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by Iqbal Syaichurrozi

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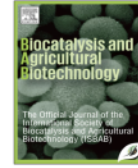
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Effect of initial pH on anaerobic co-digestion of *Salvinia molesta* and rice straw for biogas production and kinetics

Iqbal Syaichurrozi, Suhirman Suhirman, Topik Hidayat

Department of Chemical Engineering, University of Sultan Ageng Tirtayasa, Jl. Jendral Soedirman Km 3, Cilegon 42435, Indonesia



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ABSTRACT

The goal of this study was to investigate the effect of initial pH (6, 6.9 (control), 7, and 8) on biogas production from co-digestion of *Salvinia molesta* (SM) and rice straw (RS). The ratio of SM and RS was varied to be 40:60 and 0:100. This study used lab-scale batch anaerobic digesters operated at room temperature for 40 days. The results showed that ratio of 40:60 produced more total biogas yield (53.25–61.38 mL/g total solid (TS)) than ratio of 0:100 (45.98–51.20 mL/g TS) at all initial pH variations. Substrate pH during digestion in ratio of 40:60 was more stable than that in ratio of 0:100. The highest total biogas yield in ratio of 40:60 and 0:100 was obtained at initial pH of 8 (digester of P8, 61.38 mL/g TS) and 7 (digester of Q7, 51.20 mL/g TS) respectively because a large amount of volatile fatty acids (VFAs) was converted to biogas. Biogas from ratio of 40:60 contained higher methane content than that from ratio of 0:100. Furthermore, in ratio of 40:60, the methane content of biogas was not affected significantly by variation of initial pH. Surprisingly, the best condition for biogas production was achieved at ratio of 40:60 with initial pH of 8. In order to modeling the kinetics of biogas production, the Cone model was the best of all proposed models (modified Gompertz, first order kinetic, Cone model) because it resulted Mean Absolute Percentage Error (MAPE) of 8.42–18.60% while the others showed MAPE of more than 20%.

1. Introduction

To fulfill the national energy source, Indonesian government has target to decrease fossil fuels need from 92% (at year 2013) to 69% (at year 2050) and to increase renewable energy need from 8% (at year 2013) to 31% (at year 2050) (Dewan Energi Nasional, 2014; International Renewable Energy Agency, 2017; Pemerintah Republik Indonesia, 2014). Biogas, which is one of renewable energy sources, is very potential to be produced in Indonesia. As agricultural country, Indonesia has abundant aquatic weed and waste of plants that can be used as biogas feedstock such as *Salvinia molesta* (SM) and rice straw (RS) (Syaichurrozi, 2018).

SM is one of free-floating aquatic weeds that can grow more rapidly than water hyacinth. It also has higher doubling time (3–10 days) than water hyacinth (7–12 days) (Abbasi and Nipanay, 1984; Mathew et al., 2015). Some problems occurred when SM presents in agricultural system because it will (1) reduce the irrigation system efficiency, (2) reduce the fertilizer effectiveness for rice plants, (3) reduce the amount of dissolved oxygen in water (Syaichurrozi, 2018; Madsen and Wersal, 2008). Because of these problems, the total production of rice plants at harvesting season will decrease. Meanwhile, RS is a waste generated from rice fields at harvesting. Every single rice plant will generate RS as

much as 58% from its total mass (Syaichurrozi, 2018). Therefore, the amount of RS is very large (Yanli et al., 2010; Dehghani et al., 2015). Conventionally, RS are burned or discarded but it causes environmental pollutions (Ye et al., 2013).

Co-digestion concept of SM and RS is a good way to convert agricultural wastes to be biogas energy. For rural community, SM and RS are common problems in rice fields because rice farmer is their main occupation. By anaerobic co-digestion, the wastes can be utilized to produce biogas. In previous study, Syaichurrozi (2018) found that the co-digestion with SM:RS ratio of 40:60–0:100 (mass basis) was the optimum ratio range producing the total biogas yield of 103.83–113.92 mL/g volatile solid (VS). The mixture of SM and RS gives the substrate rate with good ratio of carbon per nitrogen (C/N). Beside ratio, the initial pH of substrate is also an important parameter that influences the total biogas yield (Budiyono et al., 2014b).

Initial pH is related to adaptation time for bacteria in the system (Budiyono et al., 2014b) and acid-base equilibrium of bacterial cell (Brannen and Davidson, 1993), which affect the production rate of biogas (Syaichurrozi et al., 2016b). The optimum pH range in anaerobic digestion can be 6.9–7.3 (Metcalf, 2003), 6.4–7.6 (Anderson and Yang, 1992) or 6.5–8.2 (Speece, 1996). If the pH value is lower or upper than the optimum range, biogas production rate is low.

E-mail addresses: iqbal_syaichurrozi@untirta.ac.id, iqbalsyaichurrozi@gmail.com (I. Syaichurrozi).

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In this study, authors investigated the effect of initial pH on biogas from co-digestion of SM and RS. Based on our study of literatures, this idea has not been reported by other authors yet. A few authors have reported the study of biogas production from SM. Mathew et al. (2015) compared biogas production from SM and water hyacinth at mesophilic temperature with cow dung as inoculums. O'Sullivan et al. (2010) also compared some weeds (such as SM, water hyacinth and cabomba) as biogas production feedstock. Furthermore, Syaichurrozi (2018) studied the effect of co-digestion of SM and RS on enhancement of biogas yield. Based on information above, this study was new and important for agricultural country such as Indonesia because SM and RS are very abundant and potential as biogas feedstock. This study investigated the effect of initial pH (control, 6, 7, 8) on biogas from co-digestion of SM and RS with ratio of 40:60 and 0:100. Furthermore, authors compared some popular models (modified Gompertz, first order kinetic, Cone model) to find the best model to simulate the biogas production rate. The kinetic constants obtained from the models would be discussed deeply to learn more about the effect of initial pH on anaerobic process.

2. Methods

2.1. Wastewater and inoculums

The SM and RS were obtained from rice fields in Bayah Regency (Banten Province, Indonesia). The fresh SM and RS were washed using clean water and then dried under the sun. Then, their size was reduced to be 18 mesh using a blender. Inoculums as bacteria provider was originated from fresh rumen fluid, which was waste of cow slaughterhouse in Cilegon City (Banten Province, Indonesia). The fresh rumen fluid (contained *Clostridium* sp., *Clostridium sporogenes*, *Clostridium butyricum* and rich methanogenic bacteria) used in this study was same with that used by Syaichurrozi (2018) and Syaichurrozi et al. (2016b).

