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Biohydrogen Productions from Hydrolysate of Water Hyacinth Stem (*Eichhornia crassipes*) Using Anaerobic Mixed Cultures

(Pengeluaran Biohidrogen daripada Hidrolisat Batang Keladi Bunting (*Eichhornia crassipes*) Menggunakan Kultur Campuran Anaerob)

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ABSTRACT

Response surface methodology (RSM) with central composite design (CCD) was applied to optimize key factors affecting hydrogen production (HP) from diluted acid hydrolysate of water-hyacinth stem (WHS) by heat-treated anaerobic sludge in a batch fermentation process. Key factors affecting namely substrate concentration and initial pH was investigated. The results indicated that substrate concentration and initial pH had significantly effects on HP ($p<0.05$). *A maximum HP hydrogen production rate and hydrogen yield of 182.7 mmol H_2/L, 2.81 mmol H_2/L h and 0.84 mol H_2/mol hexose were obtained under the optimum conditions i.e. substrate concentration of 4.06 g/L and initial pH of 5.81. The total energy production from the fermentative of WHS hydrolysate was 1.97 kJ.*

Keywords: Central composite design (CCD); dilute acid hydrolysis; hydrogen production; response surface methodology (RSM); water-hyacinth

ABSTRAK

Kaedah permukaan tindak balas (RSM) dan pusat reka bentuk tergubah (CCD) digunakan untuk mengoptimumkan faktor utama yang mempengaruhi pengeluaran hidrogen (HP) daripada hidrolisat asid cair batang keladi bunting (WHS) melalui enapcemar anaerobik terawat-haba di dalam proses penapaian kumpulan. Faktor utama yang mempengaruhi kepekatan substrat dan pH awal dikaji. Keputusan menunjukkan bahawa kepekatan substrat dan pH awal telah memberi kesan secara signifikan kepada HP ($p<0.05$). *Kadar pengeluaran hidrogen HP maksimum dan hasil hidrogen ialah 182.7 mmol H_2/L, 2.81 mmol H_2/L h dan 0.84 mol H_2/mol heksosa telah diperoleh pada keadaan optimum iaitu kepekatan substrat 4.06 g/L dan pH awal 5.81. Jumlah pengeluaran tenaga daripada penapaian WHS hidrolisat adalah 1.97 kJ.*

Kata kunci: Hidrolisis asid cair; kaedah permukaan tindak balas (RSM); keladi bunting; pengeluaran hidrogen; pusat reka bentuk tergubah (CCD)

INTRODUCTION

Biohydrogen is promising alternative fuel to replace fossil fuels due to the increasing of renewable energy, damaging of climate and environmental, depletion of fossil or petroleum fuel. Biohydrogen is a clean and environmentally friendly fuel, which produces water instead of greenhouse gases after combustion. Dark fermentative of hydrogen production process is more attractive due to its sustainable and less energy intensive compared to thermo-chemical and electro-chemical processes (Fangkum & Reungsang 2011; Sittijunda & Reungsang 2012). However, compared with the photosynthetic hydrogen production, the dark fermentative hydrogen generation has a higher efficiency, stability and feasibility for industrialization, simpler control requirement and lower operating costs (Fangkum & Reungsang 2011; Sittijunda & Reungsang 2012). Biohydrogen is conventionally produced from substrate containing high amount of carbohydrates such as sugarcane, starch and corn (Antonopoulou et al. 2011). However, use of these substrates significantly increases the cost of biohydrogen. In addition, these substrates are commercially feedstock of food industrial sector. Therefore, interest in second-generation processes, i.e. processes utilizing low-cost feedstock such as lignocellulosic materials (sugarcane bagasse, straw & corn stover) has emerged.

Lignocellulosic biomass, which primarily contains wood, grass, weed, agricultural by products or crops, is an ideal inexpensive, renewable and abundant resource for hydrogen production (Chong et al. 2009; Nissila et al. 2014). Direct fermentation of raw lignocellulose is typically inefficient. Hence, pre-treatments are commonly needed to saccharify lignocellulosic material into monomeric sugars, glucose and xylose, prior to biological conversion (Harmsen et al. 2010; Jung et al. 2013). Various pretreatment methods such as acid or alkaline hydrolysis were applied with the help of high temperature which possess the high cost for up-scale operation. Therefore, the possibility of pretreated lignocellulosic material at room temperature was explored in this study, in order to reduce the total cost of hydrogen production.

