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Evaluating the biomethane potential from the anaerobic co-digestion of palm oil mill effluent, food waste, and sewage sludge in Malaysia

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Abstract:

The ever-increasing organic waste generation in Malaysia is a significant contributor to greenhouse gas (GHG) emissions. However, organic wastes can be utilized to produce biogas by anaerobic digestion, which is a promising option for both energy and material recovery from organic wastes with high moisture content. Therefore, this study was formulated to investigate the feasibility of anaerobic co-digestion of three types of organic wastes generated in significantly huge quantities in Malaysia, namely Palm Oil Mill Effluent (POME), Food Waste (FW), and Sewage Sludge (SWS). The bio-methane potential (BMP) test was used to evaluate the biomethane potential from these organic wastes under mesophilic conditions to establish a stable and balanced microbial community, which may lack in mono-digestion, to improve biogas production. Comparative performance was made at different food to microorganism (F/M) ratios to investigate methane production in three groups of assays, namely A, B, and C. In groups A and B, the effect of F/M ratio variation on methane production was investigated, while in group C the effect of varying the co-substrate mixture on methane yield was examined. The findings showed that the highest methane yields achieved for mono-digestion of POME, SWS in group A were 164.44 mL-CH_4/g-$COD_{added,}$ and 65.34 mL-CH_4/g-COD_{added}, respectively, at an F/M ratio of 0.8, and 197.90 mL-CH_4/g-COD_{added} for FW in group B at an F/M ratio of 0.5. In addition, the highest methane yield achieved from the anaerobic co-digestion was at 151.47 mL-CH_4/g-COD_{added} from the co-digestion of the POME and SWS (50:50) at an F/M ratio of 1.7 in group A. Both AD and AcoD were tested to fit into two kinetic models: The Modified Gompertz and the Transfer Function models. The results showed that the modified Gompertz model had a better fit and was more adjusted to the experimental results for both AD and AcoD. The importance of this research lies in the economics of anaerobically co-digesting these abundance feedstocks and the variations in their characteristics which were found to increase their methane yield and process efficiency in anaerobic co-digestion.

Key words: Biogas; Global Warming; Waste Management; Organic Waste; Bioenergy; Substrate

1- Introduction:

The need to minimize greenhouse gas (GHG) emissions is more pressing now than it has ever been. Throughout the second half of the 20th century, energy consumption and GHG emissions have drastically increased. Global primary energy consumption increased from

27972.24 Terawatt hour in 1950 to 112416.26 Terawatt hour in 2000 (Smil 2016). The energy source used during that period was mainly fossil fuels. Thus, CO_2 emissions released increased from 3.1 gigatons carbon per year in 1960 to an average of 9.4 gigatons carbon per year in 2008–2017 (Le Quéré et al. 2018). In recent decades, these changes have sparked concerns about global climate change, particularly in light of population and economic expansion. To date, conventional waste management schemes have dominated in developed countries like Malaysia. The bulk of FW is currently deposited in landfills without being processed or separated from municipal solid waste (MSW), making it the primary source of landfill pollution (Lee et al. 2017). POME is another pollutant that emits pollutants into the atmosphere when treated in aeration ponds, adding to overall GHG emissions (Hasanudin et al. 2015; Ohimain &Izah 2014). In addition, SWS should not be left without proper treatment; otherwise, it will become a public health threat (Lowman et al. 2013).

Anaerobic digestion (AD) is a vital technology that has the potential to provide a sustainable and clean energy source while also utilizing the growing volumes of organic waste. In Malaysia, however, efficient AD is currently limited to a few types of biomass and sectors. Masnor et al. (2016) investigated the effects of acid-treated culture in POME under both mesophilic and thermophilic environments. They found that the average of 1.7 L H_2 of 2 L working volume per day was produced at 55^{o}C with a volumetric hydrogen production rate of 1.16 L/L·d. Wong et al. (2011, 2016, 2014) studied COD removal and found that methanogenesis anaerobic degradation achieved a COD reduction of 66.09 % from the AD of POME under different flow rates and hydraulic retention times. Krishnan et al. (2017) investigated the methane production from POME under different organic loading (OL) rates and temperature conditions using different reactors. The investigations revealed that the highest methane production rate and methane yield were 10.58 L-

CH_4/d and 0.11 m^3-CH_4/kg-COD, respectively, at an organic loading rate of 13.1 kg-COD/m^3/d. Osuagwu (2014) concluded that the maximum hydrogen yield for rice, fish, vegetable and mixed food waste was achieved at mesophilic conditions and pH of 5.5. Tanimu et al. (2014, 2015) studied foaming removal and methane production from FW at different C/N ratios and food to water dilution ratios and found that the maximum foam was reached at OL of 5.5 g-VS/L, and the highest cumulative biogas methane yield of 0.535 L/g-VS was achieved at OL of 3.5 g-VS/L. In addition, Seswoya et al. (2018) compared methane yield from fresh and aged FW using BMP tests and found that fresh FW had a higher ultimate methane yield and production rate. Furthermore, Seswoy and Karim (2017) studied the ultimate methane yield from SWS using BMP tests under different organic loading rates. They found that the ultimate methane yield was 588.3 mL-CH_4 /g-VS and 1244.5 mL-CH_4/g-VS at organic content 0.52 (VS/TS) and 0.68 (VS/TS), respectively. Furthermore, methane yield from SWS was studied under mesophilic conditions at organic loading of 4g/L using BMP tests. The findings showed that the cumulative methane yield did not exceed 400 mL (Ali et al. 2015; Aziz et al. 2019). Overall, research into improving anaerobic digestion processes in Malaysia is relatively limited, despite the need to scale up the technology and explore the combination of several abundant feedstocks to choose anaerobic co-digestion.

Despite substantial research on AD, technical and operational expertise for optimizing anaerobic co-digestion (AcoD) of organic wastes is still inadequate, and various technological challenges stand in the way of its adoption (Giuliano et al. 2013; Haider et al. 2015; Koch et al. 2015 and Mata-Alvarez et al. 2014). The economics of employing organic wastes in this study are important because of their quantity and dispersion in remote places compared to one other, as well as variations in their characteristics that might boost their methane production when anaerobically co-digested. As a result, the primary goal of this study was to investigate the BMP from SWS, FW, and POME as substrates under mono- and co-digestion conditions at various F/M ratios and co-substrate compositions.

