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Article

Optimization of Key Factors Affecting Methane Production from Acidic Effluent Coming from the Sugarcane Juice Hydrogen Fermentation Process

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Abstract: Response surface methodology with a central composite design was applied to optimize the key factors affecting methane production from the acidic effluent coming from the sugarcane juice hydrogen fermentation process. The parameters studied were substrate concentration, ratio of $NaHCO_3$ to substrate concentration and initial pH. The experimental results showed that substrate concentration and initial pH had significant individual ($p < 0.05$) effect on methane yield (*MY*). However, there was no interactive effect between these variables ($p > 0.05$). The maximum *MY* of 367 mL CH_4/g-volatile solid $(VS)_{added}$ was obtained at the optimum conditions of 13,823 mg-COD/L, an $NaHCO_3$ to substrate concentration ratio of 3.09 and an initial pH of 7.07. Under the optimum conditions, *MY* was enhanced 4.4-fold in comparison to raw effluent.

Keywords: effluent; methane; response surface methodology

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1. Introduction

Anaerobic digestion is a biological process known for energy recovery, especially in the form of methane, from wastewater. The recovery of biogas as well as a reduction of chemical oxygen demand (COD) in organic waste and waste stabilization are the main advantages of this process [1]. A two-stage anaerobic digestion process for producing hydrogen and methane from organic materials has been reported [2–7]. In the first stage, acidogenic bacteria convert the organic substances to hydrogen, carbon dioxide and volatile fatty acids (VFAs). Next, acetogenic and methanogenic bacteria convert VFAs into mainly carbon dioxide and methane in the second stage [7].

In our previous study, batch hydrogen fermentation of sugarcane juice by *Clostridium butyricum* was conducted in which a hydrogen yield of 3.04 mol H_2/mol sucrose was obtained [8]. 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 can cause environmental problems upon disposal due to its high COD value of 18,500 mg-COD/L [8]. VFAs are known as valuable substrates for methane production. Therefore, the possibility of using the effluent from hydrogen fermentation to produce methane by the methanogenic anaerobic sludge was explored in this study.

Environmental factors such as substrate concentration, temperature, pH and metal ions [1] have great influences on methane production. A high concentration of VFAs has been reported to inhibit methane production from VFAs by mixed anaerobic microorganisms [9]. The optimum pH range for anaerobic digestion producing methane is 6.8–7.2 [10]. The growth rate of methanogens can be greatly reduced when the pH value is less than 6.6 [11]. An excessively alkaline pH can lead to the disintegration of microbial granules and subsequent failure of the digestion process [12]. Therefore, a buffer is needed in the methane production process in order to provide the resistance to significant and rapid pH changes in the system. Buffer capacity is proportional to the concentration of bicarbonate. $NaHCO_3$ has been widely used to create a buffer system during the anaerobic digestion process [13]. Speece [14] found that an alkalinity to COD concentration ratio (w/w) of 1.2–1.6 was required to sufficiently maintain the pH at approximately 6.6 during the anaerobic digestion of carbohydrate waste to produce methane. From the aforementioned research, it is obviously seen that in order to achieve a maximum methane production, the key environmental factors should be optimized.

To facilitate the study on the interactive effect of the environmental factors, the statistical experiments could be designed by response surface methodology (RSM). RSM is a statistical model which is helpful for understanding the interactions between the parameters at varying levels and calculating the optimal level of each parameter for a response target [15]. An improvement in 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 outcomes of using this statistical approach [16,17]. Previous research have reported the optimization of the process parameters as well as investigated the independent effects of substrate concentration, ratio of $NaHCO_3$ to substrate concentration and the initial pH on methane production from cellulose and glucose [9], industrial wastewater [10] and municipal solid waste [18]. However, to the best of our knowledge, the interactive effects of these factors on methane production from the acidic effluent coming from hydrogen fermentation process of sugarcane juice have not yet been investigated.

In the present work, RSM with a central composite design (CCD) was used to optimize the process parameters affecting methane production from the acidic effluent coming from the sugarcane juice hydrogen fermentation process. The individual and interactive effects of the process parameters on methane production were also investigated. The information from this study will not only make use of this waste in a form of renewable energy, *i.e.*, methane but also to reduce the pollutants before it is released to the environment.

