Statistical Optimization of Biohydrogen Production Using Food Waste Under Thermophilic Conditions

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Abstract: In this study, optimization of biohydrogen production from food waste was investigated using response surface methodology. The fermentation was conducted in a serum bottle with 100 mL working volume. A Preliminary experiment showed that initial pH and temperature significantly influenced biohydrogen production. According to the central composite design, the optimal conditions for hydrogen yield were initial pH of 7.5 and temperature of 55.7^{o}C, while the optimal conditions for hydrogen production rate were initial pH of 7.2 and temperature of 55.6^{o}C. The maximum values for hydrogen yield and hydrogen production rate were 120 mL/g carbohydrate and 35.69 mL/h, respectively.

Keywords: Biohydrogen, hydrogen, response surface methodology, food waste.

INTRODUCTION

Hydrogen gained a lot of attention to replace fossil fuel in the energy and chemical industry. Renewable energy sources should decrease carbon dioxide emission and reduce the consumption of fossil fuel. Energy from hydrogen is most important since it is zero in carbon emission and the end product of it is pure water (Atif *et al.,* 2005) [1]. Among those of the biological processes, the dark fermentation of organic compounds is most preferable because it is technically simple and can use a wide range of organic substances (Hallenback and Benemann, 2002) [2]. The anaerobic fermentation of organic compounds involves two distinct stages: acidogenesis and methanogenesis. Hydrogen is produced as a by-product during the acidogenesis of sugars to organic acids. Hydrogen can be harvested during the acidogenesis process, leaving the remaining acidogenesis product for methanogenesis (Mizuno *et al.,* 2000) [3]. Food waste is a waste composed of raw and cooked materials including food discarded before and during food preparation. In Malaysia, food waste is the most abundant and problematic organic waste. The average amount of food waste generated was 0.8 kg/person/day and increased to 1.7 kg/person/day in major cities. Food waste makes up almost 30.84-54.04 % of municipal solid waste depending on the type of residential area (Kathirvale *et al.,* 2003) [4]. Most of the food waste is contributed by domestic and commercial kitchens. Currently, the waste management approach being employed is the landfill technique. However, this process can cause environmental damage such as increasing of methane gas and attracts on flies and vermin. Food waste is carbohydrate-rich and easily hydrosable and these characteristics make it suitable use as substrate in fermentative biohydrogen production (Han and Shin, 2004) [5].

Response surface methodology (RSM), a collection of empirical models and statistical analyses, had been used to study the effects of several factors on hydrogen production rate and hydrogen production yields (Kim *et al.,* 2004) [6]. The concept of RSM has eased the optimization process and it is also a time saving method, which minimizes the errors in determining the effects of the parameters (O-Thong *et al.,* 2008) [7]. Kim *et al.* (2004) [6] used RSM to investigate the effects of various volatile solid (VS) concentrations and the mixing ratios of two substrates, food waste and sewage sludge on biohydrogen production. The aims of this study were to determine the optimal conditions for biohydrogen production from food waste under thermophilic conditions and the relationship among the factors on biohydrogen production by using RSM.

MATERIALS AND METHODS

Seed Sludge

The seed sludge was taken from a settling tank in a local palm oil mill wastewater treatment plant at Serting Hilir, Negeri Sembilan, Malaysia. The pH and volatile suspended solids (VSS) of the palm oil mill effluent sludge were 7.26 and 84.5 g/L, respectively. The sludge was heat-treated at 80^{o}C for 20 minutes (Lin and Chang, 2004) [8] to inhibit methanogens and to selectively enrich spore forming bacteria (Mohan *et al.,* 2008) [9].

Substrate

Food wastes were taken from Serumpun College Cafeteria, Universiti Putra Malaysia, Selangor, Malaysia. The food waste was blended with water using an electric blender and then the blended food waste was filtered using a sieve with

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1876-3871/09

Table 1. Characteristics of Food Waste

Characteristics	Range	Mean
pH	6.1 - 6.4	6.25
COD (g/L)	285.8 - 376.6	331.2
Total solid (g/L)	215.7 - 295.2	255.45
Ammonia (g/L)	0.145 - 0.266	0.205
Total sugar (g/L)	49.26 - 62.10	55.68
Moisture (%)	71 - 73	72
Protein (%)	24.49 - 31.2	27.845
Fat (%)	22.98 - 28.58	25.78
Fiber (%)	1.069 - 1.34	1.2045
Ash (%)	3.99 - 6.28	5.135

an opening size of 2.00 mm (Kim *et al.,* 2004) [6]. The characteristics of the food waste are summarized in Table **1**.

