EFFECT OF SODIUM HYDROXIDE PRETREATMENT ON CHEMICAL COMPOSITION OF TREATED *ACACIA MANGIUM* USING RESPONSE SURFACE METHODOLOGY

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High cellulose biomass is required to produce high yield fermentable sugars to be used as a feedstock for biofuels production. However, the higher lignin content in biomass becomes a hindrance to a high cellulose content biomass raw material production. Therefore, an alkaline pretreatment optimised with sodium hydroxide (NaOH) is necessary to remove lignin. In this study, the optimisation was carried out using response surface methodology and the parameters were set at a temperature range of 70–100 °C, the reaction time of 1–3 hours and NaOH concentration between 1–10% w/v. The samples were analysed to obtain its chemical composition and compared with untreated *Acacia mangium*. Optimised conditions were achieved at 70 °C (temperature), 3 hours (reaction time) and 5.50% w/v (NaOH concentration) with optimized cellulose, hemicellulose and lignin removal content of 47.66, 36.16 and 12.66% and standard error of 7.90, 1.87 and 3.24% respectively. In the study, the cellulose content obtained from the NaOH treated *A. mangium* had risen to about 20.40% and had high potential to be used as a feedstock for the production of fermentable sugars.

Keywords: Lignocellulosic biomass, central composite design, analysis of variance, thermogravimetric analysis, cellulose content

INTRODUCTION

Lignocellulosic biomasses are renewable organic raw materials that are easily obtained in large amounts at low prices (Fang et al. 2010, Rawat et al. 2013). It is also part of the agricultural and forestry wastes where the accumulation of unutilised waste causes environmental problems (Gilna & Khaleel 2011, Falkoski et al. 2012). Short-term cultivation species such as *Acacia mangium* containing low lignin needs minimal fertiliser for its potential growth and is an important source for raw materials in biofuels production (Chong et al. 2013, Rawat et al. 2013). Since the 1980s, *A. mangium* plantation has been established in Malaysia with Sarawak having the largest plantation area of around 289,816 ha in 2012 and used mainly for furniture products (Redzuan et al. 2019). Nevertheless, no plantation field was developed in Malaysia for biofuels specifically for bioethanol production as an alternative to petrol.

Cellulose, hemicellulose and lignin make up about 90% of the dry weight of plant materials (Irfan et al. 2011). The decomposition of these components into simple sugars, such as glucose and xylose, is accomplished by cellulase and xylanase enzymes during enzymatic reaction (Singh et al. 2011). Cellulose is a linear polymer comprising of glucose linked to β-1,4-glucosidic bond and hemicellulose is a heteropolysaccharide consisting of β-1,4-links polysaccharide with different degrees of substitution (Praveen et al. 2012). Hemicelluloses are commonly found outside the cellulose fibre structures which act as a physical barrier that limits the cellulase enzyme to act on cellulose (Hu et al. 2011). The main challenge in the use of lignocellulosic materials is to convert its structure from complex carbohydrates into monosaccharide. Therefore, pretreatment of lignocellulosic material is crucial to enhance the decomposition of lignocellulose material by hydrolytic enzymes through the breakdown of lignin, hemicelluloses and cellulose bonds for enzymatic hydrolysis purposes (Sendelius 2005, Singh et al. 2011, Isikhuemhen et al. 2014).

Pretreatment is also an important step to remove or modify the structure of hemicellulose and lignin, reduce cellulose crystallisation, improve surface area and enhancing the hydrolysis efficiency as well as to improve its porosity (Yang et al. 2010, Irfan et al. 2011, Maeda et al. 2011) by the enzyme during the hydrolysis process for the production of fermentable sugars (Quiroz-castaneda et al. 2009, Rawat et al. 2013). Temperature, chemical concentration and treatment time affect the pretreatment process (Ruffell et al. 2010. Nur Izzati et al. 2013). Generally, after the lignocelluloses are treated, the amount of cellulose composition will increase, while the composition of hemicellulose and lignin will decrease depending on the type of pretreatment used (Sharma et al. 2016).

