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Optimization of Enzymatic Pretreatment Process for Enhanced Biogas Production from Palm Oil Mill Effluent

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Abstract

Anaerobic digestion (AD) of palm oil mill effluent (POME) for biogas production by four step processes such as hydrolysis, acidogenesis, acetogenesis and methanogenesis in which hydrolysis is pondered as a rate limiting step. To achieve high yield of biogas in the AD, enzymatic hydrolysis as the pretreatment method was applied to overcome the current limitation of the existing plants. Therefore, an optimization study was carried out to evaluate AD performance for biogas production. Among the parameters, three factors i.e. cellulase enzyme dose, pH and total suspended solids (TSS) were studied by the face-centered central composite design (FCCCD) under the response surface methodology (RSM) based on lipase pre-treated POME. The results showed that the maximum of 835 ml biogas was obtained with 85% COD removal from 50 ml of POME substrate after 10 days of operation while 290 ml biogas produced from control (without pretreatment). The optimum process conditions were at pH 5 and TSS of 1.5% and enzyme loading 200 CMC/ml. High coefficient of R^2 value of 0.994 indicated the satisfactory fit of the model.

1. INTRODUCTION

Palm oil mills are usually constructed near water channel as water is required for the operation in mills and effluent can be easily discharged to the water channel after treating. Discharging partially treated or untreated POME into water channel can cause serious damage of the water quality. According to permissible range mentioned in Malaysian Environmental Quality Order and Regulations, 1977, no industry is allowed to discharge thick brownish POME without treatment as POME create a huge pollution due to its high range of BOD and COD [1]. Treatment process of POME is a challenge for the palm oil sector. Through anaerobic treatment of POME, biogas production and COD removal could be an effective solution for oil palm indursties.

Application of enzyme is an environmental friendly and economically viable technique for the anaerobic digestion of POME as enzyme increase the rate of degradation of organic substances in POME [2], [3]. Addition of mixed enzyme showed positive result of anaerobic digestion [4]. Enzyme in anaerobic digestion plays an important role in solubilisation [5]. Akao et al. [6] reported 50% elevation of AD of citrus peel by enzymatic pre-treatment while other researchers, Ziemiński et al. [7] and Frigon et al. [8] reported 19% and 72% of increase of biogas with enzymatic pre-treatment methods with various organic wastes, respectively. These studies are showed that addition of enzymes into anaerobic digestion process could not only cut down digesting time, improve sludge digestibility, and reduce disposal costs, but also could be easily controlled, and its products were harmless to environment [9].

Although many studies are focused on the enzymatic pretreatment for enhanced biogas production by anaerobic digestion, very limited studies were found in the POME substrate especially for the lipase and cellulase enzymes. Cellulases have the ability to break down cellulose to sugar while lipase is converting lipid into free fatty acid.

Therefore these two enzymes are selected based on the POME characteristic for hydrolysis as part of pretreatment method to evaluate the enhanced biogas production through an optimization study. In this study, face centered central composite design (FCCCD) under response surface methodology (RSM) was applied to find out optimum range of variables and the interaction among the different factors or process conditions. Pre-treated POME with lipase enzyme was used as the substrate for the production of biogas by three process variables cellulase enzyme, pH and TSS.

2. MATERIALS AND METHOD

A. Sample Collection

The raw material, palm oil mill effluent (POME) was collected from an oil palm industry named West Palm Oil Mill, Banting, Selangor, Malaysia. The liquid sample was collected in a plastic container and stored at 4°C.

B. Sub-culturing, Inoculum Preparation and SSB

The microbial strain, Candida cylindracea (ATCC 14830) for lipase enzyme used in this study was purchased from the American Type Tissue Culture, USA. *C. cylindracea* was grown on PDA plates at 28°C for four days in an incubator (Incucell, Germany) and sub-cultured every two weeks. Each plate was washed with 10.0 ml sterile distilled water and the suspension was used to prepare the inoculum in the appropriate medium. Locally produced palm kernel cake (PKC) based lipase through the solid state fermentation (SSB) was used according to the methods suggested by Elgharbawy et al [10] and Salihu et al. [11].

