Heterogeneous Aspects of Acid Hydrolysis of α-Cellulose

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Abstract

Hydrolysis of α-cellulose by H_2SO_4 is a heterogeneous reaction. As such the reaction is influenced by physical factors. The hydrolysis reaction is therefore controlled not only by the reaction conditions (acid concentration and temperature) but also by the physical state of the cellulose. As evidence of this, the reaction rates measured at the high-temperature region (above 200°C) exhibited a sudden change in apparent activation energy at a certain temperature, deviating from Arrhenius law. Furthermore, α-cellulose, once it was dissolved into concentrated H_2SO_4 and reprecipitated, showed a reaction rate two orders of magnitude higher than that of untreated cellulose, about the same magnitude as cornstarch. The α-cellulose when treated with a varying level of H_2SO_4 underwent an abrupt change in physical structure (fibrous form to gelatinous form) at about 65% H_2SO_4. The sudden shift of physical structure and reaction pattern in response to acid concentration and temperature indicates that the main factor causing the change in cellulose structure is disruption of hydrogen bonding . Finding effective means of disrupting hydrogen bonding before or during the hydrolysis reaction may lead to a novel biomass saccharification process.

Index Entries: Acid hydrolysis; cellulose; hydrogen bonding; kinetics; crystallinity.

Introduction

Acid-catalyzed cellulose hydrolysis is a complex heterogeneous reaction. It involves physical factors as well as the hydrolytic chemical reaction.

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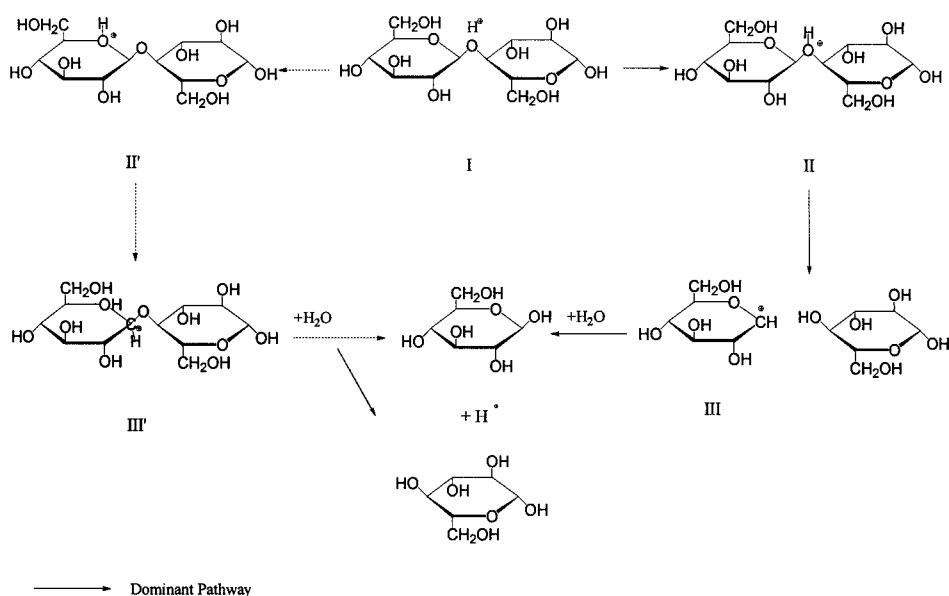
Fig. 1. Mechanism of acid catalyzed hydrolysis of β-1-4 glucan.

The molecular mechanism of acid-catalyzed hydrolysis of cellulose (cleavage of β-1-4-glycosidic bond) follows the pattern outlined in Fig. 1 *(1)*. Acid hydrolysis proceeds in three steps. The reaction starts with a proton from acid interacting rapidly with the glycosidic oxygen linking two sugar units, forming a conjugate acid. The cleavage of the C-O bond and breakdown of the conjugate acid to the cyclic carbonium ion then takes place, which adopts a half-chair conformation. After a rapid addition of water, free sugar and a proton are liberated *(2–5)*. The formation of the intermediate carbonium ion takes place more rapidly at the end than in the middle of the polysaccharide chain. In accordance with this, the yield of monosaccharides after partial hydrolysis is higher than that calculated on the basis of a random bond cleavage *(1)*.

