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Optimization of Cellulose Isolation from *Melaleuca leucadendron* Twigs by Box-Behnken Design

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Abstract. The production of *Melaleuca leucadendron* essential oil generates a lot of solid waste, since only under 2% take it out as the oil product. The solid waste contains cellulose as an important content which can be isolated by alkaline treatment of sodium hydroxide (NaOH). In this project, optimization of alkaline treatment of *M. leucadendron* twigs (MLT) waste was studied in order to obtain optimum isolated cellulose. The experiments were conducted at atmospheric pressure and boiling temperature. The 3^3 Box-Behnken design (BBD) was used to evaluate the effects of treatment time, solid loading and NaOH concentration. Quadratic model evaluation showed that the model is a prospective to describe the effects of the variables (R^2=0.8994, $p<0.05$). The significant variables were found in linear term of NaOH concentration and quadratic term of treatment time. The optimum result of the experimental variables range was predicted in treatment time, solid loading and NaOH concentrations of 60 minutes, 10%, and 1.83% respectively. In conclusion, BBD is appropriate to optimize cellulose isolation process of MLT.

Keywords: cellulose, sodium hydroxide, *Melaleuca leucadendron*, Box-Behnken Design

1. Introduction

Melaleuca leucadendron (*kayu putih*) is a plant which well-known for its essential oil products [1-2]. It is commonly grown in natural forests or plantations in Indonesia, such as in Gunungkidul, Yogyakarta [2-3]. In this area, *M. leucadendron* leaves and twigs are processed as raw materials for local essential oil plant. Since essential oil content is under 2% [1-2], the extraction process generates a lot of solid waste. The utilization of these waste is not optimum yet, since utilized as wood fuel only such as for tofu production [4]. Since the genus of *Melaleuca* is lignocellulosic biomass material, it has huge prospect as a source of useful materials i.e. bioethanol production from *M. leucadendron* shedding bark [5] and cellulose production from *Melaleuca cajuputi* wood [6]. Nanocellulose is one of cellulose form which has prospective market to develop and evolve [7]. The rise of cellulose demand will trigger the exploration of alternative raw material which *M. leucadendron* waste is one of the prospective one.

Various treatments in order to extract cellulose from biomass have been proposed; alkaline pretreatment is the favorite method among them because of its high efficiency of lignin removal and its milder condition than acid pretreatment [8]. Sodium hydroxide (NaOH) is the most common reagent for alkaline pretreatment of lignocellulosic materials. NaOH treatment has some advantages, i.e. high reaction rate, 50% hemicellulose dissolution and 60-80% delignification [8]. Since some previous study have been successfully conducted for removing lignin from sugarcane bagasse [9-11], palm midrib [12] and sansevieria fiber [13], this study will adopt the method in *M. leucadendron* twigs delignification process.

In order to optimize the process, experimental designs based on response surface method (RSM), such as Doehlert matrix (DM), central composite design (CCD) and Box-Behnken design (BBD) could be applied. Among these methods, BBD and DM are more efficient than CCD for experiment with three factors [14]. In this case, the number of experiments needed for BBD is less than the other methods [14]. Previous studies based on BBD have been successfully conducted for optimizing pretreatment of lignocellulosic such as alkaline pretreatment of rice straw [15] and cellulose extraction of jute fiber [16]. Therefore, the aim of this study is to optimize alkaline pretreatment process of *M. leucadendron* twigs to obtain optimum cellulose content by BBD.

2. Materials and methods

2.1. Materials

Melaleuca leucadendron twigs (MLT) were obtained from local essential oil factory in Gunungkidul, Yogyakarta, Indonesia. The twigs were milled and screened to obtain particles with size 16-20 mesh, then were packed in plastic bag to minimize contact with water vapor in the air.

2.2. Box-Behnken Design

The 3^3 Box-Behnken design with 3 replications at center point was selected as a method to optimize delignification process for cellulose isolation of MLT. The selected variables were time, solid loading, and NaOH concentration. The minimum and maximum levels of three variables are presented in Table 1.

Table 1. Minimum and maximum levels of the variables

Variable	Symbol		Level		
	Code	Real	-1	0	+1
Time (min)	x_1	X_1	30	45	60
Solid loading (%)	x_2	X_2	10	15	20
NaOH concentration (%)	x_3	X_3	1	1.5	2

Second-order polynomial model was used to describe the effects of the three variables. The model was given in Equation 1 below.

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

Y is the predicted response (%cellulose obtained); $ß_0$ is constant; $ß_1$, $ß_2$ and $ß_3$ are linear coefficients; $ß_{11}$, $ß_{22}$ and $ß_{33}$ are quadratic coefficients; $ß_{12}$, $ß_{13}$ and $ß_{23}$ are interactions coefficients; x_1, x_2 and x_3 are representing factors. The model evaluation with ANOVA and regression analysis was carried out using Microsoft Excel.

