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Influence of substrate to inoculum ratio (S/I) on the treatment performance of food processing wastewater containing high oil and grease (O&G) in batch mode

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ABSTRACT

Anaerobic digestion is one of the promising methods for treating high strength organic waste liquid with the recovery of energy such as biogas. The current study reports the physical–chemical characteristic of the waste, its treatment performance and methane gas production from a liquid organic substrate high in oil and grease generated from a food processing industry in Malaysia. The substrate is suitable for energy recovery as it contains high organic matter ranging between 15 and 17 g/L chemical oxygen demand (COD). The batch biodegradability test assays were used in anaerobic digestion of the food processing wastewater (FPW) for its treatment performance and methane yield at different ratios of the substrate (FPW) to inoculum (anaerobic digester sludge) (S/I) of 1.0, 1.5 and 2.0 for 54 d. From the results, S/I 1.0 ratio performance is ideal for COD, biochemical oxygen demand, total solids and volatile solids (VS) removal at 96.9%, 96.6%, 75.8%, 65.2%, respectively, showing a balanced substrate/food and microorganism (F/M ratio) in the digester that is important for the degradation of complex organic material. The maximum amount of specific biogas production rate was 228.6 mL/gVS in S/I of 1.0 treatment, with a methane gas production yield of 200.5 mL/gVS. While the minimum methane gas production yield of 67.5 mL/gVS was produced for S/I of 2.0. The specific biogas production rate was found at 84.6 mL/gVS. The higher substrate to the inoculum ratio of S/I 1.5 and 2.0 has delayed the biogas and methane production by 20 d. The results indicate that FPW anaerobic digestion is promising to treat the effluent and produce biogas containing a moderate amount of methane.

Keywords: Food processing wastewater (FPW); Methane yield; Anaerobic digestion; Treatment performance; Oil and grease (O&G)

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1. Introduction

In recent years, full-scale applications of anaerobic co-digestion of specific substrates and inoculum (sewage sludge) are identified as an environmentally sound renewable energy source [1–4]. The treatment performance in anaerobic digestion (AD) and biogas production could be recovered; thus creating energy potential through this technology. Sludge production from municipal wastewater treatment plants (MWTPs) is likely to grow with the increasing figure of treatment plants being constructed or progressed [5]. The problem is mainly due to the growing population linked to sewage networks in Malaysia, especially in the food processing industry. According to Osman [6], the sequencing batch reactor (SBR) is a popular advanced wastewater treatment used for treating industrial wastewater in Malaysia. Furthermore, the presence of inhibiting organic and chemical pollutants in the food-based effluents such as proteins, fats, oil, and grease (FOG) results in the lower production of methane gas and become inhibitory factors in anaerobic treatment [7–9].

The increasing population and rising living standards produced a rapid growth rate of food waste effluent from different origins and sources, that is, residential and commercial, industry and small-to-medium enterprises. Moreover, it has become one of the significant proportions in MWTPs. Nonetheless, due to its high biodegradability and contents of FOG, anaerobic digestion as a single substrate may encounter various potential inhibitors, including the accumulation of volatile fatty acids (VFA) in its compound [10]. The treatment of large quantities of substrates, products or waste from the food industry, agriculture, crop residues, animal waste, market waste, and organic matter of municipal solid waste for common on-site feedstock is using the designated anaerobic digesters formerly [2]. Meanwhile, substrates from various organic by-products, such as FOG from residential, slaughterhouse waste, restaurants, and industrial, are known as a typical off-site feedstock. This daily amount of substrate composition is the main factor in determining the rate of CH_4 production from the anaerobic digestion. Generally, biogas produced in AD treatment is 60% methane (CH_4), and 40% carbon dioxide (CO_2) thus can be used to generate heat supply, electricity, and further treated to be used as a vehicular fuel. Evidence has shown that increased biodegradable substrate results in a more significant amount of CH_4 gas production as usable energy inside the treatment plant [12].

High strength industrial wastewater characteristics are challenging to define and depend on the type of industrial plants such as food processing industries, chemical, agriculture, textile, paper mill, pharmaceutical, and petrochemical industries. The effluents that contain a high amount of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), ammoniacal nitrogen, total phosphorus, total suspended solids, and heavy metal contaminants affect the organic loading rate (OLR) on the wastewater generation per day [13]. Mutamim et al. [14] revealed that food manufacturing and processing wastewater contain high concentrations of several organic compounds, including carbohydrates, proteins, pectin, oils, starches, fats, vitamins, and sugars, which are responsible for high lipid concentration [15]. The metabolic pathway of anaerobic food chain degradation consists of several groups of facultative anaerobes and archaea that degrade and transform complex organic compounds (mainly carbohydrates, proteins, and lipids) into simpler organic compounds. The critical biochemical reactions in the AD process and production of CH_4 include hydrolysis, acidogenesis (acid production), acetogenesis, and methanogenesis. Methane production may occur through the consumption of acetate, H_2, CO_2, and methanol by certain microbes during the AD process [16]. Nonetheless, methane production can be delayed based on the type of substrate used for the AD process and inoculum used for the seeding process. Its low bioavailability limits the biodegradability of solid fatty residue. Long-chain fatty acids (LCFA) are the main products of lipid hydrolysis and are frequently found in wastewaters from various sources, for example, dairy industry, food processing industry, slaughterhouses, wool scouring industry, and vegetable oil/fat refineries. LCFA vary in chain length and degree of saturation, and the most abundant saturated and unsaturated LCFA present in wastewaters are palmitate ($C_{16:1}$) and oleate ($C_{18:1}$), respectively [15,17,18]. Palmitate is also a key intermediate of oleate degradation. Although anaerobic hydrolysis (lipolized) of lipids to glycerol and LCFA occurs rapidly as shown in Fig. 1, subsequent LCFA degradation via β-oxidation proceeds rather slowly [19,20].

Recently, Long et al. [8] reported that the addition of fats, oil, and grease (FOGs) to municipal anaerobic digesters could result in a significant increase in CH_4 production. Previous studies have investigated different types of organic and inorganic substrates to determine its capability for yielding biogas, such as animals' manure, high lignocellulose contain waste, industrial wastewater, and recalcitrant waste. For example, a co-digestion mixture with the substrate to inoculum S/I ratio of more than 1.0 with FOG from a meat processing plant increased the methane yield by 60% despite lag phase phenomena [21]. Likewise, methane yield was 2.6 times higher when the inoculum (municipal primary sludge and thickened waste activated sludge) was co-digested with oil and grease (O&G) wastewater from restaurants, food service providers, and residential [22]. The composition, origin, type of substrate, and chemical substances (biomass resources such as carbohydrates, proteins, fats, and lignin) influenced the biogas yield of the individual substrates.

Additionally, Muller et al. [23] reported that scum foaming could be minimized during anaerobic co-digestion of grease trap waste (GTW) at the Annacis Island wastewater treatment plant (WWTP) in Vancouver, Canada by lowering the standpipe level and modifying the operating procedure during offloading. Newer treatment methods focus on the stimulation of existing bacteria to break down FOG, rather than introducing different enzymes and bacteria. This approach utilizes optimized fermentation-based yeast proteins, micro-nutrients and specialized surfactant chemistry, dosed at only a few parts per million, to stimulate the indigenous bacteria population and accelerate natural biodegradation. This biocatalytic effect can provide various operational benefits at the WWTP, including reduced sludge production, increased nutrient uptake and improved settlement. In another study, lipase-producing

