



Effect of chemical pretreatment using sulfuric acid on biogas production from water hyacinth and kinetics



Sarto Sarto^a, Raudati Hildayati^b, Iqbal Syaichurrozi^{c,*}

^a Department of Chemical Engineering, Faculty of Engineering, Universitas Gadjah Mada, Jl. Grafika No.2, Yogyakarta, 55281, Indonesia

^b Kantor Badan Penghubung Pemerintah Provinsi Kalimantan Selatan, Jl. Biliton No.9, Jakarta Pusat, Indonesia

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

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ABSTRACT

The effect of chemical pretreatment on biogas production from water hyacinth (WH) was investigated. In pretreatment process, H₂SO₄ concentration was varied to be 0, 1, 2, 3, 4, 5 %v/v and residence time was varied to be 0, 30, 45, 60, 75, 90 min. Effluents of pretreatment were used as biogas feedstock. The results showed that pretreatment changed not only the cellulose content but also the glucose, COD (Chemical Oxygen Demand) and COD/N (COD/Nitrogen) ratio. Cellulose degradation occurred to follow first-order reaction based on the power law model. The best pretreatment condition was H₂SO₄ concentration of 5% v/v with residence time of 60 min because the slurry pretreated at that condition contained COD/N of 388.2. Anaerobic digestion of the slurry produced the biggest total biogas (424.30 mL) with the highest methane content (64.38%). The pretreatment increased total biogas 131.45% compared to without pretreatment. The measured total biogas for 90 days was fitted by using modified Gompertz, Cone, First Order model in which they resulted fitting error of 0.271–9.789%, 0.032–8.743%, 3.491–5.681% respectively. In prediction using Ratkowsky and Phenomenological model, the slurry containing optimum COD/N of 393 (total biogas 434.64 mL) was obtained with pretreatment of H₂SO₄ 5%v/v for 65 min.

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1. Introduction

Water hyacinth (WH) is one of free-floating aquatic weeds having fastest growth rate in the world. In addition, it has doubling time of 7–12 days [1]. Therefore, it can thrive in water body of rivers just in short time. The presence of WH in water bodies will block the rivers, irrigation system and pump site. Beside these, the rivers will lose their water via evapotranspiration. Also, WH will hinder the movement of aquatic organisms such as fishes and reduce the dissolved oxygen level so that the aquatic organisms will die. Moreover, WH can provide a nesting refuge for diseases [2,3].

Traditionally, Indonesian people just pile and burn WH to control the WH growth. However, the method is not effective and efficient and makes a new problem which is producing smoke polluting the air. Thus, another method has to be applied. Biological treatment using anaerobic digestion (AD) is more interesting and

has some advantages compared to the traditional method. With this method, WH will be degraded with help of bacterial activities to be biogas and biofertilizer [4–6]. The biogas can be applied as energy source to produce electrical energy for rural communities or directly applied for cooking. Whereas, the biofertilizer can be applied on paddy field so that the production of paddy can increase maximally. Furthermore, this method is cheap and easy to be operated for rural people because (i) this method does not need extensive land area, (ii) the starter (source of bacteria) can be obtained from manure or active sludge, (iii) the digesters can be operated in Indonesia's environmental temperature (without energy supply for heating).

WH is a lignocellulosic substrate that is widely spread availability in Indonesia since Indonesia is a tropical country [7]. Hence, biogas production from water hyacinth is very attracting because (i) WH is non agricultural lignocellulosic plant, (ii) WH is a low-cost raw material, (iii) WH is abundantly available. Generally, lignocellulosic substrates contain cellulose, hemicellulose and lignin. According to Putra et al. [8] and Paepatung et al. [9], WH in South-East Asian countries contains dominantly cellulose so that the hemicellulose can be neglected. Cellulose is a complex polymeric

* Corresponding author.

E-mail addresses: sarto@ugm.ac.id (S. Sarto), raudati.hildayati@gmail.com (R. Hildayati), iqbalsyaichurrozi@gmail.com, iqbal_syaichurrozi@untirta.ac.id (I. Syaichurrozi).

carbohydrate that is not easy to be degraded in AD. Besides that, lignin presents as an encrusting material and serves as protective layer providing mechanical strength [4]. Lignin makes WH is more slowly convertible. Pretreatment prior to AD effectively increases the biodegradability of WH because of decomposing the cellulose into relatively readily biodegradable components and breaking down linkage between lignin and polysaccharide (cellulose) so anaerobic bacteria can access cellulose easier. Chemical pretreatments are more attractive than physical and biological pretreatments, because they are effective and inexpensive for improving the biodegradation of lignocellulosic substrates [10].

Some authors have conducted chemical pretreatment to increase biogas production from WH. Ofoefule et al. [11] used KOH 50%w/v to treat WH before AD. Biogas yield increased from 8.48 to 9.51 L/total mass of slurry. Whereas, Patil et al. [12] used NaOH 1%v/v to increase biogas yield from WH. Biogas yield through NaOH pretreatment (followed by dried and ground, biogas yield 0.31 L/g VS) was bigger than that without pretreatment (just chopped, dried and ground, biogas yield 0.281 L/g VS). Furthermore, Dharaga and Balaji [13] pretreated WH using NaOH 1%w/w to increase biogas from co-digestion poultry feces and WH. Biswas et al. [14] conducted pretreatment of WH using H₂SO₄ 2 and 5%w/v. The results showed that concentration of 5%w/v can produce higher sugar than that of 2%w/v. However, they did not investigate the effect of the pretreatment on biogas production rate. According to Song et al. [10], acid pretreatment can produced higher methane yield than alkaline pretreatment where H₂SO₄ pretreatment had methane yield of 175.6 mL/g VS and NaOH pretreatment had methane yield of 163.5 mL/g VS. Moreover, acid pretreatment using H₂SO₄ gave better results than that using HCl and CH₃COOH. Therefore, this study used H₂SO₄ pretreatment.

To the best of our knowledge, study of the effect of pretreatment using H₂SO₄ on enhanced biogas production from WH has not been studied and reported by other authors yet. In this study, concentration of H₂SO₄ and residence time was varied in range 0–5 %v/v and 0–90 min respectively. According to Singh and Bishnoi [15], the degradation of lignocellulosic materials followed the first-order reaction. However, there was no information if the cellulose degradation in WH during sulfuric acids pretreatment followed the first-order reaction or not. Therefore, in this study, we used the power law models to find the answer. Furthermore, phenomenological model based on Arrhenius equation proposed by Dong et al. [16] was used to know that conversion of cellulose depended on reaction temperature, H₂SO₄ concentration, and residence time. By this model, we could predict at what conditions the specific conversion would be reached.

The pretreated WH was used as biogas feedstock. The measured biogas production was used to build kinetic model of biogas production through several proposed models (i.e. modified Gompertz model, first order kinetic model and cone model). Then, comparison among them was done to find which the model was the best. The kinetic parameters obtained from the models could help to explain the effect of pretreatment on biogas production deeply. Furthermore, Ratkowsky model was applied to find the optimum COD/N (COD/Nitrogen) in substrate producing the most total biogas [17]. After the optimum COD/N was obtained by Ratkowsky model, the optimum condition in sulfuric acid pretreatment could be predicted using phenomenological model. In conclusion, there was nothing studies that gave information the correlation between Ratkowsky model and phenomenological model before. Hence, this study was original and has not been reported by others yet.

2. Methods

2.1. WH and inoculums

WH was obtained from water bodies located in Village of Pulu Dadi, Sub-district of Depok, District of Sleman, Yogyakarta Province, Indonesia. The chemical composition of WH was shown in Table 1. The sludge, which was used as inoculums, was an effluent obtained from biogas installation treating cow manure located in Kebun Pendidikan dan Pengembangan Pertanian Universitas Gadjah Mada, Yogyakarta Province, Indonesia. The characteristics of the sludge were shown in Table 1.

2.2. Pretreatment process

2.2.1. Preparation of materials

WH consisted of stem, leaf and root. In this study, we used stem and leaf of WH. After collected from the water bodies, the WH was washed using clean water, and then it was cut to be ± 0.5 cm. Furthermore it was dried under the sun. Then, it was reduced in size to be 60 mesh using a blender.

2.2.2. Experimental procedures

2.2.2.1. Variation of H₂SO₄ concentration (scenario 1). The WH as much as 25 g was mixed using water with ratio 1:10 (w/w) to form slurry. Then, the H₂SO₄ with purity of 96%v/v was added to the slurry to make the concentration of H₂SO₄ in slurry to be 0 (without adding H₂SO₄ 96%v/v), 1, 2, 3, 4, 5 %v/v. The technical grade H₂SO₄ obtained from LPPT (Laboratorium Penelitian dan Pengujian Terpadu) Universitas Gadjah Mada was used in this study. Pretreatment was done using Express Portable Autoclave with Model No. ST19 at temperature of 121 °C and pressure of 2.0 atm for 60 min (residence time). Chemical compositions (cellulose, glucose, COD) were analyzed during pretreatment.

2.2.2.2. Variation of residence time (scenario 2). The best H₂SO₄ concentration from scenario 1, which had the highest cellulose reduction, glucose enhancement, and COD enhancement, was used in this scenario. Furthermore, the residence time was varied in 0, 30, 45, 60, 75, 90 min. The pretreatment process was conducted with the same procedures with section 2.2.2.1.

2.3. Anaerobic digestion

2.3.1. Preparation of substrate

The slurry resulted from pretreatment scenario 2 was used as biogas feedstock. Before the slurry was brought into digesters, water was added to the slurry until the slurry had Volatile Solid

Table 1
Characteristics of fresh WH and Sludge.

