CHAPTER VI

OPTIMIZATION OF PROCESS PARAMETERS ON METHANE PRODUCTION FROM HYDROGENOGENIC EFFLUENT BY RESPONSE SURFACE METHODOLOGY

6.1 Introduction

Anaerobic digestion is a biological process known for energy recovery, especially methane, from wastewater. Not only are a recover of biogas but also volume reduction and waste stabilization are considered as its advantages (Wang et al., 2008). A two-stage anaerobic digestion process for producing hydrogen and methane from organic material has been reported (Cooney et al., 2007; Liu et al., 2006; Ting and Lee, 2007; Ueno et al., 2007; Xie et al., 2008; Zhu et al., 2008). In the first stage, the acidogenic bacteria convert substrate to hydrogen, carbon dioxide and Volatile Fatty Acids (VFAs). Then the acetogenic and methanogens convert VFAs to mainly carbondioxide and methane in the second stage (Zhu et al., 2008).

Throughout the successful process of hydrogen production from sugarcane juice, large amounts of organic effluent were generated. The main VFAs in the effluent were butyric and acetic acids which could cause the environmental problem when disposed due to its high COD value of 18,500 mg-COD/L (Pattra et al., 2008). However, the VFAs is well know as valuable substrates for methane production. Therefore, the possibility of using hydrogenogenic effluent to produce methane by anaerobic sludge from previous hydrogen fermentation was explored in this study.

In order to effectively produce methane, there is a need to study the factors affecting methane production process e.g. substrate concentration, temperature, pH, and metal ion (Wang et al., 2008). High concentration of VFAs was reported to inhibit methane production from VFAs by mixed anaerobic microorganisms (Siegert, Banks, 2005). The growth rate of methanogen can be greatly reduced at the pH value of lower than 6.6 (Mosey, Fernandes, 1989). An excessively alkaline pH can lead to disintegration of microbial granules and subsequent failure of the digestion process (Sandberg, Ahring, 1992). The optimum pH range for anaerobic digestion producing methane is 6.8-7.2 (Ward et al., 2008). Buffer capacity is often referred to an alkalinity in anaerobic digestion system, which is the equilibrium of carbon dioxide and bicarbonate ions providing a resistance to significant and rapid changes in pH of the system. Buffer capacity is proportional to the concentration of bicarbonate, therefore, $NaHCO_3$ has been widely used to create buffer system during anaerobic digestion process (Guwy et al., 1997). Speece (1996) found that alkalinity to COD concentration ratio (w/w) of 1.2-1.6 was required for sufficiently maintained the pH to be approximately 6.6 in anaerobic digestion of carbohydrate waste to produce methane.

The independent effects of substrate concentration, ratio of $NaHCO_3$ to substrate concentration and initial pH of substrate on methane production from cellulose and glucose (Siegert, Banks, 2005), industrial wastewater (Ward et al., 2008) and municipal solid waste (Liu et al., 2008) were reported in the previous studies, though to the best of our knowledge, the interactive effects of these factors on methane production from hydrogenogenic effluent, a fermentation broth after hydrogen production, of sugarcane juice have not yet been reported. To facilitate the study on the interactive effect of those parameter, a statistical experiment design technique using the response surface methodology (RSM) is widely employed (Reddy et al., 2003; Francis et al., 2003). These techniques provide statistical models which helpful for understanding the interactions between the parameters at varying levels and calculating the optimal level of each parameter for a response target (Reddy et al., 2003). An improvement of product yield, a reduction in process variability, a closer confirmation of the output response and a reduction in the experimental time and overall costs are the results of the statistical approach (Elibol, 2004; Xiong et al., 2007).

In the present work, a central composite design (CCD) of response surface methodology (RSM) has been used to optimize the process parameters for methane production from hydrogenogenic effluent of sugarcane juice by *C. butyricum*. The individual and interactive effects of substrate concentration, $NaHCO_3$ to substrate concentration ratio and initial pH of substrate on methane production were investigated.