Water-hyacinth (*Eichhornia crassipes*) is a free floating aquatic weed, which is a widely prevalent aquatic weed in Thailand (Fan et al. 2016; Polprasert et al. 1994), constitutes a potential biomass resource for various uses (Fan et al. 2016). Water-hyacinth is a fast growing plant (Fan et al. 2016) under the climatic conditions. Daily average water-hyacinth biomass productivity is 0.26 ton of dry biomass per hectare in all seasons (Nigam 2002). Due to its fast growth and the robustness of its seeds, water-hyacinth has caused many problems in the whole river area, i.e. a reduction of fish (Gunnarsson & Petersen 2007), physical interference with fishing, obstruction of shipping routes and losses of water in irrigation systems due to higher evaporation, interference with hydroelectric schemes and increased sedimentation by trapping silt particles. It also restricts the possibilities of fishing from the shore with baskets or lines (Aweke 1993) and can cause hygienic problems (Abdelhamid & Gabr 1991). The most common use of water-hyacinth is raw material for composting and substrate for biogas (Abdelhamid & Gabr 1991) and bioethanol production (Kumar et al. 2009; Zhao et al. 2009). Water-hyacinth consists of three main fractions i.e. cellulose, hemicellulose and lignin. It has a high content of hemicellulose (30-55% of dry weight), which further pretreated and obtained hemicellulosic sugars as a by-product (Kumar et al. 2009). Dilute acid treatment of hemicellulose fraction in water-hyacinth yields a solution containing mainly xylose and glucose (Kumar et al. 2009). Glucose and xylose were reported as a substrate for producing hydrogen by various types of microorganisms such as pure culture i.e. *Thermoanaerobacter* sp. *Thermoanaerobacterium* sp. *Caldanaerobacter* sp. (Zhao et al. 2013) and *Clostridium* sp. HR-1 (Xu et al. 2010) and mixed culture (Mu et al. 2009) with the yield of 2.0 mol H_2/mol hexose.

Response surface methodology (RSM) is a combination of mathematical and statistical techniques. It used to analyze the effects of several independent variables on the response (Reungsang et al. 2013). This technique provides the optimum point, interactive effect of each variable on response, reduce the number of treatment and minimize the error in determining the effects of parameters (Fangkum & Reungsang 2011; Reungsang et al. 2013; Sittijunda & Reungsang 2012). Optimization of process parameters has been used in enhancing the activity of many microorganisms as well as hydrogen production. Therefore, in the present work, a response surface methodology (RSM) with central composite design (CCD) has been used as a tool to optimize process parameters for biohydrogen production from dilute acid hydrolysate of water-hyacinth stem (WHS) by heat-treated anaerobic sludge. This approach would not only add value to water hyacinth in a form of safe and clean energy, but also be one of the solution approaches for making use of this abundant waste.

Materials and Methods

Anaerobic seed sludge and inoculum preparation

The anaerobic granules obtained from up flow anaerobic sludge blanket (UASB) reactor were used as seed inoculum for hydrogen production. This UASB reactor was used to produce biogas from wastewater of cassava starch production process (Kalasin province, Thailand). The UASB granules were boiled at 100°C for 3 h to inactivate the hydrogentrophic methanogens (Sreela-or et al. 2011). The hydrogen-producing bacteria in UASB granules were enriched by cultivating 200 g of heat-treated UASB granules in the 100 mL serum bottle containing 5 g/L xylose and 5 g/L glucose as carbon sources and supplemented with sufficient inorganic nutrients for bacterial growth (Lin & Lay 2005). The initial pH of the enrichment media was adjusted to 5.00 using 1 M NaOH or 1 M HCl. The glass bottle was capped with rubber stopper and flushed with nitrogen gas for 20 min to create anaerobic conditions. The culture was incubated at 30±3°C for 24 h. Second successive batch cycles (10% v/v of the inoculum in the medium with 24 h cultivation per cycle) were conducted. The enriched culture obtained from the second batch cycle was further used as the seed inoculum for hydrogen production experiments. The initial cell concentration in the culture broth was 8.93±1.57 g-volatile suspended solid (VSS)/L.

