Korean J. Chem. Eng., **28**(5), 1172-1176 (2011)
DOI: 10.1007/s11814-011-0058-9

Statistical methodology for optimizing the dilute acid hydrolysis of sugarcane bagasse

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(*Received 16 December 2010 • accepted 8 March 2011*)

Abstract−In converting advanced biomass to fuel, one pretreatment that has been extensively explored is a high temperature, dilute-sulfuric acid (H_2SO_4) process. This effectively hydrolyzes the hemicellulosic portion of the lignocellulosic biomass to fermentable sugars. Our aim was to optimize the concentration of sulfuric acid and residence time to release xylose from the hemicellulose of sugarcane bagasse. According to response surface methodology (RSM), the optimum concentrations and residence time were determined. The experimental maximum yield for xylose production was found to be 78.9% at 170 °C, 0.24% acid, for 15 min, and 76.4% at 200 °C, 0.22% acid, for 6 min. The predicted maximum yield obtained for the fitted model was found to be 80.3% and 78.1% for the conditions stated above, respectively. The experimental yield was around 1.5% lower than that of the predicted yield. It was confirmed in this study that pentose sugars (xylose and arabinose) derived from hemicellulose fraction were further degraded. The statistical optimization method, which incorporates reaction time, temperature and acid concentration, did prove to provide a useful means of trading off the combined effects of these three variables on total xylose recovery yields.

Key words: Sugarcane Bagasse, Pretreatment, Sulfuric Acid, Response Surface Methodology

INTRODUCTION

In recent years, efforts have increased towards a more efficient utilization of renewable agro-industrial residues including sugarcane bagasse [1-3]. Sugarcane bagasse, the fibrous byproduct of sugar extraction from cane stalks, is generally used as a biomass fuel. The top and leaves of the sugarcane plants are typically burned off the field prior to harvest and left on the field to decompose. Sugar mills generate approximately 270 kg of bagasse (50% moisture) per metric ton of sugarcane. About 50% of this amount is enough to supply the energy required by the sugar in ethanol plants [4]. The hemicellulose content of the bagasse is approximately 25% and consists mainly of xylan. The hemicellulosic fraction of lignocellulosic residues can be hydrolyzed with dilute acids and the yield of xylose recovered depends on the acid concentration, temperature, and residence time during hydrolysis [5,6]. Previous research has indicated that the formation of the sugar degradation products furfural and 5-hydroxymethylfurfural (5-HMF) is taking place in proportion to the severity of the acid hydrolysis [7,8]. The kinetics of these reactions has been described by different models evaluated by Jacobsen and Wyman [9]. Degradation products not only lower the yield of sugar monomers but also act as fermentation inhibitors [6,10,11]. To produce fermentable hydrolyzates and to prevent high losses in yields, it is necessary to select reaction conditions that keep the generation of degradation products at a low level.

In planning acid hydrolysis conditions, optimizing through central composite design (CCD) and response surface methodology (RSM) is a common practice [12-15]. RSM is suitable for multiple factor experiments, sensitive to relationships between factors, and the ability to not only find the most suitable reaction conditions but to also forecast the response. In this research, RSM was applied to determine the optimum hydrolysis conditions. Furthermore, the extent of xylose production was evaluated at the operating conditions determined by RSM analysis. Ultimately the optimal hydrolysis conditions for the recovery of hemicellulose at the laboratory scale may potentially be scaled up to the production level.

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MATERIALS AND METHODS

1. Raw Material

Sugarcane bagasse was initially ground and passed through a 14 mesh (1.41 mm) standard Tyler sieve. The screened bagasse was used directly in sulfuric acid pretreatment studies. Some of the screened chips were ground to an average size of ~30-40 mesh (0.595-0.420 mm) using a laboratory knife mill. The powdered sugarcane bagasse was analyzed to determine total glucan and xylan, as per an NREL Chemical Analysis & Testing Standard Procedure (# 001, #002) [16]. The powdered bagasse was shown to contain 41.6% glucan and 21.8% xylan.

