

CO-DIGESTION OF GREASY WASTE AND MBR SLUDGE USING BIO-AUGMENTED PSEUDOMONAS PUTIDA: EFFECT OF BUFFERING AGENTS

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Received Date: August 28, 2020; Revised Date: April 8, 2021; Acceptance Date: May 13, 2021

Abstract

This study aims to investigate the bio-augmentation of *Pseudomonas putida* for initializing fat, oil, and grease (FOG) biodegradation in a co-digestion of the greasy waste and the membrane bioreactor (MBR) excess sludge. The MBR sludge could be an additional nitrogen source concurrently as a waste matrix fluidizer. Three rounds of a fed-batch of an HRT of 56-71 days were carried out. The first (B1) and second (B2) batches using phosphate (Na_2HPO_4) solution as a buffering system. It showed that the digesters with bio-augmented *P. putida* could remove more FOG than that of the control. In the third batch (B3), the buffering solution was changed to bicarbonate (NaHCO_3) with the interval bio-augmentation of *P. putida* in different doses; the 250 ml (B3, T-250) and the 1000 ml (B3, T-1000) of *P. putida* culture every two weeks dosing. The highest FOG removal was found in both conditions with the removal efficiency of 79.77%. This study suggests that bicarbonate was a suitable buffer in the co-digestion process for the interval bio-augmentation of *P. putida*. Moreover, it showed that a higher interval dose (B3, T-1000) of *P. putida* gave a more methane production rate of 981.08 ml/g TVS. d compared to the lower dose of B3(T-250) with 353.75 ml/g TVS. d.

Keywords: Anaerobic, Biogas, Co-digestion, Pseudomonas putida, Oil/Grease, Sludge, Waste

Introduction

Biogas is a renewable energy that promises to be a good substitution for traditional fossil fuels. Methane bio-production is a complex, multi-step process, involving many different microbial species [1,2]. Renewable energies offer an environmentally sound alternative to fossil fuels and account for a lesser contribution to climate change [1, 2]. Fats/oils/grease (FOG) waste is a common waste produced from food preparation/production by households, restaurants, schools, hospitals, and other food service facilities. FOG or greasy waste is defined as a top floatable layer of wastewater, which is high lipid content and could be separated from water by a grease trap tank. In Thailand, the grease waste-collecting from a grease trap unit has been commonly disposed of with other municipal solid wastes in landfilling. Nevertheless, the greasy waste leaking during a waste transferring process and at a transfer station create many problems, such as clogging pipes/pumping systems, interfering with an on-site wastewater treatment process [2]. Thus, greasy treatment is an alternative option for greasy waste management. Due to its characteristics particularly for high viscosity, aerobic treatment is not an appropriate option for this kind of waste.

On the contrary, anaerobic digestion of oily waste is a potential option because the organic fatty acids from lipids hydrolysis/acidogenesis are a carbon source of methanogen. Anaerobic digestion is commonly applied for treating high organic content waste, which a preferable by-product as biogas is generated. The biogas from the typical digestion gas energy recovery systems can recover the energy required for wastewater treatment plants that use the activated sludge process [1, 2].

Theoretically, lipids can generate more methane than proteins and carbohydrates [3]. Methane potential yield of lipids (1000 ml/g.VS), proteins (480 ml/g.VS), and carbohydrate (373 ml/g.VS) has been well reported [4, 5]. Nevertheless, in case some kind of waste in which their properties are not appropriate in anaerobic treatment process such as too high/low pH, too high/low C/N ratio, etc., thus a co-digestion is an optional adjustment of the waste properties of the feed. Digestion of more than one substrate in the same digester can establish positive synergism and the added nutrients can support microbial growth [6, 7]. Different materials can enhance the anaerobic digestion process due to a better carbon and nutrient balance [8, 9, 10]. For the above reason, this study proposed the treatment of greasy waste via co-digestion of bio-solids from a wastewater treatment plant namely the membrane bioreactor (MBR) sludge.

Bio-augmentation is a strategy for process intensification and high-rate methane recovery from AD in industrial wastes. The typical strains used for AD are *Clostridium*, *Acetobacterium*, *Enterobacter* and *Syntrophomonas* [9, 10]. Several species in the *Pseudomonas* genus are facultative anaerobes (e.g., *P. aeruginosa*, *P. fluorescens*, *P. denitrificans*), suggesting that the strictly aerobic *P. putida* could be designed towards a micro-aerobic or even facultative anaerobic lifestyle with a limited number of genetic modifications. The latter has been experimentally attempted several times by engineering either anaerobic fermentation or anaerobic respiration [11].

The first attempt to create a *P. putida* strain capable of anaerobic fermentation was by Sohn et al. 2014 [12]. Moreover, *Pseudomonas* sp., a lipase producer, can remove such oils at the mesophilic temperature range in a short retention time; and the increase of the oil concentration and the oil type do not affect the yield [13]. Some strains of *Pseudomonas putida* showed a high capability in crude oil degradation [14]. Thus, it was selected as a bio-augmented microbe for initiating oil/grease biodegradation in the co-digestion process in this study. The efficiency of oil/grease removal in the co-digestion of the greasy waste and the MBR sludge with and without *P. putida* bio-augmentation had been comparable. It was reported that the C: N: P ratio of *Pseudomonas* sp. varies between 52:8:1 to 163:25:1 when N:P<40:1 and N:P >40:1 in culture medium, respectively [15]. Because greasy waste contains lower nutrients including phosphorus which is an essential element for microbial growth and high VFA production from lipid biodegradation could lower the pH, thus the pH is a key factor in maintaining a good microbial consortium in anaerobic digestion. Therefore, additional phosphorus in form of buffer could help in pH balancing and more available phosphorus source in greasy waste digestion. Alternatively, NaHCO₃ is commonly used as a buffer to neutralize acid production during anaerobic digestion of refuse [16]. Thus, NaHCO₃ was used as a buffer for comparison to Na₂HPO₄ in buffering capacity during the digestion. Additionally, methane production had been measured to indicate the existence of an anaerobic process of the greasy waste mixture.

