

This is a peer-reviewed, accepted author manuscript of the following chapter: Khadaroo, S. N. B. A., Grassia, P., Gouwanda, D., He, J., & Poh, P. E. (2021). Enhancing the biogas production and the treated effluent quality via an alternative palm oil mill effluent (POME) treatment process: integration of thermal pretreatment and dewatering. *Biomass and Bioenergy*, 151, [106167]. <https://doi.org/10.1016/j.biombioe.2021.106167>

# Enhancing the biogas production and the treated effluent quality via an alternative Palm Oil Mill Effluent (POME) treatment process: Integration of thermal pretreatment and dewatering

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## Abstract

To address the limitations associated with the disposal management and the treatment process of POME, an alternative process that consists of eliminating the cooling ponds and integrating a pretreatment technology combined with a dewatering device was proposed. Since the proposed treatment process has not been tested previously, the objective of this study is to investigate the effect of thermal pretreatment and solid loading alteration via dewatering on a thermophilic continuous set up consisting of an upflow anaerobic sludge blanket – hollow centered packed bed (UASB-HCPB) bioreactor. It was established that the best performing solid loading was the 40S:60L, which is made up of 40% by volume of POME settled solids

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and 60% of clarified liquid. Thermally pretreated 40S:60L solid loading had a significantly enhanced biogas production, methane purity, chemical oxygen demand (COD), biological oxygen demand (BOD), and total suspended solids (TSS) removal efficiencies. The reactor exhibited stability owing to the low volatile fatty acid/alkalinity ratio obtained throughout the run. With a reactor capacity of 5L, thermally pretreated 40S:60L achieved a daily biogas production volume of 48L and a methane purity of 77.2%. The COD, BOD, and TSS removal efficiencies of the thermally pretreated 40S:60L solid loading were evaluated to be 97.5, 95.1, and 94.5 %. The enhanced process performance is due to the consistent solids concentration, which allowed a more effective mass transfer and transport in the medium. Meanwhile, thermal pretreatment promoted cell lysis, making the intercellular organic matter readily available for the bacteria consortium.

**Keywords:** Thermal pretreatment, Dewatering, Thermophilic Anaerobic Digestion, High-rate bioreactor, Biogas production, Microbial Community analysis.

## 1. Introduction

Malaysia's main income generator is the palm oil industry since the country is the second-leading palm oil producer worldwide. Over the past few years, questions on the sustainability of the palm oil industry have been raised. Some of these concerns are deforestation, excessive land use, water pollution, and extensive greenhouse gas emissions. Three of the latter problems are directly linked to the palm oil mill effluent (POME) treatment process. Every tonne of Crude Palm Oil (CPO) produced makes roughly 3.05 m<sup>3</sup> of POME. Likewise, every tonne of POME treated via the ponding system potentially generates about 12.4 kg of methane gas [1,2]. POME is a brown colloidal suspension of pH roughly around 4.0-5.0. POME is made up of approximately 96% water, 0.7% oil, and 5% total solids, of which 4% is accounted as suspended solids along with cellulosic material, fat, oil, and grease [3]. Alongside the odorous smell, POME can cause water pollution when discharged into watercourses (i.e., the prevalent means of disposal) due to the elevated amount of degradable organic matter represented by high chemical oxygen demand (COD) and biological oxygen demand (BOD) values of 50,000 mg/L and 25,000 mg/L, respectively.

Furthermore, POME's other physicochemical characteristics, such as the 6000 mg/L of oil and grease (O&G) content, 59,530 mg/L of suspended solids (SS), and 750 mg/L of nitrogen, can also be harmful to the aquatic ecosystem [4]. More than 85% of palm oil mills in Malaysia continue to use the ponding system, attributable to the low cost associated with the latter. The drawbacks related to the ponding system are, firstly, it requires an extensive open area of land which subsequently generates a stench, has a longer retention time, and does not include a biogas capture system, which consequently brings forth substantial greenhouse gas emission.

On the other hand, in mills with a biogas capture system, the biogas cannot be used for applications within the mill, such as on-site thermal energy and electricity generation due to

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88 unstable production of biogas low methane purity in the biogas produced. Therefore, for millers  
89 to have more incentives to install biogas facilities to reduce utility costs, it is imperative to  
90 consistently produce biogas with a higher methane composition. The biogas can then be used  
91 as a substitute fuel to replace biomass fuel and diesel in mills [1].

92 In the current treatment process of POME, as depicted in Figure 1A, raw POME, which is  
93 discharged at 90°C, is directed to the cooling ponds whereby the residual oil is scraped off the  
94 surface. In the ponds, POME is left to cool down to a suitable mesophilic temperature (35°C)  
95 prior to being sent for the anaerobic digestion to be treated. After anaerobic digestion treatment,  
96 the treated effluent from the digesters is sent to the secondary treatment, the aeration treatment,  
97 to further enhanced the treated effluent quality. Aeration treatment utilizes an excessively high  
98 amount of energy. Another disadvantage associated with the current treatment process of  
99 POME is that treated POME is discharged in watercourses, however, the treated effluent  
100 quality often does not meet the environmental standards stipulated by the Malaysian  
101 government [5,6]. The latter, therefore, causes water pollution and may adversely affect the  
102 aquatic ecosystem. Another major impediment in POME treatment is that the physicochemical  
103 characteristics of POME vary considerably from low crop season to high crop season, which  
104 significantly inhibits the anaerobic digestion process. It was found that POME's characteristics  
105 sampled at high crop season diverged by approximately 20 to 50% compared to the values  
106 recorded at low crop season [7].

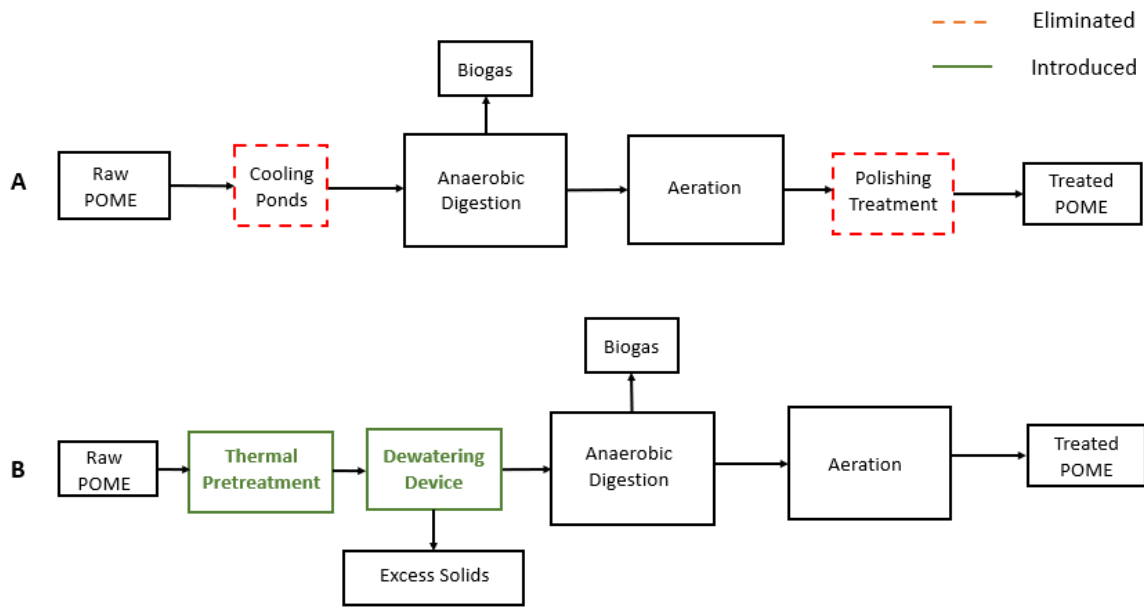


Figure 1: A- Existing POME treatment process, B- Proposed POME treatment process

To counteract these drawbacks, a substitute treatment process that has not been trialed previously was proposed, as illustrated in Figure 1B, which entails the elimination of the cooling ponds and the integration of a pretreatment technology coupled with a dewatering device, for instance, a thickener before the anaerobic digestion treatment. The pretreatment technology will contribute to ameliorating the rate-limiting step, hydrolysis, and anaerobic digestion and breaking down the lignin, long lipid, and protein chains that can inhibit anaerobic digestion [8]. The dewatering device will be placed after the oil recovery unit and before the anaerobic digesters to avoid the solid particles from floating to the surface as they are dragged by the oil moving upwards. The dewatering device will be placed in the mentioned location to prevent redundant alteration to the mill layout. This modification in the treatment process will improve the treated effluent quality of POME and counteract heat loss that can be crucial to enhance the hydrolysis step in anaerobic digestion [9]. Implementing a thickener in the POME treatment process will allow the separation of oil, liquid, and solids portions of POME. The layer of residual oil can be removed, and material that settles out deftly can be drawn out. The

124 latter will bring about a homogeneous effluent, and the sludge discharged can be sent to the  
125 secondary treatment. The proper discharge sludge to liquid ratio will be directed to the  
126 anaerobic digester, which will be operated at a higher thermophilic temperature [10]. The  
127 excess solids from the dewatering unit can be further treated by anaerobic digestion with the  
128 aim to produce A grade biosolids (to be used as fertilizers) as a valuable end product to be used  
129 in palm tree plantations since thermal pretreatment allows the treated solids to be pathogen-  
130 free [11,12]. The thickener will assist by providing a means of control on the anaerobic  
131 digesters' load and the feed composition by adjusting the amount of liquid and solids required  
132 in the medium to ensure a prominent environment for the consortium of bacteria to thrive [13].  
133 Additionally, the thickener will help regulate the digesters' load whenever there is a substantial  
134 alteration in POME's chemical properties when it fluctuates from low crop season to high crop  
135 season, thereby making the anaerobic digestion process notably more stable [14].

136 Some advantages of thermal pretreatment are increased process performance, process stability,  
137 and enhanced digestion efficiency [15]. Thermal pretreatment also improves dewaterability,  
138 further abetting the solid: liquid separation in the process [16]. The thermal pretreatment  
139 method's performance was proven to be highly reliable; it ensures the destruction of high  
140 volatile solids, lower digester volume, well-dewatered cake, high biogas yield, and pasteurized  
141 products (i.e., A class biosolids) [4]. Since POME's discharge temperature is already elevated,  
142 a lesser amount of energy is required to raise the latter to the thermal pretreatment optimal  
143 temperature. Thermal pretreatment has corroborated enhancement in biogas production,  
144 solubilization, and treated effluent quality. The energy added to the system can be easily  
145 compensated by the increase in the amount of biogas produced.

146  
147 A significant amount of research has been undertaken to improve the treatment process of  
148 POME; for example, different types of reactors have been designed (continuous stirred tank

149 reactor (CSTR), upflow anaerobic sludge blanket (UASB), and anaerobic contact digester  
150 (ACD)) [1,17–19], membrane system [20–22], photocatalytic treatment [23,24], coagulation  
151 [25–27] and many more. Some of these treatment processes are at the research stage, while  
152 some, for example, the CSTR and other high rate reactors, have been commercialized. Albeit  
153 the high rate reactors have been commercialized, they have not been widely implemented in  
154 palm oil mills due to the lack of stability, the complexity of the anaerobic digestion process,  
155 and the frequent maintenance required to ensure the proper functioning of the reactors. In order  
156 to investigate the anaerobic digestion process performance of a high rate reactor and the latter's  
157 stability, a hybrid bioreactor, the upflow anaerobic sludge blanket with a hollow centered-  
158 packed bed (UASB-HCPB) was utilized in this study; a detailed description of the bioreactor  
159 is provided in Section 2.

160 Our research group conducted a preliminary study to comprehend how thermal pretreatment,  
161 coupled with solid loading affects anaerobic digestion. Solid loadings ranging from 20% to  
162 100% solids by volume were studied in batch mode with a working volume of 100mL [28].  
163 However, the aim of this paper is to investigate the effect of the optimal solid loadings coupled  
164 with thermal pretreatment in continuous mode and at a higher capacity (5L) to potentially  
165 mimic the operating conditions applied at industrial scale.

166 Our proposed advancement in the treatment process of POME has not been studied previously,  
167 and due to a distinctly sparse amount of literature on the integration of thermal pretreatment  
168 coupled with solid loading, the purpose of this paper is to bridge the gap on the effect of thermal  
169 pretreatment and solid loading in continuous mode using a UASB-HCPB bioreactor.

## 2. Materials and Methods

### 2.1 Materials

172 POME was used as a substrate for the anaerobic digestion experiment. The latter was  
173 sampled at the Sime Darby East Oil Mill, Malaysia, where the collection site has coordinates

174 lying within 2.8843° N, 101.4369° E. At the sampling location, the temperature of POME was  
175 noted to be 65°C. The inoculum used was anaerobic seed sludge, which was also collected  
176 from the same mill. The inoculum used in the UASB-HCPB reactor was acclimatized using  
177 diluted raw POME as a substrate dosed at a pH of 7.2 with 1M NaHCO<sub>3</sub> and kept in a reactor  
178 at a temperature of 55°C [29].

## 2.2 Experimental Set up

### 2.2.1 Thermal Pretreatment

179 Raw POME from the mill was placed in a 60 L capacity tank to undertake thermal pretreatment.  
180  
181 The latter was insulated using insulating foam to prevent heat loss. The IC Basic immersion  
182 circulator (IKA, Germany) was securely placed on the tank to heat POME. The immersion  
183 circulator was set at a temperature of 120°C at a fluid recirculation rate of 1800 rpm. Once raw  
184 POME in the tank reached a temperature of 120°C, the raw POME was thermally treated for 1  
185 hr. A thermal pretreatment temperature of 120°C was chosen since thermal pretreatment carried  
186 out at temperatures lower than 150 °C was reported to be a more economical process in terms  
187 of cost rather than other pretreatment techniques [30]. The mentioned temperature was also  
188 chosen to ensure that the surge in the amount of biogas produced can deftly compensate for the  
189 energy added into the process [31,32]. To ensure that the treated POME's temperature was  
190 homogenous, an infrared thermometer was used to measure the temperature at different heights  
191 along the tank.

### 2.2.2 Dewatering Process

192  
193 Once the thermal pretreatment was completed, the treated POME was transferred to a  
194 dewatering device explicitly designed to cater to POME's rheological properties and  
195 compressive behavior [14]. Table 1 presents the dimensions and operation conditions of the  
196 dewatering device. The POME suspension was allowed to settle for 16 hrs based on the optimal  
197 residence time calculated when designing the dewatering device. After 16 hrs of settling, the  
198 temperature of the dewatered POME was measured to be 35°C. The settled solids (S) and the  
199

clarified liquid (L) were separately placed in canisters and stored at a temperature below 4°C until further use [33]. The appropriate ratio of S and L by volume was then recombined for the anaerobic digestion experiments.

**Table 1: Dewatering device specifications and operating conditions**

Diameter of the thickener/m	0.30
Height of the thickener /m	0.80
Settling Rate/ m/s	$5.81 \times 10^{-6}$
Operating Capacity/ L/d	20
Operating Temperature/ °C	65
Operating Flow rate/ m <sup>3</sup> /s	$2.31 \times 10^{-7}$
Solids Settling Flux, $q_s$ / kg/m <sup>2</sup> s	$2.36 \times 10^{-6}$
Suspension Settling Flux, $q_s$ / kg/m <sup>2</sup> s	$3.36 \times 10^{-6}$
Suspension density, $\rho_s$ / kg/m <sup>3</sup>	1100
Motor speed/ rpm	2-5

### 2.2.3 Start-Up of UASB-HCPB reactor

Poh and Chong (2014) designed a high-rate UASB-HCPB bioreactor, which consists of 2 sections. The lower part is configured like a UASB reactor, while in the second section, the middle section of the reactor constitutes of a packed bed with a cylindrical channel of 6 cm. The packing material used was rubber tubes with a hollow center of approximately 1 cm [34]. The packed bed of the UASB-HCPB reactor reduces sludge washout by capturing the biomass that does not form granules. This biomass tends to form a biofilm on the packing material to which suspended microbes adhere to produce an attached growth system. This HCPB section improves treatment efficiency since it provides a larger biomass surface area for biological treatment. In section 3, the UASB-HCPB reactor's performance is further discussed and compared to different configurations of the UASB reactor. Figure 2 below shows the process

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216 flow diagram of the proposed treatment process, along with the schematic diagram of the  
217 UASB-HCPB reactor.

218 The working volume of the UASB-HCPB reactor was 5L, and the acclimatized inoculum  
219 volume used was 20% of the working volume. The reactor's temperature was maintained at  
220 55°C using a heating water bath, which circulated hot water in the jacket of the UASB- HCPB  
221 reactor. The reactor was then filled with diluted raw POME as the substrate for the inoculum  
222 in the start-up.

