Acid Hydrolysis of Pretreated Lignocellulose from Corn Residue

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Summary

The lignocellulose (LIC) derived from the hemicellulose hydrolysis of corn residue was steeped in 15 to 25% sulfuric acid at 40 to 103°C, filtered to recover solids, and then dried in a fluidized-bed dryer to concentrate the acid. Acid concentration, steeping temperature, drying time, and temperature effects are described by the current work. Hydrolysis of the pretreated LICs gave 90% cellulose conversion with acid consumption corresponding to 1.50 g H₂SO₄/g glucose and sugar concentrations in the hydrolyzate of up to 6.5 wt% in the best cases. Kinetic parameters are presented which describe the observed rates and extent of hydrolysis. Keywords: biomass; cellulose hydrolysis; corn stover; kinetics; pretreatment lignocellulose.

INTRODUCTION

Cellulosic biomass materials have three principal components: hemicellulose, cellulose, and lignin. Hemicellulose hydrolysis with H₂SO₄ readily yields a variety of pentoses, hexoses, and acids [1], with the primary pentose being xylose. Cellulose hydrolysis is more difficult due to cellulose's crystalline structure and close association with lignin (i.e., the "lignin seal") [2]. The Madison [3,4] and TVA [5] processes which hydrolyze these materials at high temperatures (150–220°C) and acid concentrations of 0.5 to 3% cause sugar degradation (which limits yields to 60%) and restrictions in materials of construction because of corrosion. While fractionation of hemicellulose (by mild hydrolysis) from the biomass prior to cellulose hydrolysis minimizes pentose degradation, glucose degradation is still significant in the high temperature/acid environment required for cellulose hydrolysis by these processes. Grethlein et al. [6], and Church and Wooldridge [7] have suggested improvements.

A "mild," two-step hydrolysis process proposed in 1945 by Dunning and

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Lathrop [8] consists of first removing the hemicellulose fraction by hydrolysis at 98°C, using 10% H₂SO₄ and then treating the remaining lignocellulose (LIC) with concentrated (72–85 wt%) H₂SO₄ under mechanical action. The cellulose, thus pretreated, was added to water to give an 8 wt% solution which was hydrolyzed at 120 to 130°C for 10 to 20 min. Yields of 85 to 90% of theoretical were obtained. However, a subsequent pilot effort showed acid contacting had to be significantly improved if laboratory results were to be reproduced [9]. We report a modified pretreatment step consisting of steeping the biomass LIC in an acid solution, filtering the solution to recover the acid-impregnated biomass, and then drying the recovered LIC to bring the acid concentration up to solvation strength.

**MATERIALS AND METHODS**

**Biomass Materials**

Corn stover provided the lignocellulose (LIC) in our hydrolysis experiments. The cornstalks were harvested over one year prior to the experiments and contained 9.2 wt% moisture. On a dry basis, it contained 34.5 ± 0.4 wt% cellulose and 32.2 ± 0.4 wt% hemicellulose (based on three determinations each). The LIC was produced by subjecting the corn stover to a hemicellulose hydrolysis and leaching [10].

**Preparation of LIC**

Hemicellulose hydrolysis was carried out by filling a 1 L, three-necked flask with 500 mL 8 wt% sulfuric acid and 200 g biomass material. Heat was applied with a heating mantel and controlled to 100°C by using a thermowatch controller (1°F Corp., Cheltenham, PA). The reaction time was 4–5 h under mild agitation by a laboratory mixer. The contents of the flask were then cooled and filtered in a Buchner funnel to recover the solids. The solids were then rinsed with distilled water to wash off residual sugars. The LIC contained 47.9 ± 1.4 wt% cellulose (three determinations) and 3.0 ± 0.2 wt% hemicellulose (six determinations) on a dry-weight basis.

**Acid Steeping**

About 70 g of the washed LIC at 60–70 wt% moisture were placed in the 1000 mL, three-necked flask containing 500 mL 2–40 wt% sulfuric acid from 25 to 105°C, at residence times varying from 2 to 24 h. After steeping, the contents of the vessel were cooled and filtered in a Buchner funnel. The moisture content of the recovered solids was determined by difference before and after drying in a vacuum oven at 55°C. Acid content of the dried sample was determined by placing the sample in 50 to 100 mL distilled water, agitating to extract the acid, and then titrating with NaOH to an end point of pH 7.0.
Drying

The wet LIC recovered from the steeping step was dried in a fluid-bed drier (Lab Line, Melrose Park, IL). The drying temperature ranged from 40–80°C as measured by a thermocouple just before the gas (room air) entered the fluidizing chamber. The dewpoint of the air ranged from 15 to 20°C, although specific measurements of relative humidity were not made during the course of these experiments. The drying time varied from 5 to 30 min depending on: (i) the final and initial moisture contents of the material; (ii) the acid content; (iii) the drying temperature; (iv) the rate at which the material was to be dried. The drying rate was adjusted by controlling the blower speed so that all of the material was fluidized (maximum weight of 120 g, wet basis). The initial material at 60 to 80 wt% moisture was dried to 0.0 to 20 wt% moisture.

