Optimization of Reducing Sugar Production from Acid Hydrolysis of Sugarcane Bagasse by Box Behnken Design

Nukrob Narkprasom
Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai, Thailand
Email: narkprasom@hotmail.com

Rittichai Assavarachan and Pairote Wongputtisin
Faculty of Engineering and Agro-Industry, Faculty of Science, Maejo University, Chiang Mai, Thailand
Email: rittichai.assawarachan@gmail.com, pairote@mju.ac.th

***Abstract*—Sugarcane bagasse is a low-cost and abundant of bio-resources in sugar mill industry. The cellulose of bagasse can be converted into reducing sugar that has been applied in food and biological process. Statistically based Box-Behken Design (BBD) was selected for the optimization of reducing sugar production from acid hydrolysis of sugarcane bagasse. Concentration of H_2SO_4, hydrolysis time, and the ratio of raw material to solvent were identified for significant effects on reducing sugar production. Response surface method by BBD was employed to develop a mathematical model for predicting the optimal condition of acid hydrolysis process. The value of R^2 (0.981) for the presented mathematical model indicates the high correlation between experimental and predicted values. The optimization of concentration of H_2SO_4, hydrolysis time, and ratio of raw material to solvent are 27.697 %w/v, 160 min, and 1:29.827 g/ml respectively, which under this condition, it could produced 14.808% of reducing sugar from sugarcane bagasse.**

***Index Terms*—sugarcane bagasse, reducing sugar, acid hydrolysis, Box-Behnken design.**

I. Introduction

Sugarcane bagasse is a lignocellulose from agro-industry, which provides a low cost material and bioresource for the production of fuels and chemicals. Furthermore, sugar mills generate an enormously bagasse at a rate about 135 per metric ton of sugarcane [1]. The solid residue of bagasse obtained after extraction of sugarcane juice is a good raw material because of its low cost and abundance which in the part to the present, bagasse has been usually burnt to generate a boiler for steam and electricity production in the sugar mill industry [2]. Lignocellulosic materials have three major components: cellulose, hemicellulose and lignin [3]. Bagasse is a rich material of cellulose that can be converted to glucose [4] which has been applied to be a carbon source in fermentation process [5]. However, lignocellulose is a rich source of not only cellulose, but also hemicellulose, represented by groups of pentose sugar such as xylose which it can be converted to xylitol, a functional sweetener with important properties like anticarcinogenicity and low calorie [6].

Lignin in lignocellulose was eradicated by pretreatment with alkaline of NaOH [7] while, hemicellulose and cellulose of sugarcane bagasse can be readily hydrolyzed to monosaccharide in kind of reducing sugars by dilute sulfuric acid [8]. However, the concentration of reducing sugar relative with many variables such as; % w/v of sulfuric acid, hydrolysis time, and the ratio of raw material to solvent [2]. Therefore, statistical technique was the important tool to solve and determine the optimal condition of acid hydrolysis process. Statistical technique by BBD was found in many researches, which it has been applied to predict the optimization of biological, chemical, and physical processes [9] because of its reasonable design and excellent outcomes.

Thailand is agricultural country that creates a large number of wastes from agriculture. However, the study about utilization of the energy from biomass was still not available, which, unlike Brazil, India, China, Mexico, Indonesia and Colombia, that processed to produce the sugar and ethanol from agricultural wastes [7]. Therefore, optimization of reducing sugar production from acid hydrolysis is an important investigation to apply in many researches of energy and chemical food in Thailand.

II. Material and Methods

A. Pretreatment of Sample

Sugarcane bagasse was provided from Singburi sugar industry (Mitr Phol Co.). The grinded bagasse was reduced in size and increased a surface area of cellulose material by blender. The bagasse was pretreated with 15% NaOH (w/w) and incubated in a dryer at 121 °C with residence time of 25 min. The liquid fraction of lignin was eradicated by filtration; the unhydrolysed solid residue was washed with distilled water until reaching pH at 7 and then, filtrated to separate the solid. The bagasse was dried at 65 °C until the weight of sample was stable.

