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Optimization of Alkaline Pretreatment Conditions of Oil Palm Fronds in Improving the Lignocelluloses Contents for Reducing Sugar Production

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Abstract

Alkaline pretreatment by using sodium hydroxide (NaOH) is capable in removing high lignin content as well as maintaining the highest cellulose and hemicelluloses content which is a possible pretreatment for preparation of oil palm fronds (OPF) for reducing sugar production via enzymatic hydrolysis. The optimization of NaOH pretreatment conditions was carried out as designed by face centered central composite design (FCCCD) and the parameters considered for optimization include NaOH concentration (%), temperature (°C) and pretreatment time (min). Quadratic models developed for 4 responses (cellulose, hemicelluloses, lignin and reducing sugar) indicated that the optimum operating condition is 4.42% of NaOH concentration, 100°C of temperature and 58.31 min of pretreatment time where the composition of OPF at this particular condition is 42.12% cellulose, 31.93% hemicelluloses, 26.05% lignin with 2.93 mg/mL of reducing sugar through enzymatic hydrolysis. SEM imaging showed significant morphology changes of OPF structure after being pre-treated with NaOH solution.

Keywords: Enzymatic hydrolysis, Oil palm frond (OPF), Reducing sugar, Response surface methodology (RSM), Sodium hydroxide (NaOH) pretreatment.

Introduction

Lignocellulose is an attractive renewable biomass for the production of various value-added products that compose about half of the plant matter. Since lignocellulosic materials are highly abundant, plentiful and largely available in nature, it can be utilized to produce various beneficial products such as biofuels, animal feeds, enzyme, and healthy food products. At present, oil palm biomass is known as the most potentially lignocellulose biomass that can be utilized for reducing sugar production. Commonly, wastes from oil palm crops are used as animal feed but this is not the optimal economically beneficial way of manipulating the wastes. After harvesting, the wastes are usually burned or left to rot in the field. In addition, Malaysia is known as the world's leading palm oil producer and exporter, accounting for about 47% of global palm oil production and 89% of exports [1]. In 2007 alone, Malaysia was reported to produce approximately 38,256 dry kton of oil palm lignocellulosic waste, with 44% comprised of OPF [2].

Conversion of lignocellulose to reducing sugar involves two major processes which are pretreatment of raw materials followed by enzymatic hydrolysis of pretreated material into

reducing sugar. Pretreatment process plays an important role in the lignocellulosic conversion because it helps in breaking the structure of lignin and disrupting the crystalline structure of cellulose and hemicelluloses thus improving enzyme accessibility during hydrolysis process [3]. Alkaline pretreatment using sodium hydroxide (NaOH) shows great advantages in breaking down the internal structure of lignocelluloses component. Moreover, alkali can degrade the ester and glycosidic chains and alter the structure of lignin, causing cellulose swelling and partial decrystallization of cellulose [4, 5]. In many years, sodium hydroxide also has been widely studied, and it has been proven capable to break the lignin structure of the biomass, thus increasing the enzymes accessibility to degrade cellulose and hemicelluloses [6, 7].

To take full advantage of these alkaline pretreatment effects, critical process parameters such as alkaline concentration, pretreatment temperature and time must be optimized [8]. Unfortunately, there is still lack of information regarding the optimum condition of alkali pretreatment in order to increase the elimination of lignin as well as increase the amount of cellulose and hemicelluloses of OPF after pretreatment. The optimum condition of alkali pretreatment will assure higher production of reducing sugar in enzymatic hydrolysis process.

The objectives of the present study is to optimize the condition of alkali pretreatment and to find out the effects of alkali concentration, temperature and incubation time of pretreatment on the amount of lignin, hemicelluloses and cellulose content of OPF after pretreatment as well as the yield of reducing sugar after enzymatic hydrolysis process. Response Surface Methodology (RSM) based on central composite design (CCD) was applied to identify the optimal pretreatment conditions. Optimization of pretreatment condition is one of the most important processes in the development of an efficient and economical pretreatment method.

Materials and Methods

I. Sample collection

OPF waste was collected from an oil palm plantation in UTM Skudai. The samples were chopped into small pieces, thoroughly washed with water and dried before being ground and sieved to a particle size smaller than 1mm. Dried samples were stored in sealed plastic bags at room temperature. All other chemicals used in the experiment were of analytically pure grade. Enzyme used for enzymatic hydrolysis was Xylanase enzyme (NS 22083) obtained from Novozymes Company.

