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Original Article

Statistical optimization for alkali pretreatment conditions of narrow-leaf cattail by response surface methodology

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Abstract

Response surface methodology with central composite design was applied to optimize alkali pretreatment of narrow-leaf cattail (*Typha angustifolia)*. Joint effects of three independent variables; NaOH concentration (1-5%), temperature (60-100 ºC), and reaction time (30-150 min), were investigated to evaluate the increase in and the improvement of cellulosic components contained in the raw material after pretreatment. The combined optimum condition based on the cellulosic content obtained from this study is: a concentration of 5% NaOH, a reaction time of 120 min, and a temperature of 100 ºC. This result has been analyzed employing ANOVA with a second order polynomial equation. The model was found to be significant and was able to predict accurately the response of strength at less than 5% error. Under this combined optimal condition, the desirable cellulosic content in the sample increased from 38.5 to 68.3%, while the unfavorable hemicellulosic content decreased from 37.6 to 7.3%.

Keywords: response surface methodology, central composite design, narrow leaf cattail, sodium hydroxide, cellulosic pretreatment

1. Introduction

Ethanol or Ethyl alcohol, a volatile, flammable, and colorless liquid, is one of the most interested alternative fuels at present. Development and production of this fuel has been steadily increasing and its use has been promoted worldwide. Ethanol can be produced synthetically from petroleum or by microbial fermentation of sugar from biomass materials. Ethanol fuel production derived from biomass resources decreases petroleum oil requirements, and hence it helps to reduce pollution and decreases the problem of global warming. Ethanol, an oxygenated fuel that contains 35% of oxygen, can reduce the amount of particulate matters and NOx emissions from combustions. Moreover, it has opened up an opportunity in the adjustment of agro-plant production structure into an extension of plant production of economic value.

Economically feasible production of ethanol bio-fuel in Thailand has been exclusively derived from food crops that possess high sugar and starch. The use of arable land for fuel rather than for food production, and the use of a food source for fuel rather than as food, has created issues in prices and availability of traditional foods and feed. It has been reported that a more sustainable solution would be to use cellulosic feedstock (Zhang *et al*., 2010).

Lignocellulose, a common biopolymer, is considered one of the most important biomass resources for ethanol fuel production (Zhang and Cai, 2008). Lignocellulosic biomass raw materials are abundantly found in nature; be it from hardwood, softwood, grasses, plant, or agricultural residues. Lignocellulosic material contains mainly cellulose (35-50%), hemicellulose (20-35%), and lignin (10-25%). The composition varies in dependence on the type of substrate and the processing (Schacht *et al*., 2008). Cellulose in plants can be

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converted to ethanol by breaking it down into glucose follow by fermentation. Ethanol produced from lignocellulose biomass is extensively accepted as a substitute or as an additive to fossil fuel due to its negligible impact on the environment (Nlewem and Thrash, 2010). Cellulosic ethanol has a better environmental profile than starch-based ethanol, with a 90% reduction in carbon emission over gasoline as compared to 29% reduction using starch-based ethanol (Suda *et al*., 2009). Processing of cellulosic biomass into ethanol is conducted in four consecutive steps: pretreatment, hydrolysis, fermentation and dehydration (Faga *et al*., 2010).

Narrow-leaf cattail (*Typha angustifolia*) is a common wetland plant in South East Asia. Cattail contains approximately 6% protein and 50% total digestible nutrients when it is young; these are lower as it matures. In the form of lignocellulosic, cattail contains approx. 47.6% cellulose and 21.9% lignin (Zhang *et al*., 2010). Cattail utilizes solar energy effectively and grows rapidly. The nature of its rapid growth creates undesirable effect on arable lands from its encroachment. However, its rapid growth produces a large amount of biomass, and since cattail is mainly composed of lignocellulose it is also a potential renewable resource for production of ethanol fuel (Gajalakshmi *et al*., 2001; Hu and Yu, 2006).

