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Vol. 13 No. 1 (2022) 1-11
Jurnal Riset
Teknologi Pencegahan Pencemaran Industri
Journal homepage : http://www.jrtppi.id

Kementerian Perindustrian
REPUBLIK INDONESIA

The Effect of Inoculum to Substrate and Carbon to Nitrogen Ratios on the Biogas Quantity and Quality from Anaerobic Digestion of Salvinia molesta: Experimental and Kinetic Studies

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ARTICLE INFO

Article history:
Received 28 December 2021
Received in revised form 08 March 2022
Accepted 08 March 2022
Available online 27 June 2022

Keywords :
Anaerobic digestion
Biogas
C/N
I/S
Salvinia molesta

ABSTRACT

This experiment was conducted to study the effect of inoculum to substrate (I/S) and carbon to nitrogen (C/N) ratios on the biogas quantity and quality from anaerobic digestion of *Salvinia molesta* (SM). The I/S was adjusted to become 1.5, 2, 2.5 and the C/N was to become 21.5, 25, 30. Anaerobic digestion (AD) was operated during 30 days under the room pressure and temperature. The results showed that increase in I/S from 1.5 to 2.5 increased the biogas yield from 19.01 to 33.84 mL/g VS. Besides that, it increased the methane content from 52.54 to 69.01%. Furthermore, increase in C/N to 21.5 to 30 decreased the biogas yield from 33.84 to 30.85 mL/g VS and then decreased the methane content from 69.01 to 6.99%. Hence, the best condition was in the substrate with I/S of 2.5 and C/N of 21.5. The measured data was successfully predicted through the modified Gompertz with R^2 of 0.9905, while through the first order kinetic models with R^2 of 0.9476. Hence, the former gave a better prediction than the latter.

Nomenclatures:

ym	= the biogas yield potential (mL/g VS)
y(t)	= the cumulative biogas yield at t days (mL/g VS)
λ	= lag phase period (days)
μ	= the maximum biogas yield rate (mL/g VS.day)
k	= the biogas production rate constant (/day)
e	= a constant (2.718282)
t	= cumulative time for AD process (days)

1. INTRODUCTION

The application of anaerobic digestion (AD) in treating various wastes is carried out conducted by many countries in the world (Kougias & Angelidaki, 2018). This method is very powerful to treat many kinds of wastes and then produces the biogas that can be used as an alternative energy source (Iqbal Syaichurrozi, Basyir, Farraz, & Rusdi, 2020). Therefore, the AD is to be one of main research topics in Indonesia, since the country want to substitute the 33% of fossil fuel energy need with renewable energies at year of 2050 (I. Syaichurrozi, Villta, Nabilah, & Rusdi, 2019). As a tropical country, Indonesia has a variety of plants that thrive in it (Sarto, Hildayati, & Syaichurrozi, 2019). Many of them are lignocellulosic plants which are

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doi : https://10.21771/jrtppi.2022.v13.no.1.p1-11

Accreditation number : (Ristekdikti) 158/E/KPT/2021

included in weeds. One kind of weeds growing well in the country is *Salvinia molesta* (SM) (Syaichurrozi et al., 2018). It can grow floating in rivers, lakes, and rice fields with a very fast growth rate. Disadvantages arising from the presence of SM in the rivers and lakes are: (1) reducing the water volume via evapotranspiration, (2) reducing the aquatic organism movements, (3) reducing the dissolved oxygen, (4) blocking the river surface, (5) disturbing the ship track; meanwhile, in the rice fields, it can (1) reduce the fertilizer effectiveness and (2) reduce the irrigation system efficiency (Syaichurrozi, 2018; Syaichurrozi et al., 2018, 2019, 2020).

Because of its huge amount and fast growth rate, the SM can be used as a potential biogas feedstock. However, big part organic materials composing the SM are lignocellulosic compounds which are not good for AD process because it is difficult to be degraded. Acid pretreatment prior AD successfully increases the biogas yield from SM (I. Syaichurrozi et al., 2019). Furthermore, Syaichurrozi et al. (Iqbal Syaichurrozi et al., 2020) investigated the effect of initial pH (5, 6, 7, 8) and biological agent addition (*Saccharomyces cerevisiae*) on biogas yield from the SM, which was previously treated through sulfuric acid pretreatment. They found that the best condition is at initial pH of 7.0 and the yeast addition. The other affecting factors that have been not investigated yet in previous studies are inoculum to substrate (I/S) and carbon to nitrogen (C/N) ratios. Fagbohungbe et al. (Fagbohungbe, Herbert, Li, Ricketts, & Semple, 2015) reported that at I/S of 1-2 resulted a higher methane yield than I/S of 0.25-0.5 on biogas production from human faecal material. Xue et al. (Xue et al., 2020) reported that C/N ratio affected the methane yield from food wastes, in which the C/N of 25-30 resulted a higher methane yield from 20. The two ratios are important factors which are considered in AD process, but them have been investigated yet in AD of SM. Therefore, they were investigated in this current work.

