2012 International Conference on Future Environment and Energy
IPCBEE vol.28(2012) © (2012)IACSIT Press, Singapoore

Optimization of Sodium Hydroxide Pretreatment of Canola Agricultural Residues for Fermentable Sugar Production using Statistical Method

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Abstract. In our previous work, sodium hydroxide solution (1~20%) was utilized for alkaline reagent to increase the crystallinity of canola agricultural residues pretreatment. In this study, significant factors for efficient sodium hydroxide pretreatment on canola residues were optimized by using the response surface method (RSM). Based on the fundamental experiments, the real values of factors at the center (0) were determined as follows; 70 ℃ of temperature, 10% of sodium hydroxide concentration and 18 h of reaction time in the experiment design using central composition rotatable design (CCRD). A statistical model predicted that enzyme digestibility (theoretical maximum yields of sugar) and the removal yield of lignin were 59.34% and 36.38%, respectively at the following optimized reaction conditions: 73.39 ℃ of temperature, 8.95% of ammonia concentration and 21.85 h of reaction time. Saccharification was performed at 50 ℃ with Celluclast (cellulase) and Novozyme 188 (β-glucosidase) as biocatalysts in an enzyme loading test. Finally, conversion of glucose was 90.17% at the optimized conditions.

Keywords: Optimization, Response surface method, Sodium hydroxide pretreatment, Canola

1. Introduction

Production of bioenergy (biofuel) from biomass is one way to reduce both consumption of crude oil and environmental pollution. Currently, primarily used biomass were food resources such as sugarcane, corn, potato and vegetable oils for biofuel production. However, it caused several problems; the rise in food and feed price, increasing starvation, etc. and alternative biomass became necessary. Lignocellulosic biomass, such as agricultural residues (corn stover and wheat straw), wood and energy crops, is an attractive material for bioethanol fuel production since it is the most abundant reproducible resource on the Earth. Processing of lignocellulosics to bioethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation and product separation.

Alkaline pretreatment processes utilize lower temperatures and pressures compared to other pretreatment technologies. But, pretreatment time is measured in terms of hours or days rather than minutes or seconds. Unlike acidcatalyzed pretreatments, a limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the biomass by the pretreatment reactions. The characteristic of alkaline pretreatment is that it can remove the lignin without having big effects on other components.

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Response surface methodology (RSM) is a useful statistical technique which has been applied in research into complex variable processes. It employs multiple regression and correlation analyses as tools to assess the effects of two or more independent factors on the dependent variables. Its principal advantage is the reduced number of experimental runs required to generate sufficient information for a statistically acceptable result. Response surface methodology has been successfully applied in the study and optimization of fermentable sugar production with canola agricultural residues. [1-4]

In this study, canola agricultural residues were selected for biomass feedstock and focused on its alkaline pretreatment. We utilized sodium hydroxide as an alkaline catalyst and response surface methodology was used for optimize the reaction conditions.

2. Materials and Methods

2.1. Substrates, reagents and enzymes

Canola agricultural residues from Chungnam area in Korea used in this study was a kind gift from Korea Institute of Energy Research. The feedstock was ground, sieved, and the fraction within 18–40 mesh was used. Sodium hydroxide (NaOH), Cellulase (Celluclast 1.5L) and -Glucosidase (Novozyme 188) were purchased from Sigma (Sigma-Aldrich Co., USA).

2.2. Experimental design

The sodium hydroxide pretreatment experiments were performed in 250 ml pyrex sample bottle (Duran, Germany). In these experiments, Canola agricultural residues samples were soaked in specified concentration of sodium hydroxide with 1/6 solid/ liquid ratio and kept in a shaking water base (120 rpm).

In order to optimize the CCRD experimental design, a five-level-three-factors CCRD was adopted in this study, requiring 20 experiments, which included eight factorial points, six axial points, and six central points to provide information regarding the interior of the experiment region, allowing for the evaluation of the curvature. The parameters, which were selected for the study of enzymatic digestibility, and their respective levels, were as follows: reaction temperature (53.18 86.82 ℃), reaction time (7.91−28.09 h) and NaOH concentration (1.59-18.41 %). Table 1 shows the coded and uncoded independent factors (Xi), levels, and experimental design.