Component	WH	Sludge
Water content (%)	91.44	88.97
Dry Matter (DM) (%)	8.56	11.03
Ash (%/gDM)	7.63	29.15
Volatile solid (VS) (%/gDM)	92.37	70.85
Cellulose (%/gDM)	56.9	–
Glucose (%/gDM)	0.20	–
pH	–	6.9
Lignin (%/gDM) ^a	10.41	–
COD/TKN = COD/N ^b	47.4	–

Remarks: WH, water hyacinth; COD/N, Chemical Oxygen Demand/Nitrogen ratio; TKN, Total Kjeldahl Nitrogen.

^a Lignin = Cellulose/5.464 (adapted from asian WH-Paepatung et al., 2009).

^b COD/TKN = 47.4 (adapted from asian WH-Paepatung et al., 2009).

(VS) of 4%/gTMS (Total Mass of Slurry). The pH level of slurry was adjusted to be 6–8 using NaOH 1 M because this pH range was comfortable condition for anaerobic bacteria. The active sludge was added as inoculums with ratio of slurry: sludge of 75:25 with total volume of 600 mL.

2.3.2. Experiment procedures

Erlenmeyers having volume of 1000 mL were used as anaerobic digesters. The erlenmeyers were plugged using rubbers and equipped using valve for measuring biogas. The measuring biogas was conducted by liquid displacement method using U-shaped tube manometer developed by Nakagawa et al. [18]. In this method, the plastic tube with inner diameter of 0.9 cm was nailed on plywood in a U-shaped form. The plastic tube was filled half-way with water. One end was connected to biogas line connected to digesters and the other was left open. When the pressure was zero (before measuring biogas), the water levels in the two sides of the U-shaped tube were equal. When biogas measurement, the biogas pushed the water in the tube. Hence, there was a decrease in the water level (Δh) in the tube. The volume biogas was calculated using equation (1)

$$V = \frac{1}{4} \pi \times d^2 \times \Delta h \quad (1)$$

Where:

V = biogas volume (mL)

π = mathematical constant (3.14)

d = inner diameter of plastic tube (cm)

Δh = decrease in water level in manometer (cm)

Fermentation was carried out for 90 days at room temperature (28–30 °C) and at pressure of 1 atm. Biogas volume was measured every day to know daily biogas production by U-shaped tube manometer. The daily measured biogas data also could be presented to be cumulative measured biogas production. Thus, the experimental data were used to build kinetic model of biogas production from WH. Each digester was mixed manually for 1 min per day.

2.4. Analysis

The cellulose composition in solid was determined using Chesson method [19]. Dried sample as much as 1 g (W1) was mixed with 150 mL aquadest. The mixture was refluxed in a water bath at 100 °C for 1 h. Then, it was filtered and its residue was washed using hot water (± 300 mL). After that, the residue was dried in an oven to a constant weight (W2). Furthermore, it was mixed with 150 mL H₂SO₄ 1 N and refluxed in the water bath at 100 °C for 1 h. It was filtered and washed using ± 300 mL aquadest and its residue was dried (W3). The H₂SO₄ 72% as much as 10 mL was added to soak the dried residue at room temperature for 4 h. After that, H₂SO₄ 1 N as much as 150 mL was added into the mixture. Then, it was refluxed in the water bath for 1 h. The mixture was filtered and its residue was washed using ± 400 mL aquadest. The residue was heated in the oven at 105 °C to a constant weight (W4). The cellulose composition was calculated using equation (2). Cellulose composition before and after pretreatment were determined using the Chesson method. The reduction of cellulose composition in solid was calculated using equation (3) [15]. Furthermore, the reduction of lignin composition in solid was predicted using equation (4) obtained from study results of [10] in case of pretreatment using H₂SO₄. Hence, the final lignin composition was predicted using equation (5). In mass balance concept, cellulose mass and lignin mass were determined using equations (6) and (7) respectively. The difference between initial cellulose mass and final

cellulose mass was called as degraded cellulose mass. Then, the degraded lignin mass was obtained with the same way. With mass balance, we also could predict the other products mass (besides glucose). The glucose composition was determined using Nelson-Somogyi Method [20]. Glucose yield was calculated using equation (8). The yield of other products (beside glucose) was also calculated with similar concept with equation (8). The COD concentration was determined using Open Reflux Method [21]. The level of substrate pH was measured using pH meter [4]. Water content, dry matter (DM), ash content, volatile solid (VS) were measured using procedures recommended in standard method APHA [22]. Methane composition in biogas was determined using Gas Chromatography (GC) Shimadzu GC-8A, Japan, SUS Packed Column Porapak Q, 5 m \times 3 mm I.D., 50 °C column over temperature, 170 kPa inlet pressure and FID detector.

$$\text{Cellulose composition (\%/gDM)} = \frac{W3 - W4}{W1} \times 100\% \quad (2)$$

$$C_r(\%) = \frac{C_i - C_f}{C_i} \times 100\% \quad (3)$$

$$L_r(\%) = 0.308 \times C_r(\%) - 1.137, \text{ for } C_r > 0\% \quad (4)$$

$$L_r(\%) = \frac{L_i - L_f}{L_i} \times 100\% \quad (5)$$

$$\text{Cellulose mass(g)} = \text{Cellulose composition (\%/gDM)} \times \text{total DM}/100\% \quad (6)$$

$$\text{Lignin mass(g)} = \text{Lignin composition (\%/gDM)} \times \text{total DM}/100\% \quad (7)$$

$$\text{Glucose yield(\%)} = \frac{\text{final glucose mass} - \text{initial glucose mass}}{\text{initial DM mass}} \times 100\% \quad (8)$$

Where:

C_r = reduction of cellulose composition (%)

C_i = initial cellulose composition (%/gDM)

C_f = final cellulose composition (%/gDM)

L_r = reduction of lignin composition (%)

L_i = initial lignin composition (%/gDM)

L_f = final lignin composition (%/gDM)

2.5. Kinetics

2.5.1. Kinetics on pretreatment

2.5.1.1. Determining the order reaction of cellulose degradation. The degradation of cellulose during pretreatment was modeled using power law model in batch condition shown in equation (9) [23].

$$-\frac{dCA}{dt} = k_c CA^\beta \quad (9)$$

If the value of power β of 0, 1, 2 or 3, the reaction of cellulose degradation was zero-order, first-order, second-order, and third-order reaction respectively [23].

2.5.1.1.1. For zero-order reaction. Modifying equation (9) with

$\beta = 0$ to be (10)

$$-\frac{dCA}{dt} = k_c CA^0 \quad (10)$$

Rearranging equation (10) to be (11)

$$-\frac{dCA}{dt} = k_c$$

$$dCA = -k_c dt$$

$$\int_{CA0}^{CA} dCA = -k_c \int_0^t dt$$

$$CA - CA0 = -k_c t$$

$$CA = CA0 - k_c t \quad (11)$$

Equation (11) represented straight line equation $y = b + ax$ with $y = CA$ and $x = t$. Slope of straight line (a) represented the value of $(-k_c)$ and intercept of straight line (b) represented the value of $CA0$.

2.5.1.1.2. For first-order reaction. Modifying equation (9) with $\beta = 1$ to be (12)

$$-\frac{dCA}{dt} = k_c CA^1 \quad (12)$$

Rearranging equation (12) to be (13)

$$\frac{dCA}{CA} = -k_c dt$$

$$\int_{CA0}^{CA} \frac{dCA}{CA} = -k_c \int_0^t dt$$

$$\ln\left(\frac{CA}{CA0}\right) = -k_c t$$

$$\ln\left(\frac{CA0}{CA}\right) = k_c t \quad (13)$$

Equation (13) represented straight line equation $y = ax$ with $y = \ln\left(\frac{CA0}{CA}\right)$ and $x = t$. Slope of straight line (a) represented the value of (k_c)

2.5.1.1.3. For second-order reaction. Modifying equation (9) with $\beta = 2$ to be (14)

$$-\frac{dCA}{dt} = k_c CA^2 \quad (14)$$

Rearranging equation (14) to be (15)

$$\frac{dCA}{CA^2} = -k_c dt$$

$$\int_{CA0}^{CA} \frac{dCA}{CA^2} = -k_c \int_0^t dt$$

$$\frac{1}{CA} - \frac{1}{CA0} = k_c t$$

$$\frac{1}{CA} = \frac{1}{CA0} + k_c t \quad (15)$$

Equation (15) represented straight line equation $y = b + ax$ with $y = \frac{1}{CA}$ and $x = t$. Slope of straight line (a) represented the value of (k_c) and intercept of straight line (b) represented the value of $\frac{1}{CA0}$.

