Kinetic studies of hemicellulose hydrolysis of corn stover at atmospheric pressure

Xuebin Lu, Yimin Zhang[†], Ying Liang, Jing Yang, Shuting Zhang* and Eiji Suzuki**

School of Chemical Engineering and Technology, *School of Environment Science and Technology,
Tianjin University, Tianjin 300072, China
**Department of Fine Materials Engineering, Faculty of Textile Science and Technology,
Shinshu University, Nagano 386-8567, Japan

Abstract−The object of this work was to study the xylose production by hydrolysis of corn stover with diluted sulfuric acid at 100 °C. Several concentrations of H_2SO_4 (2%, 4% and 6% w/w) and reaction time (0-300 min) were evaluated. Kinetic parameters of mathematical models for predicting the concentrations of xylose, glucose and furfural in the hydrolysates were found. Optimal conditions for hydrolysis were 5.5% H_2SO_4 at 100 °C for 60 min; under these conditions, 86.7% of xylose yield and 2.82 g/g selectivity were attained, leading to liquors containing up to 18.73 g/*l* xylose, 6.64 g/*l* glucose and 0.63 g/*l* furfural. The models could be successfully used to predict the concentrations of xylose, glucose and furfural within 0-300 min under experimental acid concentrations. Furthermore, the hydrolysis process of corn stover using dilute acid could be conceived as the first stage of an integrated strategy for corn stover utilization.

Key words: Hydrolysis, Corn Stover, Xylose, Glucose, Kinetics

INTRODUCTION

Corn stover is a renewable, cheap and widely available resource which is mainly composed of cellulose, hemicellulose and lignin. Cellulose can be enzymatically hydrolyzed to its monomeric constituents and then fermented to ethanol or other products [1]. Cellulose fibers, however, are embedded in a lignin-hemicellulose network. This network retards cellulose biodegradation by cellulase. In order to remove the protecting shield of lignin-hemicellulose, and make the cellulose more readily available for enzymatic hydrolysis, corn stover must be pretreated [2]. Pretreatment is a necessary element in bioconversion of lignocellulosic materials to fuels and chemicals. The primary purpose of pretreatment is to make the lignocellulosic biomass accessible and reactive to allow high rates and yields on enzymatic hydrolysis [3]. To date, a number of pretreatment methods have been proposed and investigated, including dilute-acid [4,5], alkali [6], steam explosion [7], ammonia pretreatments [8] and other methods [9]. During pretreatment, hemicellulose may be hydrolyzed to its monomeric constituents and lignin-hemicellulose-cellulose interactions partially disrupted.

Dilute-acid at moderate temperatures effectively converts most of the hemicellulose into dissolved sugars, and increases the enzymatic digestibility of cellulose. The most widely used and tested approaches are based on dilute sulfuric acid. However, nitric acid [10], hydrochloric acid [11] and phosphoric acid [12] have also been tested. High reaction rates (compared with enzymatic process), low acid consumption, and low cost of sulfuric acid (compared with base-catalyzed pretreatments) are some of the advantages of the dilute sulfuric acid pretreatment.

Most of the xylose and a little amount of glucose can be obtained after pretreatment before mentioned. In order to increase the available of the remaining solids, cellulose must avoid being hydrolyzed to glucose. Therefore, the selectivity (xylose-glucose ratio) is an important parameter to optimize the hydrolysis conditions [13]. The hydrolysates obtained from corn stover can be used to produce hydrogen and methane by anaerobic fermentation process [14]. Thus, the hydrolysis process of corn stover using dilute acid can be conceived as the first stage of an integrated strategy for corn stover utilization. Except for xylose and glucose, other byproducts such as furfural and acetic acid are also present. For the fermentation process, the presence of byproducts in the hydrolysates can hinder or prevent a subsequent fermentation step [15]. The generation of byproducts is increased by increasing the temperature. Therefore, the dilute acid hydrolysis of corn stover at atmospheric pressure could be a promising way to use this resource. Furthermore, operating at atmospheric pressure is safer and more economic than using higher pressure [16].

Acid hydrolysis releases oligomers and monosaccharides and has already been modeled as a homogeneous reaction in which acid catalyzes the breakdown of hemicellulose to xylose followed by breakdown of xylose to furfural. Various researchers adapt Saeman's kinetics to describe the hydrolysis of hemicellulose and the formation of byproducts at high temperatures [17], but few researchers do it at atmosphere pressure. In this work, the hydrolysis of corn stover by using dilute sulfuric acid for obtaining fermentable xylose solutions at atmosphere pressure was studied. Through Seaman's model, kinetic parameters of mathematical models for predicting the concentration of xylose, glucose, and furfural were found and used to optimize the process. The experimental results and models were very useful because they can predict the concentrations of xylose, glucose and furfural within 0-300 min under experimental acid concentrations, and it allowed select several different conditions to achieve the same result.

