Research Paper

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Methane Production from the Effluent of Bio-Hydrogen Fermentation Process by Anaerobic Sludge using Statistical Method

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Abstract

The effects of substrate concentration, $CaCl_2$ concentration and initial pH of substrate on methane yield (MY) in methane fermentation from the effluent of biohydrogen production process using cassava pulp hydrolysate by anaerobic seed sludge were investigated in this study. A central composite design (CCD) and response surface methodology (RSM) were applied to determine the optimum conditions for methane production. The experimental results showed that the substrate concentrations significantly ($p < 0.05$) affected methane yield, while the $CaCl_2$ concentrations and the initial pH of substrate in the range of 200 to 600 mg/l and 5.5 to 8.5 respectively did not significantly ($p > 0.05$) affect methane yield. The interactive effects of all variables on methane yield were significant ($p < 0.05$). A maximum methane yield of 1984.47 ml $CH_4.g^{-1}VS_{added}$ was obtained under the optimum conditions, i.e., substrate concentration of 10,125 mg $COD.L^{-1}$, $CaCl_2$ concentration of 553.5 mg L^{-1} and initial pH of 7.46. Verification experiment of the estimated optimum conditions confirmed that the RSM and CCD were useful tools and relevant for optimizing the methane production from the effluent of bio-hydrogen fermentation of cassava pulp hydrolysate.

Keywords: Response surface methodology (RSM); methane production; effluent of bio-hydrogen process; central composite design (CCD); cassava pulp hydrolysate

1. Introduction

Anaerobic digestion is the process in which volatile organic materials are broken down in the absence of oxygen. This biological process produces biogas, principally composed of methane and carbon dioxide. Thus, it is a biological method for energy recovery as well as waste stabilization, and volume reduction [1]. A two-stage anaerobic digestion process for producing hydrogen and methane from organic material has been reported [2-6]. In the first stage, the acidogenic bacteria convert substrate to hydrogen, carbon dioxide and volatile fatty acids (VFAs). The main VFAs in the effluent were butyric and acetic acids which could be further converted by the acetogenic and methanogenic bacteria to mainly carbon dioxide and methane in the second stage [4]. Wang et al. [1] reported that after the hydrogen fermentation process, there remained high VFAs and large amount of undegraded organic matter in the effluent. Thus, further efficient digestion of the effluent in methane fermentation stage has become of great importance. Therefore, the possibility of effective utilization of the hydrogenogenic effluent to produce methane by UASB granule sludge was explored in this study.

There are several factors affecting the biogas production. These factors are both chemical and physical factors [7, 8]. Initial pH is an important factor that influences the activities of methanogenic bacteria, and the fermentative methane production. Methanogenic bacteria could grow at pH ranges which defined as 6.5 - 8.2 [9, 10, 13]. Wang et al. [1] and Jones et al. [11]

reported the optimum pH range for anaerobic digestion producing methane was 6.8 - 7.2, while Mosey and Fernandes [12] reported the growth rate of methanogen can be greatly reduced at the pH value of lower than 6.6. Borja et al. [14] reported that methane yield as well as VS reduction decreased remarkably when substrate concentration i.e., olive mill solid waste increased from 3 to 15 g VS L^{-1}. In the case of calcium ion concentration, the granulation of sludge was found to be enhanced with the suitable addition of calcium [15, 16]. Takashima and Speece [17] reported that calcium is required for stability of methyltransferase in methanogens and bacterial aggregation. Low concentrations of calcium (80 - 200 $mg.L^{-1}$) appeared to be beneficial for the development of granules, while high calcium concentrations (800 - 1000 $mg.L^{-1}$) induced a decline in specific activity of granule sludge [18].

