

Biogas Production from Co-Digestion Vinasse Waste and Tofu-Processing Wastewater and Kinetics

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Received: 24.04.2016 Accepted: 23.07.2016

Abstract- The biogas fermentation co-digestion vinasse waste (VW) and tofu-processing wastewater (TW) was investigated within a wide range of VW:TW of 0:100 - 100:0 (volume ratio). The VW:TW ratio of 0:100, 20:80, 40:60, 60:40, 80:20, 100:0 generated total biogas of 76.11, 159.01, 120.22, 19.96, 18.77, 10.64 mL/g COD respectively. Fitting error between measured and predicted biogas yield by using modified Gompertz model was less (1.18-9.79%) than that by using first order kinetic model (0.82-17.33%). The best variable was VW:TW of 20:80 (COD/N = 1042/7), which had kinetic constant of y_m (mL/g COD), μ (mL/g COD/day), λ (days) of 153.70, 8.49, 1.38 respectively through modified Gompertz equation. The effect of COD concentration on biogas production was successfully described by using Edward model ($R^2 = 0.95$). Meanwhile, the optimal COD/N obtained from Ratkowsky model was 414/7 ($R^2 = 0.96$). The developed equation of kinetic model of degradability nitrogen content (KMDNC) was $Nt = (Ni/\varepsilon) - \left(\frac{N}{COD}\right) \left(\left(\frac{ci}{395}\right) (yt - 395 + ym)\right)$.

Keywords- Biogas; Co-digestion; Degradability nitrogen content; Kinetic model; Tofu-processing wastewater; Vinasse

1. Introduction

Presence of Chemical Oxygen Demand (COD) and nitrogen (N) content in the substrates is the most important parameter to produce biogas optimally [1]. Substrates containing high COD will generate Volatile Fatty Acids (VFAs) in large amount. Abundant VFAs in the system causes pH drop sharply. That condition can kill methanogenic bacteria [2-3]. Whereas, nitrogen-rich substrates will generate ammonium/ammonia easily. Ammonia concentration more than 150 mg/L and ammonium concentration more than 30,000 mg/L are toxic for bacteria [3]. Anaerobic bacteria need COD as main carbon source to produce biogas and nitrogen source to build cell structure. Anaerobic bacteria especially methanogenic bacteria can

thrive in the substrates containing COD and nitrogen in good ratio. According to literature, Syaichurrozi *et al.* [4] suggested that the optimum range of COD/N ratio in anaerobic digestion was 350/7-1000/7.

Some authors added synthetic nitrogen source (such as urea) into substrates having high COD and low nitrogen content [4-5]. This concept can increase biogas production, however operation cost is increasing. Currently, other authors have developed co-digestion concept, which COD-rich substrates was mixed with nitrogen-rich substrates to get the substrates having the COD/N in optimum range. The second concept is more economically than the first concept.

Many authors have studied anaerobic co-digestion technology. O-Thong *et al.* [6] reported that co-digestion oil

palm empty fruit bunches (EFB) and palm oil mill effluent (POME) with volume ratio of 0.4:1, 0.8:1 and 2.3:1 resulted 25-32% higher methane production than digesting EFB alone. Also, Zhang *et al.* [7] conducted co-digestion concept between pig manure (PM) and dewatered sewage sludge (DSS) under mesophilic condition. The PM:DSS ratio of 2:1 (volume basic) produced the highest cumulative methane yield of 315.8 mL/g VS_{added} which was 82.4% greater than digesting DSS alone. Furthermore, Zhen *et al.* [8] stated that addition of grass *Egeria densa (E.d.)* into waste activated sludge (WAS) improved methane production greatly. Furthermore, Zheng *et al.* [9] stated that mono-digestion of switchgrass led to volatile acid accumulation and process failure, whereas co-digestion of switchgrass (SG) and dairy manure (DM) increased the buffering capacity and the fermentation efficiency. The co-digestion of SG:DM of 2:2 (volume basic) resulted 39% higher methane yield than the mono-digestion.

Vinasse waste is byproduct that is generated from bottom product of distillation unit of bioethanol industry. In the production of 1 liter bioethanol, the bioethanol industries will generate 8 – 15 liter vinasse. Vinasse contains high COD with range of 104,640 – 299,250 mg/L [4,10] and low total nitrogen with range of 153 - 4,004 mg/L [4,10-11]. Budiyo *et al.* [5] reported that COD/N ratio of vinasse was 1436/7. This ratio was out from optimum range, which was 350/7-1000/7 [4]. Therefore substrates containing high nitrogen content must be added into vinasse waste.

Liquid waste that is potential as co-digestion partner of vinasse is tofu-processing wastewater (TW). Tofu-processing wastewater is the residue generated from tofu industries [12]. Tofu is traditional oriental food produced from soybean as raw materials through some steps, i.e. soy bean grinding, cooking (boiling), filtration, protein coagulation, preservation, and packaging. During the tofu production process, especially in the filtration process, tofu-processing wastewater is generated [13]. Every tofu production of 80 kg, it will bring out 2,610 kg tofu-processing wastewater in Indonesia. Each 1 liter TW contains 82,100 mg COD and 2,100 mg total nitrogen [14]. Therefore, the COD/N ratio of TW is ~274/7. This value is lower than optimum range that allowed by Syaichurrozi *et al.* [4]. It means TW is a nitrogen-rich waste.

Based on that, in this study we investigated anaerobic co-digestion of vinasse waste (VW) and tofu-processing wastewater (TW). The utilization of TW as nitrogen source

to VW has not been reported by other authors yet. The aim of this study was to investigate the effect of VW:TW ratio of 0:100 - 100:0 to biogas production on anaerobic digestion performance in batch test. The experimental data obtained was used to make kinetic model. We compared kinetic model of biogas production using modified Gompertz equation and using first order kinetic. This comparison was important to find the better kinetic model to predict biogas production from co-digestion VW and TW. The effect of substrate concentration and COD/N ratio on biogas production was also modeled. That was done to estimate the optimum of substrate concentration and COD/N ratio that was allowed in co-digestion (VW and TW) anaerobic digestion. In addition, we also made prediction model of degradation of nitrogen-substrates as the effect of biogas generation, which it has not been developed by others yet.

2. Methods

2.1. Wastewater and Inoculums

Vinasse waste (VW) was obtained from a bioethanol industry that produced bioethanol from molasses. The bioethanol industry was located in Solo, Central Java Province, Indonesia. The VW contained 31,680 mg/L COD, 13.1 mg/L nitrogen total, pH level of 3.7. Whereas, tofu-processing wastewater (TW) was obtained from a tofu industry located in Serang, Banten Province, Indonesia. The TW contained 576 mg/L COD, 13.5 mg/L nitrogen total, pH level of 3.4. The rumen fluid was used as inoculums. In this study, rumen fluid in fresh condition was obtained from cow slaughterhouse in Serang, Banten Province, Indonesia.

2.2. Experimental Set Up

Anaerobic digesters were made from polyethylene bottles having volume of 600 mL. The bottles were plugged with rubber plug and were equipped with valve for biogas measurement. Biogas formed was measured by liquid displacement method as also has been used by the other authors [4,15-16]. In this method, each digester was connected to gas collector that was reserved cylindrical glass. The connection was done using connecting tube. Each gas collector was immersed in through of water to ensure complete sealing. Biogas formed from digesters was collected by the downward displacement of water.