223 For the control experiment, untreated raw POME of a ratio by volume of 20% settled solids  
224 and 80% of clarified liquid designated as “20S:80L” in this study was used, which provided an  
225 organic loading rate (OLR) ranging from 5.00-7.35 gCOD/Ld as shown in Figure 3. The  
226 temperature and pH of the system were maintained at 55°C and 6.8-7.2 correspondingly.  
227 Khadaroo et al. (2020) investigated the effect of different solid loadings on POME's anaerobic  
228 digestion performance when conducted in batch thermophilic mode. They observed that as the  
229 solid loading increases, the biogas production increases until the 50S:50L loading, above which  
230 the anaerobic digestion performance declined [11]. However, when the best-performing ratio,  
231 the 40S:60L, was tested with the UASB-HCPB reactor, even though the system's temperature  
232 and pH were well within the optimal conditions for anaerobic digestion, the production of  
233 biogas was sporadic and low. An explanation for this occurrence is the high protein and lipid  
234 content of POME, causing inhibition and reactor overload [35]. A comparable phenomenon  
235 was observed by Cheng et al. 2011, using a similar OLR when treating raw POME. Therefore,  
236 a lower solid loading was used in the control experiment with raw POME. A constant hydraulic  
237 retention time (HRT) of 2 days was maintained throughout the analyses, and the control  
238 experiment, along with the subsequent assays, were continuously run for 30 days.

239 In our previous preliminary study on the effect of thermal pretreatment on a range of solid  
240 loadings, the best performing ratios were found to be thermally pretreated 40S:60L and  
241 50S:50L, which achieved the highest biogas production, methane yield, and removal  
242 efficiencies in terms of COD, BOD, and total suspended solids (TSS) [28]. As optimization of  
243 the previous preliminary study, in this paper, the performance of thermally pretreated 40S:60L  
244 and 50:50L, providing an OLR ranging from 11.55-16.05 and 19.90-25.80 gCOD/Ld  
245 correspondingly, were investigated using a UASB-HCPB reactor.

246 Parameters such as the biogas production volume, the methane composition in the biogas  
247 produced, the COD, BOD, TSS removal efficiencies were monitored daily throughout the  
248 experiments. Once the methane content in the biogas produced and the COD in the effluent  
249 stream remained constant, accounting for less than 5% deviation for five consecutive days in a  
250 particular assay, the system was considered to have attained a steady-state, and the solid  
251 loadings were varied for the subsequent assays.

#### 2.2.4 Physico-chemical Analysis

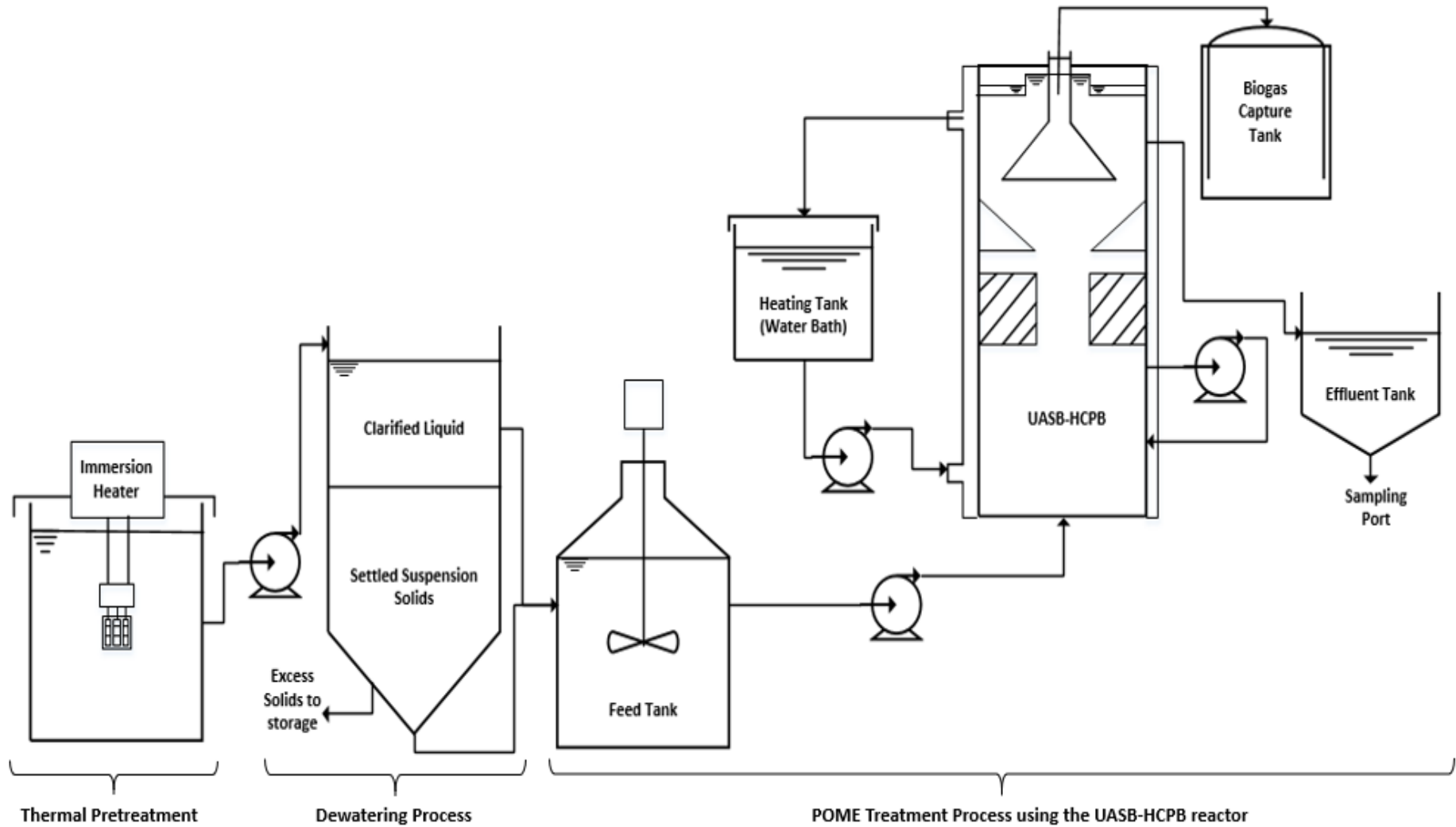
252 The chemical characterization analyses such as the COD, BOD, and TSS tests were carried out  
253 prior to and after anaerobic digestion, which was every two days to determine the quality of  
254 the effluent discharged from the UASB-HCPB reactor. The COD, BOD, and TSS tests were  
255 performed using the HACH Standard Methods 8000, 8043, and 8006, respectively, compliant  
256 with the American Public Health Association (APHA) standards. The alkalinity and the volatile  
257 fatty acids (VFA) tests were conducted as per the APHA 2320 and 5560D, respectively, and  
258 were conducted every four days. Meanwhile, the composition of biogas in terms of carbon  
259 dioxide, methane, and hydrogen sulfide concentrations was quantified using the Binder  
260 COMBIMASS Gas Analyzer. The volume of biogas was measured by water displacement in  
261 the biogas capture tank. The pH of the water in the biogas capture tank was adjusted to 2.0  
262 using 1M H<sub>2</sub>SO<sub>4</sub> to prevent carbon dioxide from dissolving into the water, thus providing a  
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264 more accurate measurement of the biogas volume when using the water displacement method

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267 Figure 2: Schematic diagram for the proposed POME treatment process

### 2.2.5 Microbial Community Analysis

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269 Once the thermally pretreated 40S:60L and 50S:50L loadings reached a steady-state, 50 mL of  
270 the digestates were collected to perform the microbial community analysis. The DNA  
271 extraction was undertaken using the Sigma-Aldrich GenElute™ Soil DNA Isolation kit  
272 (Product Number: DNB100). The DNA extraction was performed as per the standard procedure  
273 provided by Sigma-Aldrich. Once the DNA was extracted, the samples were freeze-dried prior  
274 to further testing. The hypervariable regions (V3-V4) of the 16S rRNA genes of the extracted  
275 DNA samples were amplified in a polymerase chain reaction (PCR) system (GeneAmp 9700,  
276 ABI, Foster City, CA, USA) using primers 338F and 806R for bacteria. In contrast, the archaea  
277 primers utilized were 344F and 915R. The PCR reactions were performed as follows: 3 min of  
278 denaturation at 95°C; 27 cycles of 30 s at 95°C, 30 s for annealing at 55°C, and 45 s for  
279 elongation at 72°C, and a final extension at 72°C for 10 min. The extracted DNA was purified  
280 further using the Axygen Biosciences kit (Axygen Biosciences, Union City, CA, USA), as per  
281 the manufacturer's instructions. The PCR products were sequenced with the Illumina MisSeq  
282 platform in Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Once the quality-  
283 filtered reads for sequencing of bacterial and archaeal 16S rDNA gene amplicons were obtained  
284 from the Illumina, the raw datasets were then deposited into the NCBI (National Center for  
285 Biotechnology Information) Sequence Read Archive database (BioProjectPRJNA386213).

### 2.2.6 Energy Analysis

286  
287 Some assumptions were made for the energy analysis calculations. These were, firstly, no heat  
288 is lost to the environment. Secondly, there is only a very negligible (if any) transfer of energy  
289 to the walls of the tank, which was further insulated. Thirdly, since data on the specific heat  
290 capacity of POME was restricted, it was assumed that the specific heat capacity is similar to  
291 that of water which is 4.18 J/g°C since as per the study conducted by Khadaroo et al. (2019)  
292 POME density is always equal to that of water.

### 3.0 Results and Discussion

Due to the high O&G content in POME, the anaerobic digestion of POME is significantly inhibited, which leads to complications such as reactor overload, washout of bacteria, and formation of foam and scum. The purpose of the anaerobic digestion treatment is to improve the treated effluent quality and produce biogas. However, with all the inhibitors mentioned earlier, neither the treated effluent quality nor the biogas production is consistent. As a remedy, a pretreatment technique would have to be introduced in the system. After a thorough study on different types of pretreatments such as biological, chemical, mechanical, and thermal, it was found that thermal pretreatment was the most suitable treatment for the treatment of POME [4]. The high temperature in thermal pretreatment causes the cell membranes to rupture, releasing intracellular material in the medium, which is more accessible to bacteria in the inoculum to break down and produce biogas. Thermal pretreatment also breaks down the long lipid chains, which is the building block for O&G. By breaking down the long lipid chains, thermal pretreatment prevents the formation of scum and foam, which prevents the washout of bacteria.

To avoid overload reactor and to ensure that the characteristics of POME can be controlled, a dewatering device was integrated into the system. The dewatering device allows the solids to settle and the residual oil to float forming, three distinct layers. Having several sampling ports along the height of the dewatering device, each phase (e.g., settled solids, clarified liquid and residual oil) can be individually extracted. To ensure that the characteristics of POME are maintained throughout the year, the settled solids and clarified liquid can be recombined to make up the optimal ratio of solid: liquid at which the bioreactor operates optimally. The latter does not change the composition of POME; instead, it regulates the amount of solids and liquid that go into the reactor, allowing us to control the load of the reactor at all times, even through

low and high crop season. A previous study at laboratory scale (100 mL) found that the optimal settled solid: clarified liquid ratios were 40S:60L and 50:50L[28]. However, this was undertaken in batch mode of anaerobic digestion; it is well known that batch mode anaerobic digestion results can significantly deviate from the continuous mode of anaerobic digestion since a new substrate is being fed to the reactor continuously. The batch mode of anaerobic digestion is limited in the sense that it only provides preliminary data and understanding of the mechanism of the system, and ideal conditions are set up for the system that is not realistic. In order to mimic conditions in the mills, it is imperative to study the continuous mode of anaerobic digestion to know if the proposed process is viable. This section describes and explains the results obtained when operating a 5L UASB-HCPB bioreactor with thermally pretreated 40S:60L and 50S:50L solid loadings in continuous mode of anaerobic digestion.

Table 2 summarizes the results obtained for the anaerobic digestion of untreated POME and thermally pretreated POME in batch and continuous mode. It can be established that the performance for thermally pretreated 40S:60L and 50S:50L loadings are radically more efficient in terms of COD, BOD, and TSS removal, as well as the methane yield. The control experiment was undertaken using untreated POME at 20S:80L solid loading instead of 40S:60L owing to reactor overload when using the UASB-HCPB reactor [11].

**Table 2: Performance comparison between untreated and thermally pretreated POME**

<b>Control Experiment untreated diluted POME (20S:80L) using the UASB-HCPB (5L)</b>						
	<b>Initial</b>	<b>Final</b>	-	-	-	-
COD/mg/L	20400±578	10700±651				
BOD/mg/L	11400±618	5800±687	-	-	-	-
TSS/mg/L	10300±636	6400±574	-	-	-	-
Daily Methane Yield/mLCH <sub>4</sub> /g COD <sub>removed</sub>		294± 25*	-	-	-	-
	<b>Untreated POME</b>		<b>Thermally Pretreated POME</b>			

	<b>Initial Batch mode AD (100mL)</b>	<b>Final Batch mode AD (100mL)</b>	<b>Initial Batch mode AD (100mL)</b>	<b>Final Batch mode AD (100mL)</b>	<b>Initial using UASB-HCPB (5L) **</b>	<b>Final using UASB-HCPB (5L) **</b>
<b>40S:60L Experiments</b>						
COD/mg/L	47700±120	24400±537	40800±100	8155±44	23100±180	600±92
BOD/mg/L	20600±138	13700±345	20090±130	4015±67	18000±202	920±177
TSS/mg/L	16200±113	9800±366	16000±150	3162±65	10900±107	800±104
Methane Yield/mLCH <sub>4</sub> /g COD <sub>removed</sub>		58.4± 5.2		328±9		414±17*
<b>50S:50L Experiments</b>						
COD/mg/L	49500±210	33000±352	48500±200	16789±112	30200±247	2300±110
BOD/mg/L	22100±195	16800±287	21280±125	7932±131	17800±146	1400±82
TSS/mg/L	25800±115	18200±244	20800±135	6822±156	12000±132	1800±125
Methane Yield/mLCH <sub>4</sub> /g COD <sub>removed</sub>		40.6±7.2		89.8±4.3		260±13*

AD: anaerobic digestion, \* represent the daily methane yield, \*\*continuous mode of AD

Based on the data presented in Table 1, it can be determined that the system's performance during start-up had COD, BOD, and TSS removal efficiencies of 47.5, 49.1, and 37.8%, respectively. The COD, BOD, and TSS removal efficiencies for the untreated 40S:60L using batch anaerobic digestion were 53.0, 33.4, and 39.5 %, respectively. As for thermally pretreated 40S:60L using batch anaerobic digestion, the COD, BOD, and TSS removal efficiencies were found to be 80.63, 81.01, and 80.72%, respectively, while that of the thermally pretreated 40S:60L using the UASB-HCPB with a 5L capacity were 97.4, 94.8, and 88.4 % correspondingly. Furthermore, the COD, BOD, and TSS removal efficiencies for the untreated 50S:50L were 30.0, 23.9, and 29.1 %, respectively. From the thermally pretreated 50S:50L solid loading using batch anaerobic digestion, the COD, BOD, and TSS removal efficiencies

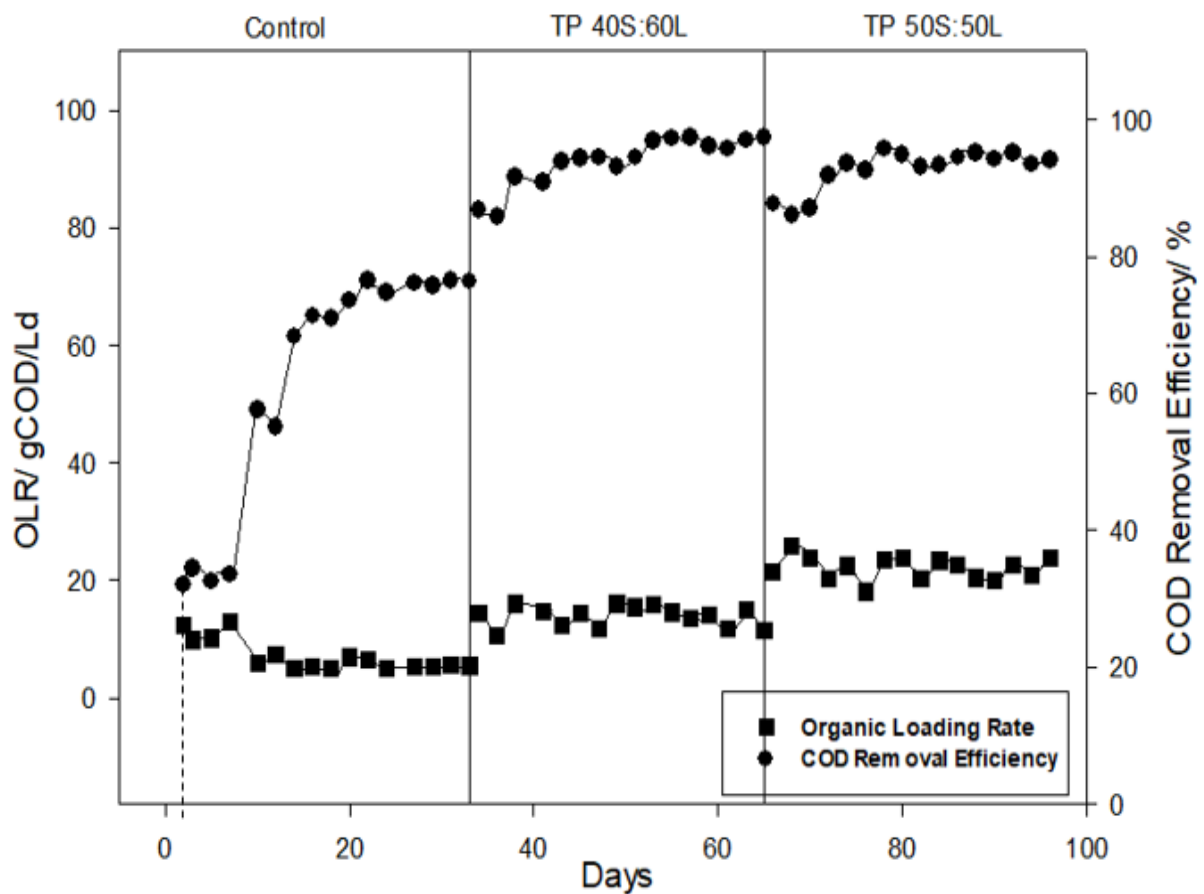
347 were 65.38, 62.72, and 67.20 % correspondingly, while that of the thermally pretreated  
348 50S:50L using the UASB-HCPB were 94.0, 92.1, and 81.6 % congruently.