2- Materials and Methods

2.1 Inoculum and Substrates collection and preparation:

FW was collected from the food canteen of Malaysia-Japan International Institute of Technology (MJIIT), University Technology Malaysia - Kuala Lumpur Campus. The waste was weighed then sorted inside plastic containers to remove non-food waste. It was then sampled according to La Cour Jansen et al. (2004) and stored at 4 ^{o}C. SWS 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 the refrigerator at 4℃, the samples were allowed to cool down to room temperature, and analysis for chemical characteristics was conducted.

The inoculum was collected from an active mesophilic anaerobic digester operating at 30 – 31 ^{o}C, treating a mixture of primary and secondary sewage sludge. The inoculum was considered suitable because the microbial composition of SWS is diverse enough to ensure that different substrates do not face nutrient limitations. The anaerobic digester was located at Bunus STP -Indah Water Konsortium in Kuala Lumpur. The inoculum was collected from an anaerobic digester sampling point. Samples were then transferred in 10 L plastic containers and were kept at 31 ^{o}C in an incubator for degassing purpose for one week (Holliger et al. 2016) before being used in BMP tests to avoid Volatile Solids (VS) influence on the results. Before mixing with the substrate, it was sieved through a 2 mm sieve to eliminate any large particles.

2.2 Analytical Methods

All analytical tests were performed in triplicates. In total, seven samples were studied namely; POME1, POME2, SWS1, SWS2, FW1, FW2, and FW3. The parameters examined in the analysis 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_5) was determined according to Standard Methods (APHA 2012) 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 (APHA 2012). Oil and Grease (O&G) was measured by extraction method using SPE-DEX 4790 Extractor System (Horizon Technology, USA). Ammoniacal Nitrogen (NH_3-N) was determined according to Standard Methods (APHA 2012) by using High Range Ammonia reagents (0-50 mg/L N) and the readings were taken using DR6000 (Hach, USA). Phosphorous was also determined according to Standard Methods (APHA 2012) by using High Range Total Phosphate reagents in the range 0-100 mg/L PO_4^{3-} (Hach, USA), and the readings were taken using DR6000 Spectrometer (Hach USA). Elemental analysis was conducted using CHNO elemental analyzer (Thermo Fisher Scientific, Flash 2000).

2.3 Experimental Set-up

2.3.1 Batch assays:

The assays were divided into three groups; A, B, and C. As shown in Table 1, each group of assays was designed with various I/S ratios, total volumes, and inoculum VSS. Furthermore, various co-substrate mixing ratios, organic loadings, and F/M ratios differed across samples and groups, as detailed in Table 2, which outlines the index for substrate ratios in each assay. Therefore, each group had a different number of assays. The test was run in triplicates in 250 mL serum bottles for each assay and the inoculum (blank control). Each serum bottle contained 100 mL of inoculum. Calculated amounts of substrates were added to each serum bottle to obtain a desired initial organic loading for each assay. In the blank control bottles, calculated amounts of distilled water were added to the inoculum so that the total volume of the assay in the blank control bottles equalled the total volume of the individual assays. It was assumed that sufficient micro-nutrients presented in the substrates; therefore, no additional micro-nutrients were added to assays. 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 aluminium caps to maintain anaerobic conditions. Bottles were manually shaken every day during the BMP test period. The bottles were kept in a general incubator (HI-162, China) at 37 ± 1 °C.

Table 1 BMP assay design parameters for groups A-C

Parameter	Unit	Group		
		A	B	C
I/S Ratio	-	3.00	2.00	2.00
Total mixture volume	mL	200.00	150.00	200.00
Inoculum VSS in mixture	mg/L	4437.33	4535.33	4481.778

Table 2 Index for F/M ratio and assay co-substrate composition

Group	Digestion Type	Substrate Composition (% COD)	Assay	pH	F/M	Organic Loading (mg/L)	COD (mg/L)
A	Mono-digestion	Inoculum (100 %)	Blank	7.63	-	-	-
		POME (100 %)	A1	7.39	0.8	578.93	3859.55
		POME (100 %)	A2	7.21	1.0	667.99	4453.33
		SWS (100 %)	A3	7.48	0.8	541.87	3612.44
		SWS (100 %)	A4	7.43	0.7	508.00	3386.67
	Co-Digestion	POME (50 %) + SWS (50 %)	A5	7.31	1.7	1176.00	7840.00
		POME (50 %) + SWS (50 %)	A6	7.35	1.1	784.00	5226.67
		POME (30 %)+SWS (70 %)	A7	7.36	1.7	1112.00	7413.33
B		Inoculum (100 %)	Blank	7.47	-	-	-

	Mono-digestion						
		FW 1 (100 %)	B1	7.36	0.5	340.15	2267.67
		FW 1 (100 %)	B2	7.23	1.0	680.30	4535.33
		FW 1 (100 %)	B3	7.26	2.0	1360.60	9070.67
		FW 2 (100 %)	B4	7.41	0.5	340.15	2267.67
		FW 2 (100 %)	B5	7.35	1.0	680.30	4535.33
		FW 2 (100 %)	B6	7.32	2.0	1360.6	9070.67
C	Mono-digestion	Inoculum (100 %)	Blank	7.63	-	-	-
		FW (100 %)	C1	7.39	0.60	403.36	2689.07
		POME (100 %)	C2	7.21	0.60	403.36	2689.07
		SWS (100 %)	C3	7.47	0.60	403.36	2689.07
	Co-digestion	FW (50 %) - POME (50 %)	C4	7.31	0.60	403.36	2689.07
		FW (50 %) – SWS (50 %)	C5	7.43	0.60	403.36	2689.07
		POME (50 %) - SWS (50 %)	C6	7.34	0.60	403.36	2689.07
		FW (33 %) - POME (67 %)	C7	7.26	0.60	403.36	2689.07
		POME (25 %) - SWS (75 %)	C8	7.43	0.60	403.36	2689.07
		FW-POME - SWS (33.33 % - 33.33 % - 33.33 %)	C9	7.35	0.60	403.36	2689.07

2.3.2 BMP data harvesting and evaluation:

The volume of biogas was calculated by measuring the headspace pressure as it was built up in bottle headspace, using a digital differential pressure gauge (SIKA, M.C., Germany), while the biogas composition was analysed using Micro GC-TCD (Agilent Technologies, USA) with Nitrogen and Argon as carrier gases. In order to maintain a constant temperature during pressure measurements and micro-GC analysis, the bottles were kept in a water bath at 37 ± 1 °C.