Table 1. Variation of VW:TW ratios

VW:TW	Substrate (mL)		Rumen fluid (mL)	COD (mg)	Total Nitrogen (mg)	COD/N
	VW (mL)	TW (mL)				
0:100	0	250	25	545.25	14.123	270/7
20:80	50	200	25	2100.45	14.103	1042/7
40:60	100	150	25	3655.65	14.083	1817/7
60:40	150	100	25	5210.85	14.063	2594/7
80:20	200	50	25	6766.05	14.043	3373/7
100:0	250	0	25	8321.25	14.023	4154/7

Remarks: VW, Vinasse Waste; TW, Tofu-processing wastewater; COD, Chemical Oxygen Demand content; N, Nitrogen total content

2.3. Experimental Design

Anaerobic digesters of experimental laboratory using 600-mL volumes were operated in batch system. Total volume of VW and TW mixing of 250 mL was put into the digesters. Rumen fluid as methanogenic bacteria provider was added into the digester as much as 10% v/v substrate. Substrates was varied at VW:TW of 0:100, 20:80, 40:60, 60:40, 80:20, 100:0. Furthermore, initial pH for all variables was adjusted 7.0 by using NaOH solution 5 N. The variable in this work can be seen in Table 1.

2.4. Experimental Procedures

Fermentation was done until no longer produced biogas at mesophilic temperature (~35 °C) and at pressure of 1 atm. According to Budiyo *et al.* [2,5], to make prediction of biogas production through modified Gompertz equation and first order kinetic, laboratory-scale batch anaerobic digesters were operated until biogas production was stop. Hence, the obtained experimental data can be used to make kinetic model of biogas production well. In this work, authors also did the same concept. Biogas formed was measured every once in two days to know biogas production by using water displacement method.

The pH of substrate in the digesters was measured by using pH meter every once in two days. Firstly, the digesters were opened. The rubber plug, which plugged the digesters, was taken. Then, substrates were taken from the digesters as much as 10 mL. After that, the digesters were plugged quickly. The pH level of substrates taken was measured by using pH meter. These procedures were done very quickly to keep the comfortable condition for methanogenic bacteria.

2.5. Kinetic Model of Biogas Production

2.5.1. Modified Gompertz model

Biogas production kinetic was modeled through modified Gompertz model [17]. Kinetic of biogas production in batch condition was assumed that had correspondence to specific growth rate of methanogenic bacteria in digesters [4,15]. The modified Gompertz equation as follows:

$$y(t) = y_m \cdot \exp \left\{ - \exp \left[\frac{\mu_e}{y_m} (\lambda - t) + 1 \right] \right\} \quad (1)$$

Where:

y(t), the cumulative biogas yield at a digestion time t days (mL/g COD); y_m, the biogas production potential (mL/g COD); μ, the maximum biogas production rate (mL/g COD/day); λ, lag phase period or minimum time to produce biogas (days); t, cumulative time for biogas production (days); e, mathematical constant (2.718282)

$$y_m = [A((COD/N) - (COD/N)_{min})]^2 \{1 - \exp[B((COD/N) - (COD/N)_{max})]\}^2 \quad (8)$$

Where:

y_m, the biogas production potential (mL/g COD); A, B, Ratkowsky parameters; COD/N, Ratio of COD/Nitrogen in substrate

Kinetic constant of y_m, λ and μ was determined by using non-linear regression with help of polymath software [4].

2.5.2. First order kinetic model

Biogas production was modeled using first order kinetic model. This model also has been used by Kafle *et al.* [18]. The first order kinetic model as follows:

$$y(t) = y_m (1 - \exp(-k \cdot t)) \quad (2)$$

Where:

y(t), the cumulative biogas yield at a digestion time t days (mL/g COD); y_m, the biogas production potential (mL/g COD); k, the biogas rate constant (/day); t, cumulative time for biogas production (days)

2.6 Kinetic Model for Substrate Concentration Effect

In this study, some models were applied to describe the inhibition effect of substrate concentration on biogas production rate. These models were Andrew Model (3), Modified Andrew Model (4), Aiba Model (5), Moser Model (6), Edward Model (7)

$$R = \frac{R_{max} S}{(K_s + S) \left(1 + \frac{S}{K_i}\right)} \quad (3)$$

$$R = \frac{R_{max} S}{K_s + S + S^2 / K_i} \quad (4)$$

$$R = \frac{R_{max} S}{K_s + S} \exp\left(\frac{-S}{K_i}\right) \quad (5)$$

$$R = \frac{R_{max} S^n}{K_s + S^n} \quad (6)$$

$$R = R_{max} S \left(\exp\left(\frac{-S}{K_i}\right) - \exp\left(\frac{-S}{K_s}\right) \right) \quad (7)$$

Where:

R, biogas production rate (mL/g COD/day); R_{max}, biogas production rate constant (mL/g COD/day); S, substrate concentration (g/L); K_s, saturation constant (g/L); K_i, inhibition constant (g/L); n, constant

2.7. Kinetic Model for COD/N Effect

The Ratkowsky model (equation 8) was applied to describe the effect of COD/N ratio on biogas production potential. The other authors have used this model usually to describe the effect of temperature and pH condition on biogas/methane/hydrogen production potential. In this study, we tried to use this model to predict the optimum COD/N ratio on biogas production from co-digestion VW and TW..

3. Results and Discussion

3.1. Biogas Production

Total biogas of VW:TW ratio of 0:100, 20:80, 40:60, 60:40, 80:20, 100:0 was 76.11, 159.01, 120.22, 19.96, 18.77, 10.64 mL/g COD respectively. Biogas production daily and cumulative for all ratios was shown in Fig.1(a) and (b). Anaerobic bacteria needed organic materials (COD content) and nitrogen source in fit ratio. The optimum COD/N ratio was in range of 350/7 – 1000/7 [4]. Nitrogen source in the substrates (such as protein and urea) was decomposed to be ammonia (NH₃)/ammonium (NH₄⁺) during fermentation process. Ammonia/ammonium in the system was utilized by anaerobic bacteria to build cell structure. Accumulation of that in large amount, however, could inhibit bacterial growth and kill anaerobic bacteria at specific concentration. Ammonia formed ammonium ions in the substrates, the extent of this was depended on the pH value. Concentration of ammonia and ammonium ions had permanent equilibrium: NH₄ ↔ NH₃ + H⁺ and NH₄⁺ + OH⁻ ↔ NH₃ + H₂O [19]. The more acid of pH substrate, the more ratio of ammonium : ammonia. Deublein and Steinhäuser [3] stated that at pH of 9.0, the ratio ammonium to ammonia is 70:30, whereas at pH of 7.0, the ratio ammonium to ammonia is 99:1. In addition, substrate pH > 9.25, ammonia is full dominant in the substrate [20] and pH < 7.0, ammonium ions is full dominant in the substrate [4]. From Fig.1(c), pH profile of all variables was lower than 7.0. Thus, ammonium inhibition was full dominant in the system. Ammonium concentration of 1,500-10,000 mg/L was start inhibition and that of 30,000 was toxic for anaerobic bacteria, especially methanogenic bacteria [19].

Organic materials (COD content) was convert into biogas through four major steps, i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis [21]. Vinasse waste contained simple organic content, such as acetic acid, lactic acid and glycerol [22]. Thus, vinasse was easy to be degraded into organic acid through acidogenesis-acetogenesis phase. In this research, substrates were contained vinasse waste and tofu-processing wastewater in various ratio. The more the vinasse presence in the substrates, the easier the organic acid (VFAs) was generated. Accumulation of VFAs caused pH substrate drop (Fig.2). In the end of fermentation, pH value of VW:TW of 0:100, 20:80, 40:60, 60:40, 80:20, 100:0 was 6.8, 5.1, 4.9, 4.0, 3.9, 3.9 respectively. Ratio of 0:100 (TW alone) had pH profile that was more stable than the others. The acid condition in substrates was not good for bacterial growth. Therefore, in the ratio of 60:40, 80:20, 100:0 (VW alone), methanogenic bacteria cannot grow well and finally death. That can be proofed from Fig.1 (a), for the variables, biogas was stopped to be produced in the 8-day fermentation.

