

## The Delignification of Pressed Pericarp Fibre is Optimised for Glucose Recovery: Using the Response Surface Technique

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**Abstract:** For the bioconversion of lignocellulosic biomass into fermentable sugars, pressed pericarp fibres (PPFs) were treated to chemical pretreatment in this work. It was investigated how well pretreatments employing an acid (HCl) and an alkaline (NaOH) reduced the lignin concentration. Compared to acid pretreatment, alkali pretreatment demonstrated superior lignin removal. Then, in order to optimise PPF delignification, more experiments were conducted using a statistical design of experiments (DoE). The percentage of lignin removal was selected as the response, with the effects of NaOH concentration, incubation time, and temperature chosen as the independent variables. The models were created using the response surface methodology (RSM) and central composite design (CCD) to correlate the impact of process variables to the response. The analysis of variance (ANOVA) revealed that temperature had the greatest impact on the delignification of PPF. For the process of delignifying PPF (54% of the lignin was removed), the ideal operating parameters were 5.96% (w/v) of NaOH, 118.02°C, and duration for 4.92 hours. In order to validate the simulated model, three sets of tests were conducted. The outcomes were consistent with the simulated values, with an average error of 1.29.

**Keywords:** pressed pericarp fibres; PPFs; delignification; chemical pretreatment; lignin; central composite design; CCD.

### 1 Introduction

During the milling of palm oil, oil palm solid residues, which comprise the shell, fibre, and kernels, are produced. These are cheap and plentiful. For every tonne of processed fresh fruit bunches (FFB), approximately 0.07 tonnes of palm shell, 0.103 tonnes of palm fibre, and 0.012 tonnes of kernels are produced as solid leftovers (Pansamut et al., 2003). Around 184.6 million tone and 9.10 billion tone of agricultural and oil palm waste are expected to be produced globally each year, respectively (Kelly-Yong et al., 2007). The production of oil palm wastes has increased yearly since 1980 as a result of the development of crop plants brought on by the rising demand for palm oil. (MPOB, 2007; Kelly-Yong et al., 2007; Nath and Das, 2003). These findings further demonstrated the importance of creating a workable strategy for utilising residue in order to save the environment and maintain a robust economy. Pressed pericarp fibre (PPF), also known as palm pressed fibre (PPF), is created by the palm oil mill after the palm fruits are screw-pressed to obtain crude palm oil (CPO). These are the fibrous leftovers from the processing and pressing of the oil palm fruits (Han et al., 2006). PPF was created in vast quantities, but it was barely partially used. They are mainly used as solid fuels to produce the necessary steam and energy for the plantations on their own, or as mulching materials to provide organic nutrients to oil palm plants in plantations (Bakar et al., 2007). Although though it hasn't yet been commercialised, the recovered fibre from PPF has been found to be a high source of carotenoid, vitamin E, and sterols (Choo et al., 1996). Choo et al. (1996) and Han et al. (2006) conducted investigations to extract and recover carotenes and coenzyme Q10. A lignocellulosic material called PPF can be converted into chemicals, cellulases, and other valuable products. It can be used to make fermentable sugars and is affordable and renewable (Mashitah et al., 1997). For the following qualities, limiting the enzymatic saccharification of insoluble cellulose is crucial: lowered reaction speed as a result of enzymes' decreased susceptibility to substrate

1. End-product inhibition number

reversible inactivation of enzymes adsorbed on cellulose surfaces

1 their inactivation on lignin fragments (Umikalsom et al., 1998).

Polysaccharides in lignocellulosic materials are associated with lignin, which makes their hydrolysis difficult. The low susceptibility of the lignocellulosic materials to hydrolysis can be improved by physical, chemical, and biological pretreatments, especially

- 1 distracting cross-linked matrix of lignin that deposited in the cellulose fibres
- 2 disrupting hydrogen bonds in crystalline cellulose
- 3 improving the porosity and surface area of cellulose for subsequent enzymatic activity (Li et al., 2010).

From literature studies, many reports were published on the pretreatment for lignocellulosic materials such as biological pretreatment, physical pretreatment, physiochemical pretreatment, chemical pretreatment and pulsed-electric-field pretreatment (Taherzadeh and Niklasson, 2004; Kumar et al., 2009; Alvira et al., 2010). Extensive pretreatment work has been done on various lignocellulosic feedstock, however, relatively little work has been done using PPF. Among the pretreatment methods, chemical and thermochemical pretreatment are currently the most effective and promising technologies for industrial applications. The combination of both the chemical pretreatment and thermochemical pretreatment were carried out by researchers and was proved to remove lignin significantly (Chang et al., 1997; Hamisan et al., 2009; Alvira et al., 2010; Evstigneev, 2010). Currently, both the acid and alkali treatment method have been chosen for degrading the lignocellulosic material for bio-ethanol production. Both of the acid and alkali pretreatment show their effectiveness depending on the substrate and the treatment conditions (Millett, Baker and Satter, 1976; Schell et al., 2003; Taherzadeh and Niklasson, 2004; Lloyd and Wyman, 2005; Saha et al., 2005; Galbe and Zacchi, 2007; Xu et al., 2007; Guo et al., 2008; Ligerio et al., 2008; Kootstra et al., 2009; Kumar et al., 2009). Generally, both acid and alkali perform different mechanism in lignin removal. During acid pretreatment, acid solubilises the hemicelluloses which disrupt the lignocellulosic components linked by covalent bonds, hydrogen bonds, and Van der Waals forces (Mosier et al., 2005; Zheng et al., 2009). As for alkali pretreatment, It was believed that the mechanism of alkaline pretreatment is to be saponification of intermolecular ester bonds cross linking xylan hemicelluloses and other components like lignin (McMillan, 1994; Taherzadeh and Karimi, 2008).