Table 1. Factors and their levels for central composite design

Variable	**Symbol**	**Coded factor levels**				
		-1.682	-1	0	1	1.682
Temperature ( )	X_1	53.18	60	70	80	86.82
Reaction time (h)	X_2	7.91	12	18	24	28.09
NaOH concentration (%)	X_3	1.59	5	10	15	18.41

2.3. Analytical method

Composition analysis of treated and untreated biomass samples was carried out according to the NREL laboratory analytical procedures. Carbohydrate analysis of the biomass was carried out in duplicate. This analysis was carried out for pretreatment liquids before and after separation of lignin. For dilute acid hydrolysis, the biomass mixture with a diluted acid solution of sulfuric acid (72%, w/w) was heated to 121°C in an autoclave. After heating and cooling, the mixture was neutralized with calcium carbonate. The supernatant of the biomass composition was then analyzed by high performance liquid chromatography (HPLC) with an Aminex HPX-87H ion exclusion column (BIO RAD). HPLC analytical conditions were as follows; column oven and refractive index detector temperature were 50℃ , 0.005 N H_2SO_4 mobile phase at a flow rate of 0.8 mL/min. [5-6]

2.4. Statistical analysis

Experimental data were analyzed via response surface methodology, in order to fit the following second order polynomial equation generated by Design-Expert 7 software (Stat-Ease Inc., USA). Second-order coefficients were generated via regression. The response was initially fitted to the factors via multiple regression. The quality of the fit of the model was evaluated using the coefficients of determination and analysis of variance. The quadratic response surface model was fitted to the following equation:

$$Y = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} X_i + \sum_{i=1}^{3} \beta_{kii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} X_i X_j \quad (1)$$

in which Y is the response factor (enzymatic digestibility), x_i is the ith independent factor, β_0 is the intercept, β_i is the first order model coefficient, β_{ii} is the quadratic coefficient for the factor i, and β_{ij} is the linear model coefficient for the interaction between factors i and j. [7]

2.5. Enzymatic digestibility

Enzymatic digestibility was determined by the NREL laboratory analytical procedure. The solid loading applied in the test was 1 g of glucan/100 ml of total reactant volume. The total solid loading in the digestibility test thus varied depending on the glucan content of the biomass sample. Hydrolysis reaction was carried out at 50 ℃, pH 4.8 (0.05 M sodium citrate buffer), 200 rpm in a shaking incubator. The digestibility was defined as the percentage glucose released after 72 h of incubation on the basis of the theoretical maximum in pretreated biomass. In all the enzymatic hydrolysis experiments, enzyme loading of 60 FPU cellulase with 30 CBU β-glucosidase/g glucan was applied. [5-6]

3. Results and Discussion

3.1. Optimization of reaction conditions by response surface methodology

Table 1 lists the experimental factor settings and results on the basis of the experimental design. All 20 of the designed experiments were conducted, and the results were analyzed via multiple regression. The coefficients of a full model were evaluated via regression analysis and tested for significance. Finally, the best fitting model was determined via regression. This showed that three linear coefficients (X_1, X_2, X_3), three quadratic coefficients (X_1^2, X_2^2, X_3^2), and three cross-product coefficients (X_1X_2, X_1X_3, X_2X_3) were significant (Table 1 and 2).

The ANOVA for the response surface quadratic model is provided in Table 3. The coefficients of the response surface model as provided by Eq. (1) were also evaluated. A p-value showed that all of the linear coefficients were more highly significant than their quadratic and cross-product terms. However, in order to minimize error, all of the coefficients were considered in the design. According to the ANOVA analysis of factors, we noted a low lack of fit. This indicates that the model does indeed represent the actual relationships of reaction parameters, which are well within the selected ranges (Table 3).

The final estimative response model equation (based on the actual value) by which the estimated enzymatic digestibility was as follows:

$$Y = 58.27 + 2.59X_1 + 1.32X_2 + 2.99X_3 - 0.25X_1X_2 + 0.29X_1X_3 + 0.26X_2X_3 - 0.25X_1^2 - 0.48X_2^2 - 2.00X_3^2 \quad (2)$$

in which Y is the response factor, enzymatic digestibility (%). X_1, X_2, and X_3 are the values of the independent factors, reaction temperature (℃), reaction time (h), NaOH concentration (%) respectively.

