

Optimization of NaOH Pretreatment for Enhancement of Biogas Production of Banana Pseudo-Stem Fiber using Response Surface Methodology

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In this paper, the NaOH pretreatment of banana pseudo-stem fiber for biogas production was investigated using a statistically designed set of experiments. A central composite design was used to identify the optimum pretreatment condition for four factors, *i.e.*, NaOH concentration, pretreatment temperature, pretreatment time, and fiber length, on biogas fermentation of banana pseudo-stem fiber. The best pretreatment condition was 7.8% NaOH, 0.2-cm fiber length, and a temperature of 48 °C for 3 days. NaOH pretreatment increased the biogas yield of banana pseudo-stem fiber. The highest biogas yield was 463.0 mL·g⁻¹VS_{added}, which was 89.2% higher than that of the control, at 244.7 mL·g⁻¹VS_{added}.

Keywords: Banana pseudo-stem fiber; NaOH pretreatment; Response surface methodology; Anaerobic fermentation; Biogas

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INTRODUCTION

Banana is recognized as an important fruit crop in tropical and subtropical countries. During the production of bananas, a large amount of byproducts are produced, including pseudo-stem, leaves, inflorescence, and rhizomes. Banana pseudo-stem (BPS) constitutes the majority of the mass of byproducts, at 120 to 165 t per hectare produced annually. Usually, BPS are crushed and left to degrade in the soil to replenish the nutrients for the next growth season, or left whole without crushing to degrade on an empty lot after the harvesting of the bananas (Padam *et al.* 2012). However, both disposal methods generate a large amount of carbon dioxide, methane, hydrogen sulfide, and associated unpleasant odors. These disposal approaches could also lead to an outbreak of banana fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense*, one of the most serious fungal diseases affecting banana production (Getha and Vikineswary 2002; Xiao *et al.* 2013). It is therefore advantageous to make use of the BPS and at the same time prevent related environmental and ecological issues. Based on previous reports, BPS can be utilized in many different processes, such as compost, paper making, rope making, production of bags, feed, and biofuels (Tock *et al.* 2010; Wei and Zhou 2001; Zuluaga *et al.* 2009). Among these options, using BPS as raw material for biogas production by anaerobic digestion technology is a very promising strategy and addresses the serious need for alternative fuel sources worldwide.

Producing clean energy from agricultural wastes to partially substitute fossil fuels has aroused worldwide attention (Chandra *et al.* 2012a; Gunaseelan 2007; Binod *et al.*

2010). Presently, China is actively promoting the development of new resources to generate biogas. Ten thousand large-scale biogas plants using industrial and agricultural wastes as raw materials are projected to be built by 2020, with biogas production reaching 14 billion m³ annually (Zhang *et al.* 2013). With the support of the National Key Technology R&D Program, the Hainan Shenzhou New Energy Construction & Development Co., Ltd in Hainan province began building a plant in 2009 and started production at the end of 2013. The proposed biogas production ability of this plant was 30,000 m³ daily, and BPS was considered as one of the main raw materials used for fermentation. Therefore, research on biogas fermentation of BPS by anaerobic digestion is of practical significance and could provide the necessary data to support the stable operation of the plant. Most reports on biogas fermentation of banana byproducts have focused on using waste bananas and banana peels (Bardiya *et al.* 1996; Clarke *et al.* 2008). Only a few studies have been conducted on biogas fermentation of BPS and focused on the biological methane potential test or co-digestion with animal manure (Tian *et al.* 2013; Zhang *et al.* 2013). The reported biogas yield for BPS is usually 221 to 325 mL·g⁻¹ TS, and the methane content of the biogas is 59% to 79% (Kalia *et al.* 2000; Ou *et al.* 2006; Pei *et al.* 2014).

Compared with wheat straw and corn straw, the high water content (about 94%) of BPS greatly increased the transportation cost and limited industrial applications. Recently, to resolve this issue, Zhang *et al.* (2013) proposed reducing the water content before transportation by squeezing the BPS, thus reducing the cost and increasing the potential transport distance. Currently, two options exist after reducing the transportation cost (Fig. 1). One is the direct production of biogas through anaerobic digestion, utilizing BPS alone or with other feedstocks. The other option is, after extraction of banana pseudo-stem fiber (BPSF) for pulping (Daud and Law 2010; Liu *et al.* 2013), the residues, mostly parenchyma cells (Chen *et al.* 2000), could be used for biogas production. However, BPSF itself is suitable for biogas production. It is uncertain whether BPSF would be better used for pulping or biogas fermentation. No relevant research has been reported on the biogas production of BPSF. Therefore, the biogas fermentation performance of BPSF was investigated in this paper.