II. Lignocelluloses composition in OPF

Characterization of OPF was analyzed according to the analytical procedure [9, 10]. The extractives of OPF were determined first by using two-step of Soxhlet extraction. First extraction was performed using water as a solvent for 8 hours. Upon completion of first extraction, the sample was weighed after being dried to a constant weight before proceeding with the next extraction.

The second extraction was carried out using ethanol for 24 h. Holocellulose content comprising of celluloses and hemicelluloses was determined by removing the lignin from the sample by chlorination method (ASTM standard). 2.5 g of free extractives sample then were heated with 150mL water at 75°C. A 0.2mL of acetic acid and 2.0g of $NaClO_2$ were added into the mixture. In every 1 h, 0.2 mL acetic acid and 1.0g $NaClO_2$ were added to the mixture

for 3 h. After complete chlorination, distilled water and acetone was used to filter and wash the slurry. The remaining residue which is actually known as holocellulose was weighed after drying in an oven at 105°C for 24 h. Then, 1 g of holocellulose was soaked with 25 mL of 17.5% NaOH solution and stirred for 40 min at 20°C. The mixture was filtered and washed using 40 mL of 10% acetic acid aqueous solution and 1 L of boiling water at two different stages. The remaining filtrate (α-cellulose) was dried in an oven before being weighed. Hemicellulose content in the sample was determined by subtracting the holocellulose content with the dry solid (α-cellulose).

III. Scanning Electron Microscopy (SEM) sample preparation

Scanning electron microscopy (SEM) analysis was conducted to view and compare the internal structure change before and after pretreatment. A sample of fresh and pretreated OPF was prepared by sputter-coated with gold prior to imaging with a scanning electron microscope.

IV. Design of experiments

The response surface methodology (RSM) applied in the study is the face-centered central composite design (FCCCD) along with three different factors. The suitable range of value of each factors needs to be identified based on previous studies in order to obtain the optimum condition for alkali pretreatment of OPF. The selected three variables including NaOH concentration, temperature and incubation time were studied at three levels (low, basal, high) denoted as -1, 0, 1. The detailed experimental design is presented in Table 1. Fifteen run of experiments with 5 replications at center points were carried out. Alkali pretreatment of OPF was conducted by performing the experiments using 500 mL Erlenmeyer flask containing OPF with the required alkali concentration solution incubated in water bath. The neutralization of each sample was done by rewashing it with distilled water a few times until it reaches a neutral pH. A quadratic model was attempted to fit the response variable so that the response variable can be correlated with the independent variables. A quadratic polynomial equation shown in Equation 1 was used to determine the relationships that exist between dependent and independent variables.

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_{i2} + \sum b_{iii} x_{i3} + \sum b_{ij} x_i x_j \qquad \text{Eq. 1}$$

where Y=predicted response, XiXj= input variables, b_0= a constant, b_i=linear coefficient, b_{ii}=square coefficient, b_{iii}=cubic coefficient and b_{ij}=interaction coefficient.

V. Enzymatic hydrolysis

The sample was dissolved in sodium acetate buffer 0.05 M, pH 5.0 in 250mL Erlenmeyer flask to a concentration of 2% (w/v). The enzymatic hydrolysis was carried out and incubated in a rotary shaker at 150 rpm at 50□C for 48 h. Samples were analyzed periodically with 24 h interval. 5 mL of samples was taken out and the enzyme reaction was stopped by boiling it for 10 min before being subjected to analyses. Duplicate samples for enzyme hydrolysis were set up for each of the experiment.

VI. Analytical method

The composition analysis of treated OPF sample was carried out according to method II. The amount of reducing sugar was determined by using 3,5-dinitrosalicyclic acid (DNS) method with xylose as a standard.

VII. Statistical analysis

Statistical software package Design Expert 6.0.4 (Stat Ease Inc., Minneapolis, USA) was used to generate the experimental data and develop the regression model. The quality of fit of the regression model is expressed as the coefficients of determination (R^2), the statistical significance is determined by Fisher's *F test*, *p-value*, *t-test*, (ANOVA) the response surface and the contour plots were all evaluated to estimate the model as well as to determine the optimum levels.

XII. Validation of the experimental model

Different combinations predicted by the point prediction feature of the statistical software package Design-Expert 6.0.4 were used to validate the FCCCD model developed. Five solutions of the 3 independent variables were evaluated experimentally and compared with the predicted results. The error analysis was computed to determine the closeness between the predicted and the experimental results.