The global kinetics of acid hydrolysis was first described by Saeman *(6)* as two pseudo-homogeneous consecutive first-order reactions. Hydrolysis of glycosidic bonds also follows a first-order reaction *(7,8)*. The first-order rate constants obey the Arrhenius equation with a modification to include the acid dependence term

$$k_i = k_{io} \times A^{mi} \times e^{-\frac{E_i}{RT}}$$

where k_{io} is the pre-exponential factor, A is the concentration of acid, m_i is an exponent indicating the acid effect, and E_i is the activation energy.

The first-order kinetic equation generally applies to reactions in homogeneous phase. Therefore, first-order reaction is justifiable for hydrolysis of oligosaccharides that are soluble in the hydrolyzing medium.

In actual hydrolysis with dilute acids, a heterogeneous reaction takes place, yielding "hydrocellulose," a product with reduced degree of polymerization (DP) but higher crystallinity *(9)*. The rate of hydrolysis of cellulose in crystalline form is one to two orders of magnitude lower than that of homogeneous hydrolysis of soluble model compounds. The hydrolysis of cellulose is strongly influenced by the degree of crystallinity and the swelling state of cellulose *(5)*. The reactivity of cellulose is also affected by mechanical disintegration and/or decrystalli-zation procedures *(5,10)*.

It is quite obvious that acid-catalyzed cellulose hydrolysis is a heterogeneous reaction in which the nonreaction factors (e.g., crystallinity, diffusion barrier, physical conformation) represent a major part of the overall resistance *(11)*. The present investigation was undertaken to verify the nature of the nonreaction resistances in cellulose hydrolysis and to provide further understanding of the heterogeneous aspects of this reaction.

Materials and Methods

α-Cellulose

Chemicals were of analytical grade purchased from Sigma-Aldrich. α-Cellulose (product no. C8002) was analyzed for sugars, moisture, and ash content according to the National Renewable Energy Laboratories (NREL) procedures *(12)*. α-Cellulose contained 92.2% glucan, 3.4% xylan, and 3.2% mannan on dry basis. Ash content was negligible.

Experimental Apparatus and Procedures

Hydrolysis of α-cellulose was performed in both batch and bed-shrinking flow through reactors. For diluteacid-catalyzed hydrolysis at high-temperature conditions, experiments were performed using sealed tubular reactors. The reactors (19.0-cm^3 internal volume) were constructed of Hastelloy C-276 tubing (0.5 in. id × 6 in. length) capped with Swagelok end fittings. Approximately 0.6 g of α-cellulose and 12 g of 0.07% H_2SO_4 solution were loaded into a reactor. The solid/liquid ratio was maintained at 1/20 in all batch operations. Two separate fluidized sand baths were used: one for preheating, the other for reaction temperature control. The reactors were first submerged into the preheating sand bath set at 30°C above the desired reaction temperature for rapid preheating. After 1.5 min, the reactors were then quickly transferred into another sand bath set at the precise desired reaction temperature. After the desired reaction time, each reactor was taken out of the sand bath and quenched in a cold-water bath. The time variation of the solid composition and the monomeric sugar concentrations in liquid were determined. Batch hydrolysis with 4% H_2SO_4 at 120°C was conducted in an autoclave using plastic-capped Pyrex glass bottles.

For treatment of α-cellulose with concentrated H_2SO_4, 3 g of cellulose was added to 50-mL solutions with varying H_2SO_4 concentrations (50–72%). The treatment was conducted at 25°C for 4 h. After treatment, distilled

water was added to dilute the acid and then the remaining or reprecipitated cellulose solid was filtered and washed. The pretreated α-cellulose was stored wet with 50–70 % moisture content for further experiments including X-ray, scanning electron microscopy (SEM), and acid hydrolysis tests.

Analytical Methods

Solid samples were analyzed for glucan content according to the NREL procedures *(12)*. Oligomeric sugars in the hydrolysate liquor were converted to monomers using 4 wt% H_2SO_4 hydrolysis at 120°C for 60 min. Sugars and other compounds were determined by high-performance liquid chromatography (HPLC) using a Bio-Rad Aminex HPX-87P column. The X-ray diffraction test on original and pretreated α-cellulose was conducted using a Rigaku X-ray D/Max-B Diffractometer. SEM was done using a Zeiss DSM 940 scanning electron microscope.

Results and Discussion

To assess the heterogeneous nature of the hydrolysis reaction, experiments were carried out beyond the range of reaction conditions normally applied for hydrolysis. The intent here was to apply reaction conditions severe enough to bring about changes of the physical structure of cellulose while the reaction is taking place.

