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Biohydrogen and Biomethane Production from Acid Pretreated Water Hyacinth and Kinetics

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Abstract

In this study, several ratios of water hyacinth to acid solution were varied from 1:10, 1:20, 1:30, and 1:40, in order to find the optimum ratio for biohydrogen and biomethane production. The pretreated water hyacinth was used as substrates to produce hydrogen in the first stage anaerobic digestion. Then the residue from hydrogen production was used as substrate for methane production in the second stage. The results showed that cumulative hydrogen production slightly increased with the substrate to acid solution (2 % v/v H_2SO_4) ratio. The highest cumulative hydrogen produced (77.47 mL with 7.75 mL H_2/gVS) at 1:40 substrate to acid solution, however the highest hydrogen content obtained at 1:20 (40.1 %). Hydrogen could not be produced from water hyacinth without acid pretreatment, only 4 mL hydrogen was detected from control case. In the second stage, methane was generated from the residue of first stage fermentation and found that the highest cumulative methane was obtained from 1:30 substrate to acid solution ratio. The methane content was not significantly different for control (53.68 %), 1:10 (54.45 %), 1:20 (53.46 %), and 1:30 (52.10 %) cases. The modified Gompertz, modified Logistic, and Cone model were best fit models for hydrogen production since the fermentation needed lag phase time before the hydrogen can be generated. The calculated lag phase time from modified Gompertz model increased from 0.0531 to 0.9775 day, and those from modified Logistic model increased from 0.3867 to 1.0305 day. On the other hand, the Cone model, first order model, and Transference function agreed very well with the second stage methane production data where lag phase time was nearly zero.

Keywords: Biohydrogen, Biomethane, Water hyacinth, Anaerobic digestion, Kinetics

Introduction

Water hyacinth is a free floating aquatic macrophyte with fast spread and crowded growth abilities. It can rapidly grow to a density as high as 60 kg/m^2 [1]. The annual average growth rate is about 50 kg dry weight/m^2 [1]. With these abilities, it creates serious problems worldwide such as destruction of eco systems, blocking the water ways, irrigation, and as a mosquito breeding place, etc. Thailand also encountered these problems from fast spreading of water hyacinth. Water hyacinth was first brought from Indonesia to Thailand in 1901 and caused several problems ever since. There was a law on controlling the growth of water hyacinths in 1913. Unfortunately, this law has been repealed in 2003 since it did not solve the problems. In the year 2016, the estimated amount of water hyacinth in the main river basins in Thailand was about 8.2 million tons [2]. Removal of water hyacinth requires high cost and labor. Thai government spent more than 10 million bahts each year to remove water hyacinth from water surface [2].

Several efforts including physical, chemical and biological methods have been tried for control and eradication of water hyacinth. But, none of these methods have been proved to be effective for permanently control of this weed. Exploit of water hyacinth is an alternative strategy for control, and limiting its spread. In Thailand, making use of water hyacinth as raw materials for handicrafts, such as handbags, hammocks, baskets, slippers, fruit trays, etc., have been developed for many years. Usage as fertilizers, animal feed stocks, and water purification medium have also been found in Thailand. Water hyacinth can also be used as solid fuel and found to have energy content of 3,956 kcal per kilogram [2]. However direct burning of water hyacinth creates air pollution problems like CO, and fine particles emission. Therefore, converting the weed biomass into hydrogen and biogas is a favorable approach for fuel production and is a better solution to control this weed.

Water hyacinth composes of 17 - 20 % cellulose, 22 - 45 % hemicelluloses, and 1 - 10 % lignin as the major component depending on its growth environment [3]. In addition, water hyacinth also contains some essential nutrients such as 1 - 3 % of nitrogen, 0.5 % of total phosphorus, and the C/N ratio of 15 - 25 [3]. These make it possible for anaerobic digestion to generate biohydrogen and biomethane. Like other lignocellulosic biomasses, pretreatment is required to make the cellulose and hemicellulose accessible for microorganism to convert them to pyruvate and finally biohydrogen and biomethane. Pretreatment facilitates the hydrogen production by degradation of cellulose crystalline structure, and reducing degree of polymerization.

Several pretreatments are available including physical, chemical, physicochemical, biological, and hybrid methods [4]. Many researchers have reported the pretreatment methods of water hyacinth such as acid [5], alkali [6], biological [6], heat treatment [7], microwave [6,7], thermo-chemical conversion [6] and microwave assisted alkali [6]. Each pretreatment method has its own advantages and disadvantages. However, some methods require sophisticated equipment, high energy consumption, and high cost. In order to lower the cost of biohydrogen production, simple and practical pretreatment methods, i.e. chemical, heat, and combined heat/chemical treatment methods, have been examined in our previous work [8].

Cao *et al.* [9] pretreated corn stover with 0.25 - 4 % v/v H_2SO_4 for 30 - 180 min in an autoclave at 121 °C and reported a maximum hydrogen yield of 3373 mL/L at 2.13 % H_2SO_4 and 105 min treatment time. Fungkum and Ruengsang [10] varied the concentration of H_2SO_4 from 0.25 - 5 % v/v and reported a hydrogen yield of 0.84 mol/mol sugar at 1 % H_2SO_4 as optimum condition. A maximum hydrogen production of 127.6 mmol H_2/L was obtained from water hyacinth pretreated with 1.31 % (v/v) H_2SO_4 [5].

In Jarusiripot *et al.* [8], water hyacinth was pretreated with 5 different methods: 1) heating at 100 °C for 30 min to 3 h, 2) chemical pretreatment with 1 - 3 % sulfuric acid, 3) alkaline pretreatment with 1 - 3 % sodium hydroxide, 4) combination of 2 % sulfuric acid and heating, and 5) combination of 2 % sodium hydroxide with heating. The results showed that water hyacinth pretreated with 2 % sulfuric acid combined with heating at 100 °C for 60 min and 2 % sodium hydroxide combined with heating at 100 °C for 60 min gave higher soluble COD of 6510 and 6992 mg/L, respectively. However, the highest hydrogen production was obtained from water hyacinth pretreated with combined acid/heat. The alkali/heat pretreatment not only produced higher soluble COD, but also produced higher VFA. Which in turns inhibited hydrogen production in the fermentation process [8].