Materials and Method

Experimental Set-up

The digester was a cylindrical shape made by an acrylic material with a working volume of 0.01413 m³ and a diameter of 11.5 cm with a total-sizing of 0.017 m³ (0.22 m x 0.68 m x 0.113 m). The gas counter was connected to the digester for counting gas production. There were 2 digesters in the experiments, the test, and the control. The mixture of two kinds of waste, the greasy waste, and the MBR sludge was employed as a feed. Both wastes were from the grease trap unit and the pilot scale-MBR unit of the cafeteria of the faculty of engineering, Kasetsart University. Generally, the greasy waste had a semi-solid form and was contaminated by a variety of small parts of food wastes. Therefore, it was blended and then analyzed for various components such as pH, total solids, total volatile solids, oil and grease, and carbon, nitrogen before experimented with. The MBR sludge was taken from the aeration tank of the pilot-scale of membrane bioreactor and stood for 30 minutes to concentrate the sludge. Table 1 shows the chemical characteristics of each type of wastes. Then, the greasy waste was mixed with the concentrated MBR sludge at a proportion to 1:10 (v/v) to obtain approximately C/N of the mixture waste in the range of 30-45 (Table 1).

Table 1. Average Characteristics of the Wastes (each n=2)

Waste	Parameters (unit % dried weight)						
	pH ^a	TS	TVS	O&G	C	N	C/N ^a
Greasy Waste	5.62	28.40	92.74	79.84	79.11	3.04	26.02
MBR Sludge	7.02	1.08	68.91	6.26	25.33	6.30	4.02
B1(C)	7.25	12.68	80.04	77.82	54.67	1.55	35.27
B1 (T)	7.24	9.64	81.37	76.67	57.13	1.6	35.71
B2 (C)	7.24	11.92	86.39	74.93	53.95	1.27	42.48
B2 (T)	7.25	9.44	83.07	74.53	54.00	1.36	39.71
B3 (T-250)	7.25	10.46	83.15	88.28	61.42	1.88	32.67
B3 (T- 1000)	7.24	10.69	81.75	89.13	62.24	1.54	40.42
Avg (mixture)	7.25	10.81	82.63	80.23	57.24	1.53	37.33
SD (mixture)	0.01	1.27	2.18	6.68	3.75	0.21	3.73

Note: C: control; T: Test with added *P. putida* 1000 ml culture initially; T-250: added 250 ml of *P. putida* culture every two weeks; T-1000: added 1000 ml of *P. putida* culture every two weeks; Avg: average; SD: standard deviation; ^a: unitless

After the waste loading into the digester, the anaerobic sludge from the anaerobic digester of the pig farm in Chonburi province was added into the digester as the anaerobic microbial seed at 5% v/v, followed by the culture of *P. putida* at 5%v/v as the lipase producing microbe [13, 14]. The operation was a fed-batch type, 3 rounds with different pH adjustment and bioaugmentation of *P. putida* (initial and interval). Two kinds of buffering systems of Na₂HPO₄ (PBS) and NaHCO₃ (BBS) were employed. The Na₂HPO₄ solution was used in the pH adjustment of the 1st (B1) and 2nd (B2) batch experiment (Table 2), while the

3rd batch (B3), a NaHCO₃ solution was used instead. The anaerobic digestion was operated at 56-71 days of the solids retention time (SRT) per batch. It was noted that bioaugmentation of *P. putida* was performed at an initial day of B1 and B2 experiments with a 1000 ml of culture, while that of B3 was an interval bio-augmented in every two weeks with the different doses of a 250 ml B3(T-250) and a 1000 ml B3(T-1000) of the culture. For B2, the remaining sludge of the B1 experiment was used as the anaerobic seed, while that of B3, the sludge of B2 was used in B3 in both digesters. Overall experimental conditions were shown in Table 2.

Table 2. Experimental Conditions

Description	Digester 1			Digester 2		
	B1 (T)	B2 (T)	B3 (T-250)	B1 (C)	B2 (C)	B3 (T-1000)
Addition of <i>P. putida</i> culture¹	1000 ml (~2.04 x10 ⁸ CFU/ml) at initial day	1000 ml (~2.04 x10 ⁸ CFU/ml) at initial day	250 ml (~0.51 x10 ⁸ CFU/ml) every two weeks	none	none	1000 ml(~2.04 x10 ⁸ CFU/ml) every two weeks
Added <i>P. putida</i> concentration in digester²	1.44 x 10 ¹⁰ CFU/L at initial day	1.44 x 10 ¹⁰ CFU/L at initial day	3.61 x 10 ⁸ CFU/L every two weeks	none	none	1.44 x 10 ¹⁰ CFU/L every two weeks
Anaerobic sludge seed (v/v)	5% (from pig farm AD)	5% of B1(T) sludge	5% of B2(T) sludge	5% (from pig farm AD)	5% of B1(C) sludge	5% of B2(T) sludge
Buffer solution	PBS	PBS	BBS	PBS	PBS	BBS
SRT, days	63	63	71	71	56	56

Note: C: control; T: Test; PBS: Na₂HPO₄; BBS: NaHCO₃; the *P. putida* addition in form of concentrated cells after centrifugation; ¹: the concentration of *P. putida* as described in [17]; ²: the calculated concentration of *P. putida* as Equation 1.

Chemicals for Buffering System

Generally, the greasy waste contains high carbon but lower macro-nutrients (N and P) for microbial growth. Because phosphorus is an essential element in bacterial growth [15], therefore, additional phosphorus in form of buffer could help in pH balance and more available phosphorus in microbial greasy waste digestion. In the study, Na₂HPO₄ (PBS) was selected as a buffer, and additional phosphorus available in the digestion process in B1 and B2 experiments. The pH of the disodium hydrogen phosphate water solution is commonly between 8.0-11.0. While the bicarbonate (NaHCO₃, BBS), a common buffer for anaerobic digestion [16], was used in the B3 experiment to compare capability in acidity tolerance. Both chemicals were prepared at 5% w/v for buffer solution. The buffer was added to the waste mixture to adjust the pH of 7.0 initially. It is noted that a mediated partial suppression of methanogenesis and acetogenesis with cation toxicity when the too high concentration of NaHCO₃ was applied > 5% [16].

