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Box-Behnken-Design based optimization strategy for alkaline pretreatment of palm oil mill effluent for producing reducing sugar

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Abstract. Alkaline pretreatment is used as a substrate prior to subsequent biological processes for palm oil mill effluent (POME) treatment. In the present study, Sodium hydroxide (NaOH) was used to recover reducing sugar from POME. Important process parameters, such as reaction time (min), reaction temperature (°C), and concentration (%) (w/v), were optimized using Box-Behnken Design in Response Surface Methodology (RSM). POME treated with NaOH yielded the reducing sugar of up to 3640.84 mg/L under the optimum conditions of 58.78 min, 77.06°C, and 2.58% (w/v) for reaction time, reaction temperature, and concentration of NaOH, respectively. The analysis of variance (ANOVA) indicated that the quadratic model for reducing sugar production had an R^2 coefficient of 0.979. Conformity testing for optimum conditions proved the validity of the model, yielding reducing sugar at a 9.35% increase, relative to untreated POME. This study verified the importance of statistical tools such as RSM for enhanced reducing sugar production from the industrial waste stream (POME) and its usefulness and efficiency in energy conversion.

1. Introduction

Renewable energy use, which involves converting waste materials into energy, is an important part of reducing greenhouse gas emissions and concomitant global warming and it is currently a limited energy source [1, 2]. For example, the bioprocessing of organic wastes from, among other things, agricultural scrap, cooking residue, fruits, and vegetables into useful bioenergy products can be used to meet energy needs and reduce agricultural waste [3, 4].

In a large agricultural sector, such as palm oil plantation, effluents from palm oil mills (POME) are a type of organic waste derived from crude palm oil (CPO) production [5]. POME is rich in lignocellulosic materials, such as cellulose, hemicellulose, and lignin [6]. The main characteristics of POME include the high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), and high pH (value) [6, 7]. The aforementioned characteristics would inhibit POME treatment and increase the duration for making the material consumable and eco-friendly for the microbial groups. Hence, a pretreatment process is needed to increase the rate of reaction and boost production capacity [5]. Response surface methodology (RSM) has been

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demonstrated to optimize the parameters of the substrate pretreatment process, such as in the cases of municipal sludge [8], sewage sludge [9], bagasse [10], and rice husk [11]. It has been reported that the predicted model obtained by RSM has been useful, with the determined coefficient of R^2 being close to unity with the significant p-value. The 3k factorial, central composite and Box-Behnken are second-order designs and are the most frequently used but among them the Box-Behnken design (BBD) is the most economical in that it requires only three-levels for each factor (setting are -1,0,1) and it has been popular in industrial research [12].

The objective of this study, therefore, is to optimize the alkaline pretreatment process by using sodium hydroxide (NaOH) on POME for maximum reducing sugar recovery by investigating the interactive effects of the process factors. The alkaline pretreatment was selected because the process could effectively reduce the hemicellulose and lignin contents [13]. In a previous study, Liu, Liu, Wang, Chang, Yin, Zhu and Lu [14] indicated an improvement in reducing sugar concentration with a lowered cellulose and hemicellulose content when the liquid to solid ratios of 5:1 and 6:1 were applied to the alkaline pretreatment of corn stalk by using NaOH and CaO. Elsewhere, Zhao, Xu, Zhang and Wang [15] reported the significant increase of lignin removal (from 14.81 to 50.16%) resulting from the increment of alkaline concentration (from 0.02 to 0.12 mol/L). Similar outcomes were reported by Jiménez, Chandel, Marcelino, Anjos, Batesttin Costa, Jose V. Bell, Pereira and da Silva [16], whereby 46.30% of lignin were removed and 8.5% of total carbohydrate content were increased after alkaline pretreatment using ammonium hydroxide. Extracellular and intracellular biopolymers, such as carbohydrates, lipids, and proteins, can be produced from the breakdown of POME's complex particulate structure from alkaline pretreatment [17]. The chemical structure of POME was destroyed by alkaline pretreatment and caused morphological changes to the cell wall and lowered crystallite and improved POME porosity [18]. For the present study, NaOH was selected because it gave the highest solubilization efficiency, relative to potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$) and magnesium hydroxide ($Mg(OH)_2$) [19, 20]. The following three independent variables were used for statistical analysis: reaction time (min), reaction temperature (°C), and NaOH concentration (%) (w/v). The Box-Behnken design was used to design experiments, build models, and determine the optimum conditions.

2. Materials and Method

The POME was collected from Felda Global Ventures (FGV) Mill, Kemahang, Tanah Merah, Kelantan, Malaysia and packed in a 20 Liter airtight container and shipped to the University of Seoul, South Korea, after which it was stored at below 4°C until further use.