2.2. Experimental set up

Lab-scale anaerobic digesters (600 mL-polyethylene bottles) were used in this study. The digesters were also used by others (Budiyono et al., 2013, 2014a; Syaichurrozi et al., 2016a, 2016b; Syaichurrozi, 2018). The polyethylene bottles were obtained from commercial market in Indonesia. The digesters were plugged by using rubbers and equipped by using valves for measuring biogas volume. Biogas that was produced during anaerobic digestion (AD) was measured by using water displacement method (Yusuf et al., 2011; Yusuf and Ify, 2011). In this method, every digester was connected with reversed cylindrical glass (as gas collector) through a connecting tube. Each reversed cylindrical glass was immersed in water to ensure complete sealing. If the valve was opened, the biogas flowed through the tube and the biogas replaced the water position in the reversed cylindrical glass. Biogas volume (mL) was recorded by the downward displacement of the water. Then, the biogas yield (mL/g TS) was obtained by dividing the biogas volume (mL) by initial total solid (g TS). The lab-scale anaerobic digester and water displacement method were shown in Fig. 1.

2.3. Experimental design

Lab-scale anaerobic digesters were run in batch system. Substrates with SM:RS ratio of 40:60 and 0:100 (mass basis, with total mixture mass of 10 g) were chosen in this study because these ratios were in optimum range of SM:RS ratio based on study of Syaichurrozi (2018). The substrate with SM:RS of 40:60 as much as 10 g contained 9.15 g total solid (TS), 2.69 g ash, 0.09 g crude lipid, 0.64 g crude protein, 3.04 g crude fiber, 5.73 g crude carbohydrate, 0.93 g lignin, 0.61 g cellulose, 0.48 g hemicelluloses, C/N ratio of 34.83. Meanwhile, the substrate with SM:RS of 0:100 as much as 10 g contained 9.45 g total solid (TS), 2.08 g ash, 0.09 g crude lipid, 0.87 g crude protein, 3.59 g crude fiber, 6.42 g crude carbohydrate, 0.77 g lignin, 0.90 g cellulose,

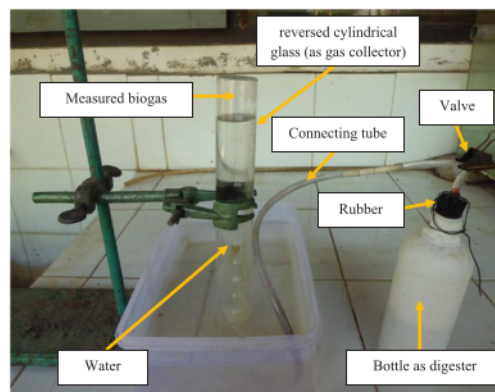


Fig. 1. The lab-scale anaerobic digester and water displacement method.

0.61 g hemicelluloses, C/N ratio of 29.50. Water was added with substrate/water (S/W) ratio of 1/16 w/v. Initial pH was varied to be control (without adjusting, 6.9), 6, 7, and 8 by using NaOH 0.01 N and HCl 0.01 N. Inoculums was added as much as 25 mL (Syaichurrozi, 2018). The origin fresh rumen fluid (inoculums) used in this study contained TS of 4%w/v. The variable in this study could be seen in Table 1.

2.4. Experimental procedures

Digestion was conducted for 40 days at room temperature ($-30\text{ }^{\circ}\text{C}$) and pressure of 1 atm. According to Membere and Sallis (2018), to build kinetic models of biogas production, the digestion was conducted during 40 days because this period was enough to determine the best variable, develop the kinetic models and predict the maximum biogas yield. In this study, authors also did the same period which was 40 days. Daily biogas volume was recorded per two days by using water displacement method. Then, cumulative biogas volume was calculated by sum all of daily biogas volume. For example, cumulative biogas volume for 40 days was obtained from sum of daily biogas volume from beginning until day 40. Furthermore, the daily and cumulative biogas yield (mL/g TS) were determined by dividing the daily and cumulative biogas volume (mL) by initial total solid (g TS). The substrate pH was recorded by using pH meter with specification of model Hanna-Digital-PHEP-98107-1, Hanna instruments, Rumania. First, rubber plugs were pulled out to open the digesters. Then, ± 10 mL substrates for recording the substrate pH were taken from the digesters and the digesters were plugged again immediately. This procedure was done very quickly to keep the suitable condition for methanogenic bacteria. This pH measurement procedure was also applied by Syaichurrozi (2018).

2.5. Chemical analysis

The ammonium ion concentration ($\text{NH}_4^+\text{-N}$) was measured through Standard Methods (APHA, 2012). The ammonia ($\text{NH}_3\text{-N}$) and volatile fatty acids (VFAs) concentration was calculated through Eq. (1) proposed by El-Mashad et al. (2004) and (2) proposed by Paul and Beauchamp (1989) respectively. Then, the total ammonia nitrogen (TAN) was calculated using Eq. (3) (Syaichurrozi, 2018). In the end of digestion, the final TS (total solid) was measured through Standard Methods (APHA, 2012), and then the TS removal was calculated through Eq. (4) (Syaichurrozi et al., 2016b). The methane percentage in biogas was recorded using GC-TCD (Gas Chromatography-Thermal Conductivity Detector) Shimadzu 8A.

Table 1
Variable in this study.

Digester code	Substrate (g)			C/N ratio	Substrate/Water (w/v)	Water (mL)	Rumen fluid (mL)	Initial pH	Total Solid (g)
	SM (g)	RS (g)	Total (g)						
P6	4	6	10	34.83	1/16	160	25	6	10.15
P6.9	4	6	10	34.83	1/16	160	25	6.9 (control)	10.15
P7	4	6	10	34.83	1/16	160	25	7	10.15
P8	4	6	10	34.83	1/16	160	25	8	10.15
Q6	0	10	10	29.50	1/16	160	25	6	10.45
Q6.9	0	10	10	29.50	1/16	160	25	6.9 (control)	10.45
Q7	0	10	10	29.50	1/16	160	25	7	10.45
Q8	0	10	10	29.50	1/16	160	25	8	10.45

Remarks: SM, *Savinia molesta*; RS, Rice straw; C/N, Carbon/nitrogen.

Table 2
The results of anaerobic digestion.