Hydrolysis Procedures

The standard hydrolysis procedure consisted of putting 1 g dried LIC in 24 mL 8 wt% sulfuric acid, adding the material to an 8 in. glass pressure tube (Ace Glass Company, Vineland, NJ), sealing it with a bottle cap, and placing the tube in an oil bath at 140°C for 2 to 40 min. The tubes were cooled by quenching in cold water. A sample was taken and centrifuged. The supernate was then neutralized with CaO to a pH of 7.0 and analyzed for glucose content by using glucose analysis (Beckman Model 2) and/or liquid chromatography [11,12]. Runs were made at solids contents ranging from 1 g/24 mL 8% H₂SO₄ (4.1 wt% solids) to 2 g/6.4 mL water or acid solution (31 wt% solids), depending on the final acid concentration desired for the hydrolysis reaction.

Experimental Design (Pretreatment)

The parameters quantified were (i) cellulose conversion to glucose; (ii) acid consumption, and (iii) sugar concentration in the final cellulose hydrolyzate. Acid consumption is a concern because of its cost and the cost of neutralizing the acid with lime. Acid consumption is reflected by the glucose-to-acid ratio (denoted glu/acid) and, in cases of incomplete cellulose hydrolysis or significant glucose degradation, the acid-to-LIC ratio (denoted acid/LIC). Pretreatment, as described here, entails acid steeping, filtering, and finally drying the acid to solvation strength (72 wt% or greater [13]). Treated LIC was then placed in water or 6% acid, heated to 100 to 140°C, and hydrolyzed. High solids and hence, sugar concentration, give upon fermentation an ethanol concentration which can be economically distilled [14,15]. Acid concentration during steeping and moisture contents of the biomass during drying are among the key parameters summarized in Table 1. The experimental design used to optimize the pretreatment step (i.e., minimum acid to give maximum conversion upon hydrolysis) is summarized in Figure 1. As part of these studies, acid-impregnated biomass of a given acid/LIC ratio (which is controlled by steep acid concentration, Fig. 2)
TABLE I
Summary of Important Design Parameters in the Pretreatment of LIC

<table>
<thead>
<tr>
<th>Process Step</th>
<th>Control Variables</th>
<th>Response Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steeping</td>
<td>Residence time</td>
<td>Acid Distribution on Biomass Acid/LIC</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>% Conversion in Solution</td>
</tr>
<tr>
<td>Filtering</td>
<td>Time</td>
<td>% Moisture on the Solids</td>
</tr>
<tr>
<td></td>
<td>Vacuum</td>
<td>Acid/LIC Ratio</td>
</tr>
<tr>
<td>Drying</td>
<td>Residence Time</td>
<td>% Acid</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>Moisture Distribution</td>
</tr>
</tbody>
</table>

OPTIMIZATION OF PRETREATMENT CONDITIONS

Lignocellulose from Hemicellulose Hydrolysis

Determine Drying Temperature & Soaking Time

Select Soaking Temperature 25–105 °C

Select H₂SO₄ Concentration in Soaking Solution 2–40 wt %

Vary Drying Time 5–30 min

Vary Hydrolysis Time 2–30 min.

Evaluate Dependent Variables Glucose Acid % Conversion

Change Pretreatment Conditions?

Hydrolysis Optimization

Fig. 1. Schematic representation of the experimental design for studying pretreatment parameters.
was divided into several samples. Each was dried to a different moisture content. Each dried sample was then further subdivided into several more subsamples for hydrolysis. Hence, the variables of LIC moisture and hydrolysis time were thus converged upon simultaneously while minimizing differences due to variations in the content of the biomass material.

