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## Evaluating the Bio-Methane Potential by Anaerobic Tri-Digestion of Palm Oil Mill Effluent, Sewage Sludge and Food Waste in Malaysia

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### ABSTRACT

The ever-increasing organic waste in Malaysia is one of the major factors of the increase in Green House Gases (GHGs) emissions. Some of the organic wastes, however, can be utilized to produce biogas by anaerobic digestion (AD), which is a promising option for both energy and material recovery from organic wastes with high moisture content. This study was formulated to investigate the feasibility of tri-digestion of three of the major organic wastes which are generated in huge quantities in Malaysia such as Sewage Sludge (SS), Palm Oil Mill Effluent (POME), and Food Waste (FW). Tri-digestion on mixture of these organic wastes was examined to establish a stable and balanced microbial community, which may be lacking in mono-digestion of a single organic waste, to improve biogas production. Batch anaerobic digestion experiment of selected samples was conducted for 33 days under mesophilic condition. The Anaerobic tri-digestion was evaluated and compared with anaerobic mono-digestion for the same samples at different mixing ratios. The experiments were designed in two groups A and B, at food to micro-organisms (F/M) ratios of 1 and 5, respectively. From the results obtained, tri-digestion of the wastes at 80:10:10 (FW:POME:SS) proportion yielded the highest biogas production of 245.04 mL CH<sub>4</sub>/g-COD at F/M ratio of 1, which was greater than the methane production in mono-digestion of food waste at the same F/M ratio. In addition, tri-digestion showed better methane yield for all the samples at F/M= 1 compared to mono-digestion for an individual substrate. The results were significantly different at F/M=5 for POME and FW as the production of methane during the first half of the test period was not stable, compared to SS which showed consistency and stability at both F/M ratios. From the results obtained, it is evident that tri-digestion of FW, POME and SS is an attractive option to be explored for improving biogas production by AD in Malaysia due to the abundance of these three organic wastes and the mesophilic conditions naturally available.

#### Keywords:

Biogas; Anaerobic Tri-Digestion; Bio-methane Potential (BMP); Malaysia

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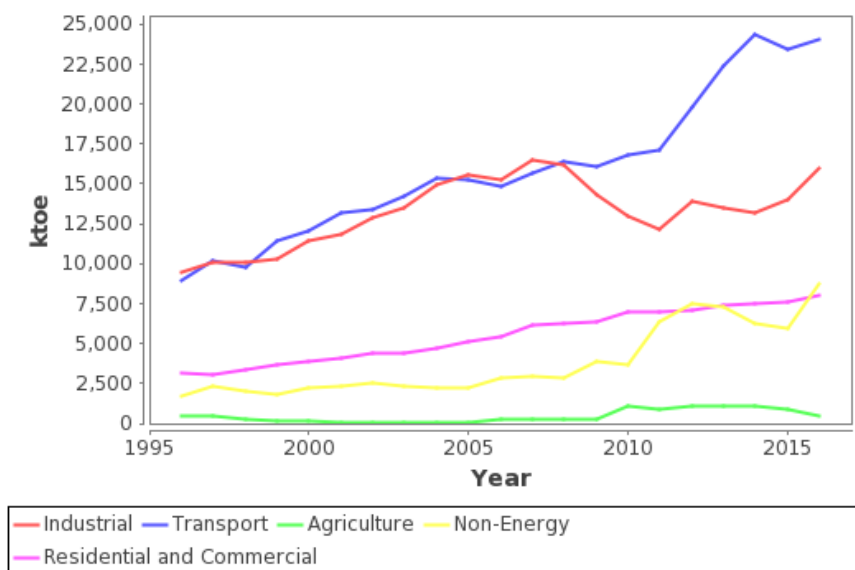
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## 1. Introduction

Solid waste management (SWM) is one of the major issues challenging development in South-east Asia including Malaysia, where the generation of municipal solid waste (MSW) is increasing at an alarming rate due to growing population and urbanization which are attributed to the industrialization of the country towards the attainment of status as developed country by 2020 [8]. Consequently, the demand for energy has dramatically increased in the last two decades where annual energy demand per capita was estimated at 2,603 kWh/person/year, 3,656 kWh/person/year and 4,549 kWh/person/year in 2000, 2010 and 2016, respectively [18]. Figure 1 illustrates the variations in total energy demand in different sectors in Malaysia for the duration of 1996–2016.

In line with the economic growth, the overall waste generation rate *per capita* increased from 0.5 kg/person/day in 1985 to 1.76 kg/person/day in 2012, and is projected to reach 2.23 kg/person/day by 2024 in large cities in Malaysia [35]. In addition, the waste composition changed and become more complex [20]. For example, the glass, plastic, and paper comprised about 34% of the waste generated *per capita* in 2005 compared to only 12% in 1975, while the percentage of food waste decreased [32]. A recent study showed that the MSW consisted of around 40% plastics waste, followed by food waste and papers at 38.2% and 21%, respectively [42]. This surpasses the capacity of the related authorities to properly manage the huge quantities of waste generated.

In order to meet the needs of energy demand and the utilization of the huge quantities of wastes generated, Malaysia needs to look for renewable resources especially from sources such as solar energy and the utilization of the huge amounts of organic waste to produce biogas.