2.5.1.1.4. For third-order reaction. Modifying equation (9) with $\beta = 3$ to be (16)

$$-\frac{dCA}{dt} = k_c CA^3 \quad (16)$$

Rearranging equation (16) to be (17)

$$\frac{dCA}{CA^3} = -k_c dt$$

$$\int_{CA0}^{CA} \frac{dCA}{CA^3} = -k_c \int_0^t dt$$

$$\frac{1}{2CA^2} - \frac{1}{2CA0^2} = k_c t$$

$$\frac{1}{2CA^2} = \frac{1}{2CA0^2} + k_c t \quad (17)$$

Equation (17) represented straight line equation $y = b + ax$ with $y = \frac{1}{2CA^2}$ and $x = t$. Slope of straight line (a) represented the value of (k_c) and intercept of straight line (b) represented the value of $\frac{1}{2CA0^2}$. Where:

CA = the concentration of cellulose at time (g/gDM)

$CA0$ = the initial of concentration of cellulose (g/gDM)

t = pretreatment time (minute)

β = reaction order

k_c = the cellulose degradation rate constant (zero-order = g/gDM.minute); first-order = /minute; second order = gDM/(g.minute), third order = gDM²/(g².minute))

2.5.1.2. Pretreatment severity. During pretreatment, a biomass was degraded. To map the degradation of the biomass, a severity parameter was proposed. A severity parameter was to combine the effects of some factors for evaluating the process of biomass pretreatment. Initially, Overend and Chornet [24] defined that a severity parameter was to relate the effects of temperature ($^{\circ}C$) and residence time (minutes) on biomass pretreatment assumed to follow first-order kinetics and obey the Arrhenius equation. That means, degradation of biomass depended on temperature and residence time during pretreatment. Furthermore, Chum et al. [25] modified the severity parameter with adding a chemical concentration factor (%v/v). The equation of modified severity parameter (M_0) developed by Chum et al. [25] was shown in equation (18).

$$M_0 = t \cdot C^m \cdot \exp\left[\frac{T_r - T_b}{\omega}\right] \quad (18)$$

Rearranging equation (18) to be (19)

$$\ln M_0 = \ln t + m \cdot \ln C + \left[\frac{T_r - T_b}{\omega} \right] \quad (19)$$

In M_0 was called modified severity factor (MSF). Furthermore, Dong et al. [16] proposed a phenomenological models based on Arrhenius equation to describe the conversion of biomass. The phenomenological model with MSF based on Arrhenius was shown in equation

$$y(t = 90d) = \left[A \left(\left(\frac{COD}{N} \right) - \left(\frac{COD}{N} \right)_{min} \right) \right]^2 \left[1 - \exp \left(B \left(\left(\frac{COD}{N} \right) - \left(\frac{COD}{N} \right)_{max} \right) \right) \right]^2 \quad (24)$$

(20) (adapted from Dong et al. [16]). Equation (20) showed that conversion degree of biomass was function of MSF (temperature, residence time, chemical concentration).

$$\alpha = 1 - \exp[-\exp(a \cdot MSF + b)] \quad (20)$$

Where:

M_0 = modified severity parameter

t = residence time (minutes)

C = H_2SO_4 concentration (%v/v)

T_r = reaction temperature ($^{\circ}C$)

T_b = base temperature ($100^{\circ}C$)

ω = severity constant

m = arbitrary constant

MSF = modified severity factor

α = degree of conversion

a, b = kinetic constant

2.5.2. Kinetics on anaerobic digestion

2.5.2.1. Kinetic of biogas production. The biogas production was modeled using some kinetic models, i.e. modified Gompertz model [26], Cone model [4], First order kinetic model [27]. Biogas production rate in batch mode were assumed that it had correspondence to specific growth rate of methanogenic bacteria during AD. Kinetic constants of y_m , λ , U , k_{hyd} , n , k were obtained through non-linear regression using polymath software 5.0 Educational Version. The equations of modified Gompertz model, cone model, and first order kinetic model were shown in equations (21)–(23) respectively.

$$y(t) = y_m \cdot \exp \left\{ - \exp \left[\frac{U \cdot e}{y_m} (\lambda - t) + 1 \right] \right\}, t \geq 0 \quad (21)$$

$$y(t) = \frac{y_m}{1 + (k_{hyd} \cdot t)^{-n}}, t > 0 \quad (22)$$

$$y(t) = y_m (1 - \exp(-k \cdot t)), t \geq 0 \quad (23)$$

Where:

$y(t)$ = the cumulative biogas at digestion time t days (mL)

y_m = the biogas production potential (mL)

U = the maximum biogas production rate (mL/day)

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

t = cumulative time for biogas production (days)

e = mathematical constant (2.718282)

k_{hyd} = hydrolysis rate constant (/day)

n = shape factor

k = the biogas production rate constant (/day)

2.5.2.2. Kinetic of Ratkowsky for predicting optimum COD/N. The Ratkowsky model was used to predict optimum COD/N ratio through plotting total biogas as function of COD/N ratio [17]. The model was shown in equation (24).

Where:

$y(t = 90d)$ = the measured total biogas for 90 day fermentation (mL)

A, B = Ratkowsky parameters

COD/N = Ratio of COD/Nitrogen in slurry

min, max = minimum, maximum

3. Results and discussions

3.1. Pretreatment

3.1.1. Effect of different concentrations (scenario 1)

The results of pretreatment using H_2SO_4 at variation of concentration were shown in Table 2. Variable of RU was slurry containing WH and water with ratio 1:10 (w/w) without pretreatment. Variable RC0 was slurry pretreated using autoclave for 60 min without H_2SO_4 concentration (0%v/v). RC1, RC2, RC3, RC4, RC5 were slurry pretreated using autoclave for 60 min with presence H_2SO_4 concentration of 1, 2, 3, 4, 5 %v/v respectively.

Pretreatment RC0 decreased cellulose composition from 56.9 to 35.5 %g/gDM with cellulose reduction of 37.61% (Table 2). With presence of H_2SO_4 , the value of cellulose reduction increased from 44.64 to 65.55% with increasing H_2SO_4 concentration from 1 to 5%v/v (Table 2). In this case, H_2SO_4 increased the rate of hydrolysis because the constant of hydrolysis reaction rate was directly proportional with ion H^+ as acid catalyst. Hence, the more the H_2SO_4 existed in slurry, the more the cellulose would be degraded.

While decreasing the cellulose, the more H_2SO_4 concentration (RC0 to RC5) increased the glucose composition in WH from 2.8 to 9.4%g/gDM (Table 2). The breaking down of the glycosidic bonds occurred in some steps. Proton (H^+), the acid catalyst, interacted quickly with glycosidic oxygen connecting two units of sugar. It formed conjugate acid. Furthermore, slow breakdown of C–O bond produced cation of cyclical carbonium. The cyclical carbonium was reacted with water (H_2O) quickly to result glucose and released the proton H^+ . These showed that during pretreatment, cellulose was degraded to be the more soluble compounds such as glucose.

Acid pretreatment changed the raw material properties through disruption of covalent bonds, hydrogen bonds, Van der Waals forces holding together the biomass components. Therefore, it caused the reduction of cellulose [28]. The higher the H_2SO_4 concentration, the more the covalent bonds, hydrogen bonds, Van der Waals forces could be ruined so that the more the cellulose was decomposed and converted into other soluble components (such as glucose). Fernandez-Cegri et al. [29] and Song et al. [10] stated that acid pretreatment (especially H_2SO_4) cannot degrade the lignin of raw materials significantly, maintaining the almost same compositions

Table 2
The results of Acid Pretreatment.

Scenario 1										
Code	H ₂ SO ₄ concentration (%v/v)	Residence time (minute)	Cellulose (%g/gDM)	Cellulose reduction (%)	Lignin (%g/gDM)	Lignin reduction (%)	Glucose (%g/gDM)	COD (mg/L)	VS (%g/gTMS)	VS enhancement (%)
RU	Unpretreatment	–	56.9	–	10.41	–	0.2	1781.1	nd	nd
RC0	0	60	35.5	37.61	9.32	10.45	2.8	2358.8	nd	nd
RC1	1	60	31.5	44.64	9.10	12.61	6.9	4658.3	nd	nd
RC2	2	60	26.8	52.90	8.83	15.16	7	7321.3	nd	nd
RC3	3	60	25.2	55.71	8.74	16.02	8.1	9192.7	nd	nd
RC4	4	60	21.4	62.39	8.53	18.08	9	12094.2	nd	nd
RC5	5	60	19.6	65.55	8.43	19.05	9.4	13675.2	nd	nd
Scenario 2										
Code	H ₂ SO ₄ concentration (%v/v)	Residence time (minute)	Cellulose (%g/gDM)	Cellulose reduction (%)	Lignin (%g/gDM)	Lignin reduction (%)	Glucose (%g/gDM)	COD (mg/L)	VS (%g/gTMS)	VS enhancement (%)
RU	Unpretreatment	–	56.9	–	10.41	–	0.2	1781.1	7.95	–
RT0	5	0	56.9	–	10.41	–	0.2	1781.1	7.95	–
RT30	5	30	25.1	55.89	8.74	16.08	8.8	13921.3	12.28	54.47
RT45	5	45	23.9	58.00	8.67	16.73	9.7	13875.0	12.34	55.22
RT60	5	60	19.92	64.99	8.44	18.88	9.1	14590.1	12.43	56.35
RT75	5	75	19.57	65.61	8.42	19.07	8.8	15199.3	12.53	57.61
RT90	5	90	11.9	79.09	7.99	23.22	8.4	15511.9	12.80	61.01

Calculations.

Cellulose reduction (%), see equation (3).

Lignin reduction (%), see equation (4).

Lignin composition (%g/gDM), see equation (5).

Remarks: DM, Dry Matter; COD, Chemical Oxygen Demand; VS, Volatile Solid; TMS, Total Mass of Slurry.

as that of the unpretreated case. It was similar with the results of this study. The highest H₂SO₄ concentration (5%v/v) just could decrease lignin composition from 10.41 to 8.43%g/gDM with lignin reduction of 19.05% (Table 2).

According to Jonsson and Martin [30], under acid pretreatment, cellulose was degraded to be oligosaccharides and disaccharides. After that, the oligosaccharides and disaccharides were degraded to be glucose. Furthermore, glucose could be degraded to be furfural, hydroxyl methyl furfural (HMF), levulinic acid and formic acid. Hence, hydrolysis of cellulose produced not only glucose but also other products such as oligosaccharides, disaccharides, furfural, HMF, levulinic acid and formic acid. In this study, we did not measure the other products composition. However using mass balance concept, we predicted the amount of the other products as shown in Table 3. Increasing H₂SO₄ concentration from 0 to 5%v/v, degradation of cellulose mass increased from 5.35 to 9.33 g and addition of glucose mass increased from 0.65 to 2.30 g. The mass obtained from difference between degraded cellulose mass and produced glucose mass was predicted as mass of the other products which was 4.70–7.03 g.

