Acetic acid by product from various fermentations of fermented biogas excess sludge and acidic sludge substrate

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Abstract: - The aim of this study was to investigate the optimum conditions for acetic acid production from various excess sludge as carbon sources for biological phosphorus removal processes. Two substrates used to produce acetic acid which were wastes from fermented biogas production system after 10-day fermentation (fermentation sludge, (FS)). The fermentation was carried in batch system with 3 substrate series. The substrates were combined with FS and acid fermentation (AF) in three different ratios; FS: AF (1:1), RS: AF (4: 1) and RS:AF (1: 4) in series 1, 2 and 3, respectively. Further investigation regarding the influence of temperature, pretreatment and the proportion of fermentation sludge (FS) and acid fermentation (AF) were also conducted. The results showed that the highest concentration of acetic acid was 1,406 mg COD/L in the reactor containing FS: AF 1: 1 (series 1) substrate after 96 hours with control conditions at 35 °C whereas similar combinations in reactor 2 and 3 produced lower concentration of acetic acid (1,018 and 792 mg/l, respectively). The Paired t-test of series 1-3 showed significant difference of acetic acid production ($p < 0.05$) for FS in series 1 and RS in series 2-3. The composition in experimental series 1 can be applied to produce acetic acid as carbon source for the biological phosphorus removal for reducing the cost of acetic acid production.

Key-Words: - Acetic acid, Acid fermentation, Biogas excess activated sludge, Biological phosphorus removal

1 Introduction

Organic carbon substances for microorganisms in the biological phosphorus removal system is required to be in a form that can be easily degraded. Among the sources, acetic acid is the often mentioned as energy source to support the release of phosphate [1] with the role in electron donation mechanism [2-3]. In most of the system, it is rather to add carbon sources in the form of acetic acid which is among the group of Volatile fatty acids (VFAs) that can be obtained from anaerobic fermentation process of organic waste. This source is usually occupied as an external carbon source for the biological phosphorus removal process (EBPR) [4-5]. Acetic acid is a crucial carbon source for biological phosphorus removal in relation to phosphorus accumulating organisms (PAOs) activity. Javier *et al.* (2012) who studied the ratio of glucose and different Sodium acetate (CH_3COONa) in Sequencing batch reactor (SBR) found that when CH_3COONa was increased to 100% and the ratio of COD: P equal to 23.5: 1, the production of acetic acid could be significantly increased [4]. In other study, Piasai *et al*. (2017) studied several types of organic carbon for phosphorus removal in the EBPR system and proposed carbon source as CH_3COONa could be the efficient form for phosphorus removal with efficiency up to 99% [5].

Mechanisms that involved in this situation are based on fact that group of microorganisms which can store phosphorus in the cell rely on CH_3COONa as the substrate for growth, thus the occurrence of this substrate affects the efficiency of phosphorus removal [5]. Nowadays, there are many excess sludge sources that generated in the treatment of municipal wastewater by activated sludge system [6] as the biological wastewater treatment systems often generates waste in excess sludge form. The removal of these wastes has become a major problem with high management cost since it takes roughly 50-70% of the operating costs in all wastewater treatment systems [7]. It is important to note that excess sludge can be treated with various methods including chemical, physical and biological treatments. Yet, the excess sludge is sediment of microorganism containing organic matter that actually potential to produce VFAs.

Acetic acid could be generated from excess sludge using many processes such as ultrasound and sediment fermentation. Those two processes are

anaerobic fermentation which remained the problem of excess sludge since those processes only reduce the amount of excess sludge [8-10]. However, the fermentation is still the promising one for treating the excess sludge [11] and obtain a large amount of acetic acid [12] with the additional advantage as the source of carbon by which the phosphorous removal can be enhanced. Adrian *et al*. (2006) have compared the removal of phosphorus using carbon sources from propionate and acetate. The finding showed that usage of acetate as carbon source can enhance the removal of phosphorous higher than that by the propionate [13]. Acidogenesis fermentation process consists of Hydrolysis, Acidogensis and Acetogenesis reactions where organic substances such as proteins, carbohydrates and fats are degraded into small moleculesand further being converted VFAs such as acetic acid, propionic acid and butyric acid. The reaction effectiveness relies on the parameters that affect microbial metabolism processes such as pH, temperature and carbon source [14]. Rademacher *et al.* (2012) found that the temperature plays an important role in the fermentation process for producing VFAs in anaerobic condition since it affects the microbial population [15]. Jiuxiao and Hui (2015) who studied the excess sludge fermentation from the municipal wastewater treatment system stated that an uncontrolled pH condition with mixing rate at 165 rpm found that the maximum concentration of VFAs was in the temperature of 55 ± 1 °C equal to 5,627.3 ± 354.6 mg COD / L and the lower temperature of 35 ± 1 °C could only enhance the production until 574.4 ± 29.2 mg COD / L after 48-hour fermentation period [11].