6.2 Materials and Methods

6.2.1 Seed inoculum preparation

Seed inoculum was anaerobic sludge obtained from the municipal anaerobic wastewater treatment plant in Ube, Yamaguchi, Japan. The sludge contains (in mg/L) Total Solids (TS), 12,000±35; Suspended Solids (SS), 10,000±51; Total Volatile Solids (TVS or VS), 8,700±40 and Volatile Suspended Solids (VSS), 7,300±100. The methanogenic bacteria in seed sludge were acclimatized by incubating seed sludge in 10 g/L glucose under anaerobic condition at 30 °C for 30 days prior the usage.

6.2.2 Hydrogenogenic effluent

The effluent from previous hydrogen fermentation of sugarcane juice by *C. butyricum* with the hydrogen yield of 3.04 mol H_2/mol sucrose (Pattra et al., 2008) was used as substrate. The main VFAs contained in the effluent were butyric and acetic acids with a high COD value of 18,500 mg-COD/L. The effluent was kept at 4°C before used in this experiment. The physical and chemical characteristics of the effluent were presented in Table 39.

Table 39 Characteristics of hydrogenogenic effluent

Total volatile solids (TVS or VS, mg/L)	2,101±1.50
Chemical oxygen demand (COD, mg/L)	18,500±12
Total organic carbon (TOC, mg/L)	7,600 ±10
Total nitrogen (TN, mg/L)	252±30
Total phosphorus (TP, mg/L)	73±6
Acetic acid (HAc, mg-COD/L)	3,390±54
Butyric acid (HBu, mg-COD/L)	13,000±13
Propionic acid (HPr, mg-COD/L)	260±20
Alkali (mg $CaCO_3$/L)	2,834±18
Cl^- (mg/L)	1,586±56

6.2.3 Experimental design

To investigate the factors affecting the methane yield (MY), three parameters i.e., substrate concentration, ratio of $NaHCO_3$ to substrate concentration and initial pH of substrate were varied. A three-level–three-factor central composite design (CCD) was applied to find out the individual and interactive effects of these 3 parameters. The levels of parameters used for optimization methane production were presented in Table 40.

Table 40 Experimental range and levels of the independent variables

Variable Label	**Range and levels**				
	-α(-1.682)	**-1**	**0**	**1**	**+α(1.682)**
X_1=Substrate concentration (mg-COD/L)	6,591	10,000	15,000	20,000	23,409
X_2=Ratio of $NaHCO_3$ to substrate concentration (0.1 g $NaHCO_3$/g COD substrate)	0.64	2.00	4.00	6.00	7.36
X_3= Initial pH of substrate	4.48	5.50	7.00	8.50	9.52

A 2^3 full factorial CCD with six axial points coded ±α and six replications of center points (zero coded level) were employed to find out the interactive effects of three variables. Twenty-runs of the experiment were required for this procedure as given in Table 3. The distance from the centre point was given by $\alpha=2^{n/4}$ (n= number of factor; α =1.682). The independent variables are coded for statistical calculation according to the following equation:

$$x_i = \frac{X_i - X_o}{\Delta X} \qquad (1)$$

Where, x_i is the independent variable coded value; X_i, is the real value of the independent variable; X_o, is the real value of the independent variable on the centre point and ΔX is the step change.

The response variable, MY, was fitted to a polynomial quadratic model in order to correlate the response variable to the independent variables. The general form of the predictive polynomial quadratic equation is as follows:

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=1}^{3} A_{ij} X_i X_j \qquad (2)$$

Where X_i are the input variables, which influence the response variable Y, A_0 is the offset term, A_i is a linear effect, A_{ii}, is a squared effect and A_{ij} is the interaction effect. The input values of X_1, X_2 and X_3 corresponding to the maximum value of Y were solved by setting the partial derivatives of the functions to zero.

The MY values were regressed with respect to three factors using the software Design Expert 7.0 (State-Ease Inc., Minneapolis, USA). Subsequently, three-dimensional (3D) response surfaces and two-dimensional (2D) contour plots were built to give visual insight into the effects of these factors on MY.

6.2.4 Experimental procedure

Methane production in batch experiment was conducted in 100 mL serum bottles containing 25 ml of acclimatized seed inoculum (VSS =7,300 mg/L) and 25 ml of substrate. The serum bottles were tightly sealed with rubber septa and aluminum caped after added with seed inoculum and substrate. The headspaces of bottles were purged with argon gas for 5 min to ensure anaerobic condition, the serum bottle was incubated at 30 ^{o}C, 150 rpm. All treatments were carried out in duplicate.