Furthermore, under acid pretreatment, lignin was degraded to be phenolic compounds (such as benzoic acid, cinnamic acid and phenolic compounds) [30]. Then, we predicted that the products of lignin degradation (phenolic compounds) were as much as 0.27–0.50 g. Hence, the yield of glucose, the other products, and phenolic compounds was 2.60–9.20%, 18.80–28.10%, and 1.09–1.98% respectively with increasing of H₂SO₄ concentration from 0 to 5%v/v.

After pretreatment, there was change of chemical composition in WH also generating new compounds. The change of that was expressed by COD level. Table 2 showed that COD level in RU was 1781.1 mg/L. Pretreatment in RC0–RC5 could increase COD level to be 2358.8–13675.2 mg/L. That proved that complex compound in WH changed to be simpler and more soluble compound.

From Tables 2 and 3, the best H₂SO₄ concentration was 5 %v/v because it resulted the highest reduction of cellulose composition (65.55%), the highest COD level (13675.2 mg/L), the highest final

glucose content (9.4 %g/gDM) and the highest glucose yield (9.20%). These results were in line with the results of Song et al. [10]. Song et al. [10] reported that H₂SO₄ with concentration from 0 to 4%w/w could decrease cellulose content of corn straw from 49.3 to 36.1 %g/gDM.

3.1.2. Effect of different residence time (scenario 2)

Based on scenario 1, the best H₂SO₄ concentration was 5 %v/v. This concentration was used in scenario 2 with variation of residence time (0, 30, 45, 60, 75, 90 min). The results of the pretreatment in scenario 2 could be seen in Table 2. The results showed that the cellulose composition decreased from 56.9 until 11.9 %g/gDM with increasing residence time from 0 until 90 min. The decreasing of cellulose composition was comparable with increasing of COD concentration during pretreatment. Glucose composition increased from 0.2 until 9.7 %g/gDM from 0 until 45 min, but it decreased from 9.7 until 8.4 %g/gDM at residence time more than 45 min. That phenomena showed that at residence time more than 45 min, the produced glucose was decomposed to be other products such as furfural, hydroxyl methyl furfural, levulinic acid and formic acid [30], so that glucose concentration in RT60–RT90 was less than RT45. That means, in RT60–RT90, the rate of glucose decomposition was bigger than the rate of glucose production. Hence, the optimum residence time to produce glucose was 45 min. The effect of various residence time on lignin content was also shown in Table 2. Its effect on lignin degradation was not significant. That was similar with study of Fernandez-Cegri et al. [29]. The highest lignin reduction was obtained at residence time of 90 min with value of 23.22% (Table 2). Furthermore, the effect of this pretreatment on VS was recorded in Table 2. The longer the residence time (from 0 until 90 min), the more the VS enhancement value from 0 to 61.01%.

Using mass balance concept, the other products produced from cellulose degradation were as much as 5.80–9.20 g (yield 23.20–36.80%). In other hand, glucose yield was increased from 8.60 to 9.50% when the residence time increased from 0 to 45 min. Above 45 min, the glucose yield decreased to be 8.20% at residence time of 90 min. Furthermore, the phenolic compounds were

Table 3
Mass balance and Yield.

Scenario 1													
Code	H ₂ SO ₄ concentration (%v/v)	Residence time (minute)	Cellulose mass (g)	Cellulose degradation (g)	Lignin mass (g)	Lignin degradation (g)	Glucose mass (g)	Products of cellulose degradation		^b Products of lignin degradation (g)	Yield of glucose (%)	Yield of products ^a (%)	Yield of products ^b (%)
								Glucose production (g)	^a Other products (g)				
RU	Unpretreatment	–	14.23	–	2.60	–	0.05	–	–	–	–	–	–
RC0	0	60	8.88	5.35	2.33	0.27	0.70	0.65	4.70	0.27	2.60	18.80	1.09
RC1	1	60	7.88	6.35	2.28	0.33	1.73	1.68	4.68	0.33	6.70	18.70	1.31
RC2	2	60	6.70	7.53	2.21	0.40	1.75	1.70	5.83	0.40	6.80	23.30	1.58
RC3	3	60	6.30	7.93	2.19	0.42	2.03	1.98	5.95	0.42	7.90	23.80	1.67
RC4	4	60	5.35	8.88	2.13	0.47	2.25	2.20	6.68	0.47	8.80	26.70	1.88
RC5	5	60	4.90	9.33	2.11	0.50	2.35	2.30	7.03	0.50	9.20	28.10	1.98
Scenario 2													
Code	H ₂ SO ₄ concentration (%v/v)	Residence time (minute)	Cellulose mass (g)	Cellulose degradation (g)	Lignin mass (g)	Lignin degradation (g)	Glucose mass (g)	Products of cellulose degradation		^b Products of lignin degradation (g)	Yield of glucose (%)	Yield of products ^a (%)	Yield of products ^b (%)
								Glucose production (g)	^a Other products (g)				
RU	Unpretreatment	–	14.23	–	2.60	–	0.05	–	–	–	–	–	–
RT0	0	14.23	–	–	2.60	–	0.05	–	–	–	–	–	–
RT30	5	30	6.28	7.95	2.19	0.42	2.20	2.15	5.80	0.42	8.60	23.20	1.67
RT45	5	45	5.98	8.25	2.17	0.44	2.43	2.38	5.88	0.44	9.50	23.50	1.74
RT60	5	60	4.98	9.25	2.11	0.49	2.28	2.23	7.02	0.49	8.90	28.08	1.97
RT75	5	75	4.89	9.33	2.11	0.50	2.20	2.15	7.18	0.50	8.60	28.73	1.99
RT90	5	90	2.98	11.25	2.00	0.61	2.10	2.05	9.20	0.61	8.20	36.80	2.42

Cellulose degradation (g) = initial cellulose mass (g) – final cellulose mass (g).

Lignin degradation (g) = initial lignin mass (g) – final lignin mass (g).

Glucose production (g) = final glucose mass (g) – initial glucose mass (g).

Remarks: HMF, hydroxyl methyl furfural; DM, Dry Matter; COD, Chemical Oxygen Demand; VS, Volatile Solid; TMS, Total Mass of Slurry.

^a Other products (oligosaccharides, disaccharides, furfural, HMF, levulinic acid, formic acid) (g) = cellulose degradation (g) – glucose production (g).

^b Products of lignin degradation (phenylic compounds such as benzoic acid, cinnamic acid and phenolic compounds) (g) = lignin degradation (g) Glucose yield = glucose production (g)/initial DM (g) × 100%.

produced with yield of 1.67–2.42%.

3.1.3. Determining order reaction using power law model

Either in scenario 1 or scenario 2, the cellulose concentration at the end of pretreatment was less than that at fresh condition (before pretreatment). It showed that degradation of cellulose occurred during pretreatment. The rate of cellulose degradation (k_c) can be calculated if the reaction order (β) could be known. Therefore, trying the zero-order, first-order, second-order, and third-order had to be done to find the best order giving the highest R^2 value.

In determining order reaction, the data of scenario 2 had to be used because scenario 2 gave correlation between concentrations of cellulose and time of pretreatment (0–90 min). equations ((11),

(13), (15) and (17) were used to determine the different k_c value with different R^2 value too. The results of plotting can be seen in Fig. 1. Based on Fig. 1, order reaction of zero, first, second, third gave the R^2 value of 0.816, 0.909, 0.862, 0.711 respectively. Hence, the reaction of cellulose degradation was first-order reaction with k_c value of 0.015/minute.

3.1.4. Pretreatment severity

During H_2SO_4 pretreatment, significant reduction of cellulose was observed. Therefore, the severity constants were determined by non-linear regression based on experimental data of cellulose conversion using equation (20). The calculation and results of non-linear regression was shown in Table 4. Furthermore, the plot of measured and predicted data of cellulose conversion against MSF

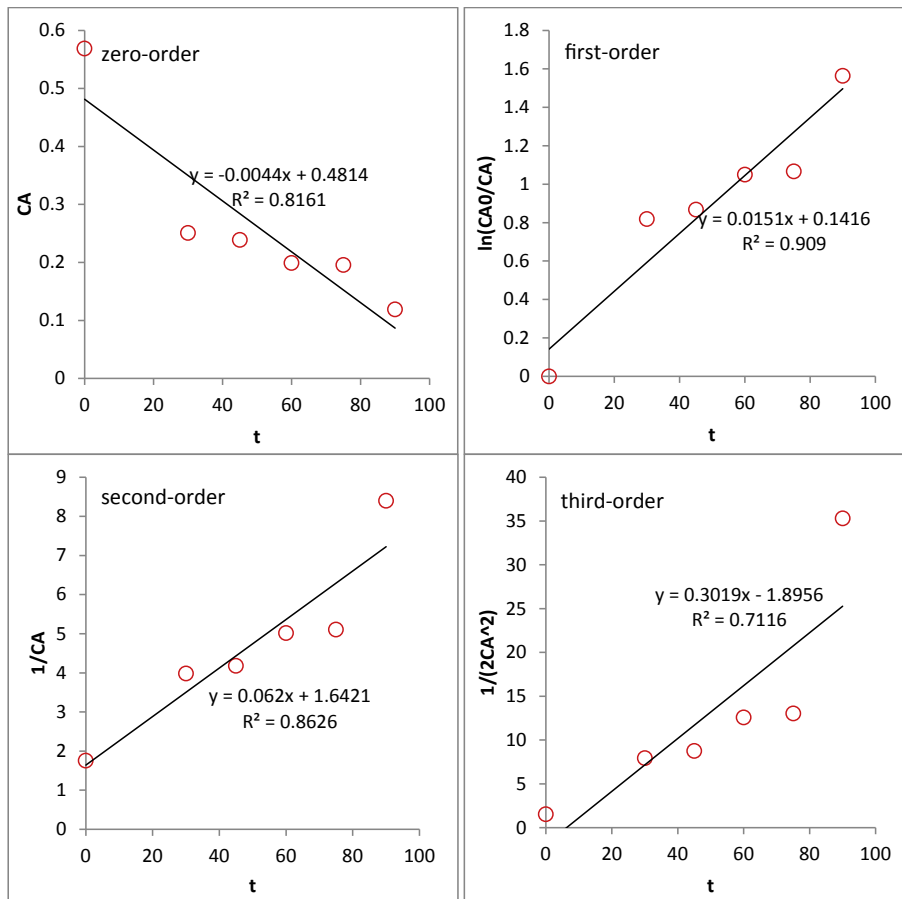


Fig. 1. Plotting zero, first, second, third-order reaction.