Possibility of excess sludge as the external carbon source for nitrogen and phosphorus removal has also been reported in other studies [16-17]. Xiong *et al*. (2018) have studied sediment fermentation from food waste to increase the removal of phosphorus in the municipal wastewater treatment system using alkaline fermentation substances as an external carbon source for the system. They found that 66.3% of VFAs of COD significantly increased nitrogen and phosphorus removal efficiency up to 78.2% and 95.2%, respectively [17]. Although these similar research have been conducted in many previous studies, the excess sludge in Thailand has rarely been explored as a carbon source for such kind of system. Therefore, excess sludge as a raw material for acetic acid production was investigated to increase the efficiency of phosphate removal for reducing the cost of carbon sources and excess sludge removal. Thus, this study aimed to investigate the acetic acid produced in different temperatures and excess sludge source ratios.

2 Problem Formulation

2.1 Sludge characteristics

Excess sludge sources which used in this study were collected from the settling tank in Conventional Activated Sludge system (CAS) for set 1 and 2, the settling tank in EBPR system for Set 3-6, swine wastewater for Set 7, and excess sludge from fermentation tank of biogas production system set 8-10. Temperature and proportion between fermentation of biogas excess sludge (FS) and acid fermentation (AF) were among the factors investigated in this study. FS was a fermented biogas sludge which stored at 4 °C after 10-day period of fermentation whereas AF was collected from acid lagoon as a part of biological biogas treatment series.

2.2 Sludge Fermentation

2.1.1 CAS

5-liter batch reactor was used to ferment excess sludge from CAS in set 1 and 2 with 35 °C [7,16,18] and 55 °C [11] temperature in the entire experiment for set 1 and 2, respectively. A sample of 50 ml condition was collected every 4 hrs and further analyzed for the occurrence of the volatile fatty acids (VFAs).

2.1.2 EBPR

Using similar reactor, the set 3 and 4 were conducted using excess sludge from EBPR. The temperature was controlled at 35 °C and 55 °C for set 3 and 4, respectively. Sampling was conducted every 4 hours by taking 50 ml of the water. In this sequence of experiments, volatile fatty acids (VFAs) were identified and quantified for further determination of acetic acid, butyric acid or propionic acid concentrations.

2.1.3 EBPR with different pH

In set 5, excess sludge from EBPR was used to understand the pH factor for the production of VFAs. The pH was varied to be 1 2 3 6 9 10 11 12 13 and 14 using 2N H_2SO_4 and 2N NaOH at 35 °C. Similarly, 50 ml sample was periodically collected every 4 hrs. Volatile fatty acids (VFAs), acetic acid, butyric acid and propionic acid were measured as the main parameter in this set as Boontian *et al.*

(2016) found that the initial treatment helped the hydrolysis reaction to occur in more effective and efficient condition [19].

2.1.4 EBPR and SM

Excess sludge from EBPR and SM were used in set 6 and 7. Both of the sludges were examined in terms of VFAs concentration in 35°C. Similar with the previous analysis, sample analysis was occupied.

2.1.5 FS and AS

FS and AS were combined in several ratios as follows; FS: AF (1: 1), RS: AF (4: 1) and RS: AF (1: 4) in set 8, 9 and 10, respectively. Sample volume 120 ml was collected every 12 hrs for measuring the volatile fatty acids (VFAs), acetic acid, butyric acid and propionic acid concentration.

2.3 Chemical analysis

Standard Method for examination of water and wastewater APHA *et al*. (2012) [20] was used for analyzing the VFAs concentration. In addition, gas chromatograph (GC) series 6890 with flame ionization detector (FID) was utilized to evaluate acetic acid, butyric acid, propionic acid ratios and concentrations in all sets of experiments [6].

3 Problem Solution

This study investigated the effect of fermentation excess sludge for produce acetic acid. The first part was about the usage of excess sludge from sedimentation tank of activated sludge system (Set 1 and 2) as carbon source. The concentration of VFA was 597 and 283 mg/L at 35 and 55 ^{o}C, respectively. As shown in Fig.1, the amount of VFAs that produced at a temperature of 35 ^{o}C was greater than at 55 ^{o}C. Using paired t-test at 95% confidence level, the VFA at a temperature of 3 5 ^{o}C (Set 1) was not significantly different ($p = 0.10 > 0.05$) with VFA from Set 2 (55 ^{o}C). It demonstrated that both temperatures were not affecting the degradation rate of the excess sludge from activated sludge at the ratio of TVS / TS equal to 0.27 because the characteristic of excess sludge was difference for degradation but it could be still degraded. It thus confirms that although temperature for degradation was high, hydrolysis and Acidogenesis in VFA production were still conducted. A different result was found by Yun *et al*. (2017) who have studied the excess sludge fermentation from the second stage sedimentation tank of the wastewater treatment system and anaerobic digestion tank [21]. At different temperatures, 35, 42 and 55 ^{o}C applied for 6 days, the VFA concentration was 1,1 195, 625and3 ,856 mg/L, respectively with ratio of TVS / TS equal to 0.73 [22]. That result represented higher organic content when it is compared to this study. The amount of VFAs that occurred in that study was 6.7 times lower 6. 7than this study. Standing in contrary, Huilei *et al*. (2012) stated that the production of VFAs depends on the SRT and temperature after conducting the experiment by increasing the temperature from 40 to 50 ^{o}C which resulting an increase of VFAs by 1.65 times while the VFAs was decreased by 1.31 times at the SRT of 120 hours [23]. Similarly, Hyun *et al*. (2015) found that during anaerobic fermentation mono-saccharides and amino acids were transformed into VFAs with dependency on the activity of acidogenic microorganisms which was enhanced by the increase of the temperature [24].