6.2.5 Analytic methods

6.2.5.1 Culture broth analysis

The concentration of TS, SS, VS, VSS, COD, TN, TP, Alkali (mg $CaCO_3$/L), Cl^- in mg/L were measured according to Standard Methods (APHA, 1989) Total organic carbon (TOC) was measured using TOC analyzer (Shimadzu TOC-5000). For VFAs analysis, 3-mL culture broth was first centrifuged at 12,000 rpm for 5 min to obtain the clarified supernatant which was then acid fixed by mixing with 0.1N HCl (ratio 1:1 v/v) and filtered through 0.45 μm membrane. Concentrations of acetic, propionic and butyric acids in the filtrate were then determined by Gas Chromatography (Model 8APF, SHIMADZU, Japan) equipped with flame ionization detector (FID) and 3m x 3.2 mm glass column packed with 30/60 mesh Unisol F-200. Injector, detector and column temperatures were 250, 250 and 140°C, respectively. Nitrogen was used as carrier gas with flow rate of 50 mL/min.

The volume of biogas was measured daily by a plunger displacement method using appropriately sized wetted glass syringes following the method of Owen et al. (1978). The components of biogas in the headspace including hydrogen, nitrogen, methane and carbon dioxide were determined with a SHIMADZU (Japan) Model GC-8APT equipped with a thermal conductivity detector (TCD). Argon was used as carrier gas with 3m x 3mm diameter stainless-steel column packed with activated charcoal (60/80 mesh). The temperatures of injector, detector and column were 50, 50 and 60°C, respectively.

Table 41 Full factorial CCD matrix of substrate concentration, ratio of $NaHCO_3$ to substrate and initial pH of substrate in coded and real values on MY

Run	Code values			Real values			MY
	x_1	x_2	x_3	X_1	X_2	X_3	(mL CH_4/g-VS_{added})
1	-1	-1	-1	10,000	2	5.5	608.06 ±4.50
2	-1	-1	1	10,000	2	8.5	1143.02 ±2.32
3	-1	1	-1	10,000	6	5.5	698.80±7.55
4	-1	1	1	10,000	6	8.5	2257.73±9.48
5	1	-1	-1	20,000	2	5.5	0.88±0.06
6	1	-1	1	20,000	2	8.5	413.88±1.67
7	1	1	-1	20,000	6	5.5	15.70±0.54
8	1	1	1	20,000	6	8.5	170.74±3.32
9	-1.682	0	0	6,591	4	7.0	1037.05±10.21
10	1.682	0	0	23,409	4	7.0	796.78±2.34
11	0	-1.682	0	15,000	0.64	7.0	1425.10±10.32
12	0	1.682	0	15,000	7.36	7.0	1434.90±7.08
13	0	0	-1.682	15,000	4	4.48	0.00±0.00
14	0	0	1.682	15,000	4	9.52	5.71±0.08
15	0	0	0	15,000	4	7.0	1776.57±3.74
16	0	0	0	15,000	4	7.0	1839.83±8.45
17	0	0	0	15,000	4	7.0	1777.12±5.24
18	0	0	0	15,000	4	7.0	1754.6±6.32
19	0	0	0	15,000	4	7.0	1820.58±9.01
20	0	0	0	15,000	4	7.0	1767.06±11.08
control	-	-	-	18,500	2.8	5.5	538.88±0.16

x_1, x_2, and x_3 were the code values and X_1,X_2 and X_3 were real values of substrate concentration, ratio of $NaHCO_3$ to substrate and initial pH of substrate, respectively.