In general, lignin is a major component in wood that prevents its use as a raw material for biotechnology product development and affects the rate of enzymatic hydrolysis (Maeda et al. 2011, Nur Izzati et al. 2013). Removal of the lignin is possible because of its solubility in alkaline solution. Decomposition of lignin can also reduce unproductive binders between celluloses (Yang et al. 2010). However, the slow decomposition process of lignocellulose material is considered less economical (Ibrahim 2008). Hence, new methods are needed to increase the fermentation of sugar through a combination of chemical and enzymatic hydrolysis processes.

The production of sugar from biomass is an important step in industrial processing, so physical and chemical pretreatment have been widely used to break down lignin bonds and separate cellulose fibres and monomers of hemicellulose components (Quiroz-castaneda et al. 2009). Removal of hemicellulose content also affects the production of fermentable sugars where more hemicellulose is removed and higher fermentable sugars will be produced (Sendelius 2005).

Alkaline pretreatment is one of the best pretreatment for delignification of lignocellulosic biomass (Nur Izzati et al. 2013) which leads to partial delignification, increasing fibre surface area through cellulose swelling and removal of hemicellulose and lignin (Chen et al. 2018). Alkaline pretreatment using sodium hydroxide (NaOH) on oil palm stem eliminates up to 19.8% of lignin content (Ibrahim 2008).

The conventional way used to optimise a multifactorial method is to resolve one factor at a time, but this is time consuming (Rawat et al. 2013, Saliman et al. 2017). Response surface methodology (RSM) has been adopted to determine the optimised parameter conditions for alkaline pretreatment process (reaction temperature, reaction time and NaOH concentration) to produce optimised cellulose composition. Thus, RSM is the fastest and easiest way to determine the effects of the various independent variables. Poplar wood pretreated using NaOH at the optimized conditions of 2.80% w/v concentration, 94 °C reaction temperature and 60 min reaction time have increased cellulose composition by 26.15% (Rawat et al. 2013). The cellulose content of treated rice straw was at approximately 42.01% with the optimised NaOH concentration of 2.96% w/v at temperature of 81.79 °C and 56.66 min reaction time (Kim and Han 2012).

Therefore, the objective of this study was to use RSM to determine the optimum pretreatment conditions to improve chemical composition of *A. mangium* with the purpose to increase the cellulose content and reduce the lignin content. The relationship among different parameters will be analysed to obtain its interacting effects on the overall process.

MATERIALS AND METHODS

Materials preparation

The raw materials (*Acacia mangium)* were obtained from FRIM Research Station, Segamat, Johor. They were cut into chips and samples of *A. mangium* were dried in an oven at 60 °C to achieve moisture content of less than 10%. The chips were ground to smaller particles with a grinder and sieved with a 250 µm mesh filter to obtain uniform 250 µm of sample size. The sample then underwent alkaline pretreatment process. Untreated *A. mangium* was used as a control and reference to the treated *A. mangium* throughout this study.

Alkaline pretreatment method

Acacia mangium was treated with a NaOH solution at a liquid to solid ratio of 1:20. About 20 g of *A. mangium* was mixed with 400 ml of NaOH solution at different concentrations of NaOH (1–10% w/v) and was treated at different reaction times (1–3 hours) with different reaction temperatures (70–100 °C). The treated samples

were filtered using a vacuum pump to separate the treated *A. mangium* sample from the liquid medium. Treated *A. mangium* was washed and rewashed using distilled water to neutralise the samples to pH 7. Lastly, the treated samples were dried in the oven at 60 °C and further characterised to determine their chemical composition and were compared against the untreated *A. mangium.*