2. Results and Discussion

2.1. Statistical Analysis and the Diagnostic Checking of the Fitted Model

CCD was used to optimize methane yield (*MY*) from the acidic effluent coming from hydrogen fermentation process of sugarcane juice. The design matrix of the variables in the coded and real value are tabulated in Table 1 with the experimental values of *MY* as response.

Table 1. 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	Substrate concentration (mg-COD/L)		$NaHCO_3$ to substrate ratio		Initial pH		*MY* (mL CH_4/g-VS $_{added}$)	
	Code	Actual	Code	Actual	Code	Actual	Observed	Predicted
1	−1.00	10,000	−1.00	2.00	1.00	8.5	270	270
2	0.00	15,000	0.00	4.00	0.00	7.0	364	366
3	−1.00	10,000	1.00	6.00	−1.00	5.5	95	97
4	0.00	15,000	−1.68	0.60	0.00	7.0	294	290
5	0.00	15,000	0.00	4.00	1.68	9.5	49	51
6	1.00	20,000	1.00	6.00	−1.00	5.5	70	74
7	0.00	15,000	1.68	7.40	0.00	7.0	262	263
8	−1.00	10,000	−1.00	2.00	−1.00	5.5	83	85
9	−1.00	10,000	1.00	6.00	1.00	8.5	208	204
10	1.00	20,000	1.00	6.00	1.00	8.5	79	84
11	1.00	20,000	−1.00	2.00	−1.00	5.5	89	94
12	0.00	15,000	0.00	4.00	0.00	7.0	359	368
13	0.00	15,000	0.00	4.00	0.00	7.0	372	368
14	1.00	20,000	−1.00	2.00	1.00	8.5	113	115
15	−1.68	6,591	0.00	4.00	0.00	7.0	223	232
16	0.00	15,000	0.00	4.00	0.00	7.0	362	368
17	0.00	15,000	0.00	4.00	0.00	7.0	376	368
18	0.00	15,000	0.00	4.00	0.00	7.0	364	368
19	1.68	23,409	0.00	4.00	0.00	7.0	246	251
20	0.00	15,000	0.00	4.00	−1.68	4.5	0	0
control	-	18,500	-	2.80	-	5.5	83	-

The predicted values of *MY* were obtained from the quadratic model and by evaluating the relationship between substrate concentration (X_1), the ratio of $NaHCO_3$ to substrate (X_2) and the initial

pH (X_3). The statistical model was developed by applying multiple regression analysis using the experimental data of *MY*, which can be given as:

$$MY = -3964.4703 + 0.0944X_1 + 106.6539X_2 + 964.7634X_3 - 0.0014X_1X_2 - 0.0041X_1X_3 - 1.87458X_2X_3 - 0.0000228X_1^2 - 9.0962X_2^2 - 62.0598X_3^2 \quad (1)$$

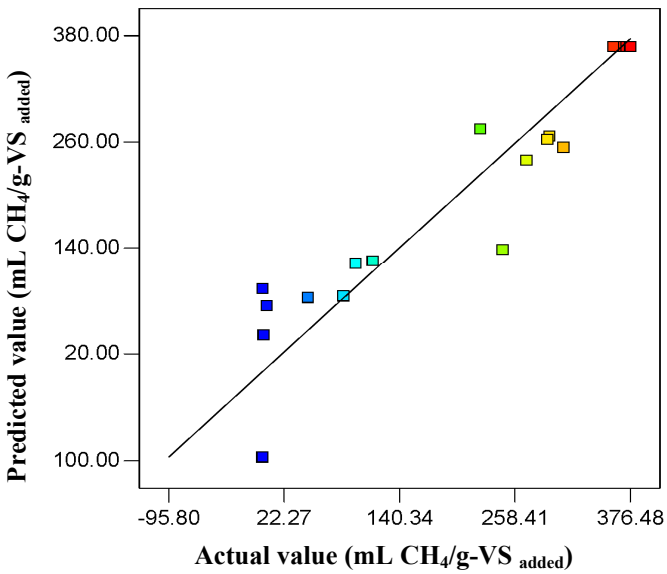
The analysis of variance (ANOVA) is necessary to determining the significance and adequacy of the model. The summary of ANOVA results presented in Table 2. The model F-value of 8.77 indicates the model is significant. In addition, the ANOVA of the quadratic regression model demonstrated that the model was highly significant ($p < 0.05$) (Table 2). The linear model terms of substrate concentration (X_1) and the initial pH (X_3) and the quadratic model terms of the substrate concentration (X_1^2) and the initial pH (X_3^2) were significant ($p < 0.05$), indicating that these two variables had an individual effect on *MY*. However, the linear model terms and quadratic model terms of the ratio of $NaHCO_3$ to substrate (X_2) were insignificant ($p > 0.05$), suggesting that there was no linear effect of this variable on *MY*. The interactive effects for all of these factors were found to be insignificant ($p > 0.05$) (Table 2). Additionally, the experimental *MY* were close to the predicted value using Equation (1) (Figure 1).