**Fig. 1.** The variations in final energy demand in Malaysia for the duration of 1996–2016 [19]

In Malaysia, the utilization of biomass resources to reduce the dependency on fossil fuels is taking place especially in the oil palm industry, which generates the most abundant biomass waste in Malaysia with a high potential for bioenergy production [6]. In addition, a wide range of biomass resources, which are high in organic contents, including waste from poultry farms, swine farms, dairy farms and sewage treatment plants are generated daily in huge quantities. Biogas from these sources is a valuable option and alternative to replace fossil fuels in electricity generation [24].

Globally, biogas produced by AD is considered as one of the most preferred methods to treat organic wastes to enhance energy production. AD also supports establishment of a sustainable waste

management system. Research on biogas production from organic wastes is comprehensive, and biogasification technologies have been developed and adopted in many countries globally such as Poland [21], Italy [7], Norway [39] in Europe, the US [37] and Canada [40], Malaysia [1, 10] and Thailand [38].

AD of single waste stream is not very effective and unstable conditions can result due to accumulation of volatile fatty acids (VFA) [13] depending mainly on the characteristics of substrates. Moreover, organic waste such as sewage sludge has relatively low C/N ratio ranging from 6:1 to 13:1, and if digested alone, inhibition by ammonia may occur, which can result in process failure [30]. Thus, the combination of different feedstocks with different C/N ratios, such as one with C/N=10 and another with C/N=30, is desired to attain better overall C/N ratio, which is necessary to overcome the drawbacks of mono-digestion, and to improve biogas production [30]. In addition to the balance in C/N ratio, mixing of different feedstocks can supply additional essential micro-nutrients, enable increased organic loading rate and improve process stability [22,27], which ultimately improves biogas yield due to positive synergistic effects of the mixed co-substrates and diluting toxic compounds associated with a particular substrate (Brown and Li 2013, Álvarez, Otero, and Lema 2010, Lo et al. 2010). Despite the attractive attempts to optimize anaerobic co-digestion (AcoD), several technological challenges associated with its implementation still persist.

AcoD of various organic substrates has been widely examined to optimize biogas production and total solids reduction [25, 28]. Al Mamun and Torii [2] examined anaerobic tri-digestion of fruit, cafeteria and vegetable wastes at four mixing ratios. Wickham *et al.*, [41] studied the co-digestion of sewage sludge and organic waste at different mixing ratios. Habiba *et al.*, [15] reported that co-digestion of fruit and vegetable wastes with activated sludge improved the efficiency compared with the mono-digestion of activated sludge. Tri- or multi-digestion will bring additional benefits to AD as it has economic value by minimizing equipment needs and maximizing organic loading capacity to co-digest multiple substrates [15, 29, 34]. Thus, the economic viability of co-digestion can be significantly enhanced, allowing the diversion of agro-industrial wastes from disposal by landfill, which leads to decreasing GHGs emissions while recovering biogas as energy source [17], achieving sustainable waste management practices [11, 23].

By considering present situation of AD of organic wastes in Malaysia, there is lack of studies which consider variations in F/M ratio in mono-digestion and co-digestion of POME, SS, and FW. To this regard, the major novelty of this study, was the anaerobic tri-digestion with three substrates. The combination of these three wastes has not been studied before in anaerobic digestion. The evaluation of such anaerobic digestion system is important, considering that it would contribute to the knowledge, management, and utilization of these waste materials. Hence, this study has been designed to determine the individual Bio-methane Potential (BMP) of three different organic wastes, which are generated in large quantities in Malaysia; namely Food Waste (FW), Palm Oil Mill Effluent (POME) and Sewage Sludge (SS). The main aim of the study was to investigate the BMP of the selected substrates under mono and tri-digestion conditions at two different F/M ratios. To provide proof of concept and identify FW: POME: SS ratios that are promising for further analysis, several biochemical parameters were measured for the purpose of experimental design and set-up. The rationale is to maximize biogas production by mixing different substrates. It seeks to convey sufficient technical details to make results fruitful for further studies.

## 2. Methodology

### 2.1 Inoculum and Substrates Collection and Preparation

FW was collected from the food canteen of Malaysia-Japan International Institute of Technology (MJIT), University Teknologi Malaysia - Kuala Lumpur Campus. FW was sampled according to the method by la Cour Jansen *et al.*, [16]. SS was collected as thickened secondary sewage sludge from the gravity settling tank from Bunus STP Indah Water Konsortium in Kuala Lumpur. Raw POME was collected from Seri Ulu Langat Palm Oil Mill Sdn Bhd processing plant in Dengkil, Selangor. All the samples were kept in plastic containers while being transferred to the lab. Before storing them in refrigerator at 4°C, the samples were allowed to cool down to room temperature, and analysis for chemical characteristics was conducted.

Inoculum was collected from a mesophilic anaerobic digester operating at 30-31°C, and treating a mixture of primary and secondary sewage sludge in Bunus STP Indah Water Konsortium in Kuala Lumpur. The inoculum was transferred and kept at 31°C in a general incubator for degassing for 5 days to avoid Volatile Solids (VS) influence on the BMP tests. Inoculum was then sieved through a 2 mm sieve to remove any large particles before transferring it to serum bottles for BMP assays.