Therefore, this research will continue to use combined acid/heat pretreatment method to solubilize water hyacinth before hydrogen production by anaerobic digestion. In this study, several ratios of water hyacinths to acid solution (g dry weight/mL acid solution) were varied from 1:10, 1:20, 1:30, and 1:40, in order to find the optimum ratio for the solubilization of water hyacinth. The pretreated water hyacinths were used as substrates to produce hydrogen in the first stage anaerobic digestion. Then the residue from hydrogen production was used as the substrate for methane production in the second stage. All biogas from the first and second stage were collected. The COD balance and the energy conversion were also compared.

Materials and methods

Water hyacinth and inoculum

Water hyacinth was harvested from a natural canal in Nakhon-Pathom province, Thailand, and used as substrate in all experiments. The collected water hyacinth was washed with tap water to remove soil and dirt, then chopped into 10 - 15 cm long, and dried at 105 °C until the weight is constant. The dried water hyacinth was ground to powder with particle size less than 2.00 mm, and kept in a zip-lock plastic bag to prevent moisture prior to use in the experiments. **Table 1** shows the characterized physicochemical properties of water hyacinth.

Inoculum for hydrogen and methane production used the sludge obtained from the upflowed anaerobic sludge blanket reactor (UASB), wastewater treatment plant of Choheng Rice Vermicelli Factory Co., Ltd., Sampran, Nakhon-Pathom province, Thailand. The obtained sludge was washed with distilled water for 3 times, left it settled and then removed excess water out. Part of sludge that used as inoculum for methane production was kept in the refrigerator at 4 °C. The rest was heated at 100 °C in an oven for 2 h in order to inactivate the methanogens and used as inoculum for hydrogen production.

Pretreatment process

In Jarusiripot *et al.* [8], the results showed that pretreatment of water hyacinth with 2 % (v/v) sulfuric acid combined with heat provided highest soluble COD and produced highest hydrogen yield. In this study, the same pretreatment method was used with varying the ratio of water hyacinth total volatile solid (g TVS) to volume (mL) of 2 % sulfuric acid solution. In each experiment, 10 g TVS of dried water hyacinth was mixed with 2 % sulfuric acid solution with ratio 1:10, 1:20, 1:30, and 1:40 in a 1000 mL digestion bottle. The slurry was heated in a 100 °C water bath for 60 min. Then, it was allowed to cool at room temperature prior to use as substrate. All chemicals used in all experiments were AR grade.

Hydrogen production

The slurry obtained from pretreatment process on each condition was used as substrate for hydrogen production in a 1000 mL digestion bottle. The pretreated sludge was added into the digester approximately 5 g total volatile solid (TVS) as the inoculum. The ratio of the substrate to inoculum was 2:1 in all conditions. The slurry pH was adjusted to 5.5 using 6 M sodium hydroxide (6 M NaOH). The total volume of slurry was adjusted to 1000 mL with distill water. The slurry was left to settle for 30 min at room temperature. After the slurry was settled, the supernatant was taken to measure soluble chemical oxygen demand COD and total volatile fatty acid (VFA). The digesters were closed with septum inserted in bottle screw caps. Nitrogen was then purged into the bottles for 15 min to create anaerobic condition in the digesters. The produced gas was measured every day using 50 mL syringe. The gas sample was taken to analyze the hydrogen content using gas chromatographic technique. The hydrogen fermentation was carried out for 15 days or until no gas was produced at room temperature (27 - 30 °C), 1 atm. All digestion bottles were shaken continuously by laboratory shaker. All experiments were run in triplicate and the average value was reported.

Methane production

The residue from hydrogen fermentation was used as substrate for methane production. After the hydrogen production was ended, 110 mL liquid samples were taken to measure soluble COD. Then, 5 g TVS of new sludge (approximately 100 mL) was added into each digester. The slurry pH was adjusted to 6.8 with 6M NaOH. The digesters were closed and purged with nitrogen for 15 min to create anaerobic condition. The biogas production was measured using 50 mL syringe and gas samples were taken every day until no gas was produced. The methane content was analyzed using gas chromatographic technique.

Analysis

COD concentration was determined using closed reflux titrimetric method [11]. pH was measured using pH meter (ADWA Instruments, CO., Ltd., model AD 1040). Moisture content, total solid (TS), and total volatile solid (TVS) were measured using procedures recommended in standard method for the examination of water and wastewater [11]. Total phosphorus (TP) and total Kjeldahl Nitrogen (TKN) were measured using Ascorbic acid method, and Macro-Kjeldahl method, respectively [11]. Hydrogen and methane composition in biogas were determined using Gas Chromatography (GC, Agilent, Model HP 6890, USA) equipped with plot Porapak column 30 m × 0.125 I.D., and TCD (Thermal Conductivity Detector) detector. Nitrogen (99.999 %) was used as carrier gas at a constant flow of 13 mL/min. The inlet, oven, and detector temperatures were set at 80, 80 and 200 °C, respectively.

Kinetic models of biogas production

In this research, the hydrogen and biomethane production were modeled using the modified Gompertz model (Eq. (1)) [12], modified logistics model (Eq. (2)) [12], Cone model (Eq. (3)) [13], first order kinetic model (Eq. (4)) [13,14], and transference function (Eq. (5)) [15], as followed;

$$Y(t) = Y_m exp\left\{-exp\left[\frac{R_m e}{Y_m}(\lambda - t) + 1\right]\right\} \tag{1}$$

$$Y(t) = \frac{Y_m}{\left\{1+exp\left(\frac{4R_m}{Y_m}(\lambda-t)+2\right)\right\}} \tag{2}$$

$$Y(t) = \frac{Y_m}{1+(k_h t)^{-n}},\ \mathrm{t} \geq 0 \tag{3}$$

$$Y(t) = Y_m[1 - exp(-kt)],\ \mathrm{t} \geq 0 \tag{4}$$

$$Y(t) = \left[1 - exp\left(-\frac{R_m(t-\lambda)}{Y_m}\right)\right] \tag{5}$$

where Y(t) is the cumulative hydrogen/methane yield at digestion time t days ($mLCH_4/g$ VS), Y_m is the hydrogen/methane yield potential (mL CH_4/g VS), R_m is the maximum hydrogen/methane production rate (mL/g VS·day), t is the digestion time (days), λ is lag phase period or minimum time to produce biogas (days), e is the mathematical constant (2.718282), k_h is the hydrolysis rate constant (/day), n is shape factor, and k is the hydrogen/methane production rate constant (/day). The kinetic parameters (Y_m, R_m, λ, k_h, and k) from these models were estimated using nonlinear least-square regression method. The statistical indicators R^2 (correlation coefficient) and root mean square error (RMSE) were calculated. Both the R^2 and RMSE (lowest value) were used to justify the goodness of fit. RMSE and R^2 were calculated using the following equations, respectively [16];