The first series of experiments on acid hydrolysis of α-cellulose was conducted at temperatures above 200°C and extremely low acid conditions (0.07%) using batch reactors. The results of these experiments are summarized in a semilog plot for α-cellulose as shown in Fig. 2, the slope of the plot representing the first-order rate constant. The first-order reaction was confirmed by the linearity of the plots for all temperatures.

If the hydrolytic reaction is the controlling resistance in the overall process, the Arrhenius equation should apply over the entire temperature range. However, the experimental data in Fig. 2 contradict this hypothesis, showing a rather abrupt increase in reaction rate between 210 and 225°C. The rate constants obtained in this experiment were also put into the Arrhenius plot, $\ln(k)$ vs $1/T$ (Fig. 3). The sudden departure of the rate constants from normal Arrhenius pattern is also seen in Fig. 3 with a breaking point near 215°C. The actual measured rate constants at 215–245°C are much higher than those predicted from the data taken over 185–205°C. For example, the measured rate constant at 235°C is 3.6 times the value extrapolated from the Arrhenius equation. We have also observed a similar behavior from hydrolysis of pretreated yellow poplar.

This finding indicates that the kinetic behavior of cellulose is strongly dependent on the physical state of the substrate. Within the low-temperature region (below 215°C), the α-cellulose is in crystalline structure; thus, it retains the kinetic behavior unique to this structure. Above 215°C, the data in Figs. 2 and 3 show a different kinetics with higher apparent activation energy (higher sensitivity to temperature). The chemical reactions are
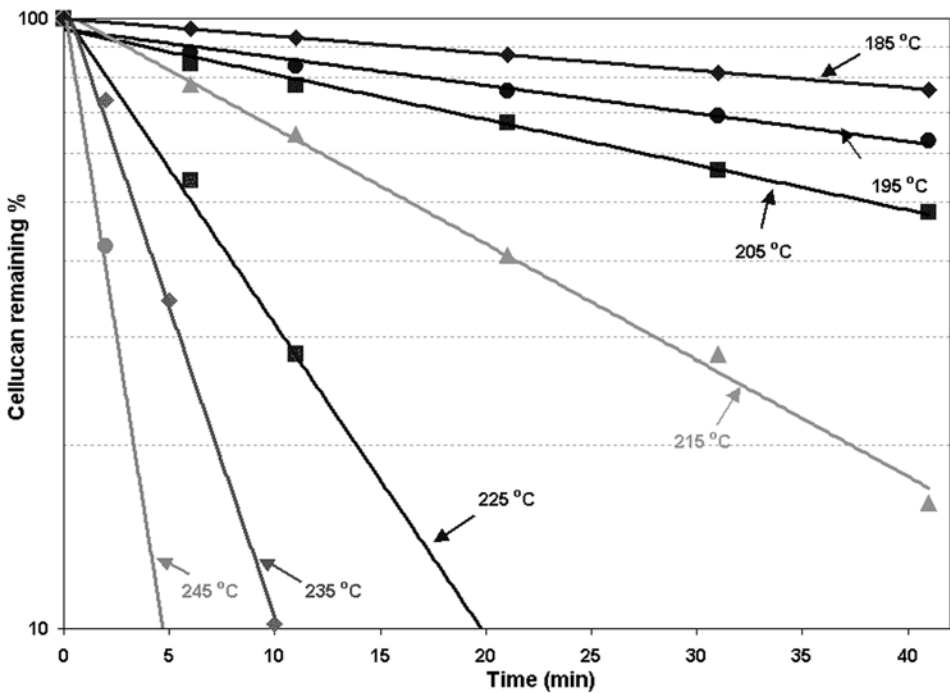
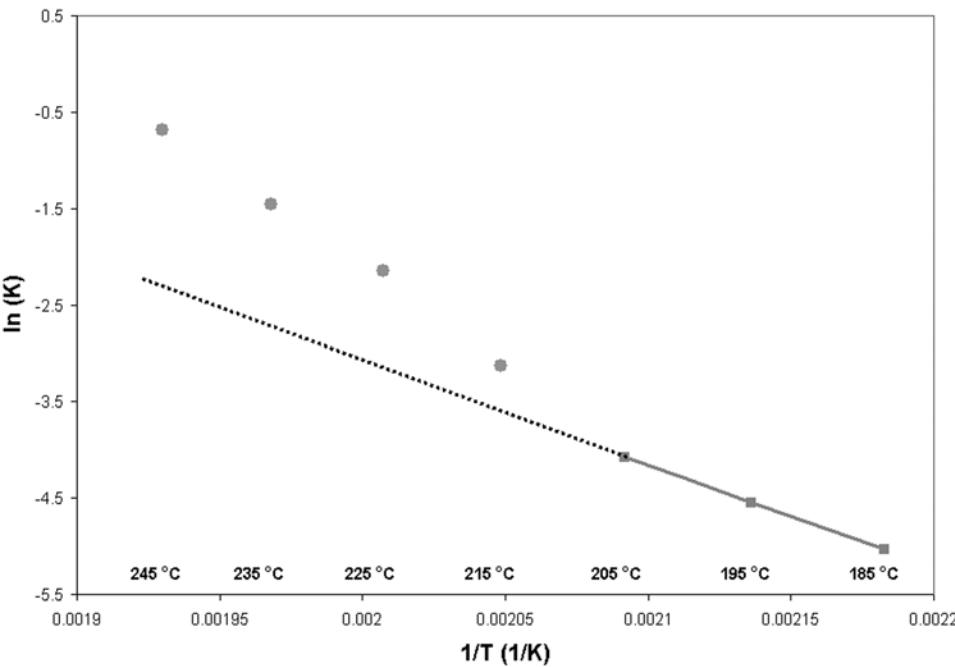Fig. 2. Effect of temperature on hydrolysis rate (0.07 % H_2SO_4, batch reactor) .