Operating Procedures

The optimization procedure using response surface methodology contains two parts. The purpose of the 2-level factorial was to determine the significant factors involved and the second part (central composite design) was used to obtain the optimal value for each significant factor. The fermentation was done in a serum bottle with the capacity of 160mL. The volume of the food waste used for the fermentation is 100 mL. To each bottle, $NaHCO_3$ was added at 0.01g/mL as a buffer to slow down the reduction of pH (O-Thong *et al.,* 2008) [7]. Subsequently, the media and the headspace of the bottles were flushed with N_2 gas for 10 minutes and the bottles were tightly sealed with aluminum seal caps and rubber septas. The bottles were incubated in a water bath for 24 hours and sampling of the biogas was done at three hour intervals time. The volume of the biogas produced was determined using a 500cc mL syringe (Owen *et al.,* 1979) [10]. The gas composition was measured and a sample from the fermentation broth was then analyzed.

Optimization Using Response Surface Methodology

2-Level Factorial Screening

The experiment for the 2-level factorial screening was done 18 runs. The variables used were chemical oxygen demand (COD) of the substrate, initial pH, temperature, and inoculum size. The Total COD of the substrate was controlled to be 100 g/L, 150 g/L and 200 g/L. The initial pH was adjusted at 5, 6 and 7 using 1 M NaOH and 1 M H_2SO_4 and the experiments were done at different incubation temperatures; 50°C, 55°C and 60°C. The inoculum sizes for the fermentation were 15%, 22.5% and 30%. The total carbohydrate range of the substrate was 25–50g/L. Table **2** shows the four variables involved in the design to evaluate their effects on biohydrogen production. Each independent variable was investigated at high (+1) and low (-1) levels. Runs of centre points were included in the design and statistical analysis was used to identify the effects of each variable. The variables having major effects on biohydrogen production were identified on the basis of confidence level above 95% ($P<0.05$).

Central Composite Design

The second part of the optimization using RSM was the central composite design (CCD) to obtain the optimal value of the tested factor. The CCD runs were developed depending on the number of factors considered for optimization. In this study, the significant factors for biohydrogen production were initial pH and temperature. The design included 5 centre points and also the variables were set at extreme levels (-2 and +2). Table **3** shows the actual and the coded levels of the variables tested for biohydrogen production. The experiment for the central composite design was done 21 times. The initial pH was adjusted to 5,6,7,8 and 9. The incubation temperatures were 45, 50, 55, 60 and 65°C. The responses obtained were statistically evaluated and models were built based on variables with confidence levels is more than 95%.

ANALYTICAL METHODS

The hydrogen, CH_4, N_2 and CO_2 contents in the biogas were measured using a gas chromatography (GC, Shimadzhu 17A) with a thermal conductivity detector (TCD) and a 1.83m × 3.18mm (inner diameter) stainless-steel column packed with Porapak Q (80/100 mesh) with molecular sieve 5A and N_2 as the carrier gas. The temperatures of the injector, detector and column were 100°C, 100°C and 50°C, respectively. The levels of organic acids (lactate, formate, acetate, propionate, n-butyrate and iso-butyrate) were analyzed using a high performance liquid chromatography (Shimadzhu LC-10AS with UV-VIS detector SPD-10A) with 4mM H_2SO_4 as mobile phase at a flowrate of 0.6 mL/min. The liquid samples were centrifuged, and the supernatants were used for the analysis of organic acids using HPLC. The carbohydrate levels was analysed using the Phenol-Sulphuric acid method (Dubois *et al.,* 1956) [11].