### 2.2 Analytical Methods

All analytical tests were performed in triplicates. The parameters determined in this test were Total Chemical Oxygen Demand (T-COD) and Soluble Chemical Oxygen Demand (S-COD) which were determined by Hach Method (Reactor Digestion Method No.: 8000, Hach, USA) using HR (20-1,500mg/L) and HR+ (200-15,000 mg/L) vials. The readings for T-COD and S-COD were taken using DR6000 Spectrometer (Hach USA). Biochemical Oxygen Demand (BOD<sub>5</sub>) was determined according to Standard Methods [5] and the initial and final readings were measured using a DO meter (Model 5000, YSI, USA). pH was determined by an ion meter (MM374, Hach, USA). Total Solids (TS) and Volatile Solids (VS) were determined according to Standard Methods [5]. Oil and Grease (O and G) was measured by extraction method using SPE-DEX 4790 Extractor System (Horizon Technology, USA). Ammonia nitrogen (NH<sub>3</sub>-N) was determined by High Range Ammonia (0-50 mg/L N) reagents and DR6000 (Hach, USA). Phosphorous was determined by High Range Total Phosphate (0-100 mg/L PO<sub>4</sub><sup>3-</sup>) reagents (Hach, USA).

### 2.3 Experimental Set-up

#### 2.3.1 Batch assays

The samples were prepared in two groups, A and B, with different substrates mixing ratios. Two F/M ratios of 1 and 5 were used for group A and B, respectively. Each group consisted of six samples. For each sample and the inoculum (blank control), the test was run in triplicates in 250 mL serum bottles. Pre-determined amounts of substrates (as shown in Table 1) were added to each of the bottles pre-filled with 100 mL of inoculum to obtain an initial organic loading of 383.31 mg-COD/L and 1916.54 mg-COD/L for groups A and B, respectively, and a final volume of 200 mL as shown in Table 2. In the control bottles, distilled water was added to the inoculum to observe the endogenous methane production of the inoculum. It was assumed that sufficient micro-nutrients were present in the substrates, therefore no micro-nutrients nor pH buffer were added to the samples. All bottles were flushed with nitrogen at 0.2 ml/min for 4 minutes before capping them with butyl rubber septa and sealing them with aluminum caps to maintain anaerobic conditions. Bottles were manually

stirred every day during the BMP test period. The bottles were kept in a general incubator (HI-162, China) at  $37 \pm 1$  °C.

In Table 1, characteristics of the substrates and the starting mixtures for each experimental ratio are shown. Although the initial pH of POME and FW alone were lower than 5 as shown in Table 3, the pH for the combined mixtures for all samples exceeded 7, except the mixtures for samples A5 and B5 where 80% (COD) of their substrates consisted of POME which has low pH, but they were still within the recommended range for AD [31].

**Table 1**

Index for substrates ratios for blank, mono-digestion and tri-digestion samples

Digestion Type	Substrate Composition (% COD)	Group A		Group B	
		Sample	pH	Sample	pH
Blank	Inoculum (100%)	Blank	7.42	Blank	7.42
Mono-digestion	FW (100%)	A1	7.38	B1	7.25
	POME (100%)	A2	7.21	B2	7.10
	SS (100%)	A3	7.38	B3	7.25
Tri-digestion	FW-POME-SS (10%-10%-80%)	A4	7.35	B4	7.15
	FW-POME-SS (10%-80%-10%)	A5	6.95	B5	6.80
	FW-POME-SS (80%-10%-10%)	A6	7.17	B6	7.11

**Table 2**

Index for substrates ratio for blank, mono-digestion and tri-digestion samples

Parameter	Unit	Value	
		Series A	Series B
F/M Ratio	mg COD/mg VSS	1.00	5.00
T-COD (substrate)	mg/L	2556.67	12783.33
Total Mixture Volume	mL	200.00	200.00
Inoculum VSS	mg/L	2557.00	2557.00

## 2.4 BMP Data Harvesting and Evaluation

Biogas production was measured as pressure built up in bottle headspace, using digital differential pressure gauge (SIKA, M.C., Germany). Biogas composition was analyzed using Micro GC-TCD (Agilent Technologies, USA) with a carrier gas of Nitrogen and Argon. Figure 2 shows the apparatus set-up for biogas analysis. The bottles were put in water bath at  $37 \pm 1$  °C while taking pressure readings and gas phase analysis by the micro-GC to keep the temperature of bottles unchanged.

Biogas volume and methane volume were calculated based on standards temperature and pressure conditions (STP; 101.3 kPa and 0 °C) using Eq. (1), (2) and (3).

$$P_T = P_i + P_{atm} \quad (1)$$

$$V_B = \left( \frac{P_T}{P_{atm}} \times V_H \right) - V_H \quad (2)$$

where,  $P_T$  is the total pressure,  $P_i$  is the pressure measured in the bottle head space,  $P_{atm}$  is the actual atmospheric pressure (all in kPa),  $V_B$  is the volume total biogas produced and  $V_H$  is the volume of the bottle head space (all in mL).