[†]To whom correspondence should be addressed.
E-mail: zhangym001@263.net

MATERIALS AND METHODS

1. Raw Materials

Corn stover collected from a local farm (Hengshui, China) was used as raw material. The corn stover was air-dried, milled, screened to select the fraction of particles with a size lower than 2.0 mm and homogenized in a single lot.

2. Pretreatment Methods

We chose the ranges of sulfuric acid concentrations as 2-6% and time course of treatment as 0-300 min because it was a similar value to those used for diluted sulfuric acid hydrolysis of other lignocellulosic substrates [9,18].

All pretreatment experiments were carried out in a glass flask reactor equipped with an impeller-type mixer with a work volume of 1,000 ml. Dilute sulfuric acid was heated to the desired temperature (100 °C) before the required amount of corn stover was placed into the reactor. All the experiments were preformed at a nominal solids concentration of 10% (w/w). Time zero for all reactions corresponded with corn stover injection. The reactor temperature was controlled with a temperature-controlled oil bath. Each reaction was terminated by placing the reactor into the cold water. After cooling, the pretreated corn stover was filtered and washed with distilled water and the total volume of the liquid was recorded.

3. Analytical Methods

Analysis of the main fractions (glucan, xylan and lignin) was carried out by performing a quantitative acid hydrolysis under standard conditions [19].

All samples were filtered through a 0.22 μm filter and diluted prior to HPLC analysis. The analysis was performed using an Agilent HPLC. Glucose, xylose and acetic acid were separated on a Zorbax carbohydrate analysis column, operating at 35 °C with 75% acetonitrile as the mobile phase at a flow rate of 1.0 ml/min, and detection was performed with an RI-detector. Furfural was separated with a Venusil XBP-C18 column by using a UV-detector set at 280 nm and operating at room temperature. The mobile phase consisted of 40% (v/v) aqueous methanol, adjusted to pH 3 with concentrated hydrochloric acid and supplied at a flow rate of 1.0 ml/min.

4. Statistical Analysis

All experiments were carried out in triplicate and data were expressed as average values. Non-linear regression analyses of experimental data were performed with a commercial optimization routine dealing with the Newton's method (Solver, Microsoft Excel 2003) by minimizing the sum of the squares of deviations between experimental and calculated data as reported previously [20].

5. Kinetic Models

A stringent kinetic study of the acid hydrolysis of lignocellulosic materials is very difficult due to several factors: (1) protection against the attacks of chemicals to the structure of the whole cells, (2) difficult access of protons to the raw material caused by lignin hydrophobicity, (3) interaction with other components, (4) presence of strong bonds in the raw material, and (5) variable exposition of hemicelluloses surface to the chemical attack along the reaction.

Satisfactory results were obtained in kinetic studies applying simplifications, such as the use of principles and laws of valid for homogeneous systems, or the assumption that the main fractions of the lignocellulosic materials do not react among them. Therefore, the use of pseudohomogeneous kinetic models in liquid phase with first order reactions was extended by Maloney [21]. These models begin with the work of Saeman for the hydrolysis of wood using sulfuric acid. In this research the hydrolysis of cellulose was studied and the following model was demonstrated to apply:

$$\text{Cellulose} \longrightarrow \text{Glucose} \longrightarrow \text{Decomposition products} \qquad (1)$$
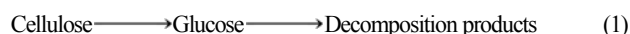
Saeman's model assumes first-order, irreversible reactions. The model of Saeman was also applied to the hydrolysis of the hemicellulosic fraction. Generalizing the model of Saeman could be applied to other homopolymers.

$$\text{Polymers} \xrightarrow{k_1} \text{Monomers} \xrightarrow{k_2} \text{Decomposition products} \qquad (2)$$
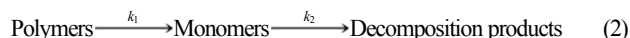
The k_1 and k_2 are the kinetic coefficients of the reactions of monomer release and decomposition, respectively, both having units of reciprocal time. Decomposition products can be furfural, hydroxymethylfurfural, etc. Solving the differential equations for an isothermal reaction, the following model predicts the concentration of monomers [22].

$$[M] = [M_0]e^{-k_2 t} + [P_0]\frac{k_1}{k_2 - k_1}(e^{-k_1 t} - e^{-k_2 t}) \qquad (3)$$

M and P are the concentrations of monomer and polymer expressed in g/l, t is time and subscript 0 indicates initial conditions. In this work, Eq. (3) was applied to model the hydrolysis of corn stover with dilute sulfuric acid.