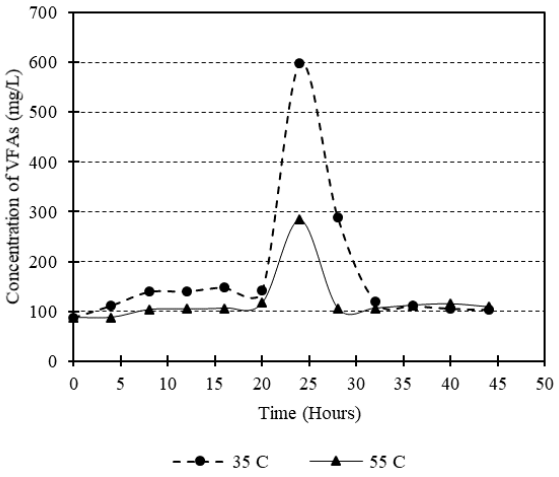

Fig.1 Concentration of VFAs from fermented excess sludge from activated sludge system (set 1-2) at temperature 35 ^{o}C and 55 ^{o}C

The effect of temperature on sludge fermentation, accelerated from the EBPR system to produce acetic acid was conducted while the production of butyric acid was found to be very low (≈ 0). The experiment in the third set produced the highest acetic acid which equals to 62 mg/L at temperature of 35 ^{o}C after 88 hours as depicted in Fig.2. Furthermore, in the set 4 experiment with SRT 44 hours at 55 ^{o}C, the concentration of acetic acid and VFAs were 55 and 108 mg/L, respectively (Fig.3). In this set, 55 ^{o}C condition took less time to produce acetic acid than at 35 ^{o}C. Paired t-test at 95% confidence level showed that both set 3 and 4 resulted acetic acid content of excess sludge from EBPR in similar concentration ($p = 0.09 > 0.05$).

However, this result proved that the temperature does not affect the excess sludge fermentation to produce acetic acid yet concentration of VFAs was considerably low. This condition of low VFAs might be due to the characteristic of excess sludge in this study. The excess sludge TS and TVS were 9,485 mg/L and

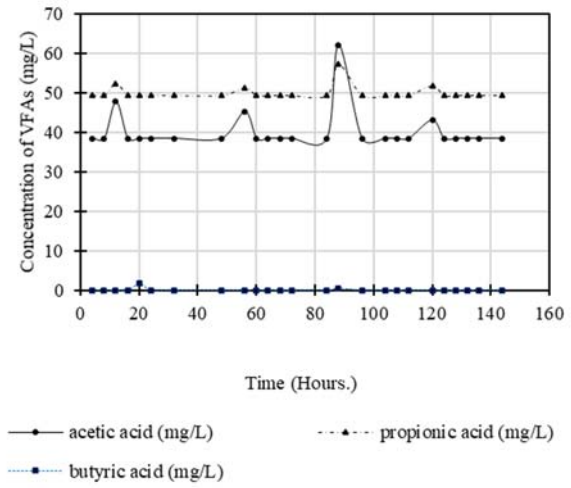

Fig.2 Concentration of acetic acid, propionic acid and butyric acid from excess sludge from EBPR at 35 ^{o}C.

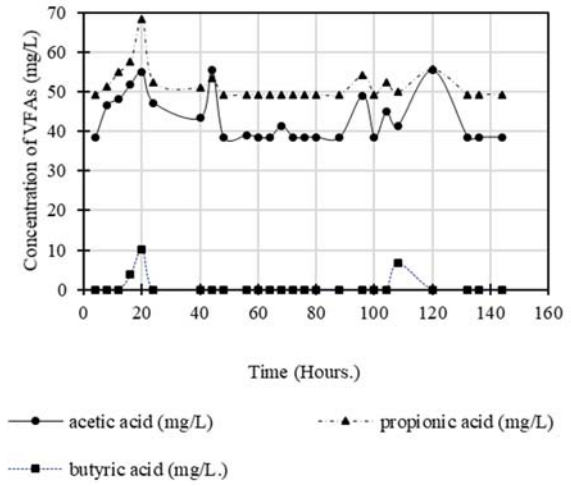

Fig.3 Concentration of acetic acid, propionic acid and butyric acid from excess sludge from EBPR at 55 ^{o}C.

1,893 mg/L, respectively resulting the 0.2 ratio of TVS/TSS. This condition indicates that the sediment of the sludge was difficult to be degraded although it was still digestible. TVS/TS ratio of set 1 and 2 was 0.27 while the ratio of set 3 and 4 was 0.20. However, the higher ratio of set 1 and 2 was likely to be digested easily than the excess sludge from the EBPR system in Set 3-4. It thus explained the reason low VFAs from excess sludge in set 3-4.