2.3. Experimental

MLT particles at selected solid loading (10, 15, 20%) were dispersed in 100 mL of NaOH solution (1, 1.5, 2%) in a pear-shaped flask. The mixture was heated until reach its boiling point in an oil bath. The reaction was stopped after the selected reaction time (30, 45, 60 min) was reached. The mixture was then filtered to obtain a solid phase. The solid phase was washed with tap water until reach neutral pH and finally washed with aquadest. The solid was dried at temperature of 105 °C until reached its constant weight. Chesson-Datta method [17] was used to analyze % cellulose ($g_{cellulose}/g_{solid}$) before and after treatments.

3. Results and discussions

3.1. Box-Behnken Design and Model Evaluation

Raw MLT contained 39.20% cellulose and after treatment, its content increased into 42.35-48.07%. The experimental and predicted results (%cellulose) based on Box-Behnken design (BBD) are shown in Table 2.

Table 2. Experimental and predicted results

Run	Code Variables			Real Variables			Response	
	x_1	x_2	x_3	X_1	X_2	X_3	Experimental	Predicted
1	-1	-1	0	30	10	1.5	47.58	47.05
2	1	-1	0	60	10	1.5	48.07	47.69
3	-1	1	0	30	20	1.5	46.15	46.53
4	1	1	0	60	20	1.5	46.38	46.92
5	-1	0	-1	30	15	1	43.65	44.02
6	1	0	-1	60	15	1	45.34	45.57
7	-1	0	1	30	15	2	47.91	47.69
8	1	0	1	60	15	2	47.54	47.16
9	0	-1	-1	45	10	1	42.35	42.51
10	0	1	-1	45	20	1	43.74	42.99
11	0	-1	1	45	10	2	45.51	46.27
12	0	1	1	45	20	2	44.66	44.50
13	0	0	0	45	15	1.5	46.08	45.19
14	0	0	0	45	15	1.5	44.24	45.19
15	0	0	0	45	15	1.5	45.25	45.19

Table 3. ANOVA results for acquired model

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	p-value	Characteristics
Model	9	36.90	4.10	4.96	0.046	Significant
Residual	5	4.13	0.83			
Total	14	41.03				

Significant at $p<0.05$ level; R^2=0.8994

Table 4. Regression analysis for acquired model

Source	Coefficients	Standard Error	t Stat	p-value	Characteristics
Constant	45.19	0.52	86.13	0.000	Significant
x_1	0.26	0.32	0.80	0.463	Not significant
x_2	-0.32	0.32	-1.00	0.362	Not significant
x_3	1.32	0.32	4.10	0.009	Significant
x_1^2	1.95	0.47	4.12	0.009	Significant
x_2^2	-0.09	0.47	-0.20	0.850	Not significant
x_3^2	-1.03	0.47	-2.18	0.081	Not significant
x_1x_2	-0.06	0.45	-0.14	0.893	Not significant
x_1x_3	-0.52	0.45	-1.14	0.306	Not significant
x_2x_3	-0.56	0.45	-1.24	0.272	Not significant

The ANOVA and regression analysis of the quadratic models is presented in Table 3 and 4. The coefficient of determination (R^2) value at 0.8994 and p-value of model<0.05, indicated that the model was appropriate to describe the phenomenon. Meanwhile, the result of model evaluation gave relation between %cellulose and three variables as shown in Equation 2. Positive coefficients indicate a linear increase in %cellulose value, while negative ones indicate a linear decrease. Overall, linear term of NaOH concentration and quadratic term of treatment time are found as significant variables as shown in Table 3.

$$Y=45.19+0.26x_1-0.32x_2+1.32x_3+1.95x_1^2-0.09x_2^2-1.03x_3^2-0.06x_1x_2-0.52x_1x_3-0.56x_2x_3 \quad (2)$$

3.2. Effects of Process Variables at Cellulose Content

The effects of each variable at selected cellulose contain were studied in this study. The 3-D response surface graphics are shown in Figure 1, 2 and 3. Each graphic shows the effects of pairing variables at observed range while another variable is fixed in zero levels. The response value was calculated based on Equation 2.