6.3 Results and discussion

6.3.1 Optimization of MY

The design matrix of the variables in the coded and real units was depicted in Table 3 with the experimental values of MY as response. MY was calculated by dividing the cumulative methane production by VS of substrate added to the bottle. The predicted values of MY were obtained from quadratic model and evaluating the relationship between substrate concentration (X_1), ratio of $NaHCO_3$ to substrate (X_2) and initial pH of substrate (X_3) as factors. The statistical model was developed by applying multiple regression analysis using the experimental data of MY, which can be given as:

$$MY = -18597.435+0.569X_1+360.593X_2+4391.152X_3-0.0179X_1X_2 \\ 0.0254X_1X_3+31.918X_2X_3-0.0000128\,X_1^2-34.850X_2^2-286.210X_3^2 \qquad (3)$$

The statistical model was tested by *F*-test, and the analysis of variance (ANOVA) for the response surface quadratic model was presented in Table 4. The ANOVA of quadratic regression model demonstrated that the model was highly significant because the *F*-test had a low probability value (Prob > $F = 0.001$) (Table 4). The regression analysis of the experimental design showed that the linear model term of substrate concentration and quadratic model term of substrate concentration were significant ($P < 0.05$) indicating that these variables had an individual effect on MY. However, the linear model term and quadratic model term of ratio of $NaHCO_3$, initial pH of substrate and interactive model terms of all variables were insignificant ($P > 0.05$) suggesting that there was no interactive effect of all three parameters on MY. A high determination coefficient, R^2, of 0.8833 could be obtained which revealed that model could explain 88.33% of variability in the response. For a good statistical model, R^2 value should be in the range of 0.75-1.0 to indicate the fit of the model (Niladevi et al., 2009). The relatively high value of R^2 indicated that the quadratic equation was able to be used instead of the experimental system under the given conditions. The plot of predicted versus experimental MY (Fig.1) depicted a correspond of x and y values which indicated that the prediction of experimental data was adequate. The second order polynomial model obtained in this study was utilized for the dependent response in order to determine the optimum conditions for methane production.

The optimum conditions for maximizing the MY calculated by this model were substrate concentration of 10,097 mg-COD/L, ratio of $NaHCO_3$ to substrate of

Table 42 Analysis of variance for quadratic polynomial model

Source	**Sum of squares**	**Coefficients**	**Mean square**	***F*-value**	***P*-value Prob > *F***
Model	968300.00	-	107,600.00	8.41	0.0013
intercept	-	-18587.43	-	-	-
X_1	14,900.00	0.569	149,000.00	11.65	0.006
X_2	72,290.73	360.593	72290.73	0.57	0.469
X_3	52,260.00	4391.153	52,260.00	4.09	0.071
X_1^2	148,300.00	-1.283E-005	148,300.00	11.59	0.007
X_1X_2	25,700.00	-0.018	25,700.00	2.01	0.187
X_1X_3	29,100.00	-0.025	29,100.00	2.28	0.162
X_2^2	28,010.00	-34.850	28,010.00	2.19	0.0.169
X_2X_3	73,346.42	31.917	73346.42	0.57	0.466
X_3^2	597,600.00	-286.210	597,600.00	46.72	<0.0001
		R^2 = 0.8833			