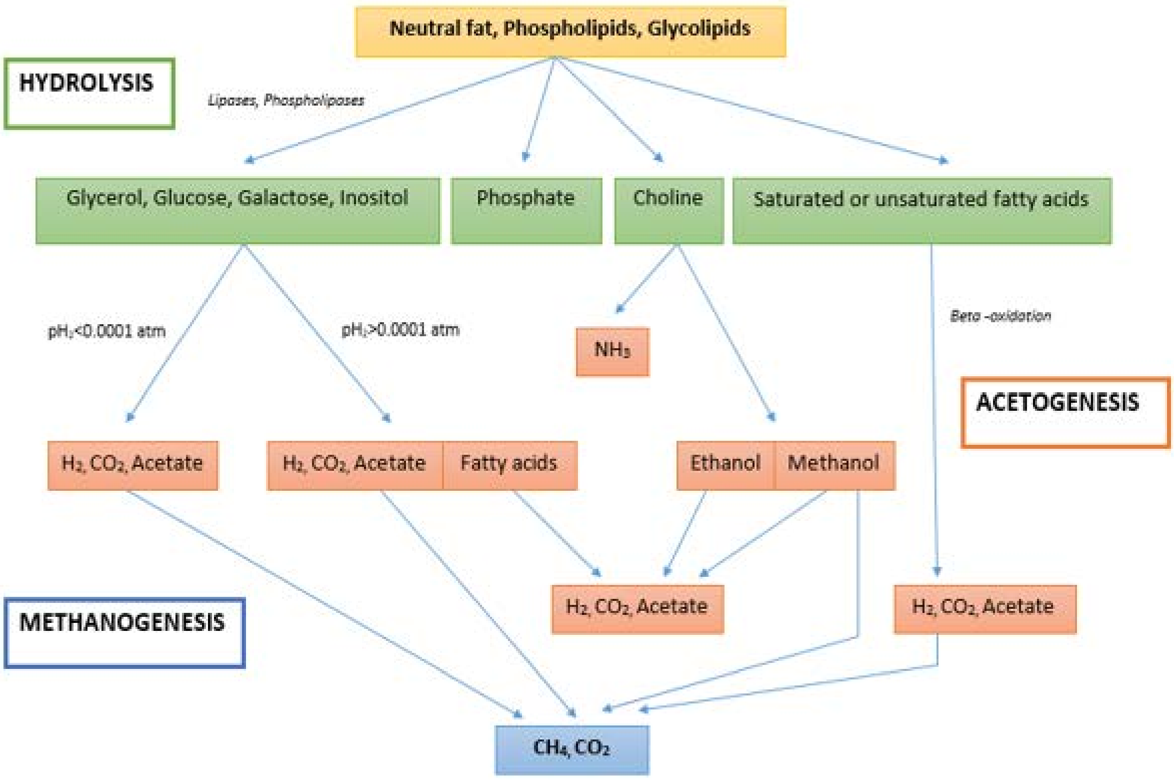

Fig. 1. Metabolic pathway of anaerobic degradation of fats [20].

microorganisms were isolated from the bakery and palm-oil-industry wastewater. The biodegradation of FOG in the isolates was tested, and up to 87.7%, FOG removal was achieved [8].

Dereli et al. [24], treated wastewater containing 11.3 ± 0.5 g/L fat, oil, and grease concentration which caused LCFA inhibition at high solid retention time in their lab-scale anaerobic membrane bioreactor system. The digestion of substrate with high O&G of 4.6–36 g/L at OLR 17 kg COD/m^{3}d, when acclimatized the sludge for long-term adaptation to LCFAs in up-flow anaerobic sludge blanket bioreactor coupled with membrane system, achieved maximum methane production and high rate methanogenesis [25]. Pellera et al. [26] conducted a study on the substrate to inoculum ratio (SIR) of 0.5 for winery waste and juice industry waste yielding 446.23 and 445.97 $NmLCH_4/gVS_{substrate}$, (VS – volatile solids) respectively. They also studied SIR of 0.25 for cotton gin waste and olive pomace yielding 267.96 and 258.65 $NmLCH_4/gVS_{substrate}$, respectively. The study concluded higher SIR delayed methane production indicating process inhibition. Anaerobic sludge was found as an adequate inoculum among tested samples, and due to its high availability, it may be considered as a manageable choice in real-scale applications. Contrarily, using landfill leachate and thickened anaerobic sludge for the same purpose showed lower efficiencies.

There are many factors to be determined before starting the batch biodegradability test procedure because the biogas production varies from one substrate to another [27]. Nonetheless, fats and proteins produce more CH_4 than carbohydrates and lignin because carbohydrate fraction of lignocellulosic biomass is degradable. In contrast, the lignin fraction is generally considered extremely difficult to be degraded during anaerobic digestion [28,29]. The carbon/nitrogen (C/N) ratio represents the relationship between the amount of nitrogen and carbon in a feedstock with a 25:1 C/N ratio that produces optimal gas production [30]. In this study, the C/N ratio of 22:1 for the substrate, which is in agreement with a previous study [31] which revealed an optimum range of C/N ratio for AD was 20–35:1. If the C/N ratio for the substrate is low, ammonia concentration could increase the AD process and delay microorganism vitality preventing anaerobic digestion [32]. A previous study suggested that the nutrients that exist in sewage are in the form that is available to the bacteria where C:N:P at 330:5:1 can be used for biomass with a high yield coefficient (e.g., degradation of volatile fatty acids) [33]. Table 1 depicts the effect of various range of ammonia-nitrogen concentrations on the AD process. The pH must occur within an optimal range of 6.8–7.2 to prevent the toxicity of ammonia because it is highly dependent on the pH changes [30,34].

Notably, Owen et al. [35] developed a simplified, secure, relatively inexpensive, and repeatable lab-scale method known as biochemical methane potential (BMP) to determine the biodegradability of organic substrates. Batch biodegradability test assay is a standard protocol to estimate biodegradability of sewage sludge in anaerobic digestion and, more importantly, to observe methane production and

Table 1
Ammonia-nitrogen concentration's effects on anaerobic digestion [30]

Effect on anaerobic digestion	NH_4^+ NH_3–N (mg/L)
Beneficial	50–200
No adverse effect	200–1,000
Inhibitory at higher pH values	1,500–3,000
Lethal	>3,000

generation based on different substrates, inoculums, and their ratios. The fundamental principle of the batch biodegradability test in this study is to mix the selected substrate and sewage sludge (inoculum), then incubate at a specific temperature and measure the biogas produced and methane yield composition. The batch biodegradability test period varies based on the rate of substrate biodegradability up to a reduction in biogas production volume [36]. Therefore, the current study investigated the biodegradability of substrates to define best practices and process efficiency before any reactor design to be fabricated [37]. The objective of this study is to evaluate the treatment performance and batch biodegradability test of food processing wastewater (FPW) under the mesophilic condition at the different substrates to the inoculum S/I ratio for the methane production. The study also wants to evaluate the sewage anaerobic sludge use as inoculum is adequate in this AD treatment, and lack of studies in the treatment performance (before and after), biogas production and different S/I ratios of batch biodegradability test is further analyzed. A lab-scale anaerobic digester will utilize the results from these batch biodegradability test studies and evaluate further on its performance, such as COD, BOD, VS, total solid (TS), total phosphorus and ammoniacal nitrogen. Fundamentally, this preliminary study is to evaluate the production of CH_4 yield and treatment performance based on the specific substrate facilitating optimization of anaerobic digesters' designs and operations.

2. Materials and methodology

The substrate, FPW, was collected from a food processing industry located at Batu Caves, Selangor, Malaysia. This factory produces varieties of fast food products such as nuggets, burgers, sausages from meat such as chicken, beef, lamb, and fish. Processes include half-cook food processing such as cooking, boiling, mincing, and seasoning. These activities produce a large amount of organic and inorganic materials, carbohydrate (starch for coating), proteins and FOG from the main ingredient, which is the animal meat. The raw sample is collected at the collection sump without any treatment. Before the analysis, the fats, oil, and grease were skimmed off from the upper layer of the substrate, and only the filtered supernatant collected was used for the batch biodegradability test. FPW was kept at 4°C in a chiller to prevent the degradation of the organic substances. In this study, anaerobically digested sewage sludge was used as an inoculum and obtained from the anaerobic sludge tank of municipal sewage treatment plant located in Kuala Lumpur and kept at 4°C.

2.1. Physical and chemical characteristics

Throughout the batch biodegradability test study, various characterization was performed to determine the physical and chemical properties of the materials before and after experiments. Table 2 displays the method for the determination of physical and chemical characterization used in this study. The measurement of TS, VS, and O&G performed according to the Standard Methods for the Examination of Water and Wastewater [38]. The characteristic of both substrate and inoculum used were analyzed following standard method APHA, and heavy metal content in the substrate was detected using inductively coupled plasma mass spectrometry (ICP-MS) and tabulated in Table 3.