The independent variable effects of substrate concentration, ratio of buffer to substrate and initial pH of substrate on methane production from cellulose and glucose, municipal solid waste and industrial wastewater were reported in the previous studies [1, 19, 20], but the interactive effects of substrate concentration, initial pH and $CaCl_2$ concentration on the methane yield from the effluent of bio-hydrogen fermentation from cassava pulp hydrolysate have not yet been reported. The main objectives of this study were to investigate the effects of substrate concentration, $CaCl_2$ concentration and initial pH of substrate on methane yield (MY) from bio-hydrogen fermentation process effluent of cassava pulp hydrolysate by anaerobic sludge. A CCD was used for designing the experiments for evaluating the effects of each factor. The predictive polynomial equation and RSM were used to obtain the optimum condition for producing methane and to explain the effect of experimental factors on the methane yield (MY).

2. Seed inoculum preparation and the effluent from hydrogen fermentation

The anaerobic microflora was obtained from a full-scale upflow anaerobic sludge blanket reactor located in Thailand. This reactor is being operated for the treatment of beer-processing wastewater and methane production. The physical and chemical characteristics of the sludge were presented in Table 1. Prior to use, the seed sludge was first washed five times with tap water, and was then sieved to remove stone, sand and other coarse matters. For inoculums preparation the sludge was enriched with synthetic medium according to Fan et al. [21] with initial pH adjusted to 7.0. Each liter of synthetic medium containing 2.0 g of NH_4HCO_3, 1.0 g of KH_2PO_4, 0.01 g of $MgSO_4.7H_2O$, 0.001 g of NaCl, 0.001 g of Na_2MoO_4.2H2O, 0.001 g of $CaCl_2.2H_2O$, 0.0015 g of $MnSO_4.H_2O$, and 0.00278 g of $FeCl_2$. Before inoculation, the culture was incubated at 36 ± 1°C and mixed on an orbital shaker rotated at 150 rpm for 24 h.

The effluent from hydrogen fermentation contained the high-strength VFAs. The main VFAs contained in the effluent were butyric and acetic acids with a high COD. The physical and chemical characteristics of the effluent were presented in Table 1. The effluent was stored at 4 °C before used as substrate in this experiment.

Experimental design and procedures

To investigate the interactive effects of three factors, viz. substrate concentration, $CaCl_2$ concentration and initial pH of substrate were varied for the experimental design in this study. A three-level–three-factor central composite design (CCD) was employed to find out the individual and interactive effects of these 3 variables. The levels of the variables and the experimental design used for optimization methane production are presented in Table 2.

Table 1. Characteristics of inoculum and effluent.

Items	Unit	Value	
		Inoculum	effluent
Total solids (TS)	(mg L^{-1})	13,500 ± 14	-
Suspended solids (SS)	(mg L^{-1}) \	12,000 ± 25	-
Total volatile solids (TVS)	(mg L^{-1})	9,700 ± 15	2,515 ± 12
Volatile suspended solids (VSS)	(mg L^{-1})	8,300 ± 27	-
Chemical oxygen demand (COD)	(mg L^{-1})	-	19,700 ± 43
Total organic carbon (TOC)	(mg L^{-1})	-	12,500 ± 15
Total nitrogen (TN)	(mg L^{-1})	-	1580 ± 22
Total phosphorus (TP)	(mg L^{-1})	-	178 ± 12
Acetic acid (HAc)	(mg COD L^{-1})	-	5,473 ± 14
Butyric acid (HBu)	(mg COD L^{-1})	-	11,490 ± 23
Propionic acid (HPr)	(mg COD L^{-1})	-	465 ± 12
Alkali	(mg $CaCO_3$ L^{-1})	-	1,537 ± 13

Table 2. Experimental levels of the independent variables used for optimization of methane production.