Organic acid was divided into two kinds which was not dissociated acid and dissociated acid. Composition of them in the substrates was depended on pH value. The more acid of pH value, the more the presence of not dissociated acid in the substrate. The presence of not dissociated acid disturbed methanogenesis phase because that organic acid was penetrated into bacterial cell and denatured protein of

bacteria [3]. Furthermore, Brannen and Davidson [23] reported that protein, nucleid acid and fosfolipid in the bacterial body were damaged by dropping pH (< 7.0). Therefore, ratio of VW:TW of 60:40, 80:20, 100:0 produced biogas in little amount which was 19.96, 18.77, 10.64 mL/g COD respectively.

In the substrate of VW:TW of 0:100 (TW alone), biogas generated was 76.11 mL/g COD. This value was more than biogas from VW:TW substrate of 60:40, 80:20 and 100:0. TW contained high-nitrogen content. Ammonia/ammonium that was generated by biological decomposition of nitrogen source caused pH substrate of stable (7.0 – 6.8), unlike pH condition in substrates of 60:40, 80:20 and 100:0 (Fig.1(c)). However, substrate of 0:100 contained COD/N ratio that was not in optimum range. Hence, biogas produced from VW:TW of 0:100 was less than that from VW:TW of 20:80 and 40:60.

Substrate with VW:TW ratio of 20:80 and 40:60 produced biogas of 159.01 and 120.22 mL/g COD respectively. The pH value of that was decreasing from 7.0 until 5.1 and 4.9 respectively. That condition was bothering bacterial activity during fermentation, but anaerobic bacteria still can adapt in this condition until 14 – 16 day of fermentation. This phenomenon was caused by final pH in 20:80 and 40:60 that was higher than final pH in substrate of 60:40, 80:20, 100:0 (4.0, 3.9, 3.9). The best ratio value was 20:80, because its COD/N ratio was the closest to the optimum range which was 1042/7 and generated biogas was the most of all variables.

Addition of vinasse waste as co-substrate of tofu-processing wastewater was caused not only COD/N substrate in good range, but also supplying micronutrients in the system. Vinasse contained micronutrients needed by anaerobic bacteria which were K⁺, Na⁺, Ca²⁺, Mg²⁺ of 6.5, 0.35, 9.0, 2.5 g/kg TS respectively. This ion increased the efficiency of organic fermentation [24]. In this research, fermentation of substrate mixing VW and TW with composition of 20-40% VW volume was better on biogas production rate than fermentation of VW or TW alone. Addition of too high amount of vinasse into the tofu-processing wastewater decreased biogas yield.

3.2. Effect of Substrate Concentration on Biogas Production

In this work, we compared some kinetic models that were appropriate to describe the substrate inhibition during fermentation. Based on experimental data, at substrate concentration of 545.25; 2100.45; 3655.65; 5210.85; 6766.05; 8321.25 mg/275 mL (Table 1) generated biogas with rate of 3.81; 7.95; 6.01; 0.99; 0.94 mL/g COD/d. Experimental biogas production rate was obtained through biogas total during fermentation divided by retention time of 20 days.

Using Andrew, Modified Andrew, Aiba, Moser and Edward model gave the fitting R² of 0.51; 0.53; 0.71; 0.43; 0.95 respectively (Fig.2 and Table 2). Hence, the best fitting R² was 0.95 which was using Edward model. The Modified Andrew model gave the more satisfactory results than Andrew model. Furthermore, Aiba and Edward model could

predict the substrate inhibition effect on biogas production rate with more appropriate than Modified Andrew. Meanwhile, the worst fitting was Moser model. The biogas production rate was corresponding with the methanogenic bacteria growth during fermentation process. The more

biogas production rate, the faster methanogenic bacteria growth, which was at substrate concentration less than 2100.45 mg COD/275 mL. At higher concentration, inhibition substrate was occurred at substrate concentration more than 2100.45 mg COD/275 mL (VW:TW = 20:80).

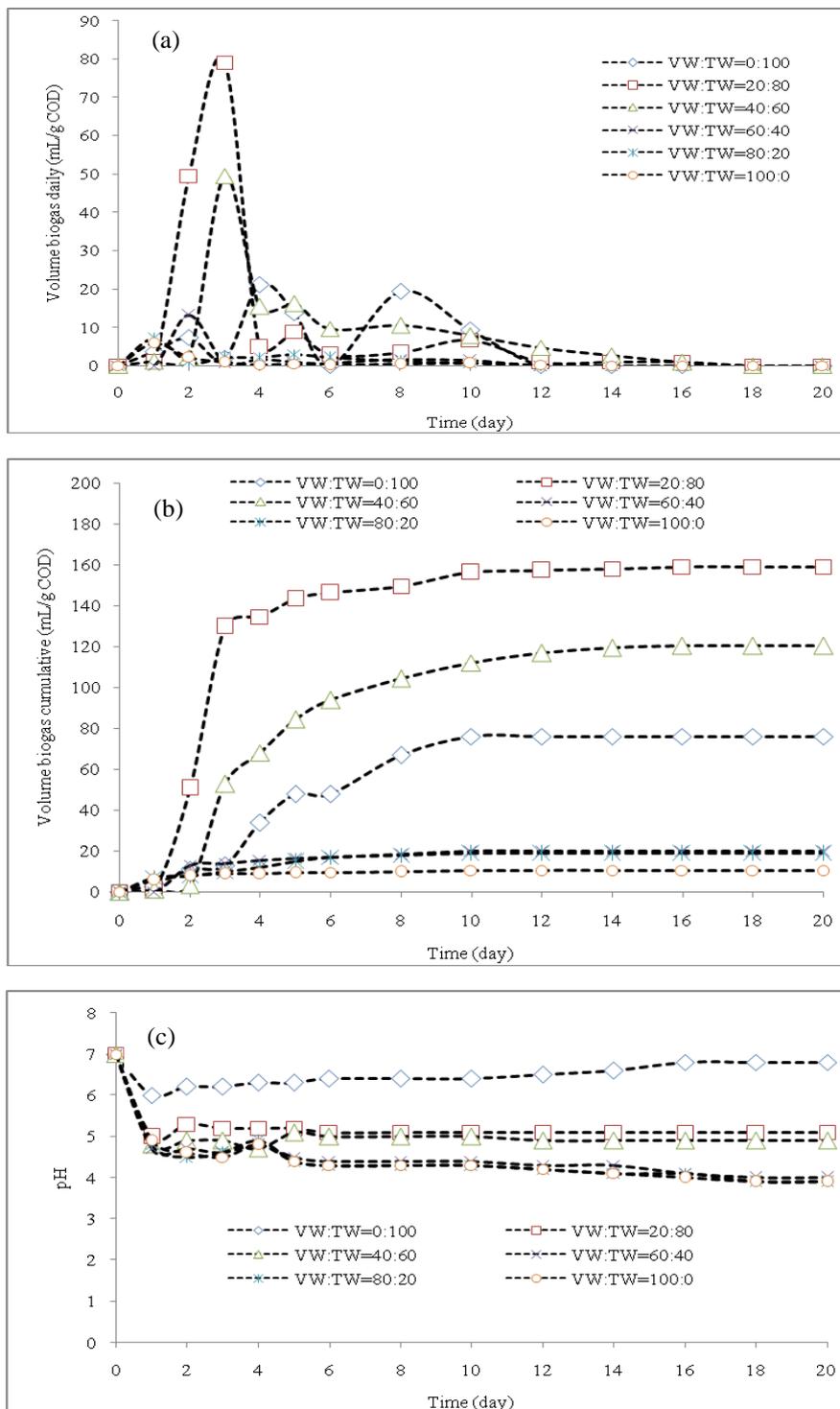


Fig. 1. Profile of (a) biogas production daily, (b) biogas production cumulative, (c) pH substrate