Modeling of biogas evolution during AD is attractive to be conducted. The popular models such as the modified Gompertz and first-order kinetic models are usually used to simulate the evolution of biogas yield as function of time. Previous studies have used the models to simulate biogas production from sheep paunch manure at variation of I/S (Lawal, Dzivama, & Wasinda, 2016) and from food wastes at variation of C/N (Xue et al., 2020). Therefore, in this work, the two models will be utilized to describe the effect of I/S and C/N ratios on biogas from the SM quantitatively. The aim of this work is to investigate the effect of I/S and C/N ratio on biogas production from the SM and simulate the biogas evolution using the two popular models.

2. METHODS

2.1. *Materials*

The SM and inoculum (cow rumen fluid), which were used in the current study were the same materials used by Syaichurrozi et al. (I. Syaichurrozi et al., 2019; Iqbal Syaichurrozi et al., 2020). The SM thrives on bodies of water in Pandeglang Regency, one of regencies in Banten Province (Indonesia). Before used as a biogas feedstock, it was pretreated using sulfuric acid with a procedure used by Syaichurrozi et al. (Iqbal Syaichurrozi et al., 2020).

2.2. *Experimental Design*

2.2.1. *Scenario 1*

The SM as much as 10 g (with C/N of 21.5) was diluted using the tap water with ratio of SM/water of 1/13 (w/v, g/mL) (based on Syaichurrozi et al. (Iqbal Syaichurrozi et al., 2020)). The initial pH level of the substrates was increased until neutral level (7.0±0.2) with addition of NaOH 1 M solution. Furthermore, the cow rumen fluid (inoculum) was added with ratio of inoculum per substrate (I/S) of 1.5, 2, 2.5 (v/w, mL/g).

2.2.2. *Scenario 2*

In this scenario, the SM/water ratio and initial pH values were similar to Scenario 1 but the inoculum/substrate ratio was adjusted based on the best ratio obtained in Scenario 1. Furthermore, the C/N ratio was varied to be 21.5, 25, 30 by glucose addition.

The characteristics of the substrates in this study are shown in Table 1.

Table 1. The characteristics of the substrates in this study

I/S	C/N	Initial $NH_3 - N$ (mg/L)	Initial $NH_4^+ - N$ (mg/L)	Initial TAN (mg/L)	Initial VFAs (mg/L)	Initial TS (g/L)	Initial VS (mg/L)
Scenario 1							
1.5	21.5	0.095	10.81	10.91	12.85	59.23	38.02
2	21.5	0.098	13.92	14.02	17.21	58.63	37.71
2.5	21.5	0.133	16.88	17.01	20.46	58.07	37.43
Scenario 2							
I/S	C/N	Initial $NH_3 - N$ (mg/L)	Initial $NH_4^+ - N$ (mg/L)	Initial TAN (mg/L)	Initial VFAs (mg/L)	Initial TS (mg/L)	Initial VS (mg/L)
2.5	21.5	0.133	16.88	17.01	20.46	58.07	37.43
2.5	25	0.133	16.88	17.01	20.46	58.07	37.43
2.5	30	0.133	16.88	17.01	20.46	58.07	37.43

