



Kinetics of Anaerobic Digestion of Dairy Fat Waste with Saponification Pre-Treatment

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ABSTRACT

Anaerobic digestion has been an attractive field of research in the era of energy crisis. Biogas, which is the product of anaerobic digestion, provides alternative energy, while at the same time it also prevents pollution due to organic waste accumulation. Among various organic wastes, dairy fat waste is a potential substrate for anaerobic digestion. Fat waste has high theoretical biogas potential because of its high lipid content. However, anaerobic digestion of organic waste with high lipid content is quite challenging. The main obstacle in anaerobic digestion of fat waste is its tendency to form insoluble floating layer on top of the liquid phase. This phenomenon hinders the access of hydrolytic bacteria to the substrate. Saponification is one of the methods to increase the solubility of the floating layer and hence to improve the availability of substrate for the bacteria. Saponification changes the lipid content into soap which has both polar and non-polar functional groups and the polar side will increase the solubility of the substrate in water. This study evaluated the effect of different dosage of base added as the reactant during saponification pre-treatment on the productivity of anaerobic digestion of dairy fat waste. The kinetics of the anaerobic digestion process was analyzed by mean of mathematical model. The variations of the alkaline dosages studied for saponification pre-treatment were 0.04 mol base/g sCOD; 0.02 mol base/g sCOD; and no pre-treatment for control reactor. This study proved that saponification increased the solubility of dairy fat waste. This result was confirmed by the hydrolysis constant value (k_H) of 0.00782/day for reactor with saponification, which was twenty times of magnitude higher than the k_H value of 0.00032/day in the reactor without saponification. However, the exposure to high pH during the saponification pre-treatment might somewhat inhibit indigenous acidogenic bacteria in the waste which results in lower methane yield in the reactors with saponification to be compared to the control reactor.

Keywords: anaerobic digestion; dairy fat waste; hydrolysis; saponification

ABSTRAK

Peruraian anaerobik merupakan salah satu bidang riset yang sangat menarik perhatian dalam era krisis energi. Biogas tidak hanya menyediakan energi alternatif, tetapi juga dapat mencegah pencemaran akibat limbah organik. Limbah lemak susu adalah substrat yang potensial untuk proses peruraian anaerobik karena memiliki potensi biogas teoritis yang tinggi akibat kandungan lemaknya yang tinggi. Namun, peruraian anaerobik dari limbah organik dengan kandungan lemak yang tinggi memiliki tantangan tersendiri. Hambatan utama dalam peruraian anaerobik dari limbah lemak susu adalah kecenderungan untuk membentuk lapisan padatan yang tidak larut dan mengapung di bagian atas fase cair. Fenomena ini menghambat akses bakteri hidrolisis terhadap substrat. Saponifikasi adalah salah satu cara untuk meningkatkan kelarutan lapisan padatan tersebut, sehingga

meningkatkan ketersediaan substrat untuk bakteri. Saponifikasi akan mengubah kandungan lemak menjadi sabun yang memiliki gugus fungsi polar maupun non-polar. Gugus fungsi yang bersifat polar akan meningkatkan kelarutan substrat dalam air. Studi ini mengevaluasi pengaruh dari berbagai dosis larutan basa yang ditambahkan sebagai reaktan selama perlakuan awal saponifikasi terhadap peruraian anaerobik limbah lemak susu. Kinetika proses peruraian anaerobik dianalisis dengan menggunakan model matematika. Variasi dosis yang diamati pengaruhnya untuk perlakuan awal saponifikasi adalah 0,04 mol basa/g sCOD; 0,02 mol basa/g sCOD; dan nol (tanpa perlakuan awal saponifikasi). Dari penelitian ini, terbukti bahwa saponifikasi berhasil meningkatkan kelarutan limbah lemak susu dan juga ditunjukkan oleh nilai konstanta hidrolisis (k_H) 0,00782/hari lebih tinggi dua puluh kali lipat dibandingkan dengan nilai k_H 0,00032/hari pada reaktor tanpa saponifikasi. Akan tetapi, penelitian ini juga mengindikasikan bahwa bakteri asidogenik bawaan substrat terhambat kinerjanya oleh paparan pH yang tinggi selama perlakuan awal saponifikasi berlangsung sehingga hasil gas metan yang diperoleh lebih rendah daripada reaktor kontrol.