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(y_i^{cal} - y_i^{exp}\right)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2}$$

$$\bar{y} = \frac{1}{n}\sum_{i=1}^{n} y_i$$

$$RSME = \sqrt{\frac{1}{n}\left(y_i^{cal} - y_i^{exp}\right)^2}$$

where, y_i^{cal} and y_i^{exp} are the calculated and experimental data. All calculations and curve fitting were performed using Microsoft Excel® 2019.

Results and discussion

Water hyacinth and inoculum characteristics

Water hyacinth characteristics are shown in **Table 1**. Volatile solid content of water hyacinth is about 84.36 % which means that the substrate is easily digestible. The initial COD:N:P ratio of the water hyacinth was approximately 345:7:1 which is higher than the optimum range (600:5:1 during startup and 300:5:1 during long term operation) for anaerobic digestion [17]. Water hyacinth contains high total nitrogen and phosphorus content. So, in this research, no external nutrient was added into the digester. The characteristics of inoculum for hydrogen and biomethane production are also shown in **Table 1**.

Table 1 Characteristics of water hyacinth and inoculums.

Parameter	Water Hyacinth (% dry weight)	Inoculum for Hydrogen Production (% dry weight)	Inoculum for Methane Production (g/100 mL)
Total Solid	92.4680 ± 0.07	10.57 ± 0.06	7.60 ± 0.35
Volatile Solid	84.3572 ± 0.11	83.55 ± 0.78	4.84 ± 0.35
Fixed Solid	15.6428 ± 0.11	16.45 ± 0.78	2.76 ± 0.24
COD (g O_2/g dry weight)	0.8958 ± 0.09	–	–
TKN	1.9264 ±0.17	–	–
TP	0.2592 ±0.06	–	–

Effect of substrate to acid solution ratio on biohydrogen and biomethane production

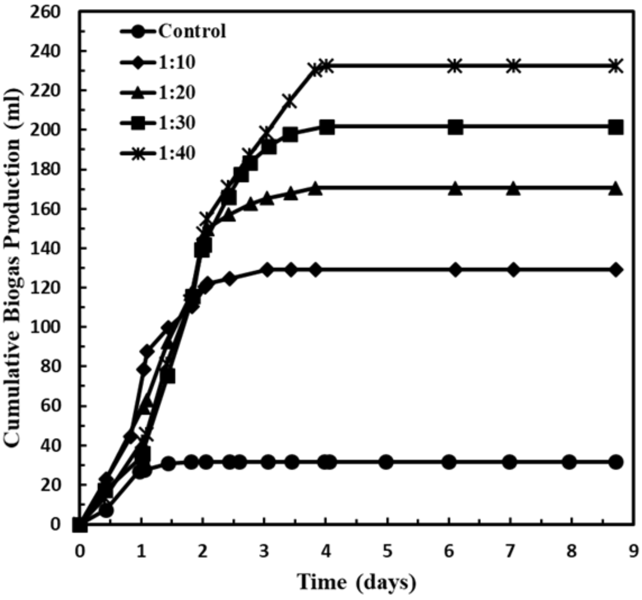
In this research, the substrate to acid solution (2 % v/v H_2SO_4) ratio was varied to see if it affected to the hydrogen and methane production. The ratios used in the experiments were ,1:10, 1:20, 1:30, 1:40 and control case which contained only water hyacinth and distilled water. In each case 10 gVS of substrate was added into each digestion bottle with the specified ratio. After pretreatment, the slurry pH was adjusted to 5.5 approximately by 6 M NaOH, and 5 gVS of inoculum for hydrogen production (UASB sludge pretreated at 100 °C in 2 h) was added into the digester. The slurry was fermented at room temperature (30 °C) and 1 atm pressure. The gas sample was also collected and measured the volume at this condition. The hydrogen content was determined using gas chromatographic technique. **Figure 1** shows the cumulative volume of produced biogas and hydrogen.

From **Figure 1**, only 48 mL of biogas and less than 4 mL of hydrogen were produced from control case which was unpretreated substrate. The biogas from control case mainly contained hydrogen sulfide and carbon dioxide, very little methane (less than 1 mL) was detected. This indicated that hydrogen cannot produced from water hyacinth without acid pretreatment. The average hydrogen content for control case is about 7.5 %. The cumulative biogas and hydrogen volume for 1:10, 1:20, 1:30 and 1:40 were 129.3 & 51.2, 170.7 & 68.4, 201.7 & 64.8 and 232.7 & 77.5 mL, respectively. The average percentage hydrogen produced were 39.6, 40.1, 32.1 and 33.2 %, respectively. Although, the cumulative biogas and hydrogen increased with the substrate to acid solution ratio, the highest hydrogen content occurred at 1:20 (40 %) and 1:10 (39.6 %) substrate to acid solution ratio. Since, the higher acid solution means higher sulfate content in the system which facilitated the sulfate reducing bacteria and probably resulted in more hydrogen sulfide content in the biogas [17]. The increase in amount of acid in the system did not enhance the hydrogen production, only acid concentration that is important as reported by Sarto *et al.* [18]. The optimum substrate to acid solution was between 1:10 to 1:20 in term of hydrogen production.