Response surface methodology and central composite design

Optimisation of alkaline pretreatment of *A. mangium* was performed using response surface methodology RSM with central composite design (CCD). Three key factors (temperature, reaction time and NaOH concentration) with a suitable range were considered based on previous studies in order to obtain optimum parameter conditions. A total of 20 experimental runs with three replicates were conducted (Table 1). A quadratic model was fitted through correlations between responses and independent variables (factors) and a quadratic polynomial equation (equation 1)

was used to determine the relationship between these variables (Jaisamut et al. 2013, Siti Sabrina et al. 2014).

$$Y = b_0 + \sum b_i x_i + \sum b_{ii} x_{i2} + \sum b_{iii} x_{i3} + \sum b_{ij} x_i x_j \quad (1)$$

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

Statistical analysis

Design Expert 8.0.6 was used to develop the model of experiment. The effect of NaOH concentration on the chemical composition (cellulose, hemicellulose and lignin) of treated *A. mangium* was analysed using the Analysis of variance (ANOVA). Verification of the predicted value was determined by repeated experiments using the optimum conditions. The percentage error was also calculated based on equation 2 to determine the closeness between the predicted and experimental results.

Table 1   CCD matrix of alkaline treatment of *Acacia mangium*

Run	Temperature (°C)	Time (hour)	NaOH Concentration (% w/v)
1	85	2	5.5
2	70	1	1.0
3	100	3	1.0
4	100	2	5.5
5	85	2	10.0
6	85	2	5.5
7	70	3	10.0
8	85	2	5.5
9	100	1	10.0
10	100	1	1.0
11	85	1	5.5
12	85	2	5.5
13	85	3	5.5
14	70	1	10.0
15	85	2	5.5
16	85	2	5.5
17	85	2	1.0
18	100	3	10.0
19	70	2	5.5
20	70	3	1.0

Percentage error =

$$\frac{\left(\left|\text{Predicted value} - \text{experimental value}\right|\right) \times 100}{\text{Predicted value}}$$

(2)

Chemical composition characterisation of *A. mangium*

Chemical compositions of untreated and treated *A. mangium* were determined using the Thermogravimetric Analysis Method (TGA). About 5 mg of *A. mangium* samples were analysed using TGA. Combustion of samples in triplicates were carried out in the absent of nitrogen gas at a heating rate of 5 °C min⁻¹ with the temperature range between 25 and 900 °C (Barneto et al. 2011).

RESULTS AND DISCUSSION

Characterisation of *A. mangium* for its chemical composition

The chemical composition (cellulose, hemicellulose and lignin) of *A. mangium* are important properties to be measured and they served as baseline data to determine the effectiveness of the pretreatment method. The cellulose, hemicellulose and lignin removal content of raw *A. mangium* in comparison with other researchers' findings are shown in Table 2.

It was found that the chemical composition of *A. mangium* used in this study was comparable to the results reported by Raphy et al. (2011). However, the cellulose content was lower and up to 20.61% difference in comparison with result reported by Yahya et al. (2010), Takazawa et al. (2018) and Mohd Hazim et al. (2018). The large difference of cellulose composition might be due to the different sources of raw material, different

age and maturity levels as well as different soil types of the *A. mangium* plantation. Chemical composition of lignocellulosic materials varies with genetics, age, location, growth conditions, anatomic structure and maturity level of the plant (Hodson et al. 1984, Irfan et al. 2011, Siti Sabrina et al. 2014, Antonopoulou et al. 2015). Lower amount of cellulose composition of *A. mangium* used in this study was optimised through pretreatment process to increase its cellulose content and to ensure effective accessibility of enzyme during enzymatic hydrolysis process to produce fermentable sugars.

Optimisation of alkaline pretreatment on *A. mangium*

The main purpose of pretreatment process is to break down complex carbohydrate polymers, remove or alter the hemicellulose and lignin content, reduce cellulose crystallisation and increase their surface area (Singh et al. 2011, Siti Sabrina et al. 2014). Alkaline pretreatment using NaOH was chosen for this study as it has been commonly used by previous researchers to remove lignin content and increase surface area (Yoo et al. 2012, Maeda et al. 2013, Uzunlu et al. 2014). Optimisation of alkaline pretreatment of *A. mangium* was carried out using RSM to obtain optimised cellulose composition.