Fig. 3. Arrhenius plot for first-order rate constants in hydrolysis of α-cellulose (185–245°C, batch reactor, 0.07% H_2SO_4).

generally more sensitive to temperature than the nonchemical reaction factors (physical factors). At high temperature (215°C), the physical factors seem to be eliminated, making the true chemical reaction the major influential factor in the overall process. The overall process therefore becomes more temperature sensitive. We believe that the sudden change in the reaction behavior (especially the activation energy) resulted from a temperature-induced disruption of the physical structure of cellulose.

Sasaki et. al. *(13,14)* reported a similar observation for cellulose hydrolysis in supercritical water. They found that cellulose is rapidly dissolved and depolymerized in supercritical water at 300–320°C with no acid. The cellulose disappearance rate under these conditions is far above the prediction by extrapolation of the Arrhenius equation from the data at 260–280°C. They reported a one-order-of-magnitude jump of hydrolysis rate when the temperature was raised from a subcritical temperature to near or above one critical temperature (approx 300°C).

To further prove the effect of physical state of α-cellulose on hydrolysis, we attempted to verify whether the kinetics is affected when the cellulose is physically altered before the reaction. Various solvents can alter the physical structure of cellulose. Some solvents including concentrated H_2SO_4 can dissolve cellulose. In the subsequent experiments, the cellulose substrate was pretreated with concentrated H_2SO_4 for 4 h at 25°C. Acid concentrations of 50, 55, 60, 65, 70, and 72% were applied. The resulting slurry/solution was then diluted with water to 4% acid. The substrates thus prepared were then subjected to further hydrolysis at 120°C and 4% acid concentration.

Figure 4 presents the hydrolysis profiles of cellulose pretreated with concentrated H_2SO_4. When pretreatment was done with 60% or less H_2SO_4, the hydrolysis of cellulose was extremely slow, basically at the same level as untreated cellulose. However, when α-cellulose was pretreated with 65% H_2SO_4 or higher, most of the cellulose was dissolved. When it was diluted with water, part of the dissolved cellulose was reprecipitated. The hydrolysis rate of the reprecipitated cellulose was two orders of magnitude higher than that of untreated α-cellulose. Beyond 65% H_2SO_4, the increase in hydrolysis rate was again gradual with respect to acid concentration. The reprecipitated cellulose was hydrolyzed at about the same rate as cornstarch under the identical hydrolysis condition. It is therefore reaffirmed that the hydrolysis reaction is indeed strongly influenced by the physical state of the cellulose. The acid hydrolysis as a rate process has two different types of resistance: reaction and physical. If so, the physical resistance is two orders of magnitude greater than the reaction resistance.