Pretreatment generally can improve the biogas yield of a lignocellulosic substrate to some extent (Chandra *et al.* 2012b; Wang *et al.* 2010; Zhang *et al.* 2011; Frigon *et al.* 2012; Quintero *et al.* 2012). Existing pretreatments for lignocellulosic substrates include physical, chemical, and biological methods. Among these methods, NaOH pretreatment is a promising approach for biogas production of lignocellulosic substrates from the point of industrial application (Salehian *et al.* 2013; Ward *et al.* 2008; Zhu *et al.* 2010). As a result, NaOH pretreatment was selected to improve the biogas yield of BPSF in this paper.

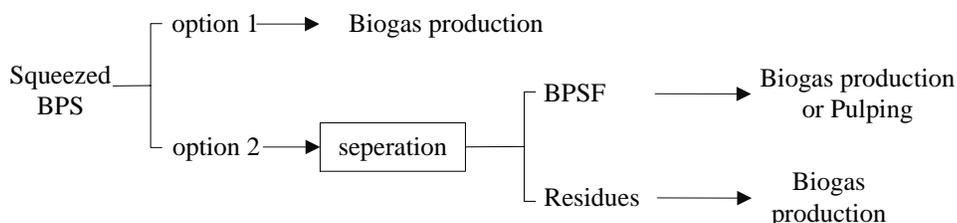


Fig. 1. Utilization options of banana pseudo-stem

The aim of this paper was to determine the biological biogas potential of BPSF with or without NaOH pretreatment, as well as the optimum NaOH pretreatment conditions.

The optimal pretreatment conditions, including NaOH concentration, pretreatment temperature, time and fiber length, were determined by response surface methodology (RSM).

EXPERIMENTAL

Materials

Fresh BPS was provided by Hainan Shenzhou. BPS was cut and milled using a disc mill (BR30-300CB KRK, Kogyo). BPSF was sieved and cut to different lengths. The preparation process is shown in Fig. 2. Samples were stored at 4 °C before anaerobic digestion. Major components of BPSF were as follows (on dry basis): cellulose, 36.9 ± 0.3% to 44.9 ± 0.5%; hemicellulose, 17.7 ± 0.7% to 22.9 ± 1.1%; acid-soluble lignin, 1.7 ± 0.1% to 1.8 ± 0.1%; and acid-insoluble lignin, 13.0 ± 0.3% to 13.9 ± 0.8%.

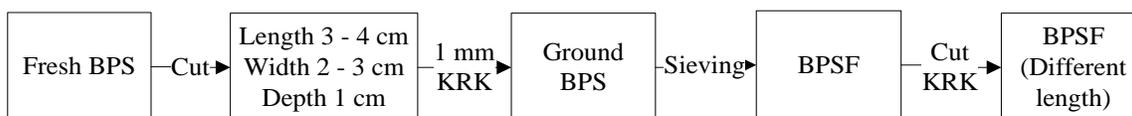


Fig. 2. Preparation process of banana pseudo-stem fiber

Inoculum, anaerobic sludge, was collected from the Gaobeidian wastewater treatment plant. The total solids (TS, based on wet solid) and volatile solids (VS, based on dry matter) of the inoculums were 6.2 ± 0.1% and 32.0 ± 0.1%, respectively. The total nitrogen (TN) was 2.4% on dry basis. The pH value of inoculum was 7.0.

NaOH Pretreatment

Samples were pretreated in 50-mL plastic bottles. The pretreatment temperature was controlled by a water bath. Wet-state NaOH pretreatment was used. The moisture content was 90% (Chandra *et al.* 2012; Zheng *et al.* 2009). NaOH doses were calculated using the following equation:

$$\text{NaOH concentration (\%)} = \frac{m_{\text{NaOH}}}{\text{dry weight of material}} \times 100 \quad (1)$$

The control sample was pretreated with water under the same conditions.

Biogas Fermentation

Alkali-pretreated BPSF was cooled to room temperature and used directly in fermentation without rinsing. The experiments were conducted in 500-mL glass bottles. The total mass and TS of the fermentation system were 400 g and 6% (w/v), respectively. The ratio of inoculum to substrate (both expressed in g of VS) was 1:1. The fermentation temperature was maintained at 37 ± 1 °C in a water bath. Urea and NaHCO₃ were used to adjust the C/N and alkalinity of the system to 25:1 and 2500 mg CaCO₃/L, respectively. Nitrogen was used before the fermentation to ensure an anaerobic environment. Reaction bottles were shaken 3 times daily to mix the samples. Biogas volume was measured using water replacement method with saturated salt solution. The daily and cumulative biogas production was recorded. The gas generation of inoculum was considered during the

calculation of biogas production. The biogas yield could be calculated using the following equation:

$$Y (\text{mL/gVS}_{\text{added}}) = \frac{\text{Biogas production}}{\text{VS}_{\text{added}}} \quad (2)$$