The experiments evaluated the relationships between temperature, reaction time and concentration of NaOH with the cellulose, hemicellulose and lignin content. Table 3 shows the ANOVA results of cellulose, hemicellulose and lignin content of treated *A. mangium*. Model F values of 69.35, 282.58 and 29.39 with p value less than 0.05 for cellulose, hemicellulose and lignin content indicated that this model was significant. The r^2 values for cellulose, hemicellulose and lignin content were 0.9842, 0.9961 and 0.9445

Table 2   Various chemical composition of *Acacia mangium*

Cellulose	Hemicellulose	Lignin	Past Research References
39.57	47.53	19.61	This study
48.10	34.20	27.20	(Takazawa et al. 2018)
49.84	36.14	17.23	(Mohd Hazim et al. 2017)
40.00	-	17.00	(Raphy et al. 2011)
45.70	34.70	31.30	(Yahya et al. 2010)
46.75	24.85	22.35	(Lim et al. 2011)

respectively showing that this model was robust with high fitness and precise (Saliman et al. 2017). A lack of fit value more than 0.05 showed that the model was not significant relative to pure error for cellulose, hemicellulose and lignin content which were 0.0518, 0.4136 and 0.0645 respectively.

The second-degree polynomial models for cellulose, hemicellulose and lignin content are given in equations 3, 4 and 5 below.

Cellulose (C_1) =
$44.291 + 0.030X_1 + 3.516X_2 + 1.493X_3 + 1.670 \times 10^{-4}X_1^2 - 0.332X_2^2 + 8.238 \times 10^{-3}X_3^2 - 0.017X_1X_2 - 0.015X_1X_3 - 0.101X_2X_3$

(3)

Hemicellulose (H_2) =
$36.255 + 0.049X_1 - 0.122X_2 - 0.349X_3 - 1.273 \times 10^{-4}X_1^2 - 0.024X_2^2 - 4.377 \times 10^{-3}X_3^2 - 7.167 \times 10^{-3}X_1X_2 + 6.926 \times 10^{-3}X_1X_3 - 0.046X_2X_3$

(4)

Lignin (L_3) =
$-33.750 + 0.902X_1 + 4.792X_2 + 1.058X_3 - 4.756 \times 10^{-3}X_1^2 - 1.375X_2^2 + 4.198 \times 10^{-3}X_3^2 + 0.024X_1X_2 - 6.130 \times 10^{-3}X_1X_3 - 0.225X_2X_3$

(5)

where X_1, X_2, and X_3 are temperature (A), reaction times (B) and concentration of NaOH (C) respectively.

Based on Table 3 and F values, temperature and NaOH concentration had the highest influences on cellulose and hemicellulose contents. However, reaction time and NaOH concentration had the highest influence on lignin removal content. Figure 1, shows the interaction effect of temperature and NaOH concentration on cellulose content. When the temperature increased from 70 to 100 °C with NaOH concentration was kept constant at 1.00% w/v, the cellulose content decreased by 0.41% (51.36 to 51.15%) and decreased by 8.31% (51.88 to 47.57%) when NaOH concentration was increased to 10.00% w/v.

If temperature and reaction time was kept constant at 70 °C and 3 hours respectively, the increase in NaOH concentration (1.00 to 10.00% w/v) increased the cellulose content by 1.01%. Based on these findings it showed that at higher temperature and higher concentration of NaOH, the cellulose was likely to dissolve. Due to the high concentrations of alkaline, the surface topography of the biomass was damaged and soluble cellulose were lost during neutralisation step, leaving behind less amount of cellulose (Siti Sabrina et al. 2014, Oushabi et al. 2017). In addition, normal alkaline pretreatment is performed at lower temperatures but longer reaction time is needed compared with just a few minutes for acid pretreatment (Jaisamut et al. 2013, Antonopoulou et al. 2015).