Trichoderma reesei RUT C-30 (ATCC 56765) for cellulase enzyme was cultured on the PDA plate as inocula source and incubated at 30°C for 8-10 days until the good sporulation was observed. After maturation each plate was washed with 25 ml of sterile water and then filtered the spore suspension with Whatman.1 filter paper. Cellulase enzyme was produced and assayed through the method described by Rashid et al [12].

C. Optimization of Biogas Production by Statistical Method

After adjusting pH, in 250 ml Erlenmeyer flask 50 ml, raw POME was pre-treated with lipase enzyme with 15 U/ml for 24 hours at room temperature (28±2°C) with 150 rpm of rotation speed. After 24 hours, cellulase enzyme was added to the pre-treated sample and put into shaker. Water displacement method was applied to measure the volume of biogas. For different TSS loading, water was added to the pre-treated sample. Response surface methodology (RSM) was used to optimize the screened variables to see the effect of variables on biogas production. Face centered central composite design (FCCCD) under RSM was employed using Design Expert software (Version 6.0.8, Stat-Ease Inc., Minneapolis, USA) to optimize the three factors: cellulase, TSS loading and pH. A set of eighteen experimental runs with four center points (Run 1, 4, 8 and 9) was generated. Variables have been studied using three different levels: low (-1), medium (0) and high (+1). The experimental design used for this study has been shown in Table 1. The remaining factors concentrations were fixed based on the results of previous experiments. Biogas and COD removal are two responses. A block digram and optimization process flow chart is shown in Figure 1.

D. Analytical analysis

The analysis of the different concentration of TSS and TS was done following the standard methods [13]. Syringe filter of 0.45μm pore size was used for the preparation of the sample for the soluble COD [11].

Table 1. Faced centered central composite design (FCCCD) experimental design for selection process conditions for biogas production and COD removal

Run	pH	TSS (%)	Cellulase (U/ml)	Biogas (ml)	COD Removal (%)
1	5.00	1.50	200	825	87.00
2	5.00	1.00	200	810	80.70
3	6.00	2.00	100	580	54.60
4	5.00	1.50	200	820	82.00
5	6.00	1.50	200	815	85.56
6	4.00	1.00	300	720	73.60
7	4.00	1.00	100	550	55.00
8	5.00	1.50	200	830	87.80
9	5.00	1.50	200	835	85.00
10	5.00	1.50	100	580	57.20
11	5.00	2.00	200	820	85.00
12	6.00	2.00	300	810	85.56
13	4.00	2.00	100	540	54.60
14	4.00	2.00	300	810	85.80
15	6.00	1.00	100	630	55.00
16	6.00	1.00	300	800	84.13
17	4.00	1.50	200	790	84.80
18	5.00	1.50	300	780	83.00

3. RESULTS AND DISCUSSION

The study was mainly focused on the optimization of the process parameter in the anaerobic digestion of POME by applying the hydrolysis process for enhanced biogas production. It was conducted by interaction the process variables through the statistical method of the face center central composite design (FCCCD) under the response surface methodology (RSM). Experiments were carried out by three process variables cellulase, pH and TSS with three level and 4 center point according to FCCCD plan and 2 response biogas and COD removal was measured (Table 1). The highest biogas and COD removal yield achieved was 835 ml and 87%, and the lowest activity observed was 540 ml and 55% respectively at run 1 and 13 (Table 1). Several regression analysis of the experimental data were employed to calculate the regression of the equation and the fitted equation was used to predict the biogas production and removal of COD. The quadratic polynomial equation provided levels of biogas production and COD removal as a function of pH, TSS and cellulase concentration which can be presented in terms of coded factors as shown in the following equation:

$$\begin{aligned} Y_1 = & 820.36 + 22.50A + 5B + 104C - 10.71A^2 + \\ & 1.79B^2 - 133.21C^2 - 15AB - -5AC + 20BC \end{aligned} \quad (1)$$

where $Y1$ is the biogas production (ml) produced as a function of the coded levels of pH (A), TSS (B) and cellulase concentration (C), respectively.