Excess sludge from the EBPR system in set 3 which fermented at 35 ^{o}C resulted acetic acid content of 62 mg/L. this value is less than the study of Jiuxiao and Hui (2015) which fermented excess sludge from the wastewater treatment system. They have found that the concentration of acetic acid could reach 191 mg/L at 35 ± 1 ^{o}C [11]. Conversely, the excess sludge

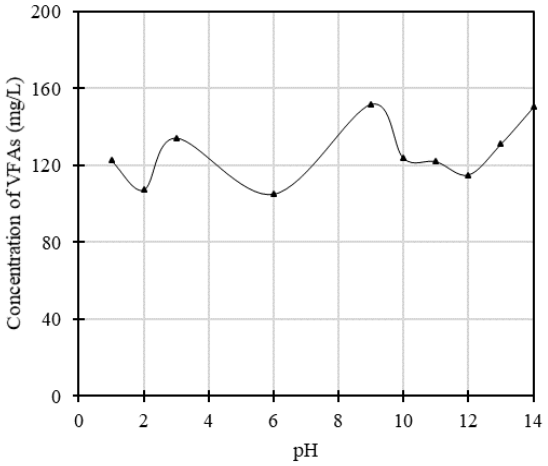

Fig.4 Concentration of VFAs in pretreatment of excess sludge from EBPR (set 5) with different pH

of EBPR in Set 4 which fermented at 55 ^{o}C produced 56 mg/L acetic acid. This concentration is also less than the study of Huibin and Sheng (2017) who fermented excess sludge at 55 ° C and later found the acetic acid production up to 615 mg/L [25]. Since the amount of easily degradable organic matter was very low, it may require initial treatment for excess sludge before. The pretreatment can enhance the VFAs in sediments below the potential to avoid low VFAs (Table 1). A study by Zhang-Wei *et al*. (2016) explained that the initial treatment using rhamnolipid in combination with anaerobic alkaline fermentation resulted the maximum SCFAs of 378 mg/L at 72 hours with pH value controlled at 10 and temperature at 35 ± 1 ° C [16].

The initial treatment of excess sludge from EBPR (set 5) was conducted by varying pH values. The highest acetic acid was found in pH 14 which was 93. 14mg/L where the propionic acid was found to be 59. 1mg/L. Moreover, acetic acid was detected to be 90. 82mg/L at pH 9. The initial sludge has the ratio of TVS / TS equal to 0.32. It was found that the amount of VFAs that appeared in these two pH levels was quite similar. The highest VFAs were 152and 150mg/L at pH 9and 14, respectively (Fig.4). The reason of that was the reduction of methanogenic organisms activity at pH 14 where high amount of solid that came from hydrolysis of

protein and fermentation. This result was similar with the study of Boontian *et al*. (2016) that found the adjustment of cassava waste with acid or alkali with additional heat could increase the production of VFAs [26].

Table 1 Concentration VFAs from different condition and concentration fo acetic acid.

Carbon source	Acetic acid	Concentration of VFAs	Conditions	Reference
Set 1 (WAS from AS)	-	597 mg/L at 24 hours.	pH not controlled at35 ^{o}C	This study
Set 2 (WAS from AS)	-	283 mg/L at 24 hours.	pH not controlled at 55 ^{o}C	
Set 3 (WAS from EBPR)	62 mg/L	120 mg/L	pH not controlled at 35 ^{o}C	
Set 4 (WAS from EBPR)	56 mg/L	133 mg/L at 20 hours.	pH not controlled at 55 ^{o}C	
Set 5 (WAS from EBPR)	93.14 mg/L	152 mg/L	pH 14	
Set 6 (WAS from EBPR)	-	574 mg/L	pH not controlled at 35 ^{o}C	
Set 7 Pig Manure	109 mg/L	439 mg/L		
Set 8 FS:AF (1: 1)	1,406 mg/L	2,505 mg/L at 96 hours.		
Set 9 RS:AF (4: 1)	1,018 mg/L	1,233 mg/L at 96 hours.		
Set 10 RS:AF (1: 4)	792 mg/L	1,227 mg/L at 108 hours.		
Excess sludge from sedimentation tank and secondary sedimentation tank	-	423.22±25.49 mg. COD/g.VSS	Ratio of excess sludge from sedimentation tank and secondary sedimentation tank (w/w: 1:1) at pH 8.9	Yun *et al*. (2017) [22]
Animal dung and wheat straw	1,394 mg/L	-	Anaerobic condition for 10 days	Maie *et al*. (2017) [27]
Cow manure and corn silage	6,444 mg/L	14,651 mg/L	Controlled 55 ^{o}C for 4 days	Cristina *et al*. (2017) [28]
secondary sedimentation tank and anaerobic fermentation tank	615 mg/L	666.5 mg/L	Controlled 55 ^{o}C for 6 days	Huibin and Sheng (2017) [25]
Sludge from trickling filter system	-	1,327 mg/L at 120 ^{o}C	Heating at 75 ^{o}C for 10 minutes	Jiabing *et al*. (2016) [29]
Pig manure	-	12.6 mg. COD/g.VSS	Controlled at 35 ^{o}C	Weiwei *et al*. (2016) [30]
Waste activated sludge	191 mg/L	574.4 mg/L at 35 ^{o}C	Mixing 165 rpm, pH 6.6 - 7.1 for 48 hours.	Jiuxiao and Hui (2015) [11]
Excess sludge from SBR (Sequencing Batch Reactor)	2,862 mg/L At pH 4	pH 4 ; 3,914 mg/L pH 6; 2,607 mg/L	Controlled 26 ^{o}C and mixing 60 rpm	Infantes *et al*. (2011) [31]