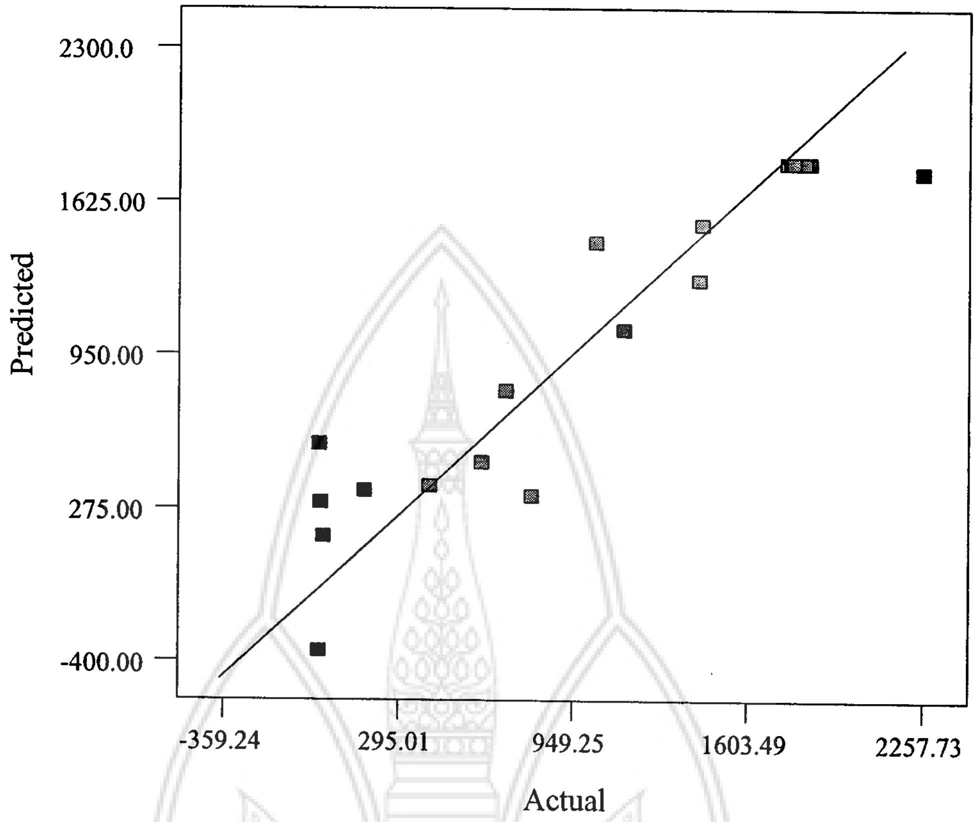


Figure 28 Predicted vs. experimental MY values.

5.53 and initial pH of substrate of 7.46. With these optimum conditions, the maximum response value (center value) for the MY of 1,789.29 mL CH_4/g-VS_{added} could be obtained in the RSM of experimental system. The maximum predicted MY at the optimum condition was 1,994.44 mL CH_4/g-VS_{added} which was close to the RSM experimental result at the center value.

Compared to the results of previous studies, the MY of 366 mL CH_4/g-VS_{added} achieved in this study was comparable to that of MY obtained from cellulose, boiled rice and fresh garbage of 356, 294 and 277 CH_4/g-VS_{added}, respectively, using one phase methane reactor (Cho et al., 1995). In addition, it was higher than the MY from the effluent of bio-hydrogen production process with the food waste as substrate of 565.76 mL CH_4/g-VS_{added} (Wang et al., 2008).

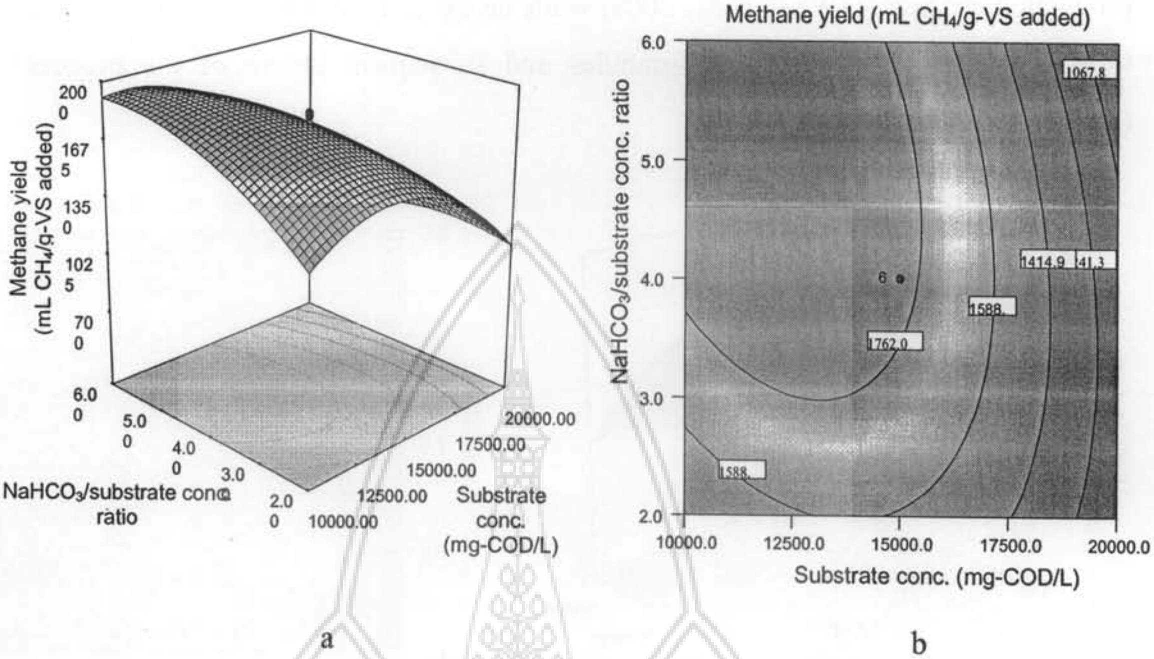
6.3.2 Effects of substrate concentration and ratio of $NaHCO_3$ to substrate on MY

