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A preliminary study: Effect of initial pH and *Saccharomyces cerevisiae* addition on biogas production from acid-pretreated *Salvinia molesta* and kinetics



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11 ABSTRACT

The aim of this study was to investigate the effect of initial pH and *Saccharomyces cerevisiae* (SC) addition on biogas yield from acid-pretreated *Salvinia molesta* (pSM). The initial pH was varied to be 5–8 for substrates without SC (D5–D8) and those with SC addition (DR5–DR8). Before used, *Salvinia molesta* (SM) was pretreated through sulfuric acid pretreatment. The SC with dose of 1 g for 10 g pSM was added. The results showed that the SC addition increased total biogas yield from 8.49–17.95 mLg⁻¹-VS (D5–D8) to 58.98–113.71 mLg⁻¹-VS (DR5–DR8). The methane content in biogas from DR5–DR8 (72.51–84.98%) was higher than that from D5–D8 (6.60–75.03%). The best variable was DR7 (initial pH of 7, SC addition) resulting the highest total biogas yield (113.71 mLg⁻¹-VS) and methane content (84.98%). The SC contributed in hydrolysis and acidogenesis phases in 14% as production. Then, the modified Gompertz model could predict biogas yield more precise than Cone and first order kinetic models. Percentage fitting error in modified Gompertz, Cone and first order kinetic models was 0.00–3.78%, 0.11–11.81% and 0.36–18.05%. The presence of SC increased the μ (biogas yield potential, mLg⁻¹-VS), increased the μ (maximum biogas production rate, mLg⁻¹-VS-d⁻¹) and decreased the τ (time, d).

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1. Introduction

Energy is to be a hot issue in Indonesia since the government has decided to decrease the fossil fuel need from 92% in year of 2013 to 69% in year of 2050 [1]. As consequence, the government will increase the renewable energy production to substitute the fossil fuel need [2]. Biogas is a renewable energy that is potential to be applied to fulfill the national energy need in the country [3].

Biogas is produced by decomposition of organic materials under anaerobic condition with help of bacterial activity [4]. *Salvinia molesta* (SM) is one of lignocellulosic plants that are potential to be used as biogas feedstock and abundantly available in Indonesia. It is a free-floating aquatic weed thriving in water bodies. It can grow quickly (doubling time 3–10 days) and has high durability to environmental changes [1,5,6]. Many problems were caused by SM i.e. blocking the water body surface, reducing the dissolved oxygen, reducing the aquatic organism movements, disturbing the ship

track and reducing the irrigation system efficiency [1]. By utilizing it as a biogas feedstock, there are two advantages that can be obtained i.e. solving the SM problems and producing the renewable energy.

Some authors have conducted studies to investigate biogas production from SM. The [6,7] compared biogas production from SM and other weeds. Furthermore, the [2] found that co-digestion of SM and rice straw produced higher biogas yield than mono-digestion with biogas yield of 113.92 mLg⁻¹-VS for the co-digestion and that of 6.30 mLg⁻¹-VS for the mono-digestion. This concept was effective enough but the rice straw is not available throughout the year because Indonesian farmers only grew rice plants in rainy season. Moreover, the [8] could increase biogas yield from SM by sulfuric acid pretreatment. However, the biogas yield was still very low (24.14 mLg⁻¹-VS). Therefore, another innovation is needed to increase biogas yield from SM.

According to Ref. [9], *Saccharomyces cerevisiae* (SC) addition could be considered to enhance biogas yield from organic substance. SC will help the anaerobic bacteria to convert complex organic compounds to be simple forms such as glucose and organic acids. Some authors have reported that the yeast is helpful in biogas production (Table 1). The [10] tried to add 3 g yeast in five digesters

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Table 1
Previous study of yeast addition on biogas production.

Waste (substrate)	Substrate: water ratio	Nutrient addition	Inoculum	Yeast	Dose of yeast	Kind of the yeast	Initial pH	Digestion time	Biogas with yeast addition	Biogas without yeast	Ref
16 Cow dung	17 400 g 5:1 (ww ⁻¹)	–	29 NI	3 g	3 g for 400 g cow dung	No specific	NI	18 14 d	6550 cm ³	38 5430 cm ³	[10]
Millet husk	5:1 (ww ⁻¹)	=	NI	3 g	3 g for 400 g millet husk	No specific	NI	14 d	5640 cm ³	5230 cm ³	[10]
Rice husk	5:1 (ww ⁻¹)	–	NI	3 g	3 g for 400 g rice husk	No specific	NI	14 d	3240 cm ³	2110 cm ³	[10]
Saw dust	5:1 (ww ⁻¹)	=	50 NI	3 g	3 g for 400 g saw dust	No specific	NI	14 d	1000 cm ³	950 cm ³	[10]
Paper waste	5:1 (ww ⁻¹)	=	NI	3 g	3 g for 400 g paper waste	No specific	NI	14 d	800 cm ³	590 cm ³	[10]
24 Tofu liquid waste	250 mL	=	Rumen liquid 10% (vv ⁻¹)	1 g	1 g for 250 mL tofu waste	SC	5	16 d	220 mL	179 mL	[9]
Tofu liquid waste	250 mL	=	Rumen liquid 10% (vv ⁻¹)	1 g	1 g for 250 mL tofu waste	SC	6	16 d	333 mL	183 mL	[9]
24 Tofu liquid waste	250 mL	–	Rumen liquid 10% (vv ⁻¹)	1 g	1 g for 250 mL tofu waste	SC	7	16 d	370 mL	237 mL	[9]
Tofu liquid waste	250 mL	–	Rumen liquid 10% (vv ⁻¹)	1 g	1 g for 250 mL tofu waste	SC	8	16 d	421 mL	275 mL	[9]
Tofu liquid waste	250 mL	–	Rumen liquid 10% (vv ⁻¹)	1 g	1 g for 250 mL tofu waste	SC	9	16 d	374 mL	263 mL	[9]
Synthetic cassava industrial waste	20 g tapioca	Urea (0.04% (ww ⁻¹ total substrate))	Rumen liquid 10% (vv ⁻¹)	0.08% (vv ⁻¹ total substrate)	1.6 g for 20 g tapioca	SC	Neutral range	45 d	212.02 mL ⁻¹ TS	155.25 mL ⁻¹ TS	[11]
Synthetic cassava starch wastewater	25 g cassava starch	Urea (0.04% (ww ⁻¹ total substrate)) Microalgae (50% (vv ⁻¹ total substrate))	Rumen liquid 10% (vv ⁻¹)	0.08% (vv ⁻¹ total substrate)	2 g for 25 g cassava starch	No specific	Neutral range	30 d	189 mL ⁻¹ TS	58.72 mL ⁻¹ TS	[12]

Remarks: NI, Not informed; SC, *Saccharomyces cerevisiae*.

treating wastes of cow dung, millet husk, rice husk, saw dust and paper waste in each digester. In the fact, it increased total biogas volume as much as 5.26–53.55%. It showed that the kind of substrate gives the different in biogas increasing. In line with [10], the other studies [11,12] reported that yeast addition could increase biogas yield as much as 36.57–221.87% in anaerobic digestion (AD) of synthetic cassava industrial waste. Furthermore, the [9] reported that presence of the yeast in anaerobic digestion of tofu liquid waste could increase biogas volume but it depended on initial pH. The best result was obtained at initial pH of 7 with biogas volume of 421 mL. Based on Table 1, effectiveness of yeast addition in enhancing biogas production depends on the kind of biogas feedstock and then value of initial pH. However, there is no information about the effect of SC addition at various initial pH values on biogas yield from SM. Therefore, it was attractive to be investigated in this study.