The methane yield in ( $L \cdot g^{-1} \cdot COD_{added}$ ) was calculated as volume of methane produced per g of

COD loaded into the bottles, and methane yield of the negative control was subtracted [4, 9, 36] according to Eq. (3) and (4) below.

$$Y_{NC} = \frac{CH_4\% \times V_C}{COD_{added}} \quad (3)$$

$$Y = \frac{CH_4\% \times V_B}{COD_{added}} - Y_{NC} \quad (4)$$

where,  $Y_{NC}$  is the methane yield from the biogas produced from control samples,  $Y$  is the methane yield ( $L \cdot g^{-1} \cdot COD_{added}$ ),  $CH_4\%$  is headspace methane concentration in percentage in gas phase of serum bottle,  $V_C$  is the gas volume of the control sample,  $COD_{added}$  is the initial mass of COD added to the bottle and  $V_{NC}$  is the methane yield of negative control ( $L \cdot g^{-1} \cdot COD_{added}$ ). Assuming the amount of solubility of methane is negligible at  $37^\circ C$ . The experiment was terminated after 33 days of biogas production when less than 5 mL of the total  $CH_4$  was produced over a day.

For statistical significance, average readings from triplicate values were taken as the results. The standard error bars were plotted based on results from triplicates using Microsoft Excel.

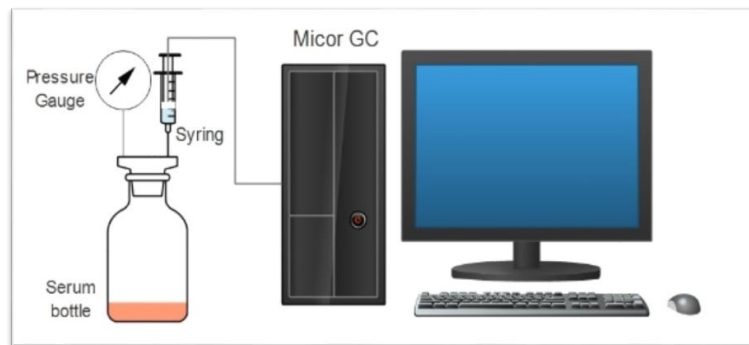


Fig. 2. Experimental setup during gas analysis

### 3. Results

#### 3.1 Characteristics of Substrates and Inoculum

The characteristics of substrates and inoculum are shown in Table 3. Based on the analysis, FW has higher TS and VS compared to POME and SS. In addition, FW and POME showed higher values for BOD compared with SS which has lower BOD value compared to its T-COD value. This indicates that FW and POME have higher biodegradability than SS. Table 4 shows the C/N ratios for the substrates. The C/N ratio of POME was determined to be 22.22, while for FW and SS were 9.58 and 5.58, respectively. The variations between the C/N ratios for the three substrates provides a good mix when co-digesting these wastes to enhance the anaerobic microorganism community. Low C/N ratios increase methane production [30], while high ratios may cause a decline in the energy and structural metabolism of the microorganisms [12].

**Table 3**  
 Physiochemical Characteristics of substrates

Parameter	Unit	Inoculum	POME	FW	SS
pH	-	7.62	4.71	4.33	6.11
Biochemical Oxygen Demand (BOD)	mg/L	-	38409.29	274274.91	2216.85
Total Chemical Oxygen Demand (T-COD)	mg/L	-	109633.30	444555.00	17333.33
Soluble Chemical Oxygen Demand (S-COD)	mg/L	-	51533.33	61300	3133.333
Nitrogen Ammonia (N-NH <sub>3</sub> )	mg/L	-	53.33	166.6667	113.33
Phosphorus (P)	mg/L	-	686.44	10272.15	148.92
Total Solids (TS)	mg/L	4420.00	84626.67	419513.3	12820.00
Volatile Solids (VS)	mg/L	3675.00	66565.00	80253.33	10103.33
Oil and Grease	mg/L	-	3370.00	48400.00	900.00

**Table 4**  
 CHNO analysis for POME, Sewage Sludge, and Food Waste

Sample	C %	H %	N %	O %	C/N
POME (Palm Oil Mill Eff.)	39.43	8.23	1.57	37.54	22.22
SS (Sewage Sludge)	31.73	7.73	5.69	28.69	5.58
FW (Food Waste)	44.73	6.16	4.67	28.61	9.58

### 3.2 Methane Yield

#### 3.2.1 Mono-digestion

The mono-digestion of substrates was carried out to investigate the BMP of FW, POME, and SS at different F/M ratios (1 and 5). The cumulative and the daily biogas yields during the anaerobic mono-digestion are shown in Figure 3(a)-(d). The tests were run for 33 days until little or no biogas production was observed. The results presented are the average net methane yield from the

As shown in Figure 3(a) and Figure 4(c), the highest methane production was from FW at 245.04 mL CH<sub>4</sub>/g-COD, and 265.18 mL CH<sub>4</sub>/g-COD at F/M ratios 1 and 5, respectively. Of the three substrates tested, FW produced the highest amount of methane. It is observed that, although the organic loading for group B substrates for FW was 5 times higher than the organic loading of group A, the increase in methane production over the same period was very little. It is shown by Figure 3(a)-(d) that for group A substrates, the methane production was high in the first few days, before starting decreasing as organic matter degraded with time. Therefore, when viewing the methane production profile in an accumulative curve, it shows normal biogas production trends. By comparing this trend with the substrates from group B, in the first days FW produced less methane, and methane production was not stable and kept fluctuating till the middle of the test period. At that point, enough TS had been destroyed; hence a convenient environment for anaerobic digestion methanogenesis bacteria to produce methane. Thus, methane production by FW reached its peak after 23 days instead of the beginning of the test. This behavior is due to the higher organic loading for group B samples.