Seeding is required to treat the wastewater biologically if the BOD_5/COD ratio is between 0.3 and 0.5 because the process will be relatively slow, as the acclimatization of the microorganism that helps in the degradation process is time-consuming. If the BOD_5/COD ratio is below

Table 2
Methods for physical and chemical analyses used in the study

No.	Parameter	Methods/Instrument
1.	pH	Portable pH meter
2.	Chemical oxygen demand (COD), mg/L	HACH Spectrophotometer
3.	Biochemical oxygen demand (BOD), mg/L	HACH method
4.	Total solid (TS), g/L	APHA method (2540B)
5.	Volatile solid (VS), g/L	APHA method (2540E)
6.	Methane gas (CH_4), %	6890N Agilent Gas Chromatography (GC-TCD)
7.	Elemental analysis (C,H,O,N,S), %	Elemental analyzer (LECO)
8.	Heavy metal content (Cu, Zn, Fe, Ni), mg/L	ICP
9.	Oil & grease (O&G), mg/L	APHA method (5520B)
10.	Ammonia-nitrogen, mg/L	Test 'N Tube salicylate method number 10023 HACH Spectrophotometer
11.	Total nitrogen, mg/L	Test 'N Tube persulfate digestion method 10071 using calorimeter HACH DR890 Spectrophotometer
12.	Total phosphorus, mg/L	HACH Spectrophotometer

Table 3
Characteristics for FPW (substrate) and anaerobically digested sewage sludge (inoculum)

Parameter	Substrate	Inoculum
pH	3.68	7.00
BOD_5 (*BOD_5/COD ratio), mg/L	10,860 (0.64*)	690
COD, mg/L	17,000	1,970
NH_3–N, mg/L	17	302
Total phosphorus, mg/L	237	563
Total solid, g/L	16.65	18.70
Volatile solid, g/L	16.25	12.75
VS/TS	0.9760	0.6818
Hydrogen, %	8.722	4.8825
Sulfur, %	2.1395	1.383
Carbon, %	54.8	32.20
Nitrogen, %	2.4685	4.51
C/N ratio	22.20	7.14
Oil & grease, mg/L	154	
Heavy metal detection: Cu (0.506mg/L), Zn (5.024mg/L), Ni (0.498mg/L) and Fe (5.094mg/L)		

0.3, biodegradation will not proceed, and the wastewater cannot be treated biologically due to wastewater characteristics that contain inhibitors (mostly toxic and refractory properties) that affect metabolic activity of bacterial seed [13,39]. Based on the VS/TS content, it shows that FPW has high energy content (97%) when compared to inoculum (68%), and this characteristic indicated that FPW might be potentially used as a substrate to produce higher methane [22,37]. Table 3 illustrates the characteristics of the substrate and inoculum utilized in this study, which kept in a tight container and stored at 4ºC. The ratio of BOD_5 to COD acts as a tool for checking the biodegradability index of wastewater influent. Raw wastewater having a BOD_5/COD ratio higher than 0.5, can easily use biological processes and biodegradable.

2.2. Experimental procedure

The batch biodegradability test in this study was adapted from Hansen et al. [40] with the guideline of VDI 4630 2016 [41] as reported by Angelidaki et al. [42] and Khairul Anuar et al. [43]. The batch biodegradability tests were conducted at mesophilic conditions (37°C ± 0.5°C) in triplicates. Samples were digested in closed serum bottles with a working volume of 100 mL, leaving 25 mL headspace. In each container, substrate (wastewater) samples were mixed with the inoculum to obtain substrate to inoculum ratio (S/I) of 1.0, 1.5, 2.0, and control (inoculum only) as shown in Table 4. There was no further modification of pH done as the initial pH ranged between 7.0 and 7.5. After mixing the samples with inoculum final pH were recorded. Next, nitrogen gas was purged for 5 min in the serum bottle to ensure the anaerobic condition, and this is called the degasification step. The batch biodegradability test serum bottles were sealed with a butyl rubber stopper and crimped with aluminum cap. Gas samples (more than 4 mL) were collected from the headspace of the reactors through the septum with an air-tight syringe. Biogas production volume was recorded daily, and the biogas sample was transferred into a Hungate tube using the water displacement method. Finally, the biogas was injected directly into the GC-TCD, where the volume of methane gas was calculated. The initial physical and chemical properties of each treatment were analyzed and tabulated in Table 4.

2.3. Analysis of biogas constituents

The substances of the biogas samples were analyzed using gas chromatography (6890N, Agilent Technologies) equipped with an HP-Molsieve 30.0 m × 530 µm × 50.0 µm nominal with a thermal conductivity detector (TCD). Argon used as a carrier gas at a flow rate of 45 mL/min. The alignment completed with a mixed standard gas composed of 5% CO_2, 2.5% O_2, 5% N_2, and 4% CH_4. The column maintained at 35°C for 7.5 min, then ramped to 230°C at 24°C/min and held at this temperature for 5 min. The temperature of the injector and detector were 100°C and 150°C, respectively, and the analysis performed in triplicates.

2.4. Theoretical yield of methane

The elemental analysis was analyzed using a LECO CHNS-932 analyzer to estimate the percentage content of carbon (C), nitrogen (N), hydrogen (H), and sulfur (S) composition. Based on the percentage of CHNS/O elements in the FPW, the chemical formula of FPW was determined to be $C_{4.563}\,H_{8.636}\,N_{0.1763}\,S_{0.067}\,O_{2.188}$. Nitrogen and sulfur contents were omitted due to their low values; thus, the molecular formula of FPW became $C_{2.085}\,H_{3.947}$ O. Theoretically, 1 mole of FPW will produce 1.3 moles of CH_4 (62%) and 0.8 moles of CO_2 (38%) based on the chemical formula of FPW using the Buswell equation [Eq. (1)] [26,44]. At standard temperature and pressure (STP), the total methane potential (TMP)

Table 4
Characteristics before batch biodegradability test treatment for all different S/I ratios

Substrate to inoculum ratio	Substrate: inoculum (S/I)	pH	BOD (mg/L)	COD (mg/L)	NH_3–N (mg/L)	PO_3–4 (mg/L)	TS (g/L)	VS (g/L)	VS/TS
Control (inoculum only)	0 mL:100 mL	7.00	690	1,970	302	563	18.70	12.75	0.68
S/I 1.0	50 mL:50 mL	7.01	1,140	3,500	172	305	18.20	14.50	0.80
S/I 1.5	60 mL:40 mL	7.00	1,650	4,600	151	270	17.40	14.85	0.85
S/I 2.0	67 mL:33 mL	7.05	1,530	5,100	110	350	17.10	15.09	0.88

calculated from Eq. (2) based on the constants from Eq. (1) to be 640.5 mL/gVS methane yield.

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right) CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \quad (1)$$

$$\text{TMP}_{\text{STP}}\left(\frac{m\text{LCH}_4}{g\text{VS}}\right) = \frac{22.4 \times 1{,}000 \times \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)}{12n + a + 16b + 14c} \quad (2)$$

3. Results and discussion

Results from the four batch tests demonstrated that the FPW substrate is all biodegradable and produced methane rapidly. Fig. 2a illustrates the initial batch serum bottle at 0 d. At the end of batch mode treatment, there was a floating scum layer observed in serum bottles S/I 1.5 and 2.0 (Fig. 2b). A possibility is that the scum layer contains LCFA that is less dense compared to water hence floats. The scum layer was not further analyzed. Based on Gerardi [30], the digester scum layer consists of grease and vegetable oil/fat matter with a specific gravity <0.1 causing the whitish substance to float on top of the water surface.

3.1. COD and BOD removal efficiency

The COD and BOD performances for all different S/I ratios are depicted in Figs. 3a and b. The results revealed that a high percentage of removal for both parameters was above 80% after batch biodegradability treatment (54 d). The highest COD removal recorded was at S/I 1.0, with 96.9% followed by 94.2% and 81.3% for S/I 1.5 and S/I 2.0, respectively. Meanwhile, for BOD_5, the highest removal was at 96.7% for S/I 1.0 and followed by 94.2% and 93.1% for S/I 1.5 and S/I 2.0, respectively. The ratio of 1.0 indicates a balance of substrate and microorganism in the digester that is important in the degradation of complex organic material. The performance of COD removal is better with the S/I ratio 1.0, where complete degradation, which showed no formation of scum layer than S/I 1.5 and 2.0 ratio. Higher S/I ratio resulted in lower removal efficiency indicates that the organic substances in too much for the available bacteria to digest. COD is an indirect measure of the amount of organic matter; thus, the results can estimate the methane yield of the substrate. Methane production can be determined from a COD mole balance because the COD removed would be converted to methane (CH_4) and carbon dioxide (CO_2). Since the high organic matter was digested in this AD process indicated by high removal of COD and BOD_5, it is believed, a high amount of methane can also be produced.