Kata kunci: hidrolisis; limbah lemak susu; peruraian anaerobik; saponifikasi

1. Introduction

There has been growing interest on renewable energy researches as an effort to replace the rapidly decreasing fossil fuel reserve. According to British Petroleum (2017), it was predicted that Indonesia's oil reserve will be depleted in the next 10.3 years while the coal reserve will be depleted in the next 59 years. One of the renewable energy sources that attracts attentions is biogas, which may be produced from anaerobic digestion of organic waste. Biogas production not only provides alternative energy, but it can also reduce the emission of organic waste into environment.

Anaerobic digestion is a process to decompose organic compounds by utilizing microbial metabolism processes that takes place in anaerobic condition (Zhang et al., 2016). This process consists of four stages, namely: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In the first stage, complex organic compounds that act as substrates for microbes are hydrolyzed into simpler compounds. The result of protein hydrolysis is its constituent amino acids, while the results of fat hydrolysis are glycerol and fatty acids. Meanwhile, hydrolysis of carbohydrates will produce sugar and alcohol compounds. The acidogenesis process breaks down these compounds into volatile fatty acids in the form of propionic acid, butyric acid, and pentanoic acid which are further decomposed into acetic acid

through the process of acetogenesis. The acetic acid is then converted to methane in the process of methanogenesis (Rasit et al., 2015).

One example of waste that has high content of organic compounds is dairy fat waste. This waste is the scrapped material from the surface of dissolved air flotation (DAF) unit in the waste treatment facility installed in a dairy industry. With the continuously increasing milk production in Indonesia up to 909,638 tons/year at 2018 (BPS, 2019), then Indonesia produces approximately twice as much of wastewater (Karadag et al., 2014) that resulted in about 18,000 tons dairy fat waste per year. High amount of fatty waste is a threat to the environment if it is not treated properly.

On the other hand, fat has the highest theoretical biogas potential compared to other organic compounds. However, the low solubility of fat in water causes the formation of insoluble floating phase so that fat bioavailability is always quite low (Halalshah et al., 2005). Several studies have been carried out to improve the bioavailability of waste with high fat content. Masse (2003) succeeded to achieve 5% reduction in the degradation time of pork slaughterhouse waste by pre-treatment using enzymatic hydrolysis and the process accomplished 80% reduction of fat content in the waste. Meanwhile, Cirne (2006) has examined the effect of bio-augmentation pre-treatment using anaerobic lipolytic bacterial strains in restaurant waste with high fat content. It was found that the pre-

treatment of enzymatic bio-augmentation succeeded in increasing the hydrolysis rate of the waste. In addition, Battimeli (2009, 2010) pre-treated the slaughterhouse waste by saponification and succeeded in increasing biogas production by 3-7%. Previous works (Cuetos et al., 2010, Cavaleiro et al., 2013) investigated the effect of pasteurization and NaOH pre-treatments of slaughterhouse waste for co-digestion with organic fraction of municipal solid waste (OFMSW). However, both studies reported that the pre-treatments were unable to increase the methane yield.

Among the pre-treatments that have been carried out, saponification is one of the simplest method and has been proven to be successful in improving the performance of anaerobic digestion of wastes with high fat content (Carrere et al., 2015). However, the kinetic model which includes the effect of saponification pre-treatment on anaerobic digestion of fatty wastes is still scarcely reported. Therefore, the objective of this study was to observe the effect of saponification with three dosages of alkali addition. Mathematical modelling was used to further elaborate the kinetics of processes with treatment variations.