Table 2. Analysis of variance for quadratic polynomial model.

Source	Sum of Squares	df	Mean Square	Coefficient Estimate	Standard Error	F Value	p-value *Prob > F*
Model	367670.4	9	40852.27	367.729	27.837	8.7688	0.0011
X_1	22466.08	1	22466.08	−40.559	18.469	4.8222	0.0528
X_2	17.622	1	17.622	−1.135	18.469	0.0037	0.9522
X_3	22709.11	1	22709.11	40.777	18.469	4.8744	0.0518
X_1X_2	1580.625	1	1580.625	−14.056	24.131	0.3392	0.5731
X_1X_3	7501.287	1	7501.287	−30.621	24.131	1.6101	0.2332
X_2X_3	253.012	1	253.012	−5.623	24.131	0.0543	0.8204
X_1^2	46799.54	1	46799.54	−56.986	17.979	10.0453	0.01
X_2^2	19078.23	1	19078.23	−36.384	17.979	4.095	0.0706
X_3^2	280988.6	1	280988.6	−139.634	17.979	60.3134	< 0.0001
$R^2 = 0.8875$; Adequate precision = 9.604; Coefficient of variation (CV) = 32.71%							

The R-squared of 0.8875 revealed that the model could explain 88.75% of the variability in the response (Table 2). For a good statistical model, the R^2 should be in the range of 0.75–1.0 which indicates a good fit of the model [19]. The relatively high value of R^2 indicated that the quadratic equation could be used instead of an experimental system under the given conditions. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this study, a ratio of 9.604 was obtained (Table 2) which indicates an adequate signal. Thus, this model can be used to navigate the design space. In addition, the low values of the coefficient of the coefficient of variation (CV) (32.17%) (Table 2) confirmed a good precision and reliability of this experiment.

Figure 1. Predicted *vs.* experimental *MY* values.



2.2. Effect of Substrate Concentration, Ratio of $NaHCO_3$ to Substrate and Initial pH on MY from Acidic Effluent Coming from Hydrogen Fermentation Process of Sugarcane Juice

The interactive effect of substrate concentration, ratio of $NaHCO_3$ to substrate and initial pH on *MY* from acidic effluent coming from hydrogen fermentation process using RSM with CCD was depicted in Figure 2. The 3D response surface and the 2D contour plots of substrate concentration and the ratio of $NaHCO_3$ to substrate on *MY* are shown in Figure 2(A), (B) with the graphical representations of the regression equation. The predicted maximum value of the substrate concentration and the ratio of $NaHCO_3$ to substrate on *MY* are indicated by the top of surface [Figure 2(A)]. The results indicate that the interactive effect of the substrate concentration and the ratio of $NaHCO_3$ to substrate on *MY* was not significant ($p > 0.05$) (Table 2).

When the $NaHCO_3$ to substrate concentration ratio and the initial pH were kept at their central values, it was found that *MY* increased with an increase in substrate concentration from 10,000 to 15,000 mg-COD/L. A further increase in the substrate concentration resulted in a decrease in *MY* [Figure 2(A,B)]. The highest *MY* of approximately 366 mL CH_4/g-VS_{added} was obtained with an initial substrate concentration of 15,000 mg-COD/L (central value) [Figure 2(A), Table 1].