Digester code	Initial pH	Total biogas yield (mL/g TS)	Final pH	Final NH ₄ ⁺ -N (mg/L)	Final NH ₃ -N (mg/L)	Final Ratio of NH ₄ ⁺ :NH ₃	Final TAN (mg/L)	Final VFAs (mg/L)	Initial TS (g)	Final TS (g)	TS removal (%)	Biogas content		
												CH ₄ (%)	CO ₂ (%)	H ₂ (%)
P6	6	53.25 ± 13.15	6.10 ± 0.10	145.80	0.15	99.9:0.1	145.95	240.60	10.15	6.8	33.00	na	na	na
P6.9	6.9	54.78 ± 11.43	6.85 ± 0.05	133.65	0.75	99.4:0.6	134.40	171.66	10.15	6.4	36.94	65.45	33.47	1.08
P7	7	58.28 ± 4.68	7.15 ± 0.05	170.10	1.90	98.9:1.1	172.00	194.14	10.15	6.8	33.00	62.64	37.19	0.17
P8	8	61.38 ± 6.60	7.30 ± 0.00	121.50	1.91	98.5:1.5	123.41	130.13	10.15	22	36.94	68.54	31.46	nd
Q6	6	49.28	6.2	119.50	0.14	99.9:0.1	119.64	194.26	10.45	na	na	na	na	na
Q6.9	6.9	45.98	6.0	133.08	0.11	99.9:0.1	133.19	226.15	10.45	na	na	19.8	48.70	2.32
Q7	7	51.20	6.1	33.95	0.03	99.9:0.1	33.98	56.02	10.45	na	na	na	na	na
Q8	8	49.38	6.2	114.20	0.14	99.9:0.1	114.34	182.84	10.45	na	na	na	na	na

Remarks: na, not analyzed; nd, not detected.

$$(NH_3 - N) = (NH_4^+ - N) \times \left[1 + \frac{10^{-pH}}{10^{-(0.1075 + \frac{2725}{T})}} \right]^{-1}, T = \text{absolute temperature, K} \quad (1)$$

$$pH = 9.43 - 2.02 \times \frac{VFAs}{[(NH_3 - N) + (NH_4^+ - N)]}, R^2 = 0.955 \quad (2)$$

$$TAN = (NH_3 - N) + (NH_4^+ - N) \quad (3)$$

$$TS \text{ Removal}(\%) = \frac{\text{initial TS} - \text{final TS}}{\text{initial TS}} \times 100\% \quad (4)$$

2.6. Kinetic model of biogas production

In this study, biogas production was modeled using some proposed kinetic models, i.e. modified Gompertz model (Eq. (5); Syaichurrozi et al., 2013), First order kinetic model (Eq. (6); Budiyo et al., 2014b), Cone model (Eq. (7); Syaichurrozi, 2018). According to Yusuf et al. (2011) and Syaichurrozi et al. (2013), kinetic of biogas production under batch system was assumed that it had correspondence to specific growth rate of methanogenic bacteria in anaerobic digester. Kinetic constants of y_m, λ, μ, k_{hyd}, n, k were determined by using non-linear regression with software of polymath 5.0 educational version.

$$y(t) = y_m \cdot \exp \left\{ - \exp \left[\frac{\mu \cdot e}{y_m} (\lambda - t) + 1 \right] \right\}, t \geq 0 \quad (5)$$

$$y(t) = y_m (1 - \exp(-k \cdot t)), t \geq 0 \quad (6)$$

$$y(t) = \frac{y_m}{1 + (k_{hyd} \cdot t)^{-n}}, t > 0 \quad (7)$$

where: y(t) = the cumulative biogas yield at digestion time t days (mL/g TS), y_m = the biogas yield potential (mL/g TS), μ = the maximum biogas yield rate (mL/g TS.day), λ = lag phase period or minimum time to produce biogas (days), t = cumulative time for biogas production

(days), e = mathematical constant (2.718282), k_{hyd} = hydrolysis rate constant (/day), n = shape factor, k = the biogas production rate constant (/day)

Furthermore, the Mean Absolute Percentage Error (MAPE) was calculated to find out which was the best model that could fit the measured biogas yield during AD. The formula of MAPE was shown in Eq. (8). If the MAPE value was less than 20%, it considered as a good model.

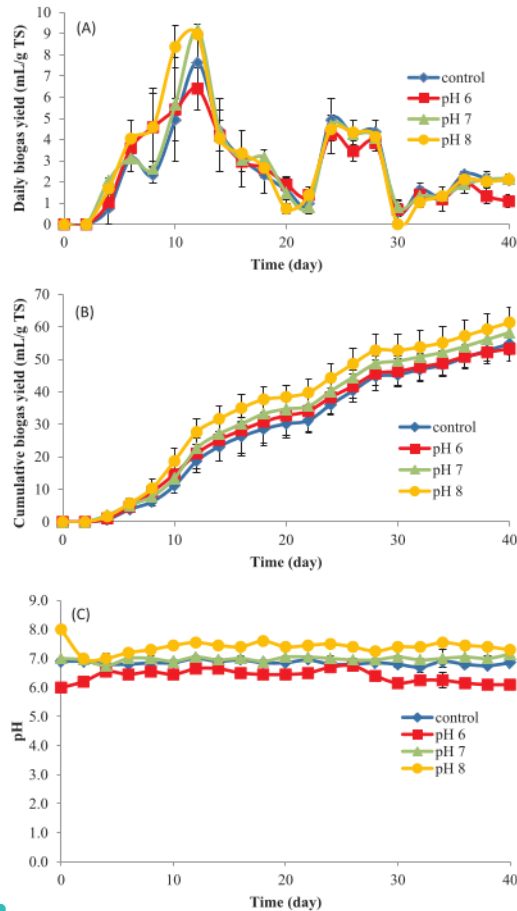
$$MAPE = \frac{1}{n} \sum_{i=1}^n \left(\frac{|\text{Measured biogas} - \text{Predicted biogas}|}{|\text{Measured biogas}|} \right) \times 100\% \quad (8)$$

3. Results and discussions

The effect of initial pH on biogas production from co-digestion of SM:RS was studied during 40 days. The results of anaerobic digestion (AD) for SM:RS ratio of 40:60 and 0:100 at various initial pH were presented in Table 2. Daily and cumulative biogas yield (Fig. 2 and Fig. 3) were recorded per two days with help of water displacement method. The ammonium, ammonia and volatile fatty acids (VFAs) were analyzed during AD (Fig. 4 and Fig. 5). They were intermediate products as results of organic matter degradation by bacterial activity. The total solid removal was measured to find out how much total solid was degraded. Furthermore, the kinetic models were applied to simulate the biogas production during AD and predict the maximum biogas that could be reached (Fig. 6). The kinetic constants in the models could help to learn further about the effect of initial pH on anaerobic process (Table 3).