Biogas volume was calculated based on standard temperature and pressure conditions (STP: 101.3 kPa and 0 °C) using equations (1) and (2) below:

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

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

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

2.4 Specific Methane Yield (SMY)

SMY is the total methane produced by the end of digestion, which was calculated by subtracting the ultimate cumulative methane production of the blank assay (mL-CH_4) from the ultimate cumulative methane production of each assay containing the substrates. Then, it was divided by the initially added amounts of T-COD of the substrates. The value obtained was subsequently normalized to STP conditions.

The methane yield in (mL/g-COD_{added}) was determined by subtracting the blank control's methane yield and dividing it by the original volume of T-COD loaded into the bottles (Angelidaki et al. 2009; Brown &Li 2013 and Sahito et al. 2014) according to equations 3 and 4 below:

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

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

Where, Y_{NC} is the methane yield from the biogas produced from control (blank) assays, *SMY* is the specific methane yield (mL/g-COD_{added}), CH_4 % is the headspace methane concentration in percentage in the gas phase of serum bottle, V_C is the gas volume of the blank control assay, COD_{added} is the initial mass of T-COD added to the bottle, and V_{NC} is the methane yield of the blank control (mL/g-COD_{added}). The amount of solubility of methane was assumed negligible at 37°C. When less than 5 mL of the total CH_4 was produced over a day, the tests for groups A-C were terminated. The tests ran between 27 and 43 days. For statistical significance, average readings from triplicate values were used.

2.5 Synergetic Effect

Methane yield from batch assays could be used to estimate the synergistic effect from co-digestion. The synergy effect accounts for the excess methane yield from co-substrates over the weighted average of the methane yield from the actual feedstocks (Li et al. 2013). Weighted experimental methane yield (weighted EMY) was calculated by equation 5.

$$Weighted\ EMY = (EMY_a\ x\ \alpha + EMY_b\ x\ \beta + EMY_n\ x\ \eta + ...)/(\alpha+\beta+\eta+...) \quad (5)$$

Where, weighted EMY is the weighted average of the experimental methane yield for co-substrates, EMY_a and EMY_b are the experimental methane yields for each co-substrate, and α and β are the T-COD fractions for each co-substrate in the co-digestion. If the difference (EMY –

weighted EMY) is greater than the standard deviation of EMY, the synergistic effect could be confirmed (Labatut et al. 2011).

2.6 Model Fitting

For each reactor in the BMP assays, two models were chosen to fit the methane production curves. The transfer function model (equation 6) was used to predict the maximum methane production based on the accumulated methane production over time and study the anaerobic digestion process as a system receiving inputs and generating outputs. However, this model has limitations, such as not predicting the conditions for maximum biological activity, lag phase and system failures. Many scholars, however, have used first-order hydrolysis models to obtain valuable interpretations about the hydrolysis kinetics (Kafle and Chen 2016). As a result, the modified Gompertz model (equation 7) was used. The Modified Gompertz is an empirical non-linear regression model which assumes that the rate of methane production is proportional to the microbial activity and substrate degradation rate with 0time (Donoso-Bravo et al. 2010). In addition, the model considers exponential growth and lag phase during methanogenic bacterial growth (I Nyoman and Seno 2010). Therefore, these two models were used to analyze the hydrolysis kinetics, the lag phase duration, and the maximum methane production. Microsoft Excel 2013 solver was used to estimate the model parameters.

Transfer function model is defined by equation 6.

$$P = P_{\circ} . \left\{ - \exp[\frac{R_m}{P_{\circ}}(t - \omega)] \right\} \quad (6)$$

Modified Gompertz model is defined by equation 7.

$$P = P_{\circ}.\exp\left\{-\exp[\frac{R_m.e}{P_{\circ}}(\omega - t) + 1]\right\} \quad (7)$$

Where,

P=Cumulative methane yield at digestion time t, (mL/g-COD)

$P_{\circ}$= Maximum methane yield of substrate (mL/g-COD)

K= Rate constant (1/d)

t= Digestion time (d)

ω = Lag phase time (d)

R_m= maximum methane production rate (mL/g-COD.d)

e = exp (1) = 2.7182

3. Results and Discussion:

3.1 Physiochemical characteristics:

Table 3 shows the average values for physicochemical characteristics and elemental analyses for feedstocks utilized as substrates. The findings revealed that samples of the same type of feedstock had varied pH concentrations. Furthermore, as indicated by the experimental findings, POME and FW were acidic, implying that they would be suitable for co-digestion with substrates with a higher pH to provide enough buffer capacity. pH is normally maintained between 6.5 and 7.5 in mesophilic systems, with a neutral pH value being ideal. The ideal pH, on the other hand, is determined by the substrate and digester type (Jain et al. 2015). The pH of FW has been recorded variously in Malaysia. For example, Ismail et al. (2009) reported pH values for FW in the range 6.1-6.4, while Tanimu et al. (2014) reported a pH of 4.4, indicating that FW is more acidic.

Because the sample collection times and conditions for the collection points were not the same, the T-COD values varied significantly between samples within the same feedstock T-COD readings for POME1 and POME2 were 44533.33 mg/L and 62966.67 mg/L, respectively, while for FW, T-COD readings for samples FW1, FW2, and FW3 were 441666.67 mg/L, 514666.67 mg/L, and 525666.67 mg/L, respectively. POME was reported in the literature as having T-COD concentrations of 70,500 mg/L (Khemkhao et al. 2015) and 32,500 mg/L (Nasrullah et al. 2020).

In this study, BOD5/COD indicated biodegradability. SWS samples showed the lowest biodegradability among the feedstocks, at around 0.1, compared to FW samples, which had the greatest biodegradability factors at around 0.7. SWS is characterized by a high VSS/VS ratio and a low S-COD/T-COD ratio, as well as limited biodegradability (Astals et al. 2013). POME had biodegradability factors at about 0.5. Other indicators for biodegradation of organic matter include ratios of S-COD/T-COD and VS/TS. Experimental data in Table 3 show variations in VS/TS ratios. The VS/TS ratio for SWS was reported in Malaysia at 0.73 (Shehu et al. 2012).

Oil and Grease concentration varied significantly between feedstocks and among the samples for the same feedstock. For instance, FW had O&G values ranged from 34,966.67 mg/L to 91,270.00 mg/L. This variation is mostly due to variances in the amount of cooking oil and fat represented in the food collected and the food source, which was from fat-rich sources such as chicken and meat. Similarly, Phosphorus and Total Nitrogen concentrations differed across samples due to the quality and conditions of the feedstock.