X-ray diffractograms were taken for the cellulose and reprecipitated cellulose. As shown in Fig. 5, the highly crystalline structure of untreated cellulose was totally disrupted and a completely different diffraction pattern with near zero crystallinity appeared after dissolution into 65% H_2SO_4 and reprecipitation. SEM photographs were taken for untreated α-cellulose and those treated with 55, 60, and 65% H_2SO_4. Untreated α-cellulose
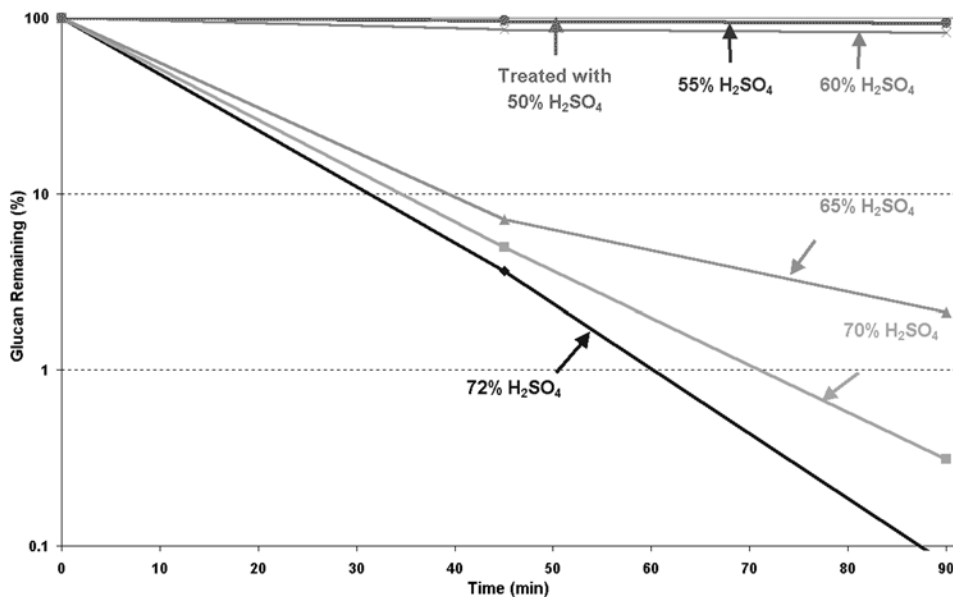
Fig. 4. Hydrolysis profiles of α-cellulose pretreated with various concentrations of H_2SO_4. Pretreatment was conducted at 25°C for 4 h. Hydrolysis was carried out at 120°C and 4% H_2SO_4.
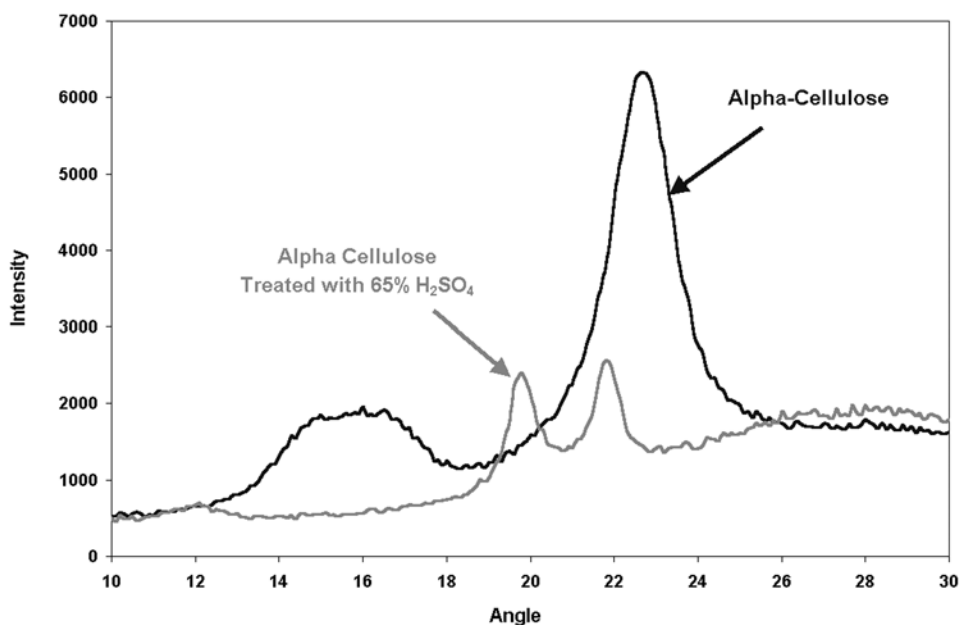