It must be emphasised that it is not always possible to transfer the results or effects of pretreatment from one type of material to another. Meaning that, one pretreatment technology that is efficient for a particular type of biomass might not work for another material. Therefore, the choice of pretreatment technology used for a particular biomass depends on its composition and the byproducts along with the pretreatment process (Taherzadeh and Niklasson, 2004; Galbe and Zacchi, 2007; Kumar et al., 2009). Since pretreatment of PPF was scarcely reported (chemical and biological) (Tong and Hamzah, 1989; Tong et al., 1993), a comparison of different pretreatment methods will be carried out in this research work in order to get reasonable and acceptable results under real process conditions. The conventional methods under chemical pretreatment (acid and alkali pretreatment) combined with thermal treatment will be carried out in this research work to investigate and compare the effectiveness of such treatments in removing the lignin. The response surface methodology (RSM) coupled with central composite design (CCD) was selected to study the effects of three pretreatment condition variables

simultaneously: chemical concentration, incubation time and temperature. The experimental results were then compared with the simulated values obtained from the proposed model.

## 2 Materials and methods

### *Substrate*

The biomass used in this study is shredded PPF. It was collected with permission from United Oil Palm Malaysia Sdn. Bhd, Nibong Tebal, Penang, Malaysia. The PPF were ground and passed through 335  $\mu\text{m}$  mesh size and kept until used.

### *Chemical pretreatment*

The PPF was delignified using physical (grounded and sieved through 335  $\mu\text{m}$  mesh size) and chemical treatment. Preliminary, the PPF was first treated with both acid and alkaline, in which two grams of untreated PPF was soaked in 200 mL of the chemical solutions (2% w/v of HCl and NaOH) for three hours. The experiments were carried out at ambient temperature (25°C), 80°C and 120°C (heating) to study the effect of temperature on chemically removal of lignin. Control was carried out by soaking the PPF in the same amount of distilled water.

All the treated PPF were filtered and washed with distilled water until no traces of acid or alkaline detected and were dried in an oven at 95°C for two days.

### *Design of experiment*

The statistical design of experiments (DoEs) is a structured and systematised method of experimentation in which all factors are varied simultaneously over a set of experimental runs (Khayet et al., 2008). In this present study, the DoE software (Design Expert version 6.0.6) is used to study the effect and the optimum value of the significant variables in PPF lignin removal. The process optimisation was done by using a RSM coupled with an analysis following CCD (Yus Azila et al., 2008; Yeong et al., 2009). RSM is a collection of mathematical and statistical techniques that are useful for the modelling and analysis of problem in which a response of interest is influenced by several variables and the objective is to optimise this response (Rodrigues et al., 2008). This method is used for fitting a model and helps to optimise the effective variables with a minimum number of experiments. Furthermore, it helps to analyse the interaction between the variables by providing a good way of visualising the parameter interaction through the surface contour (Montgomery, 2001; Yeong et al., 2009).

The CCD suggests  $2^n$  factorial runs,  $2n$  axial runs and  $n_c$  centre runs (six replicates), with  $n$  as the number of factors. The independent variables selected were

- 1 A, NaOH concentration (% w/v)
- 2 B, incubation time (h)
- 3 C, temperature (°C).

$2^3$  full factorial CCD for the three variables was employed, indicating that altogether 20 experiments (8 factorial points, 6 axial points and 6 replicates at the centre points) were required (Azargohar and Dalai, 2005; Yeong et al., 2009). The independent variables are coded to  $(-1, 1)$  interval where the low and high levels are coded as  $-1$  and  $+1$ , respectively. The axial points are located at  $(\pm\alpha, 0, 0)$ ,  $(0, \pm\alpha, 0)$  and  $(0, 0, \pm\alpha)$  where  $\alpha$  is the distance of the axial point from centre and makes the design rotatable. In this study, the  $\alpha$  value was fixed at 1.

**Table 1** Experimental independent variables

Variable	Factor code	Level and range (coded)		
		-1	0	+1
NaOH concentration, (% w/v)	A	2	4	6
Incubation time, h	B	1	3	5
Temperature, °C	C	30	75	120

**Table 2** Experiment matrix of  $2^3$  centre composite design (CCD) and results (lignin removal)

Std	Block	Factors (levels)			Response (Y)
		NaOH concentration, % (w/v) (A)	B: incubation time, h (B)	C: temperature, °C (C)	Lignin removal, % (Y1)
1	Fact	2	1	30	6.18
2	Fact	6	1	30	18.06
3	Fact	2	5	30	12.93
4	Fact	6	5	30	20.36
5	Fact	2	1	120	12.19
6	Fact	6	1	120	25.11
7	Fact	2	5	120	49.30
8	Fact	6	5	120	52.84
9	Axial	2	3	75	22.35
10	Axial	6	3	75	34.30
11	Axial	4	1	75	16.68
12	Axial	4	5	75	35.83
13	Axial	4	3	30	15.25
14	Axial	4	3	120	39.35
Repeated runs					
15	Centre	4	3	75	25.41
16	Centre	4	3	75	25.35
17	Centre	4	3	75	28.09
18	Centre	4	3	75	26.79
19	Centre	4	3	75	22.67
20	Centre	4	3	75	26.61
Mean					25.78
Mean error					±27.06
Standard deviation					2.11