Experimental Design and Data Analysis

The pretreatment conditions were optimized by central composite design (CCD) of RSM. In this study, four variables (concentration, time, temperature, and fiber length) were investigated. The range and levels of variables were selected based on the results of single-factor tests (Pei *et al.* 2014), results in the literature (Chandra *et al.* 2012; Sambusiti *et al.* 2013; Zheng *et al.* 2009), and real values from industrial-scale production. The CCD quadratic model of variables in the form of coded and actual values is given in Table 1. The value of α for this CCD was fixed at 2. The design was represented by a second-order polynomial regression model to generate contour plots,

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i < j \leq k} \beta_{ij} x_i x_j + \varepsilon \quad (3)$$

where Y , x_i , β_0 , β_i , β_{ii} , β_{ij} , and ε are the biogas yield, process factors, offset coefficient, linear coefficients, quadratic coefficients, interaction coefficients, and residuals associated with the experiments, respectively.

The regression analysis of the experimental data was performed in Design-Expert software version 8.0 to determine the coefficients of the regression equation and estimate the response surface.

Table 1. Actual Alkali-Pretreatment Conditions and the Corresponding Encoding Levels of Central Composite Design

Variable	Range and Levels				
	-2	-1	0	1	2
x_1 -Alkali concentration (% w/w)	0	3	6	9	12
x_2 -Time (d)	1	2	3	4	5
x_3 -Temperature (°C)	30	35	40	45	50
x_4 -Fiber length (cm)	0.2	1.7	3.2	4.7	6.2

Analytical Method

TS and VS were measured by the weight loss method (*Standard Methods for the Examination of Water & Wastewater* 2005). The composition of hemicellulose, cellulose, and lignin were measured using the NREL method (Sluiter *et al.* 2008). The pH was measured with a pH meter (Mettler TOLEDO 6219, Mettler Toledo International Co., Ltd. Switzerland).

Biogas composition was measured using a gas chromatograph (SP-6890, Shandong southern shandong rainbow chemical equipment co., Ltd. China). The operational conditions for chromatography were the same as in Pei *et al.* (2014).

RESULTS AND DISCUSSION

NaOH Pretreatment Effect

pH variation of samples during the NaOH pretreatment

The pH values of the samples were measured during the NaOH pretreatment and are shown in Fig. 3. For all samples, the pH values actually declined with pretreatment time (Fig. 3a), likely due to the consumption of OH⁻ (Chandra *et al.* 2012; Zheng *et al.* 2009). Furthermore, decreasing fiber length increased the speed of the pH decline under the same concentration of NaOH (Fig. 3b). For example, the pH value decreased faster for sample with shorter fiber length as seen for 3% and 6% NaOH respectively. The faster decline was probably due to better surface accessibility of the smaller fibers to the aqueous alkali solution. Results also suggested that the pH variation would lessen during the pretreatment with increasing concentration of NaOH (Fig. 3b). In samples pretreated with high doses of NaOH, the amount of OH⁻ consumed during the pretreatment was too small to cause a significant pH variation.

During the NaOH pretreatment, the pH values of the samples declined for 2 to 3 days. For example, pH of samples treated by 3% NaOH declined to 7.0, and pH of samples treated by 6%, 9%, and 12% NaOH declined to 8.3 to 9.5. Although the pH variation could indirectly reflect the alkali treatment process (Chandra *et al.* 2012; Zheng *et al.* 2009), the optimum pretreatment conditions should be determined according to the biogas fermentation results.

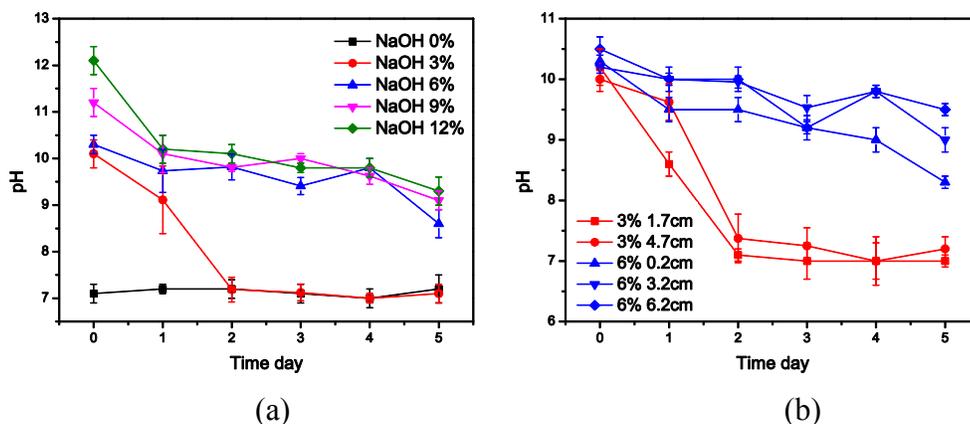


Fig. 3. pH variation of samples during the NaOH pretreatment (a: pH variation of pretreated samples with various concentrations of NaOH; b: pH variation of samples with various fiber lengths under 3% and 6% NaOH)