349 The average methane yield was determined to be  $54.8 \pm 5.2$ , and  $40.1 \pm 7.2$  mLCH<sub>4</sub>/g COD<sub>removed</sub>  
350 for the untreated 40S:60L and untreated 50S:50L in batch mode, respectively. While the  
351 methane yield evaluated for thermally pretreated 40S:60L and 50S:50L in batch mode with a  
352 100mL capacity were  $328 \pm 9$  and  $89.8 \pm 4.3$  mLCH<sub>4</sub>/g COD<sub>removed</sub>. On the other hand, the methane  
353 yield values for thermally pretreated 40S:60L and 50S:50L using the UASB-HCPB reactor in  
354 continuous mode were calculated to be  $414 \pm 17$  and  $260 \pm 13$  mLCH<sub>4</sub>/g COD<sub>removed</sub> per day. The  
355 COD, BOD, TSS, and the methane yield results from Table 1 will be further discussed in their  
356 respective sections.

### 3.1 Chemical Oxygen Demand (COD) Removal Efficiency and Organic Loading Rate (OLR)

359 Figure 3 shows the trend of COD removal efficiency with varying OLR. The OLR for the  
360 control was initially set at 12.50-13.00 gCOD/Ld corresponding to untreated 40S:60L solid  
361 loading. However, it was observed that the COD removal efficiency was meager, and the  
362 biogas production was also inhibited. Therefore, the OLR was reduced to 5.00-7.35 gCOD/Ld  
363 to see how the UASB-HCPB performs with raw diluted POME. It can be noted in Figure 2 that  
364 the COD removal efficiency increases sharply after altering the OLR. An explanation for this  
365 is the high lignin, protein, and O&G contents of untreated POME. By reducing the amount of  
366 solids, there is less agglomeration of the flocs present, allowing more effective mass transfer,  
367 better transport, and larger surface area of solids available for the consortium of bacteria, which  
368 are better sustained by more soluble substrates [37,38]. When thermally pretreated 40S:60L  
369 was used, there was no such impediment with the reactor performance; on the contrary, the  
370 results obtained were optimum. The main reason for this occurrence is due to thermal  
371 pretreatment, whereby the long lipid and protein chains were broken down into smaller, more  
372 digestible ones and enabling the release of intracellular organic matter into the medium, making

373 it readily available for the microorganisms to digest [39,40]. With an OLR ranging from 11.55-  
 374 16.05 gCOD/Ld, a COD removal efficiency of 97.5 % was achieved, making this treatment  
 375 process highly efficient. The thermally pretreated 40S:60L assay can easily meet the Malaysian  
 376 government's environmental standards, all while lowering the load on the aeration treatment,  
 377 which implies reducing the energy consumption in the aeration treatment. The OLR was further  
 378 increased to a maximum of 25.80 gCOD/Ld; it can be seen in Figure 3 that the COD removal  
 379 efficiency declined slightly to 95.1%.



380  
 381 Figure 3: COD removal efficiency and the OLR (Control Experiment:(untreated POME,20S:80L), TP  
 382 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)  
 383

### 384 3.2 Biological Oxygen Demand (BOD) Removal Efficiency

385 Similar to the COD removal efficiency, the BOD removal efficiency is dependent on the  
 386 chemical characteristics of the wastewater, which in turn depends on the amount and  
 387 composition of the organic matter. The BOD/COD ratio indicates the biodegradability of a

388 substrate, also known as the biodegradability index (BI). It measures the treatment requirement  
389 and the strength of the wastewater. If the BOD/COD ratio of the untreated wastewater is 0.5  
390 and above, also known as low strength, the wastewater is deemed to be effectively treatable by  
391 biological methods [41]. Contrarywise, a low BI indicates that the wastewater has a prevalence  
392 of toxic substances that inhibits biodegradation; this wastewater is known to be high strength  
393 [42]. The BI of POME can range from 0.36 to 0.62. In this study, the BI of POME was 0.78,  
394 which indicates that POME can be efficiently treated by anaerobic digestion.

395 Furthermore, BOD is the measure of the amount of oxygen required for the bacteria to  
396 breakdown the organic components present in water and wastewater [43]. Since treated POME  
397 is discharged in watercourses, the BOD value must be as low as possible so as to prevent  
398 microorganisms from competing with the aquatic life on the uptake of oxygen [4]. In this study,  
399 the BOD removal efficiency for the control experiment was noted to be 72.2%. It can be seen  
400 in Figure 4 that thermally pretreated 40S:60L and 50S:50L follows similar trends. However,  
401 the BOD removal efficiency of thermally pretreated 40S:60L is higher than that of thermally  
402 pretreated 50S:50L. The optimal BOD removal efficiency for thermally pretreated 40S:60L  
403 was found to be 95.1 %, while that of the thermally pretreated 50S:50L was 91.9%.

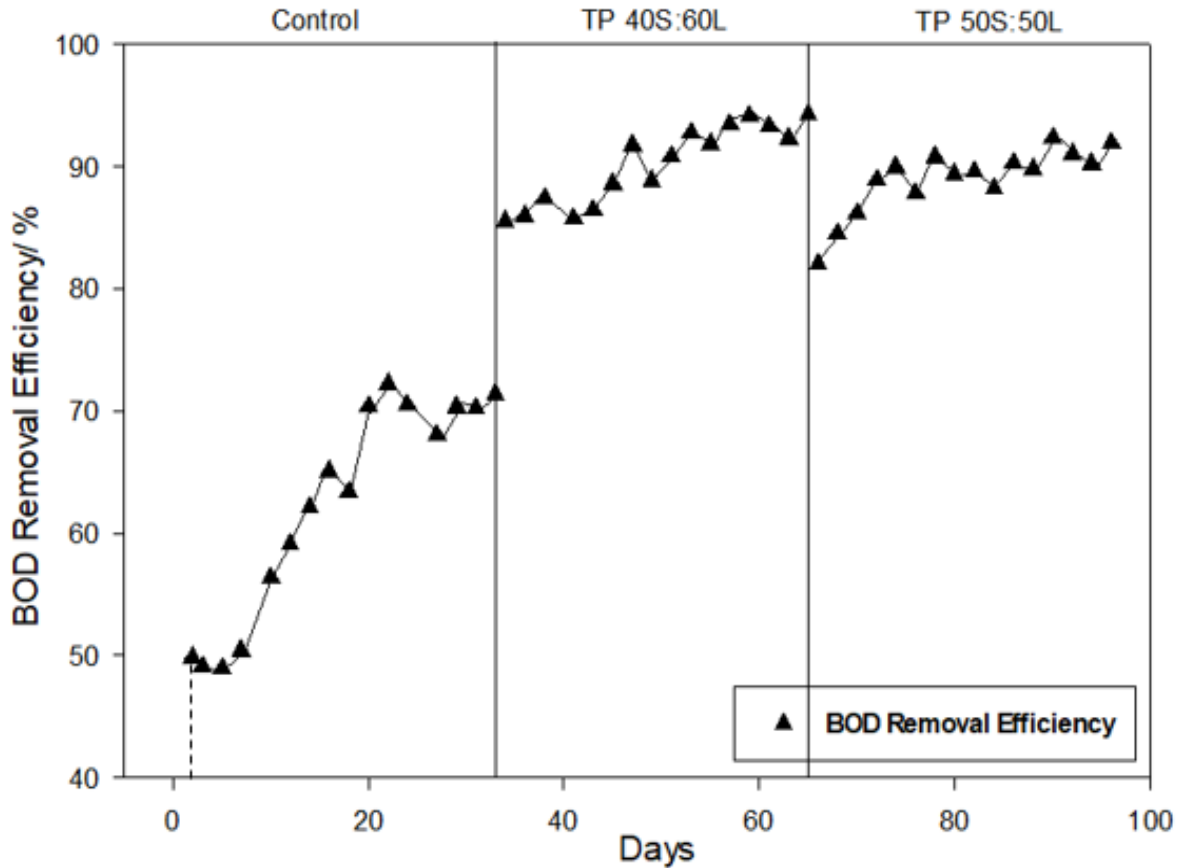
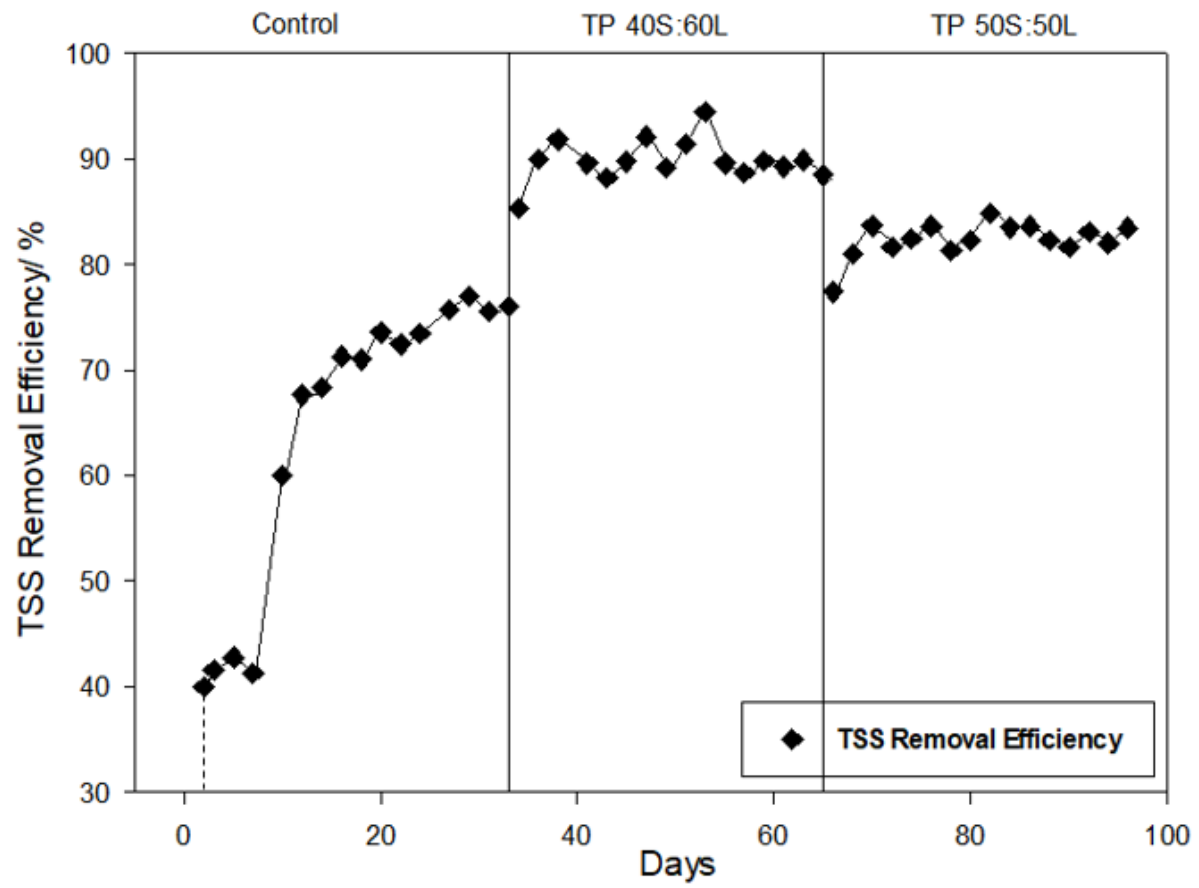


Figure 4: BOD removal efficiency (Control Experiment:(untreated POME, 20S:80L), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

### 3.3 Total Suspended Solids (TSS) Removal Efficiency

Figure 5 shows the TSS removal efficiency trend for the control, thermally treated 40S:60L and 50S:50L. TSS concentrations in both the feed and the effluent were measured to find the amount of the insoluble organic present in the POME. Additionally, TSS removal efficiency is also a good indicator of the UASB reactor's performance under different solids loading rates. The TSS concentration after thermal pretreatment was considerably reduced. It can be seen in Table 1 that the TSS concentration for the 40S:60L loading decreases from 16200 mg/L to 10900 mg/L after pretreatment alone. The TSS concentration was reduced from an average concentration of 10300 mg/L to 6400 mg/L post anaerobic digestion in the control experiment. The TSS removal efficiency for the control experiment varies from 60 to 77%.

418 Meanwhile, the treated 40S:60L solid loading, TSS concentration declined from an average  
 419 concentration of 10900 mg/L to 600 mg/L. The TSS removal efficiency for the treated 40S:60L  
 420 solid loading fluctuated between 85.0 to 94.5%. Peaks at days 47 and 53 in the TSS removal  
 421 efficiency, as shown in Figure 5, occurred due to a slightly lower initial TSS value; therefore,  
 422 the consortium of bacteria was able to breakdown almost all of the suspended solids present in  
 423 the medium. On the other hand, the TSS concentration for the treated 50S:50L solid loading  
 424 decreased from an average concentration of 12000 mg/L to 1800mg/L. The TSS removal  
 425 efficiency recorded for the latter varied from 77.5 to 87%.

