared to all the other conditions demonstrating that the solid
262content in the anaerobic digester is an essential parameter to be taken into consideration. The
263biogas production volume decreases from the 50S:50L to 100S as the solid content increases.
264The formation of a thick layer of scum was observed in the 75S:25L and 100S digesters; this
265occurs owing to a high oil content present in these ratios (Long et al., 2012). The maximum
266rates of biogas production for the 40S:60L and 50S:50L solid loadings were evaluated to be
267194.00 and 183.97 mL/d, respectively. Despite the 50S:50L solid loading having a
268comparable biogas production rate as the 40S:60L solid loading, the latter had a more
269sustained biogas production as depicted by the slopes in the graph in Figure 3 whereby

270beyond day 6, the 40S:60L solid loading was still producing biogas consistently whereas the
 271production of biogas for the 50S:50L solid loading started to decline gradually.



272

273Figure 4: Combined daily methane composition as measured by the Combimass
 274analyzer graphs for different solid: liquid ratios
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276Meanwhile, almost all the solid loadings produced less than 10% of methane in the biogas on
 277the first day except 100S for which 15.3% of methane was recorded. It is important to note
 278that the maximum methane purity tended to occur between day 5 and 8 for the 40S:60L, the
 27950S:50L, 75S:25L, and 100S, whereas for 20S:80L solid loading, it occurred much later at
 280day 16.

281The anaerobic digestion duration for the 20S:80L solid loading was found to be of
 282approximately 25 days. Duration was measured via Figure 4 as the time for methane
 283composition of the gas produced on any given day to fall to zero. The pH of the system

284ranged from the initial pH 7.23 ± 0.05 to the final pH 7.52 ± 0.03 , indicating that the medium in
285which the inoculum was thriving was at the optimal pH and did not inhibit the biogas
286production as noted by the production of biogas over a long period. It can be observed that
287the maximum biogas production occurred between day 1 to day 15, after which the biogas
288production radically declined. The initial biogas production rate was evaluated to be 66.75
289mL/d. The maximum biogas volume produced was found to be 539 ± 10 mL. Figure 4
290represents the daily methane composition; the latter shows that the maximum methane purity
291of the biogas produced was achieved from day 16-19. This is disadvantageous since it occurs
292during a period when the daily biogas production has already significantly decreased, there is
293a high proportion of methane but in a small daily volume of biogas. The methane yield
294calculated was 36.20 mL $\text{CH}_4/\text{g COD}_{\text{removed}}$ while the maximum methane purity in the biogas
295produced was $73.83\pm 2.42\%$ as indicated in Table 1 which is a significant increase in
296comparison to the methane production in the anaerobic digestion of raw POME which was
297reported to be around 35 to 54.4% methane (Chin et al., 2013b; Yacob et al., 2006). The
298initial chemical properties of the 20S:80L condition in terms of COD, BOD, TSS, and O&G
299were 37600 ± 190 , 16350 ± 152 , 9500 ± 100 , and 268 ± 23 mg/L, respectively. Meanwhile, the
300COD, BOD, TSS, and O&G of the latter after anaerobic digestion were found to be
301 14088 ± 430 , 6855 ± 287 , 4233 ± 328 and 197 ± 1 mg/L. The findings resulted in a COD, BOD,
302TSS, and O&G removal of 62.53 ± 1.45 , 58.07 ± 1.76 , 55.44 ± 3.46 , and $26.20\pm 0.46\%$
303correspondingly.

304The results obtained for the 40S:60L solid loading proved to be the best performing amongst
305the tested conditions in terms of biogas production and methane yield. The anaerobic
306digestion duration for the 40S:60L solid loading was around 20 days compared to 25 days for
307the 20S:80L solid loading and shorter retention time is advantageous when it comes to
308anaerobic digestion. The pH of the system ranged from 7.21 ± 0.02 to 7.74 ± 0.09 . This lies