NaOH pretreatment did not influence the pH of the fermentation system

The pH variations for the fermentation system before and after anaerobic digestion were also measured. Alkali-pretreated samples were directly used for biogas fermentation without further treatment. The initial pH of the fermentation systems was in the range of 7.0 to 8.5 because of the buffer capacity (2500 mg CaCO₃/L) of the system. After fermentation, the final pH values remained in the range of 7.5 to 8.5. No sudden cease of daily gas production was observed for any of the pretreatment conditions (Fig. 4), which indicated that the fermentation system did not become acidified when pretreated samples for biogas fermentation were used without neutralization. This result could be attributed to the buffering capacity of the fermentation media and the relatively low substrate

concentration (TS = 6%), which prevented the introduction of excess Na⁺ and OH⁻. Thus the alkali-pretreatment did not affect the pH stability of the overall fermentation process.

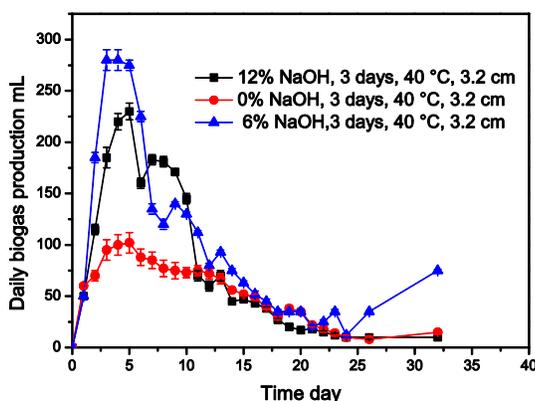


Fig. 4. Daily biogas production for representative samples

Pretreatment enhanced the degradation aspect of the fermentation process

The effect of alkali-pretreatment on anaerobic digestion was investigated. Results showed that alkali-pretreatment increased the degradation aspect of the fermentation process. After NaOH treatment, the TS and VS degradation increased from $13.3 \pm 0.4\%$ and $22.0 \pm 0.3\%$ to 16.7% and 32.9% (on average), respectively. The highest TS and VS degradation, $19.6 \pm 0.6\%$ and $47.3 \pm 0.1\%$, was obtained with 6% NaOH, 0.2 cm fiber length, 40 °C and 3 days. Pretreatment enhanced the downstream fermentation process.

Modeling of Biogas Production

To optimize the pretreatment conditions, four variables (concentration of NaOH, time, temperature, and fiber length) were selected for testing by using the RSM. Table 2 lists the experimental conditions and the final biogas yield. From these data, the following polynomial equation was obtained using multiple regression analysis and was employed to factor in the role of each variable and their second-order interactions on the biogas yield generating the predicted values in the last column. The equation for the model as a function of the coded factors is given below:

$$Y = 416.92 + 43.74x_1 + 2.82x_2 - 2.79x_3 - 20.85x_4 - 5.32x_1x_2 - 6.16x_1x_3 + 11.51x_1x_4 + 6.25x_2x_3 + 3.53x_2x_4 + 0.55x_3x_4 - 36.40x_1^2 - 22.07x_2^2 - 5.01x_3^2 + 5.31x_4^2 \quad (4)$$

ANOVA was used to determine the “goodness of fit” of the quadratic model. In this study, p-values less than 0.05 indicated that the corresponding coefficient terms were significant. The results of the simplified regression model after removing the insignificant factors are shown in Table 3.

The F value of the quadratic model was 18.77, which indicated that the developed quadratic model was highly significant ($p < 0.0001$). The regression model fitted the experimental data and could be used to appropriately explain the effects of the selected variables on biogas yield. There was a 11.50% chance that an F-value for “lack of fit” could occur due to noise. The fitness of the model was also confirmed by the regression correlation coefficient R^2 value at 0.79. In this study, the adequate precision value was 18.49, which indicated an adequate signal. A low value for the coefficient of variation (CV

= 8.97%) indicated that the experiments were precise and reliable. In short, the insignificant effect of lack of fit, the closeness of the R^2 value to 1, the adequate precision greater than 4 and the lower CV value all indicated that the developed model was suitable and appropriate to used for predicting the final yield.