DATA ANALYSIS

The Modified Gompertz Equation (1) was used to describe the hydrogen production in the batch test (Lee *et al.,* 2001) [12].

$$H = P \times \exp\left[-\exp\left\{\frac{Rm}{p}(\lambda - t)e + 1\right\}\right], \quad (1)$$

Where H is cumulative hydrogen production (mL), P is ultimate hydrogen production (mL), Rm is hydrogen production rate (mL/h), λ is lag-phase time (hours), and e is exponential 1.

In this study, the Modified Gompertz Eq. (1) was used to fit the cumulative hydrogen production to obtain H, Rm and λ. The hydrogen production yield was calculated by dividing the cumulative hydrogen by the amount of carbohydrate consumed during the fermentation.

Table 2. Coded and Real Values for Screening by 2-Level Factorial Design

	Variable	Unit	-1	0	+1
A	COD	g/L	100	150	200
B	Initial pH		5	6	7
C	Temperature	^{o}C	50	55	60
D	Inoculum Size	% (v/v)	15	22.5	30

Table 3. Coded and Real Values of Variables Selected for CCD

	Variable	Unit	-2	-1	0	+1	+2
A	Initial pH		5	6	7	8	9
B	Temperature	^{o}C	45	50	55	60	65

Eq. (2) was used to fit the experimental data obtained in the 2-level factorial design.

$$Y = X_O + X_1A + X_2B + X_3C + X_4D + X_{12}AB + X_{13}AC + X_{14}AD + X_{23}BC + X_{24}BD$$

$$+ X_{34}CD + X_{123}ABC + X_{124}ABD + X_{134}ACD + X_{234}BCD \quad (2)$$

where Y is the response, A, B, C and D are the actual values, Xo is a constant, X_1, X_2, X_3 and X_4 are the linear coefficients, X_{12}, X_{13}, X_{14}, X_{23}, X_{24}, X_{34}, X_{123}, X_{124}, X_{134} and X_{234} are the interactive coefficients.

A quadratic model (3) was used to fit the experimental data obtained in the central composite design.

$$Y = X_O + X_1A + X_2B + X_{12}AB + X_{11}A^2 + X_{22}B^2 \quad (3)$$

where *Y* is the response, A and B are the actual values, Xo is constant, X_1 and X_2 are linear coefficients, X_{12} is the interactive coefficient and X_{11} and X_{22} are quadratic coefficients.

Analysis of variance (ANOVA) was conducted to test the significance of the fitting model for the experimental data, as well as the significance of the linear terms, interactive terms and the quadratic terms. The parameters were diagnosed by correlation coefficient, R^2, 95% confidence limit, F-value and P-value. In general, the model was considered to be efficient and workable if it had a significant F-value and good R^2 (correlation coefficient). The conditions that could give maximum biohydrogen production were predicted using numerical optimization contained in the Design-Expert 7.0 software (Stat-Ease Inc.). Only the variables considered in model building were varied for prediction, other insignificant variables were maintained at constant values ('0' coded level) as in the 2-level factorial design.

RESULTS AND DISCUSSION

The hydrogen production curves from the fermentation were subjected to the eq. (1). The hydrogen production potential, *P* (mL H_2/100 mL substrate), correlation coefficient, R^2 and Rate, *Rm* (mL/day) can be obtained from the equation (1). The values of hydrogen production rate, *Rm* and yield were subjected to the response surface methodology to evaluate the relationship among the studied factors and responses. The final pHs after 24 hours incubation were in the range of 3.88 -5.56.

Two-Level Factorial Design

All of the 18 runs in the 2-level factorial design successfully produced hydrogen gas through the fermentation and the cumulative hydrogen production curves from all the runs were well-described by Eq. (1). Table **4** shows the R^2 of the hydrogen production curve obtained from Eq. (1) and results of hydrogen potential, yield and hydrogen production rate. The results showed that the correlation coefficients, R^2, for all the runs were larger than 0.9768. The maximum hydrogen production potential, yield and rate 248.9 mL/100 mL substrate, 164.3 mL/g CHO and 38.91 mL/h, respectively and the minimum were 0.8 mL/100 mL substrate, 3.5 mL/g CHO and 0.17 mL/h, respectively.