Variable	Label	Levels				
		-α(-1.682)	-1	0	1	+α(1.682)
X_1	Substrate concentration (mg COD l^{-1})	6591	10000	15000	20000	23,409
X_2	$CaCl_2$ concentration (mg l^{-1})	63.64	200.00	400.00	600.00	736.36
X_3	Initial pH of substrate	4.48	5.50	7.00	8.50	9.52

A full 2^3 factorial design with six axial points coded ±α and six replications of center points (all factors at zero level), resulting in a total 20 runs of the experiment were applied to find out the interactive effects of three variables. The procedure of the experiments is shown in Table 3. The distance from the centre point was given by $\alpha=2^{n/4}$ (for three factors n= 3; α =1.682). The independent variables in coded units were calculated 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. For predicting the optimal point, a second-order polynomial function was fitted using a polynomial quadratic equation in order to correlate the relationship between independent variables and response. The general form of the predictive polynomial quadratic equation is as follows:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{3}\sum_{j=1}^{3}\beta_{ij} X_i X_j \qquad (2)$$

Where Y is the predicated response, X_i are the input variables, which influence the response variable Y, β_0 the offset term, β_i the linear coefficients, β_{ii}, the squared coefficients and β_{ij} is the interaction effect. The quality of fit of the model equation was expressed by the coefficient of determination of R^2, and its statistical significance was determined by an F-test. The significance of the regression coefficients was tested by a t-test. The methane yield (MY) values were regressed with respect to three factors using the software Design Expert 8.0 (State-Ease Inc., Minneapolis, USA). Subsequently, two-dimensional (2D) contour plots and three-dimensional (3D) response surfaces were built to give visual insight into the effects of these factors on MY.

Experimental procedure

The batch experiments of methane production were conducted in 100 mL serum bottles using 70 mL working volume in containing with acclimatized seed inoculums (15%) and substrate. A total of 20 serum bottles with designated substrate concentration, $CaCl_2$ concentration and initial pH were simultaneously operated. Grams of inoculum, appropriate amount of substrate, distilled water, $CaCl_2$ and initial pH were operated to individual bottle according to the designation in Table 2. The bottles were tightly sealed with rubber septum and aluminum caped after added with seed inoculum and substrate. The headspaces of the bottles were flushed with argon gas to ensure anaerobic condition. The bottles were placed in a incubator shaker at 150 rpm and 35^{o}C.

Analytic methods The standard methods [22] used for analyzing the characteristics of inoculum and effluent. VFAs (acetic, propionic and butyric acids) were analyzed by using a gas chromatography (Model unisole F-200, SHIMADZU, Japan) equipped with flame ionization detector (FID) and 3m x 3.2 mm glass column packed with 30/60 mesh. Injector, detector and column temperatures were 250, 250 and 140^{o}C, respectively. Nitrogen was used as carrier gases with flow rates of 50 ml.min^{-1}.The volume of biogas was measured daily by plunger displacement method using appropriately sized wetted glass syringes following the method presented by Owen et al. [23]. The components of biogas in the headspace including hydrogen, nitrogen, methane and carbon dioxide were determined with a SHIMADZU (Japan) Model GC-2014 equipped with a thermal conductivity detector (TCD) 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^{o}C, respectively. Argon was used as carrier gas with flow rates of 50 mL.min^{-1}.

3. Results and Discussion

The design matrix of the variables in the coded and real units was depicted in Table 3 with the experimental values of methane yield (MY) as responses. MYwas 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), $CaCl_2$ concentration (X_2) and initial pH of substrate (X_3). The statistical model was developed by applying multiple regression analysis using the experimental data of methane yield (MY), which can be given as:

$$\begin{aligned} MY = & -18690.13 + 0.732X_1 + 2.376X_2 + 4176.19X_3 \\ & - 0.0248X_1X_2 + 0.0451X_1X_3 + 0.5347X_2X_3 \\ & - 0.0000120\, X_1^2 - 0.0029X_2^2 - 260.873X_3^2 \end{aligned}$$

Table 3. The central composite design matrix of substrate concentrations, $CaCl_2$ concentrations and initial pH of substrate in coded and real values on methane yield (MY).