The ability of SC to degrade lignocellulosic materials is limited, so pretreatments such as milling and chemical treatment are needed [13,14]. In this study, before the SM was used as a biogas feedstock, its size was reduced to be 18 mesh and then it was soaked in sulfuric acid solution for 2 days under room temperature (see section 2.1). By SC addition, the anaerobic bacteria and SC are presented together in the system. Because of many organisms presenting in the system, the optimum initial pH must be obtained in order to biogas yield can be produced maximally. Adjusting the initial pH is to be one of active scenarios to affect microbial activity in digesters [15]. In this study, rumen fluid was used as inoculum (see section 2.1). The acidogenic and methanogenic bacteria presented in rumen fluid. Commonly, optimum pH for collaboration between them was neutral (6–8). Meanwhile, the SC thrives in pH below 6. Hence, the optimum initial pH for all microbes is important to be founded. Furthermore, the intermediate products such as volatile fatty acids (VFAs) and total ammonia nitrogen (TAN) are important to be monitored because it is affected by initial pH [15] and SC presence [9]. Based on that, in this study, initial pH was varied to be 5, 6, 7 and 8.

Biogas production during AD is interesting to be discussed through kinetic analysis. By using it, the effect of SC addition and initial pH on the biogas yield can be explained quantitatively. Some kinetic models (modified Gompertz, Cone and first order kinetic models) were proposed to simulate the biogas production in this study. The kinetic constants of the kinetic models give important information that can explain the phenomena during AD process. Furthermore, the best kinetic model would be chosen based on the lowest fitting error.

This study was new and other authors have not studied it yet. Based on explanation above, the novelty of this study was utilization of SC as a microbial agent to increase biogas yield from acid-pretreated SM (pSM), variation of initial pH to find its optimum value in AD of pSM with or without SC addition and simulation of the biogas production using some kinetic models in this new case. The goals of this study were to investigate the effect of SC addition at various initial pH values (5–8) on biogas yield from (pSM) and simulate the biogas production using some kinetic models (modified Gompertz, Cone and first order kinetic models) to find the kinetic constants explaining the effect of SC addition on biogas yield quantitatively.

2. Methods

2.1. *Salvinia molesta*, inoculum and *Saccharomyces cerevisiae*

The SM was collected from water bodies located in Pandeglang Regency (Banten Province, Indonesia). It was the same as the SM used by a previous study [8]. It was washed using clean water and

dried under the sun. Its size was reduced using a blender and its size of 18 mesh was collected by using a screener. Then, the 18-mesh-dried SM was soaked in sulfuric acid solution with concentration $4\%v v^{-1}$ for 2 days under room temperature [8]. Furthermore, the pSM was separated from the sulfuric acid solution using a filter and then it was dried and collected to be used as the biogas feedstock. After sulfuric acid pretreatment, the pSM contained total solid (TS) 85.81%, volatile solid (VS) 63.73 %TS, crude lipid 1.30 %TS, crude protein 10.29 %TS, crude fiber 31.22 %TS, crude carbohydrate 52.14 %TS, lignin 11.49 %TS, cellulose 25 %TS, hemicellulose 11.06 %TS and carbon/nitrogen (C/N) ratio 21.50 [8]. The cow rumen fluid was used as inoculums obtained from cow slaughterhouse in Cilégon City (Banten Province, Indonesia). It contained *Clostridium* sp., *Clostridium sporogenes*, *Clostridium butyricum* and rich methanogenic bacteria [1,2]. The cow rumen fluid contained total solid $4\%w v^{-1}$ [1] and VS 70.7%TS [16]. The SC was supplied from Baker's yeast obtained from local markets in Indonesia.

2.2. Experimental set up

This study used 600-mL-polyethylene bottles as lab-scale anaerobic batch digesters that were obtained from local markets in Indonesia. To make anaerobic condition, rubbers were used to plug the digesters. The digester was equipped with 31es for measuring biogas volume. In measuring biogas volume, the water displacement method was used. In this method, the digesters were connected to a reversed cylindrical glass as a gas collector through a connecting pipe. The reversed cylindrical glass was filled fully by water. When the valve was opened, the biogas flowed through the pipe and then replaced the water volume in the reversed cylindrical glass. Biogas volume (mL) was measured by the downward displacement of the water [1,2]. The schematic diagram of the experiment is shown in Fig. 1.

2.3. Experimental design and procedure

The pSM as much as 10 g was put into the digesters. Water was added with ratio of pSM/water of 1/13 ($w v^{-1}$). This ratio was chosen based on the previous study of [8]. Variations of substrate/water of 1/7, 1/10 and 1/13 on biogas yield from SM has been conducted. The results showed that ratio of 1/13 resulted higher biogas yield than the two others (the data have not been published yet). Then, the substrate pH was adjusted to be 5, 6, 7 and 8 using NaOH 1 M or H_2SO_4 1 M. Furthermore, inoculum was added with inoculum/pSM ratio of 2.5 ($v w^{-1}$) (adapted from Ref. [1]). SC as much as 1 g was added (dose of 1 g SC for 10 g pSM). This dose approaches the previous study (dose of 1 g yeast for 12.5 g solid wastes [11,12], Table 1). The variable in this study could be seen in Table 2.

Digestion process was carried out during 30 days at room

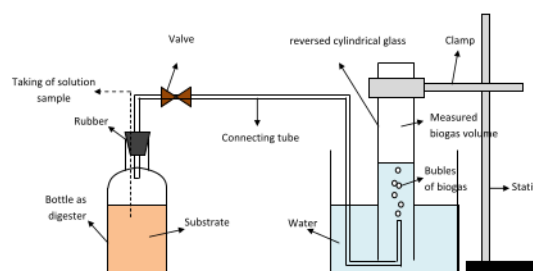


Fig. 1. The schematic diagram of the experiment.