Results and Discussion

I. Lignocelluloses characterization of OPF

The composition of OPF was analyzed for cellulose, hemicelluloses, lignin and extractives. The weight percentages of each component per gram of dry biomass are presented in Table 2. These percentages of cellulose and hemicelluloses in OPF were compared to the content of the material in other biomasses including OPF (Table 3). The lignocelluloses composition of OPF obtained by GOH *et al*. [2], which were 30.19% and 24.26%, is quite comparable with the results obtained in this study: 28.71±1.27% and 26.02±1.05 for cellulose and hemicellulose, respectively. However, it is slightly lower than that obtained by SABIHA-HANIM *et al*. [11], which were 44% and 30.4% for cellulose and hemicelluloses. This might due to the different sources of OPF used in the study and the different maturity level of the OPF plant. According to HODSON *et al.* [12], the concentration of lignin and lignocelluloses concentration vary with age, plant height-form, and anatomical structure. The maturation of living plant material does increase the lignin and lignocelluloses. Even though the cellulose and hemicelluloses content in OPF is lower than other biomass materials, the breakdown of lignocelluloses material into reducing sugar as a product still can be maximized by pretreatment in order to increase the accessibility of enzyme in the reducing sugar production.

The efficiency of enzymatic hydrolysis is dependent on the biomass digestibility. Moreover, the lignin content and crystallinity play important roles in biomass digestibility. The lignin content can be reduced and at the same time the crystallinity structure of biomass can be disintegrated by the pretreatment. In addition, pretreatment is able to disrupt the internal structure of holocellulose, increase the reaction surface and increase the porosity of biomass [13].

Table 2: Lignocelluloses composition of fresh OPF

Composition	Percentage (%)
Extractives	7.64±0.64
Lignin	37.63±0.95
Holocellulose	54.73±0.36
Hemicellulose	26.02±1.05
Cellulose	28.71±1.27

Table 3: Main composition of lignocelluloses biomass

Biomass	Composition (%)			References
	Cellulose	Hemicellulose	Lignin	
Sugarcane bagasse	40.0	24.0	25.0	[14]
Rice straw	35.0	25.0	17.0	[14]
Wheat straw	30.0	50.0	20.0	[14]
Corncob	45.0	35.0	15.0	[14]
Oil palm fronds (OPF)	44.0	30.4	15.4	[11]
Oil palm fronds (OPF)	30.19	24.26	12.96	[2]

II. SEM analysis

The effectiveness of the pretreatment in enhancing the hydrolysis process was proven by further analysis of internal structural of pretreated OPF using scanning electron microscopy (SEM). The structural modification effects after pretreatment were observed by SEM images to verify the OPF structural changes and surface characteristics caused by pretreatment.

SEM images of untreated and pretreated OPF are illustrated in Fig. 1 (i, ii). The structure of untreated OPF in Fig. 1 (i) displays rigid, organized and highly ordered fibrils compared to other pretreated OPF where their structures are distorted. The contents and structure of OPF have changed noticeably through alkaline (NaOH) treatment. This study has identified that pretreating the OPF by NaOH shows the harshest surface morphology in which the initially smooth and organized structure of untreated OPF has been severely damaged where the structure becomes roughened and loosened.

NaOH reaction on pretreatment causes the OPF surface to be destroyed and the cell internal structure exposed, generating some irregular cracks and pores. Moreover, the lignocellulosic biomass becomes “thinner and striated” under the SEM analysis after the materials had been pretreated in alkali [15]. It is believed that the lignin has degraded, hence increases the exposure of cellulose and hemicelluloses in the lignocelluloses biomass to hydrolyzing enzymes such as xylanase. The alteration of structure increases the external surface area and the porosity of the pretreated OPF, thus enhancing the enzymatic attacks in breaking down the complex sugar into simpler sugar or reducing sugar.