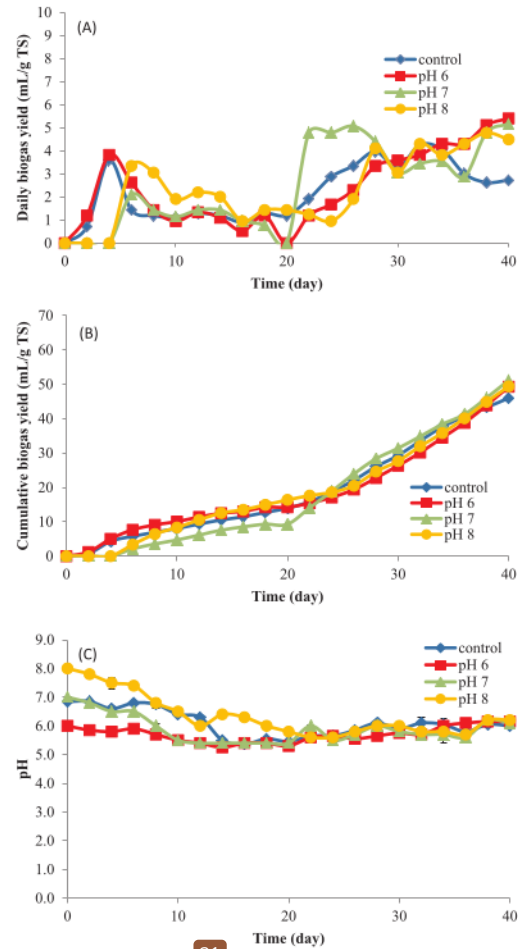
3.1. Biogas production

Organic matters of the substrates were degraded by bacterial activity and converted into biogas during AD. The daily and cumulative biogas yield (mL/g TS) from co-digestion of 40:60 at different initial pH



16 Fig. 2. Effect of initial pH on (A) daily biogas yield, (B) cumulative biogas yield, (C) pH profile in SM:RS of 40:60.

10 were shown in Fig. 2(A and B). The peak value of daily biogas yield was recorded to be 6.40 ± 0.99 , 7.64 ± 1.23 , 9.16 ± 0.30 , 8.97 ± 0.10 mL/g TS after at the same time which was twelve days of digestion from initial pH of 6, control (6.9), 7, 8 respectively (Fig. 2(A)). The daily biogas yield during AD from all digesters (P6, P6.9, P7, P8) experienced fluctuation. The biogas generation started after seeding and increased until day 12. After day 12, it decreased until approximately day 20. However, it increased again until day 28. The fluctuation of daily biogas yield might be caused by the chemical composition of substrates. The mixture substrate of 40:60 contained 3.04 g crude fiber (fiber carbohydrate) and 5.73 g crude carbohydrate. Hence, the non-fiber carbohydrate was as much as 2.69 g. The non-fiber carbohydrate was degraded more easily than the fiber carbohydrate. At period of day 0–12, biogas was generated from degradation of non-fiber carbohydrate. The longer the digestion time, the less the availability of non-fiber carbohydrate, so that at period of day 12–20, daily biogas yield decreased. Furthermore, at period of day 22–28, biogas was generated from degradation of fiber carbohydrate. Hence, this study concluded that bacteria needed a long time (approximately 20 days) to convert fiber carbohydrate into biogas. At period above 28, daily biogas yield decreased because the degradable organic compound remained in little amount. Meanwhile, the total biogas yield was 53.25 ± 13.15 , 54.78 ± 11.43 , 58.28 ± 4.68 , 61.38 ± 6.60 mL/g TS for initial pH of 6, 6.9, 7, 8 respectively (Fig. 2(B), Table 2). The total biogas yield from



31 Fig. 3. Effect of initial pH on (A) daily biogas yield, (B) cumulative biogas yield, (C) pH profile in SM:RS of 0:100.

co-digestion of 40:60 in this study (53.25 – 61.38 mL/g TS) was less than the previous study (113.92 mL/g VS or 80.40 mL/g TS) (Syaichurrozi, 2018). This difference might be caused by the S/W ratio which was 1/53 w/v (TS 5.20%) in this study and 1/7 w/v (9.67%) in previous study. Iyono et al. (2014a) stated that the optimum TS range in anaerobic digestion was 7–9%. If the range less than that, biogas production was not optimal.

Furthermore, the daily and cumulative biogas yield from co-digestion of 0:100 was shown in Fig. 3(A and B). The biogas generation started after seeding and increased until day 4–6. After that day, it decreased until approximately day 20. However, it increased again until approximately day 28. After that, the biogas generation was constant enough until the end of digestion. As same as at ratio of 40:60, the fluctuation of daily biogas yield in ratio of 0:100 was caused by the chemical compositions (non-fiber and fiber carbohydrate content).

According to Fig. 2 and Fig. 3, the digestion time (during 40 days) could be divided into two main periods i.e. day 0–20 and day 21–40. In first period (day 0–20), daily biogas yield from ratio of 40:60 was bigger than that from ratio of 0:100. In addition, the peak value of daily biogas yield from ratio of 40:60 was 6.40 – 9.16 mL/g TS obtained after 12 days (Fig. 2(A)). On the other hand, the daily biogas yield from ratio of 0:100 was below 4 mL/g TS obtained after 4 days (Fig. 3(A)). In this study, substrate of 0:100 contained 3.59 g crude fiber and 6.42 g crude