309 within the optimal pH range for sustained biogas production during anaerobic digestion is 6.8
310 to 8.0 (Iskandar et al. 2018; Choong et al. 2018). The pH of the system also corresponds to
311 the condition under which methanogens will produce methane (Lindner et al., 2015). The
312 maximum biogas volume was found to be 1431 ± 17 mL, which was nearly three times more
313 than that generated by 20S:80L solid loading, as shown in Figure 3 above. The methane yield
314 was calculated to be 58.40 mL $\text{CH}_4/\text{g COD}_{\text{removed}}$, which is about 1.6-fold higher than the
315 20S:80L solid loading. It can be observed that the maximum methane purity yield occurred
316 between day 5 to day 10. The initial COD, BOD, TSS, and O&G for the 40S:60L condition
317 were 47700 ± 120 , 20740 ± 138 , 16200 ± 113 , and 279 ± 13 mg/L, respectively. After anaerobic
318 digestion, the COD, BOD, TSS, and O&G reduced to 24377 ± 537 , 13696 ± 345 , 9788 ± 366 and
319 209 ± 2 mg/L resulting in a removal of 48.89 ± 1.12 , 32.64 ± 1.66 , 39.57 ± 2.26 and 25.05 ± 0.62 %
320 of COD, BOD, TSS, and O&G respectively.

321 For the 50S:50L solid loading, it was observed that the total biogas volume produced
322 decreased compared to the 40S:60L solid loading. The anaerobic digestion duration was
323 found to be around 18 days, which was also shorter than for the 40S:60L loading. However,
324 although the shorter anaerobic digestion duration is preferred, the overall performance of the
325 anaerobic digestion of the 50S:50L solid loading did not exceed that of the 40S:60L solid
326 loading. The total volume of biogas produced was 1322 ± 13 mL, which is approximately 107
327 mL less biogas than the 40S:60L solid loading while the methane yield was found to be 40.06
328 mL $\text{CH}_4/\text{g COD}_{\text{removed}}$. This indicates that on a full-scale application, this drop in the amount
329 of biogas produced can be substantial, therefore favoring the use of the 40S:60L solid
330 loading. The methane purity recorded was $64.26 \pm 2.7\%$ of the biogas, which is also slightly
331 less than the 40S:60L solid loading. The initial COD, BOD, TSS, and O&G for the 50S:50L
332 ratio were 49500 ± 210 , 22098 ± 195 , 15800 ± 115 , and 294 ± 25 mg/L, respectively. After
333 anaerobic digestion, the recorded COD, BOD, TSS, and O&G were 33033 ± 352 , 16799 ± 287 ,

33411155±244 and 237±1 mg/L respectively. It is noted that as the amount of solid in the
335anaerobic digesters increases, the removal efficiency in terms of COD, BOD, TSS, and O&G
336decreased. The COD, BOD, TSS, and O&G percentage removal were calculated to be
33733.26±0.71, 23.97±1.30, 29.40±1.55, and 19.24±0.45%.

338The results obtained for 75S:25L solid loading confirmed that the higher the amount of solids
339in the digester, the lower the performance of the anaerobic digestion process, albeit the
340conditions of the experiments are still within the range of what is considered the optimal in
341terms of pH and temperature (Trisakti et al., 2017). The initial rate of biogas production for
34275S:25L solid loading was recorded as 146.98 mL/d. The total biogas volume produced with
343the 75S:25L ratio was 1122.70±9.94 mL, which was approximately 309 mL less biogas than
344the 40S:60L solid loading. The methane yield was found to be 27.84 mL CH₄/g COD_{removed};
345this indicated a decrease in methane yield of slightly more than twofold compared to that of
346the 40S:60L condition. Furthermore, the purity of methane for 75S:25L was noted to be
34712.5% less than the 40S:60L loading, while the H₂S content was exceedingly elevated as
348shown in Table 1. The initial COD, BOD, TSS, and O&G for the 75S:25L condition were
34952400±145, 24950±230, 22000±162, and 322±35 mg/L, respectively. After anaerobic
350digestion, the COD, BOD, TSS, and O&G reduced to 40289±212, 18959±129, 17433±126
351and 236±1 mg/L resulting in a removal of 23.11±0.41, 24.10±0.52, 20.76±0.57 and
35226.57±0.18 % of COD, BOD, TSS, and O&G respectively. These results corroborated that
353the higher the solids loading, the less efficient is the COD, BOD, and TSS removal. However,
354the O&G removal efficiency improved compare to the 40S:60L and the 50S:50L solid
355loadings. A possible explanation for this occurrence is that some of the O&G were lost in the
356layer of scum formed on top in the 75S:25L solid loading digesters while there was no scum
357formation observed in the 20S:80L, 40S:60L, and 50S:50L loadings (Soares et al., 2019).