Table 2. Design and Experiment Results of CCD

Standard order	Concentration (%)	Time (d)	Temperature (°C)	Length (cm)	Biogas yield (mL·g ⁻¹ VS _{added})	Predicted values (mL·g ⁻¹ VS _{added})
1	3	2	35	1.7	326.7±10.3	344.5
2	9	2	35	1.7	430.2±15.8	408.9
3	3	4	35	1.7	293.9±20.2	350.1
4	9	4	35	1.7	383.9±23.6	414.6
5	3	2	45	1.7	344.7±13.3	344.5
6	9	2	45	1.7	410.7±15.7	408.9
7	3	4	45	1.7	344.8±17.6	350.1
8	9	4	45	1.7	389.6±20.1	414.6
9	3	2	35	4.7	268.2±12.6	279.8
10	9	2	35	4.7	414.5±15.5	390.3
11	3	4	35	4.7	267.0±16.3	285.4
12	9	4	35	4.7	372.9±12.2	395.9
13	3	2	45	4.7	285.2±10.4	279.8
14	9	2	45	4.7	408.4±11.5	390.3
15	3	4	45	4.7	289.8±12.7	285.4
16	9	4	45	4.7	402.9±11.3	395.9
17	0	3	40	3.2	220.4±20.7	183.9
18	12	3	40	3.2	348.9±15.6	358.9
19	6	1	40	3.2	289.2±13.5	323.2
20	6	5	40	3.2	394.8±14.7	334.5
21	6	3	30	3.2	456.6±13.5	417.2
22	6	3	50	3.2	363.8±15.7	417.2
23	6	3	40	0.2	522.7±10.3	458.9
24	6	3	40	6.2	380.2±12.2	375.5
25	6	3	40	3.2	400.6±14.4	417.2
26	6	3	40	3.2	400.2±15.3	417.2
27	6	3	40	3.2	438.4±14.2	417.2
28	6	3	40	3.2	409.2±12.4	417.2
29	6	3	40	3.2	447.8±15.5	417.2
30	6	3	40	3.2	405.3±12.5	417.2

Statistical analysis showed that alkali concentration ($P < 0.0001$) had a significant positive linear effect and a negative quadratic effect ($P = 0.0001$) on the biogas yield. Fiber length ($P < 0.05$) had a significant negative linear effect. Additionally, pretreatment time ($P = 0.0071$) had significant negative quadratic effects.

The most impactful factor was alkali concentration ($F = 41.57$), followed by fiber length ($F = 9.44$). The linear effect of pretreatment time and temperature, the quadratic effect of fiber length and temperature were insignificant. The interaction between each two variables was observed to be insignificant. The simplified second-order polynomial regression model after removing the insignificant factors is given below:

$$Y = 417.22 + 43.74x_1 + 2.82x_2 - 20.85x_4 - 36.44x_1^2 - 22.10x_2^2 \quad (5)$$

The coefficient estimates and P-values of the simplified model were also presented in Table 3.

Table 3. ANOVA Analysis for the Fitted Quadratic Model and Simplified Model^a

Source	Coefficient ^a	p-Value
ANOVA for the fitted quadratic model of biogas theoretical yield		
Model	—	0.0009
Intercept	416.92	
x ₁ -Concentration	43.74	< 0.0001
x ₂ -Time	2.82	0.7142
x ₃ -Temperature	-2.79	0.7180
x ₄ -Fiber length	-20.85	0.0147
x ₁ x ₂	-5.32	0.5747
x ₁ x ₃	-6.16	0.5166
x ₁ x ₄	11.51	0.2334
x ₂ x ₃	6.25	0.5106
x ₂ x ₄	3.53	0.7085
x ₃ x ₄	0.55	0.9537
x ₁ ²	-36.40	0.0001
x ₂ ²	-22.07	0.0071
x ₃ ²	-5.01	0.4901
x ₄ ²	5.31	0.4646
Lack of Fit		0.0608
ANOVA for the simplified model of biogas theoretical yield		
Model	—	< 0.0001
Intercept	417.22	—
x ₁ -Concentration	43.74	< 0.0001
x ₂ -Time	2.82	0.6809
x ₄ -Fiber length	-20.85	0.0052
x ₁ ²	-36.44	< 0.0001
x ₂ ²	-22.10	0.0016
Lack of Fit		0.1150

^a R² = 0.7964; adjusted R² = 0.7540; predicted R² = 0.5294; adequate precision = 18.496; CV% = 8.97.

^b degree of freedom.

RSM analysis

The effects of the four variables on biogas yield were analyzed in detail using contour plots. Figure 5 represented the effects of two independent variables on the yield while the other two variables were held constant at the central point. In Fig. 5a, the pretreatment temperature and fiber length of BPSF were fixed at the center. Figure 5a showed that biogas yield increased with increasing alkali concentration from 0% to 7% when the pretreatment time was held at a constant level (3 days). However, further increasing the alkali concentration caused a slight decline in the biogas yield. A similar trend was observed in Fig. 5b when the temperature was held at a constant level (40 °C). According to Fig. 5c, the biogas yield increased for all fiber lengths when alkali concentration increased from 3.0% to 9.0%. Also, according to the modeling results, alkali concentration was a significant factor (P < 0.0001) for biogas yield (Table 3). Generally, alkali concentration significantly affects the biogas yield of lignocellulosic materials

(Salehian *et al.* 2013; Zhu *et al.* 2010). In this study, the highest biogas yield ($522.7 \text{ mL} \cdot \text{g}^{-1} \text{VS}_{\text{added}}$) was obtained when the NaOH concentration was 6% (w/w).