Culture of *Pseudomonas Putida*

Pseudomonas putida, a member of the genus *Pseudomonas* described is a Gram-negative, non-spore-forming, straight, or slightly curved rods bacterium. It is a fast-growing bacterium found in most temperate soil and water habitats. It grows optimally at 25-30 °C and can be easily cultured in the laboratory. This study, the pure culture, *P. putida* (TISTR 1522) was ordered from the Thailand Institute of Scientific and Technological Research. Tryptic Soy Agar (TSA) is a universal medium that supports the growth of *P. putida* (TISTR 1522). It was cultured on TSA and incubated overnight at 25-30 °C, 14 h according to a description in the TISTR leaflet. Then, the isolate was grown in 1 L of Tryptic Soy Broth (TSB) to obtain the *P. putida* biomass of 1.0 at OD₆₀₀ (approximately equivalent to 2.04 x10⁸ CFU/ml [17]). Then, the biomass suspension was centrifuged at 7,000 rpm at 4 °C, 10 min. Thereafter, the cells were washed with a sterile sodium chloride solution (NaCl) (0.85% w/v) 3 times to ensure that the adsorbed culture medium on cells was almost removed. For bio-augmentation, the culture precipitate was re-suspended in a 10 ml saline solution. Then, the 10 ml of cell suspension was added into the digester at the initial/interval time of the experiments as described in Table 2. The *P. putida* concentration of the waste mixture in each condition was calculated as shown in Equation 1. All calculated concentrations of *P. putida* of each digester condition are shown in Table 2.

The concentration of *P. putida* in the waste

$$= \frac{\text{P. putida concentration, CFU/ml} \times \text{Media culture volume, ml}}{\text{A total volume of the waste in a digester, L}} \quad (1)$$

Analytical Methods

All basic parameters were determined according to standard methods [18]. Total solids and total volatile solids of the waste samples were determined using a digital weighing after drying the waste sample at 105°C and 600°C, respectively. The pH measurement of the waste samples was done using a pH meter and a pH indicator strip (non-bleeding) (Merck®) for the MBR sludge/waste mixture and the greasy waste, respectively. The pH of the waste in the digester was recorded weekly. Oil and grease were analyzed by the Soxhlet extraction method in duplicate per sample. Finally, biogas production was recorded every day. Methane concentration was determined by gas chromatography (6890 Series, Agilent). The carbon and nitrogen contents of the waste were analyzed by CHNS/O analyzer (PerkinElmer 2400).

Results and Discussion

Effects of Buffering System on pH Changes

Generally, a buffering system is required to maintain pH value within the desired range suitable for the methanogenic process because continual volatile fatty acids production during acidogenesis will lower the pH level of the waste matrix. A well-buffered anaerobic digester means the ability to provide resistance to significant and rapid changes in pH when the acidic compounds accumulated in digesters [19] of which pH<6.5 can inhibit methanogenic activity. Acidification during anaerobic digestion due to organic overloading is one of the major reasons for process failures and decreased methane productivity in anaerobic digesters [20]. In this study, the first and second batch experiments using Na₂HPO₄

as a buffering system. It showed that pH had gradually decreased within 7 weeks of operation in both digesters of which the B1(T) and B2(T)-digesters with *P. putida* had a slightly lower pH level relative to B1(C) and B2(C)-digesters in the B1 and B2 experiments (Figure 1). Nevertheless, a sharp drop of pH of T-digesters was observed at day 63 of operation in both batches. The lower pH levels in the anaerobic digester indicate more VFA production which was the by-product of organic waste degradation of acidogenic bacteria [19]. More acid compounds produced in the T-digesters were possibly influenced by *P. putida* addition. The last two weeks of operation had pH levels < 6.5, which is a general critical point for methanogenic activity [21]. Acid crisis characterized by acid accumulation and/or low pH is a common reason for the failure of anaerobic digestion (AD), which is usually applied for wastewater and waste treatment [21]. This suggests that PBS might not be enough buffering capacity for FOG digestion, particularly a longer SRT >56 days operation. When a buffering system was changed to BBS in the B3 experiment. The pH of day 56 was slightly higher than the previous batches (B1 and B2) as of 6.68 and 6.63 for B3(T-250) and B3(T-1000) digesters, respectively (Figure 1). It suggests that BBS buffer had better-buffering capacity than PBS buffer. This might be because the carbon dioxide production during B3 digestion could be additional carbonate-bicarbonate levels in the buffering system.

Additionally, the determination of volatile fatty acids and alkalinity concentration and its ratio had been carried out during the B3 experimental period (Table 3). It shows that the initial FOG waste had pH 7.1-7.2 with the VFA/ALK ratios of 1.33 and 1.22 for B3(T-250) and B3(T-1000), respectively. During B3 digestion, VFA as CH₃COOH in both conditions had increased gradually with time. The B3(T-1000) showed slightly higher VFA contents (620 to 990 mg/L as CH₃COOH) than that of B3(T-250) as of 560 to 980 mg/L as CH₃COOH). Similarly, alkalinity contents had increased with time as a result of more available bicarbonate in both digesters due to more biogas production (Figure 4 and 5). Higher alkalinity content of B3(T-1000) condition was found. As a result, its VFA/ALK ratio was lower. It reported that the VFA proper value should be not more than 2,000 mg/L as CH₃COOH [16], while alkalinity prefers 2,500-5,000 mg/L for AD operation [22].

Table 3. Average Volatile Fatty Acids, Alkalinity and VFA/ALK Ratio during B3 Experiment

Days	VFA		ALK		VFA/ALK ratio	
	(mg/L as CH ₃ COOH)		(mg/L as CaCO ₃)			
	B3(T-250)	B3(T-1000)	B3(T-250)	B3(T-1000)	B3(T-250)	B3(T-1000)
0	560	620	420	510	1.33	1.22
7	600	660	620	620	0.97	1.06
14	650	690	680	750	0.96	0.92
21	730	720	960	910	0.76	0.79
28	790	810	1140	920	0.69	0.88
35	840	870	1190	1040	0.71	0.84
42	910	900	1390	1490	0.65	0.60
49	960	940	1520	1730	0.63	0.54
56	980	990	1610	1910	0.61	0.52

Note: B3(T-250): 250 ml of *P. putida* culture was added every two weeks; B3(T-1000): 1000 ml of *P. putida* culture was added every two weeks