Rungnapa *et al*. (2015) also found the similar result. The study of the excess sludge fermentation to produce VFAs with pH 9 8 and 10 without further pH control revealed that the maximum VFAs was at pH 9with 3. 5hours of SRT [32]. Ruyi *et al*. (2016) who studied of excess sludge fermentation from wastewater treatment system found that the concentration of VFAs produced by fermentation was rapid at the experiment set with not pH controlled [33]. The study was done using 12. 9g VSS/L excess sludge and 5. 1g VSS/L seeding sludge. The experiment was conducted by mixing the sludges with ratio of seeding sludge and sediment equal to 1: 10 in 2.5-liter reaction tank and stirred 200rpm at temperature 19.5-22. 5° C 6 different sets of pH 6, 6, 8, 9, 10using no pH control. Zhu *et al*. (2015) who studied the VFAs that produced by fermentation with primary heat treatment and alkalinity using excess sludge from wastewater treatment system found that TS 75 g/L reactor produced the highest VFAs concentration, 7. 31g/L. When the excess sludge was adjusted to pH 12and stirred at 90° C for 2hours, the methanogens group was severely damaged [34]. The initial treatment of excess sludge was important in the production of VFAs which heat and alkalinity in this particular study. Those initial treatments increased the filtration resistance and viscosity of microbial sludge where in the opposite way, fermentation decreases the filtration resistance and reduce the size of the sediment. This study also tested the acetic acid production from different carbon sources using excess sludge that has high VFAs since the carbon source is highly biodegradable. The occurrence of acetic acid increased and peaked 32. 78mg VFA/g. VSS in the all set of experiments. It is important to note that this additional carbon sources can be used to ferment and it worth replaced excess sludge from pig manure and biogas production system. Here, the comparison of acetic acid produced from three sources (Fig.5): EBPR (set 6), Pig manure (SM) (set 7) and biogas production (set 8) has been conduced. After the test of latter sets, it was found that FS: AF (1: 1) had the highest acetic acid concentration, equal to 1,406. 8mg/L at 96hours (set 8). In addition, it was also known that the concentration of propionic acid was equal to 852. 81mg/L and butyric acid was 246. 25mg/L. Lastly, SM (set 7) was found to produce acetic acid equal to 109. 43 mg/L at 36hours.

The comparison of VFAs from 3 carbon sources revealed that VFAs in set 6 and 7 were 650 and 439 mg/L, respectively. The excess sludge fermentation from the EBPR system (set 6) with pH value at 7.75 produced maximum VFAs of 650 mg/L in 2 days. It was consistent with the study of Yue *et al*. (2015) who has studied the excess sludge from the SBR system of the wastewater treatment system in China [35]. The concentration of VFAs could be 118.3 mg/L for 6 days at pH 4 and by increasing the pH to 8, VFAs concentration was 1,674.4 mg/L for 6 days. Moreover, PM (set 7) was observed producing 109 mg/L acetic acid equal at 35 ^{o}C which was less than the studies of Cristina *et al*. (2017) which used carbon sources from cow dung and fermented corn that obtained 6,444 mg/L acetic acid [28]. Similarly, Maie *et al*. (2017) who investigated animal dung and wheat straw potencies successfully obtained 1,394 mg/L acetic acid [27]. The concentration of VFAs occurring in the experiment set 7 was 283.59 mg COD/g. VS which was more than the study of Jingang *et al*. (2016) who conducted similar study using pig manure that produced only 79.1 mg COD/g. VS at pH 11 for 8 days with temperature of 55 ^{o}C. It occupied 20 % of TS to obtain the highest ratio of acetic acid (80.4%) at pH 12 in 8 days [18]. The result from this set was also higher than that obtained by Weiwei *et al*. (2016) who found that the VFAs was 12.6 mg COD/g. VS. by using pig manure [30].

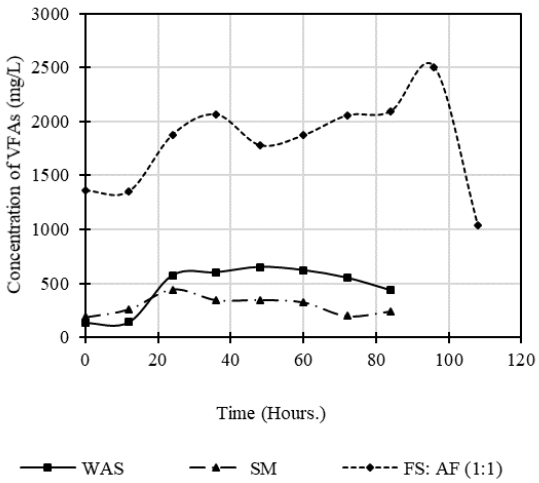

Fig.5 Concentration of VFAs from EBPR (set 6), pig manure (set 7) and FS:AF (1: 1) (set 8) in temperature 35 °C.