Table 2

Variable in this study.

Digester code	Substrate/water (wv ⁻¹)	pSM (g)	Water (mL)	Rumen fluid (mL)	Yeast (g)	Initial pH
D5	1/13	10	130	25	—	5.0 ± 0.1
D6	1/13	10	130	25	—	6.0 ± 0.1
D7	1/13	10	130	25	—	7.0 ± 0.1
D8	1/13	10	130	25	—	8.0 ± 0.1
DR5	1/13	10	130	25	1	5.0 ± 0.1
DR6	1/13	10	130	25	1	6.0 ± 0.1
DR7	1/13	10	130	25	1	7.0 ± 0.1
DR8	1/13	10	130	25	1	8.0 ± 0.1

Remarks: pSM, acid-pretreated *Salvinia molesta*.

temperature (~30 °C) and pressure of 1 atm. Biogas volume was measured through water displacement method. Furthermore, biogas yield (mLg⁻¹-VS) were calculated by dividing the biogas volume (mL) by initial volatile solid (g VS). The substrate pH level was recorded by using a digital pH meter with model of Hanna-Digital-PHEP-98107-1, Hanna instruments, Rumania [2].

2.4. Chemical analysis

The measurements of ammonium ion (NH₄⁺-N), ammonia (NH₃-N), total ammonia nitrogen (TAN) and volatile fatty acids (VFAs) concentrations during AD were done in the same method as the [1] did. Detail measurement can be seen in study of [1]. The microbial cell account was conducted by using direct microscopic count with Haemocytometer [17,18]. Furthermore, the methane content in biogas was measured using GC-TCD (Gas Chromatography-Thermal Conductivity Detector) Shimadzu 8A. The measurement can be clearly seen in study of [2].

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2.5. Kinetic model of biogas production

Measured biogas yield during AD was simulated through some kinetic models, i.e. modified Gompertz model (equation (1)), Cone model (equation (2)) and first order kinetic model (equation (3)) [2]. To obtain the value of kinetic constants of y_m, λ, μ, k_{hyd}, n, k, the non-linear regression method was used with help of Microsoft excel program.

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

$$y(t) = y_m (1 - \exp(-k \cdot t)), \text{ for } t \geq 0 \quad (2)$$

$$y(t) = \frac{y_m}{1 + (k_{\text{hyd}} \cdot t)^{-n}}, \text{ for } t > 0 \quad (3)$$

where:

y(t) = the cumulative biogas yield at digestion time t days (mLg⁻¹-VS)

y_m = the biogas yield potential (mLg⁻¹-VS)

μ = the maximum biogas production rate (mLg⁻¹-VS-d⁻¹)

λ = the lag phase period or minimum time to produce biogas (d)

t = the cumulative time for biogas production (d)

e = the mathematical constant (2.718282)

k_{hyd} = the hydrolysis rate constant (d⁻¹)

n = the shape factor

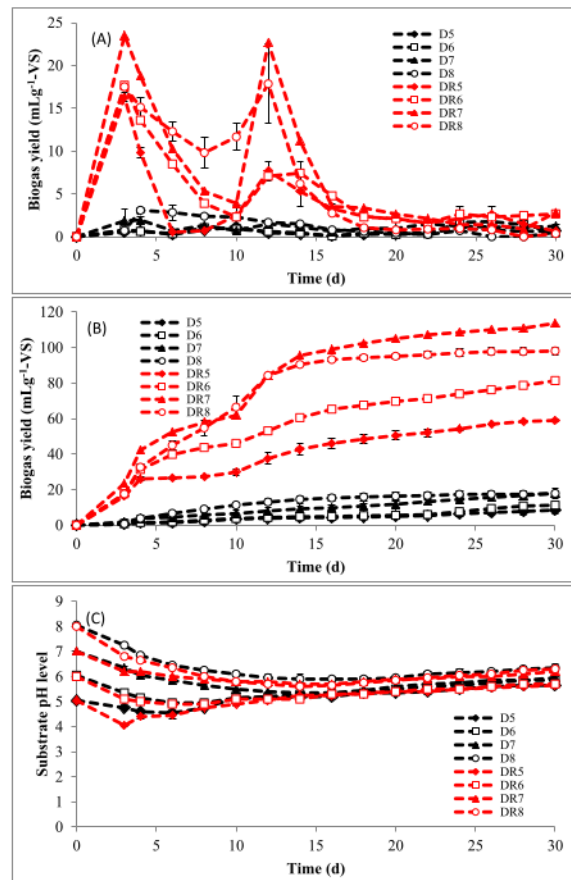
k = the biogas production rate constant (d⁻¹)

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3. Results and discussion

3.1. Effect of initial pH on biogas production without SC addition

The daily biogas yield during digestion can be seen in Fig. 2(A). The peak of daily biogas yield for D5, D6, D7 and D8 was 1.15 ± 0.27, 1.77 ± 0.88, 1.95 ± 0.35 and 3.09 ± 0.27 mLg⁻¹-VS respectively. The peak value of D7 and D8 was obtained at day 4, while that of D5 and D6 was reached more than day 4. That means initial pH of 7–8 was



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Fig. 2. Effect of initial pH and *Saccharomyces cerevisiae* addition on (A) daily biogas yield, (B) cumulative biogas yield, (C) substrate pH level during digestion. D5-D8 are digesters without SC addition with initial pH 5–8 and DR5-DR8 are digesters with SC addition with initial pH 5–8.

Table 3
The results of anaerobic digestion.

Digester code	Initial pH	Final pH	NH ₄ -N (mgL ⁻¹)	NH ₃ -N (mgL ⁻¹)	Ratio of NH ₄ : NH ₃	TAN (mgL ⁻¹)	Total VFAs (mgL ⁻¹)	Total biogas yield (mLg ⁻¹ -VS)	TS removal (%)	Biogas composition	
										CH ₄ (%)	Others (%)
D5	5.0 ± 0.1	5.7 ± 0.1	23.00	0.008	99.96:0.04	23.01	43.05	8.49 ± 1.77	na	6.60	93.40
D6	6.0 ± 0.1	5.9 ± 0.1	27.59	0.015	99.94:0.06	27.61	48.92	11.23 ± 1.68	na	59.80	40.20
D7	7.0 ± 0.1	6.0 ± 0.0	15.94	0.011	99.93:0.07	15.95	27.48	17.95 ± 2.92	63.16	69.01	30.99
D8	8.0 ± 0.1	6.4 ± 0.2	27.59	0.049	99.82:0.18	27.64	42.14	17.86 ± 1.77	na	75.03	24.97
DR5	5.0 ± 0.1	5.7 ± 0.1	152.38	0.054	99.96:0.04	152.43	285.25	58.98 ± 0.44	65.93	72.51	27.49
DR6	6.0 ± 0.1	5.7 ± 0.0	107.00	0.042	99.96:0.04	107.04	197.66	81.35 ± 0.00	72.61	79.20	20.80
DR7	7.0 ± 0.1	6.2 ± 0.0	157.90	0.198	99.87:0.13	158.10	252.80	113.71 ± 0.00	82.90	84.98	15.02
DR8	8.0 ± 0.1	6.3 ± 0.1	233.63	0.369	99.84:0.16	234.00	362.58	98.06 ± 2.03	73.94	83.65	16.35