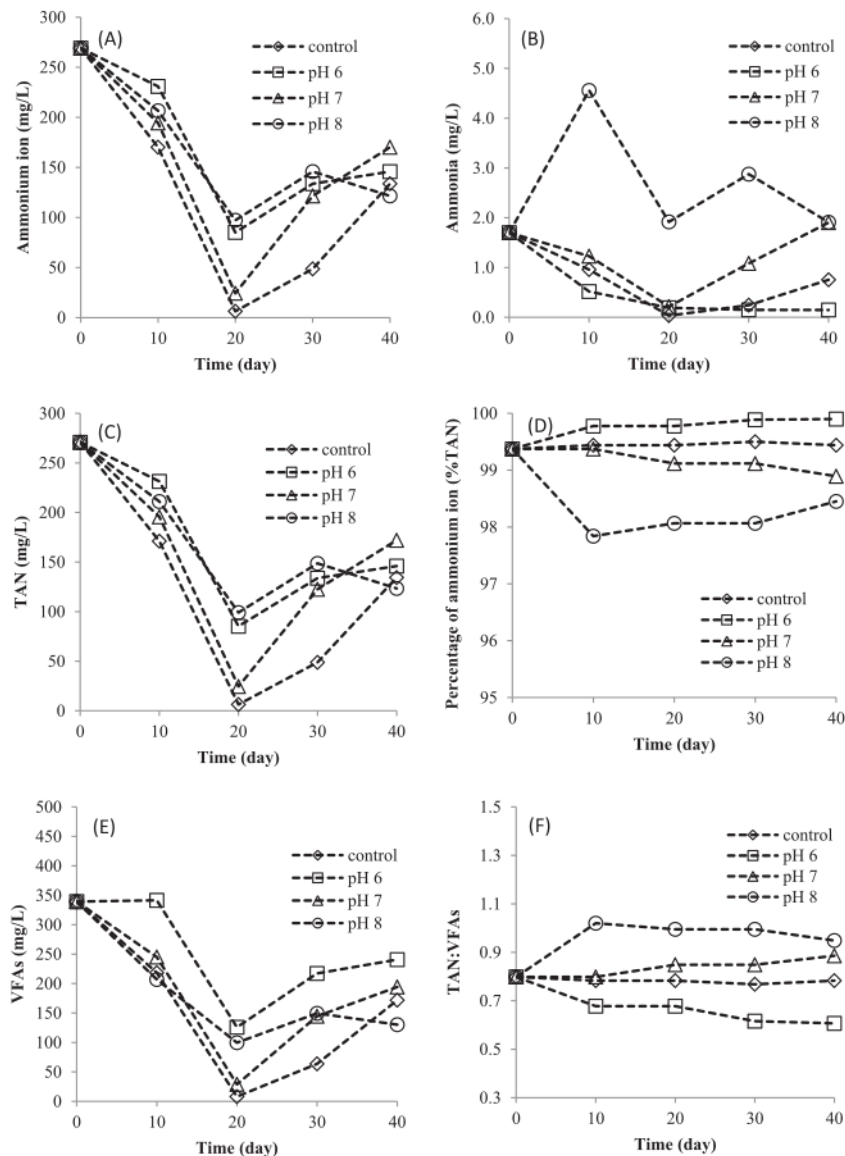


Fig. 4. Production of (A) ammonium ion, (B) ammonia, (C) total ammonia nitrogen (TAN), (D) percentage of ammonium ion in TAN, (E) volatile fatty acids (VFAs), (F) TAN:VFAs during digestion for 40 days at various initial pH in SM:RS of 40:60.

carbohydrate, so that the non-fiber carbohydrate was as much as 2.83 g. It means the substrate of 40:60 contained lower non-fiber carbohydrate (2.69 g) than substrate of 0:100 (2.83 g). Generally, the more the non-fiber carbohydrate content, the easier the substrates was degraded. In fact, biogas was easier produced from ratio of 40:60 than from ratio of 0:100. It was caused by that C/N of 34.83 (ratio of 40:60) was more suitable for biogas from lignocellulosic biomass than C/N of 29.50 (ratio of 0:100) (Syaichurrozi, 2018). The bacteria successfully converted the non-fiber carbohydrate to biogas in substrate of 40:60, whereas bacteria in substrate of 0:100 still adapted in substrate of 0:100 rather than produced biogas.

Furthermore, in period of day 21–40, daily biogas yield from ratio of 40:60 decreased after day 28, while from ratio of 0:100 the biogas was generated constantly until the end of digestion. Biogas from ratio of

40:60 was dominantly produced from fiber carbohydrate because the non-fiber carbohydrate was dominantly degraded in period of 0–20. Meanwhile, biogas from ratio of 0:100 was generated from both non-fiber and fiber carbohydrate because just a little non-fiber carbohydrate was degraded in period of day 0–20.

At all various initial pH, the total biogas yield from ratio of 40:60 (53.25–61.38 mL/g TS) was higher than that from the ratio of 0:100 (45.98–51.20 mL/g TS) (Table 2). Hence, ratio of 40:60 was better than ratio of 0:100 in various initial pH. In previous study, SM:RS ratio of 40:60 produced higher total biogas yield than ratio of 0:100 when initial pH was adjusted at level 7 (Syaichurrozi, 2018). This present study varied the initial pH in range of 6–8. The results showed that ratio of 40:60 was better than ratio of 0:100 at various initial pH with range of 6–8.

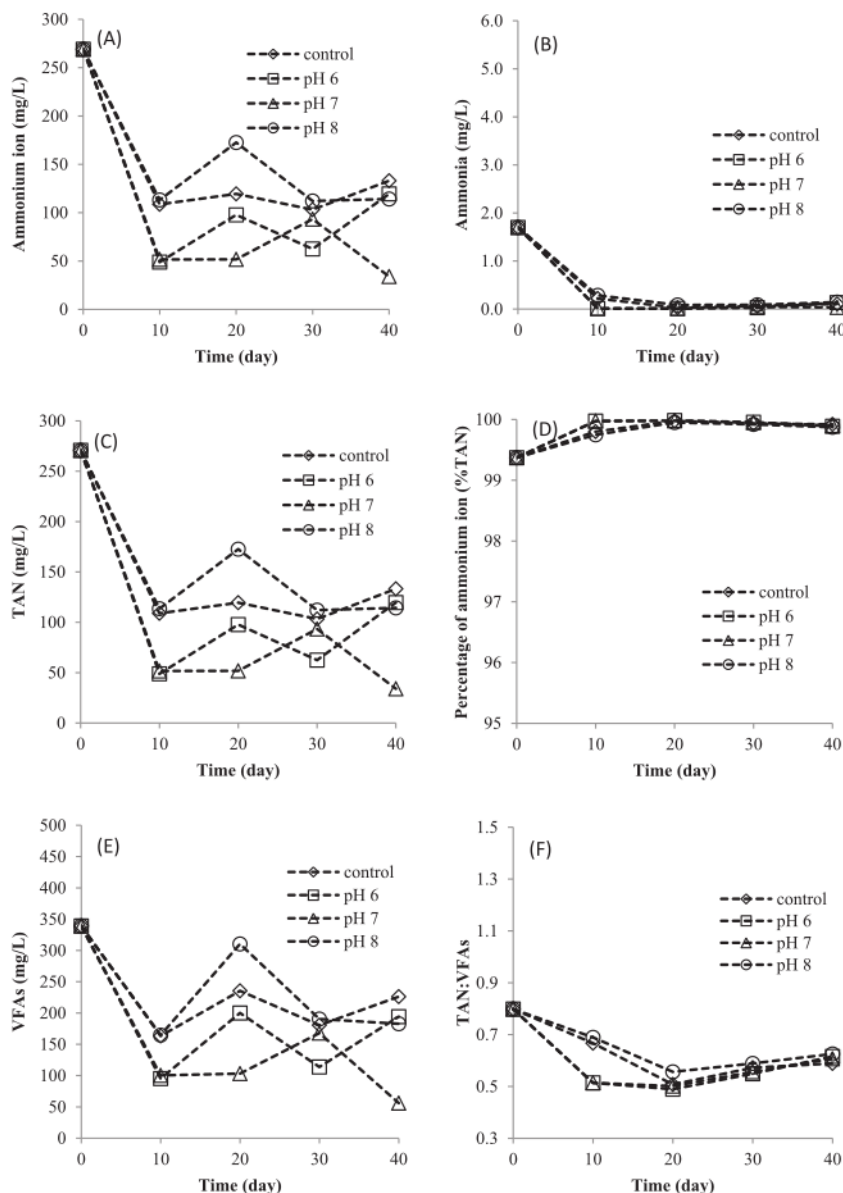


Fig. 5. Production of (A) ammonium ion, (B) ammonia, (C) total ammonia nitrogen (TAN), (D) percentage of ammonium ion in TAN, (E) volatile fatty acids (VFAs), (F) TAN:VFAs during digestion for 40 days at various initial pH in ratio of 0:100.