Steeping

A dilute acid solution gives better penetration of acid into biomass particles than an equivalent amount of acid in concentrated form. At an elevated temperature, mild cellulose depolymerization may occur to open the biomass structure and allow more effective penetration of the sulfuric acid. Depolymerization, in turn, is also a function of the residence time, and acid concentration of the steep solution. A residence time of 5 h at 95 to 103°C (Figs. 3 and 4) is optimum. At temperatures above 105°C and acid concentrations above 20 wt%, the conversion of cellulose to glucose in the steeping step ranged from 5 to 10%. This is undesirable since the glucose degrades during the subsequent drying step, and hence, causes reduced overall yields. Within this constraint the highest conversions and glu/acid ratios in the hydrolysis of dried LIC material correspond to the highest steeping temperature (Figs. 3 and 4). Hence, the data infer that the steeping step minimizes acid consumption by facilitating acid distribution on the biomass.
Fig. 3. Conversion of pretreated LIC determined by standard hydrolysis procedures (see text) as a function of acid/LIC ratio. Steeping carried out at (○) 103, (■) 70, and (▲) 40°C. Solid lines given by Eqs. (1) and (2) in the text. Corresponding correlation coefficients are 0.96 and 0.94, respectively.

Filtration

Once the steeping step has been completed, the acid-impregnated biomass solids are vacuum filtered. The filtrate, now containing some glucose, can either be reused in another steeping step or in the hemicellulose hydrolysis step [15]. The moisture content, at 60 to 80 wt%, is a function of the filtration time and amount of vacuum used, with higher acid concentrations making it more difficult.

Fig. 4. Glucose/acid ratio as a function of acid/LIC ratios for the optimum pretreatment conditions and steeping temperatures of 103°C and 40–70°C. Solid lines given by Eqs. (3) and (4) in text. Correlation coefficients are 0.91 and 0.64.
to remove the water. The residual moisture content is directly proportional to the acid content, and consequently, the acid/LIC ratio upon drying. Close control of the filtration step is needed in order to attain reproducibility in the acid/LIC ratio which follows the trend line in Figure 2.

Drying

The constant rate drying period occurs when the solid surface is covered with moisture and the drying occurs by constant vaporization of water from the surface. The falling rate period occurs when the solid surface is no longer uniformly covered with moisture and the drying rate decreases as the drying process proceeds. For a hygroscopic material such as biomass, the rate of drying in the falling rate period will be controlled by intraparticle diffusion and the particle size [16-19]. If intraparticle diffusion is controlling, the surface of the particle will become dry before the center of the particle dries. Undesirable degradation reactions can then occur at the surface while the center of the particle is still drying. Glucose adsorbed on the biomass from the steep also degrades during drying because of the high surface acid concentration [20] although the sulfuric acid is sufficiently volatile to evaporate to a small extent during an extended drying period [21]. The importance of controlled drying conditions is obvious.

Acid concentration (wet basis) also impacts the conversion as previously reported by Sakai [22]. However, the acid concentration after drying (Fig. 5) is not the only determinant for cellulose conversion, as can be seen by comparing Figures 3 and 5. An acid/LIC ratio of 0.8 and an acid concentration above 80% (after drying) are needed to attain conversions above 80%.

![Graph](image)

**Fig. 5.** Cellulose conversion as a function of acid concentration in lignocellulose steeped in (■) 15, (●) 20, and (▲) 25% H₂SO₄ at 103°C and then dried to various moisture levels.
RESULTS AND DISCUSSION

Optimum Pretreatment Conditions

The major hydrolysis variables are the solids concentration, acid/LIC ratio, reactor residence time, and temperature. The conversion is a function of acid loading on the LIC (Fig. 3). A polynomial fit of percent conversion (x) versus acid/LIC ratio (A, g/g, dry basis) for acid concentrations in the steeping step, ranging from 15 to 25% at 103°C, is given by

\[ x = 0.9625 + 147.4A - 60.45A^2 \]  
(1)

At 40 and 70°C, this relationship becomes

\[ x = 17.144 + 80.242A - 24.435A^2 \]  
(2)

The glucose/acid ratio (y) appears to be a linear function of the acid/LIC ratio (Fig. 4). The relationship at 103°C (steeping temperature) is

\[ y = 0.77188 - 0.30733A \]  
(3)

At 40 and 70°C, this becomes

\[ y = 0.61254 - 0.24371A \]  
(4)

A strong effect of steeping temperature above 70°C is indicated, thus leading to the pretreatment conditions recommended in Table II and used below.