i) Fresh OPF ii) Alkali pretreated OPF

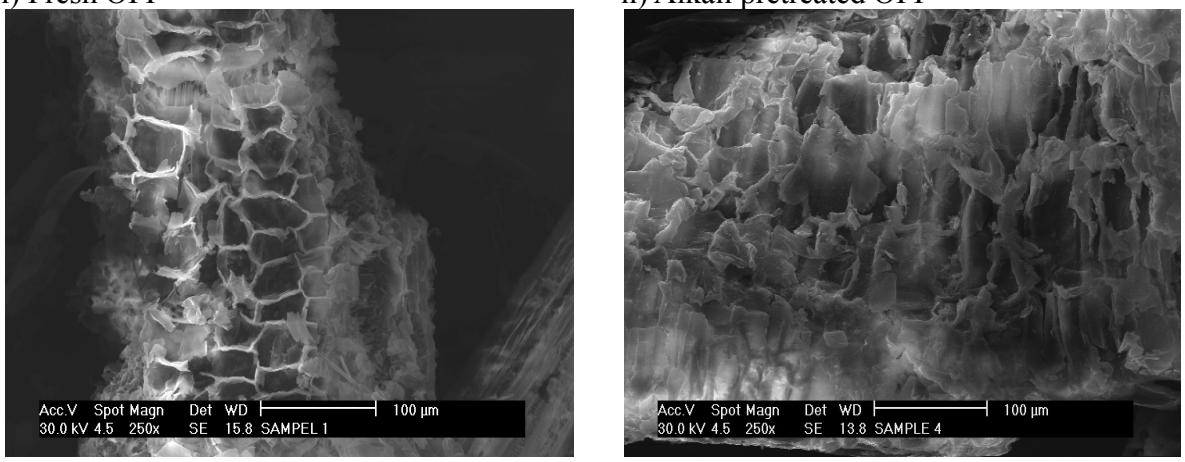

Figure 1: Scanning electronic microscopy (SEM) of OPF before and after pretreatment at constant biomass loading and chemical concentration of 0.15 g/mL and 5% (w/v), respectively with; (i) fresh or untreated OPF, (ii) alkali, NaOH.

III. Analysis of Variance (ANOVA) and Statistical Analysis

The statistical significance equation is determined by F-value and the proportion of variance explained by the model obtained is given by the multiple coefficient of determination, R^2. The effects of NaOH concentration, temperature and pretreatment time on the main composition of OPF was evaluated on a model composition corresponding on face centered central composite design which consists of 15 runs (Table 4). As the responses are obtained experimentally, analysis of variance was carried out to analyze the effect of each variable towards composition in OPF.

According to Table 4, the highest cellulose content retained was 42.78% at the pretreatment conditions of 5.25% NaOH at 100°C for 52.50 min while the lowest cellulose content was 27.39% for the pretreatment conditions of 0.50% NaOH at 50°C for 15 min. This indicates that concentration of NaOH, temperature and incubation time of pretreatment gave a high impact on the solubilization of cellulose. Meanwhile, the highest hemicelluloses content (33.94%) was found at the pretreatment conditions of 0.50% NaOH at 100°C for 90 min and the lowest (27.09%) was observed at the pretreatment conditions of 10% NaOH at 100°C for 15 min. These results showed that NaOH concentration and pretreatment time also are significant in increasing the hemicelluloses content instead of the temperature itself. Hemicelluloses content was significantly higher at lower NaOH concentration and diminished at higher NaOH concentration (10%). This was probably due to th e solubilization of hemicelluloses compound that increased when treated with concentrated NaOH.

The reducing sugar content also showed similar trends as hemicellulose contents. This might be due to the fact that the alkali pretreatment process generally utilized the lignocelluloses content at lower temperature [16]. Besides, the lignin content after the pretreatment was also affected by those three variables where the lowest residual lignin was found at NaOH concentration of 5.25%, 100°C and 52.5 min of pretreatment time. The highest lignin residual was found at 0.50% NaOH concentration, 50°C and 15 min of pretreatment time. Based on these finding, it was clearly shown that lignin can be degraded at a moderate NaOH concentration with high temperature and longer pretreatment time.

Table 4: Design matrix of CCD and the experimental results (response) obtained for alkali pretreatment

Experimental Design				Results			
				Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reducing sugar (mg/mL)
Run	NaOH conc. (%)	Temp. (°C)	Time (min)				
1	10.00	100.00	15.00	27.56	27.09	36.31	0.24
2	10.00	50.00	90.00	28.88	28.09	36.95	0.39
3	0.50	100.00	90.00	29.95	33.94	35.79	0.52
4	0.50	50.00	15.00	27.39	25.69	37.33	0.41
5	0.50	75.00	52.50	29.35	31.68	34.84	0.53
6	10.00	75.00	52.50	35.86	29.82	31.47	1.94
7	5.25	50.00	52.50	36.33	30.01	33.54	2.88
8	5.25	100.00	52.50	42.78	31.21	25.48	2.93
9	5.25	75.00	15.00	29.46	31.15	35.13	0.68
10	5.25	75.00	90.00	31.14	29.31	34.45	0.68
11	5.25	75.00	52.50	35.19	31.79	31.66	2.29
12	5.25	75.00	52.50	36.92	31.82	31.13	2.43
13	5.25	75.00	52.50	35.13	31.47	30.54	2.31
14	5.25	75.00	52.50	35.51	31.90	32.53	2.37
15	5.25	75.00	52.50	36.80	31.88	30.17	2.48