Remarks: na, not analyzed; TAN, total ammonia nitrogen; VFAs, volatile fatty acids; TAN = NH₄ + NH₃; VS, volatile solid; TS, total solid.

comfortable for bacterial activity in AD of pSM. In other word, bacteria needed a short time to adapt at initial condition with pH level of 7–8. Furthermore, cumulative biogas yield is presented in Fig. 2(B). The total biogas yield for D5, D6, D7 and D8 was 8.49 ± 1.77, 11.23 ± 1.68, 17.95 ± 2.92 and 17.86 ± 1.77 mLg⁻¹-VS respectively. Hence, increasing initial pH from 5 to 8 increased total biogas yield. Based on Table 3, initial pH of 7–8 (D7–D8) produced higher total biogas yield than that of 5–6 (D5–D6). Furthermore, the methane content in biogas formed from D7–D8 (69.01–75.03%) was also higher than that from D5–D6 (6.60–59.80%). Thus, initial pH of 7–8 was the optimal condition for biogas production from pSM. The D5 resulted the lowest methane content (6.60%). In other words, biogas from D5 contained much more by-products (especially CO₂) than methane. It was correlated with the adaptation of methanogenic bacteria in the environment. The initial pH of 5 was not good for the bacterial activity.

The substrate pH of D5, D6, D7 and D8 changed at the end of digestion, from 5 to 5.7 ± 0.1, 6 to 5.9 ± 0.1, 7 to 6.0 ± 0.0 and 8 to 6.4 ± 0.2 respectively (Table 3). For all variables, the decrease in substrate pH level occurred from first digestion time until day 8–14. Furthermore, above that day, the substrate pH level increased slowly (Fig. 2(C)). During digestion, carbohydrate was degraded to be volatile fatty acids (VFAs) and protein was degraded to be total ammonia nitrogen (TAN) [1]. TAN was sum of ammonium ion and ammonia [2]. Ammonium ion and ammonia concentration during digestion are presented in Fig. 3(A–B). Furthermore, TAN and VFAs concentration are shown in Fig. 3(C–D). The lower the ratio of TAN/VFAs, the lower the substrate pH, vice versa (Fig. 3(E)). Carbohydrate was easier to be degraded than protein, so substrate pH was always decreasing in first time of digestion because of the VFAs generation, and then it was increasing gradually because of the TAN generation [19].

Ratio between ammonium ion and ammonia depended on the substrate pH. In theory, at pH level of 7.0 and 9.0, the ratio of ammonium ion: ammonia was 99:1 and 70:30 respectively [20]. Furthermore, at substrate pH below 7.0, ammonium ion was fully dominant in the substrate [2]. The ammonium ion/TAN ratio is presented in Fig. 3(F). It showed that the lower the substrate pH (Fig. 2(C)), the higher the ammonium ion/TAN ratio (Fig. 3(F)). In other words, the lower the pH level, the higher the ammonium ion: ammonia ratio in the substrates. The final substrate pH and final ammonium ion: ammonia ratio of D5, D6, D7, D8 were 5.7 ± 0.1, 5.9 ± 0.1, 6.0 ± 0.0, 6.4 ± 0.2 and 99.96:0.04, 99.94:0.06, 99.93:0.07, 99.82:0.18 respectively (Table 3). It showed the same results with the other study of [2].

In AD, TAN concentration was divided in four effects i.e. (1) range of 50–200 mgL⁻¹ was beneficial for bacterial growth [21], (2) range of 200–1000 mgL⁻¹ was no antagonistic effect [21], (3) range of 1500–10,000 mgL⁻¹ was start to inhibit [22], (4) concentration of

30,000 mgL⁻¹ had toxicity effect for bacterial growth [22]. In this study, the TAN concentration from all variables was below 50 mgL⁻¹ (Fig. 3(C)). It means that TAN generated during digestion was in little amount and not enough as nitrogen source for bacteria.

VFAs concentration increased from day 0 to day 15. After day 15, VFAs concentration decreased. It showed that at day 0–15, VFAs production rate was higher than the biogas production rate. Then, at day 15–30, VFAs production rate was lower than the biogas production rate (Figs. 2(B) and Fig. 3(D)). The D6 and D7 (initial pH 6 and 7) resulted higher the VFAs amount than the D5 and D8 (initial pH 5 and 8) at day 15 (Fig. 3(D)). The VFAs was resulted by acidogenic bacteria which had optimum pH range of 5.5–6.5 [23]. Thus, initial pH 5 and 8 were not good condition for the bacteria. Furthermore, at day 30, the VFAs of D7 decreased more sharply than the others (Fig. 3(D)). It was correlated to methanogenic bacterial activity in which they in best performance at neutral initial pH. The bacteria could grow well at pH range of 6.5–8.2 [24] and it depended on biogas feedstock and inoculum sources. The profile of TAN production during AD was almost same with that of VFAs. It showed that C/N ratio of substrate used in this study was in ideal ratio which was 21.50. In AD, the optimum range of C/N was 20–30 [25].

During digestion, the VFAs concentration was below 60 mgL⁻¹. The good VFAs concentration for digestion process was no more than 2000 mgL⁻¹ [26]. Hence, the VFAs in this study had no inhibition effect for bacteria. However, that concentration was very low so biogas production was in little amount.

According to explanation above, the low biogas yield was caused by both low TAN and low VFAs in which they were caused by the chemical characteristic of pSM. The pSM contained complex materials (such as cellulose, hemicellulose and lignin) so that it was difficult enough to be degraded to be VFAs and TAN. Therefore, the microbial agent could be added in the substrate to help the anaerobic bacteria. In this study, SC was chosen as microbial agent. The effect of SC addition was discussion in next section.

At no SC addition, the best initial pH biogas from pSM was 7–8. It was in line with study of [1] where the best initial pH for co-digestion of SM and rice straw was 7–8. This study and study of [1] used the same rumen fluid as inoculum. The study of [27] also reported the same results, that initial pH of 7.5–8 was suitable for biogas production from tofu wastewater.