There are many reasons for the enhancement of alkali-pretreatment. Alkali-pretreatment opened the bonds between cellulose, hemicelluloses, and lignin, thus weakening the complex lignocellulosic structure allowing easier access (Zuluaga *et al.* 2009). Pretreatment most likely dissolved a portion of the lignin and hemicellulose components, producing a soluble substance and allowing more access for the hydrolysis process (Triolo *et al.* 2011; Zuluaga *et al.* 2009). The substrate porosity for pretreated material increased after alkali-pretreatment, which could improve contact between substrate and microorganism, and thus facilitated the hydrolysis, which is the first phase of biogas fermentation (Mosier *et al.* 2005; Sindhu *et al.* 2014; Yao *et al.* 2013). On the contrary, a higher concentration of alkali might result in a decline in biogas yield (Ward *et al.* 2008). Concentrations of NaOH investigated in this paper include 0%, 3%, 6%, 9% and 12%. Without considering the effects of the other pretreatment factors, the average biogas yield obtained was 220.4, 302.5, 409.1, 401.6, and 348.9 $\text{mL} \cdot \text{g}^{-1} \text{VS}_{\text{added}}$, respectively. This suggested that the biogas yield would not be significantly decreased with NaOH concentrations between 6% and 9%. However, the biogas yield for 12% NaOH was 348 $\text{mL} \cdot \text{g}^{-1} \text{VS}_{\text{added}}$ (Table 2), which was 33.4% lower than the highest sample. The negative effect of higher alkali concentration on lignocellulosic material may result from a more alkaline pH range of the fermentation system. Also extra Na^+ ions were introduced into the fermentation system, along with the possible production of toxic compounds. In this study, pretreated samples were directly used for fermentation without rinsing. However, the pH values for all tests were between 7.0 and 8.5 because of the buffering capacity of the fermentation system, which should not cause a decline in biogas yield. In addition, the pH remained stable in the appropriate range for anaerobic digestion. Extra induction of Na^+ would inhibit the activity of anaerobic microbes and thus cause a decline in biogas yield. The specific inhibitory concentration of Na^+ for microbes would be affected by the different types of sludge, salts and nutrients in the medium (Feijoo *et al.* 1995). Usually, the inhibitory concentration of Na^+ was between 3 and 16 $\text{g} \cdot \text{L}^{-1}$ (Feijoo *et al.* 1995). In this study, the highest Na^+ concentration of the fermentation system was only 1.8 $\text{g} \cdot \text{L}^{-1}$, including the Na^+ from NaHCO_3 loading ($2.5 \text{ g} \cdot \text{L}^{-1} \text{NaHCO}_3$, Na^+ , $0.68 \text{ g} \cdot \text{L}^{-1}$) and alkali pretreatment (12% of NaOH, Na^+ , $1.14 \text{ g} \cdot \text{L}^{-1}$). This concentration was relatively low and had no obvious negative effect on biogas production process. Therefore, the reason for the decline in biogas yield under high alkali concentrations may be the generation of toxic substances (Lagerkvist and Morgan-Sagastume 2012). Further experiments need to be conducted to determine the influence of the formation of toxic substances on the biogas yield.

Fiber length was the other significant factor for biogas yield ($P < 0.05$). Figure 5d shows that the biogas yield continuously increased with decreasing fiber length when pretreatment time ranged from 2 to 4 days. A similar trend was observed in Fig. 5e with pretreatment temperatures ranging from 35 to 45 °C. Shorter fiber lengths resulted in larger contact areas between fiber and aqueous alkali, higher treatment efficiency, and higher biogas yield.

The biogas yield was not significantly affected by pretreatment time ($P > 0.05$). Figure 5f shows that the biogas yield increased only slightly with increasing pretreatment time. However, increasing the pretreatment time past 3 days caused a slight decline in the biogas yield. It was previously reported that 3 days is sufficient for wet-state pretreatment to guarantee the complete chemical reaction between the substrates and NaOH (Chandra

et al. 2012; Zheng *et al.* 2009). However, further increasing the pretreatment time might result in a decline in biogas yield, which may result from the production of toxic substances (Lagerkvist and Morgan-Sagastume 2012).