The range of process variables studied is shown in Table 1. Table 2 shows the total number of 20 experiments as per CCD method. The experimental sequence was randomised in order to minimise the effects of the uncontrolled factors. Six experiments were repeated in order to estimate the experimental error. Lignin removal (%) was the only response and to study the effect of process variables. Each response was used to develop an empirical model which correlated the responses to the three process variables. The empirical model of 2 factor interaction (2FI) is presented by equation (1):

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_{12}AB + \beta_{13}AC + \beta_{23}BC \quad (1)$$

with, 1, 2 and 3 indicate coefficients for individual effect of variables 12, 13 and 23 indicate coefficients for interaction effect of variables where  $Y$  is the predicted response (lignin removal, %).  $A$ ,  $B$  and  $C$  are the coded forms of NaOH concentration, incubation time and temperature, respectively. The term  $\beta_0$  is the offset term,  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  are the linear terms and  $\beta_{12}$ ,  $\beta_{13}$  and  $\beta_{23}$  are the interaction terms.

The model terms were selected or rejected based on the P value with 95% confidence level. The results were completely analysed using analysis of variance (ANOVA), with 5% level of significance. Three-dimensional plots and their respective contour plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied. The optimum region was also identified based on the main parameters in the overlay plot.

### *Enzymatic saccharification*

Enzymatic saccharification was carried out to test the digestibility of the PPF by the cellulase enzymes. In this case, commercial cellulase mixture (Celluclast 1.5L) supplemented with  $\beta$ -glucosidase (Novozyme 188) were used at loadings of 60 FPU/g cellulose and 64 pNPGU/g cellulose, respectively. Enzymatic hydrolyses were performed at 1.5g dried treated PPF in 100 mL of 50 mM sodium acetate buffer, pH 4.8. The experiments were carried out in a shake flask incubated at 50°C and agitated at 150 rpm for a period of 72 hours. Sodium azide (2% w/v) was added to the reaction mixture to avoid bacterial contamination. Samples were withdrawn at regular time intervals for glucose analysis.

The percent of digestibility of PPF test can be determined by determining the reducing sugar concentration produced. The hydrolysis of PPF was calculated qualitatively according to the method described by Selig et al. (2008) and Umikalsom et al. (1998) with slight modification using the equation:

$$\text{hydrolysis}(\%) = \frac{\text{reducing sugars} \left( \frac{\text{g}}{\text{mL}} \right) \times 0.9}{\text{weight of total biomass}(\text{g})} \times 100\% \quad (2)$$

The correcting factor 0.9 is used to correct for the water molecule added upon hydrolysis of the cellulose polymer.

### *Analytical procedures*

#### *Determination of lignin*

The amount of lignin in PPF was determined using the method introduced as described by Thomas et al. (1998). Milled PPF (1g) was shaken with a mixture of solution containing 5 ml concentrated sulphuric acid (97%) and 50 ml hydrochloric acid (37%), in 1 L standard flask at 30°C for 20 minutes. The mixture was allowed to stand at room temperature for 16 h and then it was diluted with 450 ml of distilled water before boiling at 100°C for 10 minutes. The contents of the flasks were filtered using a vacuum filter. The acidic residues were washed to neutrality with distilled water, dried at 105°C, weighed and calcination at 450°C in a muffle furnace. After calcination, the ashed sample was cooled prior to weighing. The lignin content was calculated as follows:

$$\text{Lignin content(\%)} = \frac{\text{Weight of dried matter} - \text{Weight of ash}}{\text{Weight of original sample}} \quad (3)$$

#### *Reducing sugar determination*

Reducing sugar concentration was determined using dinitro-salicylic (DNS) method as described by Ghose (1987). 3 ml of DNS reagent was added to 1 ml aliquots of glucose standard or culture samples. The resulting coloured solutions were well mixed and placed in boiling water bath for 5 min. The solution was allowed to cool before measuring the absorbance at 540 nm against a reagent blank (0.05M citrate buffer, pH 4.8).