Water-hyacinth pretreatment

Fresh WHS was collected from Lopburi River (Lopburi province, Thailand) and washed with tap water to remove adhering dirt. Prior to use, WHS was chopped in small pieces, air dried and milled before storing at room temperature. WHS consists of (all in % (weight/wet weight)): cellulose, 27.55±0.81; hemicelluloses, 39.83±2.04; lignin, and 14.96±0.17. The dried powder of WHS approximately 15 g dry weight were added into the 250 mL flask containing 150 mL of dilute H_2SO_4 (solid (g dry weight) to liquid (mL) ratio of 1:10) and incubated in the incubator shaker at room temperature. The hydrolysis conditions was set according to the previous experiment (reaction time of 7.73 h, H_2SO_4 concentration of 1.31% (v/v) and stirring speed of 265 rpm) (Pattra & Sittijunda 2015). After hydrolysis, a solid residue was separated from the liquid phase (hydrolysate) by filtration through a thin layer cloth. Prior to being used as the substrate for hydrogen production, the hydrolysate was heated at 100°C for 15 min in order to remove or reduce the concentration of volatile components (furfural and phenolic compounds) (Kumar et al. 2009). The pH of acid hydrolysate was then adjusted to 10 by a combination of solid $Ca(OH)_2$ and 0.1% sodium sulfite. The precipitate was removed by filtration through a thin layer cloth. The resulting filtrate was re-acidified to pH 6.0 (Kumar et al. 2009) and was then concentrated at 70°C.

OPTIMIZATION OF SUBSTRATE CONCENTRATION AND INITIAL PH ON HYDROGEN PRODUCTION USING THE WHS HYDROLYSATE

RSM with CCD was used to optimize the level of initial pH (X_1) and substrate concentration (X_2) on hydrogen production from WHS hydolysate. The response variable is HP (mmol H_2/L). The levels of each factors used for optimization HP were presented in Table 1. Regression analysis was performed to estimate the response function as in (1):

$$Z = B_0 + \sum_{i=1}^{2} B_i X_i + \sum_{i=1}^{2} B_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1}^{2} B_{ij} X_i X_j. \quad (1)$$

where Z is the predicated response (HP); β_0 is a constant; β_i is the linear coefficient; β_{ii} is the squared coefficient; β_{ij} is the interaction coefficient; and X_i is the variable. The response variable (HP) was fitted using a predictive polynomial quadratic equation (1) in order to correlate the response variable to the independent variables. The statistical software Design-Expert (Demo version 7.0, Stat-Ease, Inc., Minneapolis, MN, USA) is used for regression and graphical analysis of the experimental data. The quality of fit of the quadratic model is expressed by the coefficient of determination, R^2, and its statistical significance is checked by the F-test. The conditions of each trial are shown in Table 1.

BIO-HYDROGEN PRODUCTION FROM WHS HYDROLYSATE IN BATCH EXPERIMENT

Biohydrogen production was conducted in 100 mL serum bottle using WHS hydrolysate. The fermentation medium (total volume of 60 mL) contained WHS hydrolysate, seed inoculum and inorganic nutrients solution (Lin & Lay 2005). The concentration of WHS and initial pH was adjusted according to the design (Table 1). The serum bottles were tightly sealed with rubber stopper and aluminum cap then flushed with nitrogen gas to create the anaerobic condition. Then the serum bottles were incubated at the shaking speed of 150 rpm in an orbital shaker at room temperature (30±2°C). During the incubation time, the volume of biogas was measured by plunger displacement method (Owen et al. 1978). The liquid sample was taken every day to determine the sugar residues and the concentrations of VFAs and ethanol produced. All treatments were conducted in triplicates. The fermentation process has been continued until biogas is no longer generated.

ANALYTICAL METHODS

Hydrogen content in biogas compositions were determined by GC (Shimadzu 2014, Japan) equipped with a thermal conductivity detector (TCD) and a 2 m stainless column packed with Unibeads C (60/80 mesh). The GC-TCD condition was set according to Saraphirom and Reungsang (2010). Prior to measurement of Volatile fatty acids (VFAs) and alcohol, liquid samples were centrifuged at 12000 rpm for 5 min and filtered through a 0.45 mm nylon membrane filter. The resulting filtrate was then acidified by 2 mol/L oxalic acid. Then analyze VFAs and alcohol in the resulting filtrate using GC equipped with Flam ionization detector (FID). The GC-FID conditions followed the method described by Pattra et al. (2008). Volatile suspended solids (VSS) represented as the biomass concentration was measured according to Standard Methods (APHA 1995). The sugar concentration in fermentative medium was determined according to the phenol-sulfuric acid method using sucrose as a standard (Saha & Brewer 1994).