POME followed a similar trend to that of FW for methane production. The difference is that FW was more biodegradable than POME, thus the hydrolysis stage proceeded faster than POME. It is obvious from the daily production curves that methane production from POME at F/M ratio of 5 was not stable in the first half of the total test period. Until sufficient destruction for its organic content

was achieved, the methane production again reached a peak on day 14, followed by a gradual decrease till no more methane was produced. The production of methane from POME could be enhanced by enzymatic pretreatment to improve the hydrolysis by converting complex substances in the POME into monomers towards better biogas production [43].

Differently, the methane production from SS was the lowest and the most stable compared to the trends followed by FW and POME. Methane production from SS reached its peak on the third day for both samples in in both groups A and B, and then the production decreased as the test proceeded. Since SS substrates have the lowest biodegradability compared to FW and POME, methane yield was the lowest at 110.81 mL CH<sub>4</sub>/g-COD, and 96.79 mL CH<sub>4</sub>/g-COD at F/M ratios 1 and 5, respectively. As can be observed from Figure 3(d) for the daily methane production from SS at F/M ratio of 5, the production was higher than that of POME and FW in the first few days, and this shows that SS is less sensitive to overloading compared to FW and POME.

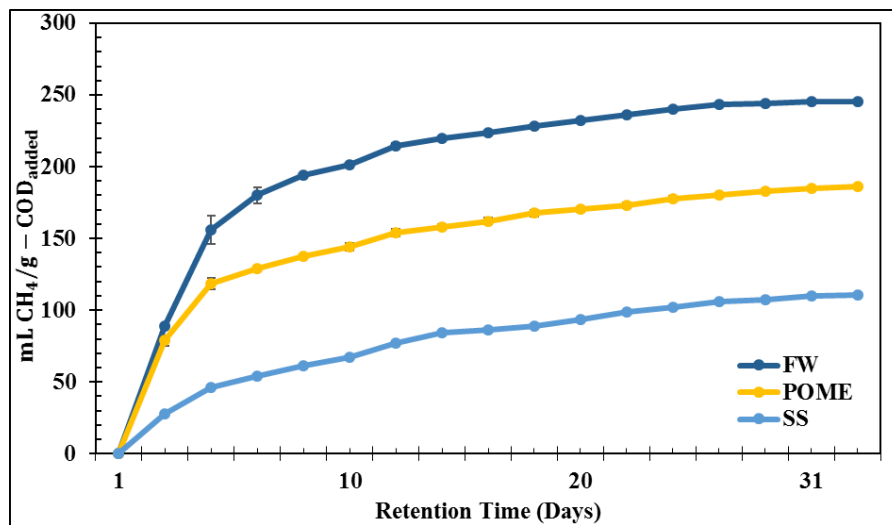


Fig. 3. (a) Cumulative methane production for mono-digestion at F/M =1

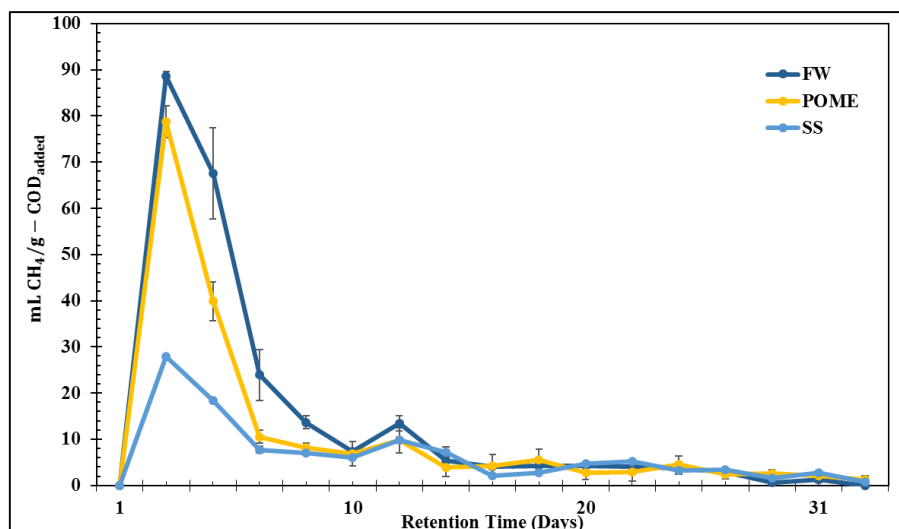


Fig. 3. (b) Daily methane production for mono-digestion at F/M =1



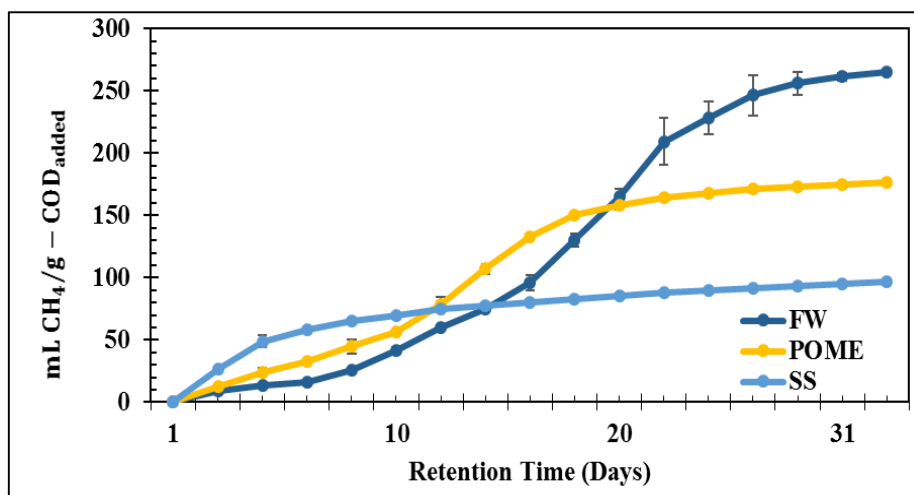


Fig. 3. (c) Cumulative methane production for mono-digestion at F/M =5

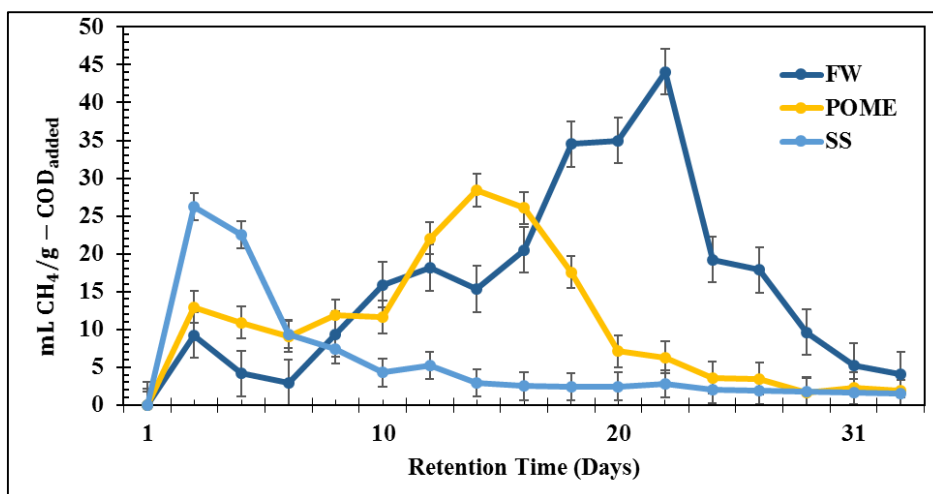


Fig. 3. (d) Daily methane production for mono-digestion at F/M =5

### 3.2.2 Tri-digestion

Figure 4(a)-(d) shows the accumulative and daily production of methane by the anaerobic tri-digestion of POME, FW, and SS. From the samples tested, sample A4 produced the highest methane yield compared to other substrates compositions and F/M ratios. Although, 80% (COD) of the sample consisted of FW, the trend of methane production in tri-digestion did not agree with the one observed by mono-digestion. In mono-digestion, methane yield from FW with high F/M ratio was higher than that of the lower ratio. But in tri-digestion, co-digesting FW with other co-substrates resulted in better yields in both scenarios (F/M ratios).