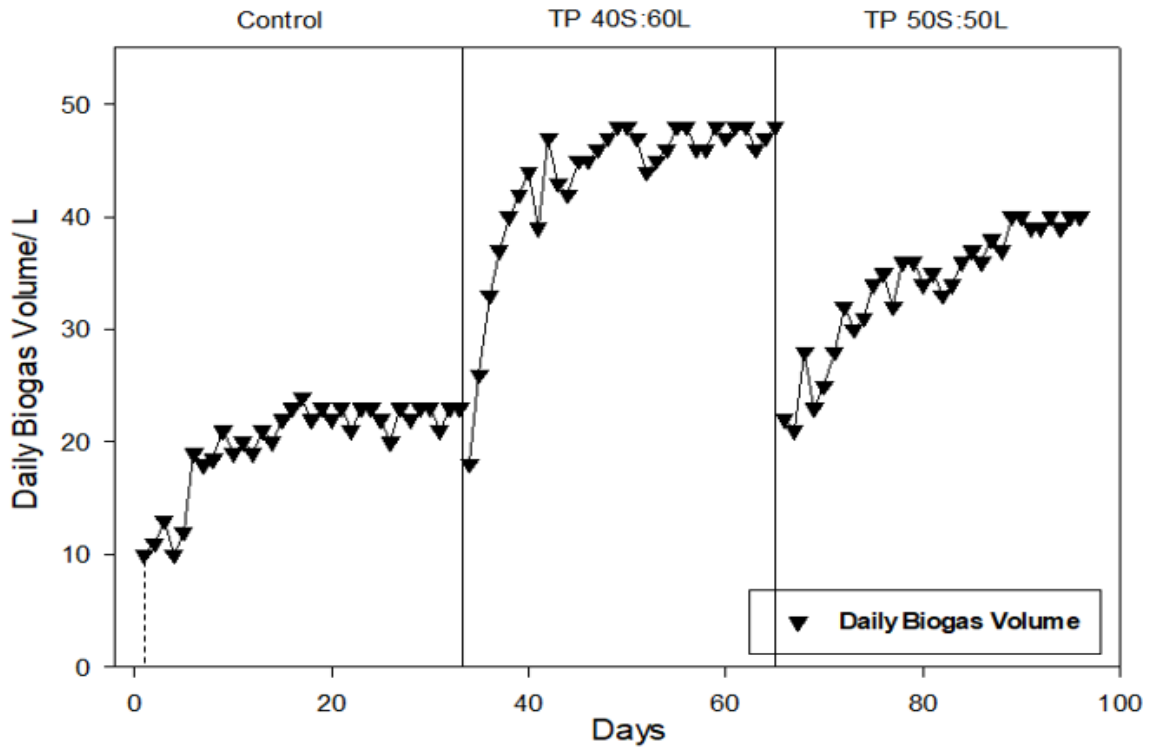


426  
 427 Figure 5: TSS removal efficiency (Control Experiment:(untreated POME, 20S:80L), TP 40S:60L:  
 428 Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)  
 429

### 3.4 Daily biogas production and methane composition in biogas

Figures 6 and 7 illustrate the trend in the volume of biogas produced daily and the biogas' methane composition, respectively. The average daily biogas volume produced during the control experiment was 23L per day, and the maximum methane purity achieved in the latter was 61.9%. In the thermally pretreated 40S:60L assay, the average daily biogas produced was 48L per day, with a methane purity of 77.8%. In comparison, the daily biogas volume produced in the thermally pretreated 50S:50L run was 40L per day, with a methane purity of 72.5%. It can be noted that the control experiment had the least amount of biogas with the lowest methane purity. An explanation for the aforementioned observation may be the limited amount of solid present in the medium. Secondly, the lignin present in POME decreases cellulose hydrolysis efficiency. The lignin component physically prevents access to the cellulose and its non-productive binding of enzymes, hindering anaerobic digestion [11,35,44]. The thermally treated conditions' enhanced performance is due to the breakdown of lignin, O&G prior to anaerobic digestion, and an improved VFA formation [45]. Since the conditions set for anaerobic digestion favor the methanogens, they can effectively and consistently convert the acetic acid into methane and carbon dioxide [46]. As for the methane yield, albeit the control produced the least biogas with the least methane purity, the methane yield was found to be  $294 \pm 25$  mLCH<sub>4</sub>/g COD<sub>removed</sub> per day. The daily methane yield calculated for thermally pretreated 40S:60L and 50S:50L were  $414 \pm 17$  and  $260 \pm 13$  mLCH<sub>4</sub>/g COD<sub>removed</sub> per day congruently. From the results obtained, it can be established that the methane yield for the thermally pretreated 40S:60L in continuous mode exceeds the theoretical 350 mLCH<sub>4</sub>/g COD<sub>removed</sub> methane yield. This occurrence can emerge when anaerobic digestion is undertaken at thermophilic conditions instead of mesophilic conditions [47]. In this study, it can be observed that the COD, BOD, TSS removal efficiencies trends are consistent with the daily biogas production volume and the methane composition.

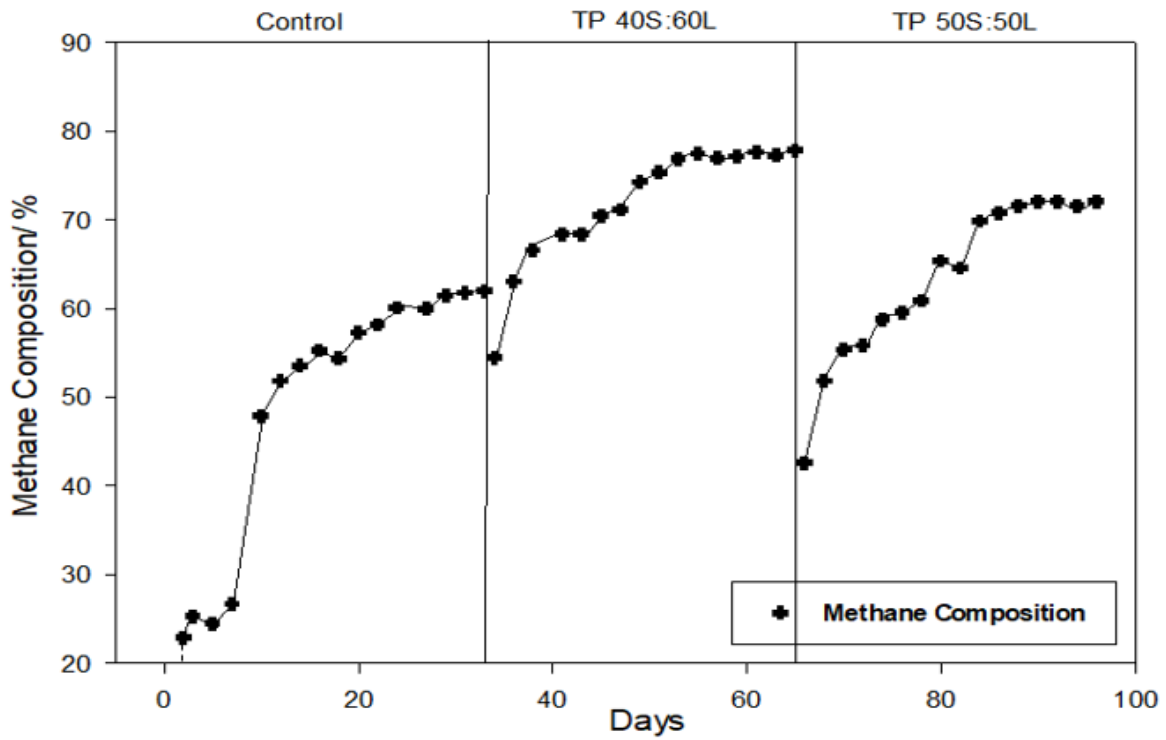
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456

457 Figure 6: Daily volume of biogas produced (Control Experiment:(untreated POME, 20S:80L), TP  
458 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

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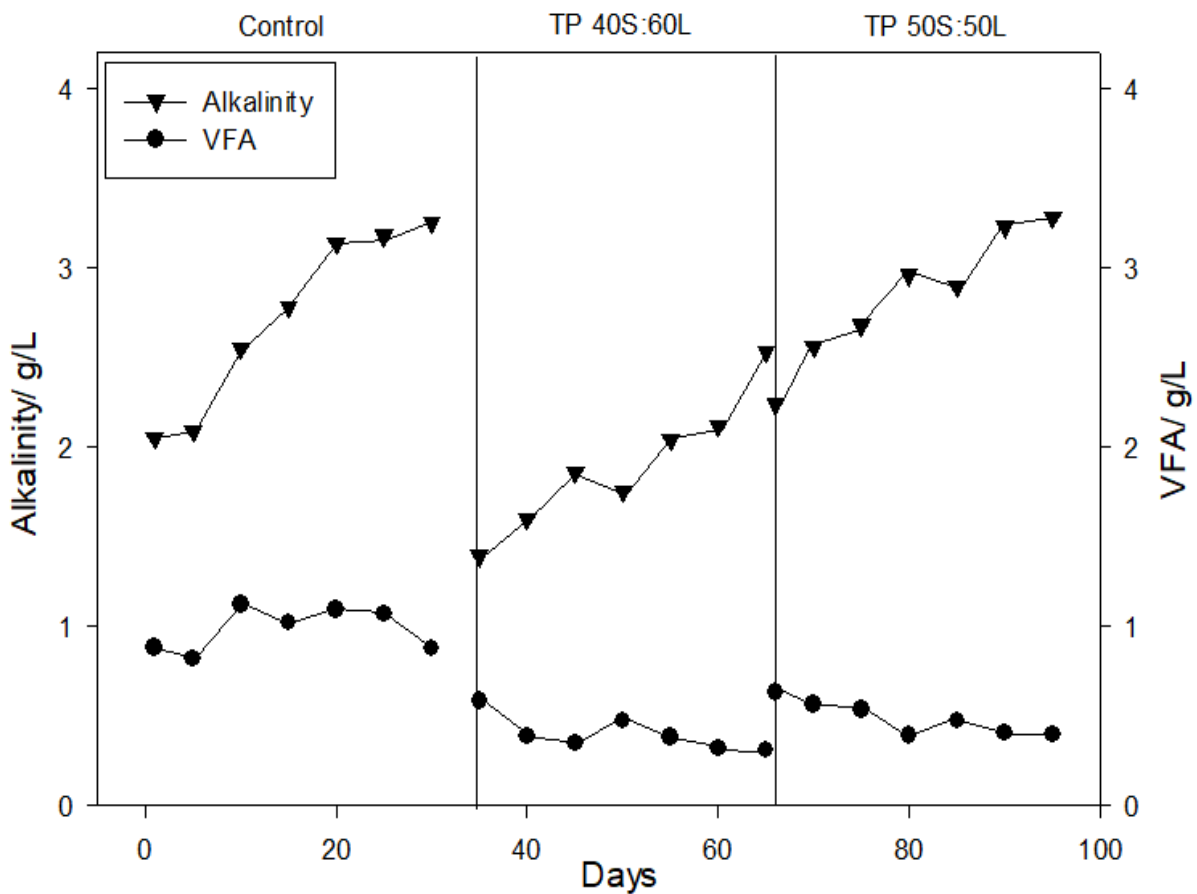
461 Figure 7: Methane composition in the biogas produced (Control Experiment:(untreated POME,  
462 20S:80L), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)  
463

### 464 3.5 Alkalinity and Volatile Fatty Acids

465 VFA is a fundamental parameter when it comes to anaerobic digestion. The accumulation of  
466 VFA in the digester brings forth an organic overload and the acidification of the digester's  
467 content, which sequentially inhibits the process [48]. The VFA concentration varies from 0.38  
468 to 1.12g/L in this study, while the alkalinity ranges from 1.39 to 3.28 g/L. Generally, the  
469 optimum alkalinity required for anaerobic digestion varies from 1 to 5 g/L. In this study, it can  
470 be corroborated that the alkalinity was well within that range [49]. Figure 8 depicts the trends  
471 for alkalinity and the VFA concentrations, which were measured every four days. Figure 8 also  
472 illustrates how the alkalinity and the VFA vary with each other. The reactor's pH, which is  
473 directly linked to the alkalinity, was recorded to be between 6.8 to 7.2 throughout the study  
474 without having to adjust the pH after day 6. It can be observed that the alkalinity of the reactor  
475 gradually increases, showing that the reactor was performing efficiently.

476 Additionally, it was noted that the VFA/alkalinity ratios of the TP 40S:60L and TP 50S:50L  
477 assays fluctuated between 0.12 to 0.36, indicating that there was sufficient buffering capacity  
478 for the reactor to be stable [50]. Moreover, the VFA/Alkalinity ratio is a parameter that  
479 designates the stability of the reactor; VFA/Alkalinity ratio less than 0.4 usually indicates that  
480 the reactor is stable [51]. It can be seen that the VFA concentrations in the pretreated runs are  
481 lower than that of the control, demonstrating that thermal pretreatment and dewatering can  
482 improve the stability of the UASB-HCPB reactor [52]. It can be observed in Figure 8 that the  
483 VFA/alkalinity ratio was lower throughout the run for the thermally pretreated 40S:60L solid  
484 loading run compared to that of the 50S:50L loading, demonstrating that the reactor was more  
485 stable in the 40S:60L pretreated assay. As mentioned before, thermal pretreatment tends to  
486 boost the VFA production, contributing to a higher methane potential as the hydrolysis step is

487 enhanced. The VFA concentration measured in thermophilic UASB reactors was reported to  
 488 be higher than 1.0g/L [53]. However, the VFA concentrations for the pretreated assays in this  
 489 study ranged from 0.3 to 0.6 g/L. An explanation for this occurrence is that the acetic acid  
 490 produced from VFA was successfully being converted to methane by the methanogens since  
 491 the conditions under which anaerobic digestion is being conducted (temp: 55°C and pH 6.8-7.2)  
 492 favors methanogens to produce methane [46,54]. The concurrence to the previously mentioned  
 493 statement can be seen through the increase in the methane composition in the biogas and biogas  
 494 production in Figures 6 and 7.



495  
 496 Figure 8: Alkanility and VFA concentrations measured (Control Experiment:(untreated POME,  
 497 20S:80L), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)  
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### 3.6 Microbial Community Analysis

In anaerobic digestion, organic matter is broken down through hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, complex molecules (organic compounds) such as carbohydrates, fats, and proteins into smaller molecules of sugar, fatty acids, and amino acids. The hydrolytic bacteria, such as *Clostridium* and *Bacillus*, are responsible for the hydrolysis process. The hydrolyzed compounds are then converted to intermediates by acidogenic bacteria, such as *Syntrophomonas*, *Pseudomonas*, and *Flavobacterium*, to form compounds such as butyric, propanoic, and acetic acid. While during acetogenesis, the intermediaries are further degraded to form acetate, carbon dioxide, and hydrogen produced by acetogenic bacteria, such as *Desulfovibrio* and *Clostridium*. To undertake the final stage, methanogenesis, two different groups of bacteria, the acetotrophic and hydrogenotrophic bacteria, are responsible for methane production [55].

The substrate degraded for biogas production depends mainly on the microbial communities present in the medium. The microbial composition is susceptible to various factors such as temperature, type of substrate, solid concentration, intermediates produced (e.g., short-chain fatty acids and ammonia), and digesters' configuration. Thermal pretreatment may also influence the metabolic pathways, diversity, and structure of microbes [56]. In the anaerobic digestion process, the function of bacterial communities is predominantly in the conversion of organic solids to VFA, carbon dioxide, and hydrogen gas. On the other hand, the archaeal communities' role is to convert acetate or carbon dioxide to methane [57]. Figure 9A depicts the bacterial community composition at the phylum level. It can be seen that for bacteria, the dominant phyla were *Firmicutes*, *Thermotogae*, and *Bacteroides* in the 40S:60L and 50S:50L samples. In the 40S:60L sample, the relative abundance recorded for *Firmicutes*, *Thermotogae*, and *Bacteroides* were 38.6, 37.2, and 4.5%, respectively, while in the 50S:50L sample, the *Firmicutes*, *Thermotogae*, and *Bacteroides* were 62.1, 27.5, and 1.1 % congruently. Meanwhile, in the unacclimatized seed sludge (USS), the dominant phyla were *Bacteroides*

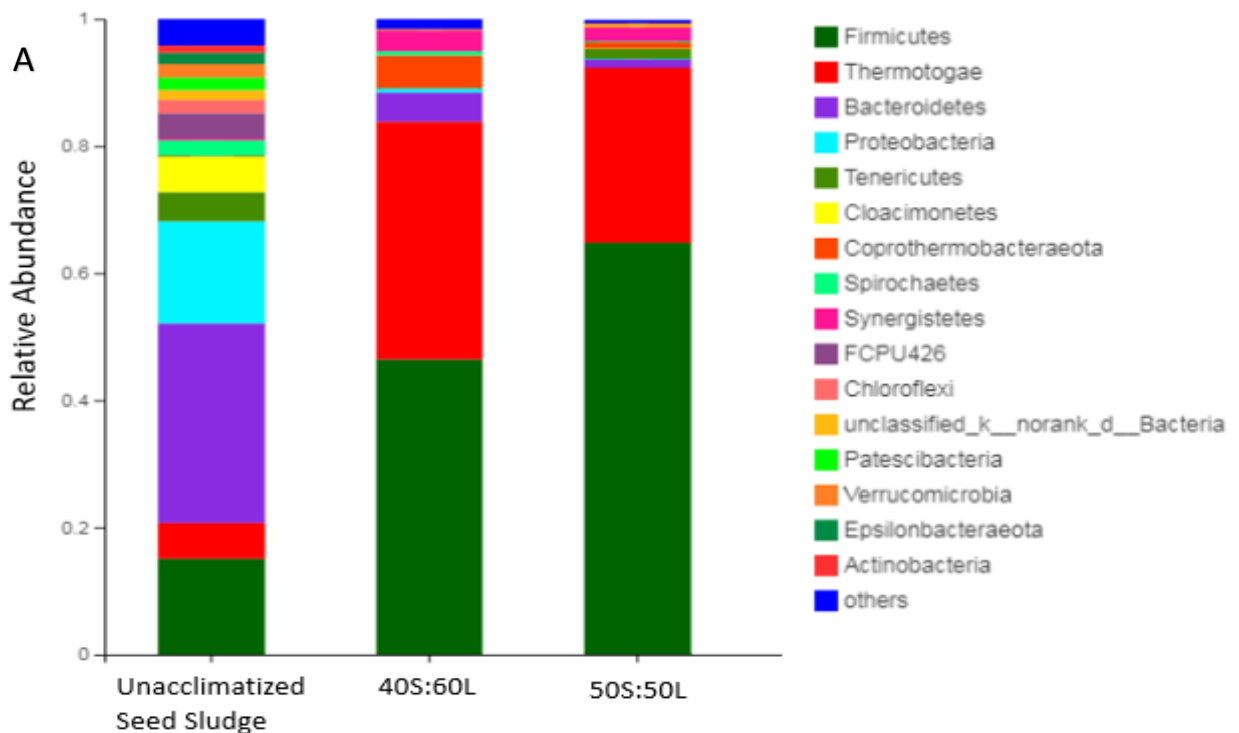
525 (27.6%), *Firmicutes* (13.5%), followed by *Thermotogae* (3.6%). *Firmicutes* produce  
526 extracellular enzymes such as protease, cellulase, and lipase to enable the hydrolysis of  
527 proteins, cellulose, lipids, and lignin. *Firmicutes* can also use butyrate and similar compounds  
528 to produce acetic acid [44,56]. Since the 50S:50L sample consists of more solids, i.e., more  
529 organic matter, it is expected to have a higher density of *Firmicutes*; this is also in concurrence  
530 with a study conducted by Wu et al. (2020) on high solids content anaerobic digestion. Within  
531 the phylum *Thermotogae*, the dominant bacterial genus was *Defluviitoga*, which typically  
532 belongs to thermophilic fermentation bacteria responsible for the degradation of complex  
533 compounds to acetate, CO<sub>2</sub>, and H<sub>2</sub> [58]. The relative abundance of *Defluviitoga* in the  
534 40S:60L and 50S:50L was 24.2% and 20.3%, respectively, while in the USS sample, the  
535 primary phylum was *Rikenellaceae DMER64* at 23.2%, which is a potential syntrophic  
536 bacteria. Alternatively, *Bacteroidetes* comprising of proteolytic bacterium are responsible for  
537 the degradation of numerous proteins to VFAs and NH<sub>4</sub>-N (Nitrogen in ammonium ion). The  
538 *Bacteroidetes* were dominant in the USS sample but not in the 40S:60 and the 50S:50L assays.  
539 Figure 9B indicates the archaeal communities' composition at the phylum level. The majority  
540 community detected from the three tested conditions was classified into the phylum  
541 *Euryarchaeota*. It can be observed that the relative abundance of *Euryarchaeota* increased at  
542 thermophilic conditions. The 40S:60L and the 50S:50L samples had a relative abundance of  
543 97.1 and 96.3%, respectively, while the USS had a 79.2% relative abundance of  
544 *Euryarchaeota*. The Archaea domain of phylum *Euryarchaeota* is constituted of Methanogenic  
545 microorganisms. Six phylogenetic orders of methanogens have been identified, the  
546 *Methanosarcinales*, *Methanobacteriales*, *Methanomicrobiales*, *Methanococcales*,  
547 *Methanopyrales*, and *Methanocellales* [59]. The three main phylogenetic orders of  
548 methanogens in this study were found to be the *methanobacteriales* in terms of

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549 *Methanothermobacter*, the *methanomicrobiales* in terms of *Methanoculleus*, and lastly, the  
550 *Methanosarcinales* in terms of *Methanosarcina*.