2. Research Methodology

2.1 Materials

The materials used in this study included the effluent of biodigester treating biodiesel waste as inoculum and milk fat waste from DAF unit (DAF float) as substrate. The effluent of biodigester treating biodiesel waste was obtained from a biodiesel plant in Gresik, East Java, while the DAF float was obtained from PT Greenfield, Malang, East Java. The characteristics of substrate and inoculum are presented in Table 1 and 2 respectively.

Saponification pre-treatment was conducted using the mixture of NaOH pellet (Merck) and KOH pellet (Merck) dissolved in demineralized water to make solutions of 1 M and 2 M, respectively. Both solutions were then mixed with the molar ratio of 25:75. Neutralization of

saponification product was carried out using HCl 37% (p.a. Merck).

Table 1. Characteristics of dairy fat waste used as substrate

Parameter	Dry weight (% w/w)
Water	91.29
Ash	1.17
Fat	0.9
Total Protein	1.18
Carbohydrate	5.48

Table 2. Characteristics of inoculum

Parameter	Value
pH	7.71
sCOD (mg/L)	765
VFA (mg/L)	184.03
TS (mg/L)	28,073.79
VS (mg/L)	16,303.05
Total anaerobic microbes count	6.1×10^6

2.2 Experimental procedures

The experiments were carried out in 2.8 L batch reactors connected to gasometers which were used to measure the volume of biogas formed by water displacement method as shown in Figure 1. The liquid used in this gasometer is 75% saturated salt water with pH 2 in accordance to the method used by Walker et al. (2009) to prevent CO₂ absorption into the water. Liquid samples were taken from the valve located at the bottom of the reactor while gas samples were withdrawn from the tube connecting the reactor and the gasometer.

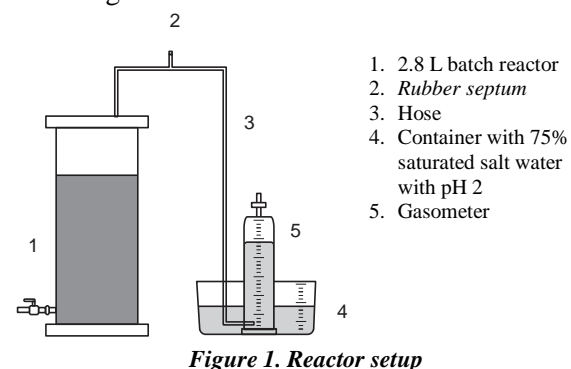


Figure 1. Reactor setup

Three reactors were run with variations in the dose of alkaline solutions used in the saponification pre-treatment as shown in Table 3. According to previous publication, the optimum alkali dosage for fat saponification was 0.04 moles of base/g sCOD (Battimelli et al., 2009,

2010). In this experiment, we tested this recommended dosage as “full saponification” and half dosage as “half saponification”. The codes of the three reactors were RNS (Reactor with No Saponification), RHS (Reactor with Half Saponification), and RFS (Reactor with Full Saponification).

Table 3. Summary of experimental setup

Reactor Name	Substrate	Inoculum
RNS	Unsaponified DAF float	Biodiesel Inoculum
RHS	Saponified DAF float with 0.02 moles of base/g sCOD	Biodiesel Inoculum
RFS	Saponified DAF float with 0.04 moles of base/g sCOD	Biodiesel Inoculum

The base used as the reactant in the saponification pre-treatment step is a mixture of NaOH and KOH with molar ratio of 25:75 in accordance to the method used by Kurnianto (2019) and Kurnianto et al. (2019). Saponification was conducted for 2 hours at 70 °C. Prior to anaerobic experiments, the pH of the saponified substrate was neutralized by adding HCl 37%, while making sure that the addition of chlorine was still below the maximum tolerable value of 8.0 (Cioabla et al., 2012). For anaerobic experiment, 1,500 ml of the substrate was mixed with 500 ml of biodiesel inoculum. The experiments were run after each reactor was flushed with N₂ gas.