358The 100S solid loading (i.e. pure settled suspension with no liquid mixed back in) was
359amongst the worst performing condition of all those tested in terms of methane yield, COD,
360BOD, and TSS. Although the anaerobic digestion duration was similar to that obtained for
36175S:25L solid loadings both around 17 days, the total biogas volume, the methane purity, and
362the removal of COD, BOD, and TSS were substantially less than for the 40S:60L solid
363loading except for the O&G removal. The cumulative biogas production was found to be
364 833 ± 17 mL, while the methane yield was 16.69 mL $\text{CH}_4/\text{g COD}_{\text{removed}}$. The initial COD,
365BOD, TSS, and O&G for the 100S solid loading were 54000 ± 250 , 25975 ± 125 , 23100 ± 190 ,
366and 649 ± 32 mg/L, respectively. Post anaerobic digestion, the COD, BOD, TSS, and O&G
367decreased to 49856 ± 568 , 20315 ± 478 , 18711 ± 658 and 446 ± 4 mg/L resulting in a removal of
368 7.67 ± 1.05 , 21.78 ± 1.84 , 19.06 ± 2.85 and 31.16 ± 0.62 % of COD, BOD, TSS, and O&G
369respectively.

370A similar trend to this study whereby the 20S:80L (3.29% TS) ratio generated less biogas in
371contrast to the 100S (7.86%TS) solid loading was in concurrence to another study by
372(Deepanraj et al., 2015) on food waste which is further discussed in section 3.2. The decline
373in biogas volume at higher TS content was explained by the limited microbial/enzymatic
374substrate contact with an increased amount of solids present in the reactor or owing to the
375competition for substrate between the methanogens and the sulfate-reducing bacteria (Yan et
376al., 2018). Meanwhile an explanation for lower biogas yield at exceedingly low TS may be
377due to the limitation in microbial biomass hydrolysis whereby given that there is insufficient
378solid in comparison to the number of bacteria found in the medium owing to the low solids
379content, the microorganisms cannot efficiently convert the solids to methane, hence, the
380biogas production lessens (Mishra et al., 2019).

381Based on the calculated average percentage deviation in the full set of measured quantities
382listed in Table 1 below, it can be observed that 20S:80L and 100S solid loadings tend to have

383the least stable anaerobic digestion process with a calculated average percentage deviation of
 3842.10%. Meanwhile, the 50S:50L and 75S:25L solid loadings tend to exhibit the most stable
 385anaerobic digestion indicated by a smaller percentage deviation of roughly around 1.00%
 386across all the quantities tested. The stability of the 40S:60L solid loading lies between that of
 387the least and the most stable ratios at a percentage deviation of 1.22%. Since the latter is the
 388best performing ratio despite its slightly lower stability, it can be established that 40S:60L
 389notably improved the anaerobic digestion performance with respect to the COD, BOD, TSS,
 390and biogas production, methane yield along with a comparatively stable anaerobic digestion
 391process for POME.

392Table 1 presents a summary of the results obtained for all the tested conditions. It can be
 393observed that the anaerobic digestion performance increases from the 20S:80L to 40S:60L
 394solid loadings in terms of biogas production, methane yield as well as COD, BOD, and TSS
 395removal after which the efficiency of these parameters decrease with increasing solid loading
 396except for the O&G. The pH of the medium for each condition was determined to be within
 397the optimal pH range for thermophilic anaerobic digestion to prevent the inhibition of
 398methane conversion by the methanogens (Appels et al., 2008; Nayono, 2009). It can also be
 399noted that the total anaerobes population at the end of anaerobic digestion decrease as the
 400solid loading increases. This is in concurrence with the amount of biogas being produce.