Eq. (4) was obtained by using Eq. (2) to fit the experimental data of the hydrogen production yield.

$$(\text{Yield}) = -190.44964 + 6.50291\ A - 124.74207\ B + 10.10027\ C - 73.06939\ D$$
$$+ 0.30885\ AB - 0.15803\ AC - 0.03679\ AD + 1.25615\ BC + 18.23742\ BD$$
$$+ 1.10039\ CD + 0.012433\ ABC - 8.47E\text{-}003\ ABD + 1.60E\text{-}003\ ACD - 0.2954\ BCD \quad (4)$$

where A,B,C and D are the actual values of substrate concentration COD, initial pH, temperature and inoculum size, respectively.

The ANOVA analysis (Table **5**) shows that the prob >F values for the model were significant for both responses (rate = $p<0.05$, yield = $p<0.05$). Correlation coefficient, R^2 for the

Table 4. Kinetics Parameters for Hydrogen Production Calculated from Eq. (1)

RUN	Cod Substrate (g/L)	Initial PH	Temperature (^oC)	Inoculum Size (% v/v)	R^2	H_2 Potential,*P* (mL)	Yield (mL/g CHO)	Rate, Rm (mL/h)
1	100	5	50	15	0.9982	26.6	19.5	5.79
2	200	5	50	15	0.9993	80.2	44.8	18.83
3	100	7	50	15	0.9981	116.4	48.2	14.27
4	200	7	50	15	0.9991	248.9	96.4	32.25
5	100	5	60	15	0.9999	49.2	64.3	10.09
6	200	5	60	15	0.9998	2.0	3.5	0.38
7	100	7	60	15	0.9995	67.8	40.9	7.65
8	200	7	60	15	0.9990	75.4	56.5	14.32
9	100	5	50	30	0.9920	50.3	28.3	8.9
10	200	5	50	30	0.9981	61.1	40.9	16.59
11	100	7	50	30	0.9998	234.1	125.6	38.78
12	200	7	50	30	0.9992	194.8	164.3	38.91
13	100	5	60	30	0.9979	26.9	28.5	8.64
14	200	5	60	30	0.9768	0.8	7.7	0.17
15	100	7	60	30	0.9987	99.5	14.65	15.59
16	200	7	60	30	0.9978	18.4	16.1	5.75
17	150	6	55	22.5	0.9989	133.5	33.9	27.00

rate was 0.9887 and the yield was 0.9930. The results of the prob>F and the correlation coefficient showed that the model was good. However, for the variables, only initial pH and temperature were significant factors ($p<0.05$) for hydrogen production potential and yield. This was in agreement with a previous report that showed temperature and initial pH gave impacts on fermentative hydrogen production individually and interactively (Wang and Wan, 2008) [13]. The maximum hydrogen production potential and yield were 248.9 mL and 164.3 mL/g CHO, respectively. The maximum biohydrogen production occurred at substrate concentration of 200 g/L COD, temperature of 50^{o}C and initial pH 7. However, for maximum hydrogen production potential, the appropriate inoculum size was 15% (v/v) while for yield it was 30% (v/v).

Central Composite Design

Table **6** shows the results of the hydrogen potential, yield and rate of hydrogen production in the central composite design. At lower temperature (<45^{o}C), the results showed that no hydrogen was produced in run 13 and 14. The other runs successfully produced hydrogen throughout the fermentation and the hydrogen production curves were fitted to the Eq. (1). All the correlation coefficients, R^2, were larger than 0.9938 as shown in Table **6**. The results of yield and rate were subjected to Eq. (3).

Effects of Initial pH and Temperature on Hydrogen Production Yield

Eq. (5) was obtained by using Eq. (3) to fit the experimental data of hydrogen production yield.

$$(\text{Yield}) = -3557.82 + 211.03A + 130.37B - 0.60AB - 11.8A^2 - 0.88B^2 \quad (5)$$

where A and B are the actual values of the initial pH and temperature, respectively.

ANOVA of the fitting model (Table **7**) shows that the model was highly significant ($p<0.01$), while the lack of fit was not significant ($p>0.05$). Correlation coefficient, R^2, was 0.8823, which could explain 88.23% of the variability of the response variable. All these show the Eq. (4) could describe the effect of temperature and initial pH on the hydrogen production yield. ANOVA of the fitting model (Table **7**) also shows the linear, quadratic and interactive effects between temperature and initial pH on hydrogen production yield. The linear and quadratic effect were significant ($p<0.05$).