Statistical analysis showed that the comparison of VFAs production of set 8 to set 6 and set 8 to 7 using Independent t-test at 95% confidence level showed that there were significant differences ($p = 0.00 < 0.05$) in both pairs. Comparison of the production of VFAs from all 3 carbon sources; EBPR SM and FS found that FS: AF (1: 1) had a higher amount of VFAs than excess sludge from EBPR and SM. The sludge fermented (FS) in 10

days was found to have higher organic content than the SM and waste activated sludge. The FS was thus proven to be transformed into VFAs by microorganisms. The acid fermentation (AF) can decompose organic matter in Acidogenesis and Acedogenesis. The production of VFA in the reactor tank of set 6 compared with set 7 showed the VFAs fermentation of the two experimental sets gave the same results in Independent t-test at 95% confidence level ($p = 0.06 > 0.05$).

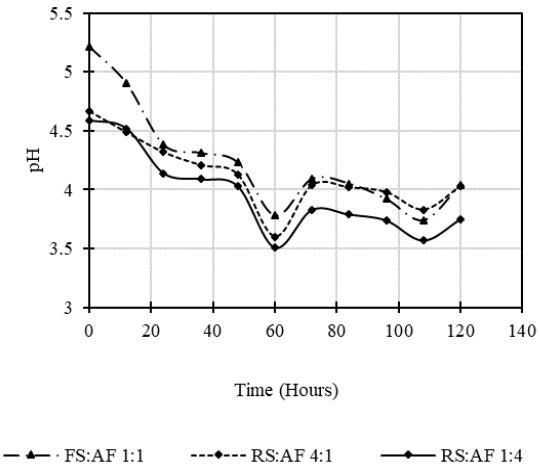

Fig.6 Effect of pH on Time (Hours) of VFAs production during the fermentation of the FS: AF (1: 1), RS: AF (4: 1), RS: AF (1: 4).

In the experiment that used raw sludge (RS) with initial pH value of 4, 5, and 6, the VFAs was 1.3-1. 8 g/L in the first day at a temperature of 35ºC. The results of the pH comparison found that the pH was constant in the range of 3.74–5. 21 as depicted in Fig.6. before slightly decreased during the first 60 hours and became more stable in the range of 3. 74 to 4. 09until the end of the experiment. This phenomenon was associated with an increase in the concentration of VFAs at the end of the experiment where it showed that120 -hour period of fermentation ended with pH at 4.04. This experiment indicated that the pH value decreased when the amount of acetic acid increased. The result was consistent with study of Ehsan *et al*. (2016) who found that the pH value decreased when acetic acid increases due to the nature of the mechanism through the formation of hydrogen bonds of acetic acid molecules and non-ion exchange of acetate ions where the acetic acid was an important form [36]. It was also similar with the study of Infantes *et al*. (2011) who studied that fermentation sludge from wastewater treatment system. They found that acetic acid decreased when the pH value increased and the temperature decreased [31]. It reflected that the pH value was a factor affecting the production of VFAs. Similarly, Yingchon *et al*. (2018) found that the pH value had the most important factor that affected the hydrolysis reaction performance of cassava starch waste. pH value of 0. 38at 100ºC was found resulting the highest VFAs in the form of acetic acid [37]. The study of Zhang-Wei *et al*. (2016) found that the initial pH affected the decomposition of organic material and Hydrolysis which was beneficial to the production of VFA [16].

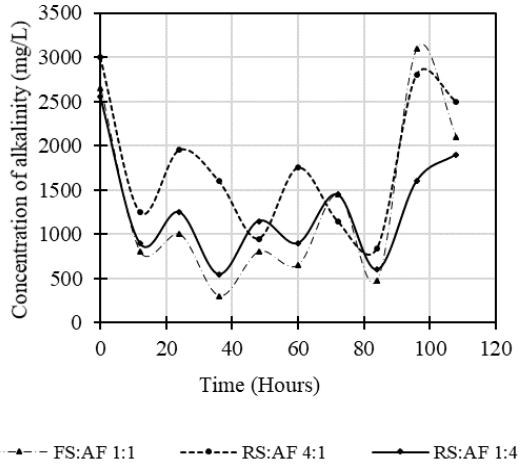

Fig.7 Effect of alkalinity (mg/L) in batch tests operated at different substrate (FS: AF (1: 1), RS: AF (4: 1), RS: AF (1: 4)) for different period time.

However, the alkaline conditions depend on the acid content. In line with that, the similar trend occurred in use of (RS: AF (4: 1) (Set 9) and RS: AF (1: 4) (Set 10) as carbon sources where the pH value decreased slowly. It thus affected the concentration of VFAs that would gradually decrease and resulted lower VFAs than use of FS: AF (1: 1) (set 8). It was found that 96 hours of FS: AF (1: 1) had the highest acetic acid (2,506 mg /L) with alkalinity equal to 840 mg/L (Fig.7). In the set 8-10, the averages of alkalinity during fermentation were 1,333 1,779 and 1,285 mg/L, respectively. Oehmen *et al*. (2007) explained that PAOs require a pH range of 7-8.5 in the growth of PAOs [38].