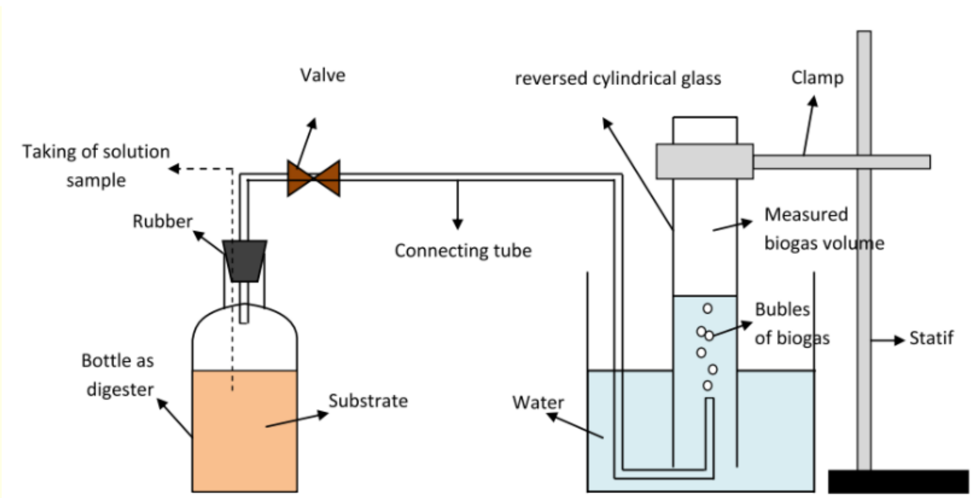


Figure 1. The laboratory anaerobic digester (Iqbal Syaichurrozi et al., 2020)

2.3. Experimental Set Up and Procedure

This study used the laboratory anaerobic digesters which were proposed by a previous study (Iqbal Syaichurrozi et al., 2020). The laboratory anaerobic digester is shown in Figure 1. The AD process was operated during 30 days with room condition (28-30 °C, 1 atm). Each digester was mixed manually one per two days. The water displacement method was applied to measure the daily biogas volume (I. Syaichurrozi, 2018). The measured biogas volume (mL) was divided by initial volatile solid mass (g VS) of substrates to get a biogas yield (mL/g VS).

2.4. Analysis

2.4.1. Liquid sample

The 10 mL of liquid sample was taken from the digesters. The pH of substrates was recorded by using a digital pH meter (model of Hanna-Digital-PHEP-98107-1) (I. Syaichurrozi et al., 2019). The ammonium (NH_4^+-N) concentration in the substrates was measured by using the Standard Method (APHA, 2012). The total solid (TS) before and after anaerobic digestion process was analyzed measured by using the Standard Method (APHA, 2012). The TS removal value was calculated by using the equation (1). The ammonia (NH_3-N) concentration was determined through the equation (2) (El-Mashad, Zeeman, Van Loon, Bot, & Lettinga, 2004). Furthermore, the total ammonia nitrogen (TAN) concentration was determined through the equation (3). The volatile fatty acids (VFAs) concentration was determined via equation (4) (Paul & Beauchamp, 1989).

2.4.2. Gas sample

The biogas volume produced during AD was collected in a gas collector. Furthermore, sample of gas was taken using a syringe for methane analysis. The methane percentage contained in the biogas was analyzed by using the GC-TCD that was same used by other authors (Iqbal Syaichurrozi et al., 2020).

$$\text{TS Removal (\%)} = \frac{\text{initial TS} - \text{final TS}}{\text{initial TS}} \times 100\% \quad (1)$$

$$(NH_3 - N) = (NH_4^+ - N)\left[1 + \frac{10^{-pH}}{10^{-\left(0.1075 + \frac{2725}{T}\right)}}\right]^{-1}, \text{T = absolute temperature, K} \quad (2)$$

$$TAN = (NH_3 - N) + (NH_4^+ - N) = (NH_4^+ - N)\left(\left[1 + \frac{10^{-pH}}{10^{-\left(0.1075 + \frac{2725}{T}\right)}}\right]^{-1} + 1\right) \quad (3)$$

$$pH = 9.43 - 2.02\,\frac{VFAs}{TAN} \quad (4)$$

2.5. Modelling

The measured biogas yield evolution was simulated via the two popular kinetic models which were the modified Gompertz model (I. Syaichurrozi, Budiyono, & Sumardiono, 2013) and the first-order kinetic model (I. Syaichurrozi, 2018). The equations of the two models were presented in equations (5) and (6) respectively. The adjustable kinetic constants of ym, λ, μ, k in the kinetic models was determined by using a non-linear regression method through Ms. Excel.

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

$$y(t) = ym\left(1 - \exp(-k.t)\right), t \geq 0 \quad (6)$$

Optimization was conducted to determine the kinetic constant values by minimizing the value of Mean Absolute Percentage Error (MAPE). The formula of MAPE was shown in the equation (7).

$$MAPE = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{|\text{Measured biogas} - \text{Predicted biogas}|}{|\text{Measured biogas}|}\right) \times 100\% \quad (7)$$

3. RESULT AND DISCUSSION

The experiments were successfully conducted with variation of I/S with values of 1.5, 2, 2.5. The experimental data obtained during experiments were biogas yield, methane percentage, TS content, substrate pH, ammonia concentration, ammonium concentration, and VFAs concentration. The kinetic models were successfully applied to simulate the experimental data. Detail results and discussions were presented below.