2.3 Analytical methods

The analysis of chemical oxygen demand (COD) and soluble chemical oxygen demand (sCOD) was conducted using colorimetric method while the analysis of volatile fatty acids (VFA) was carried out using the titrimetric method in accordance to APHA Standard Methods (Clesceri et al., 2005). For the analysis of methane content in the biogas, Gas Chromatography Shimadzu GC 8A was used with Porapak Q column, UHP helium gas carrier at 40°C column temperature, 60°C temperature detector and injector. In addition, pH measurement was conducted using Lutron PH-

208. Lastly, initial bacterial count of each reactors was measured using anaerobic total plate count method.

2.4 Mathematical modeling

The kinetic model used in this study refers to previously published work by Ramadhani et al. (2017) with some modifications. Anaerobic decomposition process is modeled into three stages, namely: 1) the hydrolysis stage to dissolve the substrate, followed by 2) the acidogenesis stage which changes the substrate into VFA, then 3) the methanogenesis stage to convert VFA into biogas. The acetogenesis stage is combined with the acidogenesis stage because the VFA measurements that was used in this research cannot differentiate the acidic products from each stage. This model was illustrated in Figure 2.

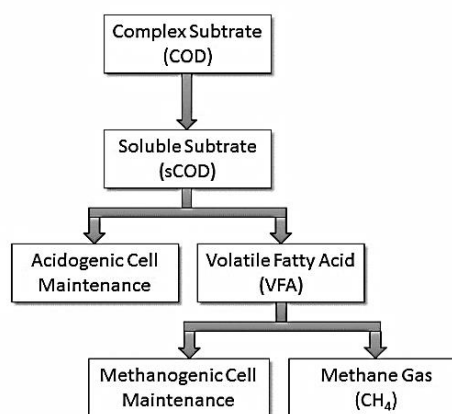


Figure 2. Simplification of anaerobic digestion stages

Acidogenic microbes are symbolized as X₁, while methanogenic microbes are as X₂. The mass balance of each microbial group is shown in Equations (1) and (2) with the constant of μ_{nett} is defined in Equation (3).

$$\frac{dX_1}{dt} = \mu_{nett1} X_1 \tag{1}$$

$$\frac{dX_2}{dt} = \mu_{nett2} X_2 \tag{2}$$

$$\mu_{nett} = \mu_g - k_d \tag{3}$$

Due to non-ideal substrate with high suspended solid content, we used Contois equation to represent the growth rate of

acidogenic and methanogenic microbes, with specific growth rate constants of μ_{g1} and μ_{g2} , respectively. The Contois expressions for μ_{g1} and μ_{g2} are shown in Equations (4) and (5).

$$\mu_{g1} = \frac{\mu_{m1}sCOD}{K_{SX1}X_1 + sCOD} \quad (4)$$

$$\mu_{g2} = \frac{\mu_{m2}VFA}{K_{SX2}X_2 + VFA} \quad (5)$$

In this study, the concentration of initial substrate (before saponification) is represented by the value of COD. Successful saponification results in hydrolyzed substrate measured as sCOD. The hydrolysis process that converts COD to sCOD is assumed to follow the equation most commonly used to represent the hydrolysis rate, i.e. first order kinetic (Vavilin et al., 1996) as shown in Equation (6).

$$\frac{dCOD}{dt} = -k_H COD \quad (6)$$

The accumulation of sCOD represented in Equation (7) is the result of COD solubilization through saponification, while the consumption of sCOD is used for VFA production and maintenance of acidogenic microbes. In Equation (7), cell maintenance is represented by the last term.

$$\frac{dsCOD}{dt} = k_H COD - \frac{1}{Y_{X_1/sCOD}} \left(\frac{dX_1}{dt} \right) - m_1 X_1 \quad (7)$$

Furthermore, for the VFA mass balance (Equation 8), VFA accumulation is produced by decomposition of sCOD, while VFA consumption is used to produce methane and for maintenance of methanogenic cells.

$$\frac{dVFA}{dt} = Y_{VFA/X_1} \left(\frac{dX_1}{dt} \right) - \frac{1}{Y_{X_2/VFA}} \left(\frac{dX_2}{dt} \right) - m_2 X_2 \quad (8)$$

Finally, the accumulation of methane is only produced by VFA consumption by methanogenic microbes as shown in Equation (9).

$$\frac{dCH_4}{dt} = Y_{CH_4/X_2} \left(\frac{dX_2}{dt} \right) \quad (9)$$

3. Results and Discussion

Table 4 shows the initial COD concentration of all three reactors. As listed in Table 4, the initial COD concentration of all reactors were approximately the same. Therefore, the comparison of sCOD concentration profiles can be performed fairly among treatments.