The pattern of either daily or cumulative biogas yield at various initial pH was similar either in ratio of 40:60 or in ratio 0:100 (Fig. 2 and Fig. 3). That means, the difference of initial pH in range 6–8 did not affect the pattern of biogas production during AD. According to Table 2, initial pH of 8 and 7 was suitable for producing biogas from co-digestion of SM:RS with ratio of 40:60 (biogas yield of 61.38 mL/g TS) and 0:100 (biogas yield of 51.20 mL/g TS) respectively. The difference of the best initial pH between them was correlated with the C/N ratio and chemical compositions in the substrates. However, commonly, these results were in line with Speece (1996), where pH level more than 6 until 8.2 can generate biogas optimally. Therefore, this study reported that the pH range of 7–8 was optimum range during anaerobic digestion of SM and RS and the best condition was at SM:RS ratio of 40:60 with

initial pH of 8. Lay et al. (2013) also reported the same conclusion with this study, where initial pH of 8 was suitable for biogas generation from tofu wastewater.

In ratio of 40:60, pH substrate in P6, P6.9, P7, and P8 was changing during digestion, from 6 to 6.10, 6.9 to 6.85, 7 to 7.15, 8 to 7.30 respectively (Table 2). All of variables had stable pH profile; that means the pH change was insignificant (Fig. 2(C)). On the other hand, the substrate pH profile in ratio of 0:100 decreased to be approximately 6 at day 20 and then it was stable in that level until the end of digestion (Fig. 3(C) and Table 2). That was caused by presence of total ammonia nitrogen (TAN) and volatile fatty acids (VFAs). VFAs was produced from decomposition of carbohydrate contents and total ammonia nitrogen (TAN) was produced from decomposition of protein contents. If

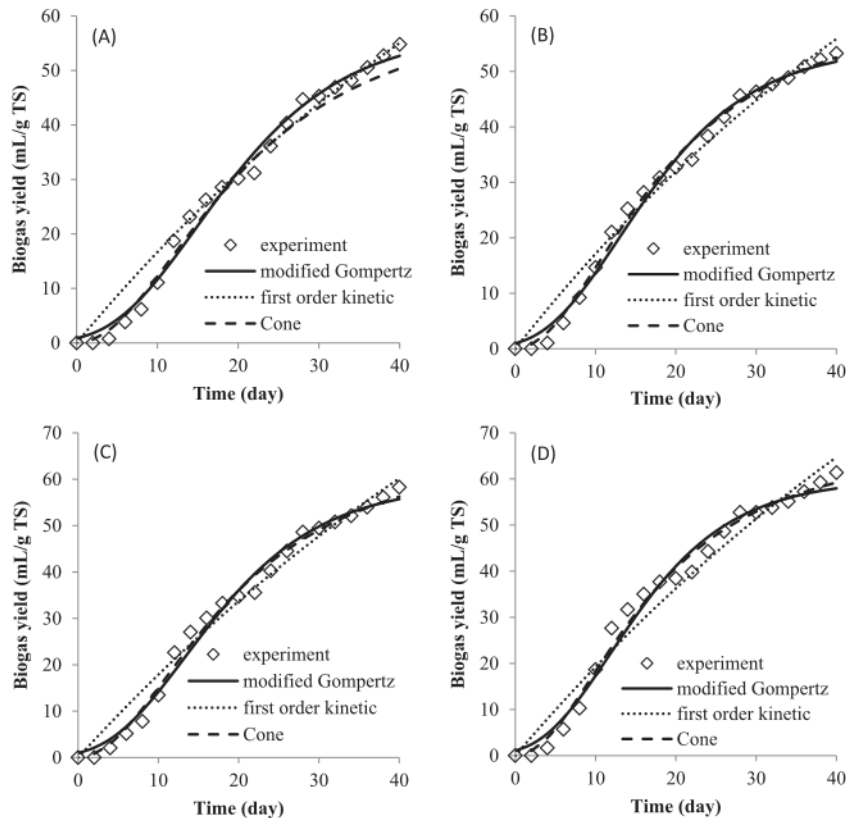


Fig. 6. Comparison of experimental data and simulation data using modified gompertz, cone and first order kinetic model in at initial pH of (A) control, (B) 6, (C) 7, (D) 8.

VFAs production rate was larger than TAN production, the substrate pH decreased. In ratio of 40:60, production rate of TAN and VFAs was almost same, so that the substrate pH was stable enough. Meanwhile, in ratio of 0:100, VFAs generated by acidogenic bacteria was not converted directly by methanogenic bacteria because methanogenic bacteria still adapted in the low C/N condition. Consequently, the TAN/VFA ratio was low and the pH substrate decreased.

The composition of methane, carbon dioxide and hydrogen in biogas was shown in Table 2. Biogas that was produced by ratio of 40:60 (62.64–68.54%) contained higher methane content than that by ratio of 0:100 (48.98%). Specifically, biogas produced by P6.9, P7, P8 contained methane of 65.45, 62.64, 68.54% respectively. Hence, variation of initial pH at neutral range had no significant effect on methane content in biogas.

3.2. Ammonium, ammonia, volatile fatty acids production

Protein as nitrogen source in substrates was degraded to be ammonium ion (NH_4^+) or ammonia (NH_3) during digestion. Ammonium ion or ammonia was utilized by anaerobic bacteria to build their cell structure. Ratio of ammonium ion to ammonia in substrates was affected by pH level. Syaichurrozi et al. (2013) reported that ammonium ion changed to be ammonia via reaction of $\text{NH}_4^+ \leftrightarrow \text{NH}_3 + \text{H}^+$ and ammonia changed to be ammonium ion via reaction of $\text{NH}_4^+ + \text{OH}^- \leftrightarrow \text{NH}_3 + \text{H}_2\text{O}$. The ratio of ammonium ion: ammonia at substrate pH of 9.0 and 7.0 was 70:30 and 99:1 respectively (Deublein and Steinhauser, 2008). When substrate pH was more than 9.25, ammonia was entirely dominant in the substrates (Markou and Georgakakis, 2011).