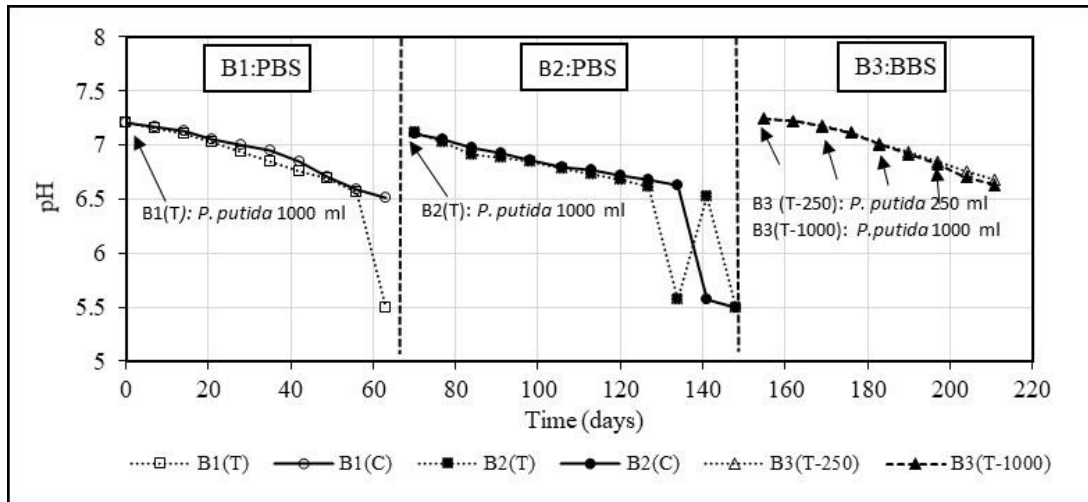


Figure 1. pH changes during digestion (Arrows indicate day of *P. putida* addition)

Note: B1(T) & B2 (T): 1000 ml of *P. putida* added; B1(C) & B2 (C): None of *P. putida*; B3 (T-250): 250 ml of *P. putida* added per two weeks; B3 (T-1000): 1000 ml of *P. putida* added per two weeks

Removal of Fat, Oil, and Grease (FOG)

Figure 2 shows the removal of FOG in all operational conditions of the co-digestion system. The FOG removal efficiency between the test digester and the control digester in the B1 experiment was different after 20 days of operation. Within 63 days of operation, only 17.72% FOG removal was found in the control digester, while that of the test digester was higher as of 31.64%. It suggests that *P. putida* addition to the co-digestion process could promote more lipid hydrolysis. The FOG removal of the digesters in the B2 experiment showed the same trend as of B1 experiment but better removal efficiencies were shown. The oil content of the control digester gradually decreased by giving 21.5% removal, while that of 46.83% in the test digester appearing after 63 days of digestion. This is because the remained digested sludge of the B1 digester might contain the acclimatized microbes of FOG biodegradability. Thus, more biodegradation of FOG was found in the B2 experiment. The decreasing pH level (Figure1) was corresponding well with the decreasing FOG contents in both digesters (Figure 2). Similarly, a previous study reported the utilization of grease waste by *Psueomonas chrysogenum* SNP5, one of the lipase producers, suggesting that it can be used for grease remediation [20].

In the B3 experiment, B3(T-250) and B3(T-1000) (added *P. putida*+BBS), sharply decreased FOG content in both digesters were found in the first week of operation. This was possible that both digesters had acclimatized anaerobic microbial consortium including *P. putida* (5% v/v of B2(T) sludge, Table 2). Additionally, FOG content in B3(T-250) had decreased faster than that of B2(T) digester even equivalent dose of *P. putida* per batch. It suggests that interval addition of *P. putida* was better than adding in once. Moreover, the FOG content of B3(T-1000) decreased faster than that of B3(T-250) during week 1 to 3 of the digestion period. It indicates that a higher dose of *P. putida* could enhance more FOG biodeterioration. Nevertheless, the final FOG removal showed no differences between both conditions by giving similarly the highest of 79.77% FOG removal. This might involve the balance of VFA/Alk ratio, caused by faster VFA production of B3(T-1000) (Table 3) during

21 days digestion gave negative effect on lipid degradation rate [23]. Because gradually decreasing pH levels in both digesters were observed throughout the experimental period (Figure 1), thus it suggests that bicarbonate was a good buffering capacity even the FOG removal in the B3 experiment was very fast relative to B1/B2 experiments which using PBS as the buffer. Finally, it concludes that the interval bio-augmentation of *P. putida* gave better efficiency in FOG removal relative to a single dose at an initial day while the dose of *P. putida* was not much influence on FOG removal. According to pH controlling and FOG removal, bio-augmentation of *P. putida* (3.61×10^8 CFU/L, Table 2), every two weeks using bicarbonate buffer is recommended for the operation of the co-digestion of greasy waste with the MBR sludge for high FOG removal efficiency.