The pH affected the percentage of not dissociated acids (NDA) and dissociated acids (DA). The lower the pH condition, the higher the NDA percentage in the system. The NDA can penetrate in the microbial cell and denature the microbial protein. The pH condition in microbial cell is in neutral range. Hence, penetration of the NDA can disturb the acid-base equilibrium in the cell [28]. This study used rumen liquid. When the rumen liquid was added into digesters, the microbes were in new environment. Hence, if initial pH

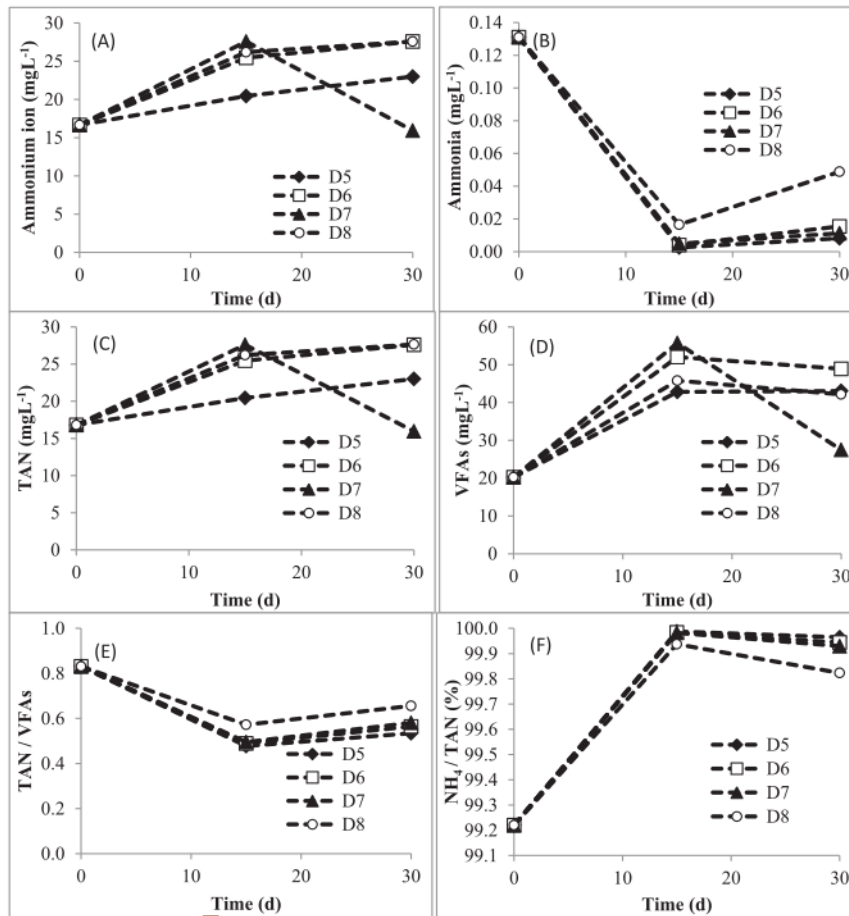


Fig. 3. Production of (A) ammonium, (B) ammonia, (C) total ammonia nitrogen (TAN), (D) volatile fatty acids (VFAs), (E) TAN/VFAs ratio, (F) NH₄/TAN ratio during digestion of D5–D8. D5–D8 are digesters without SC addition with initial pH 5–8.

is in neutral range, the microbial can adapt easily in the system [29] in which it is correlated with the acid-base equilibrium of bacterial cell [28]. Therefore, the initial pH of 7–8 was the best in this study in case of no SC addition.

3.2. Effect of initial pH on biogas production with SC addition

The daily biogas yield with SC addition (DR5–DR8) was higher than that without SC addition (Fig. 2(A)). Furthermore, the cumulative biogas yield is presented in Fig. 2(B). Total biogas yield from DR5, DR6, DR7 and DR8 was 58.98 ± 0.44 , 81.35 ± 0.00 , 113.71 ± 0.00 and 98.06 ± 2.03 mLg⁻¹-VS respectively. By SC addition (DR5–DR8), total biogas yield was increased as much as 449–624% compared to D5–D8. The rumen fluid contained some bacteria i.e. *Clostridium* sp. (hydrolysis bacteria), *Clostridium sporogenes* (acidogenic bacteria), *Clostridium butyricum* (acetogenic bacteria) and rich methanogenic bacteria. In hydrolysis phase, complex organics (carbohydrate, protein, lipid) were converted to be simple organics (glucose, amino acid, long chain volatile fatty acids) by *Clostridium* sp. Furthermore, in acidogenesis phase, the simple organics were converted to be VFAs (butyric, acetic and propionic acid) and TAN by *Clostridium sporogenes*. In acetogenesis

phase, the butyric and propionic acid were converted to be acetic acid by *Clostridium butyricum*. Finally, the acetic acid was converted to be biogas by methanogenic bacteria. On the other hand, yeast SC helped hydrolysis bacteria to hydrolyze carbohydrates and produced glucose. Furthermore, it helped acidogenic bacteria in converting glucose to be acetic acid, butyric acid and ethanol [9]. SC addition not only increased total biogas yield but also methane content in biogas (Table 3). Methane content in biogas from DR5–DR8 was 72.51–84.98%, while that from D5–D8 was only 6.60–75.03%. Methane content in DR5 (72.51%) was higher than that in D5 (6.60%). It showed that methanogenic bacteria still could produce methane at initial pH of 5 if their nutrients were available in the system. By SC presence, the main nutrients (such as glucose, VFAs, TAN, etc) for methanogenic bacteria were more abundant than in no SC addition case. However, biogas yield in DR5 was lower than the others (DR6–DR8).

Fig. 4 showed that there were different phases in biogas production from DR5–DR8. Phase 1 was exponential phase I. In this phase, biogas production was accelerated with a high speed of 6.52 – 10.57 mLg⁻¹-VS-d⁻¹. The non-fiber carbohydrate in pSM was converted to be biogas in this phase because it was easy to be degraded [1]. Phase 2 was plateau phase I. In this phase, the

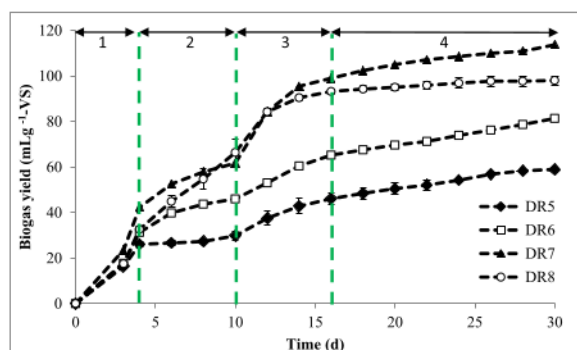


Fig. 4. Different phases of biogas production. DR5–DR8 are digesters with SC addition with initial pH 5–8.