3.2. TS and VS removal efficiency

The particulate organic and solids are the discharged TS within the supernatant. VS is a typical control parameter measured in biological treatments that approximates the organic matter presents in the waste available for degradation [30]. Based on Fig. 4a, the TS for all S/I ratios remained between 50% and 80% removal efficiency, where there was a significant organic solid and volatile reduction. Meanwhile, the VS removal was slightly lower for blank (control) at about 30% (Fig. 4b), in which higher S/I ratios resulted in less removal efficiency for both TS and VS. Nonetheless, the TS finals values for the substrate to inoculum ratio (S/I) of 1.5 and 2.0 do not pass the effluent DOE standard [45] of less than 5,000 mg/L. High FPW substrate to inoculum ratio caused incomplete organic matter degradation, which may require more than 54 d for complete degradation. Methane production is directly related to VS degradation. The methane yield increased with higher VS and less hemicellulose [46,47]. According to Gerardi [48]; however, higher VS feed to the digester could result in the formation of the more significant amount of volatile acids in the digester and severely impact on the alkalinity and pH. Referring to Fig. 4b, VS can be underestimated due to possible VFA losses during the analysis of TS [42].

3.3. Ammonia-nitrogen content

Fig. 5 shows the lowest increment recorded was S/I 1.0, with a 5.5% increment followed by 35.5% and 71.7% for S/I 1.5 and S/I 2.0, respectively. Higher substrate resulted

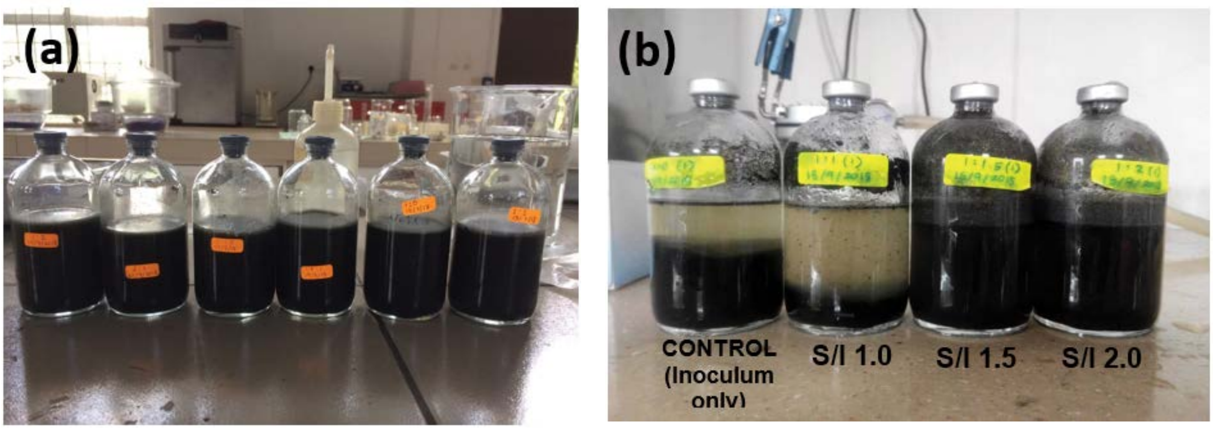

Fig. 2. (a) Day 0 and (b) day 54 of the batch biodegradability test.

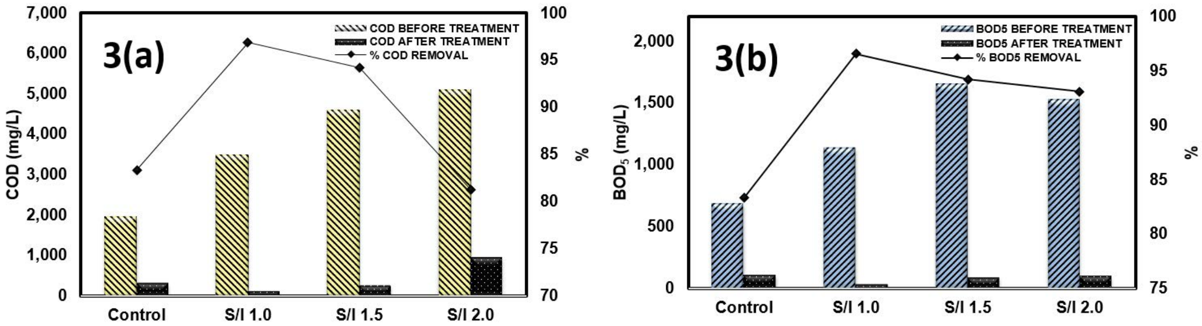

Fig. 3. (a) COD percentage removal and (b) BOD_5 percentage removal before and after batch biodegradability treatment for all S/I ratios.

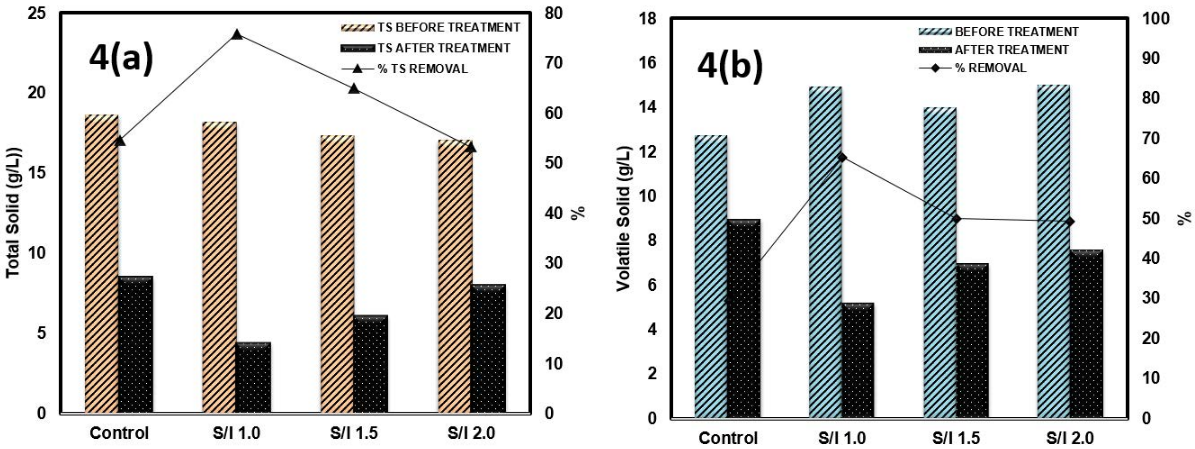

Fig. 4 (a) TS and (b) VS percentage removal before and after batch biodegradability treatment for all S/I ratios.

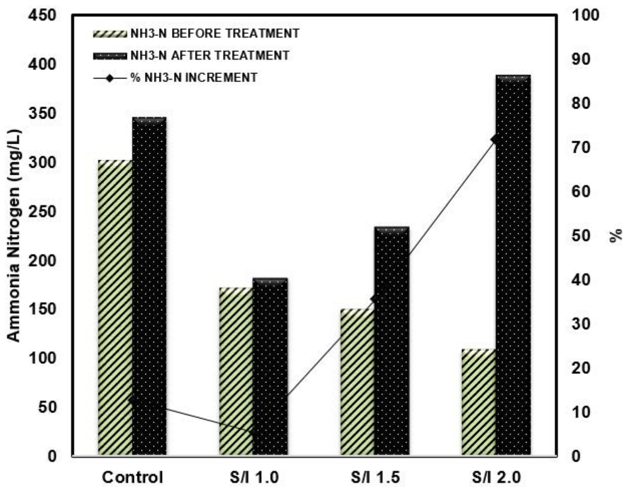

Fig. 5. Ammonia-nitrogen percentage removal before and after batch biodegradability treatment for all S/I ratios.

in higher final ammonia-nitrogen, which shows that the nutrient content in the substrate gave a significant impact on ammonia-nitrogen accumulation. It is possible that the high protein content in FPW has converted into amino acids by the microbes resulting in high ammonia-nitrogen concentration [49]. Proteins are polypeptides formed through the linkages into peptide bonds with amino acids. Food or food processing waste from chicken, meat, casein, fish, whey, cheese, and eggs contain significant amounts of protein [50]. Since the origin of the substrate used in this study was from the meat processing industry (chicken, cow, fish, and lamb) that produced meat products such as nugget, sausages, and a burger patty, the substrate may contain high lipids, carbohydrate, and proteins in its effluent. Frequently, high FPW protein content [9] would result in an augmented amount of free ammonia that causes a drop in pH, leading to methanogenic inhibition and less biogas production [28,51]. Interestingly, the control experiment demonstrated a moderately higher increment than S/I 1.0 with the value of a 12.7% increment. It is believed that the presence of high ammonia concentration in the inoculum gave this effect.