From now on, Eq. (3) will be called Saeman's model. This model has been applied to the hydrolysis of corn stover. Non-linear regression analyses were performed to obtain the kinetic parameters and constants. In addition, the results were statistically evaluated by applying the statistical one-tailed F-test to the variances. Parameters r^2 and F-test probability were obtained to establish the significance of the models.

RESULTS AND DISCUSSIONS

1. Sugars and Byproducts Formation

The composition of the main fraction of corn stover was (% dry weight basis): glucan, 43.4%; xylan, 19.0%; lignin, 21.2%. Through
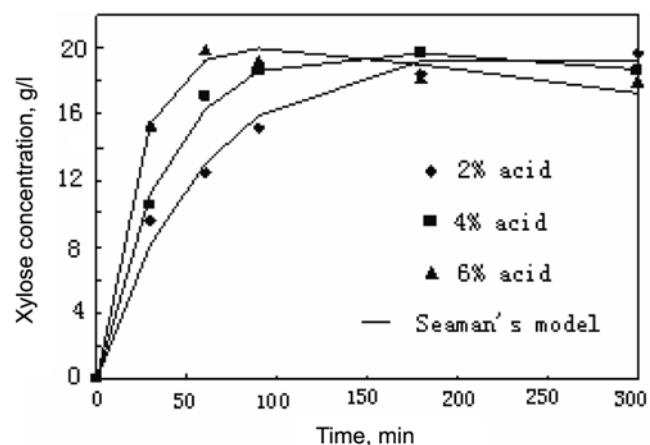

Fig. 1. Experimental and predicted dependence of xylose concentration on H_2SO_4 concentration at 100 °C.

material balance, if the conversion of xylan to xylose is quantified, the concentration of xylose can be determined. By using the solid/liquid ratio, we calculated the potential concentration of xylose as 21.6 g xylose l^{-1}.

Fig. 1 shows the xylose concentration obtained in the H_2SO_4 hydrolysis corn stover at 100 °C. The highest concentration was 19.87 g/l in the experiment carried out at 6% H_2SO_4 during 60 min. This value is close to potential concentration, indicating that this time gives almost complete hydrolysis of the xylan. With the increasing of reaction time, the concentration of xylose decreased from 18.94 g/l at 180 min to 17.78 g/l at 300 min. These results suggested that undesired secondary reactions could have been responsible for xylose decomposition to furfural.

The rate of xylose release increased with the H_2SO_4 concentration. For example, xylose concentrations in hydrolysates at 60 min of reaction were 12.64, 17.15 and 19.87 g/l using H_2SO_4 concentrations of 2%, 4% and 6%, respectively. This effect was also observed in the kinetic coefficients that were shown later.

In hydrolysis, other sugars are released, mainly glucose. Fig. 2 shows the glucose increased with reaction time and H_2SO_4 concentrations and obtained values at 300 min were 8.72, 9.77 and 10.58 g/l using H_2SO_4 concentrations of 2%, 4% and 6%, respectively. A decrease in the concentration of glucose was not observed, which
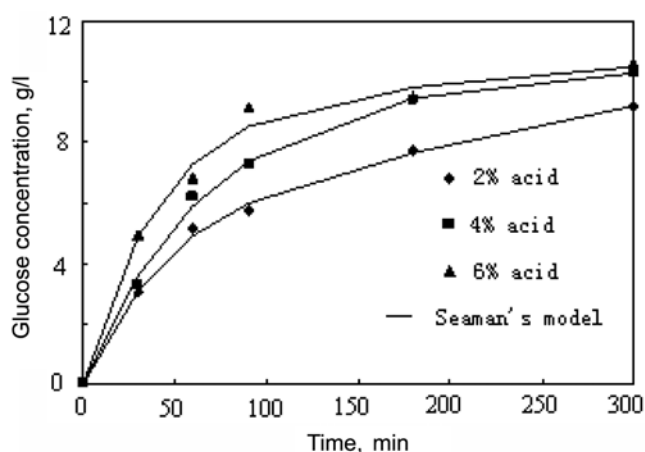