3.1. Effect of I/S ratio (Scenario 1)

Variation of I/S ratio affected the daily and cumulative biogas yields and then it was shown in Figure 2(A) and (B). The peak of daily biogas yield was obtained on days 12, 14, 18 with values of 1.70, 3.56, 4.70 mL/g VS for I/S of 1.5, 2, 2.5 respectively (Figure 2(A)). Increase in I/S from 1.5 to 2.5 successfully increased the total biogas yield from 19.01 to 33.84 mL/g VS (Figure 2(B) and Table 2). Also, ratio of I/S of 2.5 resulted biogas with high methane content which was 69.01% (Table 2).

The substrate pH profiles were same for all I/S ratios (Figure 2(C)). The different I/S ratios resulted no significant effect on the changes of substrate pH. It was still stable in neutral range until the end of process. Hence, the pH level did not disturb the microbial activity. Based on Table 3, the TAN/VFAs ratio and substrate pH had a good correlation. The bigger the TAN/VFAs ratio value in the substrates, the higher the substrate pH level in the system.

The TS removal value at I/S ratio of 2.5 was higher than the others. It showed that acidogenic bacteria grew well in the substrate, so they consumed more organic compounds (expressed as TS) to become VFAs and TAN. Furthermore, the VFAs was converted to methane by methanogenic bacteria. Meanwhile, the TAN is consumed as nitrogen source for building the cell structures. The final TAN and VFAs concentrations in the substrates were presented in Table 3. The I/S ratio of 2.5 had a lower final VFAs and TAN concentrations than the others (Table 3), however it resulted a higher TS removal than the others. It proved that methanogenic bacteria thrived and converted much of VFAs to biogas successfully at I/S of 2.5.

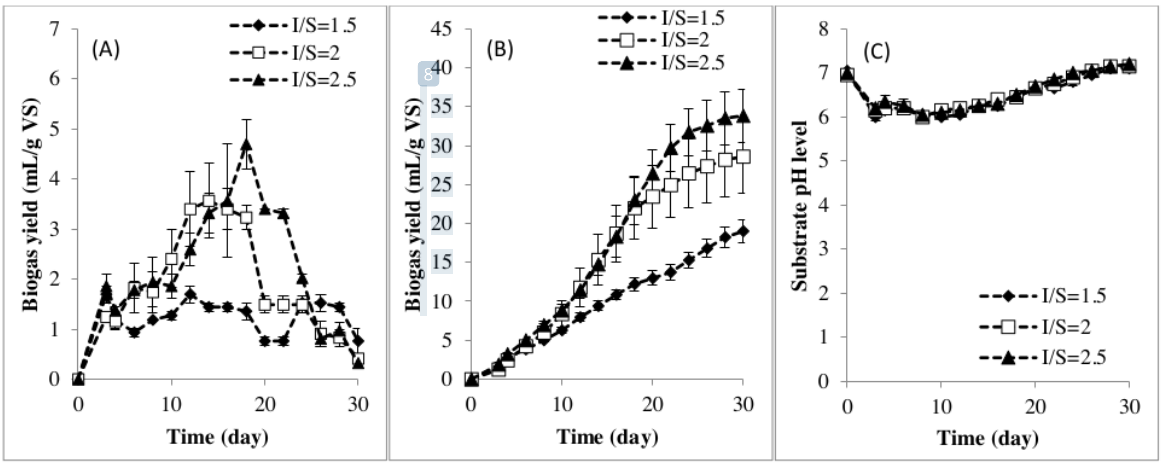


Figure 2. Profiles of (A) yield of daily biogas, (B) yield of cumulative biogas, (C) substrate pH during AD with variation of I/S ratio

Table 2. Effect of I/S ratio on total biogas yield and methane content

I/S ratio (v/w, mL/g)	C/N ratio	Initial pH	Final pH	Total biogas yield (mL/g VS)	Biogas Content		TS removal (%)
					CH_4 (%)	Others (%)	
1.5	21.5	7.0±0.1	7.1±0.1	19.01±1.53	52.54	47.46	70.01±3.41
2	21.5	7.0±0.1	7.15±0.05	28.59±4.72	71.96	28.04	70.61±2.86
2.5	21.5	7.0±0.1	7.2±0.0	33.84±3.40	69.01	30.99	76.08±8.03