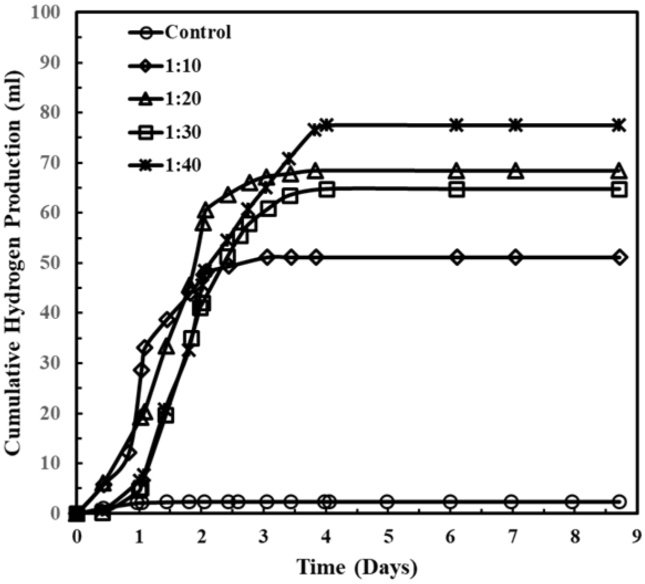
Figure 2 shows the soluble COD concentration before and after hydrogen production. The soluble COD of acid pretreated water hyacinth were doubled that of control case. After pretreatment, the soluble COD concentration did not significantly increase as the substrate to acid solution ratio increased from 1:10 to 1:40 which means that the total volume of the acid in the system did not affected to the solubilization of water hyacinth, only acid concentration that matters as reported by another researcher [18]. **Figure 2** also shows that the soluble COD concentration did not decreased after hydrogen production since there was solid substrate in the system that was hydrolyzed during hydrogen fermentation. From this Figure, soluble COD of control case was about 60 % those of acid pretreated water hyacinth, but the hydrogen yield was only about 5 % of those acid pretreated water hyacinth. This could be explained that acid pretreatment changed the raw material properties through converted cellulose to soluble components, such as glucose, which can be detected by soluble COD level. For control case, the soluble COD concentration could be reflected from the other higher molecular weight compounds instead of glucose which was not readily to digest by bacteria since mechanical pretreatment cannot removed lignin as much as acid pretreatment [18].

In the second stage, the residue from hydrogen production was used as the substrate for methane production. After hydrogen production, the remain slurry pH was adjusted to 6.8 (which is the optimum pH for methanogens [17]) by 6 M NaOH. And, 100 mL (which was equivalent to 5 g TVS) inoculum for methane production was added into the slurry. The residue slurry was fermented at room temperature (30 °C), and 1 atm pressure. The methane content was determined using gas chromatographic technique. Cumulative biogas and methane production are shown in **Figure 3**. From **Figure 3**, the cumulative biogas and methane produced in this second stage for control, 1:10, 1:20, 1:30 and 1:40 case were 1200.33 & 644.33, 1207.33 & 657.36, 1303.0 & 696.63, 1480.00 & 771.15 and 1091.67 & 526.30, respectively. The generated biogas and methane volume were highest for 1:30 case and lowest at 1:40 case. The average methane content for each case was 53.68, 54.45, 53.46, 52.10 and 48.21. In term of biogas and methane volume the highest point was at 1:30 case, however, the methane content was not significantly different from those of control, 1:20 and 1:30 cases. The optimum substrate to acid solution ratio is 1:30 for methane generation, and 1:20 for hydrogen production. Surprisingly, the control case seemed to be better than that of 1:40 case for methane production. This could be explained that, the substrate was in the system long enough for the degradation and no sulfate was added into the system. There is no competition between other organisms and methanogens which is sensitive to sulfate reducing bacteria [17].

When the total biogas yield per g TVS removed was considered, the highest yield obtained from 1:30 case was 184.27 mL/g TVS, and from control, 1:10, 1:20 and 1:40 case, were 128.9, 145.6, 158.21, 163.88 mL/g TVS, respectively (**Table 2**). In term of energy recovery, the highest energy recovery was 28.20 kJ per 10 gVS occurred at 1:30 case as well. The energy recovery for control, 1:10, 1:20 and 1:40 were 23.07, 24.00, 25.57, and 19.57 kJ/10 gVS, respectively. The energy recovery was calculated based on hydrogen energy content of 119.93 MJ/kg, and methane energy content of 55.6 MJ/kg [19]. The optimum substrate to acid solution ratio is 1:30. Although, 1:40 case generated highest hydrogen volume, the methane generated in the second stage is quite low compared to other cases. For control case, only mechanical pretreatment (dried and ground) is good enough to produce biomethane. Acid pretreatment might not be necessary for 1 stage methane production. However, the 2-stage hydrogen production before methane production might be a good strategy in term of higher energy recovery.



(a)



(b)

Figure 1 Cumulative hydrogen production from first stage fermentation were plotted with different substrate to acid solution ratios. Gas volume was measured at room temperature, 1 atm. (a) cumulative total gas production, and (b) cumulative hydrogen production.