2.1. Alkaline pretreatment

The POME samples were pretreated with NaOH with extra pure grade (DUKSAN reagent) from Duksan Pharmaceutical Co., Ltd., South Korea using a Bioprocess Control water bath (VWX18-EU) to control the temperature. The experiments were performed in 500-mL screw-cap glass bottles containing 250 mL of POME samples using Box-Behnken response surface design for a pretreatment time of 5 to 60 min, reaction temperature of 27°C to 80°C, and a concentration of NaOH between 0.1% and 3%. A mechanical stirrer was used to continuously mix the components. The pretreated samples were subjected to centrifugation at 4500 x g for 15 minutes, vacuum filtration using a glass microfiber filter mounted in a Buchner funnel, and analysis by using DNS (Dinitrosalicylic acid) method to determine the reducing sugar content. The reducing sugar in POME consists of Glucan, Xylan, and Arabinan [21], and the purpose of reducing sugar analysis was to identify the effectiveness of alkaline pretreatment on POME.

3. Results and Discussion

3.1. Model fitting and regression analysis

The experimental design performed in this study contained 17 experimental runs involving low, high, and center points, as summarized in Table 1. It was observed that reducing sugar value had ranged

between 3222.01 mg/L and 3589.88 mg/L. The highest experimental value (3589.88 mg/L) for reducing sugar value was observed in Run 13, while the lowest experimental value (3222.01 mg/L) was recorded in Run 7.

Table 1. Predicted and experimental response using Box-Behnken experimental design.

Run	Variables						Response	
	Reaction time (min) (X_1)		Reaction temperature (°C) (X_2)		NaOH concentration (%) (w/v) (X_3)		Predicted reducing sugar (mg/L)	Experimental reducing sugar (mg/L)
	Actual	Code	Actual	Code	Actual	Code	Predicted	Observed
1	5.00	-1	53.5	0	3.00	1	3337.78	3312.64
2	5.00	-1	80.0	1	1.55	0	3408.13	3413.94
3	32.5	0	80.0	1	3.00	1	3563.12	3582.44
4	32.5	0	27.0	-1	0.10	-1	3327.19	3307.87
5	5.00	-1	53.5	0	0.10	-1	3318.40	3330.02
6	60.0	1	53.5	0	0.10	-1	3339.45	3364.58
7	32.5	0	80.0	1	0.10	-1	3239.44	3222.01
8	32.5	0	53.5	0	1.55	0	3248.96	3236.77
9	5.00	-1	27.0	-1	1.55	0	3359.99	3367.70
10	32.5	0	27.0	-1	3.00	1	3244.02	3261.46
11	32.5	0	53.5	0	1.55	0	3248.96	3234.56
12	60.0	1	53.5	0	3.00	1	3560.58	3548.96
13	60.0	1	80.0	1	1.55	0	3597.59	3589.88
14	60.0	1	27.0	-1	1.55	0	3414.38	3408.56
15	32.5	0	53.5	0	1.55	0	3248.96	3248.42
16	32.5	0	53.5	0	1.55	0	3248.96	3286.48
17	32.5	0	53.5	0	1.55	0	3248.96	3238.55

* The predicted response was generated from the regression equation using ANOVA.

The model adequacy estimated for NaOH pretreatment and reducing sugar yield were tested with ANOVA. The analysis of variance involving three effects (i.e., reaction time, reaction temperature, and alkaline concentration) indicated that they were highly statistically significant. This analysis was proven by the p-value of the model, which was less than 0.0001. The p-values of A-time, B-Temp, C-NaOH concentration, AB, AC, BC, A^2, and B^2 were 0.0003, 0.0005, 0.0004, 0.0386, 0.0068, 0.0001, < 0.0001, and 0.0007, respectively, all of which are significant terms of the model. The p-value for C^2 and lack-of-fit were insignificant and they were calculated at 0.1807 and 0.2319, respectively.

Furthermore, the F-value of the model was high, which signifies that the variation in response was attributed to the model itself. An F-value of 36.94 implies that the model is significant. There is only a 0.01 % chance that this high F-value could be due to noise. The coefficient of determination (R^2) statistic indicated that the fitted model explained 97.94% of the variability in responses. In addition, the high R^2 value implies a better model accuracy.

The adjusted R^2 value of 95.29% was close to the R^2 value of 97.94%, which implied that the model was highly adequate. The lack-of-fit F-value (2.19) was negligible, meaning that there was a 23.19 % chance that it could have been due to noise and that a lack-of-fit F-value was probable. The p-value of lack-of-fit was greater than 0.10, which implied that the expected data of the model was appropriate for actual experimental response data.