In order to evaluate the variables behavior, analysis of variance of the models for four response function, along with the corresponding F-values are summarized in Table 5. A statistical analysis in Table 5 shows that Prob>F values for all responses is lower than 0.05, which indicates that there is a statistically significant relationship between the variables within 95% confidence interval. The model obtained were significant, presenting determination coefficients of R^2=0.9819, 0.9955, 0.9686 and 0.9957 for cellulose, hemicelluloses, lignin and reducing sugar respectively, which means 1.81%, 0.45% , 3.14% and 0.43% of the variability in the responses for the region studied were explained by the residue. As displayed in Table 5, the model selected for all the responses for NaOH pretreatment were proven as statistically significant with very low probability (0.0030 to <0.0001). Moreover, lack of fit (LOF) F-test was employed to determine the fitness of the model. Lack of fit is the variation of the data around the fitted model. A lack of fit value more than 0.05 implies that the model is not significant relative to the pure error. In this study, the model appeared to be adequate with no significant lack of fit with p-value for cellulose, hemicellulose, lignin and reducing sugar analysis were found to be 0.1936, 0.0772, 0.3556 and 0.0700, respectively.

Table 5: Regression equations and ANOVA results for responses parameters for NaOH pretreatment

Responses	Regression equations in terms of coded factors	Prob>F	R^2	Adj.R^2	PLOF	CV	A.P	SD
Cellulose	$36.25+3.26X_1+3.23X_2+0.84X_3-4.08X_1^2+2.87X_2^2-6.39X_3^2-0.13X_1X_2+2.92X_1X_3+3.48X_2X_3$	0.0008	0.9819	0.9493	0.1936	3.01	18.059	1.00
Hemicellulose	$31.67-0.93X_1+0.60X_2-0.92X_3-0.79X_1^2-0.93X_2^2-1.31X_3^2-3.23X_1X_2-1.21X_1X_3+0.19X_2X_3$	<0.0001	0.9955	0.9874	0.0772	0.80	41.713	0.24
Lignin	$30.96-1.68X_1-4.03X_2-0.34X_3+2.50X_1^2-1.15X_2^2+4.13X_3^2-0.12X_1X_2-3.49X_1X_3-1.72X_2X_3$	0.0030	0.9686	0.9121	0.3556	2.84	14.803	0.94
Reducing sugar	$2.33+0.70X_1+0.028X_2-4.345E\text{-}004X_3-1.03X_1^2+0.64X_2^2-1.58X_3^2-0.063X_1X_2+0.039X_1X_3+0.78X_2X_3$	<0.0001	0.9957	0.9881	0.0700	7.38	29.971	0.11

Prof>F: probability; R^2: Determination of coefficient; Adj R^2: Adjusted of determination of coefficient; PLOF: Probability lack of fit; CV: coefficient of variation; A.P: Adequate precision; SD: Standard deviation

Contour plots and response surface curves of the interaction between NaOH concentration and pretreatment time on the cellulose are shown in Fig. 2 (i). It showed that the cellulose contents increased to 42.78% as NaOH concentration was increased from 0.50% to 5.25% and extending pretreatment time from 15 min to 52.50 min. The cellulose content started to decrease to 28.88% as the NaOH concentration and time reached to the maximum point, which was 10% NaOH concentration and 90 min of the pretreatment time. Further increment in NaOH concentration, as well as longer duration of pretreatment time both negatively affects the responses by decreasing the cellulose content from 42.78% to 28.88%. High NaOH concentration (10%) tends to solubilize the cellulose and hemicellulose content that were washed out in the neutralization step, leaving small amount about 27.56% and 27.09% of cellulose and hemicelluloses. Moreover, higher alkali concentration of 12% did not only remove the lignin but degrades carbohydrates as well, hence contributing to low sugar production which was 38% from 95% of xylose obtained in 2% NaOH concentration [17].

Hemicellulose contents with varied NaOH concentration and pretreatment time presented in Fig. 2 (ii) have also experienced similar condition with the trend of cellulose content in Fig. 2 (i), where an increase in NaOH concentration and pretreatment time did not give any benefit in increasing hemicellulose content. This finding was supported by XIAO *et al*. [18] who utilized maize straw, rye straw and rice straw as a substrate using NaOH pretreatment for lignin, hemicellulose, and celluloses preparation.