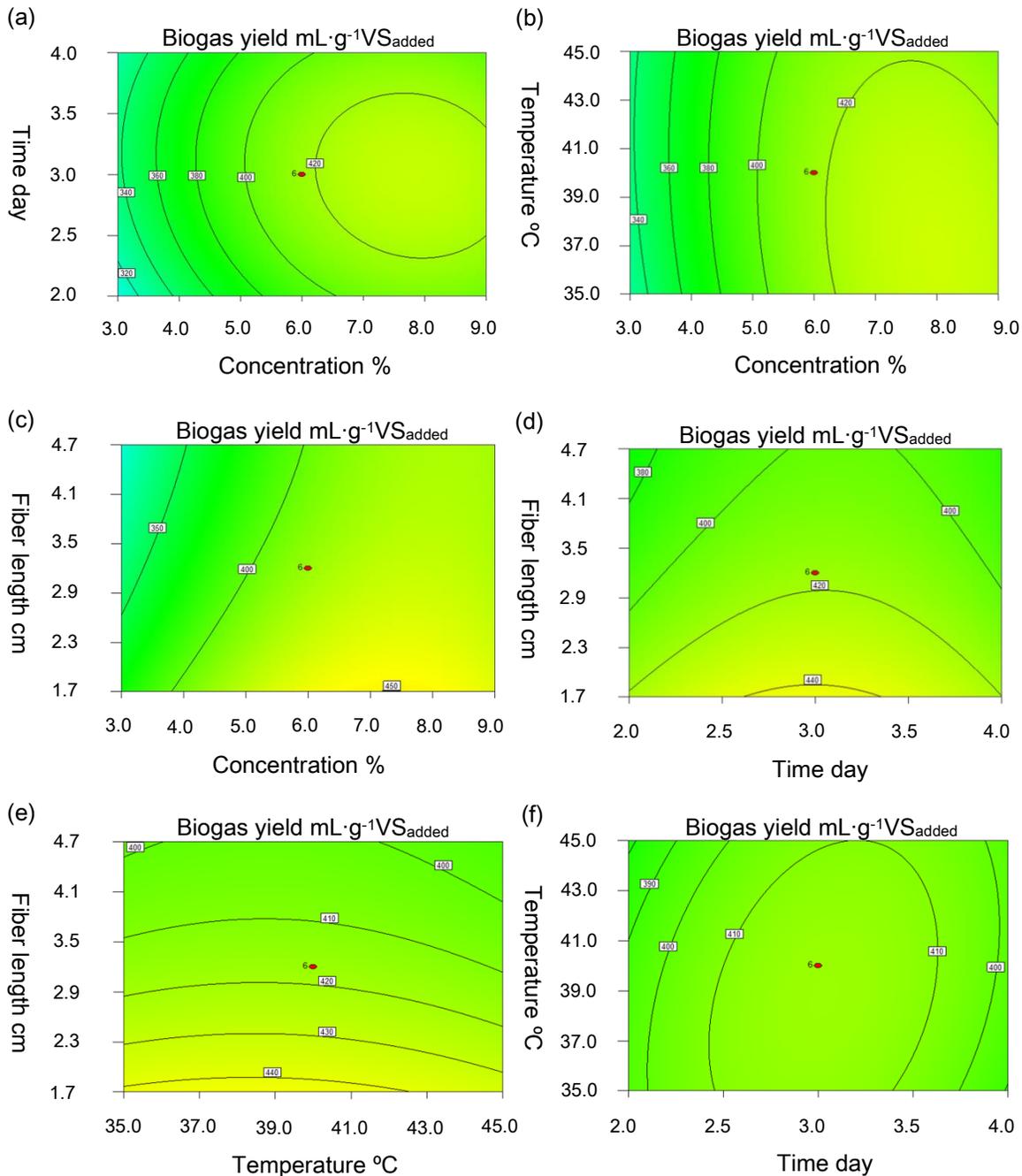


Fig. 5. Contour plots for the effects of two variables on the biogas production, holding the other two variables constant

Pretreatment temperature (35 to 50 °C) had an insignificant effect on biogas yield in this experiment ($P > 0.05$), which was in agreement with the results of previous reports (Salehian *et al.* 2013). It had been reported that the difference in biogas yield for NaOH-pretreated fibers at temperatures between 0 °C and 100 °C is negligible. In the subtropical

and tropical zones, the temperature is usually around 30 °C to 35 °C or even higher during the harvest season of banana. Therefore, for the industrial scale production, the alkali-pretreatment could be conducted in ambient temperature without heating, which could reduce the energy consumption for the pretreatment.

Anaerobic Fermentation under Optimized Pretreatment Conditions

Using Eq. 5, the model predicted a maximum biogas yield of $472.1 \text{ mL}\cdot\text{g}^{-1}\text{VS}_{\text{added}}$ under the optimum parameters of NaOH concentration at 7.8%, pretreatment for 3 days, temperature of 48 °C, and fiber length of 0.2 cm. To confirm the result predicted from the model, repeated BPSF biogas fermentation experiments under the optimum pretreatment conditions were conducted (Fig. 6a). The biogas yield under the optimum pretreatment condition was $463.0 \text{ mL}\cdot\text{g}^{-1}\text{VS}_{\text{added}}$, similar to the predicted value, and was 89.2% higher than that from the control, untreated sample. It should be pointed out that the biogas yield ($522.7 \text{ mL}\cdot\text{g}^{-1}\text{VS}_{\text{added}}$) obtained (6% NaOH, 3 days, 40 °C and 0.2 cm, Table 2) was unusually high, which could be attributed to the experimental variation. In the early stage of anaerobic digestion, the biogas production for the pretreated sample was much faster than the control. This increased rate was likely caused by the digestion of easily digestible molecules in the pretreated BPSF. This result agreed with existing studies on the alkali-pretreatment of lignocellulose materials (Chandra *et al.* 2012; Taherdanak and Zilouei 2014).