Like cellulose, temperature and NaOH concentration had highest influence on

Table 3   Statistical analysis (ANOVA) of cellulose, hemicellulose and lignin (CHL) content of treated *A. mangium*

Source	Cellulose		Hemicellulose		Lignin	
	F-value	p-value	F-value	p-value	F-value	p-value
Model	69.35	< 0.0001	282.58	< 0.0001	29.39	< 0.0001
A	230.14	< 0.0001	690.35	< 0.0001	117.08	< 0.0001
B	11.00	0.0078	1355.60	< 0.0001	0.83	0.3831
C	32.87	0.0002	233.69	< 0.0001	16.04	0.0025
A^2	0.12	0.7313	0.26	0.6195	11.85	0.0063
B^2	9.42	0.0119	0.18	0.6813	20.25	0.0011
C^2	2.38	0.1538	2.52	0.1438	0.31	0.5872
AB	16.19	0.0024	10.76	0.0083	4.37	0.0630
AC	253.94	< 0.0001	203.58	< 0.0001	6.35	0.0304
BC	51.54	< 0.0001	40.11	< 0.0001	36.81	0.0001
Lack of fit	4.96	0.0518	1.23	0.4136	3.12	0.0645

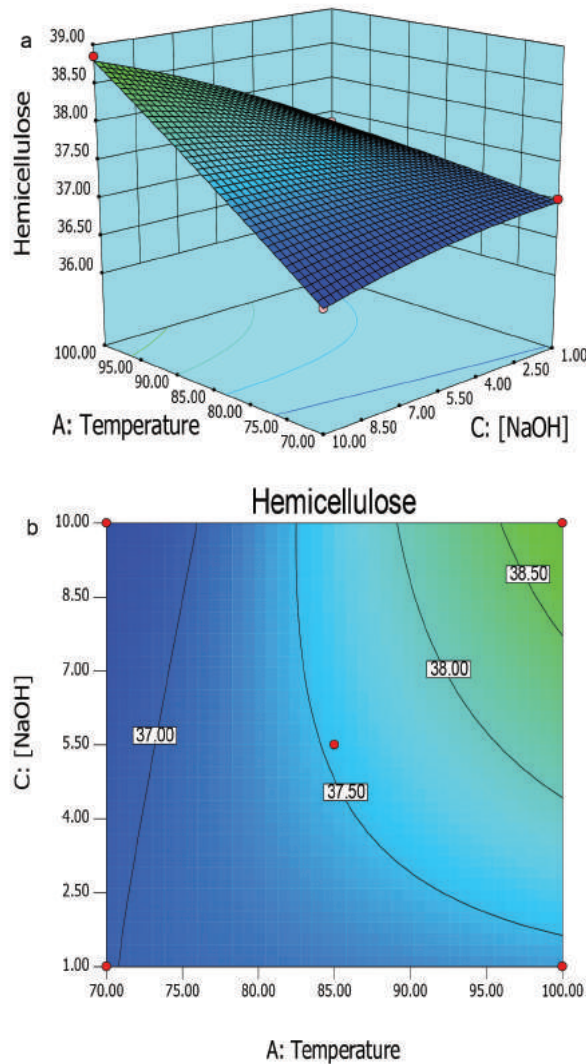A = temperature, B = reaction time, C = concentration of NaOH
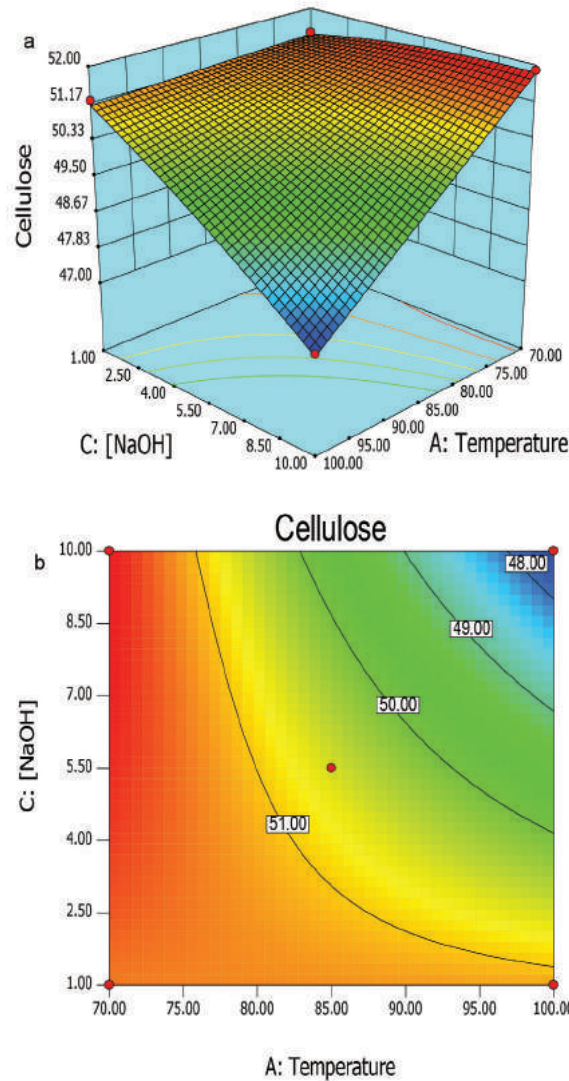
Figure 1   3D surface plot (a) and interaction effect (b) between factors
A and C on cellulose content of treated *A. mangium*