Fig. 2(iii) and (iv) show contour plots and response surface curves of the interaction between NaOH concentration and pretreatment time on the lignin content and reducing sugar production. Fig. 2(iii) revealed that along NaOH concentration from 0.50% to 10%, the lignin contents reduced steadily from 35.79% to 25.48%, followed by a huge increment to 36.95% thereafter. This showed that there was a considerable difference of lignin at low NaOH concentration compared to high NaOH concentration when treated for longer period of 90 min. In Fig. 2(iv), reducing sugar production after enzymatic hydrolysis displayed an optimum point, which was 2.93% at the center point (5.25% NaOH concentration) and started to decrease at higher NaOH concentration of 10% with longer pretreatment time of 90 min. The highest sugar production, 2.93 mg/mL was found when the sample was pretreated with mild NaOH concentration of 5.25% and pretreatment time of 52.5 min before proceeding with enzymatic hydrolysis. The increasing trend of reducing sugar recovery might be due to the lignin removal with the increased of alkali concentration from 0.50% to 5.25%.

According to ROGALINSKI *et al*. [19], the longer reaction time leads to the solubilization of biomass into glucose which then further degraded into smaller compounds such as furfural. The existence of other compounds will initiates the inhibition factors in the enzymatic hydrolysis and retards the production of reducing sugar. Therefore, an adequate time should be given during the pretreatment to increase the accessibility of cellulose by enzymes. Increased of NaOH concentration in the pretreatment assists the increase of surface area and the formation of pores, hence permitting easier enzyme access and attacks on carbohydrates for reducing sugar production [2]. However, concentrated NaOH solution higher than 5.25% and long hydrolysis time (>90 min) might cause the initially exposed cellulose tends to be solubilized and degraded into furfural compounds. The presence of furfural and others inhibitors hinders the hydrolysis process to produce fermentable sugar.

The highest reducing sugar obtained in this study was 2.93 mg/mL when the lignin content of pretreated OPF was the lowest (i.e. 25.48%), therefore indicates that delignification assisted the production of reducing sugar. Moreover, this situation was in agreement with the investigation reported by KARUNANITHY AND MUTHUKUMARAPPAN [16] that the increased in alkali concentration until 1.7% NaOH apparently increased xylose recovery by 91% due to the increase in the degree of delignification in corn stover. It can be concluded that the severity of the pretreatment condition leads to higher removal of cellulose,

hemicelluloses and lignin, therefore lower the production of reducing sugar.