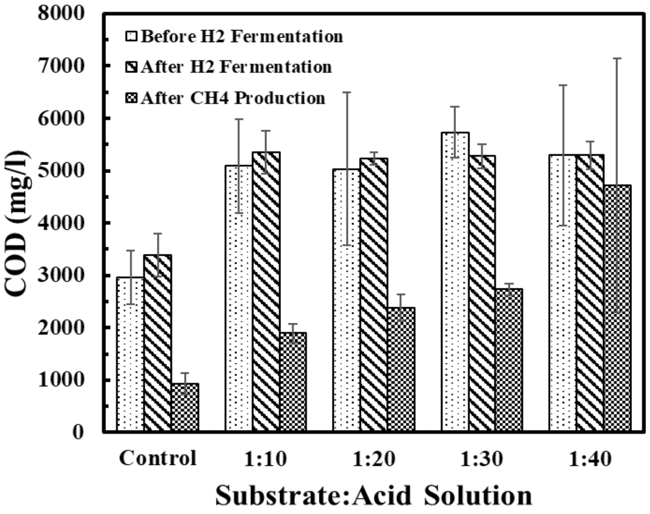
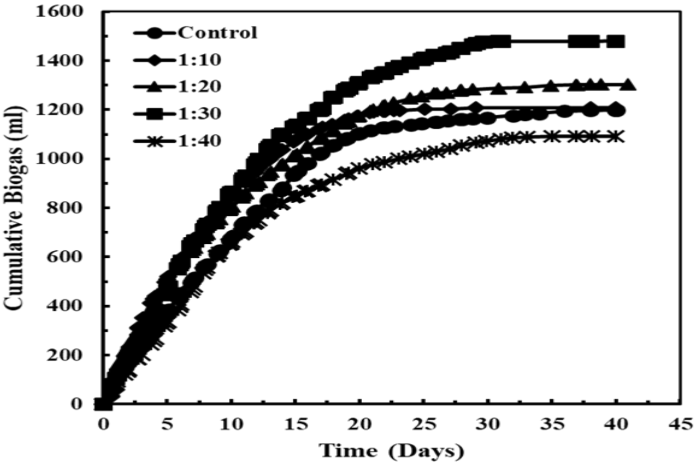
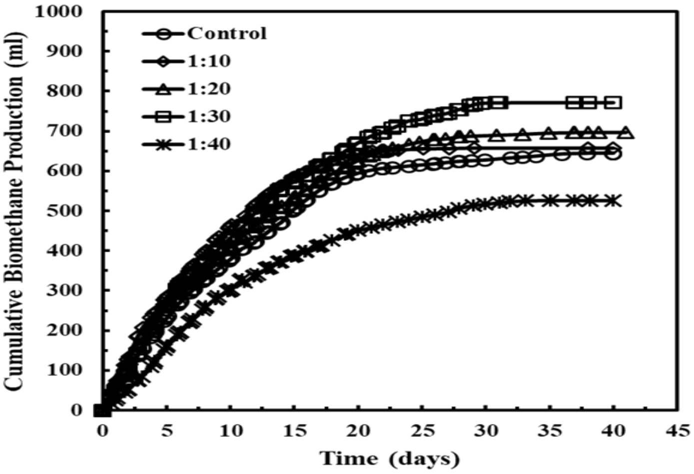


Figure 2 Soluble COD at different substrate to acid solution ratios before hydrogen fermentation, after hydrogen fermentation, and after methane production.



(a)



(b)

Figure 3 Cumulative methane production measured from second stage anaerobic fermentation versus different substrate to acid solution ratio. Gas volume was measured at room temperature, 1 atm. (a) total biogas production, (b) biomethane production.

Table 2 Solid balance and biogas generation for each case.

	Control	**1:10**	**1:20**	**1:30**	**1:40**
Input					
Amount of water hyacinth (g)	12.83	12.82	12.82	12.83	12.82
Total solid of substrate (g)	12.01	12.01	12.01	12.01	12.01
Initial volatile solid of substrate (g)	10.00	10.00	10.00	10.00	10.00
Fixed solid of substrate (g)	2.007	2.005	2.005	2.007	2.005
Total solid of inoculum for hydrogen production (g)	5.981	5.979	5.979	5.982	5.980
Volatile solid of inoculum (g)	4.997	4.996	4.995	4.998	4.996
Fixed solid of inoculum (g)	0.984	0.984	0.984	0.984	0.984
Inoculum for methane production (g)	5.985	5.985	5.985	5.985	5.985
Volatile solid of methane inoculum (g)	4.844	4.844	4.844	4.844	4.844
Fixed solid of methane inoculum (g)	1.140	1.140	1.140	1.140	1.140
Total solid input	23.97	23.97	23.97	23.97	23.97
Total volatile solid input (g)	19.84	19.84	19.84	19.84	19.84
Total fixed solid input (g)	4.131	4.129	4.129	4.131	4.129
Output					
Total solid after methane production (g)	13.47	14.46	15.07	14.85	15.89
Volatile solid after methane production (g)	10.29	10.66	10.52	10.72	11.76
Fixed solid after methane production (g)	3.180	3.804	4.129	4.131	4.129
Volatile solid removal (g)	**9.551**	**9.180**	**9.315**	**9.126**	**8.081**
Biogas					
First stage biogas volume (mL at 30 °C, 1 atm)	31.67	129.3	170.7	201.7	232.7
Hydrogen volume (mL at 30 °C, 1 atm)	2.37	51.17	68.37	64.77	77.47
Average hydrogen content (%)	7.49	39.56	40.06	32.11	33.29
Second stage biogas volume (mL at 30 °C, 1 atm)	1200	1207	1303	1480	1092
Methane volume (mL at 30 °C, 1 atm)	644.3	657.4	696.6	771.2	526.3
Average methane content (%)	53.68	54.45	53.46	52.10	48.21
Total biogas volume (mL at 30 °C, 1 atm)	1232	1337	1474	1682	1324
Total biogas yield (mL/g TVS removed)	129.0	145.6	158.2	184.3	163.9
Total energy recovery (kJ)	23.07	24.00	25.57	28.20	19.57

Kinetic model for biogas production

The experimental yields from the hydrogen production and methane production were fitted with modified Gompertz model (Eq. (1)), modified logistics model (Eq. (2)), cone model (Eq. (3)), first order kinetic model (Eq. (4)), and transference function (Eq. (5)). The parameters estimated from these equations are summarized in **Table 3** for hydrogen production and **Table 4** for methane production. From **Table 3**, the experimental yields from hydrogen production were well fitted with modified Gompertz, modified logistic, Cone, and transference function model, with the regression coefficients close to 1 (0.9-0.9997). On the contrary, the first order model gave lower regression coefficient of 0.8994, 0.8454, and 0.8831, for 1:20, 1:30, and 1:40 cases, respectively. The RMSE were also lowest for modified Gompertz, modified logistic, and Cone model, which indicated the goodness of fit. The results in **Table 3** also show that the maximum hydrogen potential increased with the acid solution ratio. However, the maximum hydrogen production rate was obtained at 1:20 and 1:30 case, which agreed with the experimental results in previous section. In order to compare the prediction results from each model, the experimental yields for 1:30 cases were plotted with the calculated yields as shown in **Figure 4**. **Figure 4** shows that Modified Gompertz model, modified logistic, and Cone model were the best fitted model for hydrogen production. These models showed the well correlated between experimental and calculated yields for hydrogen production with a linear correlation. Where, their slopes close to one and the regression correlation coefficient close to 1 as well. These indicated that the calculated values were equal to the experimental values. Many researchers also reported the best fitted of the hydrogen production with modified Gomperzt model [19-22]. Mu *et al.* [21] also found that the Logistic model and modified Gompertz equation all could be described the kinetics of substrate utilization, biomass growth and hydrogen formation which agreed very well with what finding in this research.