Fig. 2. Experimental and predicted dependence of glucose concentration on H_2SO_4 concentration at 100 °C.
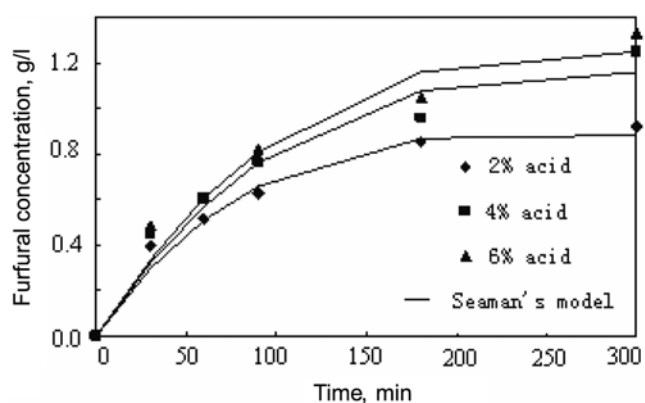

Fig. 3. Experimental and predicted dependence of furfural concentration on H_2SO_4 concentration at 100 °C.

that degradation reactions to HMF were not effective. This could be observed in the kinetic coefficients that were shown later. HMF is a growth inhibitor of microorganisms with a lethal effect much more important than furfural [23]. The low glucose concentration also implied that the glucose obtained mostly came from the hemicellulosic fraction.

Fig. 3 shows the furfural concentration generated as degradation products in the hydrolysis. The H_2SO_4 concentration enhanced the degradation reactions. The concentration of furfural increased with the improvement of acid concentration and reaction time. The maximum furfural concentration reached 1.33 g/l using 6% H_2SO_4 for 300 min which was lower enough to inhabit the microorganisms [24].

2. Kinetic Modeling of Xylose Concentration

Xylose was the main product of the dilute sulfuric acid hydrolysis of corn stover. To apply the Saeman model, the value of M_0 was 0 g/l while P_0 was determined, assuming a total conversion of xylan to xylose in the raw material without degradation, by Eq. (4):

$$[P_0] = \frac{150}{132} \frac{CXn_0/100}{WSR} 1000 = 21.6 \text{ g Xylose}/l \qquad (4)$$

CXn_0 is the initial composition for xylan (19.0 g xylan/100 g corn stover on dry basis), WSR is the liquid/solid ratio (10 g liquid/g corn stover) and 150/132 is the ratio of the stoichiometric coefficients. Table 1 shows the kinetic and statistical parameters obtained for xylose releasing and degradation in the hydrolysis of corn stover at 100 °C and Fig. 1 shows the experimental and predicted data for this hydrolysis.

The r^2 shows a good agreement between experimental and predicted data for all regressions. The statistical value of F-Test probability shows that all models were accurate in describing the experimental data.

The values of k_1 and k_2 increased with acid concentration. The values of k_1 were higher than those for k_2; it could be estimated from

Table 1. Kinetic and statistical parameters of products released in the H_2SO_4 hydrolysis of corn stover at 100 °C

	2% H_2SO_4	4% H_2SO_4	6% H_2SO_4
Xylose			
k_1 (min^{-1})	0.015724	0.024647	0.043094
k_2 (min^{-1})	0.000463	0.000555	0.000801
r^2	0.99345	0.99832	0.996271
F-Test Prob.	0.9340	0.9734	0.9829
Glucose			
k_1 (min^{-1})	0.014247	0.014896	0.024217
k_2 (min^{-1})	0.000138	−0.00023	−0.00044
$[P_0]$	8.88	9.25	9.36
r^2	0.9978	0.9988	0.9952
F-Test Prob.	0.9852	0.9857	0.9883
Furfural			
k_1 (min^{-1})	0.001487	0.001752	0.001788
k_2 (min^{-1})	0.009072	0.006364	0.006172
$[P_0]$	7.83	6.91	7.19
r^2	0.9898	0.9814	0.9840
F-Test Prob.	0.9402	0.9324	0.9272

the values that the sugar release reactions were up to 2 orders of magnitude higher than the decomposition reactions. Values of k_1/k_2 were 33.96, 44.41 and 53.80 for H_2SO_4 concentrations of 2%, 4% and 6%, respectively, indicating that xylose release reactions were always more important than decomposition reactions at low temperature. This was different from the treatments using higher temperature [25]. Treatments using phosphoric acid and nitric acid at high temperature gave ratios k_1/k_2 in the range 2.1-3.3 and 19-46, respectively [9,24]. Comparing these ratios, it was impossible to deduce that the decomposition reactions of xylose were slower by using H_2SO_4 at lower temperature.