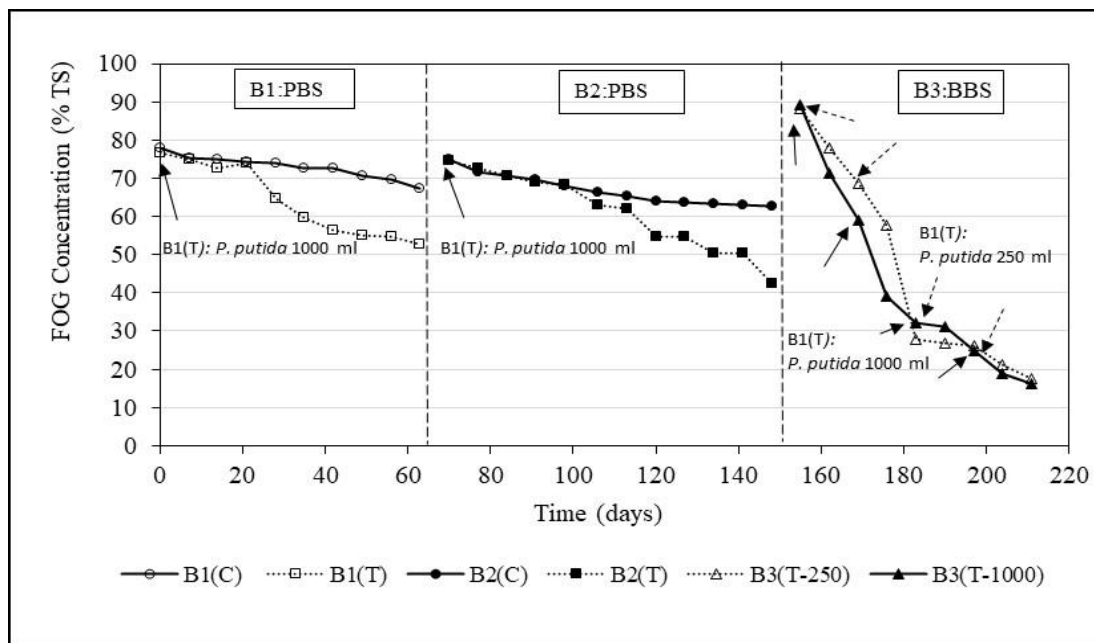


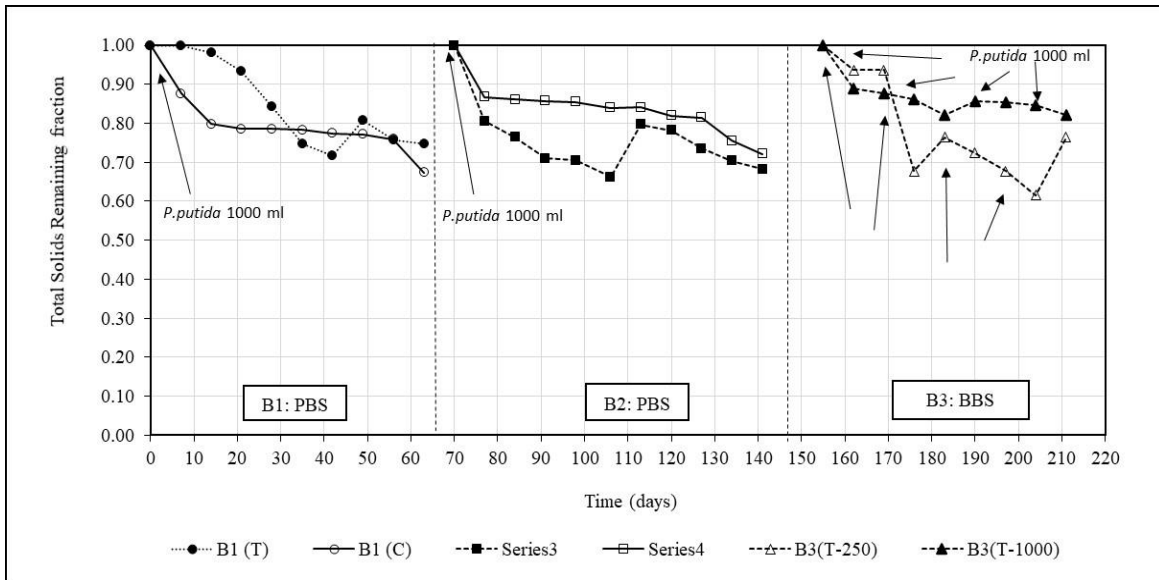
Figure 2. Changes of FOG content during digestion under different buffering system (Arrows indicate day of *P. putida* culture addition)

Note: B1(T) & B2 (T): 1000 ml of *P. putida* added; B1(C) & B2 (C): None of *P. putida*; B3 (T-250): 250 ml of *P. putida* added per two weeks; B3 (T-1000): 1000 ml of *P. putida* added per two weeks.

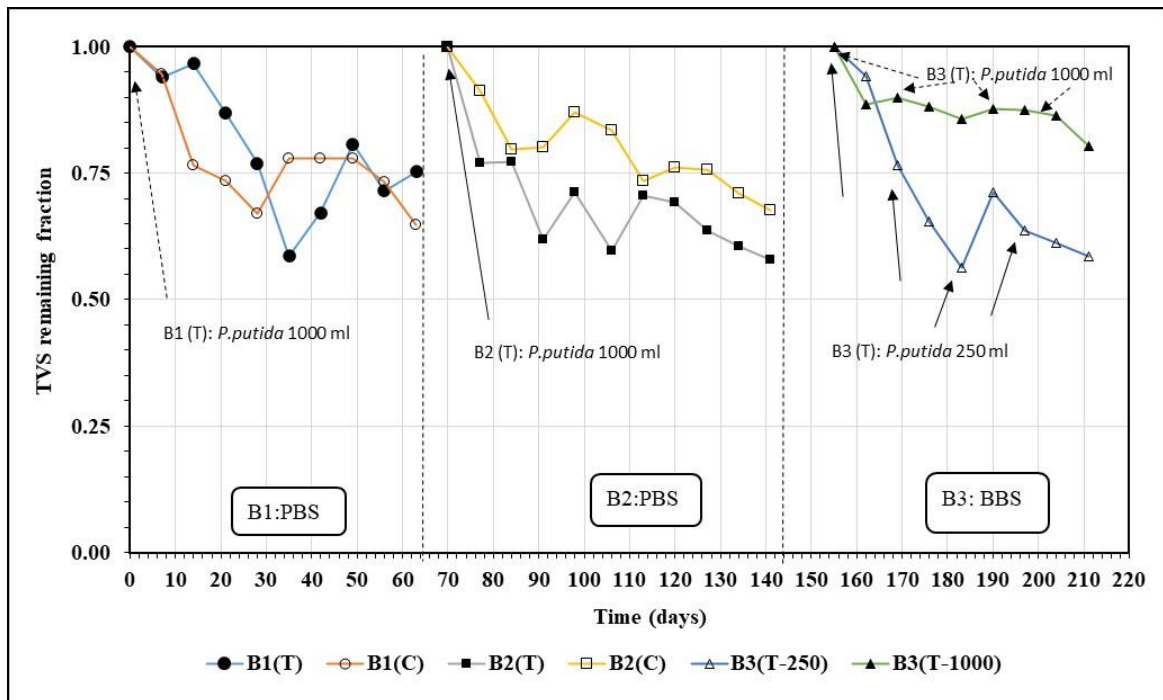
Removals of Total Solids and Total Volatile Solids