A pretreatment process and optimization for the narrow-leaf cattail is needed in order to achieve a higher cellulose fiber content product. One-factor-at-a-time optimization method is simple, but this method often fails in the search for an optimum region because joint effects of factors are not considered. Response surface methodology (RSM) is a statistical model widely used to study the aggregated effect of several variables and to seek optimum conditions for a multivariable system (Kim *et al*., 2008). RSM is also a collection of mathematical techniques useful for developing, improving, and optimizing processes in which a response of interest is influenced simultaneously by several variables, and the objective can be optimized for the best response. In addition to analyzing the effects of independent variables, the experimental methodology generates a mathematical model which describes the chemical or biological process (Baş and Boyacı, 2007). In this study, RSM based on central composite design (CCD), a design of experimental technique, was employed to identify the optimum NaOH pretreatment condition for maximizing the production of the cellulose fiber from narrow-leaf cattail. The experiments were carried out at five levels in respect to an optimal criterion. Three independent variables were chosen as independent variables, namely, NaOH concentration, temperature, and reaction time.

2. Materials and Method

2.1 Biomass preparation

Narrow-leaf cattail was gathered from Ranod, Songkhla Province, Thailand. The cattail, having a moisture content of 70-80%, was chopped then washed to remove extraneous matters (see Figure 1). It was oven-dried at 70°C for 3 days, grounded with a hammer mill and sieved to a mesh size of 1 mm. This was stored in sealed plastic bags at room temperature for further use. The stock material taken out as sample had an average total solid content of 92% dry matter; its moisture content and the ash content being analyzed by a method developed by the National Renewable Energy Laboratory's (NREL) using LAP #001 procedure and LAP # 005 procedure, respectively, similar to that conducted in the experiment by Zhang *et al*. (2011). Structural compositions of the cattail raw material obtained from analyses according to AOAC standard method (AOAC 973.18 1990) were: 38.5% celluloses, 37.6% hemicelluloses, 12.8% lignin, and 11.1% ash.

2.2 Pretreatment

Dry ground cattail was pretreated with concentrations of 1, 2, 3, 4, and 5% (w/v) of sodium hydroxide (NaOH). Individual sample was soaked in each NaOH solution in a screw-capped Erlenmeyer flask at a specimen loading of 10% (w/v) and heated at temperatures of 60, 70, 80, 90, and 100°C in a water bath at reaction times of 30, 60, 90, 120, and 150 min. After the pretreatment, each sample was recovered by filtration and washed with water until it reaches a neutralized status. This was then dried at 105°C for 24 hours and stored in a small sealed plastic bag at room temperature for use in the quantitative analysis. The sample was also analyzed using a scanning electron microscope (SEM; JSM 5800 LV, JEOL) to investigate its micrographs to determine its composition consistency characteristics after the pretreatment.

2.3 Experimental design

The response surface methodology that consists of a group of mathematical and statistical techniques can be used to define the relationships between the response and the independent variables for the effect of independent variables, alone or in combination, on the process. The relationship between the response and the input variables, Equation 1, is given by:

$$y = f(x_1, x_2, x_3, ..., x_n) + \varepsilon \tag{1}$$

where y is the response; f is the unknown function of the response; x_1, x_2, x_3,…, x_n are the independent variables, also called factors; n is the number of independent variables; and

Figure 1. Narrow-leaf cattail material: chopped and dried (left), milled and sieved (right).

å is the statistical error that represents other sources of variability not accounted for by *f*. It is generally assumed that ε is zero for this type of set up in the experiment, as with the works of Baş and Boyacı (2007) and Baboukani *et al.* (2012).

In this research, RSM was used to optimize the pretreatment reaction parameters for determination of cellulose and hemicellulose contents (responses) of narrow-leaf cattail. These experiments were based on a central composite design with a quadratic model employed to study the combined effect of three independent variables, i.e. NaOH concentration, temperature and the pretreated time. Each variable was varied at five levels (-α, -1, 0, 1, +α), as shown in Table 1. The coded and actual values of independent variables can be calculated from Equation 2, thus:

$$x_i = \frac{X_i - X_0}{\Delta X} \quad (2)$$

where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_0 is the value of X_i at the central point, and $ÄX$ is the step change (see Jabasingh and Nachiyar, 2011; Mohamad *et al*., 2011). A total of 17 experimentations had been carried out in random order according to CCD configuration.