Table 4
Pretreatment severity calculation and results.

Calculation								Results		
	Residence time, t (minutes)	H_2SO_4 concentration, C (v/v)	Tr (°C)	Tb (°C)	$\ln(t)$	$\ln(C)$	Cellulose concentration (%/gDM)	Cellulose conversion, α	Kinetic parameters	value
Scenario 1	60	1	121	100	4.094	0.000	31.5	0.446	ω	4.280
	60	2	121	100	4.094	0.693	26.8	0.529	m	0.781
	60	3	121	100	4.094	1.099	25.2	0.557	a	0.491
	60	4	121	100	4.094	1.386	21.4	0.624	b	-4.948
	60	5	121	100	4.094	1.609	19.6	0.656	R^2	0.893
Scenario 2	30	5	121	100	3.401	1.609	25.1	0.559	rmsd	0.009
	45	5	121	100	3.807	1.609	23.9	0.580		
	60	5	121	100	4.094	1.609	19.92	0.650		
	75	5	121	100	4.317	1.609	19.57	0.656		
	90	5	121	100	4.500	1.609	11.9	0.791		

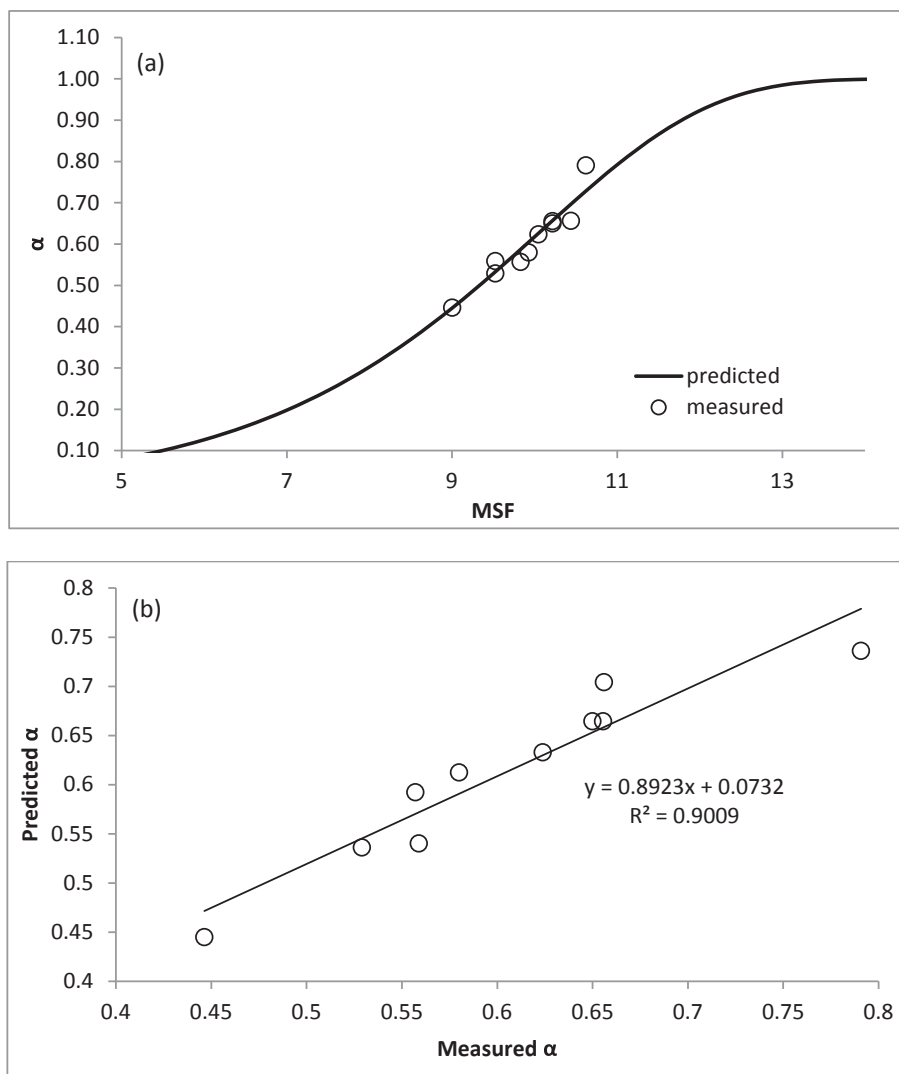


Fig. 2. Pretreatment severity (a) plotting measured α vs MSF, (b) plotting predicted vs measured cellulose conversion.

was shown in Fig. 2(a). The results showed that the model gave good degree of fitting to the measured data with high correlation coefficient ($R^2 > 0.85$). From Table 4, the ω value was much lower than 14.75 that was used as a fixed constant in conventional severity factor (SF) [15,31]. Dong et al. [16] found the value of ω was also less than 14.75. For xylan solubilization, ω was 4.11. Meanwhile for delignification, ω was 6.84. In this study, ω was 4.280 for cellulose. The different ω value was due to the different of compound that was degraded during pretreatment.

Furthermore, the value of m was 0.781 (close to 1) (Table 4). This kinetic parameter was related to reaction order with respect to acid concentration [16]. In this study, it was close to 1 that means the sulfuric acid concentration showed first-order reaction with significant effect on cellulose degradation. Silverstain et al. [31] also found the same results with this study where the value of m was 0.849 at sulfuric acid pretreatment. In other hand, Dong et al. [16] found the high value of m which was 5.95 for xylan solubilization and 3.21 for delignification when formic acid was used as solvent. That means, the different m value was due to the different of solvent used.

Fig. 2(a) showed that cellulose conversion increased when MSF increased until its value of 13.5. Moreover, at MSF of more than 13.5, the cellulose conversion was constant in value of 1. Therefore, the

perfect degradation process (conversion of 1) was obtained at MSF of 13.5. Based on Table 4, we got $MSF = \ln M_0 = \ln t + 0.781 \cdot \ln C + \frac{T_r - T_b}{4.280}$. This equation could be used to predict the particulate residence time which gave the specific cellulose conversion. For example, if the pretreatment condition was same with scenario 2 ($C = 5\%v/v$, $T_r = 121^\circ C$, $T_b = 100^\circ C$), the conversion of 0.92 obtained at MSF of 12 could be reached at $t = 342.4$ min (5.7 h). Moreover, Fig. 2(b) showed good correlation between measured and predicted conversion of cellulose expressed in straight line. That correlation had equation of $y = 0.892x + 0.073$ with good fitting of $R^2 = 0.9$.

3.2. Biogas production

In this section, the slurry from pretreatment in scenario 2, was brought to the anaerobic digester to be processed as biogas feedstock. Digester that digested slurry RT0, RT30, RT45, RT60, RT75 was called digester A, B, C, D, E respectively. The daily and cumulative biogas during AD was shown in Fig. 3. The peak value of daily biogas productions was recorded to be 13.99, 10.81, 20.35, 22.89, 21.94 mL after eight, nineteen, seventeen, nineteen, sixteen days of digester A, B, C, D, E respectively. Meanwhile, the total biogas