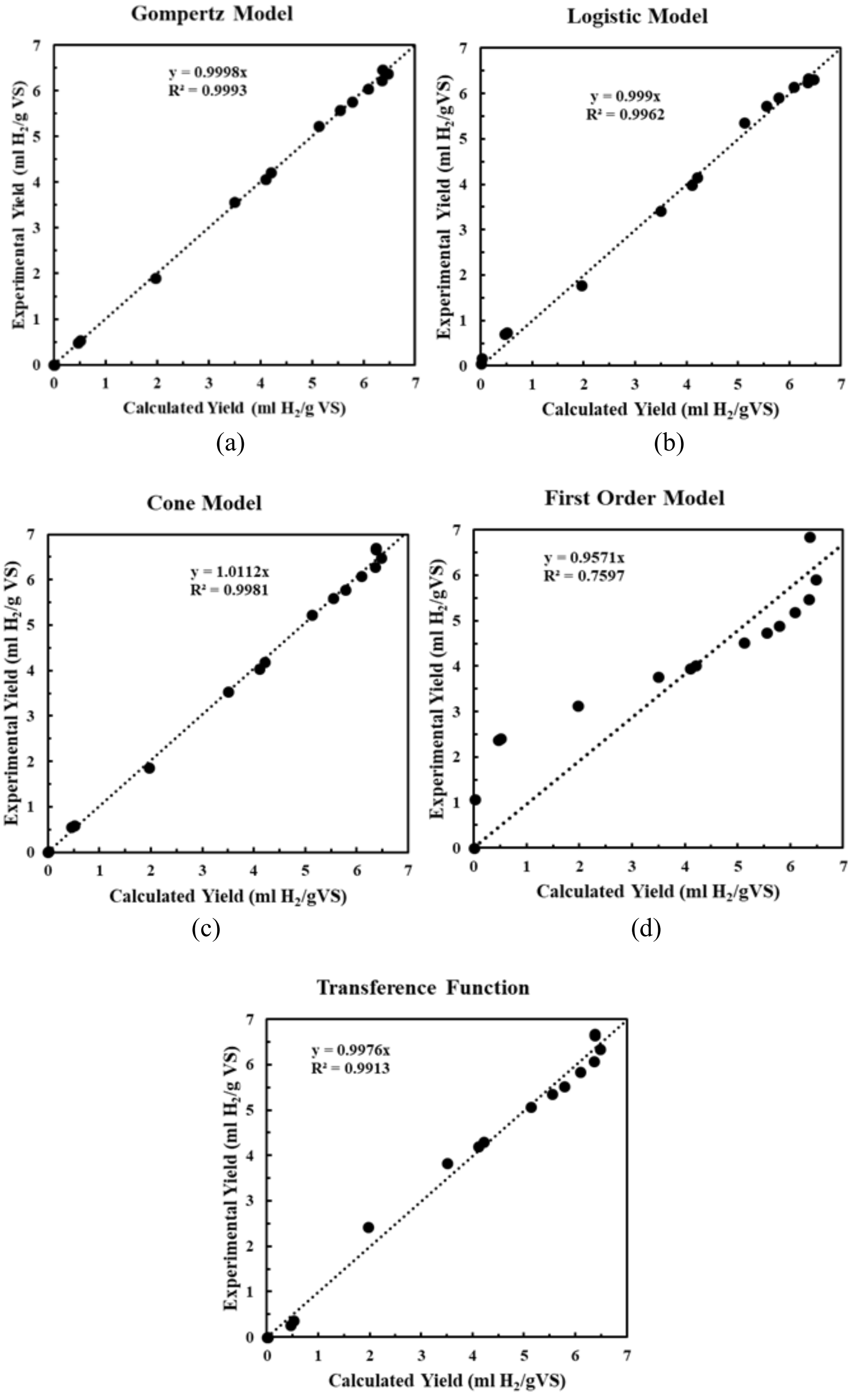
These same equations were also employed to model the formation of methane in the second stage. **Table 4** shows the model's parameters obtained from the regression. The results in **Table 4** indicate that the maximum methane yield potential was obtained at 1:30 case, but the maximum methane production rate or hydrolysis rate (in case of Cone and first order model) were highest at 1:10 substrate to acid solution ratio. **Figure 6** shows the plot between experimental methane yield and predicted methane yield at various digestion time. As can be seen from **Figure 5**, the best fitted models were Transference function, first order, and Cone model which show the linear relationship between experimental and predicted methane yield with slope close to one. These suggested that the predicted yield was equal to the experimental yield. Modified Gompertz statistically sufficient to describe the growth of bacteria and was easy to use. It has been used by many researchers to model the biomethane production [12,15,23-25]. However, some researchers reported that other models such as Cone model [13,26-28], First order Model [14,28], and Transference function [29] were fitted better with the experimental data. The modified Gompertz and logistic model assume that the specific growth rate of methanogenic organisms is directly proportional to the methane produced from the anaerobic reactor, with the growth curve seems to be sigmoidal production trend [15]. On the other hand, transference function model is used when zero lag phase time is observed and only stationary and exponential phase of biogas production is considered [29]. Since the residue from the first stage was used as substrate for biomethane production in this research, the substrate was readily to be digested and no lag phase time was required. The results agreed with those of Li *et al.* [29], Adanikina *et al.* [28], Zhen *et al.* [27], Syaichurrozi [13], and Syaichurrozi *et al.* [26].

Table 3 Results of kinetics study for hydrogen production.