The 3D response surface and the 2D contour plots of substrate concentration and ratio of $NaHCO_3$ to substrate on MY were shown in Fig. 2a-b with the graphical representations of the regression equation. The predited maximum value of substrate concentration and ratio of $NaHCO_3$ to substrate on MY was indicated at the top of surface (Fig. 2a).

The results indicated that the interactive effect of substrate concentration and ratio of $NaHCO_3$ to substrate on the MY was not significant (*P* >0.05) which confirmed by the smallest ellipse in the contour line (Fig. 2b). The shapes of contour plots, circular or elliptical, indicated a significant of the mutual interactions between the variables. Ellipse shapes of contour diagram indicated a perfect interaction between the independent variables (Muralidhar et al., 2001).

When the ratio of $NaHCO_3$ to substrate concentration and pH of initial substrate was kept at its central value, MY increased with an increase in substrate concentration from 1,000 to 1,500 mg-COD/L. Further increase in the substrate concentration resulted in a decrease of MY (Figure 29a and Figure 29b, Table 41). The highest MY of approximately 1,789.29 mL CH_4/g-VS_{added} was obtained at the initial substrate concentration of 15,000 mg-COD/L (central value) (Figure 29a, Table 41).

A decrease in MY when 23,409 mg-COD/L of substrate was used might be the result of a substrate inhibition (Table 41). An inhibitory effect of high substrate concentration generally occurs in the anaerobic digestion process depending on types of substrates and microorganisms. Borja et al. (2002) found that MY as well as VS reduction decreased remarkably when substrate concentration i.e., olive mill solid waste increased from 3 to 15 g VS/L. Murto et al. (2004) also found that the overloading of sewage sludge and pig manure (5.9 g VS/L) as a co-substrate to anaerobic digestion system resulted in the microbial inhibition and significantly reduction of methane yield.



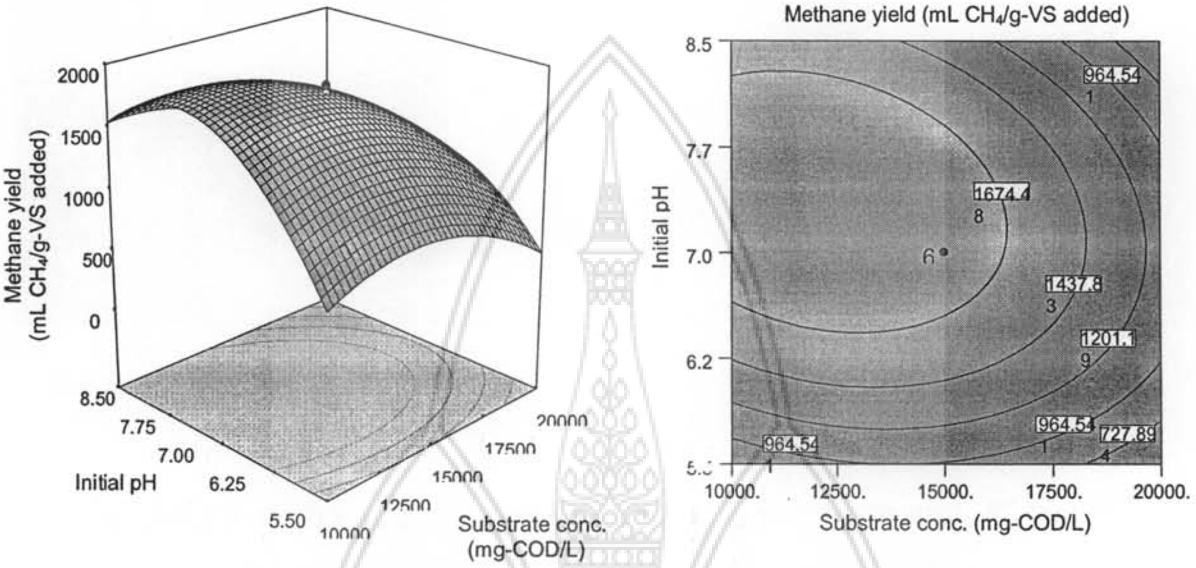
a b

Figure 29 Response surface (a) and contour plots (b) for the effects of substrate concentration and ratio of $NaHCO_3$ to substrate on the MY.