Manuscript received May 14, 2013; revised July 12, 2013.

doi: 10.12720/jomb.2.4.238-241

B. Reducing Sugar Determination by Dinitrosaclicylic Acid Method (DNS Method)

Standard curve preparation of reducing sugar was prepared using serial concentration of glucose or mannose or xylose solution (0-1000 μg/ml) in distilled water. The 500 μl of each concentration was filled into test tube and added with 500 μl of dinitrosalicylic acid (DNS) solution and subsequently boiled for 15 min, then, cooled and 4.0 ml of distilled water was added. After the homogenization of reaction mixture, the absorbance at 540 nm was measured. The relation between glucose concentration and A_{540} was plotted to determine the reducing sugar in sample solution. 500 μl of sample solution was calculated with the method as described above which was similar to standard curve preparation. After A_{540} measurement, the reducing sugar concentration was calculated by comparing to standard curve.

C. Experimental Design

Three variables of acid hydrolysis process (concentration of H_2SO_4, hydrolysis time, and ratio of raw material to solvent) were designed for the experiment according to the principle of BBD which it was applied to identify and determine the optimum condition.

TABLE I. INDEPENDENT VARIABLES AND THEIR LEVELS OF BBD

Variables	Unit	Symbol	Code level		
			-1	0	+1
Concentration of H_2SO_4	% w/v	x_1	20	30	40
Hydrolysis time	min	x_2	60	150	240
Ratio of raw material to solvent	g:ml	x_3	1:20	1:30	1:40

As shown in Table I, the three hydrolysis factors were indicated as x_1, x_2, and x_3 which prescribed into three levels, coded -1, 0, +1. Quadratic model was fitted to correlate the relationship between the independent variables and the response in order to predict the optimized condition.

$$Y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12}x_1x_2 + \beta_{13}x_1x_3 + \beta_{23}x_2x_3 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{33}x_3^2 \quad (1)$$

From (1), Y is the predicted % reducing sugar; x_1, x_2, and x_3 are the independent variables of acid hydrolysis process; β_1, β_2, and β_3 are the linear coefficients; β_{12}, β_{13}, and β_{23} are the cross-product coefficients; and β_{11}, β_{22}, and β_{33} are the second order coefficients. The quality of the fitted model was determined by the coefficient of determination R^2. The analysis regression and optimum condition were determined by using Microsoft Excel 2007. Finally, three experiments under optimal condition were repeated to verify the result.

III. OPTIMIZATION OF YIELD OF REDUCING SUGAR

Response surface optimization is more advantageous than the traditional single parameter optimization due to its time saving. 15 runs of BBD were applied to determine the optimizing of three parameters. The data were analyzed by multiple regression analysis using Microsoft Excel 2007 and the fitting a second-order equation was derived to represent the % of reducing sugar as a function of the independent variables tested. Table II shows the experimental condition and the results of reducing sugar production according to BBD, which the yield of % reducing sugar varied from 8.074 to 14.736%.

TABLE II. THE BOX BEHNKEN DESIGN WITH THREE INDEPENDENT VARIABLES OF ACID HYDROLYSIS

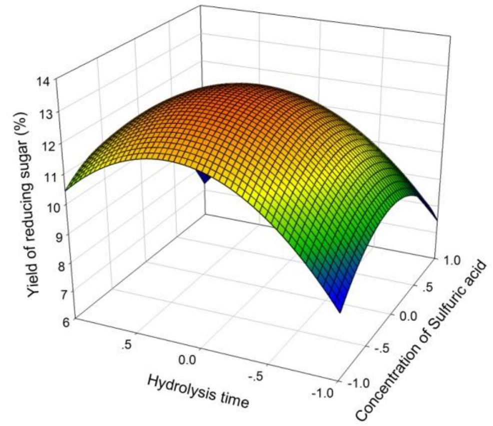
Concentration of H_2SO_4 (W/V %)	Hydrolysis time (min)	Ratio of solvent to raw material (ml/g)	Yield of reducing sugar (%)	
x_1	x_2	x_3	Experimental	Predicted
-1	-1	0	9.919	9.507
+1	-1	0	8.474	8.690
-1	+1	0	11.886	11.670
+1	+1	0	8.074	8.486
-1	0	-1	10.413	10.454
+1	0	-1	9.883	9.297
-1	0	+1	10.344	10.930
+1	0	+1	8.129	8.087
0	-1	-1	8.487	8.857
0	+1	-1	9.772	9.946
0	-1	+1	8.773	8.599
0	+1	+1	9.840	9.470
0	0	0	14.636	14.663
0	0	0	14.736	14.663
0	0	0	14.619	14.663

By applying multiple regression analysis on the experimental data, both dependent and independent variables were related by the following second-order polynomial equation. After statistical regression analysis, the determination coefficient (0.991), adjusted R^2 (0.948), predicted R^2 (0.981), and P-value (P<0.001) showed a good fit with the model. Therefore, the yield of % reducing sugar could be predicted by using the model (2).