The ammonia concentration is another parameter that must be measured throughout the characterization to get a preliminary idea of the biological performance of the AD process. According to Prockadka et al. (2012), inherent buffering capacity inside the anaerobic digester is a function of organic acids, ammonia, and bicarbonate content. It was also observed that ammonia inhibition began at about 2.5 g/L and 4 g/L for unacclimated and acclimated thermophilic methanogens, respectively (Hashimoto 1986). The inhibitory effect is also influenced by the source of inoculum and type of substrates (Chen et al. 2008). Ammoniacal Nitrogen levels for feedstocks in this study had considerably low levels (in the range 70.33 mg/L - 800 mg/L); hence the potential to cause inhibition was low. However, conditions in the reactor change as Nitrogen is being produced during the operation of the digester.

Table 3 Physiochemical characteristics and elemental analysis for feedstock

Parameter	SWS1	SWS2	POME1	POME2	FW1	FW2	FW3
pH	6.69	6.34	4.19	4.83	4.30	4.19	4.12
T-COD [mg/L]	33866.67	30033.33	44533.33	62966.67	441666.67	514666.67	525666.67
S-COD [mg/L]	1200.00	3266.67	30666.67	29100.00	118000.00	61300.00	92200.00
BOD_5 [mg/L]	3928.53	3427.13	24144.53	34783.97	325955.56	359237.34	274274.90
BOD_5/COD	0.12	0.11	0.54	0.55	0.74	0.70	0.52
TS [mg/L]	28440.25	24695.00	35897.17	41778.33	310523.33	419513.33	-
VS [mg/L]	9645.75	14581.67	8577.67	-	243150.00	80253.33	-
VS/TS	0.34	0.59	0.24	-	0.78	0.19	-
Oil and grease [mg/L]	200.00	1590.00	2490.00	7506.67	34966.67	47466.67	91270.00
Phosphorus [mg/L]	502.74	483.17	104.35	177.72	5685.01	10272.15	16750.67
NH_3-N [mg/L]	685.00	336.67	186.67	70.33	776.67	333.33	166.67
Total N [mg/L]	2193.33	1773.33	720.00	473.33	7800.00	9266.67	9780.00

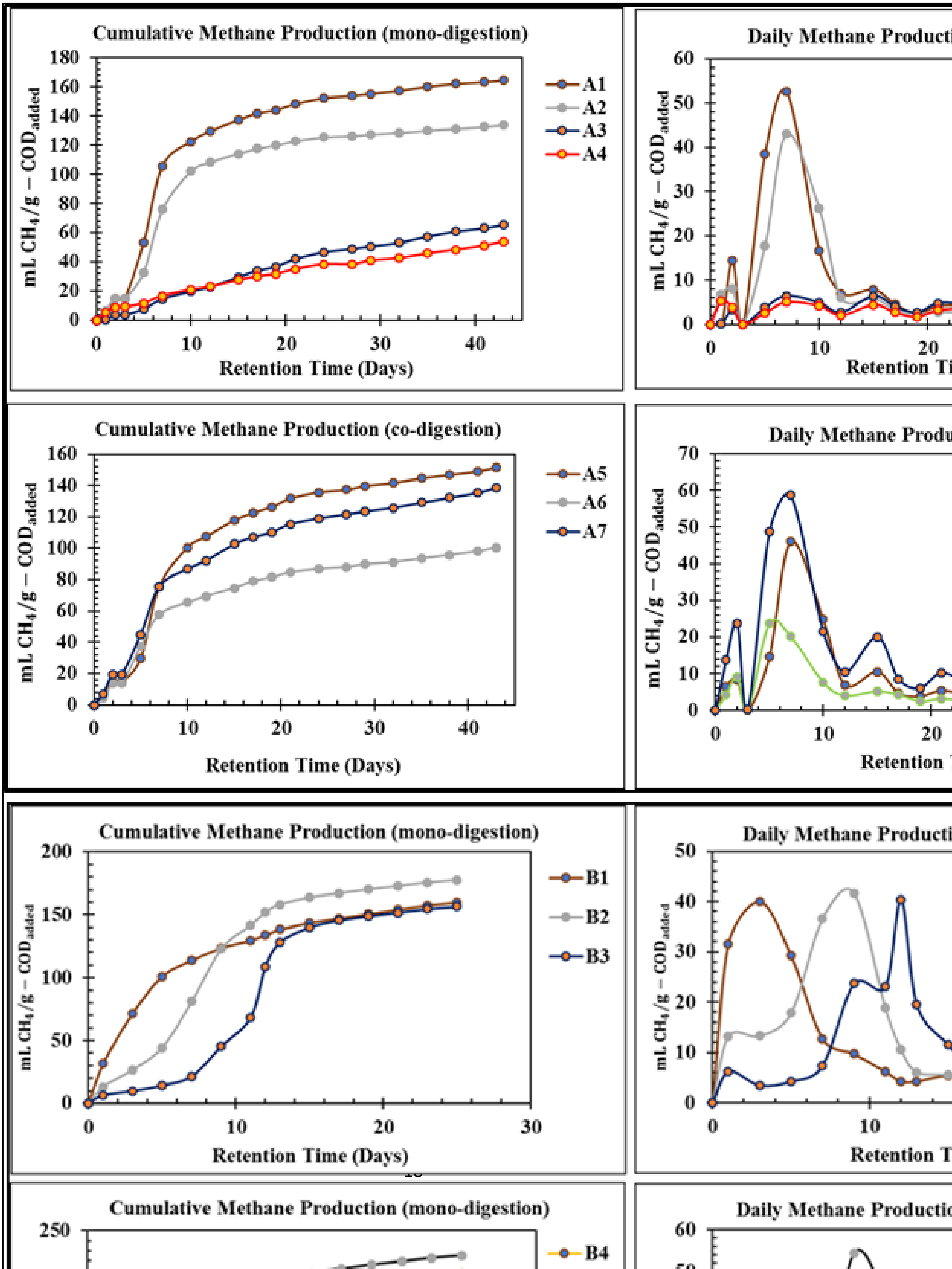
O %	26.89	27.84	35.55	34.47	25.55	33.36	28.61
N %	5.42	6.01	1.69	1.66	4.48	3.74	5.36
C %	33.66	35.76	38.74	36.88	43.96	46.70	51.92
H %	5.38	5.25	5.38	6.24	6.66	5.58	6.13
C/N	6.21	5.95	22.92	22.28	9.81	12.50	9.69

In the context of nutrients balancing in these feedstocks, POME had higher C/N ratios than SWS and FW, ranged from 20.28 to 22.92. On the other hand, SWS had C/N ratios below 10.00, ranged from 5.95 to 6.21, and FW had C/N ratios ranged between 9.69 and 12.50. Due to the variations in the C/N ratios for these feedstocks, co-digesting these wastes is a good option for enhancing the anaerobic microorganism population and supplying essential nutrients lacking in certain co-substrates. Furthermore, the balance in the C/N ratio is essential for a stable and optimum bio-methanation. Low C/N ratios promote methane production (Orhorhoro et al. 2016), while high ratios can inhibit microorganism's energy and structural metabolism (Deublein and Steinhauser 2008).