2. Experimental Apparatus

The hydrolysis experiments were performed using sealed tubular reactors. The experimental apparatus of a reaction is shown in Fig. 1. The vessels were 20 cm long, 2.54 cm diameter, and constructed out of 316 stainless steel tubing, capped at either end with Swagelok fittings, giving an internal volume of 30 cm^3. Temperature of the vessels was adjusted by immersion in oil baths filled with Heat Transfer Fluid 550 (Fisher, Pittsburgh, PA). The combined preheater, heater, and cooler assembly was 17×35×64 cm. The vessels were initially submerged into the oil bath set at 50 °C above the desired reaction temperature for rapid preheating. The vessels were then quickly transferred into a second oil bath set at the desired reac-

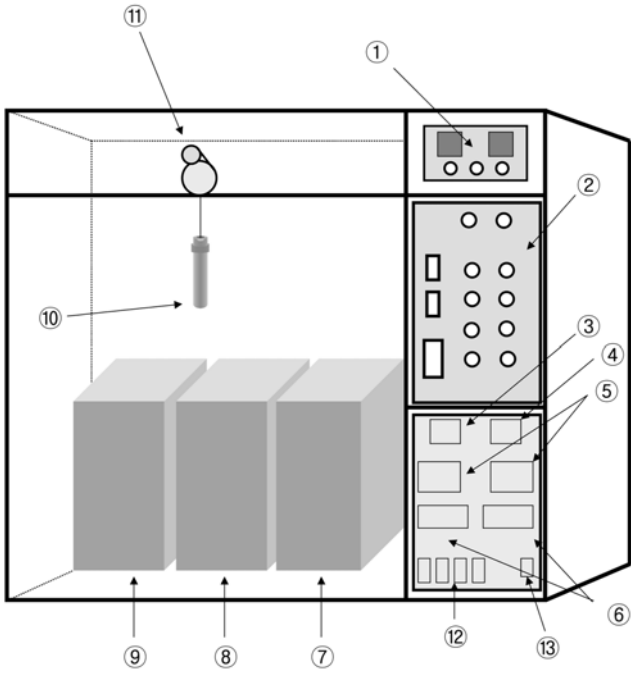

Fig. 1. Experimental apparatus of batch process: ① Timer and counter ② Operator's control box ③ Voltmeter ④ Ammeter ⑤ Digital thermo-controller ⑥ Digital indicator ⑦ Bath (Ice cooler) ⑧ Oil bath (Pre-heater) ⑨ Oil bath (Heater) ⑩ Batch reactor ⑪ Electric motor ⑫ Power switch ⑬ Main switch.

tion temperature. Two electric heaters were used, each rated at 5 KW/h with a max temperature of 300 °C. The temperature of the vessel contents was monitored by a thermocouple (KQXL-18G-12, Omega Eng. Inc., Stamford, CT) inserted into the reactor. After the reaction was finished, the vessel was quenched in an ice bath for 10 minutes.

3. Acid Hydrolysis and Design of Experiments

Bagasse samples were frozen to inhibit the growth of degradative bacteria and fungi commonly found in bagasse piles. Prior to hydrolysis, the bagasse was dried at 60 °C for 24 h and then stored in a desiccator to ensure low residual moisture content. The stainless steel reactor was loaded with 1.3 g of air-dried bagasse. Previous studies in our laboratory focused on maximizing xylose yields during dilute-acid pretreatments of advance lignocellulosic feedstocks [17]. The reaction conditions tested were temperatures (170 and 200 °C) pre-optimized from a previous study [17-20], ratio of bagasse mass to liquor mass (1 : 10), concentration of acid (H_2SO_4, 0.06-0.34%, w/v), and reaction time (1-22 min). The acid concen-

Table 2. Hydrolysis experiment design and the results of CCD at 170 °C

Run	Independent variables (coded level)		Responses; observed experimental data
	x_1	x_2	Xylose yield (%, w/w)
1	0.1 (−1)	10 (−1)	16.4884
2	0.1 (−1)	20 (1)	27.4860
3	0.2 (0)	15 (0)	77.0603
4	0.2 (0)	20 (1)	70.0855
5	0.3 (1)	10 (−1)	70.2870
6	0.3 (1)	20 (1)	57.0347
7	0.2 (0)	15 (0)	76.8410
8	0.2 (0)	15 (0)	73.1565
9	0.2 (0)	15 (0)	77.4501
10	0.06 (−1.414)	15 (0)	47.5172
11	0.2 (0)	8 (−1.414)	47.7207
12	0.2 (0)	22 (1.414)	67.0830
13	0.34 (1.414)	15 (0)	58.2386