401Table 1: Results obtained for the thermophilic anaerobic digestion of POME for
 402different solid loadings

| | 20S:80L | 40S:60L | 50S:50L | 75S:25L | 100S |
|------------------------------------|----------------|----------------|----------------|----------------|-------------|
| Dry Solids Content/ %TS | 3.29 | 4.02 | 5.25 | 6.40 | 7.86 |
| Initial pH | 7.23±0.05 | 7.21± 0.02 | 7.22±0.12 | 7.27±0.10 | 7.23±0.07 |
| Final pH | 7.52±0.03 | 7.74± 0.09 | 7.56±0.04 | 7.44±0.04 | 7.75±0.07 |

| | | | | | |
|---|---------------------|---------------------|---------------------|---------------------|---------------------|
| Cumulative Biogas Production/ mL | 539±10 | 1431±17 | 1322±13 | 1122±10 | 833±17 |
| Initial Biogas production rate/ mL/d | 66.75 | 194.00 | 183.97 | 146.98 | 106.09 |
| Maximum Methane Composition /% | 73.83±2.42 | 77.33± 1.20 | 64.26±2.71 | 57.73±1.62 | 33.17±1.30 |
| Minimum Methane Composition /% | 50.36±2.07 | 57.80±2.67 | 54.13±0.87 | 40.67±0.58 | 20.87±3.04 |
| Methane Yield/ mL CH₄/g COD_{removed} | 36.20 | 58.40 | 40.06 | 27.84 | 16.69 |
| H₂S composition/ mg/L | 204±4 | 341±16 | 560±21 | 1823±23 | 2968±52 |
| MPN Total Anaerobes/ 100mL | 1.2x10 ⁶ | 2.1x10 ⁶ | 7.5x10 ⁵ | 1.5x10 ⁵ | 9.3x10 ⁴ |
| Duration of Experiment /days | ≈25 | ≈20 | ≈18 | ≈17 | ≈17 |
| COD removal/% | 62.53±1.14 | 48.89±1.12 | 33.26±0.71 | 23.11±0.41 | 7.67±1.05 |
| BOD removal/% | 58.07±1.76 | 32.64±1.66 | 23.97±1.30 | 24.10±0.52 | 21.78±1.84 |
| TSS removal/% | 55.44±3.46 | 39.57±2.26 | 29.40±1.55 | 20.76±0.57 | 19.06±2.85 |

| | | | | | |
|------------------------------|------------|------------|------------|------------|------------|
| O&G removal/% | 26.20±0.46 | 25.05±0.62 | 19.24±0.45 | 26.57±0.18 | 31.16±0.62 |
|------------------------------|------------|------------|------------|------------|------------|

403

404 **3.2 Comparison between the effect of total solids on the**
405 **anaerobic digestion of POME and other types of substrates**

406

407 As the literature on the effect of different solid loading/ TS contents on digestion of POME is
408 rather limited, a comparison was carried out between our results on POME and findings on
409 different types of substrates to thoroughly understand the impact of TS content on the biogas
410 production and the anaerobic digestion performance overall. Since the operational conditions
411 and substrates were different, the comparisons were made relative by normalizing the
412 cumulative biogas productions and the methane yields by their highest values in their
413 corresponding data sets (Benbelkacem et al., 2015). A common observation from all the
414 literature studied was that at a lower TS concentration, the biogas production and the methane
415 yield tend to be more favorable. An explanation for this occurrence may be attributable to the
416 increase in water content and the associated more effective transport and mass transfer
417 conditions whereby the microorganisms are better sustained with soluble substrates, thus
418 making the anaerobic digestion process more efficient (Le Hyaric et al., 2012; Xu et al.,
419 2014; Zhang et al., 2018).

420

421 In a study undertaken by Wang et al. (2016) on waste activated sludge (WAS) mixed with
422 dewatered sludge collected in a domestic wastewater plant, it was observed that 4.00% TS
423 produced the maximum methane recorded to be 181.7 mL CH₄/g Volatile Solids (VS)_{added}.
424 This finding is in agreement with our study, whereby, POME at 4.02% TS (the 40S:60L solid
425 loading) achieved the highest methane yield compared to the other TS concentration. Wang et
426 al., (2016) also observed that at TS content higher than 6.00%, the biogas production
427 decreased with increasing TS concentration. This observation also concurs with the present

428 study in which it was observed that above 6.40% TS; the methane yield deteriorated
429 significantly. Another comparable behavior for POME and WAS mixed with dewatered
430 sludge was that both substrates produced the maximum amount of biogas between day 1 and
431 day 10 across all the different TS contents, after which the biogas production declined
432 progressively. Based on the results obtained, it was established that POME exhibited a
433 similar trend as WAS mixed with dewatered sludge when it comes to the effect of TS content
434 on anaerobic digestion performance.