6.3.3 Effects of initial pH of substrate and substrate concentration on MY

Figure 3a-b depicted the graphical representation visualizing the relationship between the experimental levels of substrate concentration and initial pH of substrate on MY to indicate the optimum conditions. The optimum MY was indicated on the peak of response (Figure 30a) and the smallest ellipse shapes of contour plot (Figure 30b). Results indicated that the interactive effect of substrate concentration and initial pH of substrate on MY was not significant ($P > 0.05$). However, the initial pH of substrate significantly affected the MY ($P < 0.05$). An increase in initial pH from 4.48 to 7.0 led to an increase in MY (Table 41). However, the decrease of MY could be observed when the initial pH of substrate was increased from 7 to 9.52. The greatest MY value of 1,789.29 mL CH_4/g-VS_{added} was obtained at the initial pH of 7.0 when substrate concentration and $NaHCO_3$ to substrate ratio were kept at its central value indicating that the optimum initial pH for methane production in this study was at 7.0. The methane was not produced at the initial pH of 4.48 (Table 41). The previous research reported that when pH dropped to be lower than

6.5, the methanogenic bacteria would be inhibited resulting in the decrease in methane production efficiency (Wang et al., 2008) while an excessively alkaline pH could lead to a disintegration of microbial granules and subsequent failure of the process (Sandberg, Ahring, 1992).



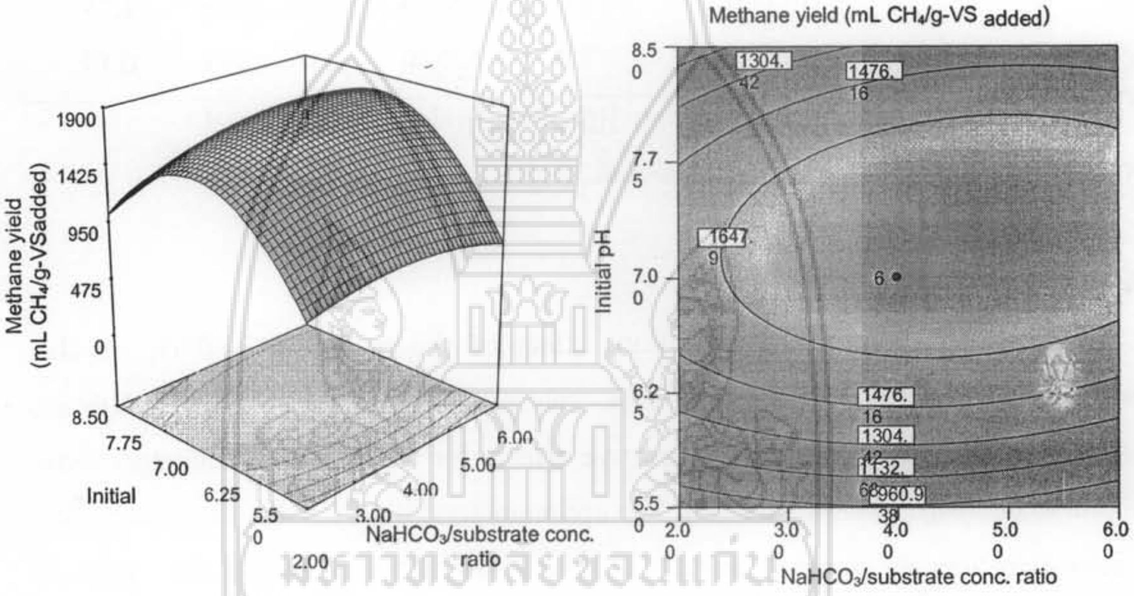
a b

Figure 30 Response surface (a) and contour plots (b) for the effects of substrate concentration and initial pH of substrate on the MY.