#### 3.2.2.1 Food waste as a main substrate

In tri-digestion, when digesting FW with other co-substrates, the methane yield was 245.77 mL CH<sub>4</sub>/g-COD at F/M=1 compared to 226.91 mL CH<sub>4</sub>/g-COD produced by the same substrate at F/M=5. By comparing between mono-digestion and Tri-digestion of FW, in mono-digestion FW produced methane yield of 245.04 mL CH<sub>4</sub>/g-COD at F/M=1 compared to 265.19 mL CH<sub>4</sub>/g-COD produced by the same substrate at F/M=5. The results for FW were mixed, and did not follow a specific trend, but the observable point is that tri-digestion at lower F/M ratio showed little

improvement. Although this improvement is very small, it tells that digesting FW with other co-substrates is a better option than mono-digestion. This is because mixing substrates with different C/N ratios enhances the microorganism's community, thus providing nutrients which would lack in mono-digestion otherwise. Optimum C/N ratios stabilize food waste conversion pathways through anaerobic digestion and composting [44].

### 3.2.2.2 POME as a main substrate

When POME was mixed with other co-substrates, the methane yield was 186.03 mL CH<sub>4</sub>/g-COD compared to 178.43 mL CH<sub>4</sub>/g-COD from mono-digestion at F/M=1. This result agrees with the trend observed in Tri-digestion of FW with other co-substrates at F/M=1. The results show improvement for methane yield at low F/M ratio in tri-digestion compared to mono-digestion. Again, at higher F/M ratio, the results were mixed. The trends in the daily methane production was fluctuating during the first half of the test period. Until enough destruction for substrates was achieved, the daily production reached a peak and then started decreasing gradually. This is due to the overloading caused by high F/M ratio. Figure 4(c) and 4(d) shows similar methane production trends for POME and FW at high F/M ratios. Both substrates under both conditions (mono-digestion and tri-digestion) produced less methane in the first half of the total test period, before reaching a peak in the second half of the test period. This is due to inhibition by high loading rates.