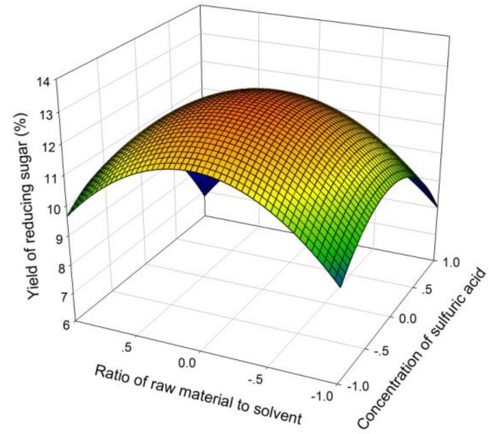
$$Y = 14.663 - x_1 + 0.49x_2 - 0.184x_3 - 0.592x_2 - 0.421x_1x_3 - 0.055x_2x_3 - 2.3x_1^2 - 2.775x_2^2 - 2.671x_3^2 \quad (2)$$

Fig. 1 Response surface plot for % of reducing sugar (a) concentration of H_2SO_4 (x_1) and hydrolysis time (x_2), (b) concentration of H_2SO_4 (x_1) and ratio of solvent to raw material (x_3), (c) hydrolysis time (x_2) and ratio of solvent to raw material (x_3)

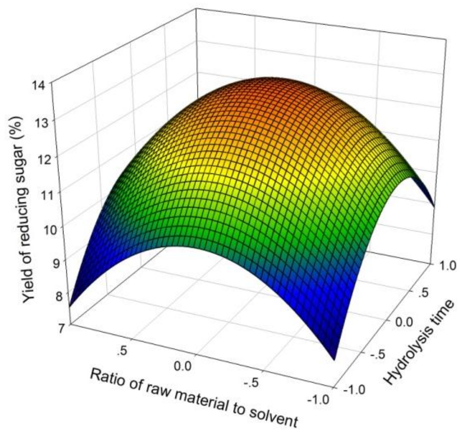
Where Y is the predicted reducing sugar yield and x_1, x_2, and x_3 are the coded values for concentration of H_2SO_4, hydrolysis time and ratio of raw material to solvent, respectively.



(a)



(b)



(c)

Figure 1.

The response surface 3D of (2) was plotted to illustrate the relationship between independent and dependent variables (Fig. 1) that the different levels of 3D graphs indicate different interactions between the variables. In these three variables, when two variables within experimental range were showed in 3D graphs, the third variable was set constant at zero. The solving of Eq. 2 by Microsoft Excel 2007 was used to select the optimal condition for maximum yield of reducing sugar. The result showed that maximum yield of reducing sugar (14.808 %) was recorded under the experimental conditions of concentration of H_2SO_4 at 27.697 %w/v, hydrolysis time of 160 min, and ratio of raw material to solvent was 1:29.827.

For validation of model, the optimum condition of acid hydrolysis was tested with three replicates to verify the prediction from the model. The average yield of % reducing sugar was 14.819±0.093, therefore the result confirmed that the response model was adequate for optimization.

This reducing sugar of sugarcane bagasse from acid hydrolysis process can applied to continue in the fermentation process for using as the cultured medium for producing food and energy of microorganism [5], [10], [11]. Therefore, this optimal condition of acid hydrolysis is useful for producer to operate the process for converting from agricultural waste to reducing sugar.

IV. Conclusion

BBD was applied to determine the optimum process parameters that gave a high yield of reducing sugar. The coefficient of predicted R^2 for the model equation was 0.981 and the probability value ($p<0.001$) demonstrated a very high significance for the regression model for predicting the response. By analyzing the second-order polynomial model, a maximum reducing sugar yield of 14.808% was obtained under the following condition: concentration of H_2SO_4 at 27.697 %w/v, hydrolysis time of 160 min, and ratio of raw material to solvent of 1:29.827. Under this condition, the mean experimental value of reducing sugar yield of 14.819±0.093 % was corresponded well with the predicted value.