A decrease in *MY* at substrate concentration of 23,409 mg-COD/L might be resulted from substrate inhibition (Table 1). An inhibitory effect of high substrate concentration generally occurs in anaerobic digestion processes, depending on the types of substrates and microorganisms. Borja *et al.* [20] found that *MY* as well as volatile solid (VS) reduction decreased remarkably when a substrate concentration *i.e.*, olive mill solid waste, increased from 3 to 15 g-VS/L. Murto *et al.* [21] found that the overloading of sewage sludge and pig manure (5.9 g-VS/L) as a co-substrate in an anaerobic digestion system resulted in microbial inhibition and a significant reduction in *MY*.

Figure 2. Response surface plots showed the effects of substrate concentration. (**A**,**B**) Ratio of $NaHCO_3$ to substrate concentration and their interactive effect on *MY* with the optimum level of initial pH (7.07); (**C**,**D**) The effects of substrate concentration, initial pH and their interactive effect on *MY* with the optimum ratio of $NaHCO_3$ to substrate concentration (3.09); (**E**,**F**) The effects of ratio of $NaHCO_3$ to substrate concentration, initial pH and their interactive effect on *MY* with the optimum level of substrate concentration (13,823 mg-COD/L).

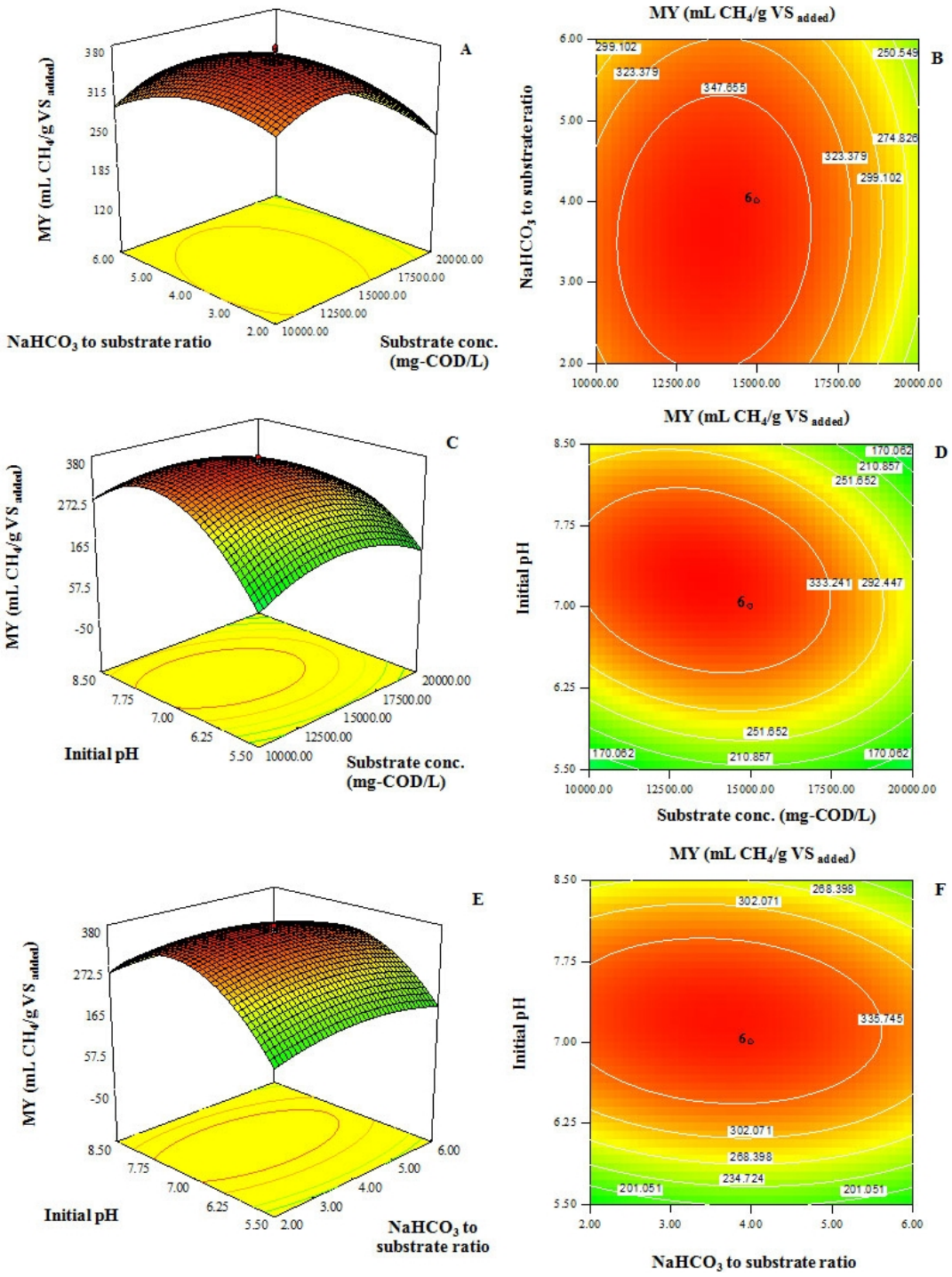


Figure 2(C,E) depicts a graphical relationship between the experimental levels of substrate concentration and the initial pH on *MY*. The results indicate that the interactive effect of substrate concentration (X_1) and the initial pH (X_2) on *MY* was not significant ($p > 0.05$) but the initial pH

significantly affected the *MY* ($p < 0.05$) (Table 2). An increase in the initial pH from 4.48 to 7.0 led to an increase in *MY* [Figure 2(C,E)].

A decrease of *MY* could be observed when the initial pH was increased from 7 to 9.52. The greatest *MY* value of 366 mL CH_4/g-VS_{added} was obtained at an initial pH of 7.0 when the substrate concentration and the $NaHCO_3$ to substrate ratio were kept at their central values, indicating that the optimum initial pH for *MY* in this study was at 7.0. Methane was not produced at an initial pH of 4.48 (Run 20, Table 1). Previous research has reported that when the pH falls below 6.5, methanogenic bacteria are inhibited, resulting in a decrease in *MY* efficiency [1], while an excessively alkaline pH could lead to the disintegration of microbial granules and subsequent failure of the process [12].