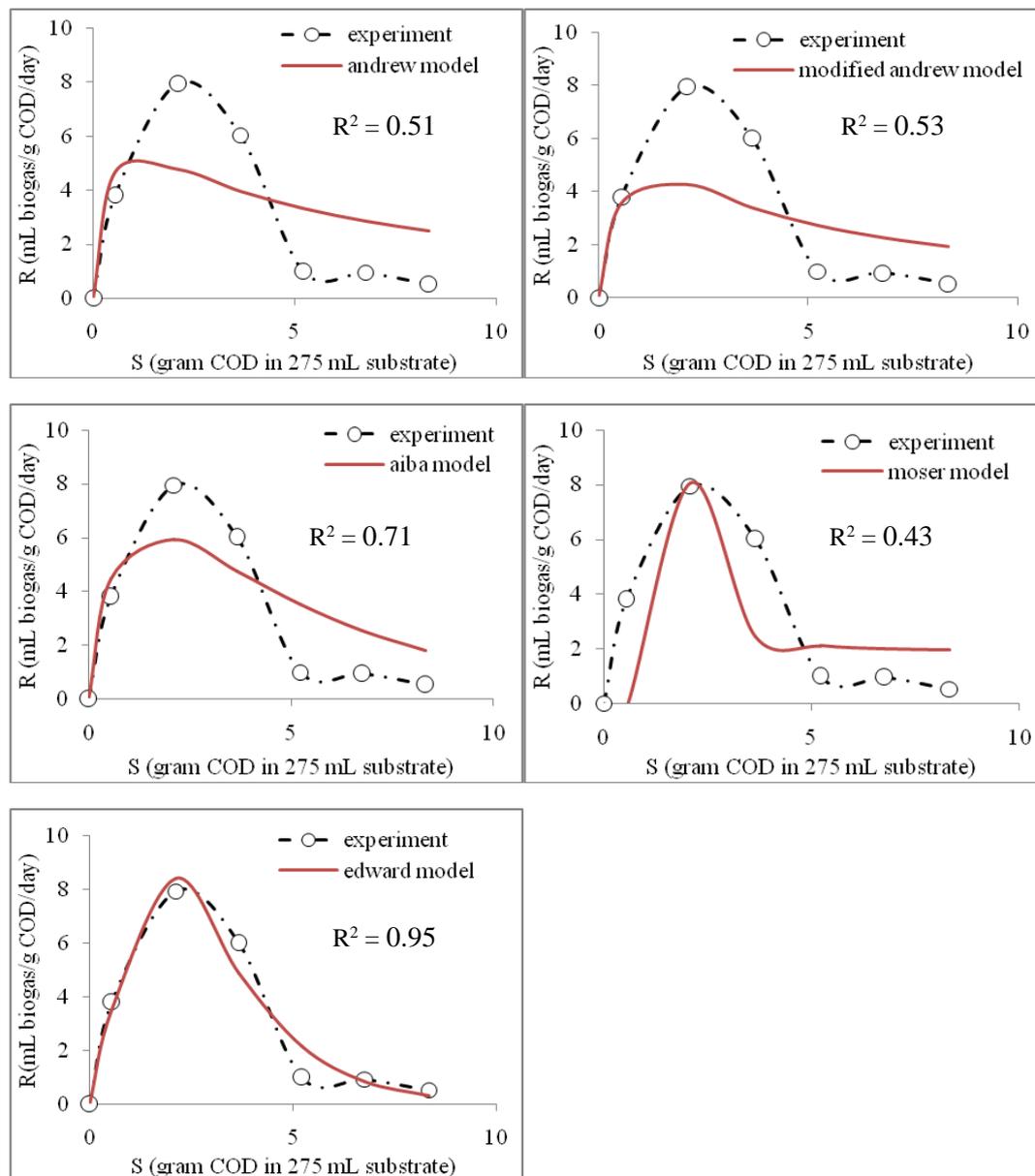


Fig. 2. Modeling in effect substrate concentration on biogas production

Table 2. Parameters of modeling for effect of substrate concentration on biogas production

Model	Parameters of modeling				R^2
	R_{max} (mL/g COD/d)	K_s (g COD/275 mL)	K_i (g COD/275 mL)	n	
Andrew	9.27	3.80	3.27		0.51
Modified Andrew	12.06	1.11	1.62		0.53
Aiba	14.34	0.99	4.25		0.71
Moser	1.87	-3.63		2.09	0.43
Edward	22.47	0.55	1.31		0.95

Remarks: R_{max} , biogas production rate constant; S , substrate concentration; K_s , saturation constant; K_i , inhibition constant; n, constant; R^2 , correlation coefficient

Chuang *et al.* [25] used Modified Andrew model to describe inhibition effect of *E.crassipes* with R^2 of 0.98-0.99. Meanwhile, Dutta *et al.* [26] found that Andrew model ($R^2 = 0.92$) was better than Aiba, Moser and Edward model. However, in this study, Edward model gave the best prediction ($R^2 = 0.95$). This difference was caused by substrate where bacteria grew and inhibitor compound. Edwards [27] compared Andrew model with Edward model to describe substrate inhibition of respiration by *Nitrosomonas* with ammonia. After simulation, Edward model give the more satisfactory prediction than Andrew model. The Edward's results were contrast with the results of Dutta and co-workers. Dutta *et al.* [26] used *Burkholderia cepacia* as microorganism and Cutin as substrate. Then, Edwards [27] also found that Edward model had the better fitting than Aiba model to describe substrate inhibition of *Candida utilis* by acetate. Meanwhile, from this study, we found that in the describing substrate inhibition of methanogenic bacteria by COD concentration, the Edward model gave the most satisfactory results. That was similar with Edward's results.

3.3. Kinetic Model of Biogas Production

3.3.1. Using modified Gompertz model

The experimental data obtained was used to make kinetic model of biogas production through modified Gompertz model. Kinetic constant of y_m , μ and λ was determined by using non-linear regression. Kinetic constants obtained were presented completely in Table 3. By plotting experimental and simulation data, we got the graph as shown in Fig.3(a).

From Table 3, substrate of VW:TW ratio of 20:80 had more value of y_m than the other variables, which was 153.70 mL/g COD. That means ratio of 20:80 generated predicted biogas in large amount compared to the other ratios. That

was due to bacterial activity in the comfortable metabolism conditions supported by the mixing feedstock.

Syaichurrozi *et al.* [4] and Budiyo *et al.* [5] reported that the more value of y_m , the more value of μ . Kinetic constant of μ is maximum biogas production rate, so that the more biogas production rate, the more total biogas formed. Substrate of 20:80 (COD/N = 1042/7) had the highest value of μ . Anaerobic bacteria needed nitrogen source to build cell, so presence nitrogen in appropriate amount in the system was the important. That caused the μ in high value.

The variable that had little value of kinetic constant of λ , needed just little time to produce biogas [4]. Zwietering *et al.* [17] reported that value of λ indicated the time that was required for bacteria to adapt in substrate condition. Based on that, bacteria in substrate with VW:TW ratio of 0:100 needed much time to adapt which was 1.63 days. In the other hand, bacteria in substrate of 80:20 and 100:0 needed less time than the other variables. TW had high proportion of proteins and fatty acids [12]. Substrate containing high proteins needed the longer time than substrate containing high carbohydrate. The high carbohydrate was contained in the vinasse so that bacteria took short time to degrade that [4]. Thus, the more presence of vinasse waste in substrate, the less time needed by anaerobic bacteria to convert organic content into biogas.