In this experiment, total solids and volatile solids were analyzed every week (Figure 3). It was found that total solids (TS) and total volatile solids (TVS) in the control digesters decreased faster in B1(C) during 20 days of the operation period, thereafter they showed gradually decreased until the end of the operation. Similarly, B2(T) showed decreasing trends as B1(T) both TS and TVS which lower remaining fraction compared to the control (B2(C)). It suggests that *P. putida* addition could promote organic matter (TVS) degradation in the co-digestion process. The TVS reduction was corresponding well with the results of FOG removal (Figure 2). Thus, it suggests that the removed TVS in the system was mainly in a form of FOG. Nevertheless, in the B3 experiment, lower TVS fractions were found for B3(T-250), while %FOG content was equal to B3(T-1000). It suggests that other forms of organic matter in B3(T-250) could be degraded concurrently with FOG. Alternatively, too high the addition of *P. putida* might result in more variety of VFA species. The severity of

long-chain fatty acids (LCFA) toxicity to microorganisms in AD is also thought to increase in the presence of a mixture of LCFAs rather than a single LCFA [2]. As a result, it might give unpreferable conditions to other microbial consortiums involving in other organic matters biodegradation.



(a)



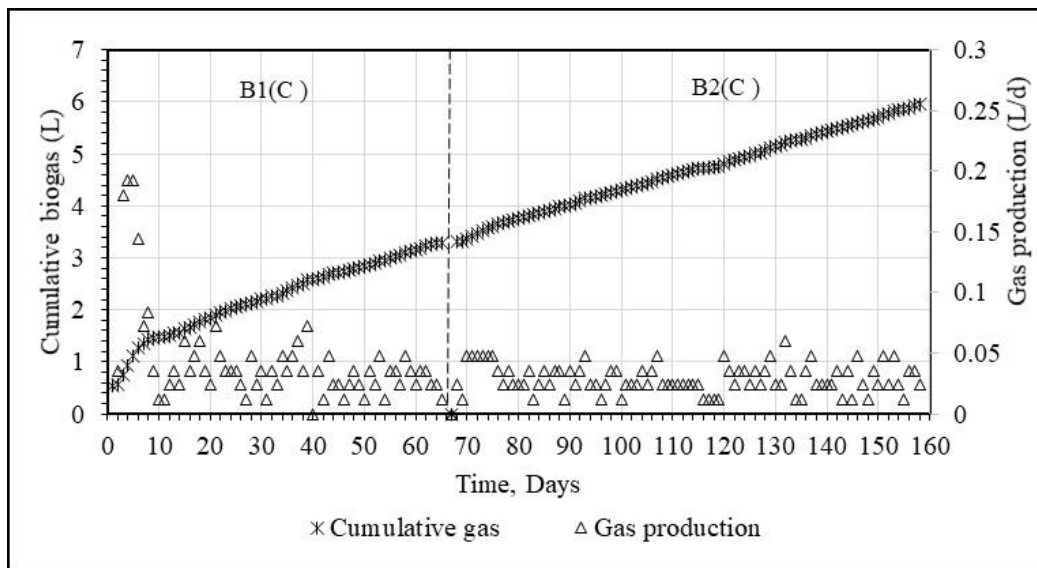
(b)

Figure 3. Changes of TS (a) and TVS (b) remaining fractions during digestion under different buffering system (Arrows indicate day of *P. putida* culture addition)

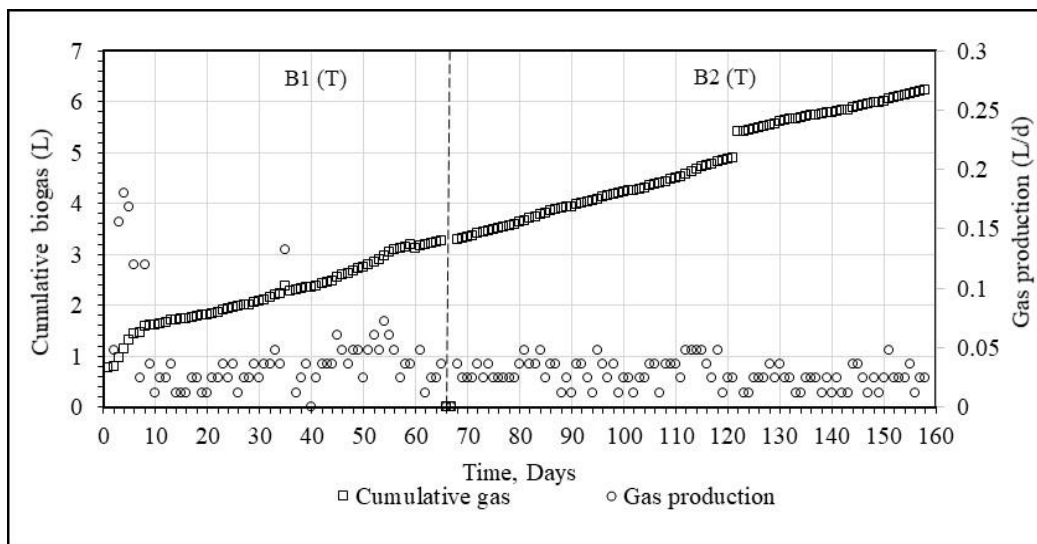
Note: B1(T) & B2 (T): 1000 ml of *P. putida* added; B1(C) & B2 (C): None of *P. putida*; B3 (T-250): 250 ml of *P. putida* added per two weeks; B3 (T-1000): 1000 ml of *P. putida* added per two weeks.

Biogas Production

Biogas is a mixture of various types of gas, including CH₄, CO₂, N₂, and H₂S [22]. The biogas production in all test batches is shown in Figure 4 to Figure 5. The results showed that there was not much difference in accumulative biogas production (5.9-6.0 L) between with or without *P. putida* condition at the end of the B1 and B2 experiments (Figure 4), but lower production as compared to that of the B3 experiment (Figure 5). Nevertheless, the gas production rate per day of B3 in both conditions (B3(T-250) and B3(T-1000)) showed a higher rate ~0.5 to 0.3 L/d than that of B1 and B2 (<0.05 L/d). It is corresponding well with the FOG removals in both conditions. It supports that the FOG biodegraded products were finally bio-transformed to biogas in the co-digestion condition.