A second order polynomial quadratic equation was fitted to evaluate the main effect and interaction of each independent variable to the response, similar to that employed by Karuppaiya *et al*. (2010), Oberoi *et al*. (2011), and Kang *et al*. (2012) using Equation 3, thus:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \sum_{j}^{k} \beta_{ij} x_i x_j \quad (3)$$

where Y is the response of cellulosic or hemicellulosic contents; β_0 is the constant coefficient; β_i, β_{ii}, β_{ij} are coefficients for the linear, quadratic and interaction effects, respectively; and x_i is the independent variable factor, representing either the NaOH concentration (g/l), or the temperature (°C), or the reaction time (min).

2.4 Statistical analysis

Statistical software package, Design-Expert 8.0.7.1 Trial Version (Stat-Ease, Inc., Minneapolis, MN, U.S.A.), was used for regression analysis of the experimental data and to plot the response surfaces. Analysis of variance (ANOVA) was used to investigate the statistical parameters.

3. Results and Discussion

3.1 SEM analysis

Figure 2 (a), (b) and (c) show, respectively, the SEM micrographs of the narrow-leaf cattail, for the untreated specimen, after pretreatment with 1% NaOH, and after pretreatment with 5% NaOH at temperature of 80°C and reaction time of 90 min. The untreated specimen exhibit higher order and compact fiber structures, while both the pretreated samples reveal higher degrees of porosity and external surface area. The pretreatment process thus could effectively decrease crystallinity of the cattail fiber similar to that reported for corn leaf by Donghai *et al*. (2006). This indicates that a large fraction of lignin and hemicellulose can be removed by alkali pretreatment. A linear reduction in weight

Table 1. Code and actual level of the three independent variables for the design of pretreatment experiment used in the CCD.

Independent Variable	Code	Code and actual factor level				
		-2	-1	0	+1	+2
NaOH concentration (% w/v)	A	1	2	3	4	5
Temperature (°C)	B	60	70	80	90	100
Reaction time (min)	C	30	60	90	120	150

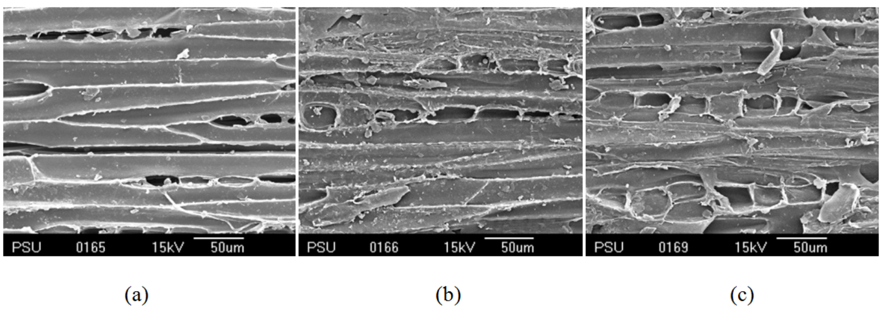


Figure 2. SEM micrographs of narrow-leaf cattail: (a) untreated, (b) 1% NaOH pretreated, and (c) 5% NaOH pretreated, at a temperature of 80ºC and reaction time of 90 min.

change, possibly due to roughening of the cattail's narrow leaf surface by formation of cavities and stripping off of the wall, as noticed in bagasse by Jabasingh and Nachiyar (2011), was observed. The surface roughness was found to be increasing as the intensity of alkali concentration increases.

3.2 RSM analysis

The experimental design runs with corresponding observed responses and predicted values for production of cellulosic and hemicellulosic fibers are presented in Table 2. These runs were randomized by the Design Expert's statistical software.