Table 4. Initial COD concentration of RNS, RHS, and RFS

Reactor name	Initial COD concentration (mg/L)
RNS	20,562
RHS	20,008
RFS	20,687

With respect to anaerobic digestion, the acidogenesis process in RHS and RFS took place at a slower rate than in RNS. This tendency is shown by the fluctuation of sCOD concentration on RHS and RFS and sharply decreasing VFA concentration in both reactors as seen in Figure 3(c). In both RHS and RFS, we did not observe VFA peaks which normally appeared in batch anaerobic process. The reason for slow rate of acidogenesis process in RHS and RFS might be the non-ideal environment for the growth of indigenous acidogenic bacteria in the substrate (after saponification). This is due to the exposure of high pH during the saponification pre-treatment. As mentioned in many studies, it has already been known that the optimum pH in acidogenesis stage is acidic (Zoetemeyer et al. 1982; Joubert and Britz, 1986; Kisaalita et al., 1987; Yu and fang, 2002) while saponification was conducted in alkaline condition. This speculation was supported by the decrease in bacteria count in substrate before and after full-dose of alkaline saponification (0.04 mol base/g sCOD) that was measured using anaerobic total plate count (TPC) method as shown in Table 5.

Table 5. Anaerobic plate count of substrate before and after saponification pre-treatment

Substrate condition	Viable bacteria count (cfu/ml)
Before saponification	4.6x10 ⁶
After saponification	1.8x10 ⁵