Table 5. ANOVA Analysis for the Hydrogen Production Potential and Yield

Source	Rate		Yield	
	F-Value	Prob >F	F-Value	Prob >F
Model	29.07	0.0026	20.4	0.0477
Substrate concentration (g/L)	3.05	0.1557	2.2	0.276
Initial pH	95.99	0.0006	64.28	0.0143
Temperature (^{o}C)	124.44	0.0004	68.49	0.0143
Inoculum size (% v/v)	8.82	0.0411	1.62	0.3305
R^2	0.9887		0.9930	

(Prob >F less than 0.05 indicate that the model terms are significant)

Table 6. Kinetics Parameters of Hydrogen Production Calculated from Eq. (1)

Run	Initial pH	Temperature (^{o}C)	R^2	H_2 Potential, *p* (mL)	Yield (mL/g CHO)	Rate, *Rm* (mL/h)
1	6	50	0.9996	97	51	5.64
2	6	50	0.9989	97	50	5.48
3	8	50	0.9994	97	87	11.36
4	8	50	0.9995	112	88	21.29
5	6	60	0.9968	64	83	8.96
6	6	60	0.9999	110	73	23.50
7	8	60	0.9999	177	117	26.05
8	8	60	0.9981	138	89	15.30
9	5	55	1.0000	12	36	2.76
10	5	55	1.0000	12	31	2.73
11	9	55	0.9999	95	82	7.44
12	9	55	0.9998	80	62	5.65
13	7	45	0	0	0	0
14	7	45	0	0	0	0
15	7	65	0.9968	16	22	3.27
16	7	65	0.9938	16	24	3.54
17	7	55	0.9988	150	93	35.69
18	7	55	0.9997	155	93	31.28
19	7	55	0.9992	145	104	31.62
20	7	55	0.9980	117	120	26.05
21	7	55	0.9996	135	78	27.30

This indicated that these terms had impact on hydrogen production yield. However, the results showed that the interactive effects between temperature and initial pH was not significant ($p>0.05$), indicating this term had little impact on hydrogen production yield. The fact that there was insignificant effect between the initial pH and temperature on hydrogen yield suggested that these factors did not affect each other.

Table 7. ANOVA of Fitting Model for Hydrogen Production Yield

Source	Sum of Squares	Degree of Freedom	Mean square	F- Value	P- Value
Model	22625.85	5	4525.17	22.49	<0.0001
A	3220.17	1	3220.17	16.00	0.0012
B	1320.17	1	11320.17	6.56	0.0217
AB	72.00	1	72.00	0.36	0.5587
A2	5103.21	1	5103.21	25.36	0.0001
B2	17795.52	1	17795.52	88.43	<0.0001
Residual	3018.72	15	201.25		
Lack of Fit	1392.02	3	464.01	3.42	0.0526
Pure Error	1626.70	12	135.56		
Cor Total	25644.57	20			

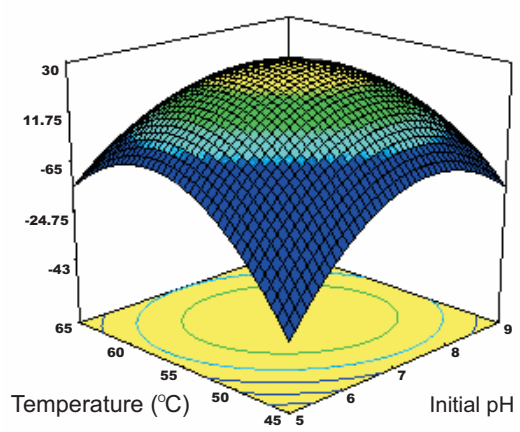

Fig. (1). Response surface plot for hydrogen production yield.