hemicellulose content (Figure 2). Interactions between temperature and NaOH concentration greatly influenced the hemicellulose content. When the temperature increased from 70 to 100 °C with NaOH concentration of 1.00% w/v, it showed an increase of hemicellulose content by 2.41% (36.99 to 37.88%) compared with 6.19% increase (36.53 to 38.79%) when NaOH concentration was at 10.00% w/v. If the temperature was set to 70 °C, an increase in NaOH concentration (1.00 to 10.00% w/v) would have resulted in a decrease in hemicellulose content by 1.24% (36.99 to 36.53%). It was found that, at lower temperature (70 °C) and higher concentration of NaOH (10.00% w/v), decrease of hemicellulose content and increase of cellulose content were observed. Treatment

temperature was an important factor that affected pretreatment either through biological or chemical process (Saliman et al. 2017).

Figure 3 shows the interaction effect between reaction time and NaOH concentration that strongly influenced the removal of lignin content. When the reaction time had increased from 1 to 3 hours with NaOH concentration at 1.00% w/v, it showed an increase of lignin content removal by 14.01% (11.56 to 13.18%) compared with 15.45% decrease (15.60 to 13.19%) when NaOH concentration was kept at 10.00% w/v. If the reaction time was set to 3 hours, the increase in NaOH concentration (1.00 to 10.00% w/v) resulted in a very small removal of lignin content by 0.08% (13.18 to 13.19%). As for lignin content, comparison of
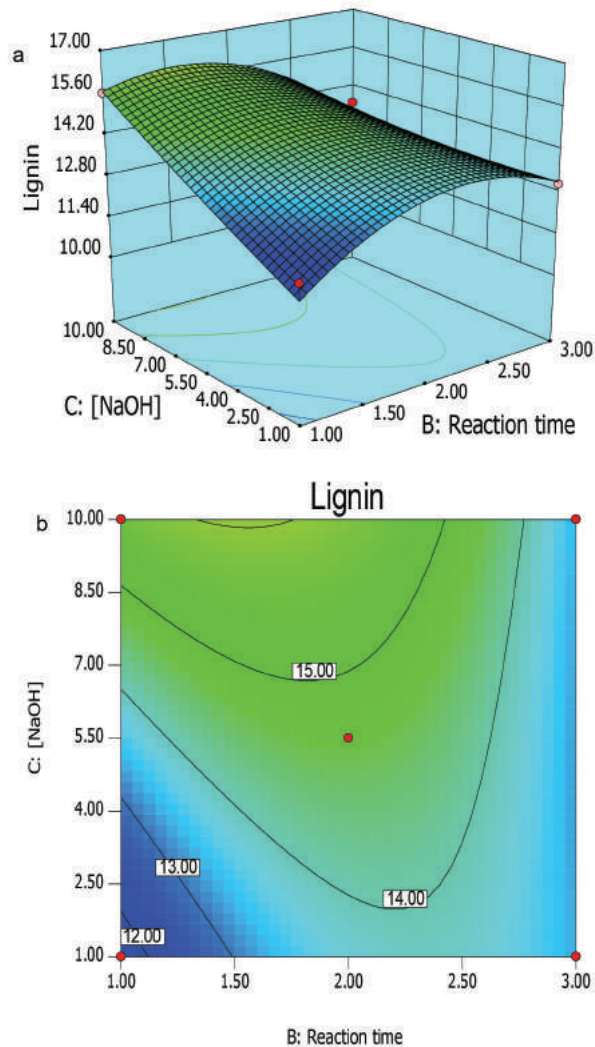
Figure 2   3D surface plot (a) and interaction effect (b) between factors
A and C on hemicellulose content of treated *A. mangium*

factors for interaction effects showed that lower temperatures (70 °C) and shorter time (1 hour) at lower alkali concentrations (1.00% v/v) could eliminate more lignin.