### 3.2.2.3 Sewage sludge as a main substrate

For SS, the trend in both mono-digestion and tri-digestion was similar. Methane yield decreased with increasing F/M ratio, and the co-digestion of SS with other substrates improved the methane yield compared with mono-digestion. This agrees with the results from tri-digestion of POME and FW as main substrates. In all samples observed, the tri-digestion showed better results than mono-digestion. The overall results for methane production from SS was consistent at both F/M ratios. Methane yield improved from 110.81 mL CH<sub>4</sub>/g-COD in mono-digestion to 132.87 mL in tri-digestion at F/M=1, and increased from 96.79 mL CH<sub>4</sub>/g-COD in mono-digestion to 118.98 mL CH<sub>4</sub>/g-COD in tri-digestion at F/M=5. One distinct trend that occurred in SS but did not occur in FW and POME was its stability during the BMP test. Although FW and POME were mixed as co-substrates with SS, which are known from the results to have unstable methane production trend in the first half of the test period, methane production from SS remained stable throughout the production period. When three substrates were mixed, a more balanced C/N ratio was achieved and the methane production improved more by adding these co-substrates at a ratio of FW: POME: SS (10:10:80).

Overall, anaerobic tri-digestion improved the methane production rate for all samples, with all samples having a single peak and the methane production stabilized within 30 days of digestion. When substrates were digested at high F/M ratio, the lag phase observed was higher than the lag phase for each substrate at lower F/M ratio.