Table 3. Effect of I/S ratio on ammonia, ammonium, TAN, and VFAs concentrations

I/S ratio (v/w, mL/g)	C/N ratio	Final NH_3-N (mg/L)	Final NH_4^+-N (mg/L)	Final TAN (mg/L)	Final VFAs (mg/L)	Final TAN/VFAs
1.5	21.5	0.206	20.85	21.06	24.29	0.87
2	21.5	0.183	16.56	16.74	18.90	0.89
2.5	21.5	0.179	14.41	14.59	16.11	0.91

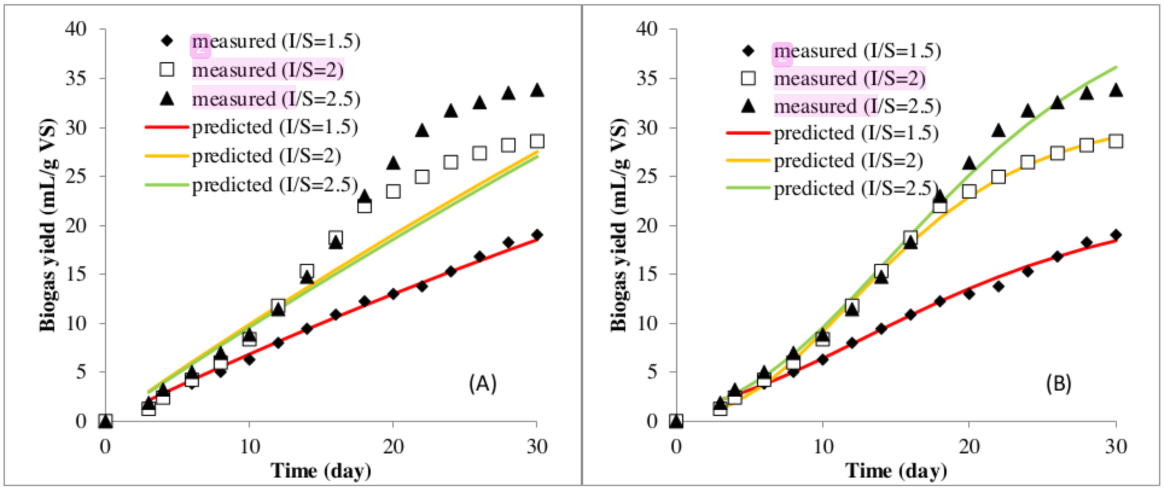


Figure 3. Plotting measured and predicted data obtained through (A) first-order kinetic model and (B) modified Gompertz model

Table 4. The kinetic constant values for Scenario 1

	I/S ratio		
	1.5	2	2.5
Modified Gompertz Model			
λ (days)	1.40	4.26	4.27
μ (mL/g VS.d)	0.74	1.58	1.62
ym (mL/g VS)	22.92	31.43	44.95
R^2	0.995	0.998	0.992
MAPE (%)	4.24	4.32	6.37
First-Order Kinetic Model			
k (/day)	0.010	0.008	0.006
ym (mL/g VS)	69.07	133.60	157.49
R^2	0.996	0.929	0.791
MAPE (%)	5.09	28.74	22.12

The measured data in scenario 1 were modeled through the two proposed models. The fitting between the measured and predicted data was shown in Figure 3. Based on kinetic constants in the modified Gompertz model, the increase in I/S increased the all kinetic constant values (λ, μ, ym). The λ showed the lag time needed by bacteria before producing biogas. The lower the I/S ratio, the lower the inoculum concentration compared to substrate concentration. In other word, the decrease in I/S from 2.5 to 1.5 will increase the substrate concentration compared to inoculum concentration. Therefore, the higher the substrate concentration (the lower I/S ratio), the lower the λ value. It means that the higher the substrate concentration, the bacteria needed the shorter lag time. This phenomena was in line with study of Budiyono et al. (Budiyono et al., 2014) where the higher the substrate concentration, the lower the λ value in biogas production from vinasse. The kinetic constant of μ showed the maximum biogas yield rate. It has good correlation with the ym value (biogas yield potential) where increase in μ will increase the ym value. Based on Table 4, ratio of I/S of 2.5 resulted the higher these values than the two others. At ratio of I/S below 2.5, which was 1.5-2, the activity of anaerobic bacteria was disturbed by the high substrate concentration in the system. The high substrate concentration will increase the osmotic pressure so that microbial cell will be broken. Therefore, although the I/S of 1.5-2 had the lower lag time (λ), these ratios resulted lower μ and ym values because the bacteria could not grow well during the AD process. According to Syaichurrozi (I. Syaichurrozi, 2018), the value of k from the first order kinetic model has negative linier correlation with the value of λ from the modified Gompertz model. It means that the higher the k value, the lower the λ value. This study also reported the same correlation (Table 4). Biologically, when the anaerobic bacteria need a short lag time (λ), they will produce biogas with high rate (k). The value of ym in the first kinetic model also increased with increase in I/S from 1.5 to 2.5.