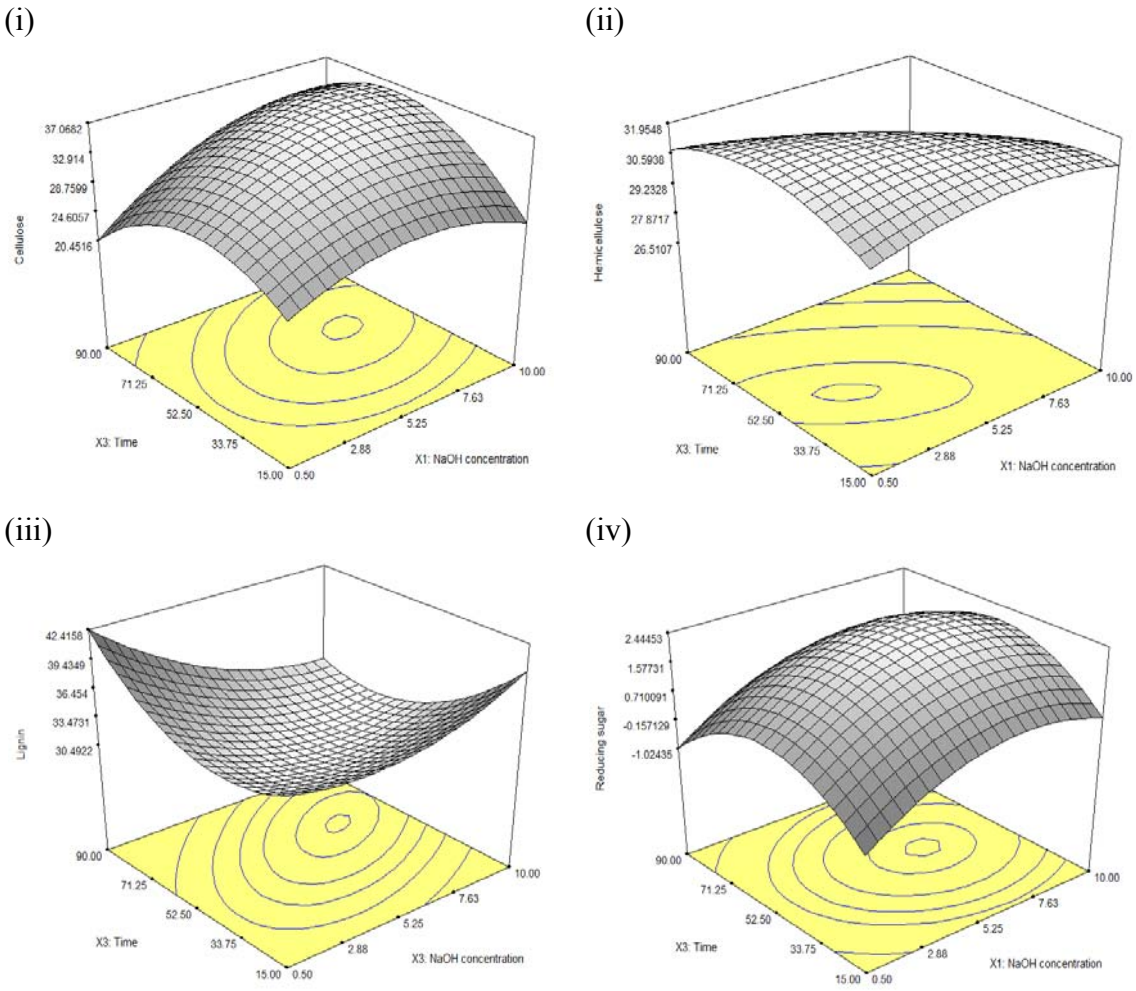

Figure 2: Response surface plot and contour lines of (i) cellulose, (ii) hemicelluloses, (iii) lignin and (iv) reducing sugar contents in pretreated OPF as a function of NaOH concentration and pretreatment time at 75°C.

Meanwhile Fig. 3 (i, ii, iii, and iv) present the 3D surface of cellulose, hemicellulose, lignin and reducing sugar contents in the pretreated OPF as a function of temperature and pretreatment time with NaOH concentration at 5.25%.

Both increased of pretreatment time and temperature (90 min and 100°C) have both positively affect the cellulose contents and lignin residual as presented in Fig. 3 (i) and Fig. 3 (iii), respectively. In contrary with the reducing sugar production shown in Fig. 3 (iv), the increase of temperature up to 100°C gave negative effects by lowering the yield to 0.24 mg/mL of reducing sugar. Moreover, increased of pretreatment temperature caused the increment in hemicelluloses content (Fig. 3 (ii)). However, longer period than 52.5 min of pretreatment time caused an adverse effect, where the hemicelluloses contents declined from 31.15% to 27.09%. Overheating at high temperature of biomass for long period probably burns the biomass surface. Hence, it was believed that the enzyme reaction was inhibited by the burnt biomass surface [10]. This situation was also reported by GOH *et al*. [2], where the temperature did not influence significantly the glucose recovery at concentrated ethanolic hot compressed water (EHCW).