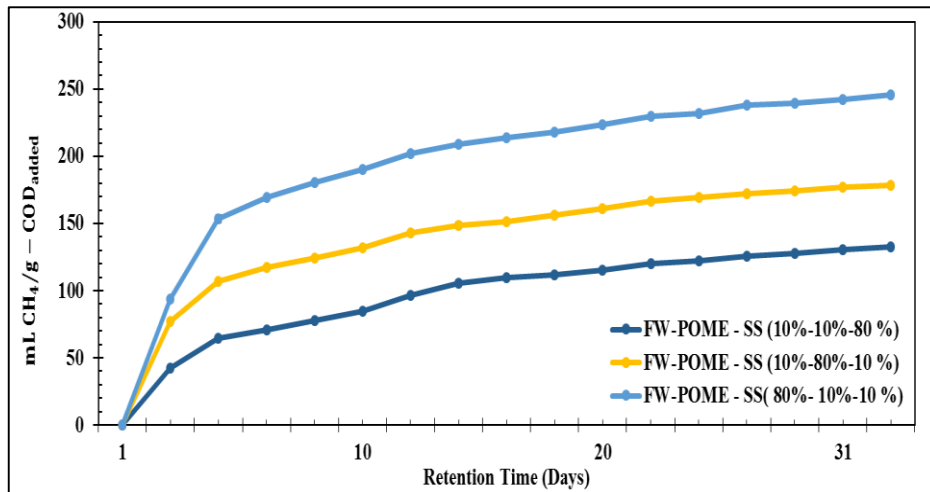


Fig. 4. (a) Cumulative methane production for tri-digestion at F/M =1

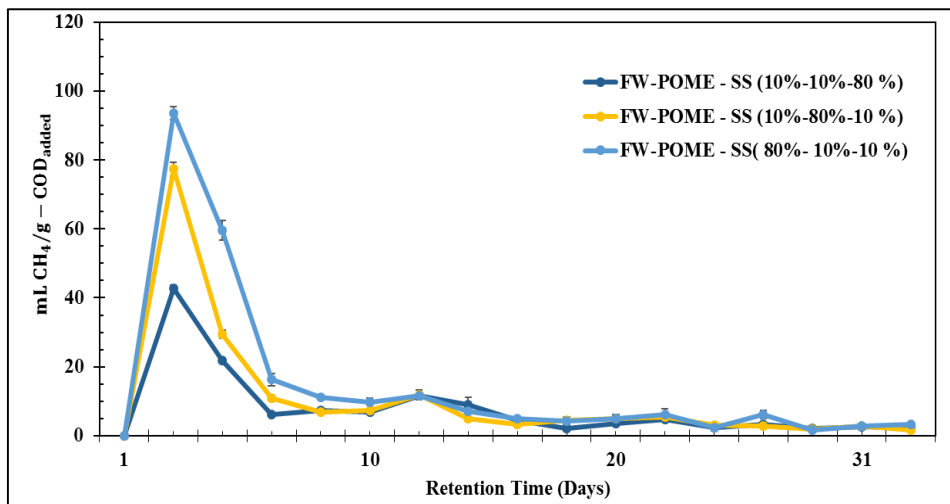


Fig. 4. (b) Daily methane production for tri-digestion at F/M =1

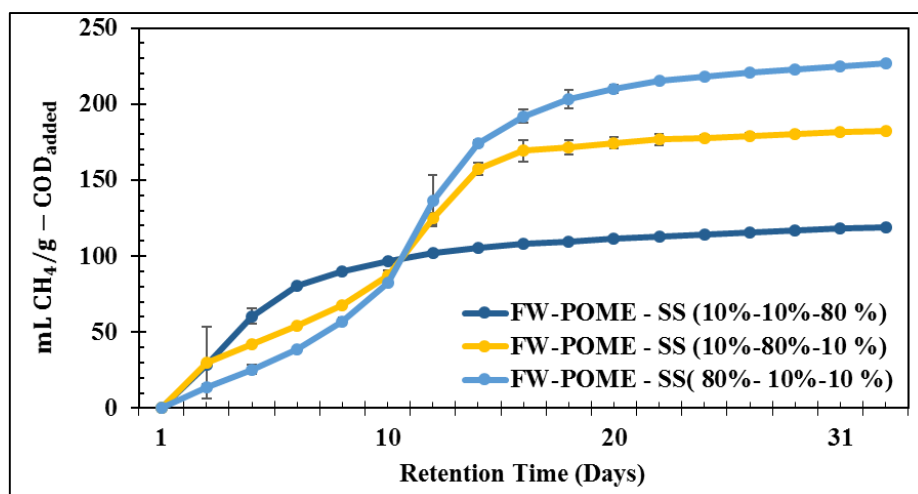


Fig. 4. (c) Cumulative methane production for tri-digestion at F/M =5

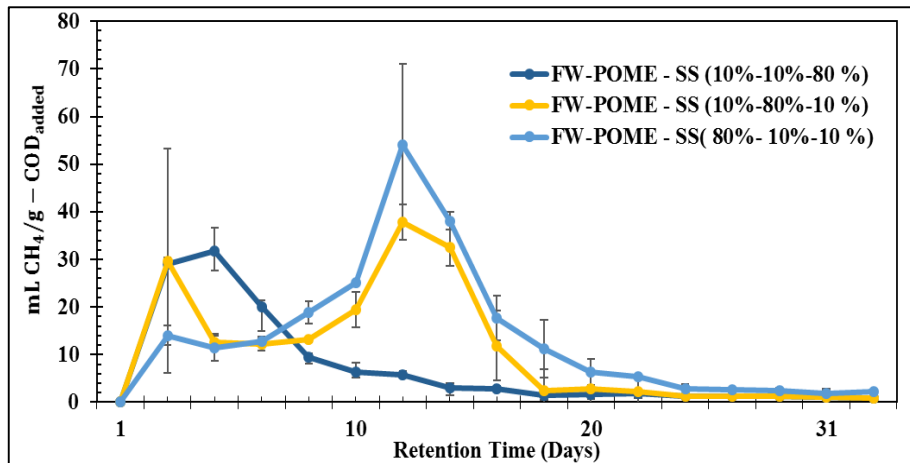


Fig. 4. (d) Daily methane production for tri-digestion at F/M =5

### 3.3 Effect of F/M Ratio on Methane Yield

The effect of increasing the F/M ratio was obvious on samples of both groups A and B. Increasing the F/M ratio by 5 times decreased methane production compared with the production at F/M=1. This is attributed to the inhibition of the anaerobic system because of organic overloading. This high organic loading resulted in excessive production and thus a build-up of volatile fatty acids [33]. For FW, it was observed that methane yields from substrates with higher F/M ratio was higher compared to the substrates with lower F/M ratio in terms of the methane volume. Although the difference in ratio was significant between the substrates for FW from group A and group B, the methane yield for the substrate with higher F/M ratio was only greater by about 20 mL CH<sub>4</sub>/g-COD.

When comparing methane yield of all three substrates by mono-digestion at F/M=1, FW has better methane yield, and this is due to the higher biodegradability of FW compared to POME and SS, which enabled its substrates to produce more methane. Similarly, at F/M=2, FW produced more methane than POME and SS. Although, it was not stable in the first half or the test period, it reached a peak in the second half and became more stable.

It is observed that SS and POME had different trends than FW when increasing their F/M to 5. It was observed that methane yield decreased as organic load increased. This is because archaea were unable to degrade high quantities of food, which led to inhibition by higher organic loads. As depicted in Figure 3(a)-(d), it was observed that the mono-digestion of POME at F/M=1 produced 186.03 mL CH<sub>4</sub>/g-COD compared to 176.77 mL CH<sub>4</sub>/g-COD produced by the same substrate at F/M=5. Similarly, 110.81 mL CH<sub>4</sub>/g-COD was produced by SS at F/M=1 compared to the production of 96.79 mL CH<sub>4</sub>/g-COD by the same substrate at F/M=5.

In tri-digestion, a similar trend was observed. By increasing F/M ratio, methane production decreased in all samples except in B1 and B4 where the quantity of FW dominated in the substrate. This is attributed to the higher biodegradability of FW compared to other substrates.

## 4. Conclusions

The study aimed at investigating the BMP of POME, FW and SS under anaerobic mono- and tri-digestion conditions at two different F/M ratios. POME, FW and SS have variations in their biodegradability physiochemical characteristics which proved to enhance methane yields when substrates are mixed in anaerobic digestion. Anaerobic tri-digestion of organic wastes is beneficial not only for improving process performance but also enhancing the methane as it balances the C/N

ratio in the reactors. The optimum condition for the anaerobic tri-digestion of the wastes tested was observed at a F/M ratio of 1 and substrates mixture at 80:10:10 (FW: POME: SS). From the results, it was observed that increasing the F/M ratio by 5 times decreases methane production due to organic overloading. This study contributes to the knowledge concerning the understanding of anaerobic tri-digestion of multiple wastes and provides crucial data concerning the characteristics of POME, SS, and FW and their potentials as energy source in Malaysia.

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