During anaerobic degradation of proteins, hydrogen-producing bacteria hydrolyze proteins into polypeptides and amino acids by secreting protease enzyme into the process. The hydrolysis is carried out by proteases excreted by microorganisms where amino acids are further brake down into volatile fatty acids (VFA), carbon dioxide (CO_2), hydrogen (H_2), ammonia, and reduced sulfur [50,52,53]. The rate of protein degradation is slower than carbohydrate and lipid degradation. Evidence has also shown that protein degradation is incomplete during the complete digestion at times [53]. The release of ammonium during the hydrolysis of the organic nitrogen compound present in the feedstock predicts protein degradation in the digestion [45,46]. The results of batch biodegradability test anaerobic assay revealed that the ammoniacal nitrogen values ranged between 180 and 390 mg/L for all S/I ratios. The result was under the threshold of inhibition, and there was no adverse effect indicating the AD is stable [34,55]. The cause of % increment ammoniacal ammonia has a relation with the anaerobes wellbeing in the AD digestion and also the type of substrate used. According to Kayhanian [32], methanogens are the least tolerant and the most likely to cease growth due to ammonia inhibition. Among the anaerobic degrading microorganisms, methanogens (Euryarchaeota) are reported to be the most affected groups by elevated ammonia levels (>1,800 mg/L) and the first to be inhibited [56–58]. As such, this resulted in low methane production due to higher S/I ratios in this study.

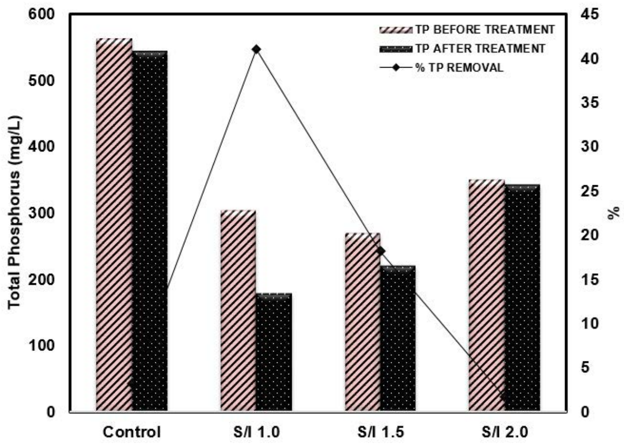

Fig. 6. Total phosphorus removal before and after batch biodegradability test for all S/I ratios.

3.4. Total phosphorus removal efficiency

Macronutrients, including nitrogen and phosphorus, are nutrients that are required in relatively large quantities by all bacteria. Microbial incorporation of phosphorus in anaerobic digestion has reported approximately 1/5 to 1/7 of that established for nitrogen [33]. Total phosphorus exists in the form of soluble and particulate phosphorus. The main components of phosphorus in wastewater exist predominantly as orthophosphate and less amount of organic phosphorus [59]. Phosphorous, similar to nitrogen, is an essential nutrient for biological metabolism. As such, the balanced existence of this element is an excellent factor for every anaerobic process [60]. Thus, total phosphorus concentration was measured before and after batch biodegradability treatment. Fig. 6 illustrates that total phosphorus concentration decreased with higher S/I ratios, and high phosphorus concentration found in control (blank) due to the origin of inoculum, which contained a high concentration of total phosphorus. The highest total phosphorus removal recorded was for S/I 1.0, with 41% removal followed by 18.1% and 1.71% for S/I 1.5 and S/I 2.0, respectively. The AD performance in the poor quality of supernatant, which may contain relatively high concentrations of nutrients (nitrogen and phosphorus). Initially, the total phosphorus was ranged between 300 and 600 mg/L and remained similar after batch biodegradability treatment, except for a slight reduction at 40% for S/I 1.0.

3.5. Specific biogas production yield and cumulative biogas production

Fig. 7 illustrates the cumulative biogas production and each S/I yield during the 54 d of conducting the assay. The accumulated biogas for control was below 100 mL. On day

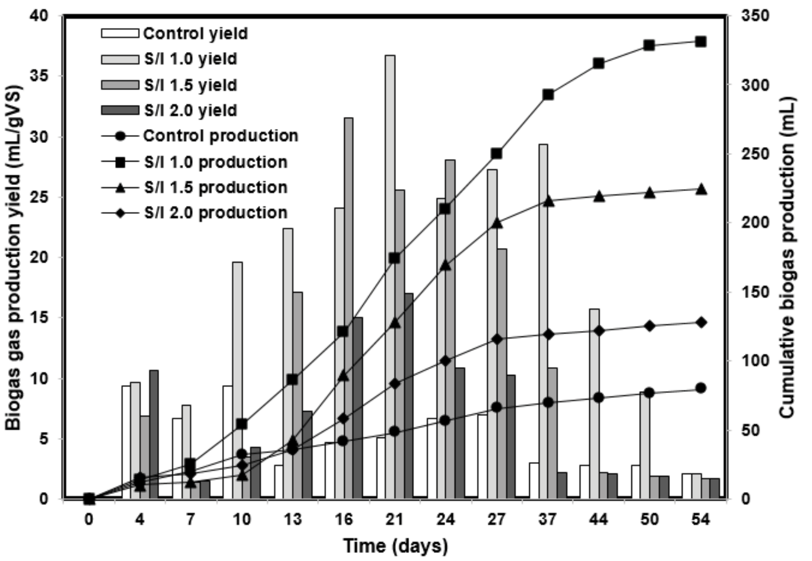

Fig. 7. Specific biogas production yield and cumulative biogas production at different S/I ratios.

10, S/I 1.0 demonstrated the rapid production of biogas then started to slow down at day 37 due to all organic matter mostly consumed by the anaerobes. S/I 1.0 accumulated the highest biogas at 331.5 mL. Meanwhile, S/I 2.0 produced the lowest biogas cumulative amount of 127.6 mL, only increased by 48.3% compared to the control sample. It can be depicted from the trend in for biogas accumulation that there was biogas production delay of 10 d for S/I ratio 1.5 and 2.0. The results could be due to the incomplete phase change from acidogenesis to the methanogenic stage [61] brought about unbalance amount of anaerobes in the inoculum and substrate. The higher ratio of FPW in S/I 1.5 and 2.0 lead to incomplete degradation of the grease; thus lower amount of biogas produced, compounded by the possible inhibition factor of LCFA, which caused poor biodegradability [21].

The degradation of lipid-rich materials (LCFA) is known as an inhibitor of methanogenic bacteria, causing the delay of methane production, hence biogas production is also affected. LCFA deposition inhibits acetoclastic and methanogenic archaea growth disrupting their metabolism, which slows down hydrolysate conversion into methane [18,62,63]. However, researchers first suggested that the mechanism for LCFA inhibition of methanogenic and acetogenic bacteria was due to a surfactant effect causing the LCFA to damage the cell membrane [64]. Multiple studies have suggested maximum concentrations of LCFAs, above which anaerobic digesters are likely to experience excessive methanogen inhibition [17,65,66].

The different substrate to inoculum S/I ratios conducted with the batch biodegradability test revealed that the VS content influenced biogas potential in FPW. The highest accumulated specific biogas yield was 228.6 mL/gVS in the substrate to the inoculum ratio of S/I 1.0, while the lowest specific biogas production rate was 84.6 mL/gVS for S/I 2.0 which shows less than 63% lower than S/I 1.0. As depicted in Fig. 7, on day 21, S/I 1.0 produced the maximum biogas yield of 36.8 mL/gVS, followed by 31.6 mL/gVS (S/I 1.5) and 17 mL/gVS (S/I 2.0). According to Bornare et al. [67] in his previous study, the decreased in biogas yield occurred when increasing the OLR. At lower OLR, a better food-to-microorganism (F/M) ratio ensured high production of biogas yield. In this study, S/I 1.0 had lower initial COD value (similar to lower OLR concept) compared to S/I of 1.5 and 2.0 which promote a better F/M ratio for the methanogenic degradation.