availability of non-fiber carbohydrate was low because a lot of that had been degraded in exponential phase I. Hence, the biogas production rate decreased. The biogas production rate in this phase was $0.62\text{--}5.63\text{ mLg}^{-1}\cdot\text{VS}\cdot\text{d}^{-1}$. Phase 3 was exponential phase II. In this phase, biogas was produced from degradation of complex organic matters (fiber carbohydrates) in pSM. It was difficult to be degraded so it could be converted into biogas after day 10. The biogas production rate in this phase was $2.71\text{--}6.22\text{ mLg}^{-1}\cdot\text{VS}\cdot\text{d}^{-1}$. Phase 4 was plateau phase II. In this phase biogas production rate was very low ($0.35\text{--}1.15\text{ mLg}^{-1}\cdot\text{VS}\cdot\text{d}^{-1}$) because of two reasons i.e. (1) the biodegradable substrate of pSM was limited and (2) substrate condition was not comfortable for microbes.

Increasing initial pH from 5 to 7 increased the total biogas yield from 58.98 ± 0.44 to $113.71 \pm 0.00\text{ mLg}^{-1}\cdot\text{VS}$. However, total biogas yield at initial pH of 8 ($98.06 \pm 2.03\text{ mLg}^{-1}\cdot\text{VS}$) was lower than that at initial pH of 7. The [30] stated that SC converted carbohydrate to be ethanol, acetic and butyric acid and their compositions depended on initial pH. Initial pH of 5 was the best for ethanol production. Composition of ethanol was lower and composition of acetic and butyric acid were higher when initial pH was higher than 5. The [9] reported that at initial pH of 5–6, SC produced ethanol in high concentration so it hampered methanogenic bacteria activity. Furthermore, at initial pH of 7–8, SC produced ethanol in low concentration that was still tolerance for methanogenic bacteria, and high acetic and butyric acids that were consumed by the bacteria to produce biogas.

By SC presence, initial pH 5 and 6 (DR5 and DR6) resulted higher the VFAs amount than initial pH 7 and 8 (DR7 and DR8) at day 15 (Fig. 5(D)). The SC has pH optimum range of 5–6 [31] and the acidogenic bacteria had optimum pH range of 5.5–6.5 [23]. The best initial pH for the collaboration SC and acidogenic bacteria was pH below 7. Furthermore, at day 30, the VFAs of DR5 and DR6 decreased more sharply but biogas yield of these was less than the others (Fig. 5(D)). It showed that much more VFAs was consumed by methanogenic bacteria to adapt in environment than to produce biogas. Meanwhile, in DR7 and DR8, the VFAs were successfully converted to biogas. As explanation above, at initial pH of 5–6, SC produced ethanol in high concentration so the methanogenic bacteria activity was disturbed. Furthermore, like in D5–D8, the TAN production profile during AD was almost same with the VFAs production profile. It was correlated to the ideal C/N ratio of pSM used in this study.

Comprehensively, DR5–DR8 produced higher VFAs than D5–D8. It means, SC successfully helped to degrade organic matters to be

acetic and butyric acid. Hence, biogas yield in DR5–DR8 was higher than that in D5–D8. In addition, the [32] reported that pH of 6 was the best condition for *Saccharomyces cerevisiae* to grow. Whereas, bacteria in rumen fluid could grow well until pH of 8. Logically, the initial pH of 7 was the best pH condition for both microorganisms together in the substrate.

The substrate pH of DR5–DR8 was almost same with D5–D8 (Fig. 2(C) and Table 3). It was caused by VFAs/TAN ratio in DR5–DR8 (Fig. 5(E)) was almost same in D5–D8 (Fig. 3(E)). Furthermore, production of TAN and VFAs during digestion in DR5–DR8 (Fig. 5(C–D)) was higher than that in D5–D8 (Fig. 3(C–D)). TAN concentration during digestion was $152.43\text{--}234.00\text{ mgL}^{-1}$ (Table 3). This TAN concentration was in beneficial level for AD process. Furthermore, the VFAs production during AD was $45.80\text{--}577.91\text{ mgL}^{-1}$. It showed that acetic and butyric acids were produced more in DR5–DR8 than in D5–D8. The higher the VFAs, the higher the biogas would be produced.

TS removal in DR7 (82.90%) was higher than that in D7 (63.16%) (Table 3). It showed that SC helped anaerobic bacteria to degrade TS to be biogas. Thus, the biogas yield from DR7 was higher than that from D7. Furthermore, Table 3 shows good correlation between total biogas yield and TS removal value. The higher the biogas was formed, the higher the TS was degraded. It was in line with study of the other studies [2,3]. Correlation between them could be expressed in straight line of $y = 4.506x - 249.1$ ($R^2 = 0.865$) with y is biogas

The best variable in this study was DR7 with total biogas yield of $113.71 \pm 0.00\text{ mLg}^{-1}\cdot\text{VS}$ and methane content of 84.98%. This value was better than mono-digestion of SM with initial pH of 7 in study of [2] where it resulted biogas yield of $6.30 \pm 0.00\text{ mLg}^{-1}\cdot\text{VS}$. Furthermore DR7 resulted biogas yield was same with co-digestion of SM and rice straw ($113.92 \pm 6.90\text{ mLg}^{-1}\cdot\text{VS}$ [2]). However, the methane content from DR7 (84.98%) was higher than that from co-digestion in the previous study (60.58%) [2]. Thus, the SC addition was very good to be used as alternative way to produce biogas maximally besides the co-digestion. Indonesian farmers only grew rice plants in rainy season so the rice straw was not available throughout the year.

3.3. Microbial growth during AD

Microbial cell during AD in digesters of D7 and DR7 was measured using the Hæmocytometer. These digesters were chosen because they resulted high biogas yield. The results of measuring microbial cell are shown in Fig. 6. The initial cell amount in D7 and DR7 was $130 \times 10^3\mu\text{L}^{-1}$ and $140 \times 10^3\mu\text{L}^{-1}$ respectively. Thus, the initial SC cell in DR7 was $10 \times 10^3\mu\text{L}^{-1}$. Based on Fig. 6, the microbial cell increased at day 4 and then decreased at day 12. At day 4, the microbes consumed non-fiber carbohydrates so they could produce biogas easily and grow well. Meanwhile, at day 12, the microbes consumed fiber carbohydrates that were not easy to be degraded. At the day, only part of microbes could survive to consume the fiber carbohydrates so the cell amount decreased. The growth profile of microbes during AD in D7 and DR7 was same. Therefore, by assuming that the growth of anaerobic bacteria cell in DR7 was same in D7, the SC cell amount in DR7 was predicted using difference between the microbial cell in DR7 with that in D7 (equation (4)).