435 In another study undertaken by Fernández et al. (2008) on the effect of substrate
436 concentration in terms of TS content of organic fraction of municipal solid waste (OFMSW)
437 during mesophilic anaerobic digestion, they noted that the methanogenic stage began at day
438 14 for 20% TS and on day 28 for 30% TS. For a working volume of 1.7 L, they observed that
439 a total methane production of 7010 mL and 5530 mL for 20% TS and 30% TS
440 correspondingly. The methane yield for 20% and 30% TS was evaluated to be 110 mL CH₄/g
441 Volatile Solid (VS)_{removed} and 70 mL CH₄/g VS_{removed}, respectively (Fernández et al., 2008). A
442 noteworthy observation made was that at even lower TS concentration, a higher biogas
443 production and methane yield was achieved. An example of the latter is the study of Dong et
444 al. (2010), who investigated the mesophilic anaerobic digestion of water sorted OFMSW.
445 They noticed that 11.0, 13.5, and 16.0% TS achieved a methane yield of 314, 283, and 273
446 mL/g VS, correspondingly with a working volume of 35L for each condition.

447 Abbassi-Guendouz et al. (2012) investigated the effect of total solids on the mesophilic
448 anaerobic digestion of municipal solid waste (MSW) at concentrations ranging from 10% TS
449 to 35% TS. They observed that as the TS concentration increased, the methane production
450 decreased. They established that 10% TS yielded the highest methane, which was recorded at
451 149.00 mL CH₄/g COD, whereas POME achieved a maximum final methane yield of 58.40
452 mL CH₄/g COD at 4.02% TS. It is important to note that MSW is substantially

453 inhomogeneous, which is a factor that can also affect its methane production (Ghanimeh et al., 2012). However, it was noted that the initial biogas production rates were similar across 454 the different TS concentrations (Abbassi-Guendouz et al., 2012). Inhibition in the methane 455 production was observed to occur at 35% TS in the mentioned study, whereas, for POME, it 456 occurred even at 6.40% TS corresponding to 75S:25L solid loading. POME has a lower 457 biogas production compared to the MSW; the latter may be explained by the lower working 458 volume of 100 mL (compared to 1.7 L and 400 mL) or owing to the high lignin and phenolic 459 compounds composition in the substrate (Abbassi-Guendouz et al., 2012; Veeresh et al., 460 2005). Veeresh et al. (2005) stated that phenolic compounds at a concentration higher than 461 750 ppm could inhibit anaerobic digestion owing to a reduced biodegradability.

463 Deepanraj et al. (2015) investigated the various TS contents ranging from 5 to 15% TS on the 464 mesophilic anaerobic digestion of food waste (FW). They observed that from 5% to 10% TS, 465 the biogas production increased after which it started to deteriorate steadily up to 15% TS. 466 Subsequently, the 5% TS sample yielded less biogas than the 15% TS sample. They stated 467 that the best performing condition was 7.5% TS at a pH of 7. In the latter condition, the 468 maximum biogas produced was recorded to be 5673 mL from 1.6 L of substrate. The other 469 reactors with 5, 10, 12.5, and 15% of total solids concentrations generated 3040, 3913, 3739, 470 and 3582 ml of biogas, respectively. In another study on FW, Liotta et al. (2014) examined 471 the influence of TS ranging from 4.5 to 19.2 % on the anaerobic digestion at mesophilic 472 temperature; they stated that the methane yield attained was 350, 335, and 207 mL CH₄/g 473 VS_{substrate} for 4.5, 12.9 and 19.2% TS respectively.

474 Liu and Lv (2016) studied the effect of TS content at different temperatures ranging from 31- 475 43°C on the anaerobic digestion of dairy manure. The investigated TS concentrations were 6, 476 8, and 10%. They observed that at temperatures higher than 37°C, as the TS content 477 increased, the digestion period shortened; we also identified this occurrence in the digestion

478of POME at 55°C. Liu and Lv (2016) stated that the best performing condition was 8% TS at
47940°C, where approximately 3800 mL biogas was attained at the end of 27 days. The
480maximum biogas was achieved around day 8 for 8% TS whereas the latter occurred after day
48110 for 6 and 10% TS. The cumulative biogas production for 6% and 10% TS was 1750 mL
482and 2600 mL, respectively, at the most favorable temperature (40°C) (Liu and Lv, 2016).