3.6. Specific methane production yield and cumulative biogas production

The amount of cumulative methane produced started to increase on the day 7 (S/I 1.0), day 10 (S/I 1.5) and day 13 (S/I 2.0) with cumulative methane production of 200.5 mL/gVS, 116.8 mL/gVS and 54.4 mL/gVS, respectively as depicted in Fig. 8. Nevertheless, after the acclimatization period, fast production of CH_4 indicates that the existence of archaea that can digest the accumulated LCFA and thus, produce methane rapidly. The extended retention time is required to complete degradation to facilitate anaerobic consortium adaptation. According to a previous study by Angelidaki et al. [68], the occurrence where higher substrates volume may decrease methane yield initially or gradually, thus delaying the entire AD process, is called lag phase phenomena. Ibrahim [69] applied a two-stage anaerobic system for the treatment of the FPW substrate resulted in the accumulation of suspended solids (SS) and floating fats in the reactor. Consequently, this inhibited the methanogenic activity, thus lowered the production of methane gas. A similar occurrence was observed in this study, where both S/I ratios of 1.5 and 2.0 containing higher substrate produced lower methane compared to S/I of 1.0.

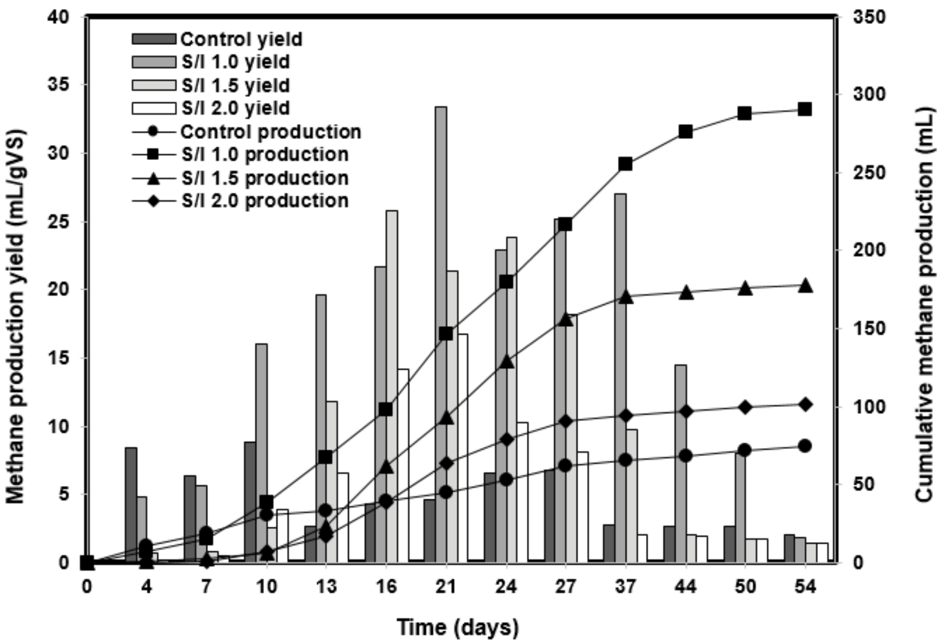

Fig. 8. Specific methane production yield and cumulative methane production at all S/I ratios.

Table 5
List of previous studies based on similar substrates and methane yield achieved

	Inoculum	Substrate	S/I ratio	Methane yield ($mLCH_4$/gVS added)
This study	Anaerobic sludge from WWTP	Food processing wastewater (FPW)	1.0	200
			1.5	120
			2.0	54
[37]	Palm oil mill sludge	Grease trap waste (GTW)	0.2	370
			0.5	500
			1.0	360
			2.0	250
			4.0	180
[71]	Anaerobic sludge from WWTP	Fat, oil and grease (aqueous fraction from GTW)	0.5	383
			1.0	63

These findings were in agreement with a previous study by Kougias et al. [70] that reported using protein and lipid wastewater could lead to a build-up of foam and affect the AD process. The methane yield was calculated by dividing methane production with the weight, gram of mixed sludge and substrate added in VS basis. Fig. 8 shows lag phase phenomena for S/I 1.5 and 2.0 degradation when the methane only started to be produced on day 10. Meanwhile, S/I 1.0 treatment methane yield starts as soon as day 4. The control treatment shows high methane production yield 6–8 mL/gVS until day 10 but reduces almost 60% on day 13 and fluctuated below 6 mL/gVS until at the end of batch biodegradability test. S/I 1.0 resulted in enhanced degradation with the highest cumulative methane yield of 200.5 mL/gVS, followed by S/I of 1.5 at 119.9 mL/gVS methane. S/I 2.0 produced the lowest methane with the methane yield of 67.5 mL/gVS. Besides, the increased of S/I ratio shows the decreased methane production. During the methanogenesis phase, acetate and H_2 were transformed into CH_4 and CO_2 by acetoclastic methanogens and hydrogen-utilizing methanogens archaea. The result from methane production indicates that S/I 1.0 is ideal for the archaea to rapidly-produce methane and carbon dioxide at the end of the process. Meanwhile, for S/I 1.5 and 2.0, the archaea were required to adapt to the slow hydrolysis process for the proteins to be degraded into simpler monomers.

Low S/I ratio indicates lower LCFA volume, thus indicating a massive adaptation of microorganism to O&G content, which will achieve higher methane yield in contrast to high S/I ratios [29]. According to Champagne et al. [71], the ideal substrate to the inoculum (S/I) ratio was 0.25–0.75 for FOG substrates, meanwhile for kitchen waste were S/I ratio 0.80–1.26 with 383 ml CH4/g VS methane production yield. It is believed that LCFA and volatile fatty acids (VFA) accumulated in the batch biodegradability test preliminary studies inhibited the methanogenesis. On that account, hydrolysis was the limiting step throughout the process, which is known as lag phase phenomena. Delay of hydrolysis might be due to the presence of inhibitor, and it is expected as LCFA accumulation since FPW has high O&G content. According to Nazaitulshila et al. [37], the biomass adaptation to LCFA is also crucial for the anaerobic treatment of lipids. Contradicting results were found by Loustarinen et al. [21], where the methane yield increased by 60% when the inoculum was co-digested with fat, oil, and grease (FOG) substrate at S/I ratio above 1.0, both originated from a meat processing plant. The bacterial colony in this study may have not fully acclimatized to degrade the FPW substrate since the inoculum used in this study was taken from a municipal treatment plant instead. A proper acclimatization period is required for the bacteria to adapt in a new environment [72]. At standard temperature and pressure (STP), the total methane potential (TMP) calculated from Eq. 2 based on the constants from Eq. (1) to be 640.5 mL/gVS methane yield, but the maximum methane yield achieved in the study was only 200.5 mL/gVS, 31.3% of the calculated value. However, the results are affected by the inhibition of methanogenic activities and the balance of F/M ratios from S/I 1.0 gave the highest accumulated methane production from other ratios. There is an agreement that during AD, 10% of the substrate is for biomass growth and transformation into heat [73,74]. Table 5 summarizes the findings of previous studies on methane yield of various food processing wastes. The studies revealed that methane content ranged between 40% and 60%. A similar trend of reduced yield at S/I ratio above 0.5 was found by Weiland [25] when using palm oil mill sludge with GTW. However, contradicting result was found by Chen et al. [54] where lower S/I ratio resulted in better yield. It is believed that the inoculum used, anaerobic sludge from MWTPs could adapt well the aqueous fraction in GTW. It is known that the MWTPs in Johor, Malaysia, also receives wastewater from establishments where the municipal wastewater is mixed with kitchen wastewater [75].

4. Conclusion

S/I 1.0 ratio was ideal for high removal of COD, BOD, TS and VS at 96.9%, 96.6%, 75.8%, 65.2%, respectively. While, the ammonia concentration lowest increment recorded was S/I 1.0, with a 5.5% increment followed by 35.5% and 71.7% for S/I 1.5 and S/I 2.0. The maximum amount of accumulated biogas produced was 331.5 mL in the S/I 1.0 coinciding with the highest methane accumulation of 290.7 mL. Only 31.3% of the theoretical yield methane production at 200.5 mL/gVS was found at the best S/I of 1.0. While the lowest methane accumulation was only 54.4 mL at S/I ratio of 2.0. The amount of cumulative methane produced started to increase on day 7 (S/I 1.0). The higher S/I of 1.5 and 2.0 delayed the biogas and methane production after 10 d. The results indicated that the anaerobic inoculum sludge from a municipal wastewater treatment plant is suitable for the treatment of FPW and a promising method to produce biogas with high methane content. Nonetheless, further studies are required to investigate the approaches to increase the hydrolysis efficiency and optimize the ultimate methane production from high O&G FPW.