The maximum hydrogen yield obtained of 120 mL/g carbohydrate consumed, at a temperature of 55.7°C and an initial pH 7.5 was higher when compared to Zhang *et al.* (2003) [14] who reported that the yield obtained was 92 mL/g carbohydrate using wastewater containing starch as a substrate at an initial pH 6.0 and temperature of 55°C by using conventional method (one-factor-at-a-time). It was suggested that food waste may contain high concentration of carbohydrate (200 g/L) and at thermophilic conditions, a high yield of hydrogen was observed. High hydrogen production was also observed under thermophilic conditions when compared to mesophilic conditions (O-Thong *et al.*, 2008) [7]. However, the biohydrogen yield obtained in this study was slightly lower compared to Kim *et al.* (2004) [6] and this might be due to the uncontrolled pH during the hydrogen fermentation.

Fig. (**1**) shows the response surface plot of the model based on Eq. (4) for hydrogen production yield. The maximum hydrogen production yield could be obtained inside the design boundary. The yield increased with the increase of the initial pH and temperature until the optimal value and then decreased with further increases of the initial pH and temperature.

Effects of Initial pH and Temperature on Hydrogen Production Rate

Eq. (6) was obtained by using Eq. (3) to fit the experimental data of hydrogen production rate.

$$(\text{Rate}) = -1347.39 + 115.87A + 34.61B - 0.46AB - 6.30A^2 - 0.28B^2 \quad (6)$$

where A and B are the actual values of initial pH and temperature, respectively.

ANOVA of the fitting model (Table **8**) shows the model was highly significant ($p<0.01$), while the lack of fit was not significant ($p>0.05$), correlation coefficient (R^2) was 0.8475, which could explain 84.75% of the variability of the response variable. Eq. (5) could describe the effects of initial pH and temperature on hydrogen production rate. ANOVA of the fitting model (Table **8**) also shows the linear effect and the quadratic effect of temperature and initial pH, and the interactive effect between temperature and initial pH. The quadratic effect of the temperature and initial pH was highly significant ($p<0.01$) indicating that these terms greatly affect the hydrogen production rate. However, the linear effect and the interactive effect between temperature and initial pH were not significant ($p>0.05$) indicating that these terms had little impacts on the hydrogen production rate.

Subsequently, the maximum hydrogen production rate obtained was 35.69 mL/h at a temperature of 55.6°C and initial pH 7.2. Wang *et al.* (2008) [13] reported that the hydrogen production rate obtained was 28.9 mL/h at a temperature of 39.3°C, initial pH 7.0 and glucose concentration of 26.8 g/L. Kim *et al.* (2004) [6] found the highest hydrogen production rate to be 24.85 mL/h using co-digestion of food waste and sewage sludge as a substrate in mesophilic conditions. A hydrogen production rate of 1.9 mL/h was reported by Zhang *et al.* (2003) [14] at initial pH 7.0 and temperature of 55°C using wastewater containing starch as a substrate. All the experiments reported were done in small scale batch mode using carbohydrate based material as a substrate.

Table 8. ANOVA of Fitting Model for Hydrogen Production Rate

Source	Sum of Squares	Degree of Freedom	Mean Square	F- Value	P- Value
Model	2511.10	5	502.22	16.67	<0.0001
A	110.51	1	110.51	3.67	0.0747
B	102.09	1	102.09	3.39	0.0855
AB	42.78	1	42.78	1.42	0.2519
A2	1435.88	1	1435.88	47.65	<0.0001
B2	1788.17	1	1788.17	59.35	<0.0001
Residual	451.97	15	30.13		
Lack of Fit	179.40	3	59.80	2.63	0.0978
Pure Error	272.57	12	22.71		
Cor Total	2963.07	20			

Wang *et al*. (2008) [13] and Zhang *et al.* (2003) [14] observed initial pH 7 as the optimal pH for hydrogen production rate using the conventional method.

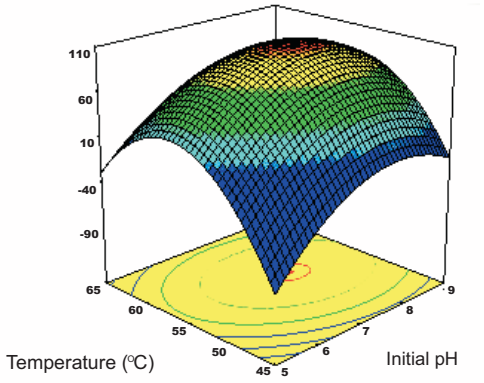

Fig. (2). Response surface plot for hydrogen production rate.