RESULTS AND DISCUSSION

OPTIMIZATION OF INITIAL PH AND SUBSTRATE CONCENTRATION ON HYDROGEN PRODUCTION USING RSM WITH CCD EXPERIMENT

The multiple regression analysis was applied on the data showed in Tables 1 and 2 and the obtained second-

TABLE 1. Full factorial CCD matrix of initial pH and initial substrate concentration on HP

Run	Variables				HP (mmol H_2/L) Observed
	Initial pH (X_1)		Substrate concentration (g/L) (X_2)		
	Code	Real	Code	Real	
1	-1.00	5.00	-1.00	1.50	90.1
2	-1.00	5.00	1.00	8.00	10.9
3	0.00	6.00	0.00	4.75	202.6
4	0.00	6.00	1.41	9.35	25.0
5	0.00	6.00	-1.41	0.15	54.6
6	1.00	7.00	1.00	8.00	26.3
7	0.00	6.00	0.00	4.75	175.0
8	0.00	6.00	0.00	4.75	185.0
9	0.00	6.00	0.00	4.75	184.5
10	0.00	6.00	0.00	4.75	191.1
11	-1.41	4.59	0.00	4.75	110.4
12	1.41	7.41	0.00	4.75	103.5
13	1.00	7.00	-1.00	1.50	73.8

order polynomial (2) could well explain the hydrogen production:

$$HP = -1646.50 + 536.30X_1 + 25.03X_2 + 0.79X_1X_2 - 46.12X_1^2 - 0.80X_2^2. \quad (2)$$

A high determination coefficient ($R^2 = 0.96$, Table 2) explained 96% of variability in the response suggesting a high significance of the model. The experimental results (Table 2) showed that an initial substrate concentration and initial pH for hydrogen production using the hydrolysate had a significantly ($p<0.05$) affected on HP and initial substrate concentration had a statistically individual effect on HP. However, the interactive effect of initial substrate concentration and initial pH had insignificantly effect ($p>0.05$) on HP. The plot of predicted versus experimental HP (Figure 1), depicts a correspondence of x and y values which indicated that the prediction of experimental data was adequate. A maximum HP of 182.7 mmol H_2/L was obtained under the optimum conditions i.e., substrate concentration of 4.06 g/L and initial pH of 5.81.

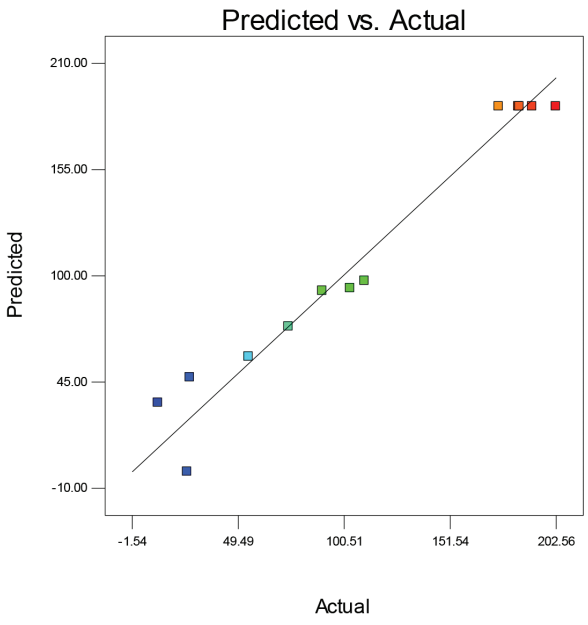

FIGURE 1. Predicted vs. experimental values of the effects of substrate concentration and initial pH of substrate on HP