3.2 Methane Profiles

3.2.1 Groups A and B: Effect of F/M ratio variation

The mono-digestion and co-digestion of feedstocks containing POME and SWS substrates were investigated in group A. Figure 1 shows the daily and cumulative methane yield profiles expressed in mL/g.COD_{added} for each batch reactor at different F/M ratios and mixture compositions. For mono-digestion, the ratios of 0.8 and 1 were used for POME (assays A1 and A2), and the ratios of 0.8 and 0.7 were used for SWS (assays A3 and A4). For the co-digestion, the F/M ratios used for the assays A5, A6, and A7 were 1.7, 1.1, and 1.7, respectively, as outlined in Table 2. Since methane production is a feature of substrate degradation, it is commonly characterized by an initial lag phase, a subsequent more rapid increasing phase, and a stabilization phase (Speece 1983). From the results, these three phases characterizing methane production were found identical for all assays, as shown by the daily methane production curves in Figure 1. Thus, despite the differences in organic loadings for each assay, as reflected by changes in F/M ratios, methane production variations throughout the production phase were similar. At first, methane was produced in the first two days, followed by a reduction in methane production on the third day. Following that, a lag phase started for all assays, and methane production continued to rise until it peaked between days 7 and 8. This trend was observed in all the batch reactors, which is explained by the inclusion of readily biodegradable matter at the start, which degraded in the first two days, while the residual organic matter took longer to degrade. Thus, the remaining organic matter in the assays, after the first peak, accounted for the second methane peak, where methane production reached a maximum daily yield on day 8. Another factor that illustrates the common pattern of methane output for all assays is the inoculum used, which affected the activity of each test by supplying the same active microorganisms in all reactors, suggesting that microbial communities had similar efficiency despite the varying composition (Wilkins et al. 2015).



Cumulative Methane Production (mono-digestion)
mL CH$_4$/g – COD$_{added}$
Retention Time (Days)
A1
A2
A3
A4
Daily Methane Producti
Retention Ti
Cumulative Methane Production (co-digestion)
A5
A6
A7
Daily Methane Produ
Retention T
Cumulative Methane Production (mono-digestion)
B1
B2
B3
Daily Methane Producti
Cumulative Methane Production (mono-digestion)
B4
Daily Methane Productio

Figure 1 Methane profiles for groups A and B

Another key finding was that increasing the F/M ratio affected methane production during mono-digestion. For POME, methane production from assays with F/M=1 was greater than that of the methane production from assays with F/M=0.8. Similarly, SWS performance was higher in assays having F/M=0.8 than in assays having F/M=0.7. As organic loading was varied, a similar pattern of methane yields was observed, with the highest yields obtained for substrates with higher loading rates (Alzate et al. 2012; Zhou et al. 2011). It was also found that SWS did not produce as much methane yield as POME at F/M=0.8. This finding supports the notion that SWS is less biodegradable than POME due to the lack of nutrients for microbial biomass to complete methanogenesis (Lim and Fox 2013; Pozdniakova et al. 2012). Furthermore, it was found that increasing the F/M ratio improved methane yield in all assays, including the co-digestion assays. However, despite the mentioned trend, the findings from assay A7, which had the same F/M ratio as assay A5 but different composition (by volume), was different. It was observed that A7 (30% POME+ 70% SWS) produced more methane than A5 (50% POME+ 50% SWS) in the first few days. This finding shows that the mixing ratio of POME and sewage sludge resulted in faster organic matter degradation.

The variance of the F/M ratio for FW was studied independently in group B utilizing two different types of FW samples. Each substrate was digested at three distinct F/M ratios (0.5, 1.0, and 2.0) to analyse the methane generation profiles over the 25-day testing. Figure 2 shows that each of the three assays (B1, B2, and B3) used to represent samples from the first type of FW had a unique methane production profile. In contrast to B2 and B3, B1 had a shorter and earlier lag phase. Methane production in B1 began on the first day and peaked on the third, following which it began to progressively normalize. This means that the AD of FW was more effective at F/M ratio of 0.5 than at F/M ratios of 1.0 and 2.0. The methane production profiles in the B4, B5, and B6 tests likewise confirm this for the second kind of FW. According to the findings shown in Table 4, raising the F/M ratio by a factor of two increased methane yield by 13% only, as shown in the methane production profiles for B2 and B3. It was found that higher organic loadings resulted in a marginally longer lag phase. This phenomenon occurs as volatile fatty acids (VFAs) accumulates due to the high organic loadings and causes acidification within the reactor. The latter condition happens as the rate of acidogenesis exceeds the rate of methanogenesis. As a result, carbohydrate breakdown happens rapidly, resulting in the production of VFAs (Zhang et al. 2014). In such a case, VFA accumulation is confirmed by the subsequent drop in pH, which inhibits methanogens (Esposito et al. 2012; Vavilin and Angelidaki 2005). Nevertheless, the phenomenon manifested in this test is most likely the outcome of reversible acidification since methane production recovered after a certain period for all relevant assays, implying eventual VFA consumption (González-Fernández; García-Encina 2009 and Kawai et al. 2014).

3.2.2 Group C: Effect of Co-substrate Mixing Ratio Variation

Assays in group C were designed to investigate the influence of varying co-substrate mixing ratios on methane yield. The tests compared anaerobic mono- and co-digestion for the three feedstock types used in this study. From Figure 2, it is found that methane production peaks occurred at the same intervals during the test period. In the first five days, methane production was sluggish, then peaked between days 5 and 8, while between days 10 and 19, methane production was minimal, resulting in a plateau. Furthermore, short production gaps occurred between days 10 and 25, which can be explained by biodegradable matter taking longer to degrade than other organic matter components within the same substrate.