Comparison to other authors, Budiyo *et al.* [5], Adiga *et al.* [28] and Zhang *et al.* [7] found that the kinetic constant of λ in manure biogas production was 4.460, 8.749 and 6.9 days respectively. Cattle manure, poultry litter and pig manure contained lignocellulosic that was difficult to be degraded through fermentation process. Because of the lignocellulosic in the manure substrate, anaerobic bacteria need a long time to adapt and produce biogas. Patil *et al.* [29] also found the high value of kinetic constant λ which was 6.625 days on biogas production from water hyacinth. Water hyacinth contained high solid lignin which was tightly surrounds cellulose and hemicellulose [30]. Thus, anaerobic bacteria cannot destroy it easily.

Table 3. Results from using modified Gompertz and first-order kinetic model

	VW:TW Ratio					
	0:100	20:80	40:60	60:40	80:20	100:0
Modified Gompertz Model						
λ (days)	1.63	1.38	1.48	0.72	0.00	0.00
μ (mL/g COD/day)	1.29	8.49	2.65	0.76	0.29	0.33
R^2	0.99	0.99	0.98	0.94	0.98	0.95
y_m -Predicted biogas yield (mL/g COD)	77.01	153.70	116.83	18.08	18.26	9.59
Measured biogas yield (mL/g COD)	76.11	159.01	120.22	19.96	18.77	10.64
Difference between measured and predicted biogas yield (%)	1.18	3.34	2.82	9.40	2.70	9.79
First-Order Kinetic Model						
k (/day)	0.13	0.33	0.17	0.35	0.27	0.59
R^2	0.94	0.91	0.93	0.92	0.98	0.98
y_m -Predicted biogas yield (mL/g COD)	89.30	162.72	130.58	19.20	18.62	9.73
Measured biogas yield (mL/g COD)	76.11	159.01	120.22	19.96	18.77	10.64
Difference between measured and predicted biogas yield (%)	17.33	2.33	8.62	3.78	0.82	8.48

Remarks: VW, Vinasse waste; TW, Tofu-processing wastewater; y_m , the biogas production potential; μ , the maximum biogas production rate; λ , lag phase period or minimum time to produce biogas; k, the biogas rate constant; R^2 , correlation coefficient

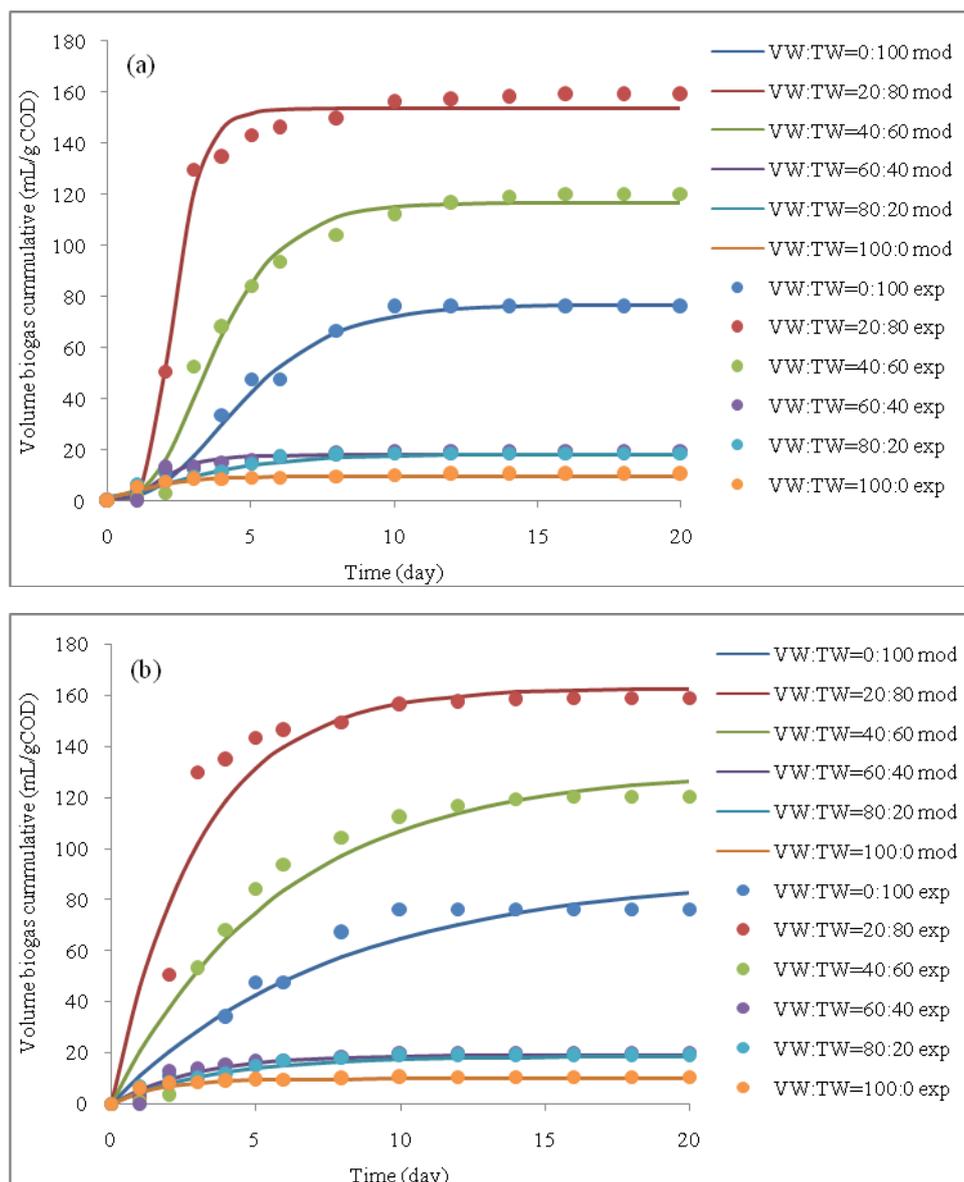


Fig. 3. Comparison of experimental data and simulation data using (a) modified Gompertz model, (b) first order kinetic model

3.3.2. Using first order kinetic model

Kinetic model of biogas production was also simulated through first order kinetic model. Kinetic constant of y_m and k was determined by using non-linear regression. Kinetic constants obtained were presented completely in Table 3. By plotting experimental data and kinetic model was obtained the graph as shown in Fig.3(b).

The term of k was a measure of the biogas production rate with time [2]. Kafle *et al.* [18] stated that, the more positive the value of k , the faster the rate of biogas generation. From Table 3, substrate of 100:0 (VW alone) had the most positive of k value (0.59/day) of all variables. According to literatures, vinasse contained simple organic compounds (such as acetic acid, lactic acid and glycerol) so much that bacteria could degrade them easily to be biogas [22]. Meanwhile substrate of 0:100 (TW alone) had the less positive of k value (0.13/day). TW contained high nitrogen and low carbohydrate content, so that anaerobic bacteria

were need a long time to degrade organic materials in TW. Furthermore, in the co-digestion of VW and TW (20:80, 40:60, 60:40, 80:20), the k value was 0.17-0.35/day.