551 There are three major pathways for methanogenesis; these are hydrogenotrophic,  
552 methylotrophic, and acetoclastic. Hydrogenotrophic methanogenesis is the most common  
553 pathway in which Class I methanogens (*Methanopyrales*, *Methanococcales*, and  
554 *Methanobacteriales*) and most of Class II methanogens (*Methanomicrobiales*,  
555 *Methanocellales*, and *Methanosarcinales*) known as hydrogenotrophs reduce CO<sub>2</sub> to CH<sub>4</sub> in  
556 six steps via the reductive acetyl-CoA or Wood–Ljungdahl pathway [60]. Subsequently,  
557 methylotrophic methanogenesis during which methane is formed from different methylated  
558 compounds such as methanol, methylamines, or methylated thiols occurs due to  
559 the *Methanomassiliicoccales*, *Methanobacteriales*, and *Methanosarcinales* methanogens. In  
560 contrast to acetoclastic methanogenesis, which involves the formation of methane from acetate,  
561 it can occur only in the order of the *Methanosarcinales* [61]. Figure 9C illustrates the  
562 composition of the archaeal communities at the genus level; in the USS sample the predominant  
563 genus was found to be *methanoculleus* with an abundance of 34.5%, followed by *methanosaeta*  
564 with a relative abundance of 20.9%. The archaeal communities for the 40S:60L and the  
565 50S:50L proved to be inherently different. In the 40S:60L sample, the dominant genus was  
566 *Methanothermobacter*, with an abundance of 55.9%. The *Methanosarcina* genus was also  
567 present with a relative abundance of 23.2%. The genus *Methanosaeta* had an abundance of  
568 12.5%, and *Methanoculleus* was also detected but at a much lower relative abundance of 4.6%.  
569 Consequently, in the 50S:50L sample, the dominant genera were *Methanoculleus*,  
570 *Methanothermobacter*, and *Methanosarcina*, with a relative abundance of 59.2, 21.7, and  
571 11.8%, respectively. It was reported that the *Methanothermobacter* is more likely to grow in  
572 an environment with VFA accumulation while the *Methanoculleus* had a higher affinity to H<sub>2</sub>  
573 (requires lower H<sub>2</sub> partial pressure) [44,62]. Based on the previous statement, it can be deduced

574 the 40S:60L sample favored the VFA formation and induced a higher H<sub>2</sub> partial pressure  
 575 providing a more favorable environment for *Methanothermobacter* to thrive. It can also be  
 576 established that the consortium of *Methanothermobacter*, *Methanosarcina*, and *Methanosaeta*  
 577 was more effective in methanogenesis since the methane purity in the 40S:60L reactor was  
 578 higher than that of the 50S:50L. However, the dominant methanogens in the anaerobic  
 579 digestion can be affected by numerous parameters, such as temperature, types of substrates, the  
 580 organic loading rate, low or high solids conditions, and different physicochemical properties  
 581 such as VFAs and H<sub>2</sub> partial pressure, NH<sub>4</sub><sup>+</sup> concentration, and concentrations of many other  
 582 different inhibitors triggered by various conditions may also bring forth an alternate archaeal  
 583 community [44,63].



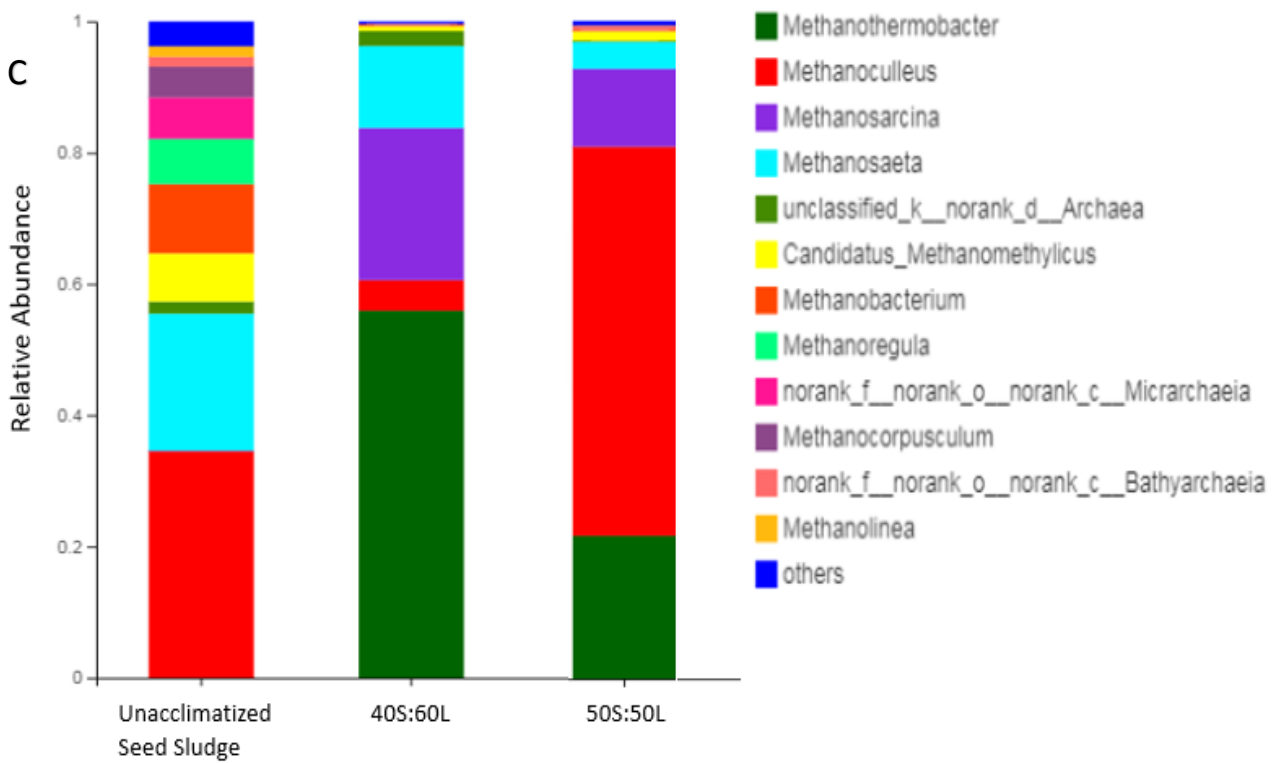
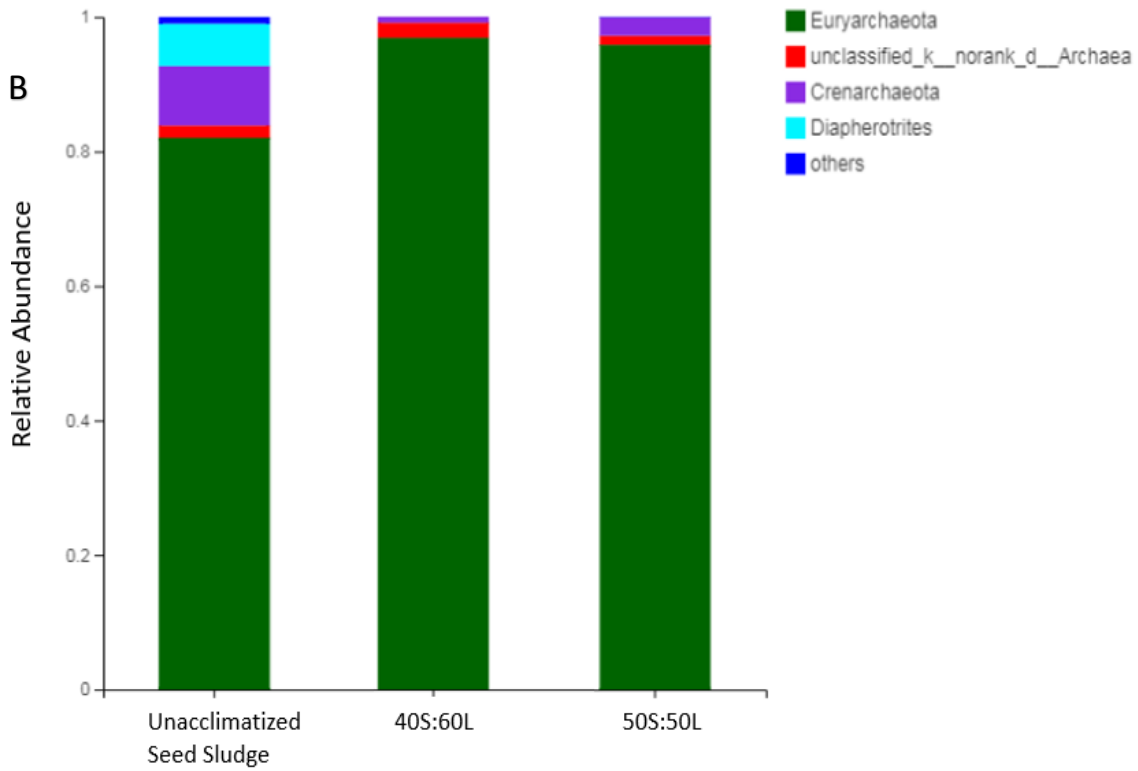


Figure 9: A-Taxonomic compositions of bacterial communities at the phylum level, B- Taxonomic compositions of archaeal communities at the phylum level, C- Taxonomic compositions of archaeal communities at the genus level.

### 3.7 Performance comparison of the UASB-HCPB to other configurations of UASB reactors

Poh and Chong 2014 studied the anaerobic digestion of POME using a 5L capacity UASB-HCPB reactor at a HRT of 5 days; they found that the optimal OLR, 6.66 gCOD/Ld, brought about a COD removal efficiency of 97.5 %, which is comparable to what we achieved in this study but at a higher OLR. They also found that at the highest OLR of 27.65 gCOD/Ld, the overall COD removal efficiency was 91.8%, while in this study, a higher percentage of 95.7% of COD removal was achieved at an OLR of 25.80 gCOD/Ld [64]. This indicates that thermal pretreatment enhances the COD removal efficiency even at higher OLR. Thermal pretreatment also effectively prevents foaming and scum formation, which arises when operating a reactor at high OLR (> 6.1 gCOD/Ld). The scum formation occurs when substrates are made up of high fat, oil, and grease content. The presence of the long-chain fatty acids instigates instability in the digester; consequently, it is more arduous for the bacteria consortium to digest the lipids in the medium [65,66]. However, when thermally pretreated, the long fatty acid chains are broken down by high temperature, and therefore no scum or foaming occurs during anaerobic digestion [4,67].

In Poh and Chong (2014) study, the optimal BOD removal efficiency of 98.2 % was achieved at an OLR of 6.66 gCOD/Ld at a HRT of 5 days. They also observed that at a HRT of 2 days with an OLR of 13.75 gCOD/Ld, which is within the 40S:60L solid loading range, the overall BOD removal efficiency was 93.1%. Poh et al. (2014) achieved the highest TSS removal efficiency of 98.3% with an OLR of 6.66 gCOD/Ld at a HRT of 5 days. However, when operated at HRT 2 days with a higher OLR (13.75 gCOD/Ld) to match that of the best performing OLR in our study, the TSS efficiency drop 86.9%. A study conducted on cattle slaughterhouse wastewater found that the maximum TSS removal efficiency varied from 72 to 85% when the UASB was operated at an OLR of 15 gCOD/Ld [68].

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618 In comparison to the study of Poh and Chong (2014), the maximum daily biogas was 36.76 L  
619 produced with an OLR of 27.65 gCOD/Ld at a HRT of 2 days when treating POME. However,  
620 the methane composition was recorded to be 57.4%. The highest methane purity of 67.5% was  
621 measured when operating the reactor with an OLR of 9.19 gCOD/Ld at a HRT of 3days. When  
622 the UASB-HCPB reactor was run at a similar optimum OLR as in our present study, 13.75  
623 gCOD/Ld, a daily biogas production of 19.4 L and 58.4% of methane was achieved [34].

624  
625 In a study conducted on Monosodium glutamate wastewater by Chen et al. (2020), they attained  
626 the highest COD efficiency removal and methane yield at an OLR of 8.0 gCOD/Ld when they  
627 operated an UASB reactor at HRT of 2 days. They also reported that the maximum favorable  
628 OLR was 18 gCOD/Ld, above which an increase in the accumulation of free ammonia and  
629 volatile fatty acids caused a decrease in the UASB reactor performance [69]. Bakraoui et al.  
630 (2020) investigated the optimal conditions for the anaerobic digestion of recycled paper mill  
631 wastewater; they observed that when operating the UASB for a HRT of 15hrs, the optimal OLR  
632 was 8.31 gCOD/Ld, which achieved a COD removal efficiency of 80.6% [48]. In another study  
633 on the anaerobic digestion of sugarcane vinasse, the optimal COD removal efficiency of 86.7%  
634 occurred when operating the reactor at an OLR of 12 gCOD/Ld [70]. Based on the  
635 aforementioned study, it can be established that the OLR and the COD removal efficiency  
636 varies from substrates to substrates. It can be inferred that at lower OLR ranging from 6.0 to  
637 16.5 gCOD/Ld, the COD removal efficiency is optimum.

638 When comparing the BOD removal efficiencies, in another study on food waste using an UASB  
639 reactor, the BOD removal efficiency obtained was 95% with a HRT of 12 days [71]. In a study  
640 on distillery wastewater with an OLR of 19 gCOD/Ld, the BOD removal efficiency was 83.1  
641 %, while at a higher OLR of 24 gCOD/Ld, the BOD removal efficiency was found to be 88.2%.