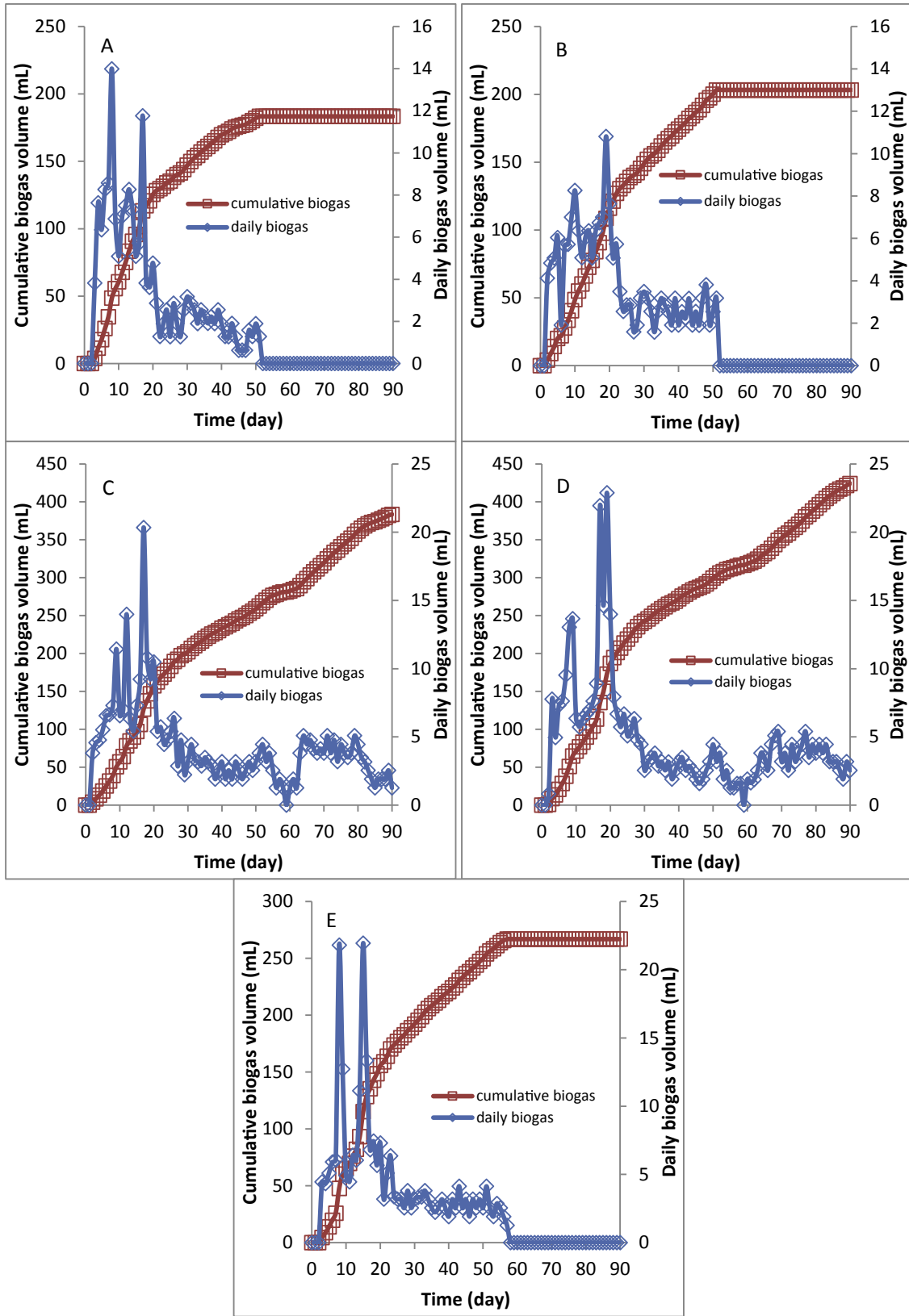


Fig. 3. The daily and cumulative biogas production for all variables.

Table 5
The Results of Anaerobic Digestion after 90 days of fermentation.

Digester	Feedstock	COD/N of feedstock	Initial VS (%g/gTMS)	Total Biogas (mL)	Increasing Total Biogas (%) Compared to Unpretreated Variable (RT0)	CH ₄ (%) in biogas
A	RT0	47.4	4	183.32	—	5.81
B	RT30	370.4	4	203.22	10.73	16.09
C	RT45	369.2	4	383.54	109.22	56.44
D	RT60	388.2	4	424.30	131.45	64.38
E	RT75	404.5	4	266.93	45.61	31.75

Note: From Table 1, unpretreated WH had COD/N of 47.4, hence RT0 contained COD/N of 47.4. The value of N was determined from $1781.1/47.4 = 37.58$ mg/L. During pretreatment N was assumed not to be changed significantly. Therefore, the COD/N of RT30, RT45, RT60, RT75 was calculated using their COD shown in Table 2 and N value of 37.58 mg/L.

Remarks: COD/N, Chemical Oxygen Demand/Nitrogen ratio; VS, Volatile Solid; CH₄, Methane.

volume was 183.32, 203.22, 383.54, 424.30, 266.93 mL from digester A, B, C, D, E respectively (Fig. 3, Table 5).

The pretreatment scenario 2 affected the difference of COD/N in the slurry. The COD/N ratios in the slurry of RT0, RT30, RT45, RT60, RT75 were 47.4, 370.4, 369.2, 388.2, 404.5 respectively (Table 5). Generally, the optimum COD/N ratio was in range of 350/7 (or 50)–1000/7 (or 143) [26]. In this study, the more the pretreatment time (from 0 until 75 min), the more the COD/N ratio of the slurry. Total biogas volume increased with increasing COD/N from 47.4 (digester A) until 388.2 (digester D). Furthermore, biogas decreased at COD/N more than it which was COD/N of 404.5 (digester E).

The value of COD/N of all variables in this study was out of the optimum range proposed by Syaichurrozi et al. [26]. From all variables, digester A had COD/N (47.4) closest to the optimum range (50–143), but it resulted the lowest total biogas. Digester A used RT0 (unpretreated WH) containing high level of cellulose. Besides that, the wall of lignin was still rigid. Also, the covalent bonds, hydrogen bonds, Van der Waals forces were still strong. Thus, digester A produced total biogas in little amount. The other variables (digester B, C, D, E) produced more total biogas than digester A. Digester E treated slurry RT75 containing the lowest cellulose level. During pretreatment, cellulose in RT75 was converted to the soluble compounds that were available to anaerobic bacteria. However, digester E resulted less total biogas than digester C and D. Digester E might contain too high COD/N ratio so that volatile fatty acids (VFAs) was easy to be produced during fermentation. Accumulation of VFAs in large amount decreased the substrate pH sharply so that bacterial activity was disturbed. Besides that, phenolic compounds might be formed by hydrolysis of lignin [30,32,33]. Although the degradation of lignin was insignificant in sulfuric acid pretreatment, the phenolic compound still could be produced during hydrolysis. The more the residence time, the more the lignin was degraded (Table 2) so that potential of phenol formation was bigger (Table 3). The other products such as furfural and HMF in large amount had also inhibitory effect on anaerobic digestion [34]. Table 3 showed that RT75 min might generated larger furfural and HMF than RT0–RT60. Therefore, slurry RT75 might contain the highest inhibitory compounds (phenolic, furfural, HMF compounds) of all slurry. These compounds were toxic and hampered the bacterial growth. Thus, biogas production was low.

Digester D resulted the highest total biogas (424.30 mL). This value was 131.45% bigger than biogas from unpretreated slurry (RT0). Slurry RT60 contained COD/N of 388.2. This COD/N ratio was the best for all variables although this value was out from optimum range proposed by Syaichurrozi et al. [26]. Syaichurrozi [4] reported that the optimum ratio of carbon/nitrogen in anaerobic digestion depended on organic materials used as feedstock. Syaichurrozi et al. [26] used vinasse, and this study used WH. Furthermore, Syaichurrozi et al. [17] stated that the best COD/N for digesting co-digestion of vinasse and tofu-processing wastewater was 1042/7

(or 149). Digester D not only produced the highest total biogas but also had biogas with the highest methane composition which was 64.38%. According to Table 5, increasing COD/N from 47.4 until 388.2 could increase the methane composition from 5.81% until 64.38%. Then, at COD/N higher than that, the methane composition decreased. That was correlated with the bacterial activity in the system. Digester D gave the comfortable condition with balance nutrients for anaerobic bacteria.

In this study, we also measured the effect of biogas production on VS removal. After fermentation time of 55 days, we measured the VS content in slurry where digester A, B, C, D, E had slurry VS of 1.92, 1.77, 1.47, 1.26, 1.50 %g/gTMS. Therefore the VS removal for digester A, B, C, D, E was 52.00, 55.88, 63.25, 68.63, 62.50% respectively. Total biogas after fermentation time of 55 days was 183.32, 203.22, 276.72, 312.08, 263.75 mL (Fig. 3). We chose fermentation time of 55 days, because there were two digesters (digester A and B) that did not produce biogas again. Based on data above, we concluded that the more the biogas was formed, the more the VS was removed. This correlation was in line with study of Syaichurrozi [4]. During anaerobic digestion, VS was converted to be biogas. The relationship between total biogas and VS removal at fermentation time of 55 days could be obtained by plotting the value of total biogas (mL) against the value of VS removal (%) to get straight line equation $y = 8.105x - 242.2$ ($R^2 = 0.99$), where y = total biogas (mL) and x = VS removal (%). With this equation, we predicted VS removal at the end of fermentation (90 days) in digester C, D, E. Total biogas at the end of fermentation in these digesters was 383.54, 424.30, 266.93 mL (Table 5). Therefore, final VS and VS removal after 90 days in digester C, D, E was predicted to be 0.91, 0.71, 1.49 %g/gTMS and 77.20, 82.23, 62.82% respectively.

3.3. Kinetic model of biogas production

The kinetic parameters (y_m , λ , U , k_{hyd} , n , k) in modified Gompertz, Cone and First order model were determined based on the best fit of the studied models and the results were summarized in Table 6. By plotting measured and predicted data, we got Fig. 4.

3.3.1. Using Modified Gompertz model

From Table 6, digester B–E (COD/N = 370.4–404.5) had more value of y_m than digester A (COD/N = 47.4). That means COD/N ratio of 370.4–404.5 generated the maximum biogas volume in larger amount (205.322–394.319 mL) than COD/N ratio of 47.4 (182.854 mL). That was due to anaerobic bacteria in the good conditions supported by the feedstock of digester B–E (RT30–RT75). Feedstock of RT30–RT75 was slurry that was pretreated using H₂SO₄ 5% v/v at residence time of 30–75 min. Pretreatment broke the lignin wall, converted cellulose to be the more soluble compounds (glucose), and increased the level of COD. Hence, biogas resulted from them was more than that from unpretreated slurry (digester A). Specifically, the digester D resulted more total biogas

Table 6
Results from using Modified Gompertz, Cone, First Order Kinetic Model.