Run	Code values			Real values			MY
	X_1	X_2	X_3	X_1	X_2	X_3	(ml CH_4.g^{-1} VS_{added})
1	-1	-1	-1	10,000	200	5.5	578.15 ± 0.09
2	-1	-1	1	10,000	200	8.5	1144.45 ± 1.39
3	-1	1	-1	10,000	600	5.5	687.25 ± 2.47
4	-1	1	1	10,000	600	8.5	2085.42 ± 0.34
5	1	-1	-1	20,000	200	5.5	1011.55 ± 0.02
6	1	-1	1	20,000	200	8.5	414.89 ± 1.27
7	1	1	-1	20,000	600	5.5	315.56 ± 0.96
8	1	1	1	20,000	600	8.5	170.44 ± 0.85
9	-1.682	0	0	6,591	400	7.0	1036.56 ± 0.75
10	1.682	0	0	23,409	400	7.0	795.74 ± 2.34
11	0	-1.682	0	15,000	63.64	7.0	1426.18 ± 2.45
12	0	1.682	0	15,000	736.360	7.0	1445.24 ± 5.42
13	0	0	-1.682	15,000	0	4.48	95.84 ± 0.05
14	0	0	1.682	15,000	400	9.52	115.71 ± 0.04
15	0	0	0	15,000	400	7.0	1774.52 ± 1.76
16	0	0	0	15,000	400	7.0	1839.84 ± 5.85
17	0	0	0	15,000	400	7.0	1785.45 ± 2.38
18	0	0	0	15,000	400	7.0	1754.65 ± 1.24
19	0	0	0	15,000	400	7.0	1819.34 ± 2.07
20	0	0	0	15,000	400	7.0	1767.55 ± 0.08

X_1, X_2, and X_3 were the code values of substrate concentration, $CaCl_2$ concentration and initial pH of substrate, respectively.

The regression analysis of the experimental design showed that the linear model terms of substrate concentration, and quadratic model terms of substrate concentration was significant and interactive model terms of all variables were significant (P < 0.05) suggesting that there was no interactive effect of all three variables on methane yield. A high determination coefficient, R^2, of 0.9637 could be obtained which revealed that model could explain 96.37 % 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 [24]. The relatively high value of R^2 indicated that the quadratic equation was able to be used for finding instead the optimal conditions of variables. The optimum conditions for maximizing the methane yield calculated by this model were substrate concentration of 10,125 mg-COD L^{-1}, $CaCl_2$ concentration of 553.5 mg L^{-1} and initial pH of substrate of 7.46. With these optimum conditions, the maximum response value (center value) for the MY of 1,790.23 mL CH_4.g^{-1} VS_{added} could be obtained in the RSM of experimental system. The maximum predicted MY at the optimum condition was 1984.47 mLCH_4.g^{-1} VS_{added} which was close to the RSM experimental result at the center value.

Effects of substrate concentration and $CaCl_2$ concentration on methane yield (MY)

The two dimensions (2D) contour plots and the three dimensions (3D) response surface of substrate concentration and $CaCl_2$ concentration on MY were shown in Fig. 1a-b. The predicted maximum value of substrate concentration and $CaCl_2$ concentration on methane yield was indicated at the top of surface (Fig. 1b). The results indicated that the $CaCl_2$ concentration on methane yield was insignificant ($P > 0.05$). However, the interactive effect of substrate concentration and $CaCl_2$ concentration on the methane yield was significant ($P < 0.05$) which confirmed in the contour line indicated a perfect interaction between the independent variables (Fig. 1a). The results accordance with Muralidhar et al. [25] who reported the shapes of contour plots were significant at circular and the shapes could be indicate the quality of interaction between the variables. Increase in the substrate concentration resulted in a decrease of methane yield when the $CaCl_2$ concentration and initial pH of substrate was kept at central value (Fig. 1a-b, Table 3). The methane yield increased remarkably with the increasing substrate concentration. The highest methane yield of approximately 1,769.54 mL $CH_4/g^{-1}VS_{added}$ was obtained at the initial substrate concentration of 15,000 mg $COD.L^1$ (central value) (Fig. 1b) The methane yield decreased when a substrate concentration increase it might be the result of a substrate inhibition (Table 3). The effect of high initial substrate concentration concentrations was prominent in the process depending on types of substrates and microorganisms. The results were in accordance with previous reports [14, 26] reported that the overloading of substrate resulted in the microbial inhibition and significantly reduction of methane yield.