Based on the experimental responses the quantity of cellulosic produced by using NaOH pretreatments on the experimental design ranged from 45.97% to 60.37%. Run No.4 and Run No. 8 yielded the maximum and the minimum cellulosic content, respectively. Hemicellulosic contents produced by the experimental design ranged from 21.51% to 35.55%, with maximum and minimum values obtaining from Run No.8 and Run No.3, respectively. Lignin content results in sample fibers for the experiment, shown also in Table 2, though could not be suitably fitted by RSM analysis equation, generally were observed to have been slightly to moderately reduced after pretreatments with NaOH. Fibers pretreated with NaOH are softer than those untreated. As reported in Sun and Cheng (2002), dilute NaOH treatment of lignocellulosic materials causes swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure. SEM micrographs of the sample fibers that show higher degrees of porosity after treatments also infer reductions of lignin content after the pretreatment process. The decrease in lignin content after pretreatments has been calculated to be up to 31.21% from that of the untreated cattail fibers.

The experimental data were analyzed using Design Expert statistical software to yield results from analysis of variance, resulting regression coefficients and regression equation. The resulted response function in terms of coded factors A, B and C, together with their corresponding coefficients, and after elimination of other insignificant terms that was derived to predict the cellulosic content is shown in Equation 4.

$$\text{Cellulosic content} = 54.41 + 5.48A + 3.31B + 0.66C + 4.46AB + 3.34BC - 2.81A^2 + 2.26B^2 - 3.31C^2 \quad (4)$$

where A is the concentration of NaOH, B is the temperature involved in the process, and C is the time of the pretreatment process. In the equation, A, B, and C are the main effects while AB, BC are the interactions, and A^2, B^2 and C^2 are the quadratic terms involved in the process.

The regression coefficients for the coded factors are tabulated in Table 3. Statistical significance of the model equation to the fitted model was evaluated by F-test in the ANOVA. The model's large F value of 97.60 and the corresponding value of probability (P)>F (<0.0001) implies that

Table 2. CCD with the observed and predicted values of the experimental response.

Run	A	B	C	Observed Cellulosic (%)	Predicted Cellulosic (%)	Observed Hemicellulosic (%)	Predicted Hemicellulosic (%)	Observed Lignin (%)
1	0.000	-2.000	0.000	52.84	53.36	29.51	30.08	10.84
2	0.000	0.000	-2.000	50.55	50.45	31.37	31.17	11.21
3	1.000	1.000	1.000	60.11	60.12	21.51	20.62	10.28
4	0.000	2.000	0.000	60.37	59.99	21.70	21.75	10.29
5	2.000	0.000	0.000	57.11	57.08	23.43	24.13	12.44
6	1.000	-1.000	1.000	53.24	52.91	30.52	29.62	8.99
7	0.000	0.000	0.000	53.82	54.41	29.59	29.55	9.83
8	-2.000	0.000	0.000	45.97	46.13	35.55	34.96	12.33
9	-1.000	1.000	-1.000	50.00	50.09	32.84	32.49	10.56
10	-1.000	-1.000	-1.000	50.93	50.68	32.19	31.83	10.93
11	0.000	0.000	2.000	51.52	51.76	28.31	27.92	11.96
12	1.000	1.000	-1.000	57.41	57.80	23.79	23.69	10.59
13	-1.000	1.000	1.000	52.28	52.41	28.77	29.42	11.91
14	-1.000	-1.000	1.000	50.09	49.66	31.51	31.64	8.83
15	1.000	-1.000	-1.000	54.08	53.92	30.41	29.80	8.90
16	0.000	0.000	0.000	54.03	54.41	29.12	29.55	9.36
17	0.000	0.000	0.000	55.25	54.41	27.61	29.55	9.72

Note: A represents NaOH concentration; B, temperature; and C, reaction time. All in coded values.

Table 3. Analysis of variance for the regression equation of cellulosic yield.