In addition, the profiles for the assays in group C had short lag phases (less than a day) and identical log phases of around six days, which is again influenced by the type of inoculum used. It was found that FW in group B had a very short log phase at F/M=0.5, but it had a more extended log phase in group C, which used a different inoculum, at F/M 0.6. The inoculums used in this study were from the same source but collected at different periods from an anaerobic digester treating sewage sludge. As a result, each inoculum sample collected contained different microorganism groups, which influenced the profiles of methane production in each community, resulting in the same pattern of methane production as seen in the methane yield profiles (Parra-Orobio et al. 2018; Wilkins et al. 2015).

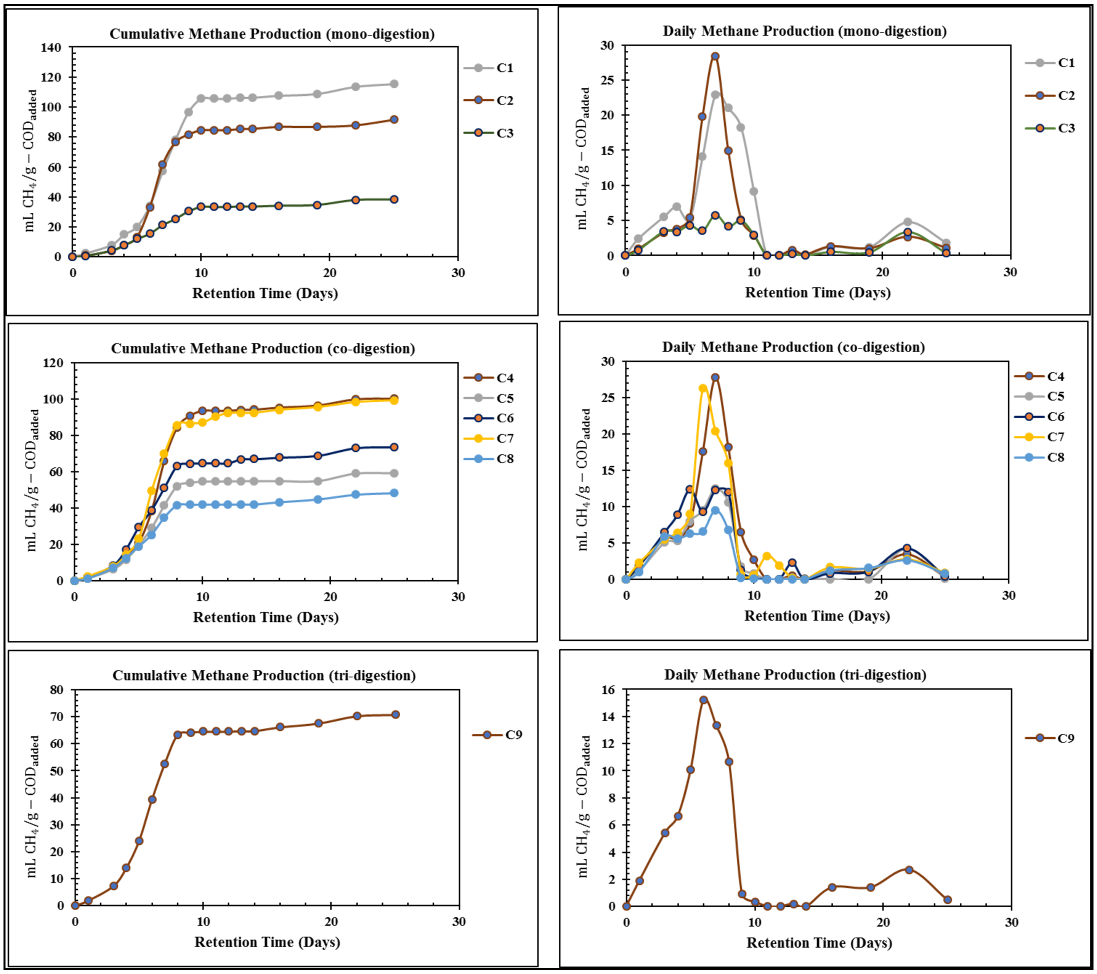


Figure 2 Methane profiles for group C (test time: 25 days)

3.4 Specific Methane Yield (SMY)

In group A, as shown in Table 4, the highest SMY was obtained for the mono-digestion of POME (A1) and the co-digestion of POME and SWS (A5) at 164.44 mL/g.COD$_{added}$ and 151.47 mL/g.COD$_{added}$, respectively. At the same time, the lowest methane yield was observed for the mono-digestion of SWS (A4) at 53.97 mL/g.COD$_{added}$. In addition, it was found that POME has a higher biodegradability than SWS, which is consistent with the findings by Sivasankari et al.

(2013). For practicality, the BMP tests were ended when the biogas yield observed was less than 1% of the total produced gas, although total organic matter decay in the reactors was not reached in this case (Alkan-Ozkaynak and Karthikeyan 2011)

In group B tests, increasing the F/M ratio from 0.5 to 2.0 did not significantly increase methane production since raising the F/M ratio indicates more organic matter is fed into the reactor, meaning that more biogas will be produced. However, if the F/M ratio in a reactor is inadequate (either low or high), the biogas produced would have a lower methane content. For assays B1, B2, and B3, methane made up about half of the biogas generated by B2 and B3. Even though B3 had four times the organic loading of B1, mono-digestion of FW at F/M=0.5 was more cost-effective and efficient than at higher F/M ratios. In assays B4 and B6, the same phenomenon was observed. Assay B6, which had a four-times higher F/M ratio than B4, yielded less methane. In addition, doubling the F/M ratio in B5 did not increase methane yield by more than 10% compared to B4. Thus, it is found that increasing the F/M for FW to higher than 0.5 resulted in overloading for the reactor, which is a related inference.

Table 4 Summary for SMY for A, B, and C assays expressed in mL-CH_4/g-COD_{added}

Assay	SMY	Assay	SMY	Assay	SMY
A1	164.44	B1	151.11	C1	90.67
A2	133.90	B2	173.36	C2	114.47
A3	65.34	B3	154.61	C3	37.59
A4	53.97	B4	197.90	C4	99.47
A5	151.47	B5	220.61	C5	58.31

A6	100.36	B6	198.60	C6	72.68
A7	138.44	-	-	C7	98.40
-	-	-	-	C8	47.34
-	-	-	-	C9	69.83

In comparison to POME and SWS, FW produced the highest methane yield in mono-digestion assays. This finding is in line with the fact that FW is more biodegradable and has a greater potential to produce methane than POME and SWS (Alsamet et al. 2019). In co-digestion, C4 (mixture of POME and Food Waste) produced more methane than the rest of the assays, while C5 and C6 produced 59.21 mL CH_4/g-COD and 73.58 mL CH_4/g-COD, respectively. Furthermore, it was found that increasing the composition of POME as a co-substrate boosted the methane yield. This finding is attributed to the balance in the C/N ratio that POME introduces as a co-substrate, whereas FW and SWS had much lower C/N ratios.