Generally, RSM model predicted the cellulose, hemicellulose and lignin removal content at 51.75, 36.85 and 13.03% respectively were achieved at optimum conditions of 70 °C (temperature), 3 hours reaction time and 5.50% w/v concentration of NaOH. The validation of results were performed using optimal conditions and the experimental values for cellulose, hemicellulose and lignin obtained were 47.66, 36.16 and 12.66% respectively, with predicted errors of 7.90, 1.87 and 3.24% respectively. It showed that the experimental results were in good agreement with the predicted values by the proposed model with less than 10% error.

Among those three factors, NaOH concentration had the highest influence on the increase of cellulose content and decreas in the lignin content. The major role of NaOH in the pretreatment was to degrade lignin by breaking up the polymer structure and increased in the biomass porosity (Irfan et al. 2011). Optimum NaOH concentration of 5.50% w/v showed that RSM could help in reducing the amount of chemical consumption and thus allowed operations at lower temperature (70 °C) instead of 100 °C that resulted in reduction of production cost and energy consumption. This finding was found agreeable with result obtained by Anita et al. (2019) which only used 1.10% acid concentration instead of 1.50% to treat oil palm empty fruit bunch at fastest reaction time (3 min) when RSM was applied.

Figure 3   3D surface plot (a) and interaction effect (b) between factors
B and C on lignin removal content of treated *A. mangium*

Effect of alkaline treated *A. mangium* on cellulose, hemicellulose and lignin content

The optimum chemical composition of cellulose, hemicellulose and lignin content in the treated *A. mangium* was compared with untreated *A. mangium* to determine the effectiveness of the pretreatment process on lignin and hemicellulose removal (Table 4). The results showed that pretreatment using alkali (NaOH) was able to reduce lignin content by 35.44%. Alkaline pretreatment increased cellulose content by 20.44% and reduced hemicellulose content by 23.92%. The alkaline treatment on lignocellulose materials had caused swelling of the biomass structure, increased surface area, reduced polymerisation and crystallisation, disrupted bond formation between lignin and carbohydrate as well as disrupted the lignin structure (Sun & Cheng 2002, Irfan et al. 2011).