	Digester				
	A	B	C	D	E
Modified Gompertz Model					
λ (days)	1.503	2.563	0.000	0.000	2.164
μ (mL/d)	6.671	6.170	5.730	7.227	7.868
R^2	0.991	0.995	0.968	0.964	0.989
y_m (mL)	182.854	205.322	380.570	394.319	267.406
Predicted biogas volume (mL)-90 d	182.823	204.859	357.438	382.765	266.754
Measured biogas volume (mL)-90 d	183.32	203.22	383.54	424.30	266.93
Difference between measured and predicted biogas volume (fitting error) (%)	0.271	0.807	6.806	9.789	0.066
Average fitting error (%)	3.548				
Cone Model					
k_{hyd} (/day)	0.068	0.051	0.024	0.026	0.051
n	1.909	1.999	1.278	1.255	1.883
R^2	0.993	0.994	0.980	0.982	0.994
y_m (mL)	189.098	219.563	481.073	536.833	289.511
Predicted biogas volume (mL)-90 d	183.378	209.668	350.009	397.590	273.816
Measured biogas volume (mL)-90 d	183.32	203.22	383.54	424.30	266.93
Difference between measured and predicted biogas volume (fitting error) (%)	0.032	3.173	8.743	6.295	2.580
Average fitting error (%)	4.164				
First-Order Kinetic Model					
k (/day)	0.048	0.035	0.017	0.021	0.035
R^2	0.981	0.977	0.985	0.979	0.979
y_m (mL)	192.473	224.033	476.324	472.972	293.037
Predicted biogas volume (mL)-90 d	189.863	214.766	370.151	401.285	280.285
Measured biogas volume (mL)-90 d	183.32	203.22	383.54	424.30	266.93
Difference between measured and predicted biogas volume (fitting error) (%)	3.569	5.681	3.491	5.424	5.003
Average fitting error (%)	4.634				

Remarks: y_m , 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; R^2 , correlation coefficient.

than the others. It was caused by the chemical properties of RT60 that were suitable for bacterial activities.

Furthermore, the value of λ presented the time required by anaerobic bacteria to adapt in the slurry before producing biogas [35,36]. Thus, the variable having low value of λ indicated that the bacteria needed a short time to produce biogas first. Based on that, bacteria in slurry of RT45 (digester C) and RT60 (digester D) needed the least time to adapt which was 0.000 days. Whereas, bacteria in slurry of RT0 (digester A), RT30 (digester B), RT75 (digester E) needed the longer time than that in digester C and D. Slurry of RT0 and RT30 still contained high cellulose so that the bacteria could not degrade them easily, finally the bacteria needed the longer time to produce the first biogas. Slurry of RT45 and RT60 was easily degraded, because it contained simple and soluble compounds. Slurry of RT75 contained the least cellulose content but it had high value of λ . The very high COD/N ratio and inhibitory compounds (phenolic, furfural, HMF compounds) in RT75 might be caused toxic for bacteria.

3.3.2. Using cone model

The predicted maximum biogas volume (y_m) of digester B-E was larger than that of digester A using unpretreated slurry (RT0). The largest value of y_m was obtained in digester D (COD/N = 388.2). The k_{hyd} indicated the hydrolysis rate of slurry. Generally, the more the carbon/nitrogen ratio of biogas feedstock, the higher the value of k_{hyd} [4]. Digester C, D, and E had the k_{hyd} of 0.024, 0.026, and 0.051/day. Digester E had the most COD/N, so that its k_{hyd} was higher than C and D. In other hand, the digester A had the most k_{hyd} value but it used slurry of RT0 (the lowest COD/N). That phenomenon was caused by the high cellulose content in RT0 so that hydrolytic bacteria could use it easily.

3.3.3. Using first order kinetic model

Digester of B-E had y_m value of 224.033–476.324 mL. Meanwhile digester A had y_m value of 192.473 mL. Hence, the slurry that

was pretreated (RT30-RT75) was good substrate for bacteria, especially RT45 (digester C) and RT60 (digester D). From Table 6, digester A (using slurry of RT0, unpretreated WH) had the most k value (0.048/day) of all variables. Meanwhile digester B-E (using slurry of RT30-RT75) had the less k value (0.017–0.035/day). Generally, the more value of y_m , the value of k in first order kinetic model [4]. However, in this study, digester A having the lowest value of y_m had the highest value of k . This phenomenon was similar with the study of Budiyo et al. [36]. This phenomenon occurred when bacteria produced biogas just for the least period of fermentation time. From Fig. 2, biogas production was stop at time fermentation of 51day for digester A, in contrary it from other digester was stop at more than 51 day.

3.3.4. Comparison the modified Gompertz, cone, and first order kinetic model

The difference between the measured and predicted biogas volume for 90 days observed in modified Gompertz model was 0.271–9.789% (average 3.548%), in Cone model was 0.032–8.743% (average 4.164%), in First order kinetic model was 3.491–5.424% (average 4.634%) (Table 6). Clearly, all of proposed models fitted the actual evolution of biogas production successfully because they gave fitting error less than 10%. Their fitting error value was also strongly verified by their high R^2 which was 0.964–0.995 for modified Gompertz model, 0.980–0.994 for Cone model and 0.977–0.985 for first order kinetic model. According to Syaichurrozi [4], the first order kinetic had the good fitting if biogas could be produced at the first of fermentation (short time of adaptation). In this study, biogas was produced in the first of fermentation time and the value of λ was just 0.000–2.563 days. Hence, the first order kinetic was suitable to be applied in this case. The COD/N for digester B-E was very high. The COD/N was similar with C/N. Syaichurrozi et al. [4] stated that the more C/N of substrates, the easier the substrate degraded to be biogas so that the value of λ was short. Furthermore, carbon could express the carbohydrate and

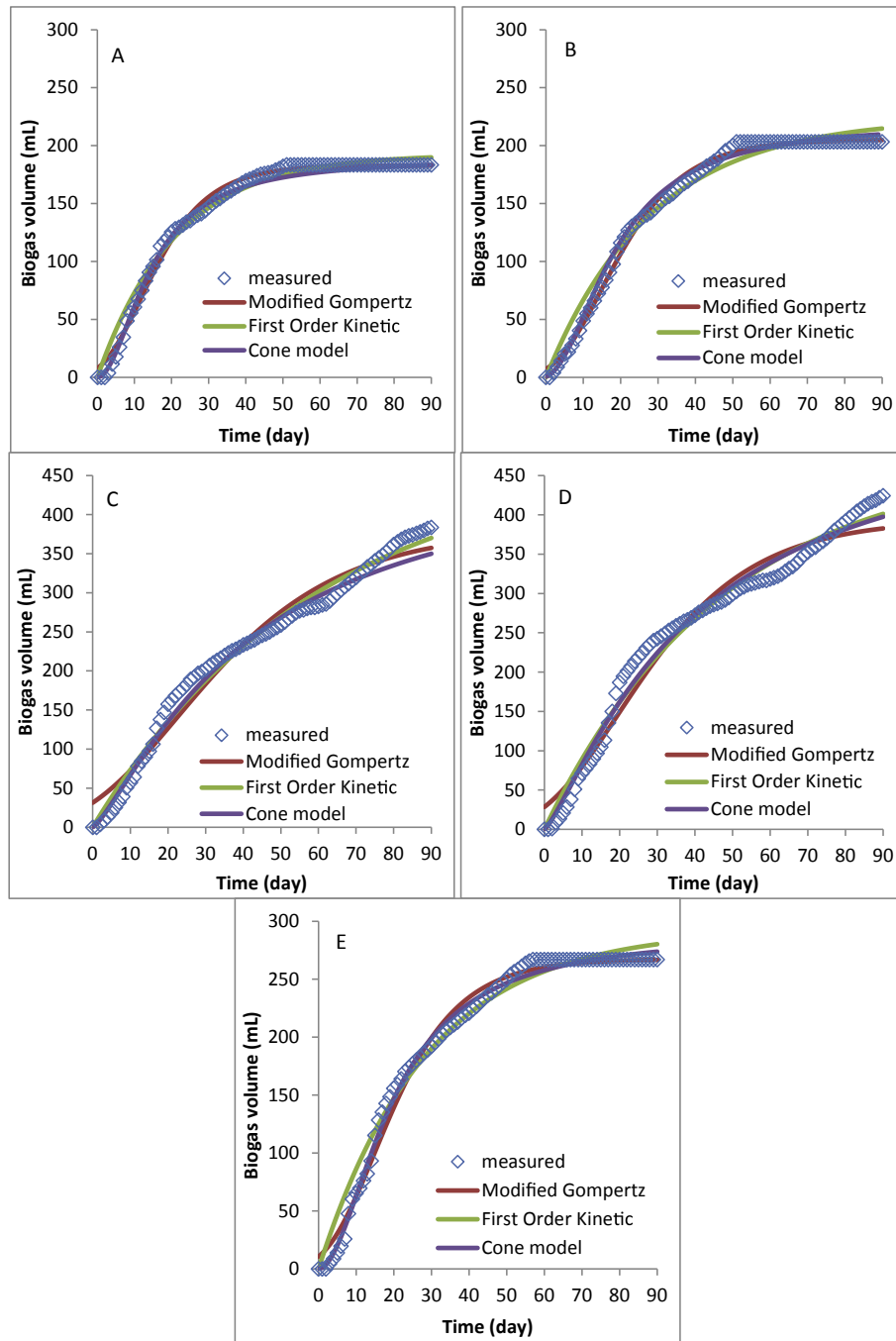


Fig. 4. Comparison of Measured Data and Predicted Data obtained from Modified Gompertz, Cone and First Order Kinetic Model for all Variables.

nitrogen could express the protein. Therefore, if substrates had high value of carbohydrate/protein, it means the substrates had high value of COD/N ratio or C/N ratio. Substrate containing high carbohydrate needed the shorter time to be degraded than substrate containing high protein [17]. Meanwhile, digester A had low COD/N but it still had short lag time (low λ). It was caused by the low lignin content of WH used in this research (Table 1). Hence, first order kinetic was also available to be applied on biogas produced from digester A.