Reaction Kinetics of Pretreated LIC

The pretreatment and reaction mechanism which takes place for a lignocellulosic material appears to be complex. We have chosen to empirically represent the pretreatment in terms of native cellulose, C, which is the fraction of potential glucose where potential glucose equals cellulose \( x_{180/162} \). C is converted to a more reactive form \( C^* \). The reactivity of C is low; untreated LIC gives a maximum conversion of 0.8% at 100°C and 6.8% at 140°C in 16% acid. Sae- man's approach [3] was used with \( C^* \) representing the substrate concentration,

| TABLE II |
| Pretreatment Conditions for LIC Used in Kinetics Studies |
| Steeping Time | 5 hours |
| Steeping Temperature | 100–103°C |
| \( \text{H}_2\text{SO}_4 \) in Steeping Solution | 20 wt% \( \frac{g \text{ acid}}{g \text{ acid} + g \text{ water}} \times 100 \) |
| Drying Temperature | 80°C |
| Drying Time | 9 – 11 min. |
$G$ representing glucose, and $D$ the glucose degradation products:

$$C^* \xrightarrow{k_1} G \xrightarrow{k_2} D$$  \hspace{1cm} (5)

It is assumed that the reaction is independent of water concentration (i.e., excess water is present). The rate expressions are

$$\frac{dC^*}{dt} = -k_1 C^* \quad \text{and} \quad \frac{dG}{dt} = k_1 C^* - k_2 G$$  \hspace{1cm} (6)

where

$$k_i = k'_i \exp(-E_i/RT)$$  \hspace{1cm} (7)

Solution of Eq. (6) (integrating factor of $e^{-k_1 t}$) for $G = 0$ at $t = 0$ gives

$$G = C_0^* \left( \frac{k_1}{k_1 - k_2} \right) \left[ \exp(-k_2 t) - \exp(-k_1 t) \right]$$  \hspace{1cm} (8)

$C_0^*$ is a function of all of the variables (see Table I) encountered in the pretreatment and was determined from the data in Figure 3. This approach assumes that the maximum conversions shown in Figure 3 represent the corresponding concentration of $C_0^*$ at the indicated optimum pretreatment conditions. The conversion data in Figure 3 were obtained at a reaction temperature of 140°C with a solids concentration of 4.1 wt% (i.e., 1 g pretreated LIC in 24 mL). Thus $C_0^*$ is the maximum potential glucose initially present in pretreated LIC. The

![Graph](image)

Fig. 6. Hydrolysis time course (plotted as normalized yield) for pretreated cellulose at 100°C and acid concentrations of 10 and 16%. Solid lines represent nonlinear least-squares fit of Eq. (8) to the data. $k_1$ values of 0.0166 and 0.0203 at 10 and 16% acid, respectively, and $k_2$ values of 0.00114 and 0.00064. Correlation coefficients for the fit are 0.97 in both cases.
cellulose ($C' - C_2$) which is assumed to remain in its original state after pretreatment, is taken to be unreactive (as a first approximation) at the hydrolysis conditions used here.

The kinetics for the cellulose hydrolysis of pretreated corn stover LIC were determined at 31 wt% (LIC + acid solids), 10% acid (2 g solids/6.4 mL water) to 16% acid (2 g/6.4 mL 6% H₂SO₄), and a reaction temperature range of 100 to 140°C (for LIC with an initial cellulose fraction, $C_0$, of 0.502 and $C_2$ defined as above). Figure 6 compares the fit of Eq. (8) to the data for a reaction temperature of 100°C and acid concentrations of 10 and 16 wt%. The time values used in Eq. (8) are 3 min less than the values on the time axis in Figures 6 and 7 in order to approximate the time lag required for heating the samples from room temperature to reaction temperature. Figure 7 compares the fit of Eq. (8)

![Graph](image)

**Fig. 7.** Normalized yield (fractional conversion of $C'$) vs hydrolysis time for reaction temperatures of 100 to 140°C and acid concentrations of 10 and 16%. Solid lines represent nonlinear least-squares fit of Eq. (8) to data at the conditions indicated. Correlation coefficients for the fit at 10% acid and 100, 120, and 140°C are $R^2 = 0.97, 0.99$, and $0.88$, respectively in (a) and at 16% acid and 100, 120, and 140°C are $R^2 = 0.97, 0.95$, and $0.78$, respectively, in (b).
Fig. 8. Kinetic constant for the hydrolysis of reactive cellulose, $k_1$, vs reciprocal temperature at acid concentrations of 10 and 16%.

at three different temperatures, 100, 120, and 140°C and two acid concentrations. The maximum sugar concentrations obtained in these experiments were in range of 6.0 to 6.5 wt%.