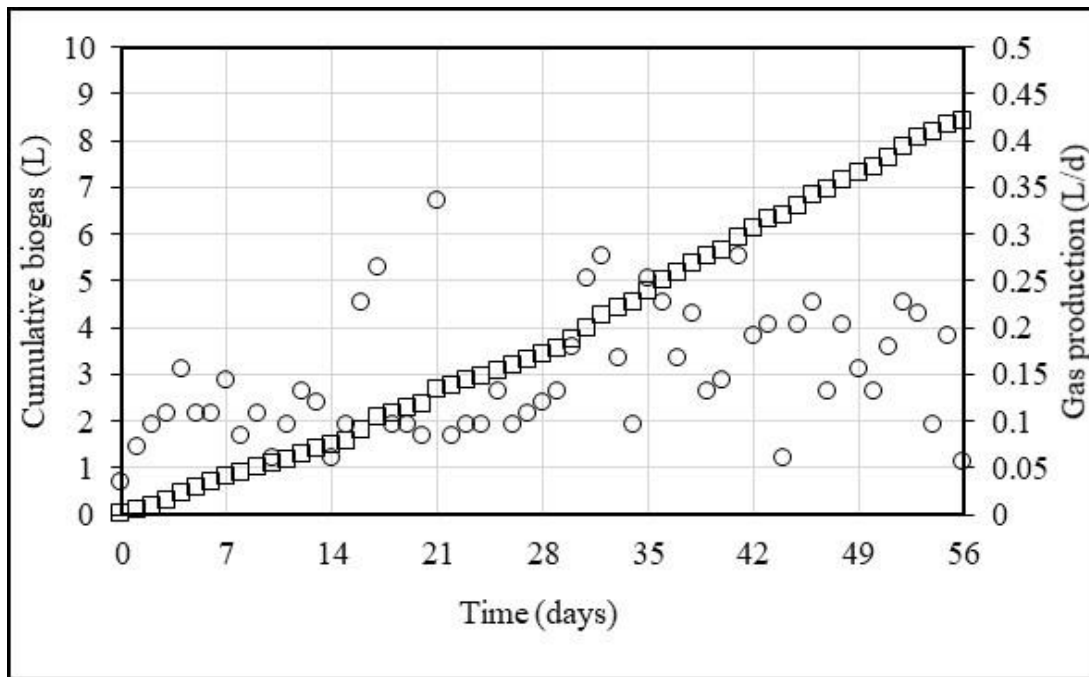
(a)



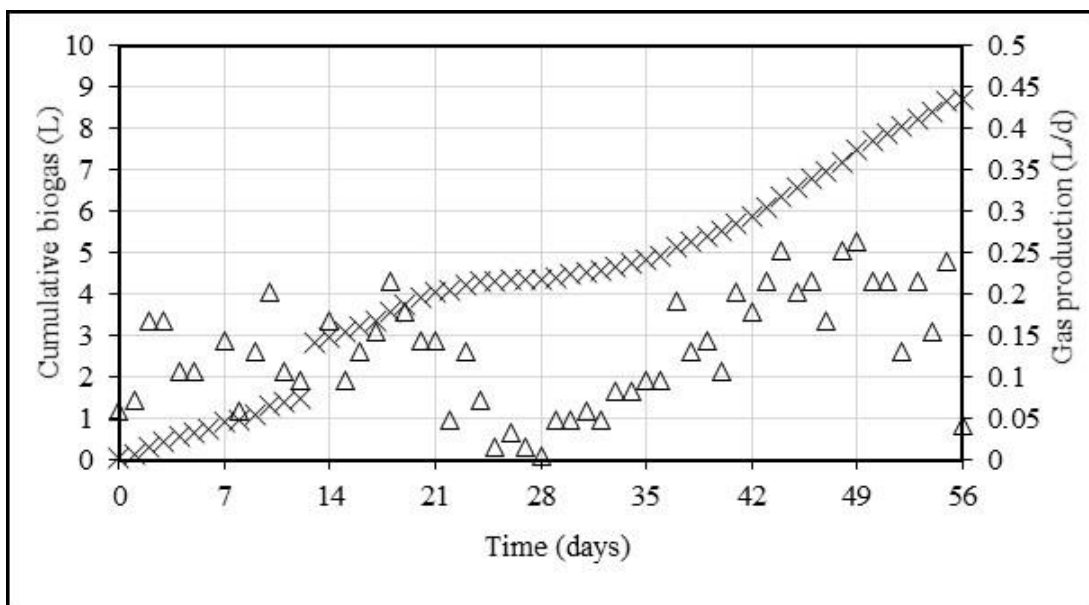
(b)

Figure 4. Biogas production in B1, B2 digesters

Note: B1(T) & B2 (T): 1000 ml of *P. putida* added at initial day; B1(C) & B2 (C): None of *P. putida* addition.



(a)



(b)

Figure 5. Gas production in B3(T-250); square: cumulative gas; circle: gas production (a) and B3(T-1000); cross: cumulative gas; triangle: gas production (b)

Note: B3 (T-250): 250 ml of *P. putida* added every two weeks; B3 (T-1000): 1000 ml of *P. putida* added every two weeks.

Changes in Methane Concentration and Production Rates

In B1 experiment, methane concentration gradually increased with time which corresponding to the decreasing of FOG content. After 30 days of operation, the methane concentration in the test digester was higher than that of the control digester. However, the methane concentration of methane reached the same level (25%) at 63 days of operation in both digesters. When the waste mixture was fed in the B2 experiment, methane concentration was

continually increasing up to 35% at 148 days of operation (Figure 6). It showed that the methane concentration started at a high level of 30% in both digesters. As mentioned above, there was the acclimatized sludge remaining from the B1 experiment used as the additional seed for the B2 start-up. Thus, the methane concentration of the test digester was higher than that of the control in some weeks but it was no significant difference in methane concentration at the end of operation which indicates no interference of *P. putida* addition on methanogenic activity. When a buffer solution was changed to bicarbonate, the methane concentration of B3(T-1000) was about 58% which was higher than that of B3(T-250) on the last day of operation. Because the FOG content was continually removed after 20 days of operation, it suggests that methane-producing in the B3 experiment might be from FOG degradation rather than the bio-solids degradation of the MBR sludge. Table 4 shows the methane production rate in each condition compared to previous studies. It is seen that the methane production rate in the digester with *P. putida* addition had a higher production rate. The highest production was found in B3(T-1000) with 961.08 ml/g TVS. d equivalent to 202.95 ml/g FOG. d. This value is higher than the reports of previous studies with a variety of mixture of FOG with various types of wastes such as thickening sludge of WWTP, sewage sludge, sedimentation tank sludge with the production rate of methane of 159 to 681 ml/g VS added, [23, 24, 25, 26]. This result suggests that a higher dose of *P. putida*, B3(T-1000), gave more methane production than that of the lower dose of *P. putida* (B3, T-250). It suggests that more *P. putida* concentration could better enhance methane formation in this co-digestion system. Alternatively, considering the cost of the operation, the 250 mL of *P. putida* addition every two weeks is more interesting in terms of equivalent FOG removal as the higher dose condition. Nevertheless, a lower methane production rate (353.75 ml/g TVS. d) was found in B3(T-250) compared to the higher dose (B3(T-1000)).