A low coefficient of variation (CV) value of 0.79 indicated that the experiments were performed with great accuracy and reliability, while an adequate precision of 17.5659 suggests that the appropriate signal with a ratio above 4.0 is desirable. The observed and predicted yield of reducing sugar from the alkaline pretreatment is plotted in Figure 1(a). A reasonable correlation could be made

between the experimental and predicted values; the modified R^2 is 97.90%. The model development by response surface model in this study was, therefore, considered satisfactory to predict the reducing sugar recovery from POME [22]. Similar high predicted and adjusted R^2 values of 0.9881 and 0.9980, respectively, were reported by Lv, Xiong, Li, Chen, Xiao, Zhang, Li, Gong, Lin and Liu [23] in their study on vacuum-assisted alkaline pretreatment of sugarcane bagasse. They observed the maximum sugar yield of 0.5146 g/g when the composition (%) of Glucan, Xylan, Lignin, and solid recovery rate (%) were set at 59.43%, 22.51%, 8.15%, and 59.80%, respectively. Kamalini, Muthusamy, Ramapriya, Muthusamy and Pugazhendhi [24] reported the R^2 of 0.9952 and adjusted R^2 of 0.9907, where the predicted R^2 of 0.9841 was in reasonable agreement with the adjusted R^2 (0.9907). The observed value of R^2 (0.9952) indicated that total variation in reducing sugar (xylose) yield was attributed to the studied pretreatment parameters (i.e., solid to liquid ratio, reaction time, microwave frequency, and NaOH concentration). These findings verified the quality of the model developed by the BBD experiment that fitted the data adequately and was close to the predicted value.

3.2. The effects of alkaline pretreatment parameters on the reducing sugar content

The three-dimensional plot of the response surface displays the visual relationship between independent and dependent variables as shown in Figure 1(b), Figure 1(c) and Figure 1(d). As can be seen in Figure 1(b), POME's reducing sugar content ranges from 3200 mg/L to over 3750 mg/L, depending on the changes of reaction time (min) and reaction temperature (°C), as well as their interaction. Moreover, the reducing sugar content had increased to the maximum value of 3500 mg/L with an increase in reaction temperature from 54.6°C to 80°C. The longer alkaline pretreatment time also had a positive impact on reducing sugar content, which is comparable with a previous study [25]. The interaction of both reaction temperature (°C) and reaction time (min) yielded an increment of reducing sugar content from 3200 mg/L to the maximum value of 3750 mg/L. However, Kassim and Bhattacharya [26] reported that the combination of pretreatment at higher temperatures and increased reaction time proved disadvantageous for reducing sugar production.

Nevertheless, a combined short reaction time with lower NaOH concentration was unfavorable for reducing sugar production when the reaction time was within the range of 16 to 49 min, as shown in Figure 1(c). It was indicated that the maximum reducing sugar (3750 mg/L) was predicted when the reaction time and NaOH concentration were at 60 min and 3% (w/v), respectively. The extracellular biopolymers hydrolyzed by the addition of NaOH resulting in the release of intracellular material externally [27]. This phenomenon was also observed by Shahabazuddin, Sarat Chandra, Meena, Sukumaran, Shetty and Mudliar [28]; specifically, they observed the release of 5.2 times more reducing sugar with alkaline loading was increased. Nikzad, Movagharnejad, Talebnia, Aghaiy and Mighani [29] also indicated a higher glucose yield could be facilitated by increasing the reaction time and through the addition of NaOH loading.

Figure 1(d) depicts that the alkaline pretreatment process for reducing sugar recovery is significantly dependent on NaOH concentration and reaction temperature when the reaction time is fixed, which is demonstrated by the low p-value (0.0001). The higher reducing sugar recovery occurred at the NaOH concentration of 1.84% (w/v) and a temperature above 58.8°C. The increment of both NaOH concentration and temperature has enhanced the reducing sugar recovery. The higher pretreatment temperature could also improve the solubilization of organic substances [30, 31].

3.3. Experimental validation of model prediction values

Prediction of the optimum condition was made via the desirability function provided by the software (Design Expert). The optimum process parameters was determined to be a reaction time of 58.78 min, a temperature of 77.06°C and a NaOH concentration of 2.58% (w/v). Under these conditions, it was found that the predicted response of reducing sugar was 3708.13 mg/L. The conformity experiment (average of three replicates) using the aforementioned optimized parameters yielded a value of 3640.84 mg/L, which was close to the predicted value.