$x_1=(X_1-0.2)/1$, $x_2=(X_2-15)/5$
x_1=acid concentration (%, w/v)
x_2=reaction time (minutes)

Table 3. Hydrolysis experiment design and the results of CCD at 200 °C

Run	Independent variables (coded level)		Responses; observed experimental data
	x_1	x_2	Xylose yield (%, w/w)
1	0.1 (−1)	3 (−1)	20.1048
2	0.1 (−1)	9 (1)	50.5081
3	0.2 (0)	3 (−1)	57.9752
4	0.2 (0)	6 (0)	78.5565
5	0.2 (0)	9 (1)	69.2937
6	0.3 (1)	3 (−1)	72.5384
7	0.3 (1)	6 (0)	56.8944
8	0.3 (1)	9 (1)	46.5873
9	0.2 (0)	6 (0)	73.6933
10	0.2 (0)	6 (0)	76.8599
11	0.2 (0)	6 (0)	75.7973
12	0.06 (−1.414)	6 (0)	31.2372
13	0.34 (1.414)	6 (0)	63.5060

$x_1=(X_1-0.2)/1$, $x_2=(X_2-6)/3$
x_1=acid concentration (%, w/v)
x_2=reaction time (minutes)

Table 1. The coded and uncoded values of factors in CCD

Temp.	Factors	Symbol	Coded variable level				
			−1.414	−1	0	1	1.414
170 °C	Acid conc. (%)	X_1	0.06	0.1	0.2	0.3	0.34
	Reaction time (min)	X_2	8	10	15	20	22
200 °C	Acid conc. (%)	X_1	0.06	0.1	0.2	0.3	0.34
	Reaction time (min)	X_2	1	3	6	9	11

tration and residence time for the experimental design are given in Tables 1. A total of 13 runs were made, with runs 4, 7-9 (Table 2) and 3, 9-11 (Table 3), as four replications.

4. Response Surface Methodology

Data analysis was carried out using R package (version 2.8.1, Vienna University, Vienna, Austria) in Statistical Analysis System (Macintosh and VAX/VMS). The range and the level of the variables both coded values and natural values investigated in this study are given in Table 1. The xylose yield was considered as the dependent variable or response (Y). To fit the empirical second-order polynomial model, a CCD with five coded levels was performed. The model proposed for the response (Y) was [13]:

$$Y=A_0+A_1X_1+A_2X_2+A_3X_3+A_{12}X_1X_2+A_{13}X_1X_3 +A_{23}X_2X_3+A_{11}X_1^2+A_{22}X_2^2+A_{33}X_3^2 \quad (1)$$

where Y is the response variable, A_0, A_i, A_{ii}, and A_{ij} are the regression coefficients variables for intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are independent variables. The model was evaluated in terms of statistically significant coefficients, R^2 and P-value.

5. Chemical Analyses

Xylose was analyzed by high performance liquid chromatography equipped with refractive index and UV detection (Breeze HPLC system, Waters Co., Milford, MA, USA), using the Aminex HPX-87H column (Bio-Rad, Hercules, CA). The column was operated with a 5 mM sulfuric acid mobile phase at 0.6 mL/min. and 60 °C. Samples were filtered through 0.22 μm syringe filters prior to injection.

RESULTS AND DISCUSSION

1. The Central Composite Design (CCD) and Response Surface Analysis

A response surface design has been broadly applied in searching for optimum conditions for multivariable systems and in the analyses of interaction variables. The xylose yield was further optimized by using a Box-Wilson CCD with four-star points and four replicates at the center point for each of acid concentration (X_1) and residence time (X_2) at constant high temperature. The coded and uncoded values of factors in the CCD are shown in Table 1. Experimental design and results are shown in Tables 2 and 3.