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References

[1] K. Hagos, J. Zong, D. Li, C. Liu, X. Lu, Anaerobic co-digestion process for biogas production: progress, challenges and perspectives, Renewable Sustainable Energy Rev., 76 (2017) 1485–1496.
[2] P. Kumaran, D. Hephzibah, R. Sivasankari, N. Saifuddin, A.H. Shamsuddin, A review on industrial scale anaerobic digestion systems deployment in Malaysia: opportunities and challenges, Renewable Sustainable Energy Rev., 56 (2016) 929–940.
[3] M. Maaz, M. Yasin, M. Aslam, G. Kumar, A.E. Atabani, M. Idrees, F. Anjum, F. Jamil, R. Ahmad, A.L. Khan, G. Lesage, M. Heran, J. Kim, Anaerobic membrane bioreactors for wastewater treatment: novel configurations, fouling control and energy considerations, Bioresour. Technol., 283 (2019) 358–372.
[4] H.H.P. Fang, Environmental Anaerobic Technology–Applications and New Developments, Imperial College Press, 2010.
[5] Z. Zahan, M.Z. Othman, W. Rajendram, Anaerobic codigestion of municipal wastewater treatment plant sludge with food waste: a case study, Biomed Res. Int., 2016 (2016) 1–13.
[6] M.F. Osman, Personal Communication, Selangor, Malaysia, August 2018.
[7] J. Kuo, J. Dow, Biogas production from anaerobic digestion of food waste and relevant air quality implications, J. Air Waste Manage. Assoc., 67 (2017) 1000–1011.
[8] J.H. Long, T.N. Aziz, F.L. de los Reyes III, J.J. Ducoste, Anaerobic co-digestion of fat, oil, and grease (FOG): a review of gas production and process limitations, Process Saf. Environ. Prot., 90 (2012) 231–245.
[9] E. Klaucans, K. Sams, Problems with fat, oil, and grease (FOG) in food industry wastewaters and recovered FOG recycling methods using anaerobic co-digestion: a short review, Key Eng. Mater., 762 (2018) 61–68.
[10] D. Brown, Y. Li, Solid state anaerobic co-digestion of yard waste and food waste for biogas production, Bioresour. Technol., 127 (2013) 275–280.
[11] T. Forster-Carneiro, M. Pérez, L.I. Romero, Influence of total solid and inoculum contents on performance of anaerobic reactors treating food waste, Bioresour. Technol., 99 (2008) 6994–7002.
[12] A. Mudhoo, Biogas Production: Pretreatment for Enhanced Anaerobic Digestio, Scrivener Publisher, New Jersey, 2012.
[13] S.B. Mahat, R. Omar, A. Idris, S.M. Mustapa Kamal, A.I. Mohd Idris, Dynamic membrane applications in anaerobic and aerobic digestion for industrial wastewater: a mini review, Food Bioprod. Process., 112 (2018) 150–168.
[14] N.S.A. Mutamim, Z.Z. Noor, M.A.A. Hassan, A. Yuniarto, G. Olsson, Membrane bioreactor: applications and limitations in treating high strength industrial wastewater, Chem. Eng. J., 225 (2013) 109–119.
[15] N. Rasit, A. Idris, R. Harun, W.A.W.A.K. Ghani, Effects of lipid inhibition on biogas production of anaerobic digestion from oily effluents and sludges: an overview, Renewable Sustainable Energy Rev., 45 (2015) 351–358.
[16] M.H. Gerardi, Wastewater Bacteria, John Wiley & Sons, Inc., Pennsylvania, 2006.
[17] M.M. Alves, J.A.M. Vieira, R.M.A. Pereira, M.A. Pereira, M. Mota, Effects of lipids and oleic acid on biomass development in anaerobic fixed-bed reactors, Part II: oleic acid toxicity and biodegradability, Water Res., 35 (2001) 264–270.
[18] R. Rodríguez-méndez, Y.L. Bihan, F. Béline, P. Lessard, Long chain fatty acids (LCFA) evolution for inhibition forecasting during anaerobic treatment of lipid-rich wastes : case of milk-fed veal slaughterhouse waste, Waste Manage., 67 (2017) 51–58.
[19] D.Z. Sousa, M.A. Pereira, A.J.M. Stams, M.M. Alves, H. Smidt, Microbial communities involved in anaerobic degradation of unsaturated or saturated long-chain fatty acids, Appl. Environ. Microbiol., 73 (2007) 1054–1064.
[20] C. Gallert, J. Winter, Environmental Biotechnology: Bacterial Metabolism in Wastewater Treatment Systems, John Wiley and Sons, 2005, pp. 1–48.
[21] S. Luostarinen, S. Luste, M. Sillanpää, Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant, Bioresour. Technol., 100 (2009) 79–85.
[22] J.C. Kabouris, U. Tezel, S.G. Pavlostathis, M. Engelmann, J. Dulaney, R.A. Gillette, A.C. Todd, Methane recovery from the anaerobic codigestion of municipal sludge and FOG, Bioresour. Technol., 100 (2009) 3701–3705.
[23] C. Muller, P. Lam, E. Lin, T. Chapman, D. Devin-Clark, J. Belknap-Williamson, S. Krugel, Co-digestion at Annacis Island WWTP: metro Vancouver's path to renewable energy and greenhouse gas emissions reductions, Proc. Water Environ. Fed., 14 (2010) 2706–2722.
[24] R.K. Dereli, B. Heffernan, A. Grelot, F.P. Van Der Zee, J.B. Van Lier, Influence of high lipid containing wastewater on filtration performance and fouling in AnMBRs operated at different solids retention times, Sep. Purif. Technol., 139 (2015) 43–52.
[25] C. Ramos, A. García, V. Diez, Performance of an AnMBR pilot plant treating high-strength lipid wastewater: biological and filtration processes, Water Res., 67 (2014) 203–215.
[26] F.M. Pellera, E. Gidarakos, Effect of substrate to inoculum ratio and inoculum type on the biochemical methane potential of solid agroindustrial waste, J. Environ. Chem. Eng., 4 (2016) 3217–3229.
[27] P. Weiland, Biogas production : current state and perspectives, Appl. Microbiol. Biotechnol., 85 (2010) 849–860.
[28] R.M. Jingura, R. Kamusoko, Methods for determination of biomethane potential of feedstocks: a review, Biofuel Res. J., 4 (2017) 573–586.
[29] D.G. Mulat, J. Dibdiakova, S.J. Horn, Microbial biogas production from hydrolysis lignin: insight into lignin structural changes, Biotechnol. Biofuels, 11 (2018) 1–16.
[30] M.H. Gerardi, The Microbiology of Anaerobic Digesters: Mixing, John Wiley & Sons, Inc., New Jersey, 2003, p. 117–119.
[31] E. Kwietniewska, J. Tys, Process characteristics, inhibition factors and methane yields of anaerobic digestion process, with particular focus on microalgal biomass fermentation, Renewable Sustainable Energy Rev., 34 (2014) 491–500.
[32] M. Kayhanian, Performance of a high-solids anaerobic digestion process under various ammonia concentrations, J. Chem. Technol. Biotechnol., 59 (1994) 349–352.
[33] C.A.L. Chernicharo, Biological Wastewater Treatment Series Vol. 4: Anaerobic Reactors, London, New York, 2007.
[34] P.L. Mccarty, Anaerobic Waste Treatment Fundamentals Part Three: Toxic Materials and their Control, Public Work, Stanford, 1964, pp. 91–94.
[35] W.F. Owen, D.C. Stuckey, J.B. Healy, L.Y. Young, P.L. McCarty, Bioassay for monitoring biochemical methane potential and anaerobic toxicity, Water Res., 13 (1979) 485–492.