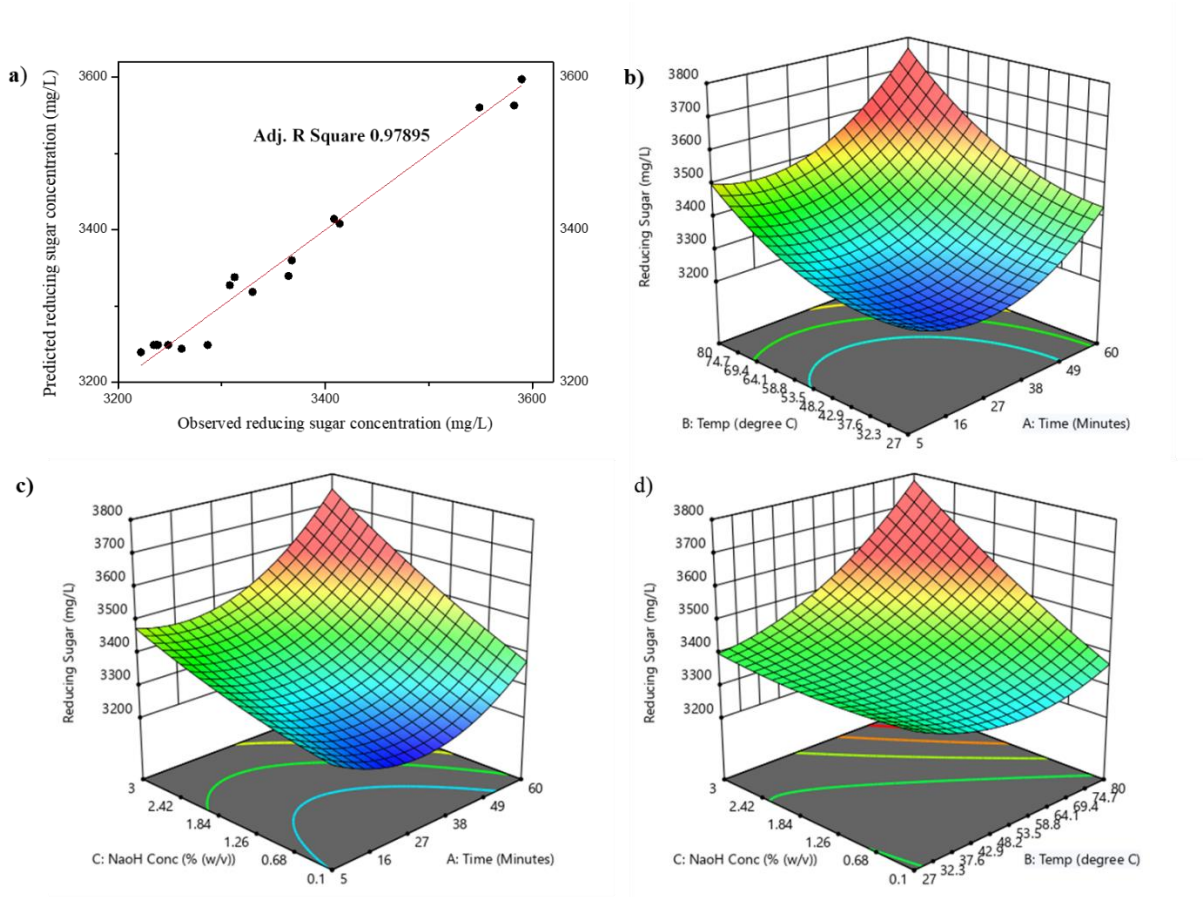

Figure 1. a) Parity plot to demonstrate the observed and predicted values for reducing sugar (mg/L) from the sodium hydroxide pretreatment of POME are distributed. b) The interaction between independent variables of time (min) and temperature (°C) on reducing sugar recovery from POME with alkaline pretreatment. c) The interaction between the independent variables of time (min) and NaOH concentration (%) (w/v) on reducing sugar recovery from POME with alkaline pretreatment. d) The interaction between independent variables of NaOH concentration (%) (w/v) and temperature (°C) on reducing sugar recovery from POME with alkaline pretreatment

4. Conclusion

The results of the statistical analysis indicated that the output from the pretreatment was significantly affected by all factors; essentially, there was a favorable interaction between the system variables and a resultant increase of the yield from the reaction. The response surface methodology for optimization indicated that the optimum value for the pretreatment process yielded 3640.84 mg/L of reducing sugar and relative to the untreated POME, reducing sugar context increased by 9.35%. Therefore, this finding justifies that the optimization of the pretreatment process via the Box-Behnken design technique could facilitate the subsequent fermentation process of biofuels such as biohydrogen and biomethane production, in achieving higher yield from organic wastes.

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