Table 4. Analysis of variance (ANOVA) for the second-order model at 170 and 200 °C using RSREG analysis[a]

Temp. (°C)	Regression	Freedom	Sum of square	F value	P_r>F
170	Regression	5	4203.21	545.80	0.00051
	Residual	3	11.96	-	-
	Pure error	3	11.96	-	-
	R^2		0.9972		
200	Regression	5	2498.19	37.24	0.03620
	Residual	3	131.30	-	-
	Pure error	1	11.83	-	-
	R^2		0.9501		

[a]Analysis of variance from "R version 2.8.1" statistics program

2. Regression Equation, R^2, and P_r Value of Model

The coded values of the independent variables are given in Table 1. The second-order regression equations show the dependence of xylose recovery on the two different reaction parameters. The parameters of the equation were obtained by multiple regression analysis of the experimental data at 170 °C (Eq. (2)) and 200 °C (Eq. (3)). The following polynomial equation explains the experimental data:

$$Y \text{ (xylose yield, \% @ 170 °C)}=76.13+20.87x_1 -0.56x_2-27.83x_1^2-5.48x_2^2-6.06x_1x^2 \quad (2)$$

$$Y \text{ (xylose yield, \% @ 200 °C)}=73.09+10.61x_1 +2.63x_2-20.75x_1^2-6.23x_2^2-14.09x_1x^2 \quad (3)$$

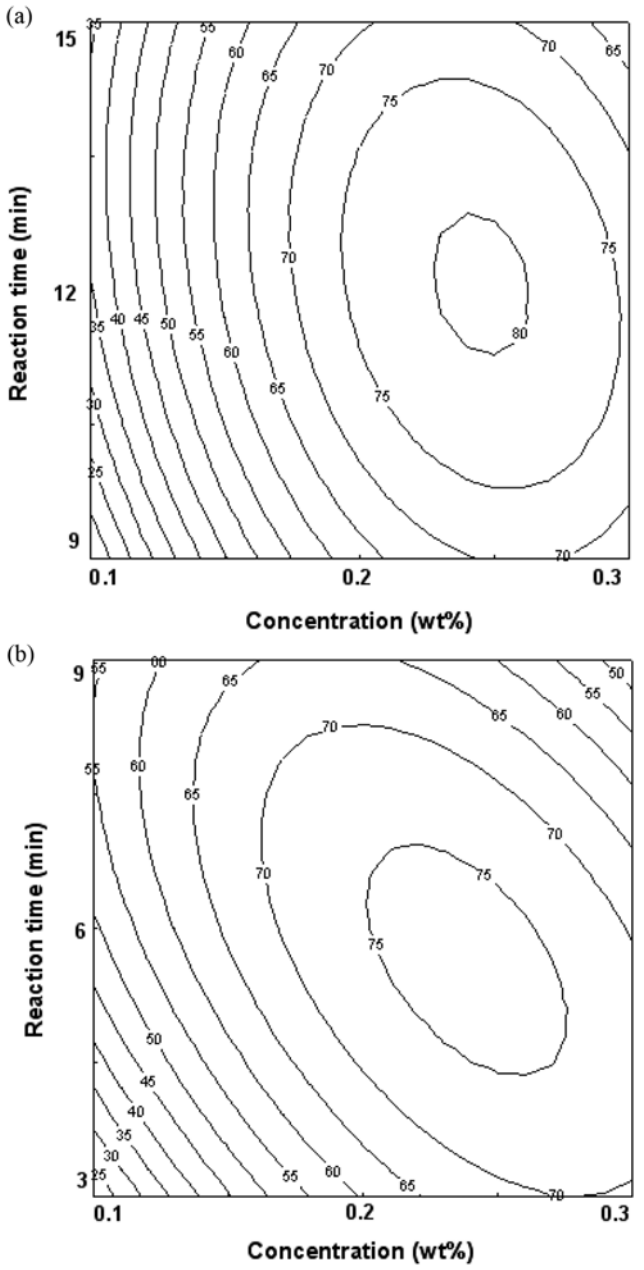

Fig. 2. Contour plot for xylose production in hydrolyzate at various acid concentrations and residence times at 170 °C (a) and 200 °C (b).

where Y is the predicted response, x_1 the coded value of variable X_1 (acid concentration), and x_2 the coded value of variable X_2 (reaction time). These equations were optimized by iteration method using R package (version 2.8.1, Vienna University, Vienna, Austria). The statistical significance of the second-order model equation was checked by an F-test (ANOVA). R^2 values for the responses in the models are given in Table 4. The ANOVA results show that this model was appropriate since the R^2 value was over 90%. Also, the statistics' P-value for the overall regression is significant at the 5% level, which further supports that the model is adequate in approximating the response surface of the experimental design.