The three assays C3, C5, and C8, contained SWS T-COD content at 50% and above in the feed. C3, which was in mono-digestion, had the lowest biomethane yield among the assays in group C but the highest percentage of methane. This observation indicates that AD of SWS at F/M = 0.6 proceeded at more stable conditions than FW. In addition, AD of SWS at F/M = 0.6 had a better methane percentage compared with AD at F/M ratios of 0.7 and 0.8 in group A.

C9 did not significantly improve the biomethane yield compared with the rest of the assays in the same group. This suggests that this combination should be investigated more to prove if it is more favorable than co-digesting two feedstocks alone.

3.4 Synergistic Effect

Synergism can be viewed as an additional methane yield resulting from the co-digestion of different substrates over the weighted average of the specific methane yield from the individual substrates (Labatut et al. 2011). Co-digestion of certain substrates can produce a synergistic effect that results from the availability of additional trace elements, alkalinity, or nutrients in which another substrate is lacking. Hence, they improve biodegradability resulting in higher biomethane production. The calculation of synergetic effects was explained in section 2.5. The effect exists if the difference between EMY and weighted EMY is less than the standard deviation. However, when methane yield is lower than the weighted EMY in the assay, this is evidence for the antagonism effect, resulting from pH inhibition, ammonia toxicity, or high volatile acid concentration (Labatut et al. 2011). Table 5 summarizes the calculated synergistic effects from the assays run in co-digestion., which suggest that the results were mixed. It is observed that all assays from group A showed a synergistic effect while those in group C did not show. This phenomenon could be due to the type of inoculum used in each group and how diverse their microbial communities were, which eventually enhanced the AD process. In addition, it could be attributed to the I/S ratio, which is higher for group A (3/1) than group C (2/1). Asante-Sackey et al. (2018) found that the highest biogas potential was recorded at an inoculum to feedstock ratio of 3:1.

Table 5 Summary of synergetic effects

Sample	EMY	Weighted EMY	Difference	SD	Synergistic effect
A2	133.91	84.53	49.38	0.03	Synergetic
A4	53.97	34.98	18.99	0.27	Synergetic
A5	151.47	114.89	36.58	0.12	Synergetic
A6	100.37	77.35	23.01	0.12	Synergetic
A7	138.44	95.07	43.37	0.22	Synergetic
C4	99.47	102.57	-3.10	0.06	Non-Synergetic
C5	58.31	64.14	-5.83	0.10	Non-Synergetic
C6	72.68	76.03	-3.36	0.27	Non-Synergetic
C7	98.40	106.62	-8.22	0.11	Non-Synergetic
C8	47.34	50.87	-3.52	0.07	Non-Synergetic
C9	69.83	80.10	-10.28	0.13	Non-Synergetic

3.5 Post Characterization

Post-characterization focused on physiochemical characteristics for assays at the end of the BMP tests. The analysis included pH, Ammoniacal Nitrogen, and the removal of TS, VS, T-COD, and S-COD. For technical reasons, during the experiments' running, the post-characterization for group B assays was not conducted.

Figure 3 shows the post characteristics for the digestate for groups A and C analysed after BMP tests were ended. For group A, pH values for all assays fell between 7.0 to 7.5, as seen in graph (a-1) in Figure 3. Similarly, for group C, as shown in graph (b-1) in Figure 3, the pH range was between 6.93 and 7.07, suggesting that the buffer capacity at the end of the BMP test was adequate and that there was no indication of VFA accumulation or inhibition (Cheah et al. 2019). Furthermore, Ammoniacal Nitrogen concentrations fell below 500 mg/L for most reactors in group A and all reactors in group B, well below the inhibitory level of 2 g/L. During AD, ammonia is formed, and if it reaches 2 g/L, it inhibits methanogenesis (Chen et al. 2016), thus affecting methane production.

Graphs b-2, b-3, c-2, and c-3 in Figure 3 show the reductions in T-COD, S-COD, TS, and VS. In terms of T-COD reduction, assay A1 had the highest reduction rate at 84.39%, while the lowest reduction rate was observed in assay A4 at a rate of 77.81%. For TS, the reduction for all assays was above 90%, with assay A1 being the highest at a reduction rate of 95.82%. In addition, the VS reduction rates were 74.42% and 83.76% for assays A5 and A6, respectively. Overall, the post-characteristics for group A assays showed reasonable reduction rates exceeding 75% for T-COD and solids, and methane composition in biogas produced was within the typical range, 50%-70% (Baltrėnas and Misevičius 2015). Similarly, the high reduction rates in assays C1, C5, and C7 indicate faster substrate degradation.

Moreover, it was observed that reductions in COD and solids were the lowest in assays containing 50% or more SWS. These observations supplemented previous findings regarding SWS low biodegradability (Zhang et al. 2019). Overall, in mono-digestion assays, FW reported reduction rates of 98.72%, 98.65%, and 99.08% for T-COD, TS, and VS, respectively, while for POME, the reduction rates were 81.44%, 77.10%, and 74.35% for T-COD, TS, and VS, respectively.