Source	df	Coefficient Estimate	Standard Error	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	8	54.41	0.29	222.39	27.80	97.60	< 0.0001
A-NaOH	1	5.48	0.27	119.93	119.93	421.08	< 0.0001
B-Temperature	1	3.31	0.27	43.88	43.88	154.08	< 0.0001
C-Time	1	0.66	0.27	1.72	1.72	6.04	0.0395
AB	1	4.46	0.75	9.95	9.95	34.94	0.0004
BC	1	3.34	0.75	5.57	5.57	19.55	0.0022
A^2	1	-2.81	0.49	9.53	9.53	33.45	0.0004
B^2	1	2.26	0.49	6.18	6.18	21.71	0.0016
C^2	1	-3.31	0.49	13.29	13.29	46.65	0.0001
Residual	8			2.28	0.28		
Lack of Fit	6			1.08	0.18	0.30	0.8949
Pure Error	2			1.20	0.60		
Cor Total	16			224.67			

$R^2 = 0.9899$ Adjusted $R^2 = 0.9797$

the model is significant. Normally, a p-value of less than 0.05 will indicate that a model is statistically valid and acceptable. The linear terms A and B were found to be the more significant factors in the regression, having p-values of <0.0001, comparing to C with a p-value of 0.0395. The interaction terms and the quadratic coefficient terms AB, BC, A^2, B^2 and C^2 were also found to be significant factors. In this model, the high R^2 value of 0.9899 and also that of the adjusted R^2 of 0.9797 indicate a close agreement between the experimental results and the theoretical values predicted by the model, the model is capable of explaining 98.99% of the variation in the response. The high p-value for the lack of fit test indicates the high level of insignificance of the error, and further confirms that the model fits suitably the data.

For the hemicellulosic content equation, this is described, after eliminating all insignificant terms, as a simultaneous function of the three independent variables, presented in Equation 5.

$$\text{Hemicellulosic content} = 29.55 - 5.41A - 4.17B - 1.63C - 6.77AB - 2.89BC - 3.63B^2 \quad (5)$$

as with earlier detailed, A denotes concentration of NaOH, B the temperature, and C the time of pretreatment process. A, B, C are the main effects; AB, BC the interactions; and B^2 the quadratic term involved in the process.

Again, statistical significance was evaluated by F-test and the results are tabulated in Table 4. The model yields an F value of 51.12 and a probability value (P)> F (<0.0001), that implies the significance of the model. The linear terms A and B are significant factors with p-values less than 0.0001, and are relatively more significant than C. However, C, with p-value of 0.0045, is still well within the significance limit. The interaction and quadratic coefficient terms, AB, BC and B^2 are also of significance with p-values less than 0.05. The Coefficient of determination (R^2) for the model is 0.9684, and the adjusted R^2 0.9495, indicating that the model is good and is capable of explaining 96.84% of the variation in response. The high p-value for the lack of fit test reinforces once again the insignificance of the error in the model.

3.3 Model analysis

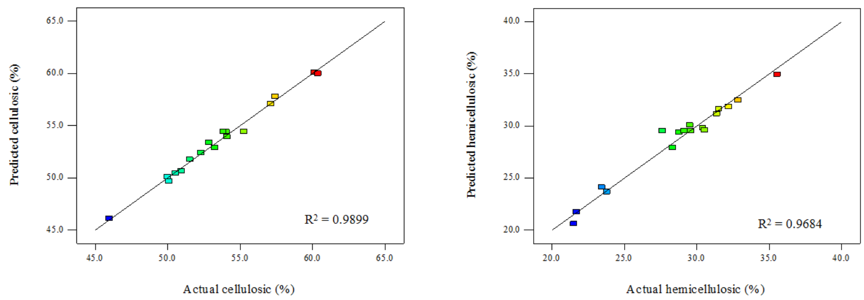
Figure 3 depicts the plots of actual response values obtained from the experimental results versus the predicted response values based on the quadratic model equations for cellulosic content (A) and hemicellulosic content (B). Similar trend of close correlations at high significance can be seen from both plots.

Figure 4 and 5 are plots of two-dimensional contours and three-dimensional response surfaces after pretreatments. These plots were made to illustrate results from the model equations in the investigations of interactions from two chosen independent variables, while the third variable is kept constant at its central point value, on the cellulosic content in Figure 4, and the hemicellulosic content in Figure 5, and to visualize the optimal results from these variables for a desired response. In both figures, the two independent variables in (A) and (B) are the NaOH concentration and the temperature, while in (C) and (D) these are the temperature and the time. The 2D contours and the 3D response surface plots in the case of cellulosic content were derived using Equation 4, and for the case of hemicellulose, Equation 5.