Meanwhile, when substrate pH was less than 7.0, ammonium ion was fully dominant in the substrate (Syaichurrozi, 2018). In ratio of 40:60, the concentration of ammonium ion (6.30–269.10 mg/L, Fig. 4(A)) was more than the concentration of ammonia (0.04–4.56 mg/L, Fig. 4(B)) in all digester (P6, P6.9, P7, P8). Total ammonia nitrogen (TAN) was calculated by ammonium ion + ammonia (Fig. 4(C)). Furthermore, percentage of ammonium ion in TAN was presented in Fig. 4(D). It showed that the higher the substrate pH (Fig. 2(C)), the lower the percentage of ammonium ion in TAN (Fig. 4(D)). It means that the higher the substrate pH, the lower the ammonium ion: ammonia ratio in the substrates. Furthermore, the final substrate pH of P6, P6.9, P7, P8 was 6.10, 6.85, 7.15, 7.30 respectively and then final ratio of ammonium ion: ammonia in P6, P6.9, P7, P8 was 99.9:0.1, 99.4:0.6, 98.9:1.1, 98.5:1.5 respectively (Table 2). These results were in line with the results of Syaichurrozi (2018). Meanwhile, in ratio of 0:100, the concentration of ammonium ion (33.95–269.10 mg/L, Fig. 5(A)) was more than the concentration of ammonia (0.01–1.70 mg/L, Fig. 5(B)) in all digesters (Q6, Q6.9, Q7, Q8). Percentage of ammonium ion in TAN was similar during digestion (Fig. 5(D)). It was correlated with the substrate pH profile (Fig. 3(C)) which was almost same until the end. The final ratio of ammonium ion: ammonia in Q6, Q6.9, Q7, Q8 was same (value of 99.9:0.1, Table 2). It was caused by the final substrate pH of all variables was almost same (6.0–6.2, Table 2).

Rajagopal et al. (2013) reported that substrates containing TAN of 50–200 mg/L were beneficial for bacterial growth. Moreover, substrates containing TAN of 200–1000 mg/L had no antagonistic effect (Rajagopal et al., 2013). On the other hand, TAN in range of 1500–10,000 mg/L would start inhibition and by level of 30,000 had

Table 3
Results from using Modified Gompertz, First Order Kinetic, Cone Model for co-digestion with SM:RS ratio of 40:60.

	Initial pH			
	Control (6.9)	6	7	8
Modified Gompertz Model				
λ (days)	4.54	3.75	3.94	3.39
μ (mL/g TS.d)	2.06	2.17	2.31	2.62
R^2	0.989	0.991	0.989	0.985
y_m (mL/g TS)	57.40	54.65	59.13	60.05
Predicted biogas (mL/g TS)-40 d	52.68	51.77	55.72	57.96
Measured biogas (mL/g TS)-40 d	54.78	53.25	58.28	61.38
MAPE (%)	24.09	18.69	11.15	16.18
First-Order Kinetic Model				
k (/day)	0.01	0.01	0.01	0.01
R^2	0.972	0.978	0.976	0.967
y_m (mL/g TS)	131.93	126.85	156.98	167.87
Predicted biogas (mL/g TS)-40 d	55.04	55.85	60.16	64.71
Measured biogas (mL/g TS)-40 d	54.78	53.25	58.28	61.38
MAPE (%)	57.86	40.21	26.46	30.73
Cone Model				
k_{hyd} (/day)	0.05	0.05	0.05	0.06
n	1.98	1.98	1.97	1.97
R^2	0.984	0.994	0.991	0.989
y_m (mL/g TS)	64.08	63.83	69.64	70.23
Predicted biogas (mL/g TS)-40 d	50.34	52.49	56.23	59.23
Measured biogas (mL/g TS)-40 d	54.78	53.25	58.28	61.38
MAPE (%)	18.60	13.31	8.42	12.60

Remarks: y_m , the biogas yield potential; μ , the maximum biogas yield rate; λ , lag phase period or minimum time to produce biogas; k_{hyd} , hydrolysis rate constant; n , shape factor; k , the biogas rate constant; R^2 , correlation coefficient; MAPE, Mean Absolute Percentage Error.

toxicity effect for bacterial growth (Sung and Liu, 2003). In this study, TAN concentration from all variables was below 250 mg/L (Fig. 4(C) and Fig. 5(C)). It could be concluded that TAN generated during digestion in this study had not negative effect for all variables.

Volatile fatty acids (VFAs) were produced from degradation of carbon source (carbohydrate) by acidogenic bacteria (Budiyono et al., 2013). Production of VFAs in large amount would decrease substrate pH sharply. The low substrate pH disturbed the stability of anaerobic digestion that depended upon the maintenance of a delicate biochemical balance between the acidogenic and methanogenic microorganisms (Rajagopal et al., 2013; Syaichurrozi, 2018). In ratio of 40:60, the VFAs concentration at P6 was higher than that at the others. Whereas, digester P8 had the least VFAs concentration (Fig. 4(E)). It showed that the large amount of VFAs was converted to biogas at P8. Thus, the total biogas yield at P8 (61.38 mL/g TS) was the highest of all digester (P6, P6.9, P7, P8). However, in ratio of 0:100, digester Q7 had the least VFAs concentration (Fig. 5(E)). This digester also produced the highest biogas yield (51.20 mL/g TS). Hence, initial pH of 7 was suitable in ratio of 0:100. Comprehensively, the best variable was ratio of 40:60 with initial pH of 8 because it resulted the highest biogas yield and highest methane content (Table 2). Lay et al. (2013) also reported that initial pH of 8 was suitable for biogas generation in case of tofu wastewater as feedstock. At that initial pH, a large amount of VFAs was converted to be biogas.

The final VFAs in ratio of 40:60 and 0:100 were 130.13–240.60 and 56.02–226.15 mg/L respectively (Table 2). The ratio of 40:60 had higher final VFAs than ratio 0:100 because ratio of 40:60 had higher C/N value than ratio of 0:100 (Table 1). Higher C/N ratio would produce more VFAs (Mata-Alvarez et al., 2000). However, the substrate pH in ratio of 40:60 was more stable than that in ratio of 0:100. That was caused by TAN/VFAs ratio. Therefore, the TAN/VFAs ratio was important to be presented (Fig. 4(F) and Fig. 5(F)). Based on these figures, the TAN/VFAs ratio in 40:60 was stable and the TAN/VFAs ratio in 0:100 decreased. Decreasing of TAN/VFAs ratio indicated that production rate of TAN was lower than production of VFAs so that the

substrate pH decreased (Fig. 3(C)).