3.2. Effect of C/N ratio (Scenario 2)

The effect of C/N ratio on AD of SM was discussed in this section. The SM contained C/N value of 21.5. Glucose was added to increase the C/N to become 25 and 30. The daily and cumulative biogas yields are shown in Figure 4(A) and (B). Furthermore, the substrate pH profiles are presented in Figure 4(C). The C/N of 25-30 resulted the higher daily biogas yields than the C/N of 21.5 in the first ten day. However, after day ten, daily biogas yields at C/N of 21.5 was more than those at C/N of 25-30. At C/N of 25-30 (glucose addition), biogas was easier produced at the first digestion time than that at C/N of 21.5 (without glucose addition). Glucose is simple carbon-organic compound that is easily converted to be VFAs. Furthermore, the methanogenic bacteria consumed the VFAs and then produced biogas. However, the higher VFAs concentration at C/N of 25-30 than at C/N of 21.5 caused the substrate pH more drop at the former C/N. Based on Figure 4(C), clearly, C/N ratio of 25-30 resulted VFAs in high concentration in the first digestion time which that is shown by the pH profiles.

The increase of C/N from 21.5 to 25 could increase the total biogas yield from 33.84 to 41.70 mL/g VS (Table 5). Further increasing the C/N decreased the total biogas yield from 41.70 to 30.85 mL/g VS (Table 5). Although the C/N of 25 resulted the higher total biogas yield than C/N of 21.5, the biogas quality from the latter was better than the former. Methane content from C/N of 21.5 and 25 was 69.01 and 34.08 % respectively. That was caused by the low pH level during AD at C/N of 25 in which the substrate pH was drop from day two to the end of AD process. The methanogenic bacteria activity was not good in pH below 5. Therefore, the biogas quality at C/N of 25 was not good. In the other side, the substrate pH at C/N of 21.5 was stable enough during AD. Table 6 showed that the VFA concentration at C/N of 25-30 was much higher than that at C/N of 21.5. TS removal values at C/N of 21.5, 25, 30 was 76.08, 48.70, 55.72% respectively (Table 6). It showed that C/N of 21.5 was good condition not only for methanogenic bacteria but also the acidogenic bacteria.

Table 5. Effect of C/N on total biogas yield and methane content

I/S ratio (v/w, mL/g)	C/N ratio	Initial pH	Final pH	Total biogas yield (mL/g VS)	Biogas Content		TS removal (%)
					CH_4 (%)	Others (%)	
2.5	21.5	7.0±0.1	7.2±0.0	33.84±3.40	69.01	30.99	76.08±8.03
2.5	25	7.0±0.1	3.8±0.1	41.70±20.32	34.08	65.92	48.70
2.5	30	7.0±0.1	3.9±0.0	30.85±14.82	6.99	93.01	55.72