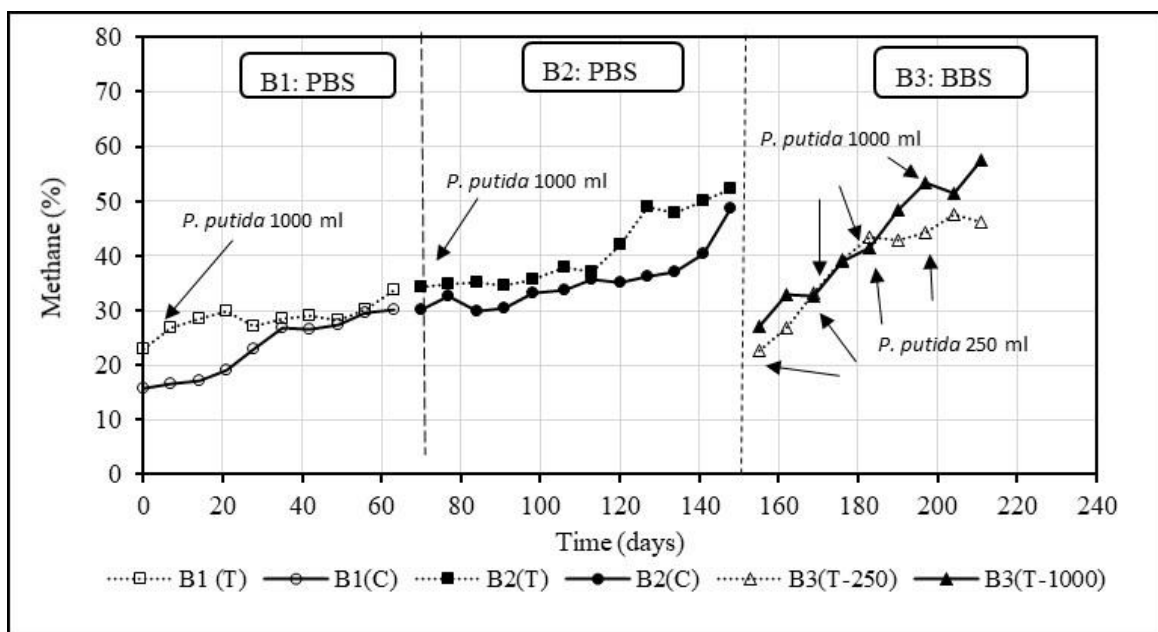


Figure 6. Changes of methane concentration in the digesters using PHB and NaHCO_3 as a buffering solution (Arrows indicate the addition of *P. putida*)

Note: B1(T) & B2 (T): 1000 ml of *P. putida* added; B1(C) & B2 (C): None of *P. putida*; B3 (T-250): 250 ml of *P. putida* added per two weeks; B3 (T-1000): 1000 ml of *P. putida* added per two weeks.

Table 4. Methane Yield in Each Condition

Conditions	CH ₄ (ml/g TS. d)	CH ₄ (ml/g TVS. d)	CH ₄ (ml/g FOG. d)	Reference
B1(T) with SRT 63 day	181.79	189.42	108.67	This study
B1(C) with SRT 63 day	84.67	102.23	86.51	This study
B2(T) with SRT 71 day	216.04	196.92	170.58	This study
B2(C) with SRT 71 day	123.49	163.33	128.98	This study
B3(T-250) with SRT 56 day	296.02	353.75	156.02	This study
B3(T-1000) with SRT 56 day	848.11	961.08	202.95	This study
Sewage thickening sludge +FOG In two phase CSTR with SRT 13 days	-	159-551 ml biogas/g VS added	-	[23]
WWTP sludge +grease trap sludge in methane potential reactor	-	325-681 ml CH ₄ /g VS added	-	[24]
WWTP sludge +grease trap sludge in batch fed once per day with SRT 10-13 days	-	271-344 ml CH ₄ /g VS added	-	[25]
WWTP sludge +grease trap sludge in CSTR with SRT 16 days		278-463 ml CH ₄ /g VS added		[26]

Note: The calculation of methane yield based on %CH₄ of the last day operation and cumulative methane; conditions: B1(T) & B2 (T): 1000 ml of *P. putida* added; B1(C) & B2 (C): None of *P. putida*; B3 (T-250): 250 ml of *P. putida* added per two weeks; B3 (T-1000): 1000 ml of *P. putida* added per two weeks; CSTR: Continuously stirred tank reactor.

Conclusions

A bio-augmentation of *P. putida* showed better efficiency in the removal of FOG contents in the anaerobic co-digestion of greasy waste and the membrane bioreactor (MBR) excess sludge. However, there was no difference in FOG removal between the low and high dose of *P. putida*, while the interval bioaugmentation gave faster FOG biodegradation than a single dose initially. The co-digestion using bicarbonate as a buffering system showed better pH controlling than phosphate buffer. Nevertheless, interval *P. putida* augmentation with a higher dose gave more methane production rate. According to pH controlling and FOG removal, *P. putida* bio-augmentation at 250 ml every two weeks using bicarbonate as a buffer is suggested as the operational condition of the co-digestion of greasy waste and the MBR sludge.

Acknowledgments

This study was financially supported by the Collaborative Research Program (CR) under the ASEAN University Network/Southeast Asia Engineering Education Development Network (AUN/SEED-Net).

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