**Table 3** Lignin content of treated and untreated PPF at different operation condition

	<i>Solvent concentration (% w/v)</i>	<i>Solvent</i>	<i>Temperature (°C)</i>	<i>Incubation time (min)</i>	<i>% lignin</i>	<i>% removal</i>
1	2	NaOH	Room	180	35.42 ± 1.407	14.07
2	2	HCl	Room	180	32.65 ± 0.872	20.79
3	Distilled water		Room	180	40.52 ± 0.86	1.69
4	2	NaOH	80	180	26.53 ± 1.667	35.64
5	2	HCl	80	180	32.76 ± 1.923	20.52
6	Distilled water		80	180	37.21 ± 0.541	9.73
7	2	NaOH	120	180	18.38 ± 0.989	55.41
8	2	HCl	120	180	31.40 ± 1.011	23.82
9	Distilled water		120	180	31.28 ± 0.628	24.11
10		Raw PPF			41.22 ± 1.08	

### **3 Results and discussions**

#### *Effect of the various treatments on chemical composition of PPF*

Table 3 showed the lignin content and lignin removal of treated and untreated PPF by using basic and acid solutions. Delignification treatment with both HCl and NaOH were compared and found that NaOH gave higher lignin removal at 55.41% as compared to

HCl at 23.83% at 120°C with 2% (w/v) solution after 180 minutes. Lignin is rich in oxygen-bearing functional group (e.g., phenolic-OH, carbonyl and carboxyl), therefore it has greater solubility in alkaline solution but hardly soluble in acid media (Umikalsom et al., 1998; Essington, 2004; Chen et al., 2009; Hamisan et al., 2009). In fact, the alkali could remove the acetate group and various uronic acid substitution (which lower the accessibility of the enzyme to the hemicelluloses and cellulose surface) from hemicelluloses and reduced the steric hindrance of hydrolytic enzymes, hence enhancing the carbohydrate digestibility, accordingly (Chang et al., 1997; Mosier et al., 2005).

For the effect of temperature, NaOH gave 14.07% of lignin removal at ambient temperature (25°C) and the efficiency was increased with the increased of temperature to 80°C giving 35.64 % of lignin removal. The delignification of NaOH was even higher when the mixture was autoclaved at 120°C for the same duration. This phenomenon showed that temperature increment could enhance the lignin removal efficiency. Young et al. (1985) reported that heat treatment at a temperature above 100°C managed to alter the physical nature of lignin. Previous experimental results showed that thermal treatment could degrade the hemicelluloses and affected the lignin polymer through depolymerisation and recondensation reactions such as cleavage of  $\beta$ -aryl-ether linkages (Brosse et al., 2010). Since lignin easily dissolved in alkaline solutions (Umikalsom et al., 1998; Essington, 2004; Chen et al., 2009; Hamisan et al., 2009), therefore by expanding its surface, the molecule became very accessible for oxidation (Umikalsom et al., 1998).

#### *Response surface modelling of lignin removal (%) after NaOH pretreatment*

Table 2 also shows the values of the response (lignin removal in %) against each experiment conducted as per CCD. The CCD was used to develop correlation between the three process variables (factors A, B and C) to one response (Y1). Experimental run 15–20 at the centre point were used to check the reproducibility and to estimate an experimental error. The lignin removal results were input into the Design Expert software for further analysis. Examination of the Fit Summary output revealed that the 2FI model is statistically significant for the response.

**Table 4** ANOVA for the 2FI models for lignin removal

Responses	Model terms	Sum of squares	Mean square	F value	Prob > F
Lignin removal, Y1	Model [equation (2a) and (2b)]	2,631.13	438.52	98.61	< 0.0001 <sup>a</sup>
	A	227.53	227.53	51.16	< 0.0001
	B	865.82	865.82	194.7	< 0.0001
	C	1,123.80	1,123.30	252.71	< 0.0001
	AB	23.95	23.95	5.39	0.0372
	AC	1.02	1.02	0.23	0.6395
	BC	389.01	389.01	87.48	< 0.0001
	Lack of fit	40.83	5.10	1.5	0.3395 <sup>b</sup>

Notes: <sup>a</sup>Significant under 95% level of confidence

<sup>b</sup>Not significant relative to the pure error due to noise.

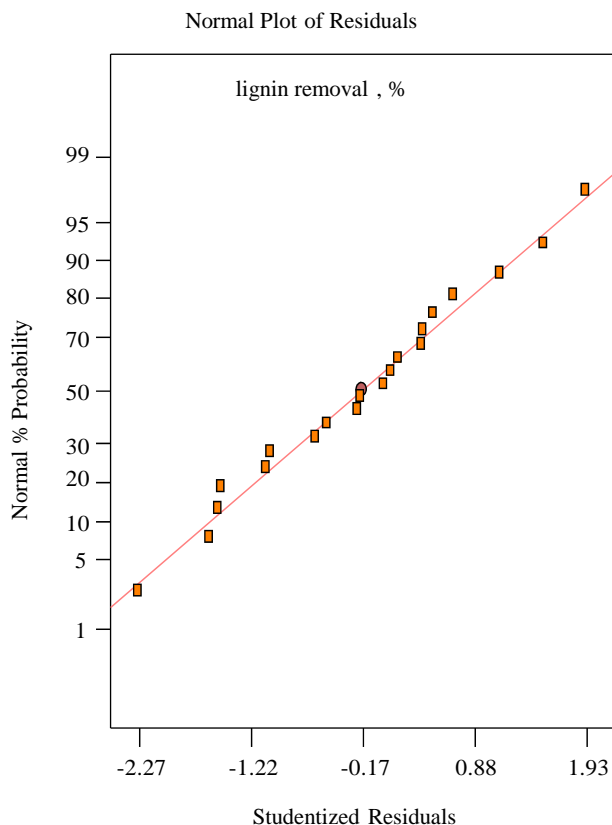
In order to ensure a good model, test for significance of the regression model, test for significance on individual model coefficients and test for lack-of-fit need to be

performed. The ANOVA for  $2^3$  full CCD design of the response,  $Y_1$  is presented in Table 4. The model proposed by the software for lignin removal in terms of coded factors and actual factors are presented in equation 4(a) and equation 4(b) respectively. The positive sign in front of the terms indicates synergistic effect while the negative sign indicates antagonistic effect.