Fig. (**2**) shows the response surface plot of the model based on Eq. (5) for hydrogen production rate. The maximum hydrogen production rate could be achieved within the design boundary. The hydrogen production rate increased with the increase of initial pH and temperature until the optimal level, and then the hydrogen production rate decreased with further increases of initial pH and temperature.

Organic Acids Production

Fermentative biohydrogen normally is related with organic acids production throughout the fermentation. Hydrogen can be produced either from carbohydrates, proteins and lipids during the acidogenesis and the acetogenesis phases concurrently with the production of organic acids (Mohan *et al.*, 2008) [9]. There is a reduction of carbohydrate concentration of about 7 % to 36 % which shows that the microorganism used the carbohydrates for the production of hydrogen instead of using the proteins and lipids. Lay *et al.*, (2003) [15] reported that proteins and lipids could hardly produce hydrogen. The production of organics acids during fermentative biohydrogen is important to assess the fermentation process. Furthermore, the organic acids produced can be used as substrates for photosynthetic biohydrogen production (Kim *et al*., 2004) [6]. Table **9** shows the production of organic acids after fermentative hydrogen production for 24 hours. The table shows that the organic acids produced were lactic acid followed by acetic acid and n-butyric acid. Among the organic acids, lactic acid was the highest organic acid produced for each run. The highest organic acids production was 24.33 g/L at a temperature of 50^{o}C, substrate concentration of 200 g/L, initial pH 7.0 and inoculum size of 15% (v/v). Normally, butyric and acetic acids were the organic acids that favor the production of biohydrogen, and lactic acid was known to disfavor hydrogen production (O-Thong *et al*., 2008, Monika *et al*., 2009 and Ren *et al*., 2006) [7,16,17]. The presence of high lactic acid concentration corresponded with low production of hydrogen.

CONCLUSIONS

Temperature and initial pH had significant impacts on fermentative biohydrogen production individually and interactively according to the 2-level factorial design study. The central composite design study showed the optimal conditions for biohydrogen production yield to be initial pH 7.5 and temperature of 55.7^{o}C. The maximum hydrogen yield was 120 mL/g CHO. The maximum hydrogen production rate of 35.69 mL/h was obtained at initial pH 7.2 and temperature of 55.6^{o}C. The response surface methodology was useful for optimizing the biohydrogen production process and the predicted values under the optimized conditions were highly reproducible. The Modified Gompertz equation was successfully described the progress of cumulative biohydrogen production in the experiment.

ACKNOWLEDGEMENT

The authors would like to thank the Ministry of Science, Technology, and Innovation (MOSTI), Malaysia for the grant (R4GA 05/01/07-0195R4).

Table 9. Organic Acids Produced from Biohydrogen Fermentation after 24 Hours

Runs	Sample				Organic Acids		
	Cod Substrate (g/L)	Initial pH	Temperature (^{o}C)	Inoculum Size (% v/v)	Lactic Acid	Acetic Acid	Iso-Butyric Acid
1	100	5	50	15	5.54	0.64	0.87
2	200	5	50	15	10.22	3.04	-
3	100	7	50	15	4.60	3.10	1.51
4	200	7	50	15	15.52	8.81	-
5	100	5	60	15	2.69	2.48	2.30
6	200	5	60	15	2.83	1.32	-
7	100	7	60	15	2.34	1.33	1.03
8	200	7	60	15	5.61	2.94	-
9	100	5	50	30	3.18	1.07	4.11
10	200	5	50	30	12.47	5.62	-
11	100	7	50	30	1.30	2.28	3.47
12	200	7	50	30	13.41	7.38	-
13	100	5	60	30	1.13	1.15	0.89
14	200	5	60	30	3.85	1.13	-
15	100	7	60	30	3.38	1.53	0.21
16	200	7	60	30	9.15	1.92	-
17	150	6	55	22.5	3.18	3.14	-

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Received: August 06, 2008 Revised: October 26, 2009 Accepted: November 03, 2009