In Figure 4, the fixed central point value for the plots in (A) and (B) is the time of 80 min, and the fixed value in (C) and (D) for NaOH concentration is 3%. It can be seen that

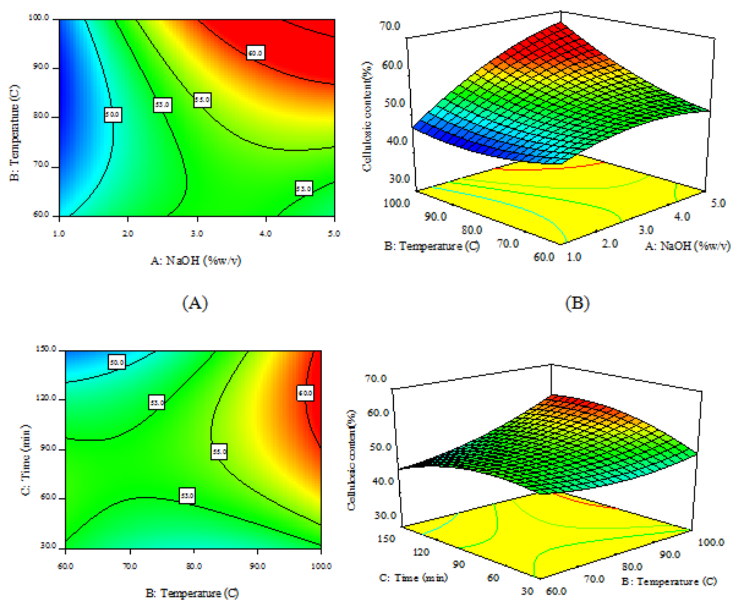
Table 4. Analysis of variance for the regression equation of hemicellulosic yield.

Source	df	Coefficient Estimate	Standard Error	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	6	29.55	0.27	244.99	40.83	51.12	< 0.0001
A-NaOH	1	-5.41	0.45	117.27	117.27	146.83	< 0.0001
B-Temperature	1	-4.17	0.45	69.44	69.44	86.94	< 0.0001
C-Time	1	-1.63	0.45	10.61	10.61	13.29	0.0045
AB	1	-6.77	1.26	22.94	22.94	28.73	0.0003
BC	1	-2.89	1.26	4.19	4.19	5.24	0.0451
B^2	1	-3.63	0.72	20.54	20.54	25.72	0.0005
Residual	10			7.99	0.80		
Lack of Fit	8			5.84	0.73	0.68	0.7145
Pure Error	2			2.15	1.07		
Cor Total	16			252.97			
$R^2 = 0.9684$ Adjusted $R^2 = 0.9495$							



(A) (B)

Figure 3. Experimental result versus mathematical model prediction: (A) cellulosic content, and (B) hemicellulosic content.



(A) (B)

(C) (D)

Figure 4. Contour and 3D response surface plot showing interactions between variables affecting production of cellulosic content: (A), (B) interaction of NaOH concentration vs. temperature; (C), (D) interaction of temperature vs. time.