Acknowledgment

Our work was supported by Faculty of Engineering and Agro-Industry, Maejo University. The authors would like to thank our undergraduate researchers, Miss Watsana Howong and Mr. Anuphong Sangphung for collecting and analyzing the data of this experiment.

References

[1] M. Brienzo, A. F. Siqueira, and A. M. F. Milagres, "Search for optimum conditions of sugarcane bagasse hemicellulose extraction," *Biochemical Engineering Journal*, vol. 46, pp. 199-204, October 2009.

[2] L. Mesa, E. Gonzalez, I. Romero, E. Ruiz, *et al.*, "Comparison of process configurations for ethanol production from two-step pretreated sugarcane bagasse," *Chemical Engineering Journal*, vol. 175, pp. 185-191, November 2011.

[3] A. A. Silva, H. Inoue, T. Endo, S. Yano, *et al.*, "Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation," *Bioresource Technology*, vol. 101, pp. 7402-7409, October 2010.

[4] G. Siqueira, A. Varnai, A. Ferraz, and A. M. F. Milagres, "Enhancement of cellulose hydrolysis in sugarcane bagasse by the selective removal of lignin with sodium chlorite," *Applied Energy*, vol. 102, pp. 399-402, February 2012.

[5] J. R. A. Santos, M. S. Lucena, N. B. Gusmao, and E. R. Gouveia, "Optimization of ethanol production by saccharomyces cerevisiae UFPEDA 1238 in simultaneous saccharification and fermentation of delignified sugarcane bagasse," *Industrial Crops and Products*, vol. 36, pp. 584-588, March 2012.

[6] A. R. Chong, J. A. Ramirez, G. Garrote, and M. Vazquez, “Hydrolysis of sugar cane bagasse using nitric acid: A kinetic assessment,” *Journal of Food Engineering*, vol. 61, pp. 143-152, February 2004.
[7] C. A. Cardona, J. A. Quintero, and I. C. Paz, “Production of bioethanol from sugarcane bagasse: Status and perspectives,” *Bioresource Technology*, vol. 101, pp. 4754-4766, July 2010.
[8] K. K. Cheng, B. Y. Cai, J. A. Zhang, H. Z. Ling, *et al.*, “Sugarcane bagasse hemicellulose hydrolysate for ethanol production by acid recovery process,” *Biochemical Engineering Journal*, vol. 38, pp. 105-109, January 2008.
[9] H. Gao and W. Y. Gu, “Optimization of polysaccharide and ergosterol production from agaricus brasiliensis by fermentation process,” *Biochemical Engineering Journal*, vol. 33, pp. 202-210, March 2007.
[10] P. Laopaiboon, A. Thani, V. Leelavatcharamas, and L. Laopaiboon, “Acid hydrolysis of sugarcane bagasse for lactic acid production,” *Bioresource Technology*, vol. 101, pp. 1036-1043, February 2010.
[11] G. J. M. Rocha, C. Martin, I. B. Soares, A. M. S. Maior, *et al.*, “Dilute mixed-acid pretreatment of sugarcane bagasse for ethanol production,” *Biomass and Bioenergy*, vol. 35, pp. 663-670, February 2011.

Nukrob Narkprasom was born in 1980, Thailand. He received Ph.D. degree in Food Engineering from National Pingtung University of Science and Technology, Taiwan, in 2012. He is a lecturer at Maejo University, Chiang Mai, Thailand. His research interests about optimization in engineering and agro-industry, waste management, design and development of food and agricultural machinery.

Rittichai Assavarachan was born in 1978, Thailand. He received Ph. D. degree in Food Engineering and Biotechnology from Asian Institute of Technology, Thailand. He is an assistance professor at Maejo University. His research interests about thermal processing, drying technology, pre-cooling and vacuum cooling.

Pairote Wongputtisin was born in 1979, Thailand. He received Ph. D. degree in Biotechnology from Chiang Mai University, Thailand. He is a lecturer at Maejo University, Chiang Mai, Thailand. His research interests about fermentation technology, enzyme technology and functional food.