The optimal values of the selected variables were obtained by solving the regression equation (Eq. (1)) using Design-Expert 7 software. The optimal conditions for sodium hydroxide pretreatment estimated by the model equation were as follows: X_1 = 79.39℃, X_2 = 8.95%, and X_3 = 21.85 h (Figure 1). The theoretical enzymatic digestibility under the above conditions was Y = 59.34%.

Table 2. Central composite rotatable second-order design, experimental and estimated data for five-level-three-factors response surface analysis

Std.	Run	Temperature X_1	Reaction time X_2	NaOH concentration X_3	Enzymatic digestibility (%)
1	1	-1	-1	-1	51.71
2	10	1	-1	-1	56.45
3	20	-1	1	-1	54.31
4	14	1	1	-1	59.38
5	3	-1	-1	1	53.57
6	13	1	-1	1	60.81
7	8	-1	1	1	58.54
8	4	1	1	1	63.45
9	5	-1.68	0	0	52.47
10	19	1.68	0	0	60.44
11	6	0	-1.68	0	57.08
12	7	0	1.68	0	60
13	11	0	0	-1.68	43.7
14	2	0	0	1.68	59.34
15	9	0	0	0	57.88
16	17	0	0	0	59.81
17	15	0	0	0	57.8
18	12	0	0	0	58.53
19	18	0	0	0	57.5
20	16	0	0	0	58.5

Table 3. ANOVA for response surface quadratic model analysis of variance table

Source	Sum of squares	DF	Mean square	F-Value	Prob > F
Model	303.767	9	33.752	7.423	0.0021
X_1	91.573	1	91.573	20.138	0.0012
X_2	23.859	1	23.859	5.247	0.0450
X_3	122.029	1	122.029	26.836	0.0004
X_{12}	0.500	1	0.500	0.110	0.7470
X_{13}	0.684	1	0.684	0.151	0.7062
X_{23}	0.541	1	0.541	0.119	0.7373
X_1^2	0.937	1	0.937	0.206	0.6596
X_2^2	3.351	1	3.351	0.737	0.4108
X_3^2	57.632	1	57.632	12.674	0.0052
Residual	45.472	10	4.547		
Lack of Fit	42.041	5	8.408	12.252	0.0078
Pure Error	3.431	5	0.686		
Cor. Total	349.239	19			

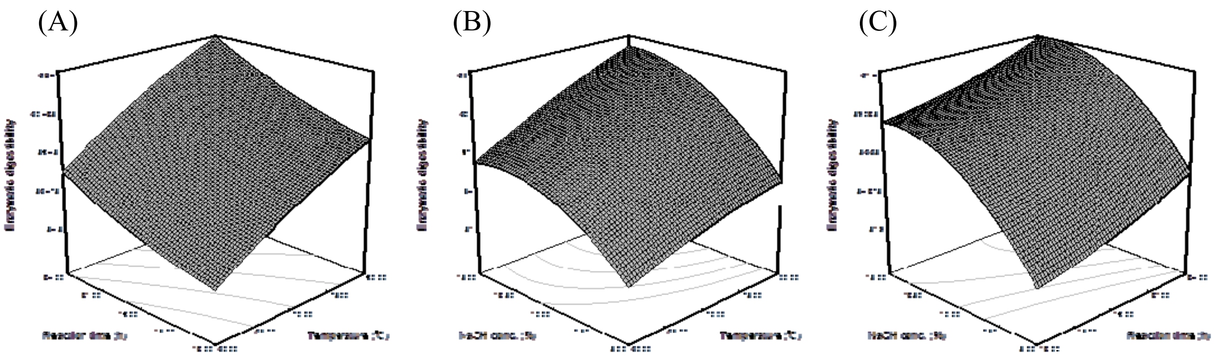

Figure 1. Response surface plots representing the effect of (A) temperature and reaction time, (B) temperature and NaOH concentration, (C) reaction time and NaOH concentration. Other factor is constant at zero levels.