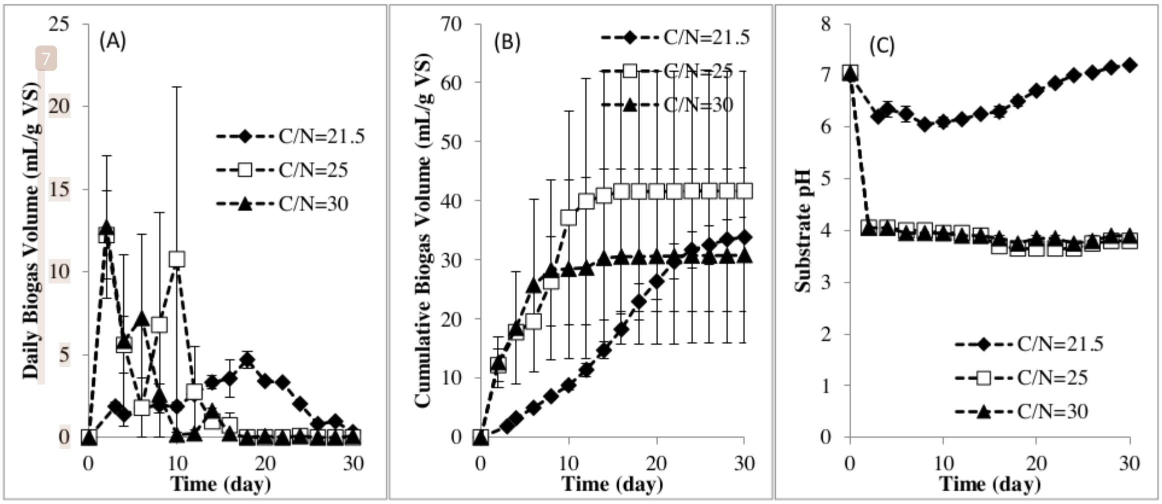


Figure 4. Profiles of (A) yield of daily biogas, (B) yield of cumulative biogas, (C) substrate pH during AD with variation of C/N ratio

Table 6. Effect of C/N on ammonia, ammonium, TAN and VFAs concentrations

I/S ratio (v/w, mL/g)	C/N ratio	Final NH_3-N (mg/L)	Final NH_4^+-N (mg/L)	Final TAN (mg/L)	Final VFAs (mg/L)	Final TAN/VFAs
2.5	21.50	0.179	14.41	14.59	16.11	0.91
2.5	25	0.0005	85.68	85.68	234.56	0.37
2.5	30	0.0003	54.26	54.26	148.54	0.37

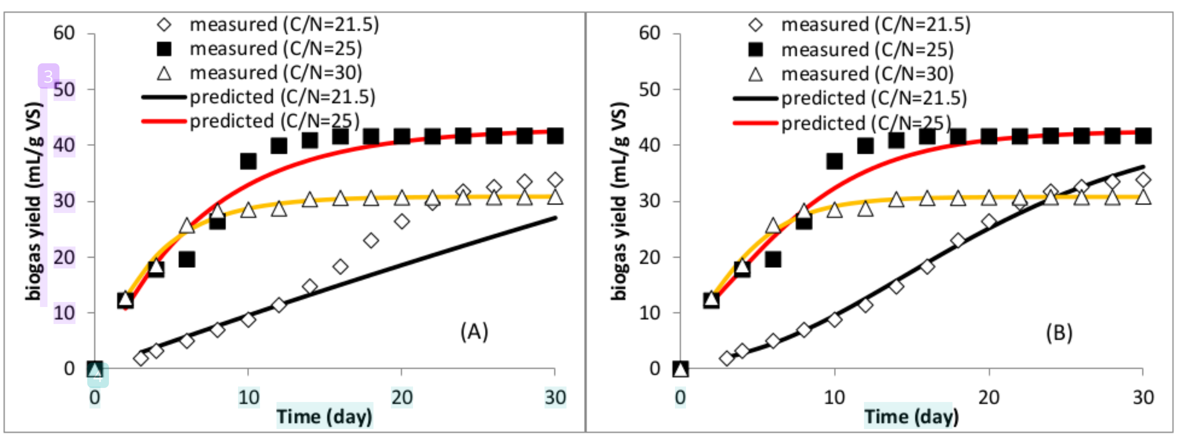
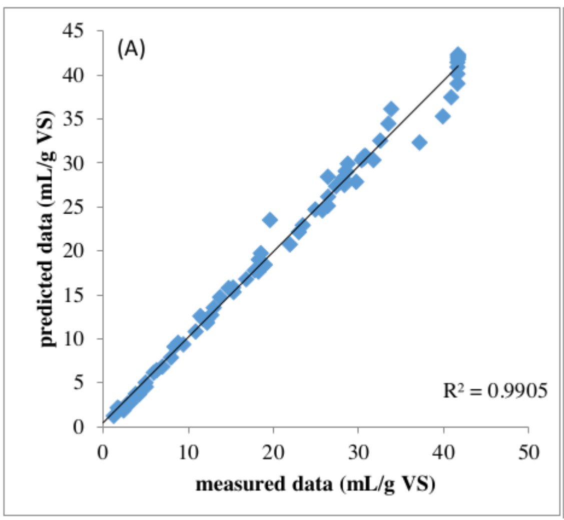


Figure 5. Plotting measured and predicted data obtained through (A) first order kinetic model and (B) modified Gompertz model