In addition, the gaseous components were measured in the biogas fermentation process (Fig. 6b). The methane content for the alkali-pretreated sample remained stable between 55.5% and 63.9% after fermenting for 3 to 4 days which was slightly higher than that of the control (50.9% -56.23%).

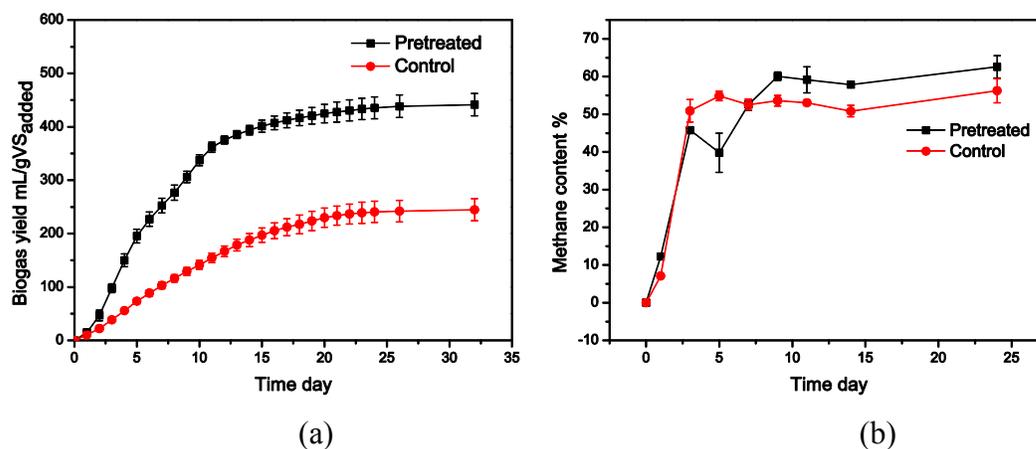


Fig. 6. Biogas yield and methane content of BPSF under optimized pretreatment condition. (a)The biogas yield of pretreated and unpretreated samples; (b)The methane content of pretreated and unpretreated samples

NaOH pretreatment can improve the biodegradability and biogas yield of biomass material (Raposo *et al.* 2006; Salehian *et al.* 2013; Ward *et al.* 2008; Zhu *et al.* 2010). Studies have shown that pretreatment of lignocellulosic substrates, such as corn stover and rice straw, with NaOH increased biogas production by 32% to 87.5% (Chandra *et al.* 2012; Zheng *et al.* 2009). In this paper, the highest biogas yield for the optimal pretreatment condition was 89.2% higher than that from the control. This result was higher than previous reports on biogas production from BPS (Kalia *et al.* 2000; Ou *et al.* 2006). Compared with

other existing reports on the alkali-pretreatment of biomass materials, the biogas yield discussed in this paper was relatively high (Chandra *et al.* 2012; Zheng *et al.* 2009).

BPS has much potential to be a biomass material for biogas production. Further efforts still need to be made to improve the availability of BPS for industrial application after reducing the transportation cost. Extract BPSF for pulping and using the residues for biogas production could be another choice (Fig. 1). Data obtained from this paper provided a better understanding of the biogas yield and pretreatment of BPSF. These data can be used along with data on pulping to determine the best utilization of BPSF. Further work still needs to be done to optimize the technological route of BPS for industrial applications.

CONCLUSIONS

1. Banana pseudo-stem fiber (BPSF) could be considered as a potential substrate for the biogas production for its favorable biogas fermentation performance. NaOH pretreatment was successfully used to increase the biogas yield of BPSF to 463.0 mL·g⁻¹VS_{added}, which was 89.2% higher than that of the control.
2. Among the pretreatment factors within the test ranges in this paper, only NaOH concentration and fiber length had significant effect on the biogas yield. Based on the climate condition of subtropical and tropical zones, and consideration of industrial application, ambient temperature and 3 days are recommended for the NaOH pretreatment.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Science and Technology of China, National Key Technologies R&D Program (Grant No. 2012BAC18B01), Science and Technology Program for Public Wellbeing (Grant No. 2013GS460202-3) and the International S&T Cooperation Program (Grant No.2010DFB64040). We would like to thank Ruitao Zha for his analytical and technical help.

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Article submitted: April 8, 2014; Peer review completed: June 1, 2014; Revised version
received: June 15, 2014; Accepted: June 18, 2014; Published: July 10, 2014.