	Substrate: Acid Solution Ratio (g/mL solution)				
	Control	**1:10**	**1:20**	**1:30**	**1:40**
Modified Gompertz Model					
Hydrogen yield potential, Y_m (mL H_2/g VS)	0.2374	5.0983	6.9247	6.4619	7.7857
Maximum hydrogen production rate, R_m (mL H_2/g VS·day)	0.2947	4.4209	4.6272	4.2067	4.0048
Lag phase time, λ (days)	0.0531	0.4305	0.6430	0.9755	0.9162
R^2	0.9997	0.9855	0.9913	0.9993	0.9963
RMSE	0.0011	0.2055	0.2228	0.0655	0.1751
Predicted hydrogen yield (mL H_2/g VS	0.2374	5.0983	6.9247	6.4619	7.7853
Measured hydrogen Yield (mL H_2/g VS)	0.2374	5.1168	6.8370	6.3721	7.7468
Modified Logistic Model					
Hydrogen yield potential, Ym (mL H_2/g VS)	0.2338	5.0260	6.8537	6.3263	7.6273
Maximum hydrogen production rate, Rm (mL H_2/g VS/day)	2.6786	4.4028	4.3739	4.2375	4.0003
Lag phase time, λ (days)	0.3867	0.4462	0.6482	1.0305	0.9647
R^2	0.9836	0.9822	0.9960	0.9963	0.9911
RMSE	0.0076	0.2275	0.1516	0.1482	0.2724
Predicted hydrogen yield (mL H_2/g VS	0.2338	5.0260	6.8537	6.3263	7.6273
Measured hydrogen yield (mL H_2/g VS)	0.2374	5.1168	6.8370	6.3721	7.7468
Cone Model					
Hydrogen yield potential, Y_m (mL H_2/g VS)	0.2385	5.1303	6.9842	6.7012	7.8904
Hydrolysis rate constant, k_h (/day)	2.2049	0.9899	0.7163	0.5598	0.5272
Shape factor, n	2.7482	3.6664	3.7472	4.1998	3.7378
R^2	0.9994	0.9866	0.9893	0.9995	0.9960
RMSE	0.0014	0.1978	0.2467	0.0532	0.1818
Predicted hydrogen yield (mL H_2/g VS)	0.2384	5.1284	6.9769	6.6925	7.8640
Measured hydrogen yield (mL H_2/g VS)	0.2374	5.1168	6.8370	6.3721	7.7468
First Order Model					
Hydrogen yield potential, Ym (mL H_2/g VS)	0.2396	5.4256	7.4810	7.6479	9.2569
Hydrogen production rate constant (/day)	1.8637	0.7696	0.5564	0.3690	0.3199
R^2	0.9875	0.9176	0.8994	0.8454	0.8831
RMSE	0.0066	0.4901	0.7568	0.9583	0.9864
Predicted hydrogen yield (mL H_2/g VS)	0.2396	5.4190	7.4220	7.3407	8.6861
Measured hydrogen yield (mL H_2/g VS)	0.2374	5.1168	6.8370	6.3721	7.7468
Transference Function					
Hydrogen yield potential, Y_m (mL H2/g VS)	0.2376	5.2460	6.9718	6.6812	8.0384
Maximum hydrogen production rate, R_m (mL H_2/ g VS · day)	0.6791	6.4613	9.2631	6.5297	6.3775
Lag phase time, λ (day)	0.2100	0.3952	0.8255	0.9641	0.9608
R^2	0.9997	0.9708	0.9851	0.9963	0.9912
RMSE	0.0009	0.2918	0.2909	0.2264	0.2702
Predicted hydrogen yield (mL H_2/g VS)	0.2376	5.2458	6.9716	6.6778	8.0211
Measured hydrogen yield (mL H_2/g VS)	0.2374	5.1168	6.8370	6.3721	7.7468

Table 4 Results of kinetics study for biomethane production.

	Substrate: Acid Solution Ratio (g/mL solution)				
	Control	**1:10**	**1:20**	**1:30**	**1:40**
Modified Gompertz Model					
Methane yield potential, Y_m (mL CH_4/g VS)	63.8684	65.6900	69.1377	76.2820	51.2480
Maximum methane production rate, R_m (mL H_2/g VS·day)	4.0158	5.1940	4.2910	4.4390	3.0378
Lag phase time, λ (days)	0	0	0	0	0.0729
R^2	0.9922	0.9918	0.9875	0.9996	0.9923
RMSE	1.6978	1.7334	2.1954	2.0250	1.3208
Predicted methane yield (mL CH_4/g VS)	68.6323	65.6569	68.9499	75.9107	51.0236
Measured methane yield (mL CH_4/g VS)	64.4116	65.7357	69.6629	77.1147	52.6305
Modified Logistic Model					
Methane yield potential, Ym (mL CH_4/g VS)	62.6659	64.3975	67.9401	73.9638	49.5610
Maximum methane production rate, Rm (mL CH_4/g VS/day)	3.8091	4.9218	4.0528	4.2393	2.9594
Lag phase time, λ (days)	0	0	0	0	0.2014
R^2	0.9984	0.9847	0.9816	0.9958	0.9793
RSME	2.3526	2.3671	2.6652	2.8619	2.1699
Predicted methane yield (mL CH_4/g VS)	62.6382	64.3951	67.9114	73.9066	49.5336
Measured methane yield (mL CH_4/g VS)	64.4116	65.7357	69.6629	77.1147	52.6305
Cone Model					
Methane yield potential, Y_m (mL CH_4/g VS)	76.5031	78.5208	89.0974	110.8860	62.9096
Hydrolysis rate constant, k_h (/day)	0.1061	0.1354	0.0935	0.0702	0.0943
Shape factor, n	1.3745	1.3565	1.1856	1.1561	1.4280
R^2	0.9930	0.9921	0.9924	0.9991	0.9994
RMSE	1.6088	1.7074	1.7150	0.6292	0.3660
Predicted methane yield (mL CH_4/g VS)	67.2770	71.3055	74.0294	85.0799	54.6868
Measured methane yield (mL CH_4/g VS)	64.4116	65.7357	69.6629	77.1147	52.6305
First Order Model					
Methane yield potential, Ym (mL CH_4/g VS)	69.6936	71.2679	75.0579	89.5212	59.6195
Methane production rate constant (/day)	0.0827	0.1062	0.0834	0.0676	0.0685
R^2	0.9934	0.9950	0.9959	0.9992	0.9947
RMSE	1.5610	1.3577	1.2627	0.6124	1.0942
Predicted methane yield (mL CH_4/g VS)	67.1429	70.2482	72.5867	83.5099	55.7658
Measured methane yield (mL CH_4/g VS)	64.4116	65.7357	69.6629	77.1147	52.6305
Transference Function					
Methane yield potential, Y_m (mL CH_4/g VS)	68.4556	70.4806	75.0579	85.2066	56.6143
Maximum methane production rate, R_m (mL CH_4/ g VS·day)	6.1582	7.9063	6.2581	6.4794	4.6327
Lag phase time, λ (day)	0.4433	0.2444	0	0.3004	0.7992
R^2	0.9949	0.9842	0.9959	0.9980	0.9986
RMSE	1.3736	1.2618	1.2627	0.9715	0.5622
Predicted methane yield (mL CH_4/g VS)	66.5064	69.6634	72.5867	81.0349	54.3187
Measured methane yield (mL CH_4/g VS)	64.4116	65.7357	69.6629	77.1147	52.6305