The difference of k value was caused by composition substrates that were used as feedstock. Zhang *et al.* [7] stated that k value on biogas production from co-digestion of pig manure (PM) and dewatered sewage sludge (DSS) was 0.044-0.094 days. The pig manure contained high cellulose, hemi-cellulose and lignin so that the degradation rate of those and biogas production rate were slow. Whereas, biogas production from fish waste had k value of 0.017-0.040/days [18]. Raposo *et al.* [31] stated that degradation of substrate containing high protein and fats needed more time than that of substrate containing high carbohydrates. Fish waste (FW) contained very high fats [18], so that the value of k was just 0.017-0.040/day. Meanwhile, Zhen *et al.* [8] found that k value obtained from co-digestion of waste activated sludge (WAS) and *Egeria densa* (*E.d.*) was 0.175 – 0.200/days.

3.3.3. Comparison the modified Gompertz model and first order kinetic model

The comparison between this study and other studies can be seen in Table 4. Kafle *et al.* [18] reported that organic waste such as fish waste (pacific saury, mackerel, cuttle fish waste) contained high protein and fat. Meanwhile, brewery grain waste (BGW) and bread waste (BW) contained high nitrogen free extract (NFE) and low protein and fat. During fermentation process, acidogenesis bacteria converted

substrates containing high fat content into LCFAs in large amount. Accumulation LCFAs inhibited the methanogenic bacterial growth, so that the bacteria needed a long time to adapt (lag phase). Biogas generation from FW needed lag time (λ) of 17.2-24.2 days [18]. Whereas, biogas production from BGW containing high nitrogen free extract needed lag time of 18.2 days and BW containing low protein and fat needed lag time of 9.1 days. Also, Sunflower oil cake (SuOC) contained low fat, so that methanogenic bacteria produce biogas just in short lag time [31].

Table 4. Comparison between this result and other results in modified and first order kinetic model to predict biogas yield

Substrate	Difference between measured and predicted biogas (%)		References
	Modified Gompertz	First Order Kinetic	
Brewery grain waste	3.2	19.5	Kafle et al. (2012)
Bread waste	1.6	9.2	Kafle et al. (2012)
Pacific saury fish waste	0.7	13.6	Kafle et al. (2012)
Mackerel fish waste	6.1	29.8	Kafle et al. (2012)
Cuttle fish waste	13.7	37.1	Kafle et al. (2012)
Sunflower oil cake	-	≤ 10	Raposo et al. (2009)
Apple waste (AW)	2.5	12	Kafle and Kim (2013)
Swine manure (SM)	1.9-2.7	5.3-9.8	Kafle and Kim (2013)
Co-digestion of AW and SM	1.3-3.4	4.6-18.1	Kafle and Kim (2013)
Vinasse	0.76-3.14	1.54-7.5	Budiyono et al. (2014)
Pig manure (PM)	0.8	14.2	Zhang et al.(2014)
Dewatered sewage sludge (DSS)	3.7	1.5	Zhang et al.(2014)
Co-digestion of PM and DSS	0.0-0.8	1.5-10.6	Zhang et al.(2014)
Co-digestion of WAS and <i>E.d.</i>	4.4-7.3%	4.0-7.1%	Zhen et al. (2015)
Vinasse	9.79	8.48	This study
Tofu-processing wastewater	1.18	17.33	This study
Co-digestion of vinase and tofu-processing wastewater	2.70-9.40	0.82-8.62	This study

The first order kinetic model gave the satisfactory result with error of 10% or less only if the lag period needed by bacteria was very short [31]. Kafle *et al.* [18] reported that degradation of high protein and fat substrate needed a long lag time and high carbohydrate substrate needed a short time. In this study, at substrate of VW:TW of 0:100 (TW alone), the lag time was the longest (λ was 1.63 days) than the others substrate, so that the fitting error at first order kinetic was the biggest which was 17.33 %. The TW contains high proportion of proteins and fatty acids [12]. Meanwhile, with the vinasse addition into TW substrate (20:80, 40:60, 60:40, 80:20, 100:0), the lag time (λ) was shorter (0 – 1.48 days), so that the fitting error at first order kinetic became smaller (0.82 – 8.62%). With that value, first order kinetic model was allowed to be used in predicting biogas production from co-digestion VW and TW, but the model was not appropriate to describe biogas generation from substrate of TW alone (fitting error > 10%). Whereas, modified Gompertz model gave the satisfactory result in predicting biogas production for all variables (fitting error 1.18 – 9.79%). Zhang *et al.* [7] found the same results with this study. Digesting of pig manure (PM) had the fitting error at first order kinetic of 14.2% ($VS_{PM}:VS_{DSS}$ of 1:0). This was caused by organic content in the manure (such as cellulose, hemi-cellulose, lignin) so that the lag time (λ) was long (6.9 days). At the addition of dewatered sewage sludge (DSS) into the manure

($VS_{PM}:VS_{DSS}$ of 2:1, 1:1, 1:2, 0:1), the fitting error at first order kinetic was better (1.5-10.6%). Furthermore, prediction of methane yield by using modified Gompertz model gave the good fitting for all $VS_{PM}:VS_{DSS}$ ratio (0-3.7%). Meanwhile, Kafle and Kim [32] also reported that modified Gompertz equation gave the better fitting (1.3-3.4%) than first order kinetic (4.6-18.1%) on digestion biogas production from co- apple waste and swine manure.

Moreover, Budiyono *et al.* [2] and Zhen *et al.* [8] reported that both first order kinetic and modified Gompertz model gave the good fitting (error <10%). Budiyono *et al.* (2014) predicted biogas yield from vinasse waste. Biogas generated at the first time of vinasse fermentation (λ of 0 – 2.24 days) because vinasse contained high carbohydrate content. Zhen *et al.* [8] also found that methane yield from co-digestion of waste activated sludge (WAS) and *Egeria densa* (*E.d.*) could be predicted through both first order kinetic (fitting error of 4.0-7.1%) and modified Gompertz model (fitting error of 4.4 – 7.3%). The wastes contained high carbohydrate and low protein contents.

3.4. Effect of COD/N Ratio on Biogas Production

By plotting experimental data and Ratkowsky model kinetic of COD/N effect was obtained the graph as shown in Fig.4 ($R^2 = 0.96$). Meanwhile, Table 5 summarized the

biogas production during fermentation process at variation of COD/N ratio in the substrates. Biogas production potential (y_m) was obtained from modified Gompertz model, because it was more better fitting than first order kinetic model. From the modeling, the value of y_m increased with increasing COD/N_{min} to COD/N_{opt} and then decreased with further increasing COD/N from COD/N_{opt} to COD/N_{max}. The COD/N_{opt} obtained was 414/7 (Table 5). From all COD/N ratios obtained from the model, COD/N of 414/7 was found as the best ratio. This value was in optimum range allowed (350/7 – 1000/7) by Speece [33]. Meanwhile, the other ratios were out of the optimum range.

Syaichurrozi *et al.* [4] found that ratio of COD/N at 400/7 – 700/7 had the higher μ value (which was 13.331 –

15.2010 mL/g COD/day) than COD/N > 1000/7 (which was 12.817 mL/g COD/day). Meanwhile, the kinetic value of λ at 400/7 – 700/7 was less (0.213 – 0.315/days) than that at COD/N > 1000/7 (0.345/days). At the COD/N of 1436/7, the ammonium requirement is not fulfilled to do activity for degradation of COD content, which was a shortcoming of -980 mg/L [4]. In this study, the vinasse addition of more than 20%, the COD/N value was more than 1436/7 (Table 5). Thus, anaerobic bacteria were experiencing a shortage of ammonium more than -980 mg/L. That means COD/N ratio in fit value was good condition for anaerobic bacteria, so that the bacteria needed a short time to adapt and produce biogas in high rate.