The models predicted that maximum xylose concentration (19.91 g/l) was achieved with 5.5% H_2SO_4 for 90 min; higher xylose concentrations could not be obtained even if longer reaction time was used. This was because the degradation reaction decreased xylose concentration under those conditions. The value of maximum xylose concentration was 19.91 g/l, which represented that 92.2% xylose yield was obtained. This xylose yield was higher than reported before [26,27].

A generalized model for predicting xylose produced by the hydrolysis was developed. Kinetic parameters k_1 and k_2 were correlated with the sulfuric acid concentration (C) by means of the empirical Eq. (5), where j was 1 or 2, k_0 and n were the regression parameters and C was the acid concentration expressed as % (w/w).

$$k_j = k_0 C^n \tag{5}$$

Table 2. Generalized models for predicting kinetic parameters as a function of sulfuric acid concentration

Products	Models		r^2
Xylose	$k_1 = 0.0081C^{0.89}$	(6)	0.9775
Xylose	$k_2 = 0.0003C^{0.48}$	(7)	0.9400
Glucose	$k_1 = 0.0098C^{0.44}$	(8)	0.9363
Glucose	$k_2 = -0.00018C$	(9)	0.8699
Furfural	$k_1 = 0.001332C^{0.18}$	(10)	0.9630
Furfural	$k_2 = 0.011397 (1/C^{0.37})$	(11)	0.9548
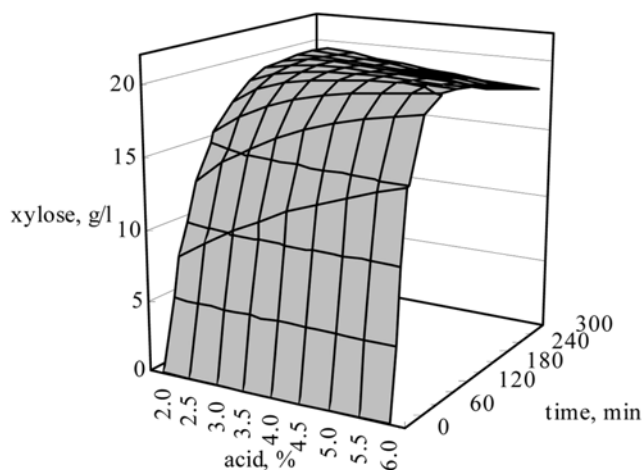
Fig. 4. Prediction of the generalized model for the dependence of xylose concentration on the H_2SO_4 concentration and reaction time.

The k_1 correlated with H_2SO_4 concentration as shown in Eq. (6) of Table 2, and k_2 could be determined by Eq. (7) of the same table. Therefore, combining Eqs. (6) and (7) of Table 2 with Saeman's model, it was possible to predict the xylose concentration for any time and acid concentration in the range of study (0-300 min and 2-6% acid concentration). Fig. 4 shows how the generalized models predicted the dependence of xylose concentration on the acid concentration and time using the parameters found for Saeman's model. This kind of response surface was very useful because it allowed selection of several different conditions to achieve the same result. For example, Fig. 4 shows that 17.0 g xylose/l could be achieved by using 2.5% H_2SO_4 for 90 min or 4% H_2SO_4 for 60 min. Therefore, it was possible to select conditions leading to the highest xylose concentration and the minimum degradation products concentrations by comparing response surfaces for different products of the corn stover. The response surface could be also used to obtain the optimum xylose concentration. The model predicted a maximum xylose concentration using 5.5% H_2SO_4 for 90 min. Under these conditions, 19.91 g xylose/l was predicted. And more than 75% xylose yield was obtained when the acid concentration was lower than 3.5% and time was longer than 90 min, or when the acid concentration was higher than 3.5% and time was longer than 60 min.

3. Kinetic Modeling of Glucose Concentration

Glucose is a secondary product obtained in the hydrolysis of corn stover. The kinetic modeling of glucose concentration can be obtained by using similar equations to those for modeling xylose concentration. However, $[P_0]$ cannot be obtained experimentally because glucose comes from both cellulose and hemicelluloses. It is not possible to determine how much has been hydrolyzed from each type. Therefore, $[P_0]$ has been introduced as a new regression parameter.