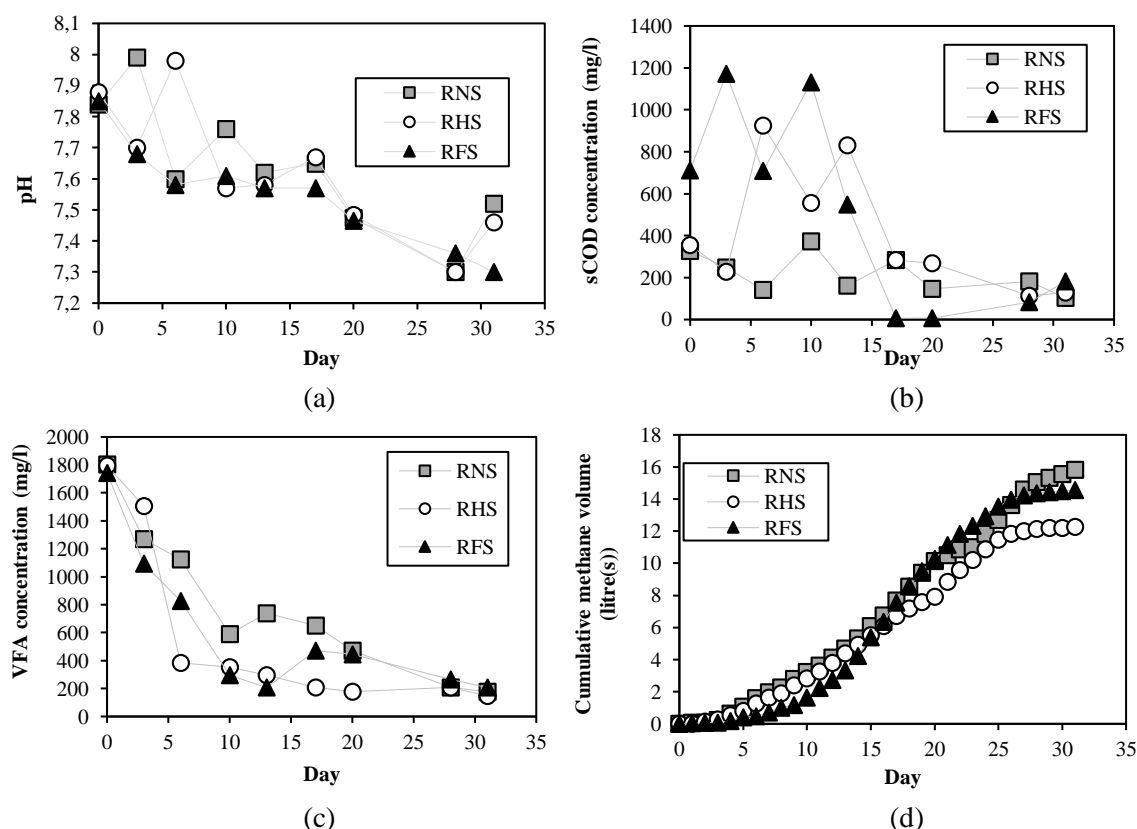


Figure 3. Data of: (a) pH values, (b) sCOD concentration, (c) VFA concentration, and (d) cumulative methane production

At the end of the experiment, the COD concentration of all three reactors were also measured as shown in Table 6. The COD removal in RFS is the highest followed by RHS and RNS respectively. This also proved that saponification pre-treatment managed to increase bio-availability of the fatty substrate.

On the other hand, Figure 3(d) shows that the methane gas production of RNS and RFS was almost the same (15.8 liters and 14.6 liters, respectively) on day 30, while the methane production of RHS was below both of them. Even though RFS gas production was somewhat lower than RNS, from the perspective of waste treatment, RFS performance was better than RNS because it managed to decompose twice as much COD as RNS. In this study, we also considered the possibility of light metal ion inhibition. According to a published work (Deublein and Steinhauser, 2008), the inhibiting concentrations of Na⁺ and K⁺ ions are 5,000-30,000 mg/l and 2,500-5,000 mg/l respectively. The total concentration of Na⁺ and K⁺ ions added to the saponified reactors for saponification pre-treatment are summarized in Table 7.

Table 6. Percentage of COD removal of RNS, RHS, and RFS

Reactor name	Final COD (mg/l)	COD removal (%)
RNS	15,632	23.98%
RHS	11,625	41.90%
RFS	8,637	58.25%

Table 7. Summarization of Na⁺ and K⁺ ions concentration in RHS and RFS

Reactor name	Na ⁺ concentration (mg/l)	K ⁺ concentration (mg/l)
RHS	457.125	2,325.4
RFS	914.25	4,650.75

It was found that in RFS, the concentration of K⁺ ions exceeded the aforementioned inhibiting concentration limit while it was not the case in RHS. This may be the reason as to why the initial methane production rate in RFS was notably lower than the other reactors.

The data of sCOD concentration, VFA concentration, and methane concentration was also fitted to the kinetic model using MATLAB. The results of data fitting for RNS, RHS, and RFS were summarized in Figure 4(a)-(c), Figure 5(a)-(c), and Figure 6(a)-(c).