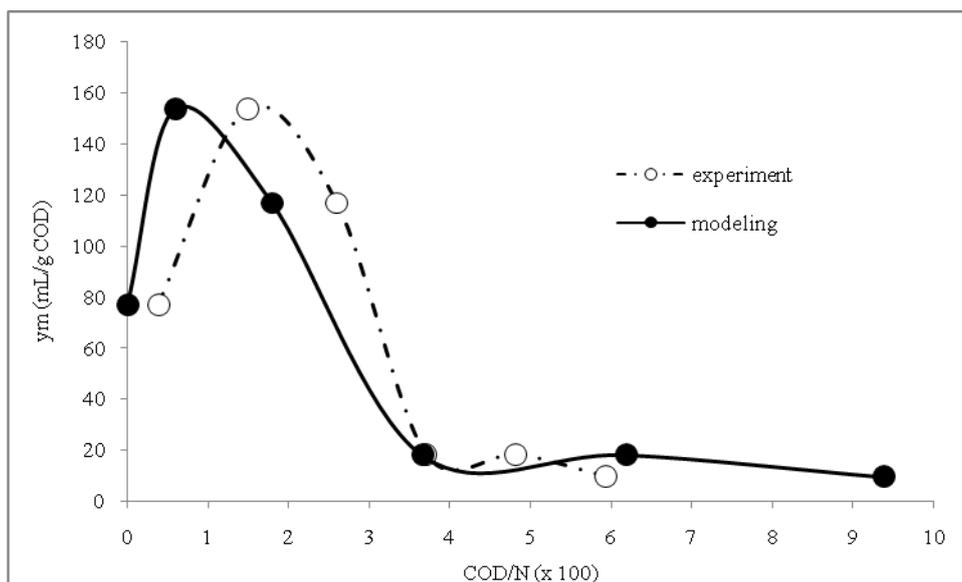


Fig. 4. Effect of COD/N on biogas production potential

Table 5. The expanded Ratkowsky model for biogas production

COD/N experiment (x100)	COD/N modeling (x 100)	y_m (mL/g COD)
0.39 {270/7}	0	77.01
1.49 {1042/7}	0.59 {414/7}	153.70
2.60 {1817/7}	1.79 {1256/7}	116.83
3.71 {2594/7}	3.66 {2560/7}	18.08
4.82 {3373/7}	6.18 {4328/7}	18.26
5.93 {4154/7}	9.38 {6565/7}	9.59
Parameters of the expanded Ratkowsky model for biogas production		
A	1.63	
(COD/N) _{min}	0.00	
B	-0.47	
(COD/N) _{max}	5.08	
R ²	0.96	
Optimal COD/N (x100)	0.59 {414/7}	

Remarks: COD/N, ratio of COD and Nitrogen content; y_m , the biogas production potential obtained through modified Gompertz equation; A,B, Ratkowsky constant; R², correlation coefficient

3.5. Development of Kinetic Model for Degradation of Nitrogen Content (KMDNC)

The biogas production rate was modeled by using modified Gompertz equation. This equation can describe biogas production with good fitting. Furthermore,

Syaichurrozi *et al.* [4] developed kinetic model of biodegradability organic materials rate (KMBOM). However, the kinetic model just predicted the degradation of COD content during fermentation process. Hence, in this study we developed the kinetic model for degradation of

nitrogen content (KMDNC) through modified Gompertz model.

From Fig.5, can be written equation of

$$\frac{Co - Ct}{Co} = \frac{yt}{ym} \tag{9}$$

$$yt = \left(\frac{Co - Ct}{Co} \right) ym \tag{10}$$

Where, Co is total COD that can be removed; Ct is COD at any time; yt is biogas yield at any time; ym is biogas yield maximum.

Modified Gompertz equation:

$$yt = ym \exp \left\{ - \exp \left[\frac{\mu \cdot \epsilon}{ym} (\lambda - t) + 1 \right] \right\} \tag{1}$$

Furthermore, Syaichurrozi *et al.* [4] used equation (9), (10), (1) to make biodegradability organics materials (COD) rate:

$$Ct = Co - \exp \left\{ - \exp \left[\frac{\mu \cdot \epsilon}{ym} (\lambda - t) + 1 \right] \right\} Co \tag{11}$$

$$\left(Co - \exp \left\{ - \exp \left[\frac{\mu \cdot \epsilon}{ym} (\lambda - t) + 1 \right] \right\} Co \right) + Ce = Co - \left(\frac{COD}{N} \right) (No - Nt) \tag{15}$$

$$\left(\frac{N}{COD} \right) \left(\left(\exp \left\{ - \exp \left[\frac{\mu \cdot \epsilon}{ym} (\lambda - t) + 1 \right] \right\} Co \right) - Ce \right) = (No - Nt) \tag{16}$$

$$Nt = No - \left(\frac{N}{COD} \right) \left(\left(\exp \left\{ - \exp \left[\frac{\mu \cdot \epsilon}{ym} (\lambda - t) + 1 \right] \right\} Co \right) - Ce \right) \tag{17}$$

Where, Nt is total nitrogen at any time (g), No is total nitrogen in influent substrates (g), (N/COD) is ratio of nitrogen and COD in influent substrates, Co is total COD that can be removed (g) (Co = Ci - Ce, Ci is influent COD (g), Ce is effluent COD (g)), ym is biogas production potential (mL/g COD), μ is maximum biogas production rate (mL/g COD.day), λ is lag phase period or minimum time to produce biogas (days), t is cumulative time for biogas production (days) and e is mathematical constant (2.718282).

Equation of (17) can be changed in simple model, with substituting equation (1) to (17)

$$Nt = No - \left(\frac{N}{COD} \right) \left(\left(\frac{yt}{ym} \right) Co - Ce \right) \tag{18}$$

Speece [33] state that 1 gram COD can be converted to 395 mL biogas at temperature of 35 °C and pressure of 1 atm. Meanwhile, this study also was conducted in the same condition (~35 °C, 1 atm). From information above, if “a” gram COD was degraded completely in the anaerobic system, which would be converted into a × 395 mL. Hence, COD that was removed or converted into biogas can be predicted with mathematical calculation: Biogas yield production (mL/g COD) × ε = Biogas yield if all of organic content can be degraded (mL/g COD).

$$Nt = (Ni/\epsilon) - \left(\frac{N}{COD} \right) \left(\left(\frac{yt}{ym} \right) \left(\frac{Ci \cdot ym}{395} \right) - \left(Ci - \frac{Ci \cdot ym}{395} \right) \right) \tag{27}$$

$$Nt = (Ni/\epsilon) - \left(\frac{N}{COD} \right) \left(\left(\frac{ci}{395} \right) (yt - 395 + ym) \right) \tag{28}$$

In the end of fermentation process, substrate still contained amount of organic materials called COD effluent (Ce), so equation (11) become:

$$Ct = \left(Co - \exp \left\{ - \exp \left[\frac{\mu \cdot \epsilon}{ym} (\lambda - t) + 1 \right] \right\} Co \right) + Ce \tag{12}$$