Figure 2 depicts the graphical representation of the relationship between the experimental levels of substrate concentration and initial pH on HP. The HP produced at the optimum condition was indicated on the peak of response (Figure 2) and the smallest ellipse shapes of contour plot (Figure 2). The results indicated that the initial substrate concentration significantly affected the HP ($p<0.05$). An increase in initial pH from 5 to 6.0 led to an increase in HP (Table 1). However, the decrease in HP could be observed when the initial pH was increased from 6 to 7. The greatest HP value of 202.6 mmol H_2/L was obtained at the initial pH of 6.0 when initial substrate concentration were kept at its central value indicating that the optimum initial pH for HP in this study was at 6.0. At a weak acid condition (an initial pH of 5.5-6.5), hydrogen producing bacteria such as *Clostridia* species can be activated to extrude the express proton from cytoplasm to facilitate the resumption of the cell growth as well as producing hydrogen which normally exhibited at the exponential growth phase of this species (Jianlong & Wei 2009; Phowan & Danvirutai 2014). The results were consistent with the previous study which found that pH in the range of weak acid was an optimum pH for hydrogen production by anaerobic fermentative bacteria (Jianlong & Wei 2009; Phowan & Danvirutai 2014; Sivagurunathan et al. 2016).

When the pH was kept at its central value, HP increased with an increase in substrate concentration from 1.5 to 4.75 g/L. Further increase the substrate concentration resulted in a decrease in HP (Figure 2 & Table 1). The highest HP of approximately 202.6 mmol H_2/L was obtained at the initial substrate concentration of 4.75 g/L (central value) (Figure 2, Table 1). The results showed that the change in initial total sugar concentration remarkably affected the production of hydrogen. Although increase in total sugar concentration could enhance the hydrogen production, but the higher total sugar concentration also results in the accumulation of soluble metabolites product and a drop in pH which can inhibit the growth of microorganisms (Sivagurunathan et al. 2016). In addition, the increase in total sugar concentration could increase the partial pressure of hydrogen in the headspace of reactor resulting in the inhibition of hydrogen production caused from the

TABLE 2. Analysis of variance for quadratic polynomial model of initial pH and substrate concentration on HP

Source	Sum of squares	Coefficient	DF	Standard Error	*F* -value	*P*-value Prob > *F*
Model	57,023.21	187.63	5	8.26	33.45	<0.0001
X_1	14.31	-1.34	1	6.53	0.042	0.84
X_2	3,548.93	-21.06	1	6.53	10.41	0.01
X_1^2	14796.56	-46.12	1	7.00	43.39	0.0003
X_1X_2	249.53	-7.90	1	9.23	0.73	0.42
X_2^2	44,178.33	-79.69	1	7.00	129.56	<0.0001
$R^2 = 0.96$						

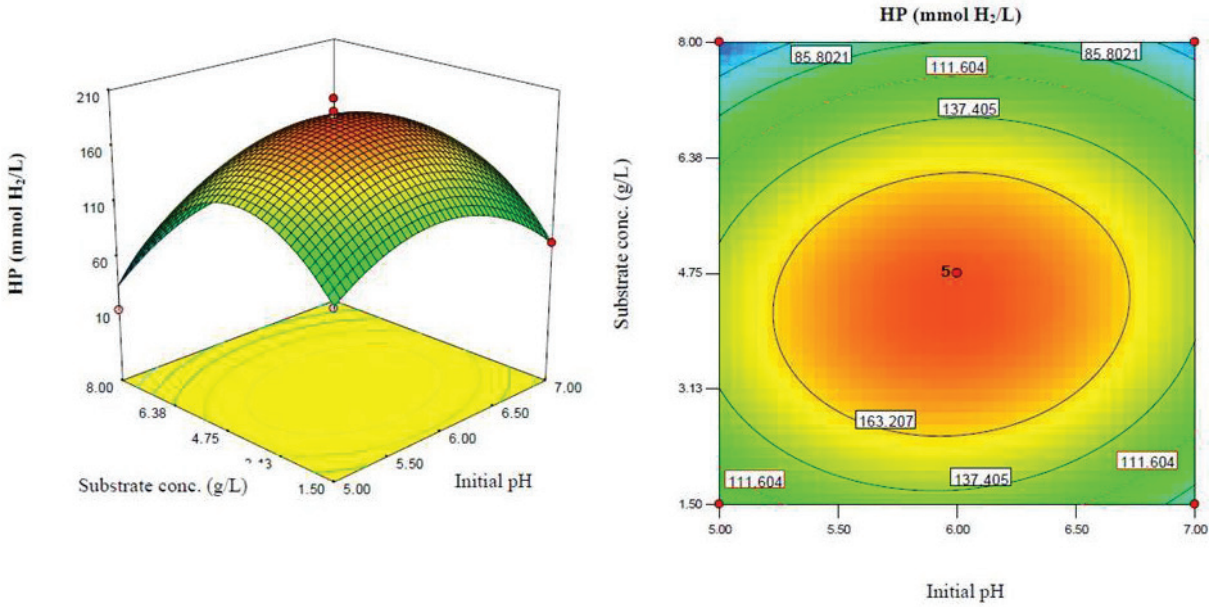

FIGURE 2. Response surface and contour plots for the effects of substrate concentration and initial pH of substrate on HP