Effects of initial pH of substrate and substrate concentration on methane yield (MY)

The relationship between the substrate concentration and initial pH of substrate on methane yield (MY) depicted in Fig. 2a-b. The results indicated that the initial pH of substrate on MY was insignificant ($P > 0.05$) and the substrate concentration significantly affected the MY ($P < 0.05$). However, the interactive effect of substrate concentration and initial pH of substrate on MY was significant ($P < 0.05$) (Table 4). The observations in this study are findings that increasing pH from 4.48 to 7.0 results in an increase in methane. However, methane yield was decreased when the initial pH of substrate was increased from 7.0 to 9.52. The optimum initial pH for methane production in this study was at 7.0 which was obtained the highest methane yield of 1,769.54 $mLCH_4.g^{-1}VS_{added}$ when substrate concentration and $CaCl_2$ concentration were kept at the central value. The methane was produced at the low level when the initial pH of 4.48 and 9.52 (Table 3). The results agree with Wang et al. [1] who reported the excessive of alkaline pH could inhibit the activity of methanogenic bacteria; it might be resulting in the decrease in methane production efficiency.

Effects of $CaCl_2$ concentration and initial pH of substrate on methane yield (MY)

The interaction between $CaCl_2$ concentration and initial pH of substrate on methane yield (MY) was investigated as shown in Fig.3. The Fig. 3b depicted the top of the surface was the optimum value of $CaCl_2$ concentration and initial pH of substrate of methane yield. The MY increased with an increase in the $CaCl_2$ concentration when substrate concentration and pH of initial substrate was kept at its central value. The highest MY of approximately 1,769.54 mL $CH_4.g^{-1}VS_{added}$ was obtained at the $CaCl_2$ concentration at the central value (400 $mL.L^{-1}$). The analysis of variance indicated that $CaCl_2$ concentration and initial pH had an individual insignificant on methane yield ($P > 0.05$). However, their interactive effects were significant on the MY ($P < 0.05$). The results of individual effects of variables indicating that substrate concentration was also more important factor in methane production from the effluent of bio-hydrogen fermentation process than the $CaCl_2$ concentration and initial pH of substrate. However, Ca^{2+}

may possibly enhance the anaerobic bacteria activity by increasing the biomass granulation. The reports of Yu et al. [27] presented that the granulation and the biomass accumulation was improved with addition of calcium in the reactor.