Comparison between this study and other studies was shown in Table 7. The results of this study were similar with other studies. Syaichurrozi et al. [35] also reported that modified Gompertz

(fitting error 0.316–3.254%) and Cone model (0.193–2.809%) could be used to predict biogas production from tofu wastewater. Furthermore, biogas production from Co-digestion of waste activated sludge and *Egeria dense* (E.d.) was also successfully simulated using modified Gompertz (fitting error 4.4–7.3%), Cone (1.0–2.8%) and First order kinetic (4.0–7.1%) [36]. Beside these, Budiyo et al. [27] stated that modified Gompertz (fitting error 0.76–3.14%) and First order kinetic (1.54–7.5%) gave good fitting on biogas production from vinasse because biogas was generated at the first time of fermentation (λ of 0–2.24 days). The vinasse, WAS and E.d. contained high carbohydrate and low protein compositions. Hence, digesting the substrates produced biogas easily that means biogas

Table 7

Comparison between this result and other results in modified Gompertz, first order kinetic and Cone model to predict biogas yield.

Substrate	Difference between measured and predicted biogas (%)			References
	Modified Gompertz	First Order Kinetic	Cone Model	
Tofu wastewater	0.316–3.254	–	0.193–2.809	[35]
Co-digestion of waste activated sludge and <i>Egeria dense</i>	4.4–7.3	4.0–7.1	1.0–2.8	[36]
Chicken manure	–	2.77–70.88	0.54–9.62	[38]
Rabbit manure	–	0.22–3.06	6.74–8.98	[38]
Brewery grain waste	3.2	19.5	–	[39]
Bread waste	1.6	9.2	–	[39]
Pacific saury fish waste	0.7	13.6	–	[39]
Mackerel fish waste	6.1	29.8	–	[39]
Apple waste	2.5	12	–	[40]
Swine manure	1.9–2.7	5.3–9.8	–	[40]
Co-digestion of apple waste and swine manure	1.3–3.4	4.6–18.1	–	[40]
Vinasse	0.76–3.14	1.54–7.5	–	[27]
Co-digestion of pig manure and dewatered sewage sludge	0.0–3.7	1.5–14.2	–	[37]
Co-digestion vinasse and tofu-processing wastewater	1.18–9.79	0.82–17.33	–	[17]
Co-digestion of <i>Salvinia molesta</i> and rice straw	0.96–6.45	1.97–15.25	0.14–3.52	[4]
Water hyacinth	0.271–9.789	0.032–8.743	3.491–5.424	This study

was formed in first time of fermentation. In conclusion, biogas produced from these substrates could be predicted using the three models.

In other hand, some authors reported the different results (Table 7). Zhang et al. [37] declared that biogas production from AD of pig manure and dewatered sewage sludge could not be predicted well by the first order kinetic model with error of 1.5–14.2%. In other hand, the modified Gompertz resulted the good fitting with error of 0.0–3.7%. Liu et al. [38] reported that simulation of biogas production from chicken manure and rabbit manure by using cone model resulted fitting error below 10%. In Cone model equation, there was the shape factor “n”, so that cone model had high flexibility in modeling various pattern of measured cumulative biogas production. However, first order kinetic could not predict the biogas production from chicken manure well (fitting error up to 70.88%). Biogas from fish wastes (such as brewery grain waste, bread waste, pacific saury fish waste, mackerel fish waste) could be predicted using modified Gompertz model with low fitting error, however that could not be predicted using first order kinetic [39]. These was caused by that the substrates used by Zhang et al. [37], Liu et al. [38], Kafle et al. [39] contained more protein compared to carbohydrate. Degradation of substrates containing high protein needed longer time, so that first order kinetic was not suitable in their case. Furthermore, Kafle and Kim [40], Syaichurrozi et al. [17], Syaichurrozi [4] also found the same conclusion with Kafle et al. [39].

3.4. Kinetic model of Ratkowsky

By plotting measured data and predicted data obtained from Ratkowsky model, the kinetic of COD/N effect on biogas production was obtained and shown in Fig. 5. This model just used total biogas from digester B, C, D, E. Digester A was not included because it produced biogas with very low methane content. Fig. 5(a) showed the results of predicted total biogas from four points of the COD/N in this study (370.4 (digester B), 369.2 (digester C), 388.2 (digester D), 404.5 (digester E)). Non-linear regression was used to determine the kinetic parameter value. The value of kinetic parameters was $A = 0.223$, $B = 0.145$, $COD/N_{min} = 292.959$, $COD/N_{max} = 411.799$. Furthermore, we made a graph (Fig. 5(b)) showing the correlation between predicted total biogas and COD/N from $COD/N = 292$ until $COD/N = 412$. The more the COD/N level from COD/N_{min} to COD/N_{opt} , the more the total biogas was obtained but further increasing COD/N level from COD/N_{opt} to COD/N_{max}

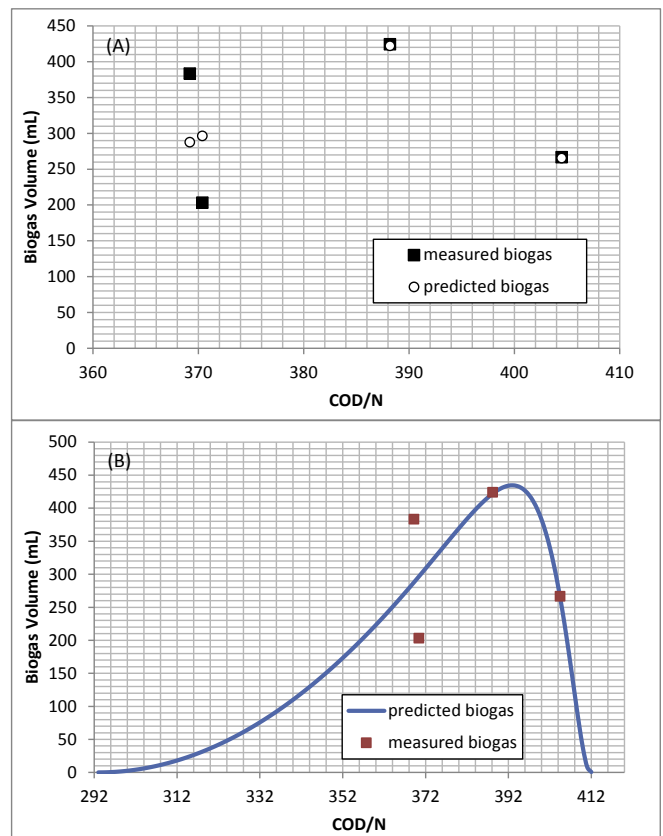


Fig. 5. Result of Ratkowsky model (a) fitting between biogas (measured and predicted) vs experimental COD/N, (b) predicted biogas production as function of COD/N.

decreased the total biogas. The predicted COD/N_{opt} was 393 which produced predicted total biogas of 434.64 mL.

Based on Tables 2 and 5, the longer the residence time, the more the COD concentration and the more COD/N ratio in the slurry. The relationship between residence time and COD/N ratio in slurry could be obtained by plotting the value of residence time (minutes) against the value of COD/N ratio to get straight line equation $y = 0.808x + 340.6$ ($R^2 = 0.88$), where $y = COD/N$ and $x =$ residence time (minutes). With the equation, we could predict the residence time that changed the COD/N ratio in slurry to be 393 (COD/N_{opt}).

From calculation, the residence time to get the COD/ N_{opt} was 64.85 min \approx 65 min. Therefore, in prediction, the optimum condition of pretreatment to produce biogas maximally (434.64 mL) was H_2SO_4 pretreatment using concentration of 5%v/v during 65 min. Furthermore, by using equation (20) and its kinetics parameters value (shown in Table 4), pretreatment at the optimum condition resulted slurry with cellulose content of 18.29 %g/gDM (cellulose conversion of 0.6785).

4. Conclusion

At scenario 1, the H_2SO_4 concentration was varied to be 0, 1, 2, 3, 4, 5 %v/v and at scenario 2, residence time was varied to be 0, 30, 45, 60, 75, 90 min. Cellulose contained in WH after pretreatment at scenario 1 was 35.5, 31.5, 26.8, 25.2, 21.4, 19.6 %g/gDM using H_2SO_4 concentration of 0, 1, 2, 3, 4, 5 %v/v respectively. The best H_2SO_4 concentration was 5%v/v. Furthermore, cellulose contained in WH after pretreatment at scenario 2 was 56.9, 25.1, 23.9, 19.92, 19.57, 11.9 %g/gDM using residence time of 0, 30, 45, 60, 75, 90 min at fixed H_2SO_4 concentration of 5%v/v. Based on the power law model, degradation of cellulose occurred following first-order reaction. The phenomenological model with MSF based on Arrhenius showed that conversion of cellulose depended on temperature, residence time and sulfuric acid concentration. Slurry that was pretreated using H_2SO_4 concentration of 5%v/v and residence time of 60 min resulted the highest total biogas (424.30 mL) and the highest methane content (64.38%). Simulation using modified Gompertz, Cone, and First Order model fitted the measured biogas production successfully with low error of 0.271–9.789%, 0.032–8.743%, and 3.491–5.681% respectively. The three proposed models were suitable to be used in this case because of its low fitting error (less than 10%). Furthermore, based on Ratkowsky model, the predicted optimum COD/N ratio was 393 and it could produce total biogas of 434.64 mL. By using the phenomenological model, the optimum condition of pretreatment resulting slurry with COD/N of 393 was H_2SO_4 concentration of 5%v/v, temperature of 121 °C, residence time of 65 min.

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