Cellulose content of oil palm frond increased by 46.71% and the lignin content decreased by 31.53% after being treated with alkali at 100 °C for 58.31 min with a NaOH concentration of 4.42% w/v (Siti Sabrina et al. 2014). Poplar wood cellulose and hemicellulose content increased by 26.15 and 14.20% and lignin removal content decreased by 12.70% after treatment with NaOH at a concentration of 2.80% w/v, at 94 °C for one hour reaction time (Rawat et al. 2013). Meanwhile, lignin content of wheat straw was reduced by 62.50% after treatment with alkali at an optimum temperature of 80 °C, reaction time of 39 min using a mixture of 0.18 g NaOH and 0.016 g lime per gram of raw material (rice straw) (Jaisamut et al. 2013). Table 5 shows

Table 4   Chemical composition of untreated and treated *A. mangium*

Raw material	Chemical composition (%)		
	Cellulose	Hemicellulose	Lignin
A. mangium (untreated)	39.57 [0.10]	47.53 [0.20]	19.61 [0.69]
A. mangium (treated)	47.66 [0.20]	36.16 [0.11]	12.66 [0.61]
Per cent difference	+20.44	-23.92	-35.44

(+) = increase; (-) = decrease; [ ] = standard deviation

Table 5   Comparison of chemical composition of lignocellulosic biomass treated with alkaline (NaOH)

Raw material	Before treatment			After treatment			References
	C	H	L	C	H	L	
A. mangium	39.57	47.53	19.61	47.66	36.16	12.66	This study
Sugarcane baggase	39.70	28.00	23.02	55.10	18.10	8.70	(Sornlake et al. 2017)
Sugarcane baggase	52.80	19.10	22.10	59.20	22.30	11.40	(Visser et al. 2013)
Oil palm frond	28.71	26.02	37.63	42.12	31.93	26.05	(Siti Sabrina et al. 2014)
Poplar wood	45.50	16.20	26.80	57.14	18.50	14.10	(Rawat et al. 2013)
Mix wheat straw & cotton stalk	38.50	22.00	18.95	40.02	15.06	10.25	(Sharma et al. 2016)

C = cellulose, H = hemicelluloses, L = lignin

the chemical composition of various types of biomass in comparison with findings obtained in this study. The comparison was made for the lignocellulosic biomass before and after the pretreatment with NaOH.

The effect of pretreatment varies from one biomass to another depending on its chemical composition and morphology structure (Dar & Phutela 2019). Besides, pretreated biomass demands less fermentation time and low energy consumption during fermentable sugars production as compared with untreated biomass (Chen et al. 2018). Due to this reason, it was important to firstly determine the optimised condition in order to get the optimum product yield at low production cost. Alkaline pretreatment using NaOH significantly affected the removal of lignin and hemicellulose contents which up to 36.00 and 44.90% respectively (Antonopoulou et al. 2015). Up to 27.40% of lignin was removed after pretreatment of oil palm mesocarp fibre at temperature of 70 °C for two hours using 6.00% w/v NaOH concentration (Nur Izzati et al. 2013). Results from this study showed almost similar observations with other researchers although different types of lignocellulosic biomass materials were used.

CONCLUSIONS

Alkaline pretreatment is the best way to get rid of lignin thus increases cellulose content. Optimised conditions of alkaline pretreatment was achieved at 70 °C, 3 hours reaction time by using 5.50% w/v NaOH concentration yielding an increase in cellulose content by 20.44% and a decrease in hemicellulose and lignin contents by 23.92 and 35.44% respectively. The ANOVA results indicated that the model and all independent parameters were statistically significant at 95% of confidence level. Besides, alkaline pretreatment of *A. mangium* shown to be predictable with desirability of 0.93 that was able to increase the amount of cellulose. Adoption of RSM to determine the optimum chemical composition helps in reduction of the pretreatment cost at the early processing stage. This is achieved when RSM managed to identify the desirable optimum conditions thus obtaining the optimised yield of fermentable sugars during enzymatic hydrolysis process.

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