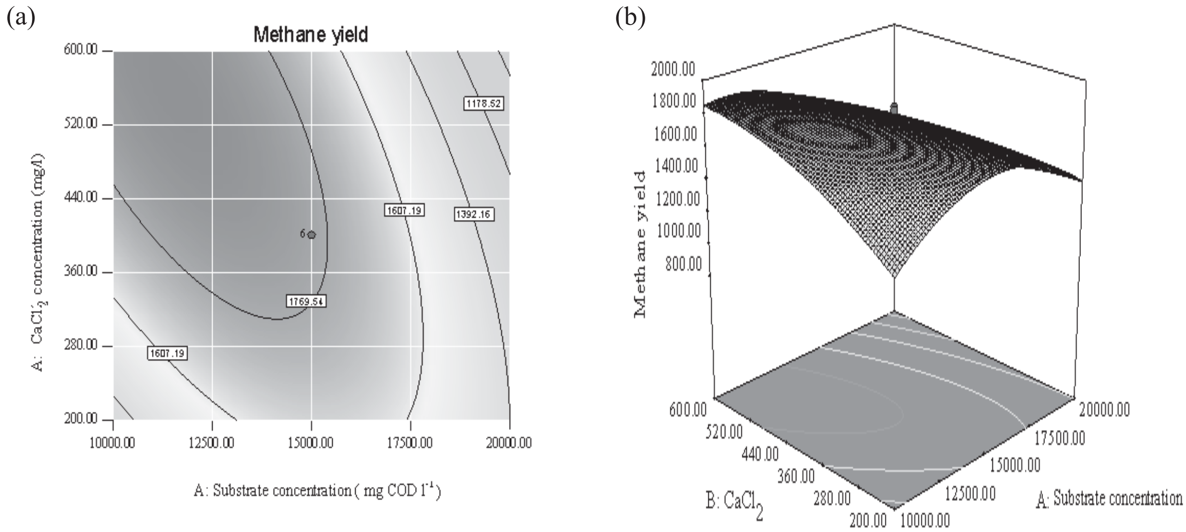

Fig. 1. The effects of substrate concentration and $CaCl_2$ concentration on the methane yield (MY) (a) Contour plots and (b) response surface.

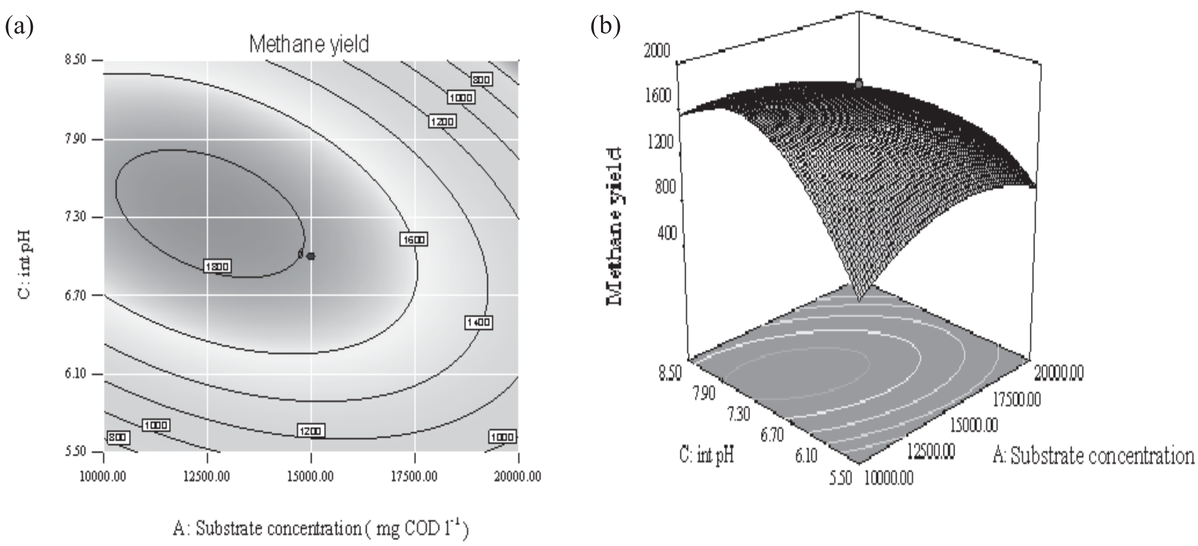

Fig. 2. The effects of substrate concentration and initial pH of substrate on the methane yield (MY) (a) Contour plots and (b) response surface.