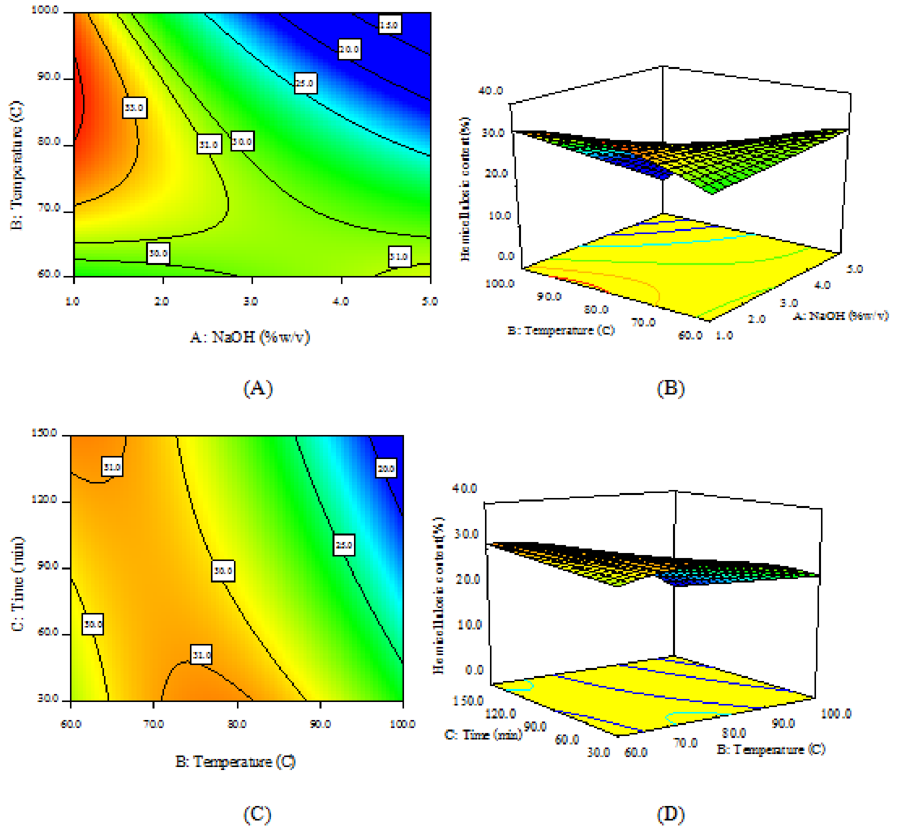


Figure 5. Contour and 3D response surface plot, showing interactions between variables affecting production of hemicellulosic content: (A), (B) interaction of NaOH concentration vs. temperature; (C), (D) interaction of temperature vs. time.

interactions between temperature versus NaOH concentration, as well as that between time versus temperature, significantly influence the amount of cellulosic content produced.

When all illustrations are visualized together as an overall, it is evident that the cellulosic content increases and reaches an optimal level when the NaOH concentration is 5% w/v, at a temperature of 100°C under a pretreatment time of 120 min, to produce a maximum cellulosic content of 68.3%.

In Figure 5, the fixed central point values for all hemicellulosic content plots shown are correspondingly the same as detailed in Figure 4. Lowering of hemicellulosic content can be achieved with increasing NaOH concentration together with increasing temperature, as illustrated in Figure 5 (A) and (B). In (C) and (D), it can be seen that at the desirable higher temperature, particularly at 100°C, the hemicellulosic content decreases with increasing pretreatment time. At 5% NaOH concentration, 100°C temperature, and 120 min pretreatment time, as established in the maximum derivation of cellulosic content, the amount of hemicellulosic content level is 7.3%.

4. Conclusions

Ethanol is an environmental-friendly alternative fuel and its derivations are mostly from raw agricultural products. Narrow-leaf cattail is a fast-growing wild plant that has a potential to be used as a raw material. However, higher cellulosic content for good production of ethanol from the plant is needed. Thus, an appropriate pretreatment process, together with its optimal condition, would be useful.

In this research, response surface methodology (RSM) had been used for optimization of pretreatment conditions of the narrow-leaf cattail. The highest cellulosic content from predicted conditions obtained using experimental result data are: NaOH concentration of 5% w/v, temperature of 100°C, and residence pretreatment time of 120 min. Under the combined conditions, the desirable cellulosic content increased from 38.5% to 68.3%, an additional increase of 29.8%, and the undesirable hemicellulose decreased from 37.6% to 7.3%, an additional decrease of 30.3%. The process had also favorably reduced the amount of lignin fiber. This optimization had been verified to be of significant model-experiment relationships; with an R^2 value of 0.9899 for the model prediction of cellulosic content, and an R^2 value of 0.9684 for that of the hemicellulosic content.

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