The interaction between the $NaHCO_3$ to substrate ratio and the initial pH on *MY* was presented in Figure 2(E,F). The optimum value of the $NaHCO_3$ to substrate ratio and the initial pH for *MY* is indicated on the top of the surface [Figure 2(E)]. The *MY* increased with an increase in the ratio of $NaHCO_3$ to substrate, when the substrate concentration and the initial pH were kept at their central values.

In anaerobic digestion processes, carbon dioxide produced by microorganisms often leads to weak acid condition in aqueous anaerobic systems. Therefore, sufficient bicarbonate alkalinity is required for neutralization [13]. The effect of the alkalinity to substrate ratio is important in the anaerobic digestion process, depending on the type of substrate and microorganism. 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 the anaerobic digestion process of carbohydrate waste [14].

The optimum conditions for maximizing the *MY* calculated by the obtained model [Equation (1)] were a substrate concentration of 13,823 mg-COD/L, a ratio of $NaHCO_3$ to substrate of 3.09 and an initial pH of 7.07. Under the optimum conditions, the predicted maximum *MY* of 367 mL CH_4/g-VS_{added} was obtained from the quadratic regression model. The average maximum observed *MY* at the optimum condition was 366 mL CH_4/g-VS_{added}, which was close to the RSM experimental result at the center value (Figure 1 and Table 1).

2.3. Confirmation Experiments and Sufficiency of the Models

The sufficiency of the predicted response was examined by conducting three additional experiments. The experimental conditions for the substrate concentration, the $NaHCO_3$ to substrate ratio and the initial pH as well as the experimental results of *MY* with the predicted values obtained from the second-order model are shown in Table 3. The predicted value for *MY* calculated from the polynomial quadratic Equation 1 for runs 21, 22 and 23 were 269, 331 and 332 mL CH_4/g-VS_{added}, respectively. These values were close to the predicted values using CCD. Results confirmed that the RSM with CCD analysis was a useful technique to optimize the *MY* from the effluent of hydrogen production process.