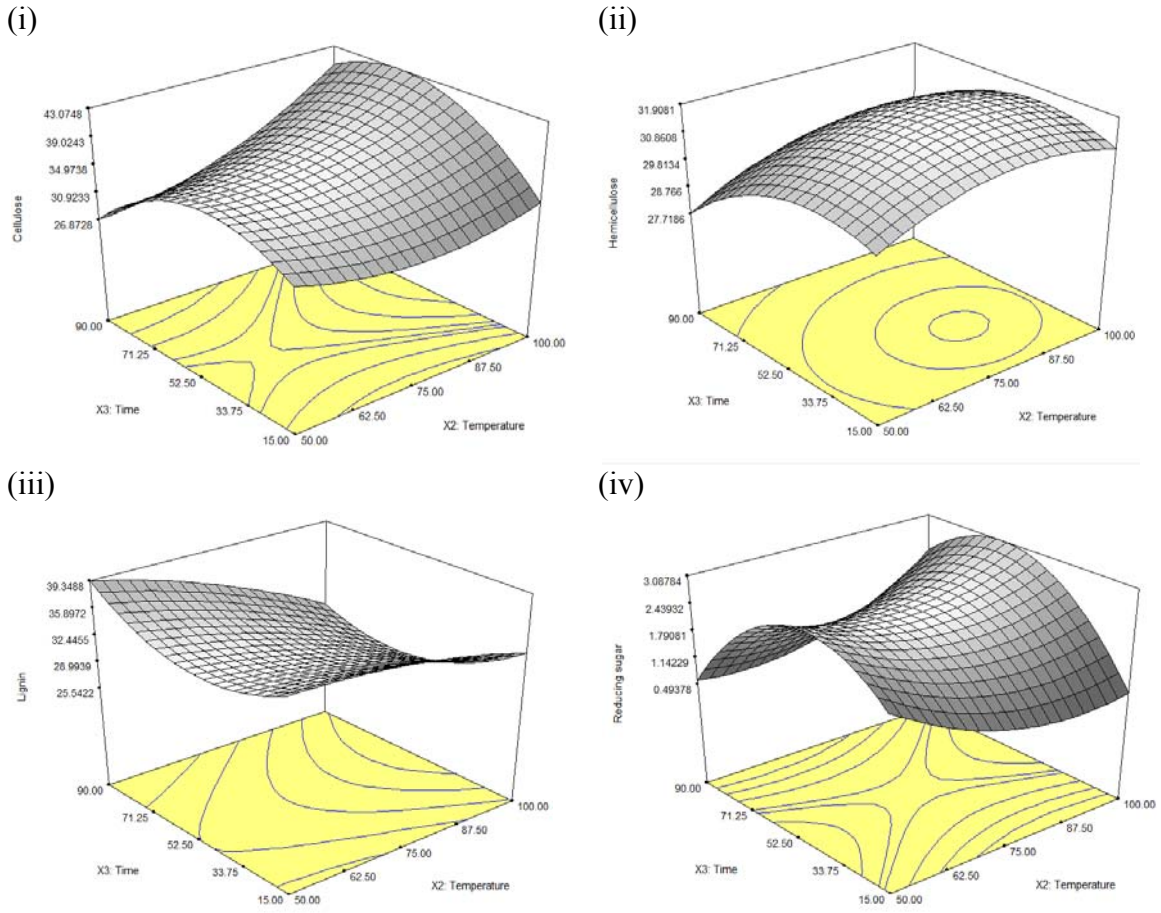

Figure 3: Response surface plot and contour lines of (i) cellulose, (ii) hemicelluloses, (iii) lignin and (iv) reducing sugar contents in pretreated OPF as a function of temperature and pretreatment time at 5.25% NaOH concentration.

IV Prediction and Verification of Optimization Point

Following the design and analysis of the experimental data obtained, numerical optimization was carried out for NaOH pretreated OPF. The criteria of the variables were set accordingly and the responses including cellulose, hemicellulose and reducing sugar contents were set to maximum, while lignin contents were set to minimum.

Table 6 indicates the predicted and experimental value for fresh OPF treated by NaOH suggested by the model with a desirability value of 0.911. By implementing the experimental condition of 4.42% NaOH concentration, 100°C and 58.31 min of pretreatment time, the highest predicted value of cellulose, hemicelluloses and reducing sugar were 42.12%, 31.93% and 2.93 mg/mL respectively. Meanwhile, the lowest predicted lignin content is 26.05%. Based on the predicted and experimental results presented, the experimental values were in good agreement with the predicted values proposed by the model with error of less than 10%.

Table 6: Results of verification experiments at optimum process condition

Responses	Predicted	Experimental	Predicted error (%)
Cellulose (%)	42.12	43.30	2.81
Hemicellulose (%)	31.93	30.19	5.46
Lignin (%)	26.05	26.51	1.76
Reducing sugar (mg/mL)	2.93	2.95	0.68
Desirability		0.911	

Conclusion

The optimization using central composite design (CCD) combined with response surface methodology (RSM) was successfully applied to determine the relationship between the response and the independent variables. The results obtained from this study are highly beneficial in determining the optimum condition of NaOH pretreatment in treating the fresh OPF before proceeding with enzymatic hydrolysis process to ensure maximum yield of reducing sugar obtained from pretreated OPF. ANOVA and response surface plots provide a clear view of how NaOH concentration, temperature and pretreatment time affect the contents of cellulose, hemicelluloses and lignin in pretreated OPF. Pretreatment of OPF using NaOH is shown to be predictable with desirability of 0.911 since it is able to increase the amount of cellulose and hemicelluloses as well as reducing the lignin contents. Moreover, the experimental findings in NaOH pretreatment of OPF are in good agreement with the model predictions with less than 10% thus signifying the adequacy of the models employed in optimizing the NaOH pretreatment conditions parameters. By implementing the pretreatment process under the optimum condition, lengthy reaction time and high amount of chemical usage (NaOH) can be avoided, therefore reducing the pretreatment cost as well as preparing an optimum pretreated OPF for enzymatic hydrolysis process.

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