Table 7. The kinetic constant values for Scenario 2

	C/N ratio		
	21.5	25	30
Modified Gompertz Model			
λ (days)	4.27	-1.92	-1.27
μ (mL/g VS.d)	1.62	3.01	3.89
ym (mL/g VS)	44.95	42.59	30.77
R^2	0.992	0.987	0.951
MAPE (%)	6.37	5.30	1.37
First-Order Kinetic Model			
k (/day)	0.006	0.145	0.266
ym (mL/g VS)	157.49	43.03	30.82
R^2	0.791	0.984	0.937
MAPE (%)	22.12	7.10	1.56



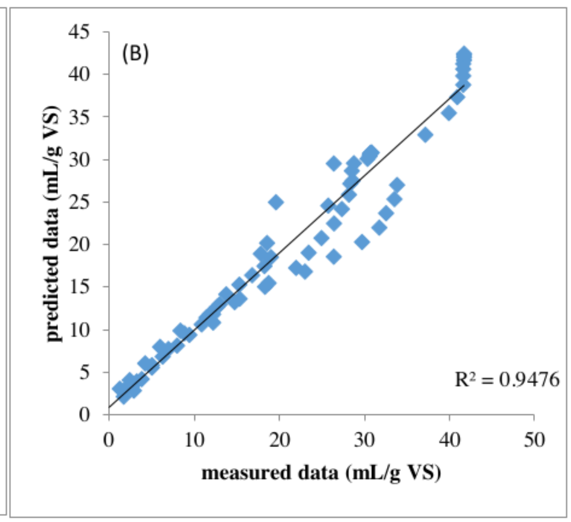


Figure 6. Plotting between all measured biogas yield data and all predicted biogas yield data from (A) modified Gompertz model and (B) first order kinetic model

The measured data in scenario 2 were also modeled through the kinetic models. The fitting between the predicted and measured data was presented in the Figure 5. The obtained kinetic constants are presented in Table 7. The increase in the C/N from 21.5 to 30 decreased the λ value. Substrates with high carbon (glucose) were converted to be VFAs easily. Furthermore, the abundant VFAs were converted to biogas easily. Therefore, the lag time (λ) decreased with increased the C/N ratio. However, further the digestion process, the abundant VFAs decreased the pH value (below 5). It hampered the methanogenic bacteria activity, so that the μ and ym decreased with increase in C/N from 21.5 to 30. In this section, commonly, the value of k from the first order kinetic model has negative linier correlation with the value of λ from the modified Gompertz model.

3.3. Comparison the modified Gompertz and first order kinetic models

The kinetic models were successfully applied to simulate the measured data of biogas yield as function of time. Based on Table 4 and Table 7, the former resulted the MAPE value of 1.37-6.37% and the latter resulted the MAPE value of 1.56-28.74%. Furthermore, the Figure 6 proved that the modified Gompertz resulted the higher R^2 value than the first order kinetic with value of 0,9905 for the former and 0.9476 for the latter. Hence, the modified Gompertz model gave better prediction than the other.

4. CONCLUSION

The experiment of AD process with variation of I/S and C/N ratios during 30 days. In scenario 1, the increase in I/S from 1.5 to 2.5 increased the biogas yield from 19.01 to 33.84 mL/g VS and the methane content from 52.54 to 69.01%. The I/S ratio of 2.5 resulted the higher the TS removal but the lower the final VFAs. It proved that the acidogenic and methanogenic bacteria were in good condition. TS was consumed by acidogenic bacteria and resulted the VFAs. Furthermore, the VFAs was converted to biogas by methanogenic bacteria easily. In scenario 2, increase in C/N to 21.5 to 30 decreased the biogas yield from 33.84 to 30.85 mL/g VS and decreased the methane content from 69.01 to 6.99%. The higher C/N (25-30) was not good for AD because VFAs was produced in high amount so that the pH level was drop (below 5). The biogas yield evolutions as function of time were simulated with R^2 of 0.9905 and 0.9476 through the modified Gompertz model and the first-order kinetic model respectively. Hence, the former was better than the latter.

ACKNOWLEDGEMENT

All authors thanks profusely to the Lembaga Penelitian dan Pengabdian Masyarakat in University of Sultan Ageng Tirtayasa due to the Grant Research of Penelitian Dosen Madya with contract number of 324/UN43.9/PP/KT/2017 (Nomor SP DIPA-042.01.2.401017/2017).

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