Therefore, the alkalinity value is important because it is a buffer that helps to resist the change of pH in the production system of each type of sediment VFAs. It was also found that the highest amount of VFAs was acetic acid followed by butyric acid and propionic acid during the decomposition process by the deformation of SCOD containing VFAs and compounds with small molecules. In this study, it was found that the amount of VFAs that produced using carbon sources FS: AF 1: 1 slowly increased from 12 to 96 hours

(Fig.8). Concentration of VFAs in experiments using RS: AF (4: 1) and RS: AF (1: 4) were ranging in 341-1,362 mg/L at 96 and 108 hours, respectively. In the study of the production of VFAs, the proportion of each type of organic substances are important. VFAs are organic substances with 2-5 carbon atoms, such as acetic acid, propionic acid and butyric acid. It was found that over 36 hours of FS: AF 1: 1 in Series 8 produced 2.5 g/L VFAs. The results indicated the Hydrolysis, Acitogenesis and Acedogenesis reactions were conducted. Using excess sludge that has been left for 10 days as a carbon source, it has been found that the amount of acetic acid was greater than propionic acid.

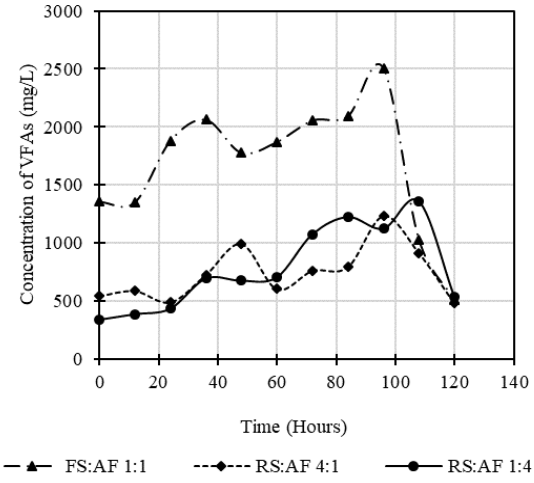

Fig.8 VFAs in batch tests operated at different substrates ratios FS: AF (1: 1), RS: AF (4: 1) and RS: AF (1: 4).

It was different from the previous results from Huijun *et al*. (2016) [8]. In their study, acetic acid and propionic acid were dominant VFAs. Acetic acid is a group of VFAs that can be easily digested by microorganisms which may reduce the concentration since acetic acid is the last product that generated by anaerobic fermentation before being converted into methane in the process called methanogenesis conducted by the group of Methanogens [8]

The experiment of FS: AF 1: 1(set 8) at 96 hours revealed that the highest acetic acid content was 1. 4g COD/L and VFAs was 2,506 mg/L (Fig.9). Experimental results showed that the hydrolysis reaction of Acitogenesis and Acidogenesis could occur quickly. Compared with study from Ana *et al*. (2010) who found that the amount of VFAs increased from 3, 500 to 8, 700 mg/L when fermentation of excess sludge that had been fermented for 4days with acetic acid content equal to 41% and propionic acid equal to 36%, the result of this study was low since the result of this previous study occurred during the first 36hours of excess sludge fermentation [39]. The acetic acid produced by fermentation can be used as a carbon source for biological nutrient removal systems. Guojing *et al*. (2018) studied the removal of phosphorus in the EBPR system and found that the efficiency of phosphorus removal was as high as 90.2% when it used ratio of 100/0% acetate/glycerol [40]. Similarly, Piasai *et al*. (2017) studied the use of acetate as a carbon source in the EBPR system and found that acetate content was appropriate for the growth of PAOs [41]. Therefore, the production of acetic acid in set 8can be used as a carbon source for biological phosphorus removal.

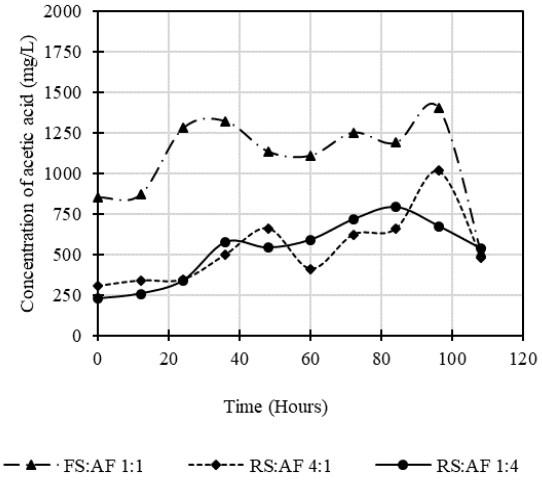

Fig.9 Acetic acid in batch tests operated at different substrates ratios FS: AF (1:1), RS: AF (4:1) and RS: AF (1:4).

Propionic acid was an intermediate product between the decomposition of organic matter. The acid brakes down slowly compared with acetic acid. The results showed that the highest amount of propionic acid was produced in batch 8 (FS: AF (1: 1)) which was 853 mg/L at 96 hours. Set 9 and 10 also produced the acid but in the lower amout (341 mg/L and 612 mg/L, respectively) in 108 hours (Fig.10). This result was consistent with the study of Yongqing *et al*. (2011) who found that the increase in nutrient removal efficiency and reduced the amount of sludge were accelerated by anaerobic anoxic oxic (A^2O) system. The reaction was 19.7 % Acidification and it resulted the VFAs occurred with a high proportion of acetic acid and propionic acid when it was used as a carbon source for the A^2O system. It was found that nitrogen and phosphorus removal efficiencies were up to 80.1 and 90%,

respectively [42]. The study of Maite *et al*. (2017) deliberated the SBR system using carbon sources from propionate and acetate and found that propionate could be the best for eliminating phosphorus. It was also found that the amount of PAOs was arguably high when it used propionate as a carbon source [27]. Satoh *et al*. (1996) who studied the effects of carbon on biological phosphorus removal found that the rate of phosphorus evaporation in anaerobic conditions was reaching the highest rate when it used acetate and propionate as carbon sources [43]. Therefore, it is potential for the set 8 experiment to use acetic acid and propionic acid for carbon source for biological phosphorus removal systems.