$$\text{lignin removal}(\%), Y_1(\text{coded factors}) = 25.78 + 4.77A + 9.30B + 10.60C - 1.73AB - 0.36AC + 6.97BC \quad (4a)$$

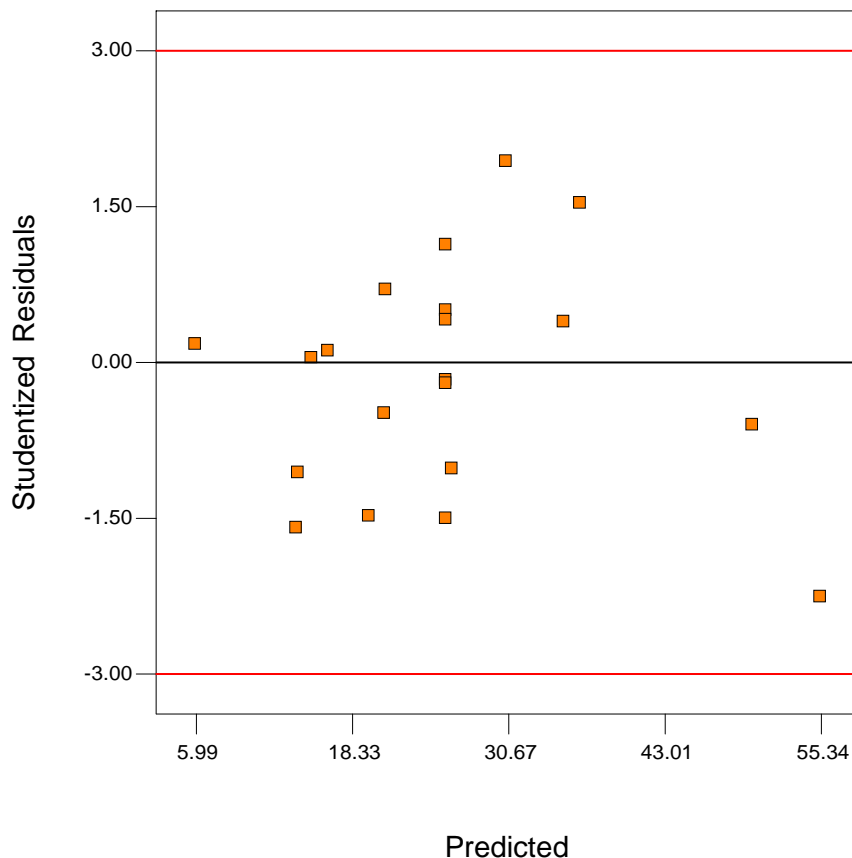
$$\begin{aligned} \text{lignin removal}(\%), Y_1(\text{coded factors}) = & -4.331 + 3.981 \times \text{NaOH concentration} \\ & + 0.572 \times \text{incubation time} + 0.019 \\ & \times \text{temperature} - 0.433 \\ & \times \text{NaOH concentration} \times \text{incubation time} \quad (4b) \\ & - 3.973 \times 10^{-3} \times \text{NaOH concentration} \\ & \times \text{temperature} + 0.0775 \times \text{incubation time} \\ & \times \text{temperature} \end{aligned}$$

**Figure 1** Normal probability plot of residual for lignin removal (see online version for colours)





**Figure 2** Plot of residual versus predicted response for lignin removal (see online version for colours)



This model can be used to predict the lignin removal after treatment within the limits of the experiment where the model is subjected to 2% (w/v) < NaOH concentration < 6% (w/v), 1 h < Incubation time < 5 h and 30°C < incubation temperature < 120°C. The normal probability plot of the residuals and the plot of the residuals versus the predicted response for lignin removal are shown in Figure 1 and Figure 2. A check on the plot in Figure 1 revealed that the residuals generally fall on a straight line implying that errors are distributed normally, and thus, support adequacy of the least-square fit. Figure 2 revealed that it has no obvious pattern and unusual structure. It also shows equal scatter above and below the  $x$ -axis. This implies that the model proposed are adequate and there is no reason to suspect any violation of the independence or constant variance assumption.

From Table 4, the 'Model  $F$  value' of 98.61 implies that the model is significant under 95% level of confidence. Meanwhile, the value of 'Prob >  $F$ ' less than 0.05 indicates that the model terms are significant. Meaning that the terms in the model have significant effect on the response. In this case,  $A$ ,  $B$ ,  $C$ ,  $AB$  and  $BC$  were found to be significant model terms while  $AC$  was insignificant model term for lignin removal after treatment. However, the insignificant model term was included in Equation (3a) and (3b) to obtain a hierarchy model.