483

484To summarize the findings of this study, it was found that the biogas production increased
485from the 20S:80L (3.29% TS) to 40S:60L (4.02% TS) and decreased from 50S:50L (5.25%
486TS) to 100S (7.86% TS). In the study, we found that the best performing ratio (40S:60L) lies
487below the solid: liquid ratio of raw POME which ranges from 55S:45L to 85S:15L. Higher
488solid loadings produced an adverse effect on the biogas production, the methane yield as well
489as the methane purity in the biogas produced. The latter may occur owing to poor transport
490and mass transfer conditions and an elevated amount of hydrogen sulfide gas produced at the
491higher solid loadings due to the presence of sulfate-reducing bacteria (Yan et al., 2018). The
492removal efficiency of COD, BOD, and TSS deteriorated as the solid loading increased. An
493interesting observation was that the O&G removal efficiency decreased the 20S:80L to
49450S:50L but steadily increases from the latter to 100S solid loadings. It was also observed
495from the cumulative biogas production volume graphs, based on the size of error bars that as
496the solid content increases the stability of the anaerobic digestion process increases.

497This study demonstrates that with the ability to control the digesters' solid loading, specific
498desired outcomes can be achieved. For example, if the main aim of the anaerobic digestion
499process is to produce biogas with higher methane purity, then a lower solids loading is
500required whereas if the objective is to treat the effluent such that the end products are more
501manageable then higher solid contents may be more desirable i.e. to produce fertilizers. This
502study indicates significant potential in the customization of the anaerobic digesters' load to
503enhance the anaerobic digestion process to cater for different industrial requirements. The

504 implementation of a solid-liquid separation stage (e.g., a thickener) into the treatment process
505 of POME should allow a consistent treatment process and a means of control on the
506 characteristics of POME, although the latter fluctuates from high crop to low crop season
507 (Poh et al., 2010).

508 4. Conclusion

509

510 A higher solid content may not necessarily signify a better anaerobic digestion process
511 performance; contrariwise, this study indicated the opposite. It was observed that the methane
512 yield per mass of COD removed, and the biogas production increased between the 20S:80L
513 to 40S:60L loadings, after which it steadily decreased with higher solid loadings. It was
514 observed that the COD, BOD, and TSS removal efficiencies progressively deteriorate as the
515 solid loading increases. The O&G removal efficiency steadily decreases from 20S:80L to
516 50S:50L; however, it increases for 75S:25L and 100S solid loadings. It was established that
517 the best performing solid loading is the 40S:60L which produced 1431 ± 17 mL of biogas,
518 having a methane yield of 58.40 mL CH_4/g COD and removal efficiencies of COD, BOD,
519 TSS and O&G of 48.89 ± 1.12 , 32.64 ± 1.66 , 39.57 ± 2.26 and 25.05 ± 0.62 %, respectively.
520 Based on this study, we now know that since the optimal ratio lies below the solid: liquid
521 ratio of raw POME, the suggested way to operate the thickener is therefore to run a fraction of
522 POME through the thickener and mix the clarified liquid from that thickener with the remainder of the
523 raw POME to reach the targeted solids fraction. This study shows that the anaerobic digestion
524 process for POME can be customized through the solid loading of the bioreactor to produce
525 the desired outcome, whether it is in terms of enhanced biogas production or manageable end
526 products. Implementing a solid-liquid separation step in the process to control the solid
527 loading, thereby has tremendous potential for improving the process.

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529

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533 Nomenclature

| | | |
|-----|-------|---|
| 534 | APHA | American Public Health Association |
| 535 | ASTM | American Society for Testing Material |
| 536 | BOD | Biological Oxygen Demand |
| 537 | COD | Chemical Oxygen Demand |
| 538 | FW | Food Waste |
| 539 | L | Liquid |
| 540 | MPN | Most Probable Number |
| 541 | MSW | Municipal Solid Waste |
| 542 | OFMSW | Organic Fraction of Municipal Solid Waste |
| 543 | O&G | Oil and Grease |
| 544 | POME | Palm Oil Mill Effluent |
| 545 | S | Solid |
| 546 | TS | Total Solids |
| 547 | TSS | Total Suspended Solids |
| 548 | VS | Volatile Solids |
| 549 | WAS | Waste Activated Sludge |

550 6. References

551

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