Analysis of variance (ANOVA) was used to assess the adequacy of the statistical model for biogas production (Ta-

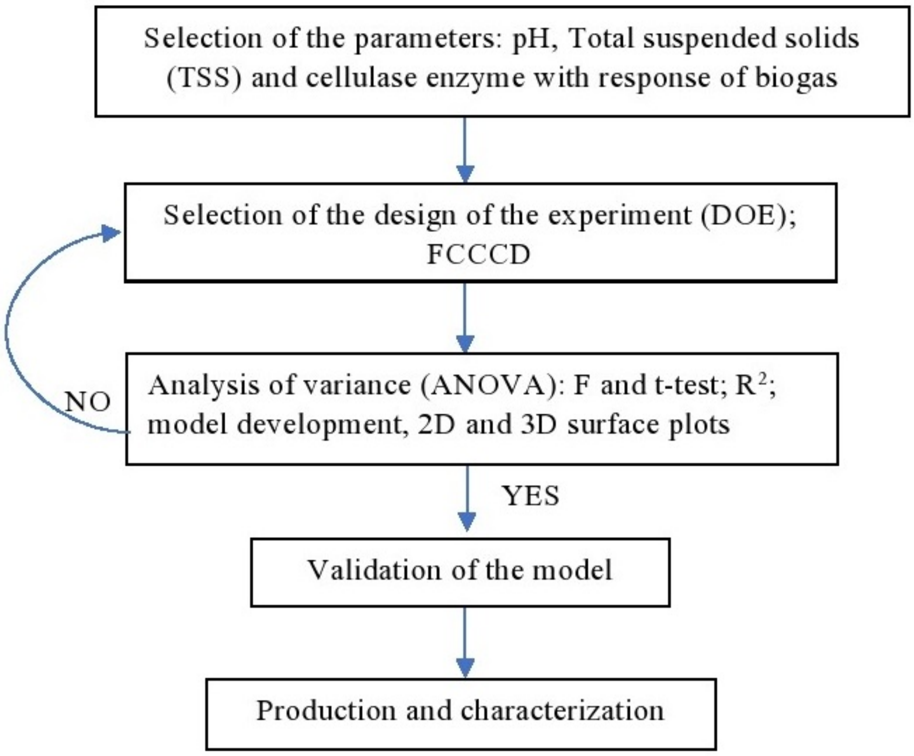

Figure 1. A block diagram and optimization process flow chart for biogas production

ble 2. The F value of 157.42 biogas production, p-value of < 0.0001 indicated that the selected quadratic models were significant. p-value was also used to determine the significance of each coefficient, and used as an indication to examine the interaction strength between each coefficient that was independent. p-value of less than 0.05 implies that model terms are significant, while values greater than 0.1 means insignificant model terms. For biogas production, the terms C and C^2 were found to be significant model and it influenced the overall biogas production remarkably. Meanwhile, based on the F-values of the main factors studied, the cellulase concentration showed the highest value, denoting that it presented the strongest influence on biogas production, while the pH showed the least pronounced effect for both yield. The lack of fit F-value of 4.92 for biogas implied that the lack of fit was not significant relative to the pure error. Non-significant lack of fit indicates that the model fits adequately.

For biogas production, the efficiency of the model was demonstrated by the high value of R^2 0.99 and adjusted R^2 0.98, which indicated better correlation between the actual and predicted values. The signal to noise ratio was measured by the adequate precision, in which a ratio greater than 4 is considered a requirement for desirable models and the model showed a ratio of 31.93. The CV for the biogas production was 1.62, which was within the acceptable range.