Table 1 shows the kinetic and statistical parameters for the glucose releasing and degradation in the hydrolysis of corn stover at 100 °C and Fig. 2 shows the experimental and predicted data for this hydrolysis. The statistical parameters demonstrated that Saeman's model fitted very well. It predicted that the value of $[P_0]$ was around 9 g/l, which represented 20.7% of total glucan in corn stover. This result was higher than sorghum straw at 122 °C [28]. Through these models, a maximum concentration of 10.26 g glucose/l was predicted. This concentration was achieved by using 6% H_2SO_4 for 300 min.

The value of k_1 increased with the H_2SO_4 concentration, while k_2 was negative when H_2SO_4 concentration was 4% and 6%. The value of the ratio of k_1/k_2 of glucose was 103.2 using H_2SO_4 concentration of 2%, indicating that the decomposition reaction of glucose was negligible. Table 1 shows the rate of glucose release is a little lower than the rate of xylose release. This is very interesting because the main decomposition product of glucose is HMF, a great microbial growth inhibitor, and the main application of the hydrolysates is its use as fermentation media.

A generalized model for predicting glucose released was also developed. Eqs. (8) and (9) of Table 2 show the dependence of kinetic parameters on H_2SO_4 concentration. The k_2 could be considered as zero, suggesting that sugar degradation reactions occurred mainly in xylose. Eq. (8) could be considered well fitted ($r^2=0.9363$), but Eq. (9) was less accurate ($r^2=0.8699$).

Fig. 5 shows how the generalized model predicted the dependence of glucose concentration on the H_2SO_4 concentration and time.

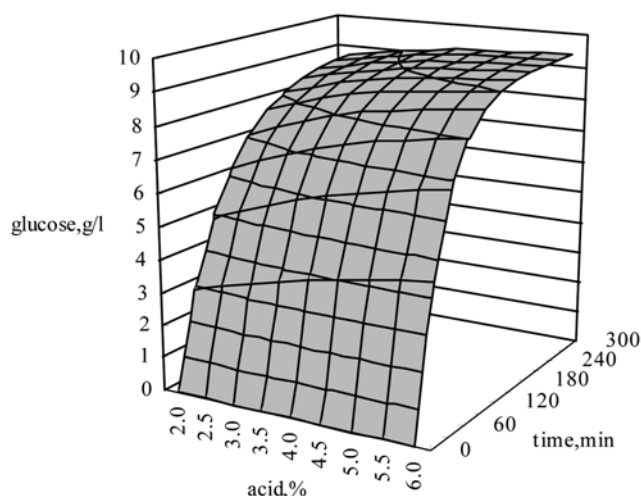**Fig. 5. Prediction of the generalized model for the dependence of glucose concentration on the H₂SO₄ concentration and reaction time.**

Fig. 6. Prediction of the generalized model for the dependence of furfural concentration on the H₂SO₄ concentration and reaction time.

It could be observed that a continuous increase of glucose concentration with the time course and no clear dependence on H_2SO_4 concentration. The model predicted a maximum glucose concentration (10.26 g glucose/l) at the most severe conditions (6% H_2SO_4 and 300 min), but less than 8 g/l was obtained after time less than 90 min for any H_2SO_4 concentration. In the pretreatment process, glucose concentration increased with the increase in acid concentration and reaction time. However, as high levels of glucose could inhibit the fermentation process of some microorganisms, so the selectivity was an important parameter to optimize the hydrolysis conditions. Therefore, the condition with highest selectivity and higher xylose yield was selected which was 5.5% H_2SO_4 at 100 °C for 60 min; under these conditions, 86.7% of xylose yield and 2.82 g/g selective was attained. The generalized models proposed here were useful tools for selecting operational conditions with lower glucose concentration and higher xylose concentration.

4. Kinetic Modeling of Furfural Concentration

In the hydrolysis of corn stover, furfural is the main degradation product. Saeman's model can be used empirically to model furfural data, although it was developed for the conversion of lignocellulosic polymers to monomers. In this case, [P_0] was the potential furfural concentration; k_1 was the furfural generation rate and k_2 was the furfural decomposition rate. Table 1 shows the kinetic and statistical parameters fitting the model for the furfural in the hydrolysis of corn stover at 100 °C, and Fig. 3 shows the experimental and predicted data for furfural in these hydrolyses. [P_0] varied slightly in the range 6.91-7.83 g/l, and the average value was 7.31 g/l. The statistical parameters confirmed that the model fitted very well.