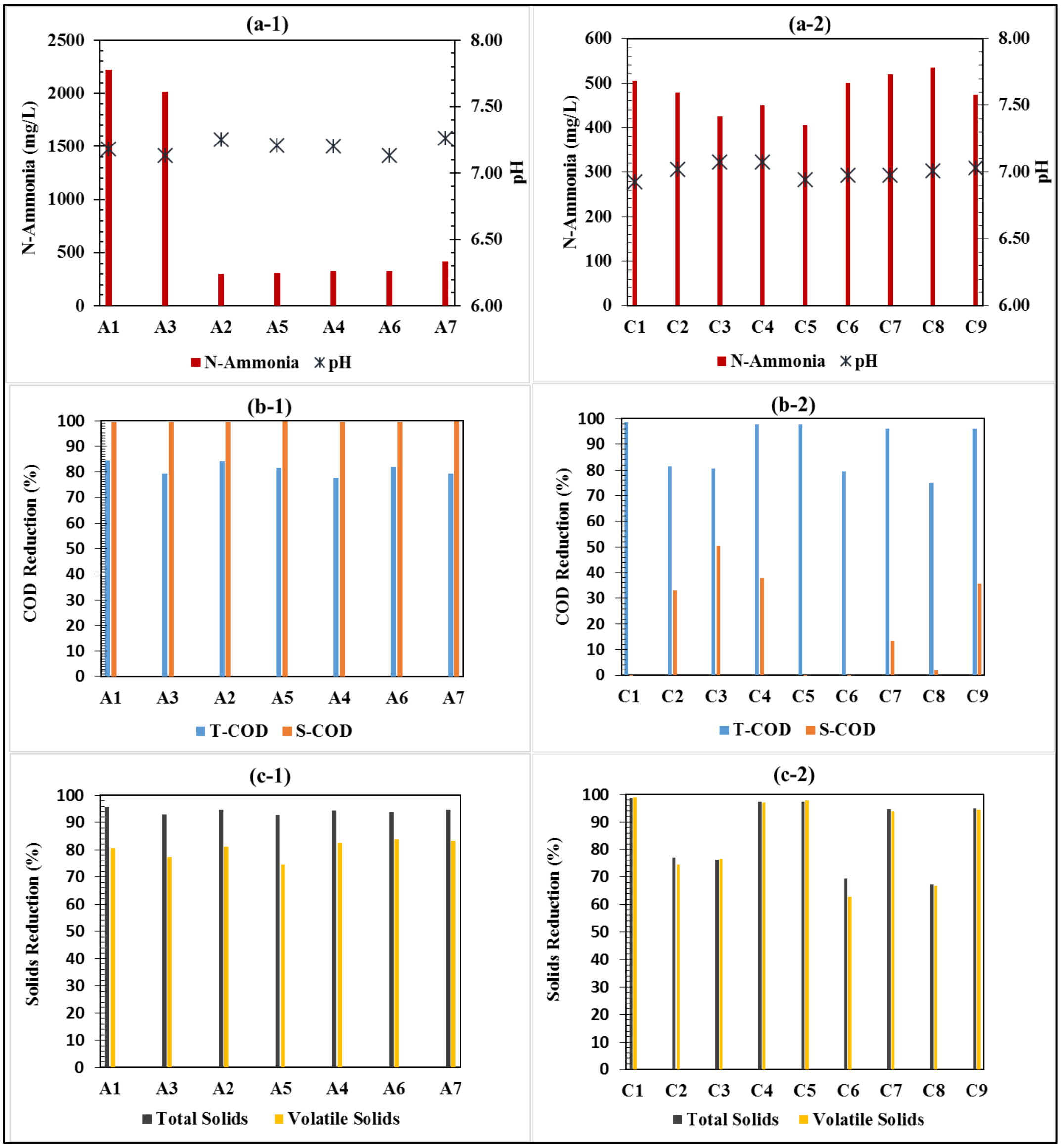


Figure 3 Post-characteristics for groups A and C assays for: (a) Ammoniacal nitrogen (N-Ammonia) and pH, (b) COD Reduction, and (c) Solids Reduction

3.6 Kinetics and Model Fitting

After obtaining the cumulative biogas production curves from batch reactors, the modified Gompertz and transfer function models were used to determine biogas production potential (P), maximum rate of biogas production (R_m), and duration of the lag phase (λ). These two particular models were chosen to fit the experimental data for their suitability for anaerobic co-digestion in previous studies (Li et al. 2012; Zahan et al. 2018).

By fitting the experimental data to the two kinetic models, it is found that both models match methane profiles well, with a few variations showing a significantly different pattern in assays A2, A4, A5, and A6 when the data fitted using the transfer function model. The modified Gompertz model, on the other hand, was the perfect fit for all reactors. The minor discrepancies between the predicted and measured values suggest that the models accurately predicted the reactor behaviour (Raposo et al. 2009).

Tables S-1 and S-2 in the supplementary file summarize parameters obtained from the modelling process by the Gompertz model and the Transfer Function model, respectively, for the methane yields from assays in groups A, B, and C. In addition, the modelling curves for each group are presented in Figures S-1 to S-4 in the supplementary file. The two models were demonstrated to be proper tools for evaluating the parameters of AD and AcoD.

Conclusion:

The study aimed to investigate the BMP of POME, FW, and SWS in anaerobic mono- and co-digestion conditions under various F/M and co-substrate mixing ratios. Feedstocks used in this study had variations in characteristics. With increasing organic loading, FW demonstrated a longer lag phase and lower methane yields, but SWS showed improvement in methane yields and a more stable AD process. In addition, when combined with FW and SWS, POME boosted methane yield by balancing the microbial population for co-substrates in the reactor by adding and delivering nutrients that would otherwise be lacking. As a result, AcoD of these feedstocks, which are produced in substantial amounts daily, has been shown to improve methane yield and process performance by balancing the C/N ratio. Furthermore, this work identifies a significant gap in the technical knowledge concerning the AcoD of multiple wastes and provides sets of data characterizations for various organic wastes, their biomethane potential, and kinetic parameters.

LIST OF ABBREVIATIONS

AcoD	-	Anaerobic Co-Digestion
AD	-	Anaerobic Digestion
BOD	-	Biochemical Oxygen Demand
BMP	-	Biomethane Potential
C	-	Carbon
C/N	-	Carbon to Nitrogen Ratio
COD	-	Chemical Oxygen Demand
EMY	-	Experimental Methane Yield
F/M	-	Food to Microorganisms Ratio
FW	-	Food Waste
H	-	Hydrogen
I/S	-	Inoculum to Substrate Ratio
MSW	-	Municipal Solid Waste
N	-	Nitrogen
NH_3N	-	Ammoniacal Nitrogen
O	-	Oxygen
OL	-	Organic Loading
POME	-	Palm Oil Mill Effluent

S-COD	-	Soluble COD
SD	-	Standard Deviation
SMY	-	Specific Methane Yield
SS	-	Suspended Solid
STP	-	Standard Temperature and Pressure
SWS	-	Sewage Sludge
T-COD	-	Total COD
TP	-	Total Phosphorous
TSS	-	Total Suspended Solid
TVS	-	Total Volatile Solid
VFA	-	Volatile Fatty Acid
VS	-	Volatile Solid
VSS	-	Volatile Suspended Solids

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests in this study.

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Authors' Contributions

MAA conducted the experiment in biomethane potential and conducted data analysis

MG planned and supervised the study

NMM participated in data analysis

SAA participated in sample collection and characterization

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