642 From the studies mentioned above, it can be established that the BOD removal efficiency is  
1  
2 643 predominantly dependant on the nature of the wastewater [41].  
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5 644 Meanwhile, when examining the TSS removal efficiencies of different substrates, in a study  
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7 645 on the slaughterhouse wastewater, it was reported that the highest TSS removal efficiency was  
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9 646 98% using an OLR of 6.99 g total organic carbon (TOC)/Ld; nevertheless, when operated at  
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11 647 an OLR of 14.25 gTOC/Ld, the TSS removal efficiency declined to 85% [51]. The decline in  
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13 648 TSS removal efficiency from the 40S:60L to 50S:50L solid loading is due to the increase in  
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15 649 TSS concentration in the 50S:50L solid loading along with less effective mass transfer through  
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17 650 the medium [37,72].  
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23 651 When studying the biogas production and the methane composition, in a study using an UASB  
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25 652 reactor of 1.5 L capacity, it was reported that using an OLR of 2.9 gVS/Ld for the treatment of  
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27 653 POME, the daily biogas production was recorded to be 1.2 L with a methane composition of  
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29 654 66%. Nonetheless, when an OLR of 5.8 gVS/Ld was applied, the daily biogas produced was  
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31 655 two-fold higher, 2.6L, with a decrease in the methane composition of 61% [35]. In another  
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33 656 study conducted on POME using a 4.7 L UASB reactor, the maximum daily biogas volume  
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35 657 recorded was 12.4 L when operated at an OLR of 2.2 gCOD/Ld at 10.4 days HRT. In the same  
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37 658 study, when the reactor was operated at a higher OLR of 11.5 gCOD/Ld with a HRT of 4 days,  
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39 659 the volume of biogas produced decreased significantly to 6.3 L with a methane composition of  
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41 660 55% [73].  
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48 661 Based on the studies mentioned above, it can be concluded that the results obtained in our study  
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50 662 are the most efficient in terms of biogas production along with methane composition, even with  
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52 663 a lower HRT of 2 days; this is owing to the thermal pretreatment coupled with the optimal solid  
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54 664 loading. Thermal pretreatment enabled the use of a higher OLR without compromising the  
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56 665 biogas production and methane purity.  
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666 Table 2 demonstrates the performance of different conditions using an UASB reactor compared  
 667 to that of an UASB-HCPB reactor. All in all, it can be seen that operating the bioreactors at  
 668 thermophilic temperature yields the best results in terms of COD removal and methane  
 669 composition in the biogas produced.

670 Table 3: Performance of the UASB-HCPB compared to other UASB bioreactors  
 671 configurations.  
 672

	Substrates	Temperature/ °C	OLR/ (gCOD/Ld)	COD removal/ %	Methane / %	HRT/ days	References
<b>UASB- HCPB</b>	POME	55	11.55-16.05	97.5	77.8	2.0	Current study
	POME	55	6.66	97.5	60.0	5.0	[64]
<b>UASB</b>	POME	35	4.5-12.5	94.9	48.2	4.0	[74]
	POME	37	2.2	96.0	65.0	10.4	[73]
	POME	55	2.9 (gVS/Ld)	95.5	66.0	5.0	[35]
	POME	55	5.8 (gVS/Ld)	92.5	61.0	5.0	[35]
	Monosodium glutamate wastewater	35	8.0	97.9	67.5	1.0	[69]
	Domestic sludge	35	0.213	86.0	70.0	2.5	[75]
	Vinasse	32	0.2-7.5	82.0	58.0	2.8	[76]
	Dairy wastewater	28-32	0.72-4.32	83.5	70.0	0.83	[77]

673  
 674 From Table 2, a common operational characteristic is that at lower OLR that is less than 8  
 675 gCOD/Ld, the COD removal tends to be optimal with the exception of the present study. The  
 676 treatment condition used in this study allowed an increase in OLR without affecting the COD  
 677 removal efficiency. This implies that more effluent can be treated, all while providing a higher

678 methane purity as well as enhanced treated effluent quality. With such a highly efficient  
 679 primary treatment performance, the load on the secondary treatment, the aeration treatment  
 680 will be drastically diminished, extensively reducing the process's energy consumption. As  
 681 aforementioned, the performance of the bioreactors varies from substrate to substrate. The only  
 682 study that attained better COD removal was Chen et al. (2020), with a COD removal of 97.9%.  
 683 However, the methane composition of the biogas was considerably lower than that achieved in  
 684 this study.

### 3.8 Energy Analysis case study on a medium-capacity plant

686 Assuming that the amount of treated POME in a medium capacity plant, such as Kilang  
 687 Kosfarm Sdn, Bhd, Pahang, Malaysia, is 567.4 m<sup>3</sup> per day [78]. Electricity used for  
 688 thermophilic anaerobic digestion and thermal pretreatment was found to be 1.61x10<sup>4</sup> and  
 689 1.53x10<sup>4</sup> kWh/d, respectively. These values were calculated by scaling up the laboratory-scale  
 690 experimental results obtained. The assumptions listed in section 2.2.6 were used to compute  
 691 the energy generated and converting it to electricity, the electricity consumed, and the excess  
 692 electricity which can be used for other operations in the mill.

693 Table 4: Energy analysis case study on a medium-capacity plant

	<b>Mesophilic AD without Pretreatment</b> [78]	<b>Thermophilic AD without Pretreatment</b> [(Control:20S:80L) this study]	<b>Thermophilic AD with Pretreated 40S:60L</b> [this study]	<b>Thermophilic AD with Pretreated 50S:50L</b> [this study]
<b>Biogas produced/m<sup>3</sup>/d</b>	28.36	2.61x10 <sup>3</sup>	5.45x10 <sup>3</sup>	4.53x10 <sup>3</sup>
<b>Percentage CH<sub>4</sub> in biogas/%</b>	55.0	61.9	77.8	72.5
<b>CH<sub>4</sub> produced/m<sup>3</sup>/d</b>	15.6	1.62x10 <sup>3</sup>	4.24x10 <sup>3</sup>	3.29x10 <sup>3</sup>
<b>Energy produced from CH<sub>4</sub>/kJ/d</b>	5.78x10 <sup>5</sup>	5.98x10 <sup>7</sup>	1.57x10 <sup>8</sup>	1.22x10 <sup>8</sup>
<b>Electricity generated/kWh/d</b>	1.60x10 <sup>2</sup>	1.66x10 <sup>4</sup>	4.35x10 <sup>4</sup>	3.38x10 <sup>4</sup>

<b>Electricity consumed/kWh/d</b>	----	$1.61 \times 10^4$	$3.15 \times 10^4$	$3.15 \times 10^4$
<b>Extra Electricity generated/kWh/d</b>	----	$4.71 \times 10^2$	$1.21 \times 10^4$	$2.34 \times 10^3$
<b>Extra electricity generated compared to conventional process/ fold</b>	----	2.9	75.1	14.6

AD: anaerobic digestion

From Table 3, it can be seen that thermophilic anaerobic digestion can generate approximately three times more electricity than the current treatment process. While with the proposed treatment process, which consists of the integration of thermal pretreatment and with an optimal solid loading of 40S:60L, the total amount of extra electricity generated is 75-fold higher than that of the current treatment. On the other hand, the thermally pretreated 50S:50L solid loading generates 14.6 times more electricity than that of the existing treatment process. The high electricity generation is due to much higher biogas production and a higher methane purity, which is where the main difference lies in performance compared to the pretreated 50S:50L loading. When extrapolating to a plant scale, the results can be substantial in terms of electricity generated. The above results demonstrate that the integration of thermal pretreatment and dewatering can significantly enhance POME's treatment process, all while making it more sustainable.

Table 5: Electricity generation of the proposed treatment process compared to other pretreatment technologies

	<b>Thermally Pretreated 40S:60L</b> [this study]	<b>Advanced Oxidation (H<sub>2</sub>O<sub>2</sub>: dose 1%)</b> [79]	<b>Microwave Irradiation (2,450 MHz, 700W, 3 min)</b> [80]	<b>Ultrasonication (20Hz, 500W, 16.2 min, 0.88 W/mL, 30°C)</b> [81]
<b>CH<sub>4</sub> produced/m<sup>3</sup>/d</b>	$4.24 \times 10^3$	$1.12 \times 10^1$	$2.19 \times 10^3$	$3.93 \times 10^2$

<b>Energy produced from CH<sub>4</sub>/kJ/d</b>	1.57x10 <sup>8</sup>	4.14x10 <sup>5</sup>	8.12x10 <sup>7</sup>	1.45x10 <sup>7</sup>
<b>Electricity generated/kWh/d</b>	4.35x10 <sup>4</sup>	1.15x10 <sup>2</sup>	2.25x10 <sup>4</sup>	4.05x10 <sup>3</sup>

For consistent results, values from the literature were upscaled to a medium-capacity plant (567.4 m<sup>3</sup>/d)

The pretreatment technologies were chosen based on their applicability to the POME treatment process, that is, if they are feasible technically and economically, as well as how widely they have been researched. The advanced oxidation process has widely been studied for POME treatment, both as pretreatment and polishing treatment. Alternatively, Microwave irradiation and ultrasonication were deemed suitable pretreatment techniques for POME treatment as it does not compromise the treated effluent quality like chemical pretreatment does [4]. Table 4 shows the potential electricity that can be generated using the chosen techniques in a medium capacity plant. It can be noted that the least performant technique in terms of electricity generation is the advanced oxidation method, followed by ultrasonication and microwave irradiation systems. Our proposed treatment process is predicted to generate over 370 times more electricity than the advanced oxidation technique. Compared with the ultrasonication and microwave irradiation treatments, our proposed treatment process can produce 10 and 2 times more electricity than the ultrasonication and microwave irradiation methods, congruently.

Furthermore, excess biogas can be purified to be utilized off-site. The biogas can be converted to electricity to power residences in rural areas that are off the grid. This excess biogas can also be compressed in the form of Bio-compressed natural gas (CNG) to be used for domestic applications such as cooking and heating. Most households in Malaysia presently use liquefied natural gas (LNG) as their heat source for cooking. However, LNG is expensive due to the

1 730 many processes involved and the technology used for LNG processing. Meanwhile, for the  
2 731 cooking application, biogas in the form of CNG is good enough to handle the demand as a heat  
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4 732 source for cooking. Bio-CNG can be distributed by trucks to residential areas where the gas  
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7 733 can be delivered in storage cylinders. The Bio-CNG can also be transferred to the fuel stations  
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10 734 through existing pipelines [82].

## 11 12 13 735 4 Conclusion

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18 737 Thermal pretreatment and solid loading can significantly enhance the treatment process of  
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20 738 POME. From this study, it can be established that with the appropriate treatment conditions  
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23 739 (i.e., thermal pretreatment and optimum solid loading), a higher OLR does not affect the COD  
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25 740 removal efficiency nor the methane composition. It was observed that the optimal solid loading  
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28 741 was thermally pretreated 40S:60L, which corresponded to an OLR ranging from 11.55 to 16.05  
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30 742 gCOD/Ld. The latter achieved a daily biogas production volume of 48L along with a methane  
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33 743 purity of 77.2%. The COD, BOD, and TSS removal efficiencies of the thermally pretreated  
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35 744 40S:60L solid loading were 97.5, 95.1, and 94.5 %, respectively. The overall reduction in  
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38 745 removal efficiencies and biogas production for the treated 50S:50L solid loading is due to a  
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40 746 higher solids content, which tends to make the mass transfer and transport within the medium  
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43 747 less effective and subsequently reduces surface contact with the consortium of bacteria. With  
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45 748 such a high efficiency anaerobic digestion process, the amount of time in secondary treatment  
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48 749 (aeration treatment) to fully treat the effluent for it to meet the environmental standards is  
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50 750 substantially diminished; this is highly advantageous since the energy consumption in the  
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52 751 aeration process is also drastically reduced, and no further treatments (polishing treatments)  
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55 752 will be required.

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## 755 5 Acknowledgment

756

757 The authors acknowledge financial support from Monash University Malaysia, the Royal  
758 Academy of Engineering under the Newton Research Collaboration Programme (Project  
759 NRCP1516/4/34) and non-financial support in terms of resources for the microbial community  
760 testing from Key Project of Research and Development of Sichuan Province (No.  
761 2019YFS0480).

## 762 Nomenclature

763

764	ACD	Anaerobic Contact Digester
765	AD	Anaerobic Digestion
766	APHA	American Public Health Association
767	ASTM	American Society for Testing Material
768	BI	Biodegradability Index
769	BOD	Biological Oxygen Demand
770	CNG	Compressed Natural Gas
771	COD	Chemical Oxygen Demand
772	CPO	Crude Palm Oil
773	CSTR	Continuous Stirred Tank Reactor
774	HRT	Hydraulic Retention Time
775	L	Liquid (Clarified Liquid)
776	NCBI	National Center for Biotechnology Information
777	OLR	Organic Loading Rate
778	PCR	Polymerase Chain Reaction
779	POME	Palm Oil Mill Effluent
780	S	Solid (Settled Solids)
781	TOC	Total Organic Carbon
782	TSS	Total Suspended Solids
783	UASB	Upflow Anaerobic Sludge Blanket
784	UASB-HCPB	Upflow Anaerobic Sludge Blanket- Hollow Center Pack Bed
785	USS	Unacclimatised Seed Sludge
786	VFA	Volatile Fatty Acids

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Table 1: Dewatering device specifications and operating conditions

Diameter of the thickener/m	0.30
Height of the thickener /m	0.80
Settling Rate/ m/s	$5.81 \times 10^{-6}$
Operating Capacity/ L/d	20
Operating Temperature/ °C	65
Operating Flow rate/ m <sup>3</sup> /s	$2.31 \times 10^{-7}$
Solids Settling Flux, $q$ / kg/m <sup>2</sup> s	$2.36 \times 10^{-6}$
Suspension Settling Flux, $q_s$ / kg/m <sup>2</sup> s	$3.36 \times 10^{-6}$
Suspension density, $\rho_s$ / kg/m <sup>3</sup>	1100
Motor speed/ rpm	2-5

Table 2: Performance comparison between untreated and thermally pretreated POME

<b>Control Experiment untreated diluted POME (20S:80L) using the UASB-HCPB</b>						
	<b>Initial</b>	<b>Final</b>	-	-	-	-
COD/mg/L	20400±578	10700±651				
BOD/mg/L	11400±618	5800±687	-	-	-	-
TSS/mg/L	10300±636	6400±574	-	-	-	-
Daily Methane Yield/mLCH <sub>4</sub> /g COD <sub>removed</sub>		2.05± 0.86	-	-	-	-
	<b>Untreated POME</b>		<b>Thermally Pretreated POME</b>			
	<b>Initial Batch AD (100mL)</b>	<b>Final Batch AD (100mL)</b>	<b>Initial Batch AD (100mL)</b>	<b>Final Batch AD (100mL)</b>	<b>Initial using UASB-HCPB (5L)</b>	<b>Final using UASB-HCPB (5L)</b>
<b>40S:60L Experiments</b>						
COD/mg/L	47700±120	24400±537	40800±100	8155±44	23100±180	600±92
BOD/mg/L	20600±138	13700±345	20090±130	4015±67	18000±202	920±177
TSS/mg/L	16200±113	9800±366	16000±150	3162±65	10900±107	800±104
Daily Methane Yield/mLCH <sub>4</sub> /g COD <sub>removed</sub>		37.8± 5.2		52.4±3.7		80.0±1.5
<b>50S:50L Experiments</b>						
COD/mg/L	49500±210	33000±352	48500±200	16789±112	30200±247	2300±110
BOD/mg/L	22100±195	16800±287	21280±125	7932±131	17800±146	1400±82
TSS/mg/L	25800±115	18200±244	20800±135	6822±156	12000±132	1800±125
Daily Methane Yield/mLCH <sub>4</sub> /g COD <sub>removed</sub>		12.6±7.2		15.2±4.3		19.0±2.6

AD: anaerobic digestion

Table 3: Performance of the UASB-HCPB compared to other UASB bioreactors configurations

	Substrates	Temperature/°C	OLR/ (gCOD/Ld)	COD removal/ %	Methane/ %	HRT/ days	References
<b>UASB-HCPB</b>	POME	55	11.55-16.05	97.5	77.8	2.0	Current study
	POME	55	6.66	97.5	60.0	5.0	[37]
<b>UASB</b>	POME	35	4.5-12.5	94.9	48.2	4.0	[58]
	POME	37	2.2	96.0	65.0	10.4	[52]
	POME	55	2.9 (gVS/Ld)	95.5	66.0	5.0	[31]
	POME	55	5.8 (gVS/Ld)	92.5	61.0	5.0	[31]
	Monosodium glutamate wastewater	35	8.0	97.9	67.5	1.0	[41]
	Domestic sludge	35	0.213	86.0	70.0	2.5	[59]
	Vinasse	32	0.2-7.5	82.0	58.0	2.8	[60]
	Dairy wastewater	28-32	0.72-4.32	83.5	70.0	0.83	[61]

Table 4: Energy analysis case study on a medium-capacity plant

	<b>Mesophilic AD without Pretreatment [62]</b>	<b>Thermophilic AD without Pretreatment</b>	<b>Thermophilic AD with Pretreated 40S:60L</b>	<b>Thermophilic AD with Pretreated 50S:50L</b>
<b>Biogas produced/m<sup>3</sup>/d</b>	28.36	2.61x10 <sup>3</sup>	5.45x10 <sup>3</sup>	4.53x10 <sup>3</sup>
<b>Percentage CH<sub>4</sub> in biogas/%</b>	55.0	61.9	77.8	72.5
<b>CH<sub>4</sub> produced/m<sup>3</sup>/d</b>	15.6	1.62x10 <sup>3</sup>	4.24x10 <sup>3</sup>	3.29x10 <sup>3</sup>
<b>Energy produced from CH<sub>4</sub>/kJ/d</b>	5.78x10 <sup>5</sup>	5.98x10 <sup>7</sup>	1.57x10 <sup>8</sup>	1.22x10 <sup>8</sup>
<b>Electricity generated/kWh/d</b>	1.60x10 <sup>2</sup>	1.66x10 <sup>4</sup>	4.35x10 <sup>4</sup>	3.38x10 <sup>4</sup>
<b>Electricity consumed/kWh/d</b>	----	1.61x10 <sup>4</sup>	3.15x10 <sup>4</sup>	3.15x10 <sup>4</sup>
<b>Extra Electricity generated/kWh/d</b>	----	4.71x10 <sup>2</sup>	1.21x10 <sup>4</sup>	2.34x10 <sup>3</sup>
<b>Extra electricity generated compared to conventional process/ fold</b>	----	2.9	75.1	14.6