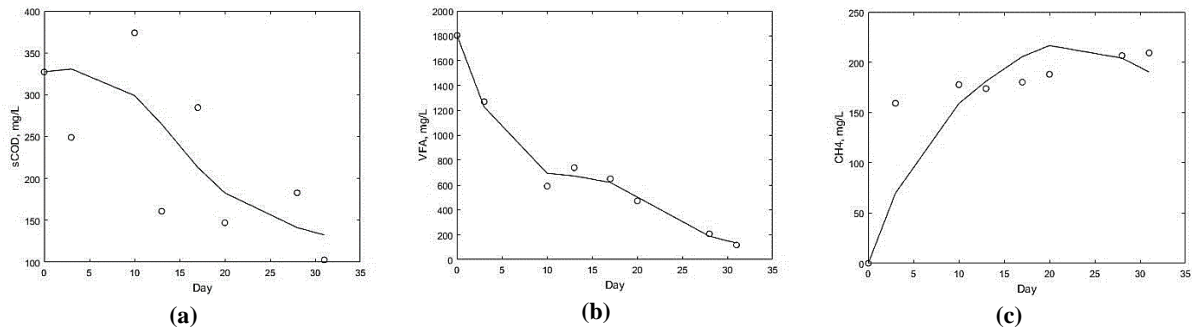


Figure 4. Data fitting of RNS: (a) sCOD; (b) VFA; and (c) CH₄

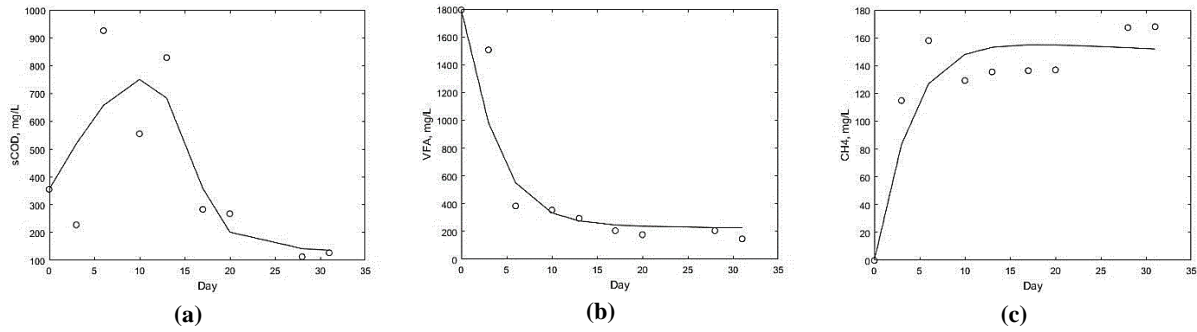


Figure 5. Data fitting of RHS: (a) sCOD; (b) VFA; and (c) CH₄

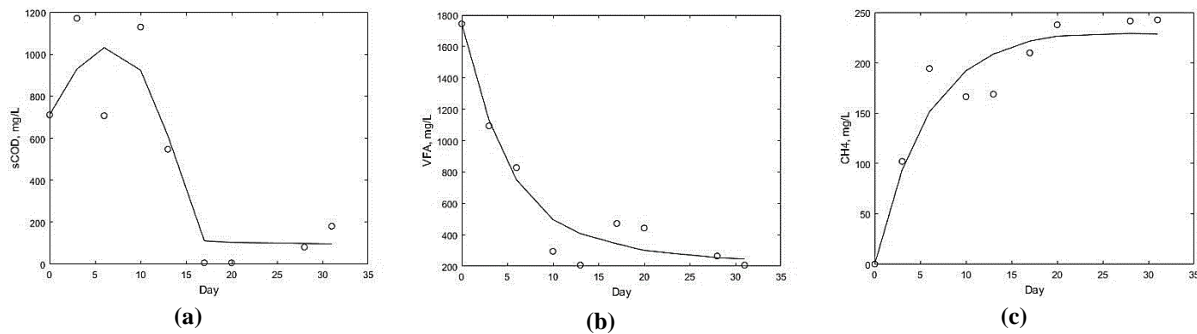


Figure 6. Data fitting of RFS: (a) sCOD; (b) VFA; and (c) CH₄

Table 8. Kinetic constants from the kinetic model developed in this study

Kinetic constants	Value of constants			Unit
	RNS	RHS	RFS	
k_H	0.000320	0.003062	0.007819	day ⁻¹
μ_{m1}	4.4404	3.6292	2.9524	day ⁻¹
μ_{m2}	2.0979	8.3197	10.4899	day ⁻¹
k_{d1}	4.2586	3.3538	2.8317	day ⁻¹
k_{d2}	0.03624	0.04118	0.0358	day ⁻¹
K_{SX1}	0.05	0.022	0.02	mg sCOD/mg cell
K_{SX2}	350	60	70	mg VFA/mg cell
m_1	0.09146	0.11543	0.7877	mg sCOD/(mg cell.day)
m_2	0.5846	0.004195	0.001205	mg VFA/(mg cell.day)
$Y_{XI/sCOD}$	1.0398	1.9216	0.1284	mg cell/mg sCOD
$Y_{VFA/XI}$	9.2309	0.005727	0.4112	mg VFA/mg cell
$Y_{X2/VFA}$	0.029460	0.53746	0.78369	mg cell/mg VFA
$Y_{CH4/X2}$	3.6948	0.1916	0.1903	mg CH ₄ /mg cell