The kinetic constants determined from the experimental data presented in Figure 7, by using Eq. (8) and a nonlinear regression, were then graphed on an Arrhenius-type plot (Fig. 8). The values of the kinetic parameters, thus obtained, are given in Tables III and IV. The effect of acid concentration is not removed from the preexponential factor because only two acid concentrations have been studied so far. Our estimate of the activation energy, $E_i$, for the hydrolysis of $C^*$ is in good agreement with the value of 25,400 cal/mol reported by Kobayashi and Sakai [23]. At 115°C and a 10% acid concentration, we estimate a rate

<table>
<thead>
<tr>
<th>Acid Conc.</th>
<th>$k_i \text{ min}^{-1}$</th>
<th>$E_i \text{ cal/mol}$</th>
<th>$k_i \text{ min}^{-1}$</th>
<th>$E_i \text{ cal/mol}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>$6.00 \times 10^{15}$</td>
<td>30,200</td>
<td>$3.21 \times 10^{8}$</td>
<td>19,540</td>
</tr>
<tr>
<td>16%</td>
<td>$4.84 \times 10^{14}$</td>
<td>27,800</td>
<td>$7.66 \times 10^{11}$</td>
<td>25,600</td>
</tr>
</tbody>
</table>

*$k_i = k_i^* \exp(-E_i/RT)$ for $i = 1,2$; $R = 1.987 \text{ cal/mol} \cdot ^\circ \text{K}$. 

**TABLE III**

Summary of Values of Constants Obtained by Fitting Eq. (8) to the Data in Figure 7.


<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Hydrolysis Acid Conc.</th>
<th>Observed Value (Fig. 7)</th>
<th>Calc. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k₁</td>
<td>100</td>
<td>16</td>
<td>0.0203</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>16</td>
<td>0.233</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>16</td>
<td>0.772</td>
</tr>
<tr>
<td>k₂</td>
<td>100</td>
<td>10</td>
<td>0.0116</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>10</td>
<td>0.0911</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>10</td>
<td>0.605</td>
</tr>
<tr>
<td>k₂</td>
<td>100</td>
<td>16</td>
<td>0.00084</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>16</td>
<td>0.00545</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>16</td>
<td>0.0183</td>
</tr>
<tr>
<td>k₂</td>
<td>100</td>
<td>10</td>
<td>0.00114</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>10</td>
<td>0.00421</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>10</td>
<td>0.0147</td>
</tr>
</tbody>
</table>

*Calculated based on data given in Table III.

constant, $k_1$ of 0.0570 min$^{-1}$ which is in between values of 0.195 min$^{-1}$ for soluble glucose polymer and 0.000294 min$^{-1}$ for cellulose [23]. When we attempted to determine the kinetic constant for glucose degradation, $k_2$, by fitting Eq. (8) to the experimental data, $k_2$ had little effect on the time course compared to $k_1$. Statistically this result [24] also indicated that glucose degradation has only a small effect in the region of our experiments making it difficult to determine values of $k_2$. Data of Hong et al. [20], at 100°C and 10% acid, of Wright [25] and Bhandari et al. [26], indicated glucose degradation is small at 140°C and acid concentrations of less than 3%.

Figure 9 is a plot of conversion versus acid/LIC ratio comparing the bagasse data of Ladisch et al. [10], to corn stover. The two grasses yield similar results for cellulose hydrolysis when the LICs are steeped at 103°C and dried as explained previously. For bagasse the dependence of conversion on acid/LIC ratio, at optimum pretreatment conditions, is given by

$$\%\text{CONV} = 17.139 + 112.51 A - 27.283 A^2$$  (9)
Fig. 9. (a) Comparison of bagasse LIC (■) and corn stover (▲) LIC cellulose hydrolysis. (b) Conversion of bagasse (■) and corn stover (▲) LIC in a cellulose hydrolysis vs acid/LIC ratio at optimum pretreatment conditions (see text) and a steeping temperature of 103°C. Solid lines given by Eqs. (1) (for corn stover LIC) and (9) (for bagasse LIC) in text.

The LIC from both grasses was pretreated in the same manner, the only apparent difference being the slightly higher acid on the pretreated bagasse (0.689 acid/LIC ratio vs 0.653 acid/LIC ratio for corn stover). From Figure 9 it is apparent that both grasses exhibit similar kinetics, and both materials reacted rapidly, with the maximum glucose yield occurring between 10 and 15 min and then slowly decreasing as the hydrolysis time is increased.

CONCLUSIONS

The approach presented here examined steeping to obtain enhanced acid distribution in lignocellulose in a manner which can be scaled up. The key parameters are (1) acid impregnation by steeping in 15 to 25% H₂SO₄ instead of applying a concentrated acid using mechanical action; (2) steeping at elevated temperatures, and (3) filtration followed by evaporation of the residual water in a fluidized bed dryer to bring the acid back to solvation strength. High sugar concentrations can be achieved in the cellulose hydrolyzate with only small effects on the
cellulose hydrolysis kinetics. A first-order kinetic model fits the experimental data over the range of conditions considered. The kinetic parameters determined here for corn stover LIIC may also be applicable to the LIICs produced from other biomass materials which are pretreated in the same manner.

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