A generalized model for predicting furfural concentration generation was also developed. Eqs. (10) and (11) of Table 2 show the dependence of kinetic parameters on H_2SO_4 concentrations. The k_2 shows an inverse dependence on acid concentration, suggesting that decomposition reactions of furfural were decreased at high H_2SO_4 concentrations. This may be that the accumulation of decomposition byproducts inhibits the decomposition reaction of furfural. Both equations could be considered well fitted (r^2=0.9630 and r^2=0.9548,
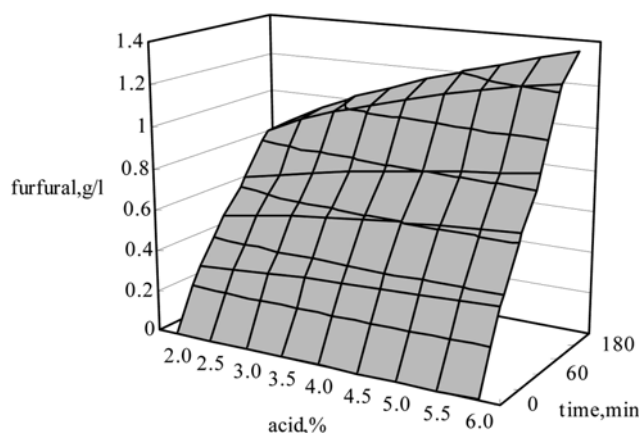
respectively).

Fig. 6 shows how the generalized model predicted the dependence of furfural concentration on the H_2SO_4 concentration and time. The model shows that an interaction between acid concentration and time existed. At this temperature, the model predicted a continuous increase in the value of furfural concentration and time. In some circumstances, furfural is an inhibitor of microorganism growth [15,29]. Therefore, if the hydrolysates are prepared for fermentation, conditions that minimize the furfural concentration should be selected.

Except for furfural, acetic acid is also generated in the hydrolysis of the acetyl groups of the hemicelluloses. The release rate of acetic acid became lower after 60 min, while the acetic acid concentration was not decreased indicating that no decomposition reactions occur (data not shown). Acetic acid can be an inhibitor of microbial growth from 4 to 10 g/l because it goes through the cellular membranes and decreases intracellular pH, which affects the metabolism of the microorganism [30-32]. In all our experiments, the concentration of furfural was lower than 3.5 g/l, which is significantly lower than the toxic effect.

5. Overall Optimization

For pretreatment corn stover using dilute sulfuric acid, one of the aims of the process is to obtain higher xylose yields. However, in order to increase the availability of the remaining solids, glucose must be controlled to produce. Thus, the selectivity is an important parameter to optimize the hydrolysis conditions. The optimal working conditions were defined by using the following criteria: (a) xylose yield could not be lower than 75%; (b) highest selectivity was attained.

Based on these conditions, optimal conditions for hydrolysis hemicellulose of corn stover were found. This operational condition was achieved at 5.5% H_2SO_4 at 100 °C for 60 min and the models predicted 86.7% of xylose yield and 2.82 g g^{-1} selectivity, leading to liquors containing up to 18.73 g/l xylose, 6.64 g/l glucose and 0.63 g/l furfural. The developed models are useful tools for further technical and economic studies. The application of H_2SO_4 for the hydrolysis of corn stover showed a very good selectivity when compared with other methods.

CONCLUSIONS

Corn stover is a renewable, cheap and widely available resource. It contains about 20% xylan, which is a promising source of xylose. Dilute sulfuric acid pretreatment was effective in solubilizing hemicellulose in corn stover to xylose. Kinetics of hydrolysates from corn stover at atmosphere pressure was investigated in this study, and Saeman's model was used to describe the concentrations of xylose, glucose and furfural in the hydrolysates. Experimental results showed that about 86.7% of the maximum possible concentration of xylose and 2.82 g/g selective could be obtained in the pretreatment process, leading to liquors containing up to 18.73 g/l xylose, 6.64 g/l glucose and 0.63 g/l furfural under the operating conditions of 5.5% acid concentration and 60 min at 100 °C. The hydrolysates obtained from corn stover can be used to produce hydrogen and methane by anaerobic fermentation process. Therefore, this study can be conceived as the first stage of an integrated strategy for corn stover utilization.