The data fitting produced kinetics constants for every reactor as summarized in Table 8. It could be seen that the k_H value of RNS was the lowest of the three (about one tenth of the k_H in RHS), followed by RHS and RFS respectively. This trend confirmed that saponification was significantly increasing the solubility of the substrate.

Furthermore, it can be seen that the values of μ_{m1} (4.440 day⁻¹) in RNS was higher than those in both RHS and RFS. Meanwhile, the value of m_1 increased with the increase of alkaline dosage added in the pre-treatment. Both of these data are in good accordance to the aforementioned assumption about inhibition of anaerobic bacteria caused by the exposure of high pH during the saponification pre-treatment. This finding suggests the necessity of further study to

overcome the alkaline inhibition resulted from the saponification pre-treatment so that the biogas production from pre-treated fatty wastes can be maximized.

4. Conclusions

The consequences caused by saponification pre-treatment on anaerobic digestion of dairy fat waste has been investigated in the present work. The results of this study confirm that saponification pre-treatment on dairy fat waste increases COD solubility which leads to the increase in COD removal. A mathematical model has been constructed to elaborate the kinetics of the anaerobic digestion of the pre-treated fat waste. The constants obtained by data fitting on the mathematical model further confirms that the value of k_H representing the solubilization rate of the substrate, was the lowest in RNS (the one without saponification), which was 0.00031 day^{-1} . The highest k_H value of 0.00782 day^{-1} was observed in RFS (saponification with full alkaline dosage). The values of constants obtained from fitting the mathematical model with experimental data indicate that anaerobic bacteria were inhibited during the saponification pre-treatment. As a result, in terms of gas production, the anaerobic process in reactor with saponification pre-treatment (RHS and RFS) showed somewhat lower performance than the anaerobic process in reactor without saponification (RNS). Further study is needed to eliminate the disadvantages regarding process inhibition caused by the alkaline addition.

Notation

k_H	= first order hydrolysis kinetic constant, day^{-1}
μ_{m1}	= maximum acidogenic microbial growth rate constant, day^{-1}
μ_{m2}	= maximum methanogenic microbial growth rate constant, day^{-1}
k_{d1}	= acidogenic microbial death rate constant, day^{-1}
k_{d2}	= methanogenic microbial death rate constant, day^{-1}
K_{SX1}	= half saturation constant of

	acidogenic microbe, $\text{mg sCOD}/(\text{mg acidogenic cell})$
K_{SX2}	= half saturation constant of methanogenic microbe, $\text{mg sCOD}/(\text{mg methanogenic cell})$
m_1	= acidogenic microbe maintenance constant, $\text{mg sCOD}/(\text{mg acidogenic cell.day})$
m_2	= methanogenic microbe maintenance constant, $\text{mg sCOD}/(\text{mg methanogenic cell.day})$
$Y_{X1/sCOD}$	= mass yield of acidogenic microbe produced per unit of sCOD ($\text{mg acidogenic cell}/\text{mg sCOD}$)
$Y_{VFA/X1}$	= mass yield of VFA formed per unit of acidogenic microbe ($\text{mg VFA}/\text{mg acidogenic cell}$)
$Y_{X2/NFA}$	= mass yield of methanogenic microbe produced per unit of sCOD ($\text{mg methanogenic cell}/\text{mg VFA}$)
$Y_{CH4/X2}$	= mass yield of CH_4 formed per unit of methanogenic microbe ($\text{mg VFA}/\text{mg methanogenic cell}$)

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