3. Xylose Yield at Optimum Condition Based on RSM

Contour plots for the estimated xylose production surface were constructed over the independent variables (X_1 and X_2) at 170 and 200 °C. Fig. 2 shows the optimum conditions for both temperature conditions (residence time: 15.4 min, xylose yield: 80. 3% at 170 °C, Fig. 3(a)) and (residence time: 6.2 min, xylose yield: 78.1% at 200 °C, Fig. 3(b)) based on the contour plot. Fig. 3 shows the residence time course of xylose production in the hydrolyzates. The xylose yield would be 78.9% and 76.4% at 170 and 200 °C, respectively, under the optimum conditions. The experimental optimum matches exactly with the predicted optimum (Fig. 3). Optimization and comparison of multiple variables has been successfully carried out in the current study using response surface methodology.

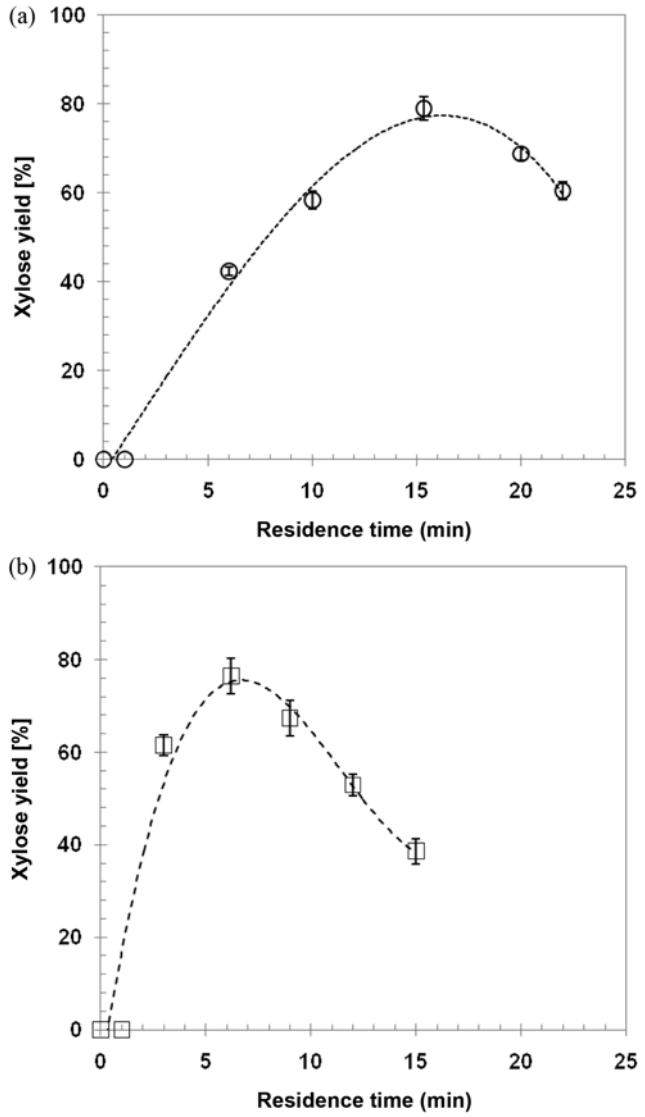

Fig. 3. Xylose yield for acid hydrolysis of solids resulting from sugarcane bagasse under optimum conditions determined through RSM: (a) 0.24% (w/v) acid concentration at 170 °C. (b) 0.22% acid concentration at 200 °C.

CONCLUSION

Acid hydrolysis of sugarcane bagasse has been widely studied as a good method of degrading xylan. It was reported that the average range of xylose recovery was 70-75% in hydrolyzate through dilute acid pretreatment [21-23]. This investigation has clearly established the effect of acid pretreatment conditions on the recovery of xylose. Following RSM optimization, the maximum recovery yield of xylose obtained was 78.9% and 76.4% at 170 and 200 °C, respectively, under the optimum conditions. Approximately 3.9% more xylose than previously reported was obtained in the hydrolyzate. The optimum pretreated conditions of sugarcane bagasse in this study were 0.24% (w/v) concentration of acid, 170 °C reaction temperature and 15 min of reaction time determined by the experimental results and the fitted models. Further investigation is needed to evaluate the compositional properties of the sugar degradation products, lignin degradation products, and aliphatic acids under the conditions.

ACKNOWLEDGEMENTS

The authors thank Dr. G. Peter van Walsum for his internal review of this paper, many thought-provoking comments, and valuable discussion.

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