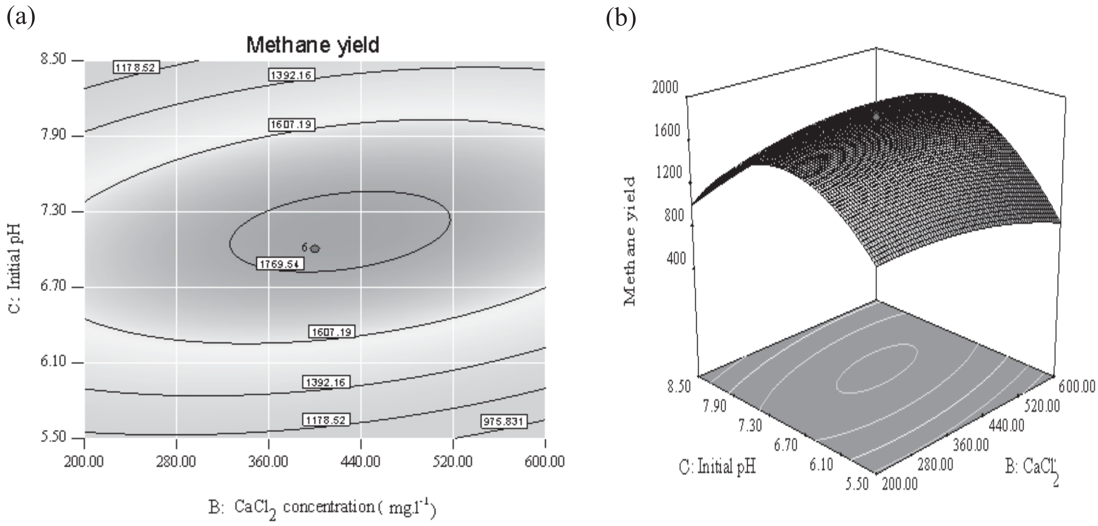

Fig. 3. The effects of $CaCl_2$ concentration and initial pH of substrate on the methane yield (MY) (a) Contour plots and (b) response surface.

Table 4. Analysis of variance for quadratic polynomial model.

Source	Sum of squares	coefficients	Mean square	*F* -value	*P*-value Prob > *F*
Model	8.226E+006	-	9.140E+005	29.51	< 0.001
intercept	-	-18690.13	-	-	-
X_1	6.537E+005	0.732	6.537E+005	21.11	0.0010
X_2	1469.93	2.376	1469.93	0.047	0.8319
X_3	1.155E+005	4,176.20	1.155E+005	3.73	0.0822
X_1^2	1.301E+006	-1.202E-005	1.301E+006	42.01	< 0.0001
X_1X_2	4.953E+005	-2.488E-004	4.953E+005	15.99	0.0025
X_1X_3	9.155E+005	-0.045	9.155E+005	29.56	0.0003
X_2^2	1.965E+005	-2.919E-003	1.965E+005	6.34	0.0304
X_2X_3	2.059E+005	0.53475	2.059E+005	6.65	0.0275
X_3^2	4.965E+006	-260.873	4.965E+006	160.34	< 0.0001
R^2 = 0.9637					

4. Conclusions

This work focused on the effects of substrate concentration, $CaCl_2$ concentration and initial pH of substrate on methane yield (MY) in methane fermentation from the effluent of bio-hydrogen production process using cassava pulp hydrolysate by anaerobic seed sludge for improving the methane yield using the statistical methodology. Results demonstrated significant effect of the model (p<0.05). However, only substrate concentration had significant individual effect on methane yield. The interactive effects of all factors were found to be significant ($p < 0.05$). Maximum methane yield (MY) of 1984.47 ml $CH_4.g^{-1}VS_{added}$ was obtained under the optimum condition of substrate concentration 10,125 mg $COD.L^{-1}$, $CaCl_2$ concentration of 553.5 $mg.L^{-1}$ and initial pH of 7.46. CCD and RSM were useful to optimize the methane production process in this study successfully.

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Biography:

Dr. Naphat Phowan obtained her B.Sc (Biotechnology) from Thammasat University in 2001. M.Sc (Biotechnology) from Chulalongkorn University in 2005 and Ph.D (Biotechnology) from Khon Kaen University in 2010. She joined and worked as instructor at Srinakharinwirot University since 2010-present.

Research interests:

Her research interests are mathematic and statistical modeling, aspects of sustainable developments and renewable & sustainable energy.