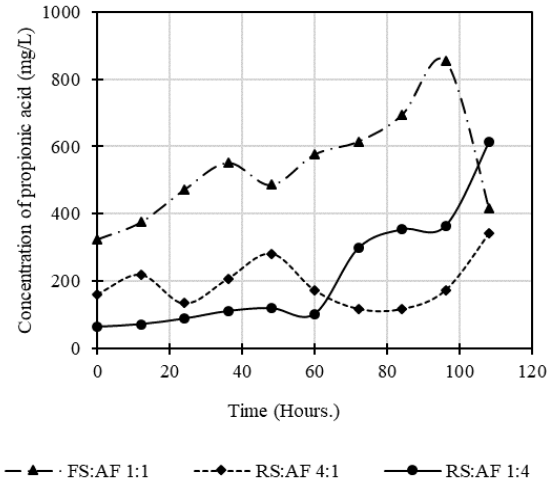

Fig.10 Propionic acid in batch tests operated at different substrates FS: AF (1: 1), RS: AF (4: 1) and RS: AF (1: 4).

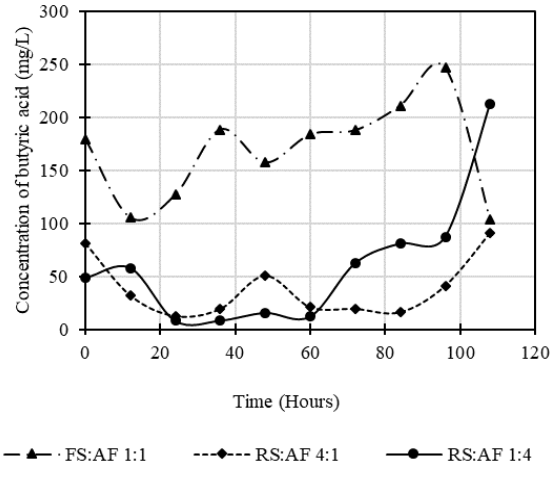

Fig.11 Butyric acid in batch tests operated using different substrates FS: AF (1: 1), RS: AF (4: 1) and RS: AF (1: 4).

The study of the concentration of butyric acid in set 8-10 showed that the carbon source in set 8 (FS: AF (1: 1)) had the highest butyric acid content that equal to 246 mg/L at 96 hours where pH was equal to 3.92. It was followed by RS: AF (1: 4) that produced 212 mg COD/L butyric acid at 108 hours with pH value 3.57. Lastly, RS: AF (4: 1) had the lowest amount of butyric acid of 91 mg COD/L at 108 hours with pH value 3.83 (Fig.11). The study of Rustrian *et al*. (1996) found that acetate and butyrate were the best carbon sources for phosphorus removal and propionate resulted lower phosphorus removal efficiency than the former carbon sources [44]. Their results showed that the highest amount of VFAs was acetic acid and followed propionic acid. It was different from the study of Huijun *et al*. (2016) which found that acetic acid and the most abundant butyric acid were produced when pH value was higher [8]. This study tried to establish suitable fermentation conditions for acetic acid production using the external carbon source for biological phosphorus removal systems. This study found that the set 8 experiment could be used as a carbon source for the biological phosphorus removal system as it had the highest acetic acid that suitable for the growth of PAOs in biological phosphorus removal systems.

4 Conclusion

Acetic acid contents from fermentation at temperature of 35 and 55 ^{o}C were not statistically significant ($p = 0.09 > 0.05$) in all experiments from set 1 to set 7. It was also found that optimum conditions for excess sludge fermentation to produce acetic acid was FS: AF (1: 1) in set 8 which resulted 1,406 mg/L acetic acid as final concentration in condition of 120 rpm mixing rate for 96 hours at 35 ^{o}C. The highest acetic acid was also detected in RS: AF (4: 1) (set 9) and RS: AF (1: 4) (set 10) where the acetic acid concentrations were equal to 1,018 and 792 mg/L, respectively. The comparison of acetic acid production of set 8 to set 9 and set 8 to 10 using Paired t-test at 95% confidence level showed that there were significant differences ($p = 0.00 < 0.05$) in both pairs. Nevertheless, the level of acetic acid produced of set 8 to set 9 was not significant different ($p = 0.44 > 0.05$). It was also discovered that in the experiment set 8 to 10, the amount of acetic acid was greater than propionic acid. This study elucidated that the set 8 experiment condition with its carbon source is very potential for the biological phosphorus removal system as it had the highest acetic acid which

suitable for the growth of PAOs in biological phosphorus removal systems.

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