Figure 2(a) and (b) depicted the interaction between TSS and pH on biogas production when the pH was set at the central point. The response plot showed a good interaction between the factors on biogas production. This suggested that the optimum conditions were well defined and the effect of the interaction between these factors. Result showed that the increased in biogas production resulted from the increase in pH to optimum values, and TSS further increase led to a decrease in the production. Both of these factors were the important variables for biogas production and considered as indicators of effectiveness and economic performance.

The results showed the effect of pH and cellulase concentration on biogas production at high level of cellulase concentration. The shape of the 3D response surface curves showed a less interaction between these tested variables. Cellulase concentration exhibited quadratic effects on the response were cellulase concentration increased up 200 ml to about 250 ml concentration followed by its further increase. However, pH demonstrated a linear effect on biogas production. The pH shown to have a less effect on biogas production as compared to the cellulase concentration, for the range covered in this study [12]. Figure 3(a) and (b) showed the effect of pH and cellulase concentration on biogas production at high level of cellulase concentration. The shape of the response surface curves showed a less interaction between these tested variables. Cellulase concentration exhibited quadratic effects on the response, were, cellulase

Table 2. Analysis of variance (ANOVA) based on the FCCCD experimental runs for biogas production

Source	Sum of Squares	DF	Mean Square	F-value	Prob>F	Remark
Model	2.149E+005	9	22643.28	157.42	<0.0001	significant
A, pH	2259.00	1	5062.50	35.20	0.0473	
B, TSS	2560	1	250.00	1.74	0.0371	
C, Cellulase	1.254E+005	1	1.082E+005	751.95	<0.0001	
A^2	5.08	1	311.06	2.16	0.9142	
B^2	726.05	1	8.64	0.060	0.2202	
C^2	43271.21	1	48086.06	334.30	<0.0001	
AB	312.5	1	1800	12.51	0.4083	
AC	112.5	1	200	1.39	0.6148	
BC	1012.5	1	3200	22.25	0.1549	
Residual	3283.63	8	143.84			
Lack of Fit	3214.88	5	205.14	4.92	0.0101	Not significant

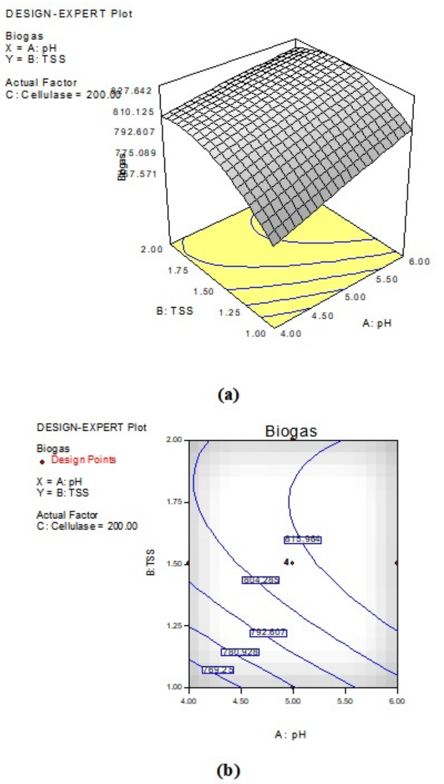

Figure 2. (a) 3D response surface plot for the optimization of biogas production based on pH and Total Suspended Solids (TSS) ; (b) Contour plot of interaction effect between pH and TSS on biogas production