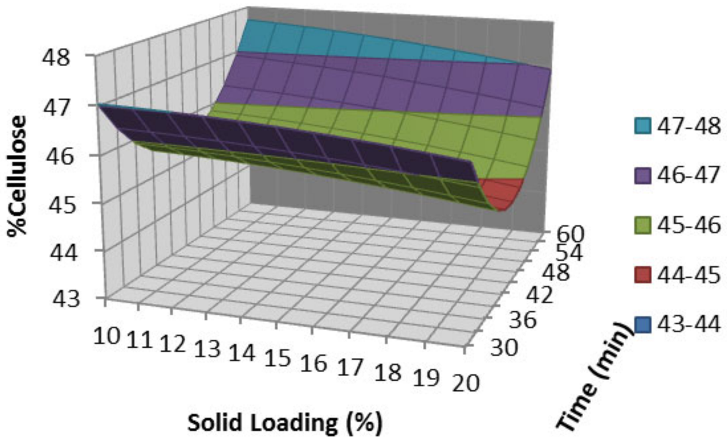

Figure 1. Effect of interaction between solid loading and time

Figure 1 shows the effects of solid loading, time and their interactions with the cellulose content. The cellulose content slightly increased as solid loading decreased. The trend was in line with Equation 2 which the coefficient of solid loading is in a negative value, means has negative effect on cellulose content. Trend of treatment time was different with solid loading. It has a negative peak which means quadratic term has important role. This phenomenon was in line with the regression analysis results in Table 4 which quadratic term of time (x_1^2) has a significant effect. Meanwhile, the interaction between solid loading and time was not significant and was in line with the previous result conducted by Velmurugan and Muthukumar [10].

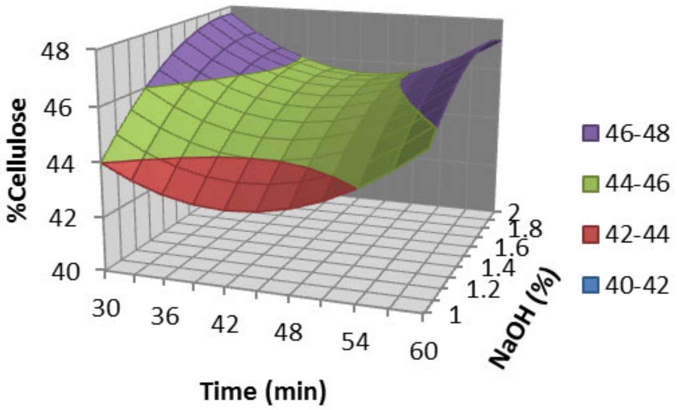

Figure 2. Effect of interaction between time and NaOH concentration

The effects of interaction between time and NaOH concentration is presented in Figure 2. As mentioned before, the time has significant at quadratic term. Meanwhile, NaOH concentration has a positive coefficient which means the increasing of cellulose content increases in line with NaOH concentration increase. This result was supported by the previous studies [12-13]. The insignificant interaction between time and NaOH concentration based on Table 4 is similar with previous results by Velmuragan and Muthukumar [10].

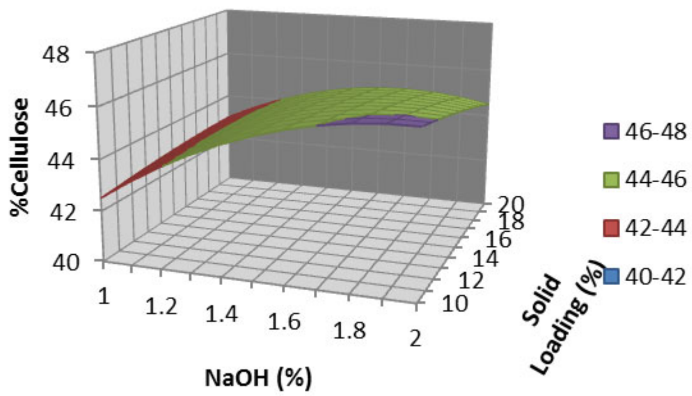

Figure 3. Effect of interaction between NaOH concentration and solid loading

The Interactive effect was found in the interaction between NaOH concentration and solid loading. Figure 3 shows a positive peak in the range of 1.6-2% NaOH concentration and 10-12% solid loading. The low value of solid loading and high value of NaOH concentration means the ratio between NaOH and MLT is high, thus the probability NaOH to react with lignin in MLT become higher. Therefore, decreasing of solid loading and increasing of NaOH concentration was the key factors to optimize cellulose content value.

The prediction of optimum condition for the maximum value of cellulose content was conducted by Solver program in Microsoft Excel. Range value of the optimum condition was set based on experiment value i.e. 30-60 minutes of time, 10-20% of solid loading and 1-2% of NaOH concentration. Maximum predicted the value of cellulose content was 48.14% at a condition of 60 minutes, 10% solid loading and 1.83% NaOH concentration.

4. Conclusion

Utilization of Box-Behnken design (BBD) for optimization of cellulose isolation from *Melaleuca leucadendron* twigs (MLT) has been studied. The Quadratic model has significant conformity between experimental and predicted value with a coefficient of determination value (R^2) at 0.8994 and $p<0.05$. Linear term of NaOH concentration and quadratic term of time was found as significant variables. The predicted optimum condition was found at the condition of 60 minutes, 10% solid loading and 1.83% NaOH concentration with a result of 48.14% cellulose content. Overall, BBD could be adequate to optimize the process of cellulose isolation from MLT.

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