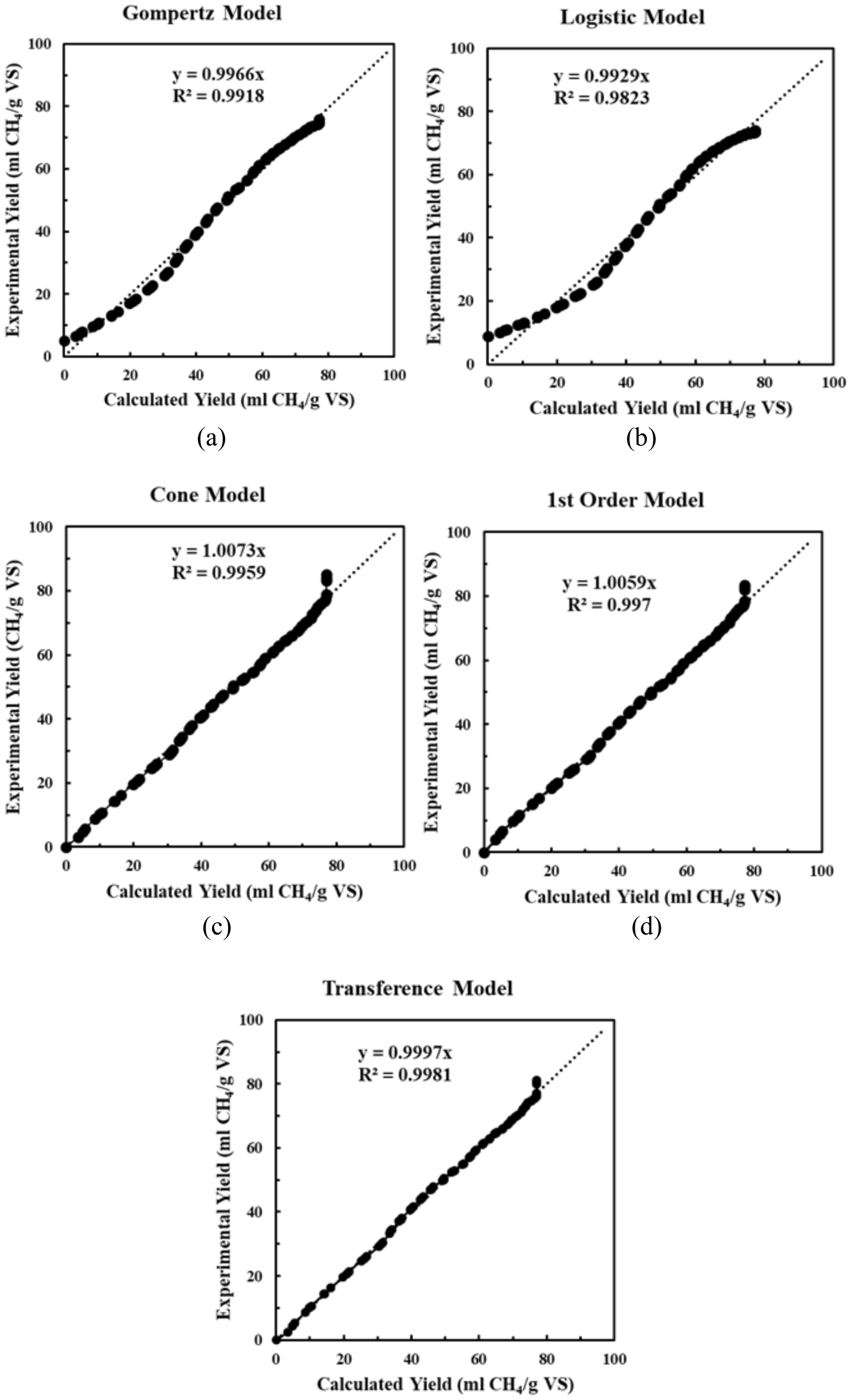


(a) (b)

(c) (d)

(e)

Figure 4 Experimental hydrogen yields were plotted with calculated yields at different fermentation time for 1:30 substrate to acid solution ratio, from (a) modified Gompertz, (b) modified logistic, (c) Cone, (d) First order, and (e) Transference function model.



(a) (b)

(c) (d)

(d)

Figure 5 Experimental methane yields were plotted with calculated yields at different fermentation time from for 1:30 substrate to acid solution ratio, from (a) modified Gompertz, (b) modified logistic, (c) Cone, (d) First order, and (e) Transference function model.

Conclusions

The results showed that the cumulative hydrogen production slightly increased with the substrate to acid solution (2 % v/v H_2SO_4) ratio. The highest cumulative hydrogen produced (77.47 mL with 7.75 mL H_2/gVS) at 1:40 substrate to acid solution, however the highest hydrogen content obtained at 1:20 (40.1 %). Hydrogen could not be produced from water hyacinth without acid pretreatment, only 4 mL hydrogen was detected from control case. In the second stage, methane was generated from the residue of first stage fermentation and found that the highest cumulative methane was obtained from 1:30 substrate to acid solution ratio. The methane content was not significantly different for control (53.68 %), 1:10 (54.45 %), 1:20 (53.46 %) and 1:30 (52.10 %) case. The modified Gompertz, modified Logistic, and Cone model were the best described models for hydrogen production since the fermentation needed lag phase time before the hydrogen can be generated. The calculated lag phase time from modified Gompertz model increased from 0.0531 to 0.9775 day, and lag phase time from modified Logistic model increased from 0.3867 to 1.0305 day. On the other hand, the Cone model, first order model, and Transference function were well described the second stage methane production with nearly zero lag phase time.

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