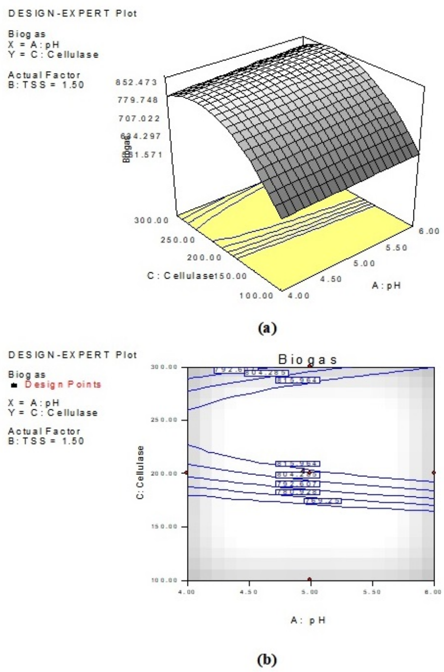

Figure 3. (a) 3D response surface curve of the interaction effects of pH and Cellulase for biogas production. (b) Contour plot of interaction effect between pH and Cellulase on biogas production

concentration increased up 200 ml to about 250 ml concentration followed by its further increase. However, pH demonstrated a linear effect on biogas production. The pH shown to have a less effect on biogas production as compared to the cellulase concentration, for the range covered in this study.

Figure 4 a) and (b) represented the interaction between TSS and cellulase concentration at fixed pH at 5.00. Cellulase concentration exhibited good effect at high dose on the biogas production, while the effect of TSS was medium level compared to cellulase concentration towards the higher design point of the variable produced a greater response [12].

Yang et al. [4] reported the addition of mixed enzyme (α-amylase and protease) has the more significant effect than that of single enzyme on the biogas production. Sonakya et al. [14] reported 7-14% increase of methane production by using damaged wheat grains treated cellulase, protease, and α-amylase prior to AD. Frigon et al. [8] reported that 72% increase of methane gas with high loading of pectate-lyase and 53% decrease with lower loading after 78 days incubation using switch grass as a feedstock. These result showed lower loading of enzyme resulted inhibition of enzyme while higher loading of enzyme showed a significant positive result. Bruni et al. [15] reported 34% increased of yields of methane production treated with laccase and cellulases and hemicellulases mixture.

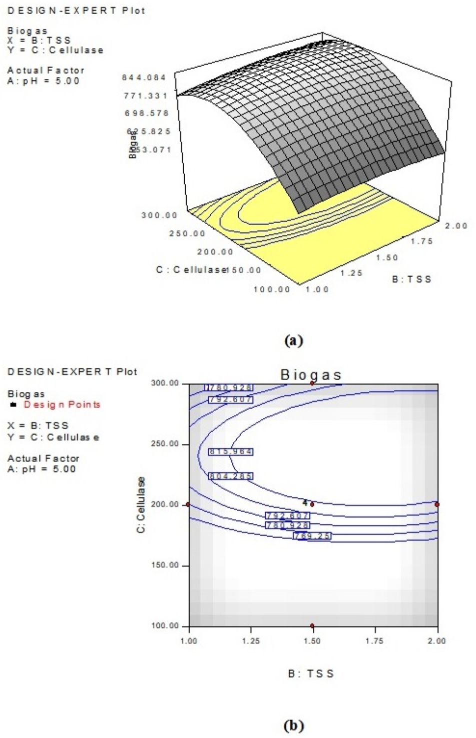

Figure 4. (a) 3D response surface curve of the interaction effects of TSS and Cellulase for biogas production. (b) Contour plot of interaction effect between TSS and Cellulase on biogas production

The optimum process conditions are determined by applying the model equation within the ranges of the experimental dsign and tested for validation. The results showed that the validatin results support the predicted model with 8-12% error which is condiered as a very good predicted model.

4. CONCLUSION

The results conclude that the application of cellulase and lipase enzyme in different process conditions showed an effective method of the pretreatment for enhanced biogas production as compared to the control (without pretreatment). Cellulase showed more effect on biogas production than other two parameters, TSS and pH during statistical optimization experiments. The optimization of the process conditions i.e. pH 5, TSS 1.5% (w/w) and CMC celluase of 200U/ml showed the maximum biogas production of 835 ml in the anaerobic digestion of POME. The findings of this study provide that application of locally produced enzyme in hydrolysis of POME is an efficient technique to produce biogas and COD removal for anaerobic digestion of POME.

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