AD: anaerobic digestion

Table 5: Electricity generation of the proposed treatment process compared to other pretreatment technologies

	<b>Thermally Pretreated 40S:60L [this study]</b>	<b>Advanced Oxidation (H<sub>2</sub>O<sub>2</sub>: dose 1%) [77]</b>	<b>Microwave Irradiation (2,450 MHz, 700W, 3 min) [78]</b>	<b>Ultrasonication (20Hz, 500W, 16.2 min, 0.88 W/mL, 30°C) [79]</b>
<b>CH<sub>4</sub> produced/m<sup>3</sup>/d</b>	4.24x10 <sup>3</sup>	1.12x10 <sup>1</sup>	2.19x10 <sup>3</sup>	3.93x10 <sup>2</sup>
<b>Energy produced from CH<sub>4</sub>/kJ/d</b>	1.57x10 <sup>8</sup>	4.14x10 <sup>5</sup>	8.12x10 <sup>7</sup>	1.45x10 <sup>7</sup>
<b>Electricity generated/kWh/d</b>	4.35x10 <sup>4</sup>	1.15x10 <sup>2</sup>	2.25x10 <sup>4</sup>	4.05x10 <sup>3</sup>

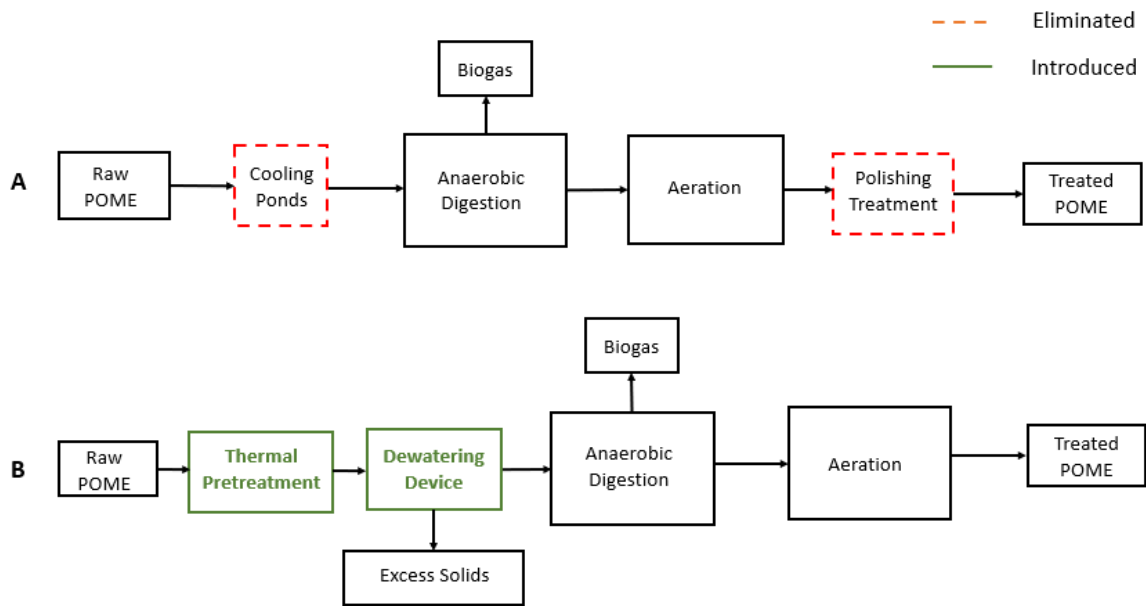


Figure 1: A- Existing POME treatment process, B- Proposed POME treatment process

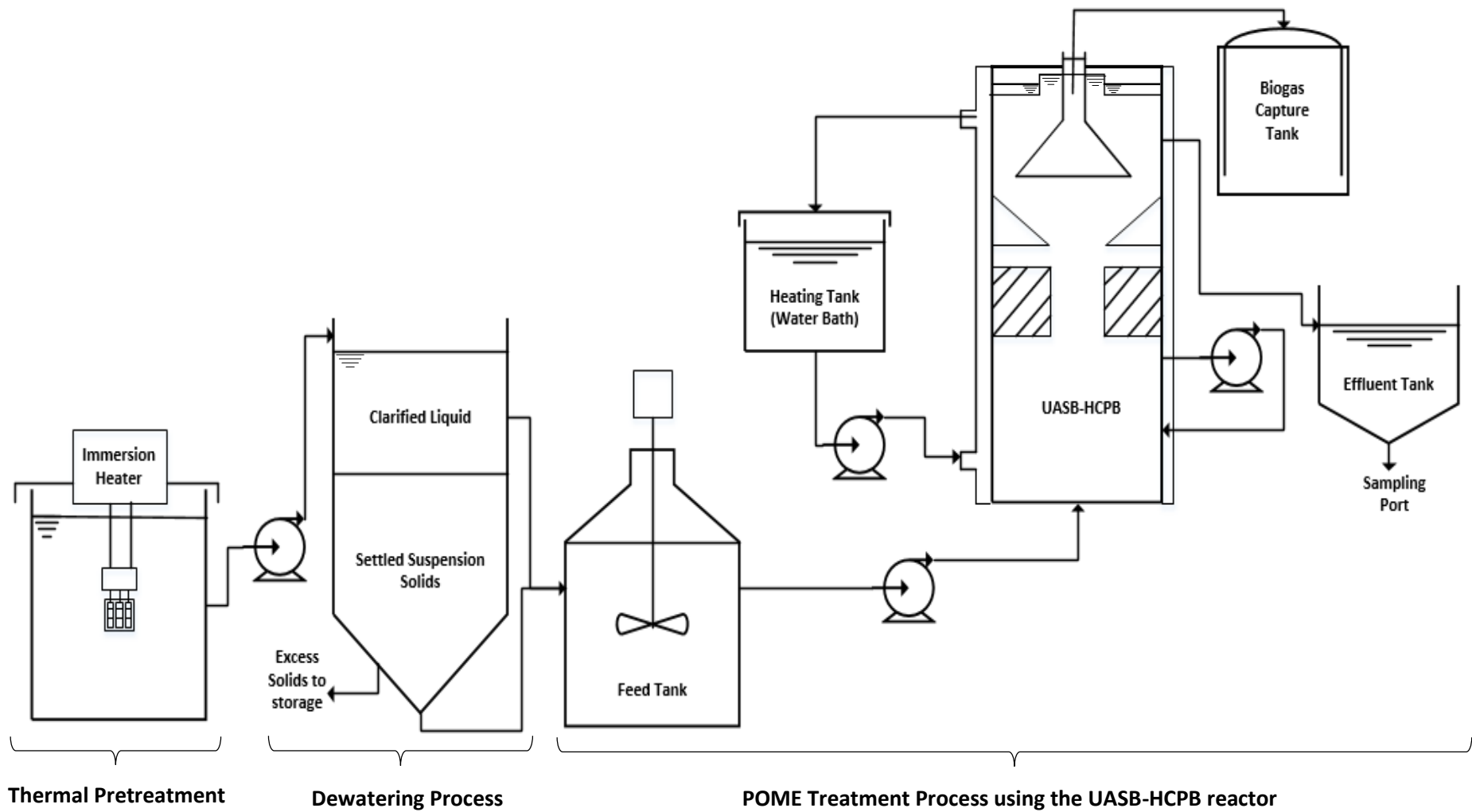


Figure 2: Schematic diagram of the novel POME treatment process

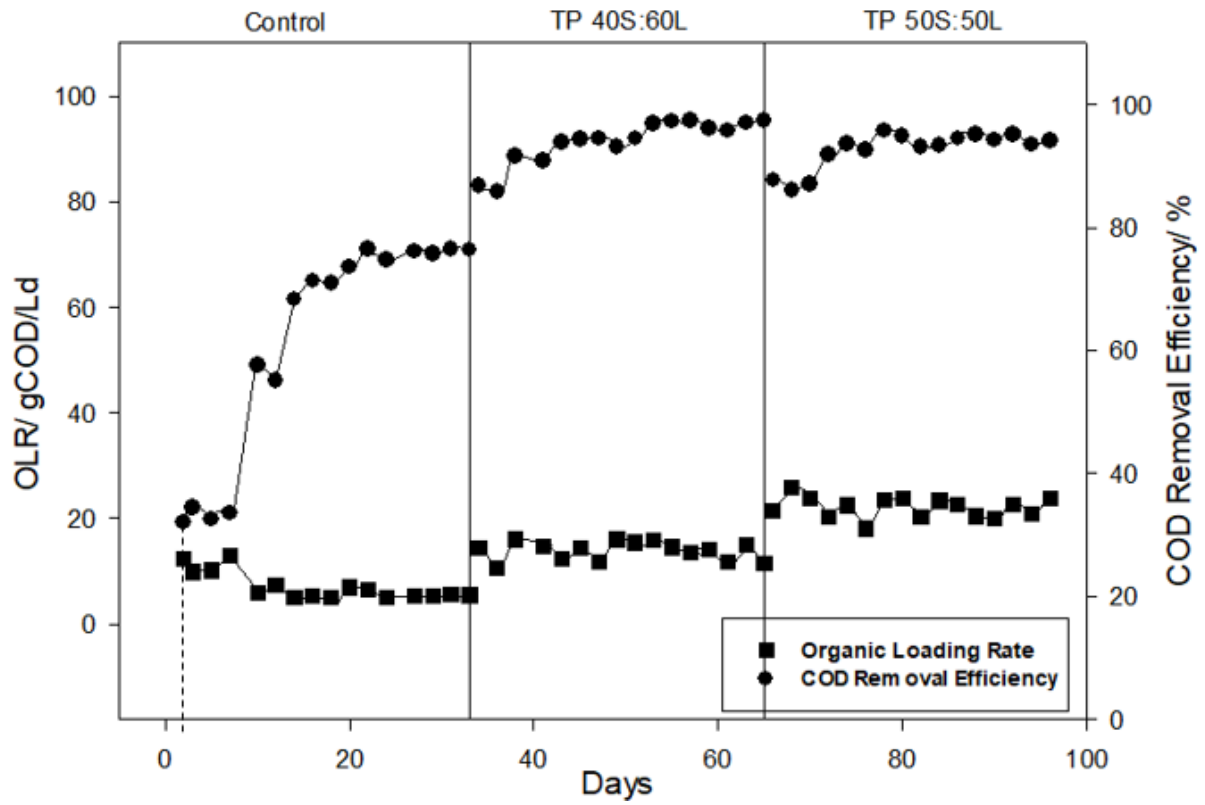


Figure 3: COD removal efficiency and the OLR (Control Experiment:(untreated POME), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

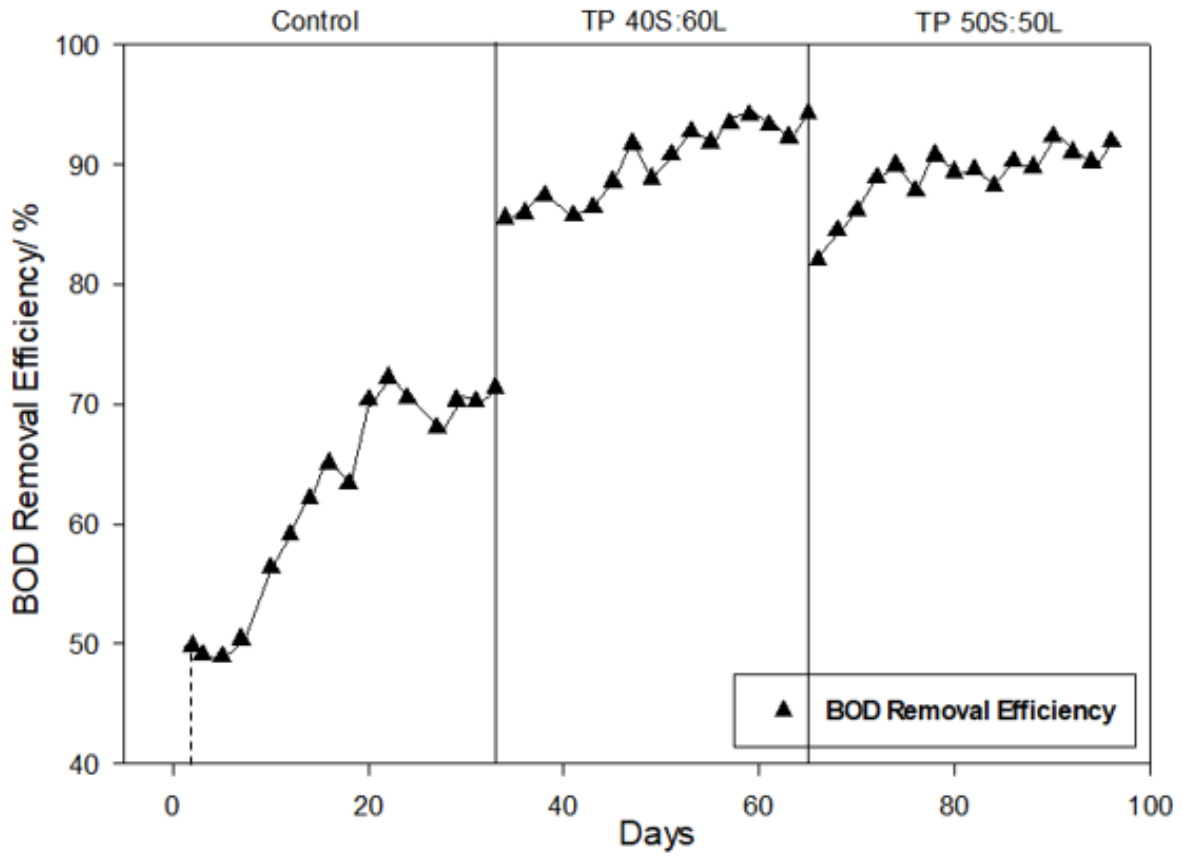


Figure 4: BOD removal efficiency (Control Experiment:(untreated POME), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

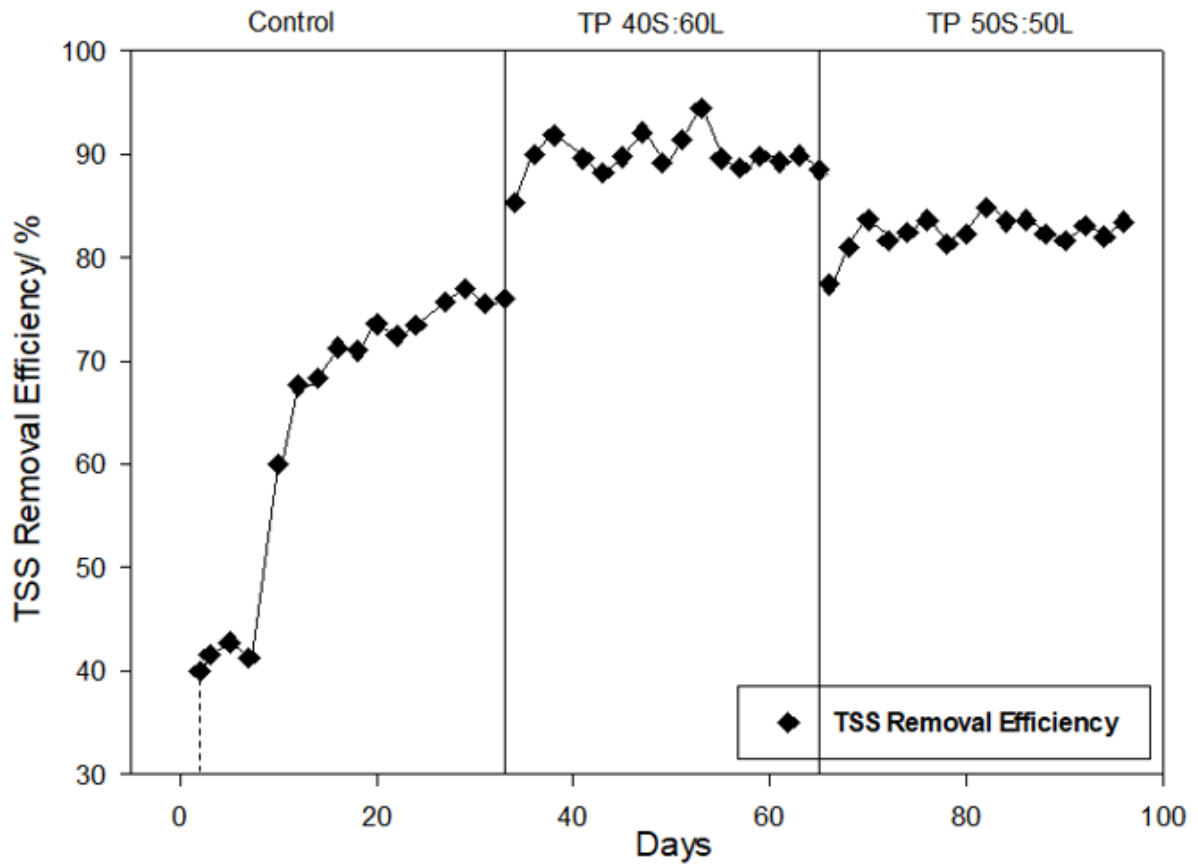


Figure 5: TSS removal efficiency (Control Experiment:(untreated POME), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