3.3. Total solid removal

Total solid (TS) in the substrates were converted to biogas in AD. Amount of TS value degraded by bacteria was called as TS removal. The TS removal for P6, P6.9, P7, P8 was 33.00, 36.94, 33.00, 36.94% (Table 2). Based on Table 2, variation of initial pH did not give significant effect on TS removal. It correlated with the total biogas and VFAs that were produced. In AD, there were two important bacteria in biogas production which were acidogenic and methanogenic bacteria. The organic compound (TS) was converted to be VFAs by acidogenic bacteria. Furthermore, the VFAs were converted to be biogas by methanogenic bacteria. The activity of them was usually linear with their products. Hence, the more the activity of acidogenic bacteria, the VFAs in substrate would be higher. Also, the more the activity of methanogenic bacteria, the more the biogas was resulted. In this study, not only the total biogas for all variables but also the VFAs production during AD was almost same. Hence, the TS removal was almost same.

3.4. Kinetic model

In this kinetic model analysis, authors focused on biogas production from ratio of 40:60 because it resulted higher biogas yield and higher methane content than ratio of 0:100. Three proposed models were used to simulate biogas production during AD.

3.4.1. Using modified Gompertz Model

The kinetic parameters in modified Gompertz model (y_m , μ and λ) were determined based on the fitting of the studied models and the results were shown in Table 3. By plotting measured and predicted (simulation) data, we had the graph as shown in Fig. 6. From Table 3, digester P8 (initial pH 8) had more value of y_m than variable of P6, P6.9 and P7 (initial pH 6–7). It means initial pH 8 generated the maximum biogas yield in large amount (60.05 mL/g TS) compared to initial pH 6–7 (54.65–59.13 mL/g TS). Digester P8 provided the comfortable metabolism conditions for anaerobic bacteria. Furthermore, kinetic parameter of μ presented maximum biogas production rate. Commonly, higher value of y_m would increase the value of μ in modified Gompertz model. It means that the more the biogas production rate, the more the total biogas was formed (Syaichurrozi et al., 2013). In this model, P8 had highest value of μ (2.62 mL/g TS.d).

Kinetic parameter of λ presented the time required by anaerobic bacteria to adapt in the substrates before the bacteria produced biogas (Syaichurrozi et al., 2016a, 2016b). Therefore, the digester having low value of λ indicated that bacteria needed just little adaptation (lag) time in the digester. Based on that, bacteria in P8 needed the least time to adapt which was 3.39 days, while bacteria in P6, P6.9, and P7 needed longer time than P8 with value of 3.75–4.54 days. It proved that initial pH of 8 was suitable for anaerobic bacteria so that the bacteria did not need a long time to adapt in substrates.

3.4.2. Using Cone model

The kinetic parameters in Cone model (y_m , k_{hyd} , n) were estimated based on the fitting of the studied models and the results were shown in Table 3. To further verify the observations, the predicted data of biogas from Cone model were plotted against the measured data, as presented in Fig. 6. The predicted maximum biogas total (y_m) of P8 (70.23 mL/g TS) was larger than that of P6, P6.9, P7 (63.83–69.64 mL/g TS). The k_{hyd} indicated the hydrolysis rate of organic matters (Sarto et al., 2019). Table 3 showed the correlation in which the less the value of λ obtained from modified Gompertz, the value of k_{hyd} obtained from Cone model was higher. Digester P8 had the lowest value of λ (3.39 days) and the highest value of k_{hyd} (0.06/day), which means P8 provided more comfortable condition to degrade organic matters than others. Hence, bacteria needed shorter time to adapt, and hydrolysis phase was carried

out in high rate.

3.4.3. Using ²⁹ first order kinetic model

The kinetic parameters ¹ first order kinetic model (γ and k) were estimated based on fitting of the studied models and the results were shown in Table 3. Fig. 6 showed the plotting between measured data and predicted data. Digester P8 had γ value of 167.87 mL/g TS and the others (P6, P6.9, P7) had γ value of 126.85–156.98 mL/g TS. Hence, the P8 was the best initial pH for bacterial activity. Kafle et al. (2012) stated that higher value of k would increase the rate of biogas production. All digesters (P6, P6.9, P7, P8) had the same value of k (0.01/day) (Table 3). It showed that the rate of biogas production almost same or the difference of k value was not seen.

3.4.4. Comparison ³⁴ the modified Gompertz, Cone, and first order kinetic model

The mean absolute percentage error (MAPE) of prediction in biogas production for 40 days observed in modified Gompertz model ranged from 11.15% to 24.09%, in Cone model ranged from 8.42% to 18.60%, while in first order kinetic model ranged from 26.46% to 57.86% (Table 3). According to Kivak (2014), the prediction error between measured and predicted values had to be below 20%. Clearly, the Cone model best fitted the actual evolution of biogas production, which was also strongly supported by its high correlation coefficient (R^2). The value of γ in all proposed model was different (Table 3). It was related to accuracy of the models in prediction. Based on the prediction error, the γ value ⁴³ which was resulted by Cone model was the most accurate.

Germeç et al. (2018) reported that modified Gompertz model could predict lactic acid productions as a results of *Lactobacillus casei* activity in bioreactor with high correlation activity $R^2 = 0.99$. In this study, the model also predicted the biogas production with high $R^2 = 0.985–0.991$. Commonly, the modified Gompertz was very popular in product formation rate or cell growth rate. On the other hand, many authors did not use Cone model to simulate the product formation especially biogas, because of its low familiarity ³⁹. In this study, Cone model resulted better prediction than modified Gompertz model (Table 3). The results of this study was in line with Syaichurrozi (2018) and Zhen et al. (2015), where modified Gompertz and Cone model could predict biogas production better than first order kinetic; especially, Cone model was the best. Therefore, with this study, the authors proposed the Cone model as a potential model in biogas formation because of its high precision.

4. Conclusion

Co-digestion ratio with SM:RS ratio of 40:60 and 0:100 carried out at various initial pH (6–8). The ratio of 40:60 produced higher total biogas yield (53.25–61.38 mL/g TS) than ratio of 0:100 (45.98–51.20 mL/g TS). Variation of initial pH had no significant effect on pattern of daily biogas production, substrate pH profile, TAN production profile and VFAs production profile. The best initial pH in ratio of 40:60 and 0:100 were 8 and 7 respectively. However, comprehensively, the best condition was ratio of 40:60 with initial pH of 8 because it resulted the highest biogas yield (61.38 mL/g TS). In ratio of 40:60, variation of initial pH did not affected the methane content of biogas significantly. Simulation using Cone model resulted the best prediction of all proposed models (modified Gompertz, first order kinetic, Cone model) with Mean Absolute Percentage Error (MAPE) of less than 20% (8.42–18.60%) whereas the others resulted MAPE of more than 20%.

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