The 'lack of fit' value was calculated from the experimental error and residuals. The 'lack of fit  $F$  value' of 1.5 indicates that the lack of fit was not significant relative to the pure error due to noise. For a model to be reliable, the result or data should be predicted with reasonable accuracy by the model and tested with the experimental data. The value of coefficient of determination for the current model,  $R^2$  is 0.979 while comparing the experimental results, which means, the experimented value is 97.9% similar to predicted result generated by the model. Since the value were higher than 80%, it showed that there was a good agreement between the experimental data and predicted values (Yeong et al., 2009; Wee et al., 2010; Bhatti et al., 2011). Therefore, the suggested model for lignin removal after treatment in equation 4(a) and equation 4(b) is valid in the present study, and could be used to predict the lignin removal for a selected value of NaOH concentration, incubation time and temperature, respectively.

Based on Table 4, temperature ( $C$ ) shows the highest  $F$  value of 252.71 which indicated that it had the most significant effect on the lignin removal after treatment compared to the other two factors, NaOH concentration ( $A$ ) and incubation time ( $B$ ). The significant factors were ranked based in the value of  $F$ -ratio. The larger the magnitude of  $F$ -value and correspondingly the smaller the 'Prob >  $F$ ' value, the more significant is the corresponding coefficient. Thus, in this study, the ranking is as follows:  $C > B > BC > A > AB$ .

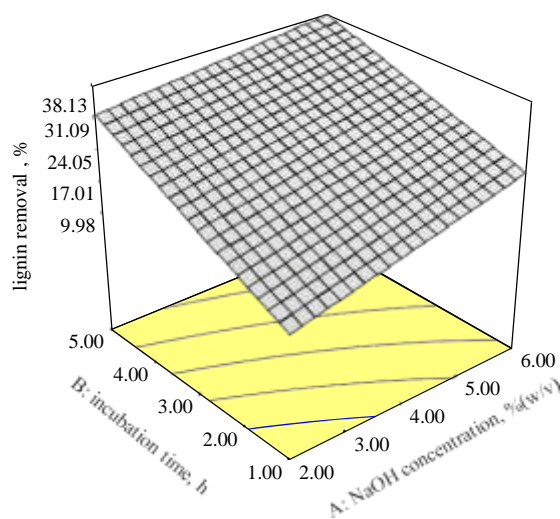
The three dimensional surface and contour plots demonstrated the effect of different process variables on lignin removal and they are depicted in Figure 3 and Figure 4. The 3D surface graph for the lignin removal has a planar profile in accordance to the 2FI model and is shown in Figure 3(a) and Figure 4(a) whilst the contour plots for the response surface for lignin removal is shown in Figure 3(b) and Figure 4(b).

The effect of the NaOH concentration ( $A$ ) and incubation time ( $B$ ), while keeping temperature at the middle level (75°C) is clearly seen in Figure 3. Lignin removal is obviously increased with the increasing of both the NaOH concentration (from 2% w/v to 6% w/v) and incubation time (from 1 hour to 5 hours). The maximum lignin removal of 38% is observed when both the NaOH concentration (6% w/v) and incubation time (five hours) are the highest. This showed that the lignin removal is increased when the NaOH concentration and incubation time is increased. This observation consistent with the results reported by Tran and Chambers (1987). Lignin is rich in oxygen-bearing functional group (e.g., acetyl group, phenolic group, carbonyl and etc.) which was always associated with hemicelluloses through covalent bonding (Essington, 2004). The higher the NaOH concentration, the more the  $\text{OH}^-$  that attacks the phenolic groups of lignin, thus improve the solubility of lignin in NaOH solution. Meanwhile, since the lignin's structure appeared in the form of unorganised macromolecular polymers (Evstigneev, 2010), it can be assumed that a longer incubation time is needed for the NaOH to penetrate into the inner layer of the polymers. Therefore, longer incubation time provide longer interaction time between the NaOH and lignin which then enhanced the delignification process.

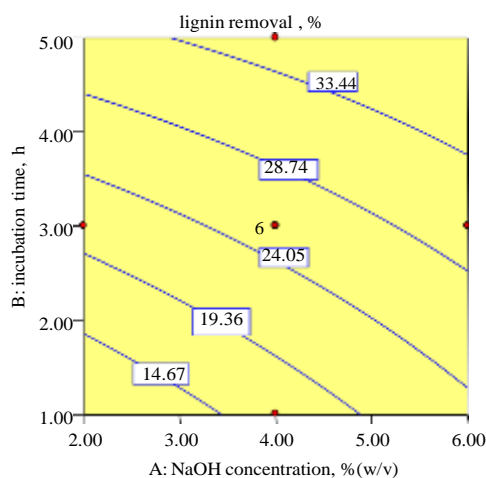
The surface and contour plots of the incubation time and temperature on the lignin removal are represented in Figure 4(a) and Figure 4(b). The lignin removal rose from 13% at 30°C and incubation time of one hour to 53% at 120°C and incubation time of five hours when the NaOH concentration was 4% (w/v). This showed that lignin removal is increased with the increased of incubation time and temperature. Thus indicates that, all the three factors are significant to the delignification process. Among all, temperature serves as the most significant factor in delignification process, followed by incubation time and NaOH concentration. Lignin is a highly-branched, three dimensional polymer

with a wide variety of functional groups providing active centres for chemical and biological interactions (El Mansouri and Salvad $\tilde{a}$ <sup>3</sup>, 2007). Due to the highly-branched macromolecular matrix structure of lignin, it might end up in steric hindrances which limit the collision between the hydroxide ions from NaOH and the active site of the related functional groups. Therefore, by adding the concentration of NaOH alone did not seem to improve the delignification process as much as with the aiding of higher temperature and longer incubation time. These finding is consistent with that reported in literature (Chang et al., 1997). Furthermore, it was reported that heat treatment was able to cleave the  $\beta$ -aryl-ether linkages between the polymers which might break the steric resistant and provided higher chances of collision between NaOH and its corresponding active site (Liu et al., 2004).