Speece [33] state that anaerobic bacteria required “a” g nitrogen (N) to convert “b” g COD into biogas. It means, during fermentation process, the more the biogas produced, the more the degradation of COD and nitrogen in the substrates. Hence, Fig.5 can be deduced that:

$$\frac{Co - Ct}{No - Nt} = \left(\frac{COD}{N} \right) \tag{13}$$

$$Ct = Co - \left(\frac{COD}{N} \right) (No - Nt) \tag{14}$$

Substituting equation (12) to (14). Hence we obtained equation (15), (16), (17)

$$ym \cdot \epsilon = 395 \tag{19}$$

$$\epsilon = 395/ym \tag{20}$$

Value of ε was ratio between COD influent (Ci) and COD removed (Co), ε = Ci/Co. Therefore, the value of COD removed (Co) can be found by Ci/ε

$$Co = Ci/\epsilon \tag{21}$$

$$Co = (Ci \cdot ym)/395 \tag{22}$$

Meanwhile, Co = Ci - Ce, so Ce = Ci - Co

$$Ce = Ci - Ci/\epsilon \tag{23}$$

$$Ce = Ci - (Ci \cdot ym)/395 \tag{24}$$

During fermentation, anaerobic bacteria destroyed COD content into biogas. In biogas generating process, the bacteria also consumed nitrogen source in the system. Hence, the value of Ci/Co was equal with Ni/No

$$\frac{Ci}{Co} = \frac{Ni}{No} = \epsilon \tag{25}$$

$$No = Ni/\epsilon \tag{26}$$

Thus, equation (22), (24) and (26) was substituted to equation (18). Hence we got equation (27) and (28)

Equation (28) can be used to get prediction of degradability nitrogen content during fermentation process in the digesters. The curve kinetic model of degradability nitrogen content for all variables can be seen in Fig.6. At the prediction, final nitrogen content for variable 0:100, 20:80,

40:60, 60:40, 80:20, 100:0 was 11.34, 8.80, 1.00, 1.35, 1.34, 13.72 mg respectively. Thus, %Nitrogen removal was 19.71, 37.57, 28.71, 4.15, 4.49, 2.17% respectively for all variables. This value showed that the more biogas was generated, the more nitrogen content was removed (Fig.3 and Fig.6).

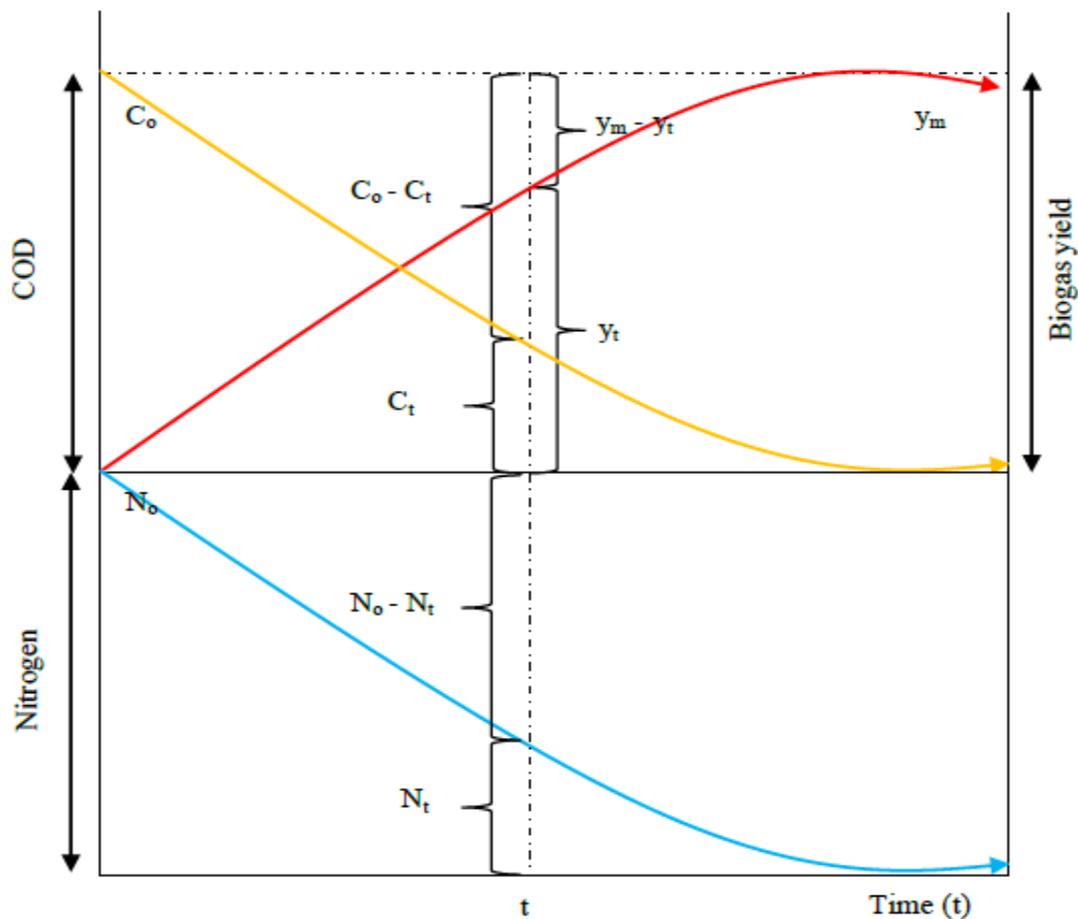


Fig. 5. Substrate transformation into biogas during anaerobic degradation

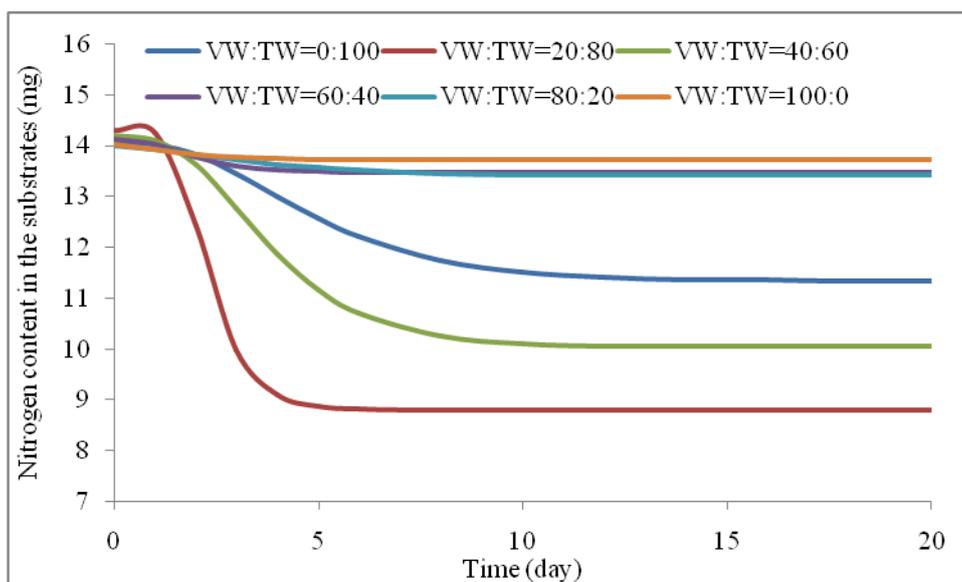


Fig. 6. Predicting kinetic model of degradability nitrogen content in the substrates

4. Conclusion

Variables of VW:TW of 0:100, 20:80, 40:60, 60:40, 80:20, 100:0 generated total biogas of 76.11, 159.01, 120.22, 19.96, 18.77, 10.64 mL/g COD respectively. The fitting error between measured and predicted biogas yield by using modified Gompertz was 1.18-9.79% and by using first order kinetic was 0.82-17.33%. The best variable of VW:TW was 20:80. The Edward model gave the best fitting ($R^2 = 0.95$) in predicting the effect of substrate concentration on biogas production. The optimal COD/N obtained through Ratkowsky model was 414/7. The development of kinetic model for degradation of nitrogen content (KMDNC) was

$$Nt = (Ni/\varepsilon) - \left(\frac{N}{COD}\right) \left(\left(\frac{ci}{395}\right) (yt - 395 + ym)\right).$$

Acknowledgements

The authors thank to LPPM Untirta and Ministry of Research, Technology and Higher Education via Hibah Dosen Pemula 2015 grant for financial support.

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