The *MY* of 366 mL CH_4/g-VS_{added} achieved in this study was comparable to the *MY* obtained from cellulose, boiled rice and fresh garbage of 356, 294 and 277 mL CH_4/g-VS_{added}, respectively, in a single-phase methane reactor [22]. The optimization could improve a methane production by 4.4 fold in comparison to the raw effluent (Table 1). However, the *MY* obtained was lower than that obtained

from the effluent of the bio-hydrogen production process from food waste which was of 565.76 mL CH_4/g-VS_{added} [1]. Such a discrepancy might be due to the different in inoculums types and substrate used.

Table 3. Predicted and measured values of the confirmation experiments.

Run	Variables						*MY* (mL CH_4/g-VS_{added})		Bias [a] (%)
	Substrate concentration (mg-COD/L)		$NaHCO_3$ and substrate ratio		Initial pH				
	Code	Actual	Code	Actual	Code	Actual	Predicted	Measured	
21	0.00	15,000	0.00	4.00	1.00	8.5	269	266	0.97
22	0.00	15,000	1.00	6.00	0.00	7.0	331	327	1.01
23	0.00	15,000	−1.00	2.00	0.00	7.0	332	331	0.33

[a] Bias was calculated using the equation: [(predicted value − measured value)/predicted value] × 100 [22].

2.4. Energy Analysis

Total energy production from sugarcane juice was calculated based on the hydrogen production and methane yield (mL $H_2/L_{substrate}$ and mL CH_4/kg-$VS_{substrate}$, respectively), relative density of hydrogen and methane (0.089 kg-H_2/m^3-H_2 and 0.72 kg-CH_4/m^3-CH_4, respectively) as well as the heating values of hydrogen and methane (121 MJ/kg-H_2, 50 MJ/kg-CH_4, respectively) [23]. From our previous experiment, sugarcane juice was used to produce hydrogen and the highest hydrogen yield (HY) of 2.29 L $H_2/L_{substrate}$ was obtained under the optimum replacement ratio of 50% (v/v) [8], Therefore, the energy production from hydrogen was [2.29 × (0.089 × 121)] = 24.66 kJ. In this study, the acidic effluent coming from hydrogen fermentation process of sugarcane juice was used to produce methane and the maximum *MY* of 331 mL CH_4/ g-VS_{added} was obtained under the optimum conditions. Thus, the energy production from methane was [(331 × (0.72 × 50)] = 11.92 MJ. Hence, the net energy generated from this sequential process (hydrogen production in the first phase and methane production in the second phase) was 11.94 MJ. The results suggested that the acidic effluent coming from hydrogen fermentation process of sugarcane juice is worth to be used for methane production. In addition, a sequential fermentation process would give a higher energy production than a production of hydrogen or methane alone.

3. Experimental Section

3.1. Methanogenic Anaerobic Seed Sludge

Methanogenic anaerobic seed sludge was taken from the municipal anaerobic wastewater treatment plant in Ube (Yamaguchi, Japan). Total solid (TS), suspended solids (SS) and volatile suspended solid (VSS) of methanogenic seed sludge were (all in mg/L) 12,000 ± 35, 10,000 ± 51, and 7,300 ± 100, respectively. The methanogenic bacteria in the seed sludge were acclimatized by incubating the seed sludge in 10 g/L glucose under anaerobic conditions at 30 °C for 30 days prior the usage.

3.2. Substrate

The effluent from hydrogen fermentation of sugarcane juice by *C. butyricum* with a HY of 3.04 mol H_2/mol sucrose [8] was used as the substrate. The main VFAs in this effluent were butyric and acetic acids with a high COD value of 18,500 mg-COD/L. The pH of the effluent from hydrogen fermentation was 5.5. The effluent was kept at 4 °C before used in this experiment. The physical and chemical characteristics of the effluent are presented in Table 4.

Table 4. Characteristics of the acidic effluent coming from hydrogen fermentation process of sugarcane juice.