**Figure 3** Effect concentration of NaOH and incubation time on lignin removal at temperature of 75°C, (a) three-dimensional plot (b) contour plot (see online version for colours)

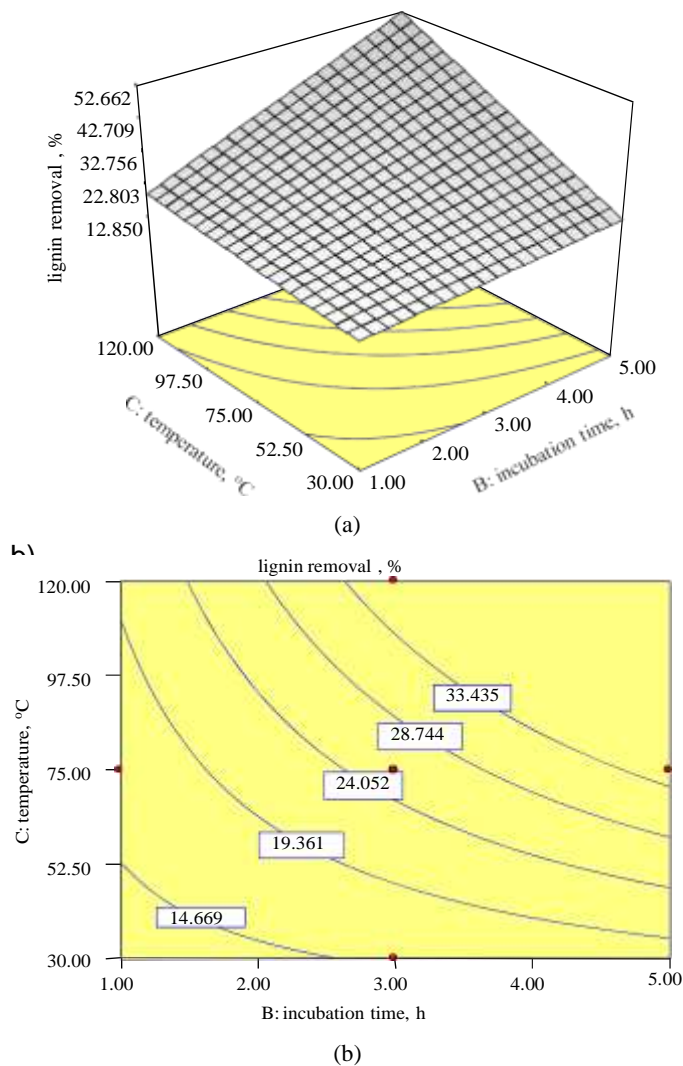


(a)



(b)

**Figure 4** Effect of incubation time and temperature on lignin removal at NaOH concentration of 4% (w/v), (a) three-dimensional plot (b) contour plot (see online version for colours)



### *Optimisation studies*

There are many methods which are available to optimise a process. In the present study, numerical optimisation was chosen. Numerical optimisation presents a comprehensive and up-to-date description of the most effective method in optimisation (Wee et al., 2010). The numerical optimisation was carried out by using the optimisation function embedded in a regression analysis program (Design Expert version 6.0.6) which searches for a combination of factors level that simultaneously meet the requirements placed on each of the responses and factors. The software combines the individual desirability into a single number that lied between 0 and 1 which represented the closeness of a response

to its ideal value. Generally, the total desirability is defined as a geometric mean of the individual desirability (Raissi, 2009):

$$D = d_1 \times d_2 \times \dots \times d_k \quad (5)$$

where  $D$  is the total desirability and  $d_i$  is the  $i^{\text{th}}$  desirability,  $i = 1, 2, \dots, k$ . The nearer the responses approaches the ideal intervals or ideal values, the nearer the desirability to 1. The optimum condition for the three independent variables in this delignification process which are NaOH concentration, incubation time and temperature were investigated. Table 5 presents the 10 solutions generated by Design Expert software. By default, the solution was sorted from best to worst. Thus the optimum condition in Solution 1 was chosen for further process studies.

**Table 5** Optimum condition for three different set of goals obtained from DoE for the lignin removal

Number	NaOH concentration, % (w/v)	Incubation time, h	Temperature, °C	Lignin removal, %	Desirability
1	5.96	4.92	118.02	53.95	1 <sup>a</sup>
2	5.42	4.9	119.06	53.49	1
3	4.34	5	119.88	53.04	1
4	5.97	4.94	115.28	53.10	1
5	5.9	4.98	114.51	52.99	1
6	4.79	4.98	119.32	53.33	1
7	5.1	4.94	118.68	53.16	1
8	4.94	4.92	119.46	53.10	1
9	3.39	5	120	51.83	0.978
10	2.51	5	120	50.66	0.953

Note: <sup>a</sup>Selected as optimum point as suggested by the software.