[36] I.M. Nasir, Anaerobic Digestion for Cattle Manure Treatment and its Kinetic Modelling in an Oscillatory Flow Biogas Reactor, Universiti Putra Malaysia (UPM) Thesis, Malaysia, 2016.
[37] R. Nazaitulshila, A. Idris, R. Harun, W.A.W.A.K. Ghani, The influence of inoculum to substrate ratio on the biochemical methane potential of fat, oil, and grease in batch anaerobic assays, Energy Sources Part A, 37 (2015) 590–597.
[38] APHA, Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, Washington, DC, 2005.
[39] A.V. Leena, C. Meiaraj, N. Balasundaram, BOD/COD a measure of dairy waste treatment efficiency–a case study, IOSR J. Mech. Civ. Eng., 13 (2016) 107–114.
[40] T.L. Hansen, J.E.Schmidt, I. Angelidaki, E. Marca, J.C. Jansen, H. Monsbaek, T.H. Christensen, Method for determination of methane potentials of solid organic waste, Waste Manage., 24 (2004) 393–400.
[41] C. Holliger, M.S. Alves, D. Andrade, I. Angelidaki, S. Astals, U. Baier, C. Bougrier, P. Buffière, M. Carballa, V. de Wilde, F. Ebertseder, B. Fernández, E. Ficara, I. Fotidis, J.-C. Frigon, H. Fruteau, D.S.M. Ghasimi, G. Hack, M. Hartel, J. Heerenklage, I.S. Horváth, P. Jenicek, K. Koch, J. Krautwald, J. Lizasoain, J. Liu, L. Mosberger, M. Nistor, H. Oechsner, J. Oliveira, M. Paterson, A. Pauss, P. Sébastien, I. Porqueddu, F. Raposo, T. Ribeiro, F. Rüsch, S. Strömberg, M. Torrijos, M.H.A. van Eekert, H. Wedwitschka, I. Wierinck, Towards a standardization of biomethane potential tests, Water Sci. Technol., 74 (2016) 2515–2522.
[42] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, J.B. van Lier, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays, Water Sci. Technol., 59 (2009) 927–934.
[43] N. Khairul Anuar, H. Che Man, S. Idrus, N.N. Nik Daud, Biochemical methane potential (BMP) from anaerobic co-digestion of sewage sludge and decanter cake, IOP Conf. Ser. Mater. Sci. Eng., 368 (2018).
[44] A.M. Buswell, S.L. Neave, Laboratory Studies of Sludge Digestion, Bulletin No. 30, Illinois Division of State Water Survey, 1930.
[45] EQA, Sewage Characteristics and Effluent Discharge Requirements, Volume 4, Section 3, Malaysian Sewerage Industry Guidelines, Environmetal Quality Act, Malaysia, 1974.
[46] L. Moody, R. Burns, W. Wu-Haan, R. Spajić, Use of Biochemical Methane Potential (BMP) Assays for Predicting and Enhancing Anaerobic Digester Performance, Proceedings of the 44th Croatian and the 4th International Symposium on Agriculture, Opatija, Croatia, 16-20 February 2009.
[47] W.J. Gao, X. Qu, K.T. Leung, B.Q. Liao, Influence of temperature and temperature shock on sludge properties, cake layer structure, and membrane fouling in a submerged anaerobic membrane bioreactor, J. Membr. Sci., 421–422 (2012) 131–144.
[48] M.H. Gerardi, Wastewater Bioaugmentation and Biostimulation, DEStech Publications Inc., Pennsylvania, 2016.
[49] G.N. Demirer, S. Chen, Two-phase anaerobic digestion of unscreened dairy manure, Process Biochem., 40 (2005) 3542–3549.
[50] N.H.M. Yasin, T. Mumtaz, M.A. Hassan, N.A. Rahman, Food waste and food processing waste for biohydrogen production: a review, J. Environ. Manage., 130 (2013) 375–385.
[51] A. Khalid, M. Arshad, M. Anjum, T. Mahmood, L. Dawson, The anaerobic digestion of solid organic waste, Waste Manage., 31 (2011) 1737–1744.
[52] J.P.Y. Jokela, J.A. Rintala, Anaerobic solubilisation of nitrogen from municipal solid waste (MSW), Rev. Environ. Sci. Biotechnol., 2 (2003) 67–77.
[53] G. Vidal, A. Carvalho, R. Méndez, J.M. Lema, Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters, Bioresour. Technol., 74 (2000) 231–239.
[54] K. Fricke, H. Santen, R. Wallmann, A. Hu, N. Dichtl, N.O. Dinitrous, Operating problems in anaerobic digestion plants resulting from nitrogen in MSW, Waste Manage., 27 (2007) 30–43.
[55] O. Yenigün, B. Demirel, Ammonia inhibition in anaerobic digestion: a review, Process Biochem., 48 (2013) 901–911.
[56] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: a review, Bioresour. Technol., 99 (2008) 4044–4064.
[57] M. Kayhanian, Ammonia inhibition in high-solids biogasification : an overview and practical solutions, Environ. Technol., 20 (1999) 355–365.
[58] Q. Niu, T. Hojo, W. Qiao, H. Qiang, Y.Y. Li, Characterization of methanogenesis, acidogenesis and hydrolysis in thermophilic methane fermentation of chicken manure, Chem. Eng. J., 244 (2014) 587–596.
[59] K. Shahot, A. Idris, R. Omar, H.M. Yusoff, Review on biofilm processes for wastewater treatment, Life Sci. J., 11 (2014) 1–13.
[60] R.P. Singh, S. Kumar, C.S. Ojha, Nutrient requirement for UASB process: a review, Biochem. Eng. J., 3 (1999) 35–54.
[61] P.H.L. Nguyen, P. Kuruparan, C. Visvanathan, Anaerobic digestion of municipal solid waste as a treatment prior to landfill, Bioresour. Technol., 98 (2007) 380–387.
[62] I.H. Franke-whittle, A. Walter, C. Ebner, H. Insam, Investigation into the effect of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic communities, Waste Manage., 34 (2014) 2080–2089.
[63] S.A. Silva, A.F. Salvador, A.J. Cavaleiro, M.A. Pereira, A.J.M. Stams, M.M. Alves, D.Z. Sousa, Toxicity of long chain fatty acids towards acetate conversion by *Methanosaeta concilii* and *Methanosarcina mazei*, Microbiol. Biotechnol., 9 (2016) 514–518.
[64] H. Galbraith, T.B. Miller, Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts, J. Appl. Microbiol., 36 (1973) 647–658.
[65] H.S. Shin, S.H. Kim, C.Y. Lee, S.Y. Nam, Inhibitory effects of long-chain fatty acids on VFA degradation and β-oxidation, Water Sci. Technol., 47 (2003) 139–146.
[66] M.A. Pereira, A. Cavaleiro, M. Mota, M.S. Alves, Accumulation of long chain fatty acids onto anaerobic sludge under steady state and shock loading conditions : effect on acetogenic and methanogenic activity, Water Sci. Technol., 48 (2003) 33–40.
[67] J.B. Bornare, U.S. Adhyapak, G.P. Minde, V.K. Raman, V.S. Sapkal, R.S. Sapkal, Submerged anaerobic membrane bioreactor for wastewater treatment and energy generation, Water Sci. Technol., 71 (2015) 1654–1660.
[68] I. Angelidaki, J.E. Schmidt, L. Ellegaard, B.K. Ahring, Methods an automatic system for simultaneous monitoring of gas evolution in multiple closed vessels, J. Microbiol. Methods, 33 (1998) 93–100.
[69] M.S.S. Ibrahim, Treatment of Food Processing Industrial Wastewater Using Two Stages Anaerobic System, Thesis MSc. Faculty of Civil and Environmental Engineering Universiti Tun Hussein Onn Malaysia, Malaysia, 2014.
[70] P.G. Kougias, K. Boe, S.O.-Thong, L.A. Kristensen, I. Angelidaki, Anaerobic digestion foaming in full-scale biogas plants : a survey on causes and solutions, Water Sci. Technol., 69 (2014) 889–895.
[71] C. Li, P. Champagne, B.C. Anderson, Evaluating and modeling biogas production from municipal fat, oil, and grease and synthetic kitchen waste in anaerobic co-digestions, Bioresour. Technol., 102 (2011) 9471–9480.
[72] R. Alkarimiah, S.B. Mahat, A. Yuzir, M.F.M. Din, S. Chelliapan, Performance of an innovative multi-stage anaerobic reactor during start-up period, Afr. J. Biotechnol., 10 (2011) 11294–11302.
[73] R.P. Rodrigues, D.P. Rodrigues, A. Klepacz-Smolka, R.C. Martins, M.J. Quina, Comparative analysis of methods and models for predicting biochemical methane potential of various organic substrates, Sci. Total Environ., 649 (2019) 1599–1608.
[74] J. Filer, H.H. Ding, S. Chang, Biochemical methane potential (BMP) assay method for anaerobic digestion research, Water, 11 (2019).
[75] M.S.S. Ibrahim, A. Aziz, A. Latiff, Z. Daud, Preliminary study : treatment of food industry wastewater using two-phase anaerobic treatment system, Int. J. Integr. Eng., 5 (2013) 1–7.