$$\text{Predicted SC cell in DR7} = \text{microbial cell in DR7} - \text{microbial cell in D7} \quad (4)$$

Based on Fig. 6, the SC cell amount increased from $10 \times 10^3\mu\text{L}^{-1}$

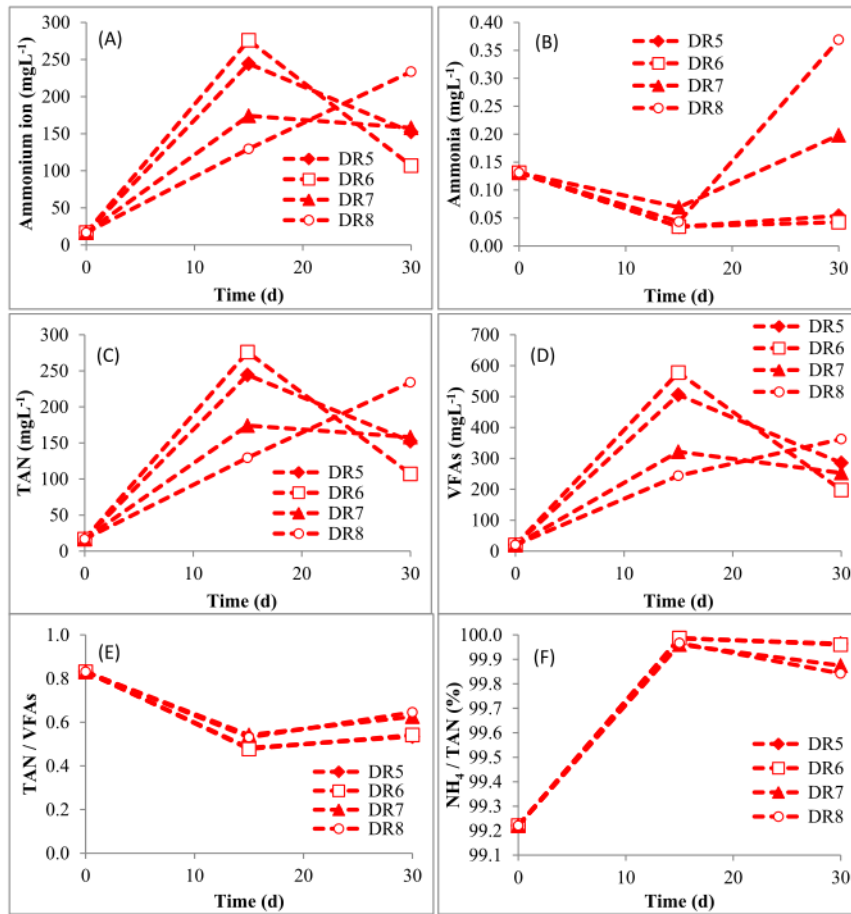


Fig. 5. Production of (A) ammonium, (B) ammonia, (C) total ammonia nitrogen (TAN), (D) volatile fatty acids (VFAs), (E) TAN/VFAs ratio, (F) NH₄/TAN ratio during digestion of DR5-DR8. DR5-DR8 are digesters with SC addition with initial pH 5–8.

(at day 0) to $350 \times 10^3 \mu\text{L}^{-1}$ (at day 4) and then decreased to $100 \times 10^3 \mu\text{L}^{-1}$ (at day 12). The decrease in SC cell was caused by the limit of nutrients at the day.

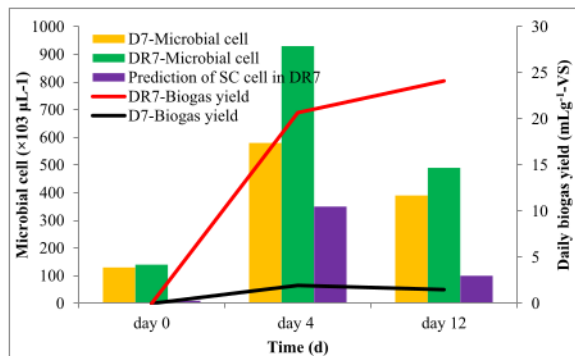


Fig. 6. Microbial cell amount during AD. D7 is a digester without SC addition with initial pH 7 and DR7 is a digester with SC addition with initial pH 7.

3.4. Kinetics

3.4.1. Using modified Gompertz model

Kinetic constants of y_m , μ and λ are successfully obtained and shown in Table 4. Furthermore, Fig.S1 shows a plotting between measured and predicted data (in the supplementary material). In Table 4, DR5-DR8 had more value of y_m than D5-D8. It showed that SC addition would generate the biogas yield potential in large amount ($63.67\text{--}112.26 \text{ mLg}^{-1}\text{VS}$) compared to no SC addition ($17.51\text{--}40.20 \text{ mLg}^{-1}\text{VS}$). The best variable was DR7 because it produced the highest y_m value. Furthermore, μ value presented maximum biogas production rate. In theory, the higher the y_m value, the higher the μ value. It means that the more the biogas production rate, the more the biogas yield potential would be formed [33]. They of D5-D7 and DR5-DR7 were in line in that theory. Surprisingly, there was interesting phenomena in D8 and DR8. The y_m value at initial pH of 8 was lower than that at initial pH of 7, but μ value at initial pH of 8 was higher than that at initial pH of 7. At initial pH of 8, biogas was produced in high rate from first digestion time until day 16, but y_m was very low after that day (Fig.S1). Hence, at initial pH of 8, the maximum biogas production rate was higher although the biogas yield potential was lower than

Table 4
Results from using modified Gompertz, Cone and first order kinetic models.