In order to check the accuracy of the optimum condition generated by the software, additional three experiments were carried out at the optimum condition (Solution 1). Table 6 shows the results of the three repeated experiments. The experimental values of the three responses were comparable with the values predicted by the software. The mean error was 1.289, showing that there was a good agreement between experimental and predicted values.

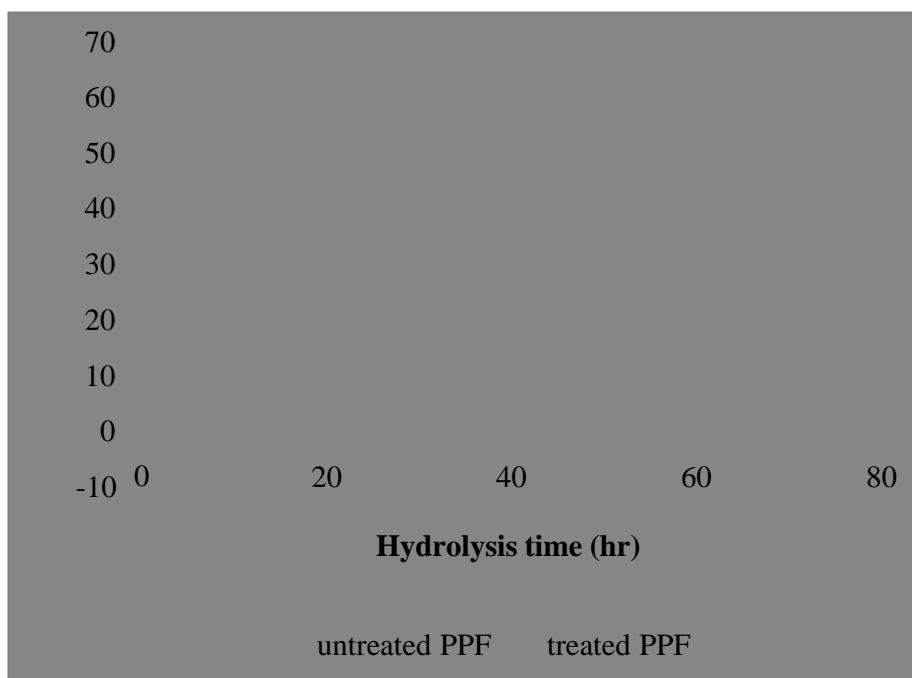
**Table 6** Verification experiments under optimum condition over lignin removal after pretreatment

Run	Lignin removal (%)		
	Experimental	Predicted (DoE)	Error
1	52.04	53.949	-1.912
2	55.29	53.949	1.344
3	50.65	53.949	-3.299
Mean error		1.289	
Standard deviation ( $\sigma$ )		2.384	

### *Enzymatic saccharification*

The digestibility test of the three sets of treated PPF was examined through enzymatic saccharification and average was taken to compare with the untreated PPF. Figure 5 shows that treated PPF yielded higher PPF hydrolysis than that of original untreated PPF with an increase of about 40%. It is being reported that the PPF digestibility is proportional to lignin removal since the fibrous structure of the biomass has been swelled and broken down that leads to a bigger accessibility of cellulose polymer for enzymatic saccharification (Huijgen et al., 2008). In this current study, we achieved 53% of lignin removal and a hydrolysis improvement of 40% , which shows an improvement when compare to current biological pretreatment which removed 27% lignin and 6% of hydrolysis improvement after two months of treatment period (Tong et al., 1993). However, this performance shows less attractive when compare with the performance reported by the same treatment method on oil palm empty fruit bunch (OPEFB), reaching 94% of lignin removal after 3 days treatment period (Hamisan et al., 2009) and gained 76% increment of hydrolysis after 4 hours treatment period (Umikalsom et al., 1998). This phenomenon shows that NaOH pretreatment although showed high efficiency in OPEFB pretreatment, did not give the same efficiency in PPF pretreatment and this statement is consistent with what is being concluded elsewhere (Taherzadeh and Niklasson, 2004; Galbe and Zacchi, 2007; Kumar et al., 2009). Therefore, more researches on PPF pretreatment should be examined in order to find a highly efficient technology for lignin removal as well as increase the digestibility of PPF.

**Figure 5** Profile of hydrolysis of treated and untreated PPF (pretreatment condition: 5.96 w/v% of NaOH, 4.92 h and 118.02°C) (see online version for colours)



#### 4 Conclusions

The pretreatment of PPF with alkaline (NaOH) was found to be more efficient in removing lignin removal as compared to acid (HCl). The application of statistical DoEs to study the pretreatment condition of the more effective chemical (NaOH) has been successfully demonstrated in the present work. A RSM coupled with CCD helped in obtaining the optimum condition and to study the interaction between the tested factor for NaOH pretreatment (NaOH concentration, incubation time and temperature). The variance of analysis (ANOVA) showed that the temperature played the most significant effect on lignin removal, followed by incubation time and NaOH concentration. The predicted value (54% of lignin removal) with the optimal condition at 5.96% (w/v) NaOH, 118.02°C and 4.92 incubation time generated from the model proposed by RSM was validated with the experimental results and was found to be in agreement with an average error of  $\pm 1.29$ .

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