NOMENCLATURE

[   ] : concentration
P   : polymer (corn stover)
M   : product studied (xylose, glucose and furfural)
C   : sulfuric acid concentration [%, w/w]
CXn_0 : the initial composition for xylan
WSR : the liquid/solid ratio
k_0 : regression parameter
k_1 : rate of generation reaction [min^{-1}]
k_2 : rate of the decomposition reaction [min^{-1}]
n   : regression parameter
t   : time [min]

REFERENCES

1. K. D. Oh and C. Kim, *Korean J. Chem. Eng.*, **4**, 105 (1987).
2. A. Esteghlalian, A. G. Hashimoto, J. J. Fenske and M. H. Penner, *Bioresour. Technol.*, **59**, 129 (1997).
3. C. Y. Park, Y. W. Ryu and C. Kim, *Korean J. Chem. Eng.*, **18**, 475 (2001).
4. Y. Y. Lee, Y. M. Zhu and R. T. Elander, *Appl. Biochem. Biotechnol.*, **124**, 1045 (2005).
5. J. H. Choi and S. B. Kim, *Korean J. Chem. Eng.*, **11**, 178 (1994).
6. S. Kim and M. T. Holtzapple, *Bioresour. Technol.*, **96**, 1994 (2005).
7. K. Ohgren, O. Bengtsson, M. F. Gorwa-Grauslund, M. Galbe, B. Hahn-Hagerdal and G. Zacchi, *J. Biotechnol.*, **126**, 488 (2006).
8. H. H. Yoon, *Korean J. Chem. Eng.*, **15**, 631 (1998).
9. K. K. Oh, Y. S. Kim, H. H. Yoon and B. S. Tae, *J. Ind. Eng. Chem.*, **8**, 64 (2001).
10. A. Rodríguez-Chong, J. A. Ramírez and G. Garrote, *J. Food Eng.*, **61**, 143 (2004).
11. G. Bustos, J. A. Ramírez, G. Garrote and M. Vazquez, *Appl. Biochem. Biotech.*, **104**, 51 (2003).
12. W. Carvalho, M. A. Batista and L. Canilha, *J. Chem. Technol. Biotechnol.*, **79**, 1308 (2004).
13. I. C. Roberto, S. I. Mussatto and R. C. L. B. Rodrigues, *Industrial Crops and Products*, **17**, 171 (2003).
14. D. Liu, D. Liu, R. J. Zeng and I. Angelidaki, *Water Res.*, **40**, 2230 (2006).
15. Y. Sun and J. J. Cheng, *Bioresour. Technol.*, **96**, 1599 (2005).
16. S. J. Tellez-luis, J. A. Ramirez and M. Vazquez, *J. Sci. Food Agric.*, **82**, 505 (2002).
17. N. S. Mosier, C. M. Ladisch and M. R. Ladisch, *Biotechnol. Bioeng.*, **79**, 610 (2002).
18. S. J. Tellez-Luis, J. A. Ramirez and M. Vazquez, *J. Food Eng.*, **52**, 285 (2002).
19. A. Sluiter, *Determination of structural carbohydrates and lignin in biomass*, National Renewable Energy Laboratory (NREL), Golden, CO (2005).
20. G. Garrote, H. Dominguez and J. C. Parajo, *Process Biochem.*, **36**, 571 (2001).
21. M. T. Maloney, T. W. Chapman and A. J. Baker, *Biotechnol. Bioeng.*, **27**, 355 (1985).
22. S. H. A. Rahman, J. P. Choudhury and A. L. Ahmad, *Biochem. Eng. J.*, **30**, 97 (2006).
23. S. Larsson, E. Palmqvist and B. Hahn-Hagerdal, *Enzyme Microb. Tech.*, **24**, 151 (1999).
24. S. Gamez, J. J. Gonzalez-Cabriales, J. A. Ramirez, G. Carrote and M. Vazquez, *J. Food Eng.*, **74**, 78 (2006).
25. R. Aguilar, J. A. Ramirez, G. Carrote and M. Vazquez, *J. Food Eng.*, **55**, 309 (2002).
26. N. Bhandari, D. G. Macdonald and N. N. Bakhshi, *Biotechnol. Bioeng.*, **26**, 320 (1984).
27. T. A. Lloyd and C. E. Wyman, *Bioresour. Technol.*, **96**, 1967 (2005).
28. A. Herrerat, S. J. Tellez-Luis, J. A. Ramirez and M. Vazquez, *J. Cereal Sci.*, **37**, 267 (2003).
29. T. Walther, P. Hensirisak and F. A. Agblevor, *Bioresour. Technol.*, **76**, 213 (2001).
30. H. G. Lawford and J. D. Rousseau, *Appl. Biochem. Biotech.*, **70-72**, 161 (1998).
31. B. Maiorella, H. W. Blanch and C. R. Wilke, *Biotech. Bioeng.*, **125**, 103 (1983).
32. C. Van Zyl, B. A. Prior and J. C. Du Preez, *Enzyme Microb. Technol.*, **13**, 82 (1991).