switching of hydrogen production pathway to solvent production pathway (Table 3) (Jianlong & Wei 2009; Sivagurunathan et al. 2016).

CONFIRMATION EXPERIMENTS AND SUFFICIENCY OF THE HP MODELS

Four confirmation experiments were conducted in this study. The experimental conditions for initial pH and initial substrate concentration, experimental results of HP and hydrogen production rate (HPR) were shown in the Table 4. The results of experiment are in close agreement with the predicted values (bias less than 5%) which indicated that the obtained model was applicable for hydrogen production from WHS hydrolysate by heat-treated anaerobic sludge. Hydrogen production, hydrogen production rate (HPR) and hydrogen yield (HY) of 182.7 mmol H_2/L, 2.81 mmol H_2/L h and 0.84 mol H_2/mol hexose, respectively, were obtained. The optimal conditions were initial pH of 5.81 and total sugar concentration of 4.06 g/L. The hydrogen production obtained from this study (182.7 mmol H_2/L,) was lowest compared with the study of Cheng and Chang (2011) (Table 5). However, it's was higher compared with the HY obtained using xylose as the feedstock (Long et al. 2010). The maximum HY obtained from this study was compared low with those reported in literatures (Table 5). This disciplinary might be due to the different inoculums

TABLE 3. pH change and total sugar degradation and soluble metabolites distribution at the final incubation time

Run	pH		Total sugar degradation[a] (%)	Soluble metabolites (mg/L)					
	Initial	Final		HAc	HBu	HPr	EtOH	HBu/HAc	HBu/TVFAs (%)
1	5.01	4.07	88.79	3.88	2.03	8.64	2491.31	0.52	13.97
2	5.02	4.12	95.67	4.75	1.23	4.10	4316.46	0.26	12.21
3	6.13	4.27	91.31	7.46	24.75	3.94	470.50	3.32	68.47
4	6.03	4.13	94.31	6.22	1.45	5.57	4297.23	0.23	10.95
5	6.12	4.40	92.08	2.69	3.00	0.10	309.53	1.11	51.78
6	7.03	4.20	93.41	15.73	8.96	0.16	4177.17	0.57	36.06
7	6.05	4.38	87.91	7.26	23.95	1.83	996.06	3.30	72.49
8	6.05	4.18	83.42	6.94	22.29	2.83	1158.90	3.21	69.53
9	6.14	4.54	89.68	6.82	22.26	3.33	1340.98	3.26	68.68
10	6.12	4.41	92.77	6.91	24.57	2.62	1432.94	3.56	72.05
11	4.63	3.58	82.51	5.17	3.84	0.58	3762.31	0.74	40.04
12	7.44	4.42	94.94	13.60	32.06	3.47	1705.94	2.36	65.25
13	7.03	4.44	82.44	13.46	14.99	0.93	1547.66	1.11	51.03

HAc: acetic acid; HBu: normal butyric acid; HPr: propionic acid; EtOH: ethanol; TVFAs (total volatile fatty acids) = HAc+HBu+HPr.
[a]Calculated by $(TS_i-TS_f)/TS_i$ x 100%, where TS_i and TS_f denote initial and final total sugar concentration, respectively

TABLE 4. Predicted and measured HP value, HPR and bias in the confirmation experiments

Run	Variables				HP (mmol H_2/L)		Bias [a] (%)	Hydrogen production rate (HPR) (mmol H_2/L h)
	Initial pH (X_1)		Substrate concentration (g/L) (X_2)		Predicted	Measured		
	Code	Real	Code	Real				
14	-1.00	5	0.00	4.75	142.9	140.7	1.38	1.57
15	0.00	6	1.00	8.00	86.9	84.5	2.78	1.25
16	1.00	7	0.00	4.75	140.2	136.5	2.63	1.74
17	optimum	5.81	optimum	4.06	187.4	182.7	2.49	2.81

[a]Bias was calculated using the equation: [(predicted value – experimental value)/predicted value] × 100

type and operation condition. The results from this study showed potential of hydrogen production from WHS hydrolysate by heat-treated anaerobic sludge.