3.2. Fermentable sugar yield

The sodium hydroxide pretreatment was performed on the evaluated condition (73.39 of temperature, 8.95% of ammonia concentration and 21.85 h of reaction time) and the composition was analyzed, also. The composition of non-pretreated canola residue (control) was determined to be 31% of glucan, 18.6% of XMG (xylan + mannan + galactan) and 20% of lignin. The glucan composition was increased 31 to 59% by optimized pretreatment (Figure 2). And the removal yield of lignin was 36.38% (data not shown). Finally, the glucose conversion by enzymatic hydrolysis was significantly increased 48 to 90%. This demonstrated that response surface methodology with appropriate experimental design can be effectively applied to the optimization of the pretreatment process. This may provide useful information regarding the development of economic and efficient.

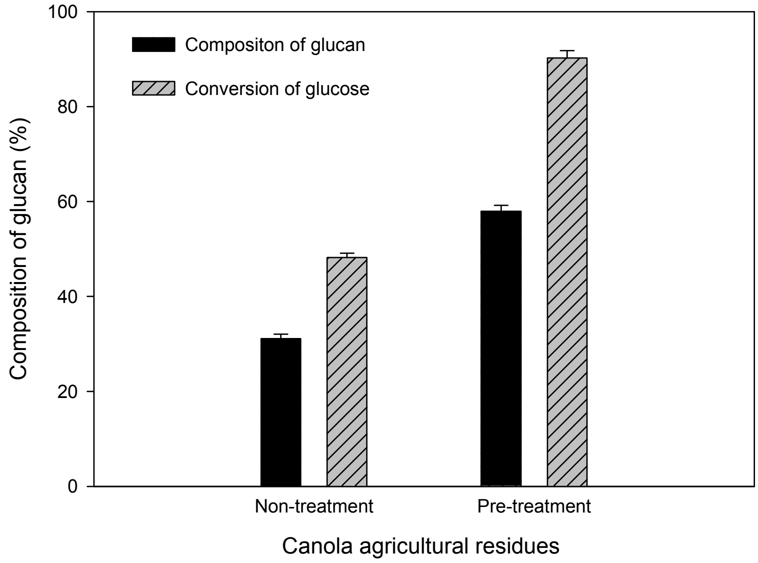

Figure 2. The glucan composition and conversion of canola agricultural residues

4. Acknowledgements

This Study was supported by Technology Development Program (309016-5) for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea and the Advanced Biomass R&D Center (ABC-2010-0029799) of Korea Grant funded by the Ministry of Education, Science and Technology.

5. References

[1] R. Gupta, T. H. Kim and Y. Y. Lee. Substrate dependency and effect of xylanase supplementation on enzymatic hydrolysis of ammonia-treated biomass. *Appl. Biochem. Biotechnol*. 2007, **148**;59–70.

[2] S. B. Kim, J. S. Kim, J. H. Lee, S. W. Kang, C. Park and S. W. Kim. Pretreatment of rice straw by proton beam irradiation for efficient enzyme digestibility. *Appl Biochem Biotechnol*. 2011, **164**;1183–1191

[3] S. B. Kim, J. H. Lee, K. K. Oh, S. J. Lee, J. Y. Lee, J. S. Kim and S. W. Kim. Dilute Acid Pretreatment of Barley Straw and Its Saccharification and Fermentation. *Biotechnology and Bioprocess Engineering*. 2011, **16**;725–732

[4] T. S. Jeong and K. K. Oh. Optimization of fermentable sugar production from rape straw through hydrothermal acid pretreatment. *Bioresource Technology*. 2011,**102**;9261-9266

[5] L. Brown, R Torget. Enzymatic Saccharification of Lignocellulosic Biomass. Laboratory Analytical Procedures No. 9, Chemical Analysis and Testing Task. *National Renewable Energy Laboratory*, 2004.

[6] R. Gupta and Y. Y. Lee. Investigation of biomass degradation mechanism in pretreatment of switchgrass by aqueous ammonia and sodium hydroxide. *Bioresource Technology*. 2010, **101**;8185 8191

[7] G. T. Jeong, H. S. Yang and D. H. Park. Optimization of transesterification of animal fat ester using response surface methodology. *Bioresource Technology*. 2009, **100**; 25 30