Chracteristics	Concentration
Chemical oxygen demand (mg-COD/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
Chloride (Cl^-) (mg/L)	1,586 ± 56
Volatile solids (VS, mg/L)	5,870 ± 26

3.3. Experimental Design

The effects of three key environmental factors including substrate concentration, the ratio of $NaHCO_3$ to substrate concentration and the initial pH on *MY* were investigated using CCD. The levels of the factors used for *MY* optimization are presented in Table 5. Twenty runs of the experiment were required for this procedure, as given in Table 1. Control is the acidic effluent coming from hydrogen fermentation of sugarcane juice without any adjustments. The independent variables were coded for statistical calculations according to the following equation:

$$x_i = X_I - X_0/\Delta X \quad (2)$$

where X_I is the independent variable coded value, X_I is the real value of the independent variable, X_0 is the real value of the independent variable at the center 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:

$$Y = \beta_0 + \sum\beta_i X_i + \sum\beta_{ii} X_i^2 + \sum\beta_{ii} X_{ij} \quad (3)$$

where Y is the predicted response (*MY*), β_0 is the offset term, β_i is the linear coefficient, β_{ii} is the squared coefficient, β_{ij} is the interaction coefficient, and X_i is the input variables. The response variable (*MY*) was fitted using a predictive polynomial quadratic equation [Equation (3)] in order to correlate the response variable to the independent variables [24]. The statistical software Design-Expert (Demo version 7.0, Stat-Ease, Inc., Minneapolis, MN, USA) was used for regression and graphical analysis of the experimental data. 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*.

3.4. Batch Fermentation

Batch fermentation for methane production was conducted in 100 mL serum bottles with a working volume of 50 mL. The fermentation media contained the following constituents dissolved in distilled water: inoculum (10% v/v), and different concentrations of the components that needed to be optimized *i.e.*, substrate concentration, the ratio of $NaHCO_3$ to substrate concentration (Table 1). The initial pH of fermentation media was adjusted using 5 N NaOH or 3 N HCl according to the experimental design (Table 1).

Table 5. Experimental range and levels of the independent variables.

Variables	Range and levels				
	−α (−1.682)	**Low (−1)**	**Central (0)**	**High (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	0.64	2.00	4.00	6.00	7.36
X_3 = Initial pH of substrate	4.48	5.50	7.00	8.50	9.52

All serum bottles were tightly sealed with rubber septa and aluminum cap after the seed inoculum and substrate were added. The headspace of the bottles was purged with argon gas for 5 min to ensure anaerobic conditions. The serum bottles were incubated at 30 °C on a horizontal shaker at 150 rpm. All treatments were carried out in triplicates.

3.5. Analytic Methods

Concentrations of TS, SS, VS, VSS, COD, total nitrogen (TN), total phosphorus (TP) and Cl^- were measured according to standard methods [25]. Total organic carbon (TOC) was measured using a TOC analyzer (Shimadzu TOC-5000). For VFAs analysis, 3 mL of fermentation broth was first centrifuged at 12,000 rpm for 5 min to obtain clarified supernatants which were then acid fixed by mixing with 0.1 N HCl (ratio 1:1 v/v) and filtered through a 0.45 μm nylon membrane. The concentrations of VFAs in the filtrate were determined by gas chromatography (GC, Model 8APF, Shimadzu, Japan) equipped with a flame ionization detector (FID) and a 3 m × 3.2 mm glass column packed with 30/60 Unisol F-200 mesh. The operation conditions were set according to Hasyim *et al.* [26]. The volume of biogas was measured daily by the plunger displacement method using appropriately sized wetted glass syringes [27]. The components of biogas in the headspace, including hydrogen, nitrogen, methane and carbon dioxide, were determined by GC (Model GC-8APT, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD). The operation conditions were set according to Hasyim *et al.* [26].

4. Conclusions

Only the substrate concentration and the initial pH had significant individual effects on *MY*. The interactive effects for all of these factors were found to be insignificant ($p > 0.05$). The optimum conditions for maximizing *MY* were a substrate concentration of 13,823 mg-COD/L, a $NaHCO_3$ to substrate ratio of 3.09 and an initial pH of 7.07 in which a maximum *MY* of 367 mL CH_4/gVS_{added} was obtained. Under the optimum conditions the *MY* was 4.4-fold greater than the raw effluent (control). The model validation experiment confirmed that the *MY* from the experimental data was close to the

predicted data suggesting the adequacy of the model. Net energy generated from the sequential process (hydrogen production in the first phase and methane production in the second phase) was 11.94 MJ, which suggested that the acidic effluent coming from the sugarcane juice hydrogen fermentation is worthy of use for methane production.

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