METABOLIC PRODUCTS AT THE END OF HYDROGEN PRODUCTION FROM WHS HYDROLYSATE

Anaerobic hydrogen production is always accompanied with VFAs production. The results of production of soluble metabolite products (SMP) including HBu, HAc, HPr and EtOH during hydrogen fermentation were summarized in Table 3. The main VFAs type in the fermentative broth was butyrate (accounting for 72.49% of total VFAs) in which the ratio of HBu/TVFAs was highest in fermentative broth at central value (run 7) while acetate is the second VFAs type in central value (run 7). The previous research also reported that hydrogen production by heat-treated anaerobic sludge was essentially butyrate-type fermentation and can be described by (3) (Fangkum & Reungsang 2011).

$$3C_5H_{10}O_5 + 4C_6H_{12}O_6 + 3H_2O \longrightarrow 5C_4H_8O_2 + 3C_2H_4O_2 + 13CO_2 + 16H_2 \quad (3)$$

Ethanol could be observed in the fermented broth at the end of incubation (Table 3) indicating the occurrence of solvent phase which could be taken place when VFAs were accumulated in the hydrogen production system using hydrogen producing bacteria (Lin & Lay 2004). The formation of solvent such as ethanol in metabolic pathway of *Clostridia* species was due to a development of new enzyme system at a drop of pH to approximately 4 in order to be able to survive at a low pH (Vazquez et al. 2015). Hence, the results implied that the pH control at an optimum value is needed in order to obtain high hydrogen produced.

ENERGY PRODUCTION

The total energy production from WHS hydrolysate can be calculated based on the hydrogen production (mL H_2/L), relative density of hydrogen (0.089 kg-H_2/m^3-H_2) as well as the heating values of hydrogen (121 MJ/kg-H_2). Thus the energy prouction from WHS hydrolysate is as follows:

TABLE 5. Hydrogen Production (HP) and hydrogen yield (HY) in this experiment and literature search

Substrate/ pretreatment	Microorganism	Optimum conditions	HP (mmol H_2/L)	HY (mol H_2/mol hexose)	Reference
Wheat straw (hydrothermal pretreatment)	Enrichment culture	70°C	-	1.59	Kongjan & Angelidaki 2010
Wheat straw (hydrothermal pretreatment)	Enrichment culture	70°C	-	2.56	Kongjan et al. 2010
Sugarcane bagasse (Acid and bacteria)	*C. butyricum*	35°C, pH7.50	-	1.08	Lo et al. 2011
Bagasse (alkaline and enzymatic)	*C. pasteurianum* CH4	35°C, Endo-nutrient	1420	0.96	Cheng & Chang 2011 Long et al. 2010
Xylose	*Enterobacter* sp. CN1	16.15 g/L xylose, 250.17 mg/L $FeSO_4$, 2.54 g/L peptone	51.33	-	This study
WHS (Acid)	Heat-treat sludge	4.06 g/total sugar, pH5.81, room temp. (30°C)	182.7	0.84	

Energy from H_2 = 182.7 mL H_2/L × [0.089 kg-H_2/ m^3-H_2 × 1 m^3-H_2/1000000 mL H_2] × [121 MJ/ kg-H_2] = 0.00197 MJ
= 1.97 kJ.

Therefore, the total energy generated from WHS hydrolysate was 1.97 kJ.

CONCLUSION

The factor affecting hydrogen production (HP) from diluted acid hydrolysate of water-hyacinth stem (WHS) by heat-treated anaerobic sludge was optimized by RMS with CCD. This results showed a potential application for converting water hyacinth stem into biohydrogen by heat-treated anaerobic sludge. The initial pH and substrate concentration were significantly affected on hydrogen production. The maximum HP and HPR of 182.7 mmol H_2/L and 2.81 mmol H_2/L h were obtained at 4.06 g/L substrate concentration and initial pH of 5.81. The main VFAs type in the fermentation broth was butyrate and following with acetate. This indicated that the main hydrogen production process was butyrate type fermentation. Under the optimum condition, the total energy generated from the fermentative process was 1.97 kJ.

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