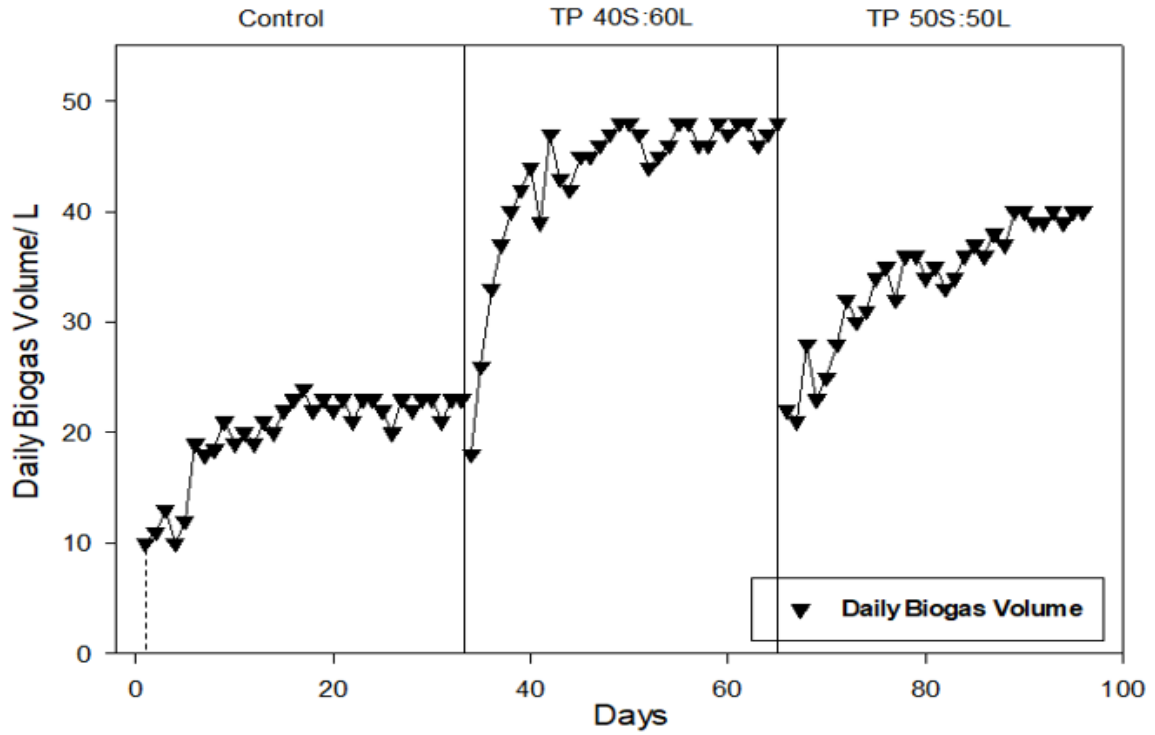


Figure 6: Daily volume of biogas produced (Control Experiment:(untreated POME), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

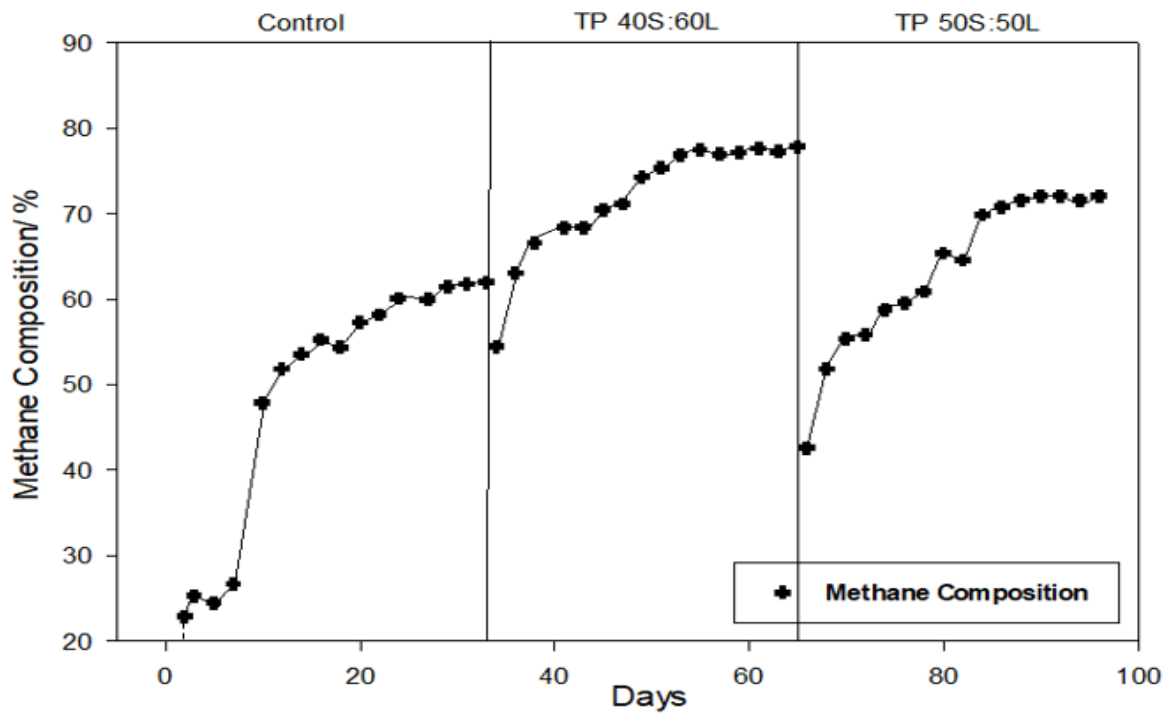


Figure 7: Methane composition in the biogas produced (Control Experiment:(untreated POME), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)

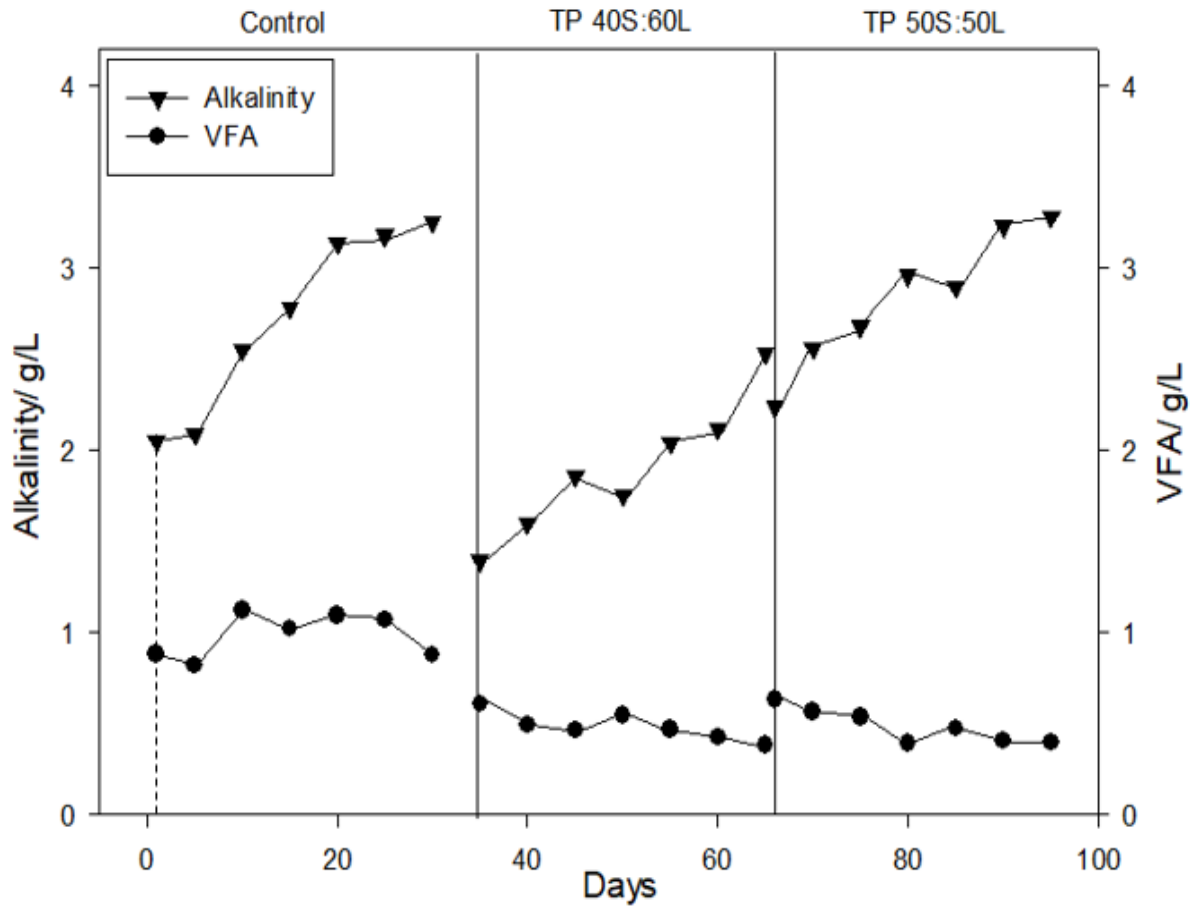
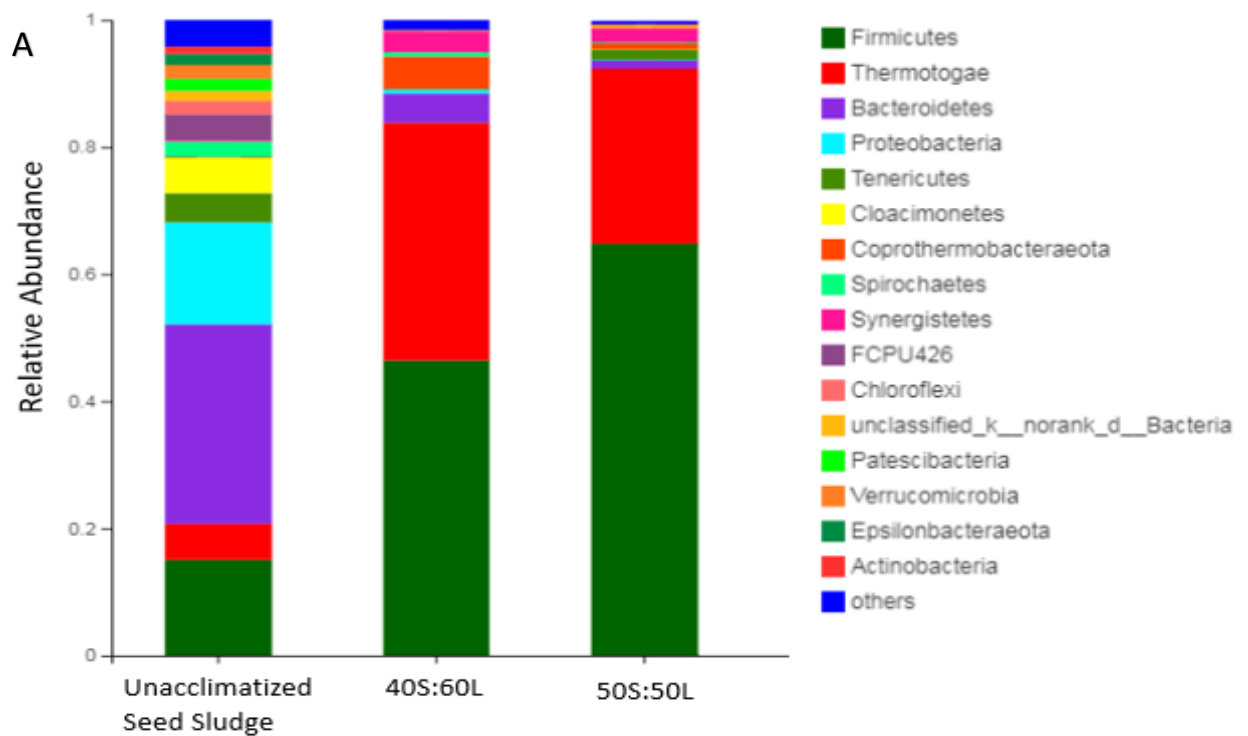


Figure 8: Alkanility and VFA concentrations measured (Control Experiment:(untreated POME), TP 40S:60L: Thermally Pretreated POME, TP 50S:50L: Thermally Pretreated POME)



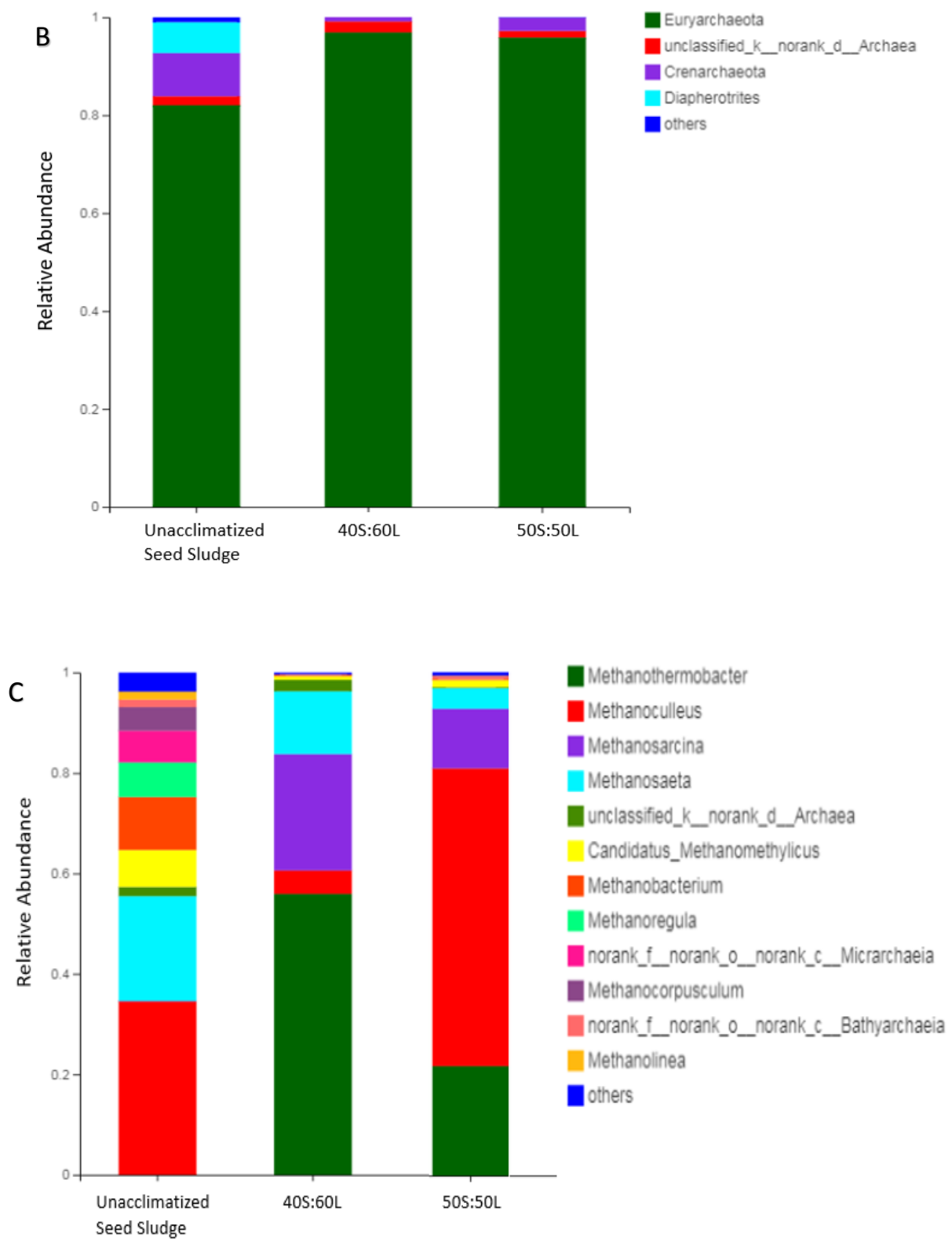


Figure 9: A-Taxonomic compositions of bacterial communities at the phylum level, B- Taxonomic compositions of archaeal communities at phylum level, C- Taxonomic compositions of archaeal communities at genus level.