6.3.4 Effects of $NaHCO_3$ to substrate ratio and initial pH of substrate on MY

The interaction between $NaHCO_3$ to substrate ratio and initial pH of substrate on MY was examined in this study. The optimum value of $NaHCO_3$ to substrate ratio and initial pH of substrate of MY were indicated on the top of the surface (Figure 31a). The MY increased with an increase in the ratio of $NaHCO_3$ to substrate when substrate concentration and pH of initial substrate was kept at its central value. The highest MY of approximately 1,789.29 mL CH_4/g-VS_{added} were obtained at the ratio of $NaHCO_3$ to substrate of 4 (central value). The effect of $NaHCO_3$ to substrate ratio was weaker than substrate concentration and initial pH of substrate on MY and was statistically insignificant ($P > 0.05$). Results suggested that the interactive effect of $NaHCO_3$ to substrate ratio and initial pH of substrate on MY was not significant (P >0.05) which confirmed by the smallest circular contour plot (Figure 31b).

In anaerobic digestion processes, carbon dioxide produced via microorganisms often lead to the weak acid conditions in aqueous anaerobic systems. Therefore, sufficient bicarbonate alkalinity was required for neutralization (Guwy et al., 1997). The effect of alkalinity to substrate ratio would be occurred in the anaerobic digestion process depending on types of substrates and microorganisms. It was found that the ratio of alkalinity to COD concentration in a substrate requirement was 1.2-1.6 g $CaCO_3$/g influent COD which would be sufficient to maintain the pH above 6.6 in anaerobic digestion process of carbohydrate waste (Speece, 1996).



a

b

Figure 31 Response surface (a) and contour plots (b) for the effects of $NaHCO_3$ to substrate ratio and initial pH of substrate on MY.

6.3.5 Confirmation experiments and sufficiency of the models

The sufficiency of the predicted response was examined by conducting three additional experiments. The experimental conditions for substrate concentration, $NaHCO_3$ to substrate ratio and initial pH of substrate and experimental results of MY with the predicted values from the second-order model and the experimental values were shown in the Table 5. The predicted value for MY calculated from the polynomial quadratic equation (3) at run of 21, 22 and 23 were 1,342.83, 1,724.54 and 1,579.03 mL CH_4/g-VS_{added}, respectively. These values were close to the experimental results using the CCD which confirmed that the RSM with CCD analysis were a useful technique to optimize the methane production from hydrogenogenic effluent of hydrogen production by *C. butyricum* using sugarcane juice as substrate.

Table 43 Predicted and measured values of the confirmation experiments

Run	**Code values**			**Real values**			**MY (mL CH_4/g-VS_{added})**		**Bias%**[a]
	X1	**X2**	**X3**	**X_1**	**X_2**	**X_3**	**Predicted**	**Measured**	
21	0	0	1	15,000	4	8.5	1,342.83	1,325.60±43	1.28
22	0	1	0	15,000	6	7.0	1,724.54	1,694.42±57	1.75
23	0	-1	0	15,000	2	7.0	1,579.03	1,576.05±3	0.19

[a] Bias was calculated using the equation: [(predicted value – experimental value)/predicted value] x 100 (Cuetos et al., 2007).

6.4 Conclusions

Results demonstrated significant effect of the model ($p < 0.05$) on the improvement of MY. However, only substrate concentration had significant individual effect on MY. The interactive effects for all of these parameters were found to be insignificant ($p > 0.05$). The optimum conditions for methane production obtained in this study were substrate concentration of 10,097 mg-COD/L, ratio of $NaHCO_3$ to substrate of 5.53 and initial pH of substrate of 7.46 which gave the maximum response value for MY of 1,994.44 mL CH_4/g-VS_{added}. The model validation experiment confirmed that the MY from experimental data was close to the predicted data by using CCD and RSM. This confirmed that the RSM with CCD analysis are a useful technique to optimize the methane production from hydrogenogenic effluent.

Acknowledgements

The authors would like to thank Research Group for Development of Microbial Hydrogen Production Process from Biomass, the Commission on Higher Education, Thailand for the financial support of this study.

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