	Digester							
	D5	D6	D7	D8	DR5	DR6	DR7	DR8
Modified Gompertz model								
λ (d)	5.31	7.35	0.66	1.23	-3.46	-2.49	0.07	1.26
μ (mLg ⁻¹ -VS-d ⁻¹)	0.34	0.50	0.62	1.34	2.50	3.92	7.80	9.48
R ²	0.96	0.96	0.98	0.99	0.96	0.97	0.98	0.99
ym (mLg ⁻¹ -VS)	24.76	32.25	40.20	17.51	63.67	81.36	112.26	97.95
MAD	0.37	0.52	0.43	0.28	1.89	2.73	3.02	1.88
Predicted biogas yield (mLg ⁻¹ -VS)-30 d	8.49	11.23	18.09	17.39	58.98	78.27	111.19	97.81
Measured biogas yield (mLg ⁻¹ -VS)-30 d	8.49	11.23	17.95	17.86	58.98	81.35	113.71	98.06
Difference between measured and predicted biogas yield (%)	0.00	0.00	0.81	2.63	0.00	3.78	2.22	0.25
Cone model								
k_{hyd} (d ⁻¹)	0.04	0.02	0.02	0.12	0.08	0.09	0.14	0.17
n	1.41	1.28	1.13	2.01	1.12	1.09	1.64	2.28
R ²	0.96	0.93	0.99	0.99	0.95	0.98	0.97	0.97
ym (mLg ⁻¹ -VS)	13.94	35.60	50.54	19.20	79.80	106.46	124.90	101.06
MAD	0.39	0.62	0.40	0.23	1.83	1.67	2.97	2.59
Predicted biogas yield (mLg ⁻¹ -VS)-30 d	7.49	10.38	17.23	17.84	58.35	79.52	113.58	98.59
Measured biogas yield (mLg ⁻¹ -VS)-30 d	8.49	11.23	17.95	17.86	58.98	81.35	113.71	98.06
Difference between measured and predicted biogas yield (%)	11.81	7.57	3.99	0.11	1.06	2.25	0.11	0.54
First order kinetic model								
k (d ⁻¹)	0.01	0.01	0.01	0.09	0.07	0.09	0.10	0.10
R ²	0.98	0.93	0.99	0.96	0.97	0.99	0.98	0.97
ym (mLg ⁻¹ -VS)	24.76	32.14	55.26	19.52	66.61	84.02	119.22	107.70
MAD	0.32	0.60	0.32	0.71	1.70	1.74	3.14	4.00
Predicted biogas yield (mLg ⁻¹ -VS)-30 d	7.91	9.37	17.63	18.07	58.98	78.61	113.71	101.63
Measured biogas yield (mLg ⁻¹ -VS)-30 d	8.49	11.23	17.95	17.86	58.98	81.35	113.71	98.06
Difference between measured and predicted biogas yield (%)	4.02	18.05	0.36	2.69	0.97	1.49	1.42	2.70

Remarks: ym, the biogas production potential; μ , the maximum biogas production rate; λ , lag phase period or minimum time to produce biogas; k_{hyd} , hydrolysis rate constant; n, shape factor; k, the biogas rate constant; R², correlation coefficient; MAD, Mean Absolute Deviation.

that at initial pH of 7. That phenomenon was in line with study of [29]. The λ value presented the time required (lag time) by anaerobic bacteria to adapt in the substrates before biogas was formed [9]. SC addition successfully decreased λ value from 1.23-7.35 d to (-3.46)-1.26 d. The negative value of λ (below 0) is not surprising because it represented that microbe no need lag time to produce biogas; in other words, the lag time was 0 day. Previous study [8] also reported that the negative value of λ could be obtained in modeling when the biogas was very easy to be produced in first fermentation.

3.4.2. Using Cone model

Kinetic constants of ym, k_{hyd} and n are successfully obtained and shown in Table 4. Furthermore, the predicted data are plotted against the measured data, as shown in Fig. S2 (in the supplementary material). The ym value of DR5-DR8 (79.80-124.90 mLg⁻¹-VS) was larger than that of D5-D8 (13.94-19.20 mLg⁻¹-VS). The k_{hyd} presented the hydrolysis rate of organic matters [3]. Table 4 shows the good correlation between λ and k_{hyd} in that the less the λ value obtained from modified Gompertz, the higher the k_{hyd} value obtained from Cone model. This means, bacteria needed shorter time to adapt so that hydrolysis phase was carried out in high rate. Presence of SC in the substrate successfully increased the hydrolysis rate (k_{hyd}) from 0.02-0.12 d⁻¹ (without SC addition) to 0.08-0.17 d⁻¹ (with SC addition).

3.4.3. Using first order kinetic model

Kinetic constants of ym and k are successfully obtained and shown in Table 4. Fig.S3 shows the plotting between measured and predicted data (in the supplementary material). The ym value of DR5-DR8 (79.80-124.90 mLg⁻¹-VS) was larger than that of D5-D8 (13.94-19.20 mLg⁻¹-VS). Furthermore, the k value of DR5-DR8 (0.07-0.10 d⁻¹) was larger than that of D5-D8 (0.01-0.09 d⁻¹). The k presented the biogas production rate constant. The higher the k

value, the faster the biogas was produced [34]. This result showed that SC increased the k value because SC helped in hydrolysis and acidogenesis phases so that production of VFAs was in large amount. Finally, biogas was easy to be produced.

3.4.4. Comparison among the modified Gompertz, Cone and first order kinetic models

For all proposed models, the predicted maximum biogas potential (ym) increased with increased the initial pH from 5 until 7. Furthermore, at initial pH of 8, the ym decreased. The difference between the measured and predicted data (fitting error) after 30 days observed in modified Gompertz model was 0.00-3.78%, in Cone model was 0.11-11.81%, in first order kinetic model was 0.36-18.05% (Table 4). Clearly, among the proposed models, modified Gompertz model was the best model in fitting the actual evolution of biogas production because it had fitting error below 10%, which was also strongly supported by its high correlation coefficient (R² of 0.96-0.99). Meanwhile, Cone and first order kinetic model had R² of 0.93-0.99.

4. Conclusion

The SC addition successfully increased total biogas yield from pSM from 8.49-17.95 mLg⁻¹-VS (D5-D8) to 58.98-113.71 mLg⁻¹-VS (DR5-DR8). In other word, SC addition increased total biogas yield 449-624% compared with no SC addition. The TAN and VFAs production was higher in DR5-DR8 than in D5-D8. Thus, biogas was formed in large amount. Also, the methane content in biogas was higher in DR5-DR8 than in D5-D8. The optimum pH value for the case either with or without SC addition was 7. However, the best variable was DR7 (initial pH 7 with SC addition) because it produced the highest total biogas yield (113.71 mLg⁻¹-VS) and highest methane content (84.98%). Of all proposed models, the modified Gompertz model was the best in predicting biogas yield. Fitting error value in modified Gompertz, Cone and first order

kinetic model was 0.00–3.78%, 0.11–11.81% and 0.36–18.05% respectively. The presence of SC increased the γ_m (biogas yield potential, $\text{mLg}^{-1}\text{-VS}$), increased the μ (maximum biogas production rate, $\text{mLg}^{-1}\text{-VS-d}^{-1}$) and decreased the λ (lag time, d).

CRediT authorship contribution statement

Iqbal Syaichurrozi: Conceptualization, Methodology, Software, Validation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **M. Fakhri Basyir**: Methodology, Software, Formal analysis, Investigation, Data curation. **Rafi Muhammad Farraz**: Methodology, Software, Formal analysis, Investigation, Data curation. **Rusdi Rusdianto**: Conceptualization, Methodology, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

There are no competing interests to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.energy.2020.118226>.

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