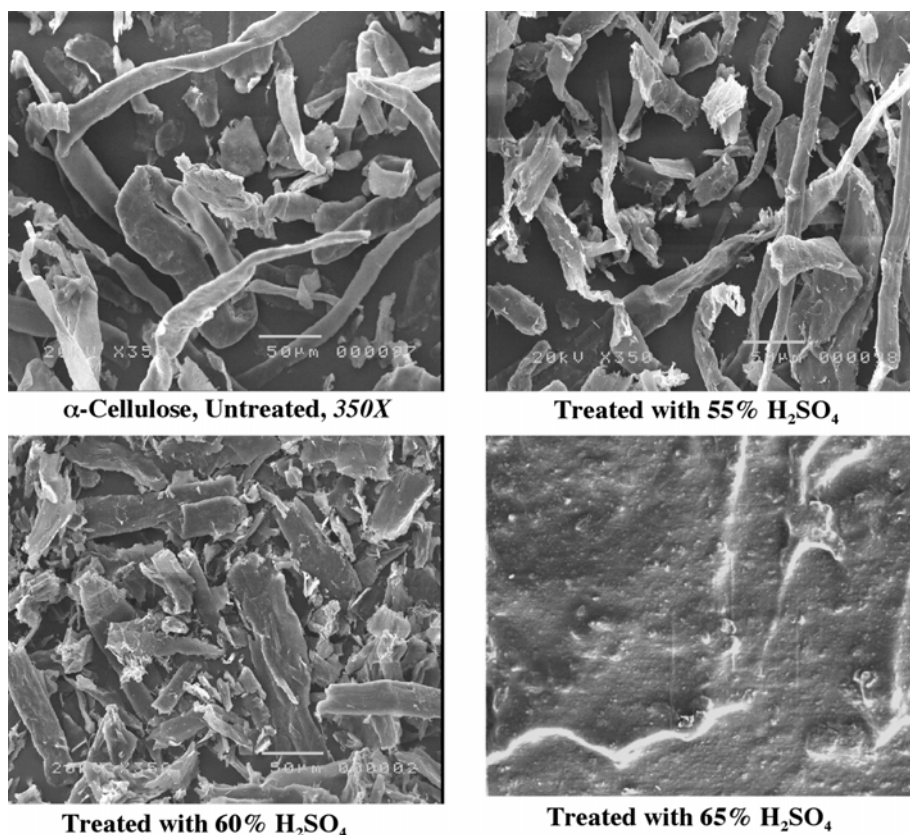Fig. 5. X-ray diffractograms of α-cellulose (original and treated with 65% H_2SO_4).

α-Cellulose, Untreated, *350X*   Treated with 55% H_2SO_4

Treated with 60% H_2SO_4 Treated with 65% H_2SO_4

Fig. 6. SEM photographs of α-cellulose treated with different levels of concentrated H_2SO_4.

and samples treated with 55 and 60% acid are seen to retain the original fibrous structure although the fibers were broken into smaller fragments by acid treatment (Fig. 6). However, the sample treated with 65% acid shows a completely different picture. The original fibrous form of cellulose disappeared and changed into a gel-like substance. When the cellulose fibers are dissolved into concentrated acid, the bundles of glucan chains are separated into multiple single chains. As the acid is diluted, the dissolved glucan chains reassociate. When this happens, the glucan chains do not go back to the original orderly structured fibrillar form but form an irregular bundle. We note that the change in cellulose structure owing to acid treatment is gradual to a certain point (60% acid in this case) and then undergoes a drastic change beyond that point. At higher temperature, it requires less concentrated acid to undergo this drastic change. For example, at 70°C, it requires only 50% H_2SO_4. We mentioned earlier a similar behavior with respect to temperature: a drastic increase in hydrolysis rate at a certain temperature. We have confirmed that these sudden changes in kinetic behavior and crystallinity are owing to structural changes in the cellulose.

The cellulose structure is closely related with the hydrogen bonding existing inside the cellulose chains. The existence of hydrogen bonds in cellulose molecule is well documented *(1)*. The hydrogen bonding exists within a single chain of glucan (intramolecular hydrogen bonding) and between the adjacent glucan chains (intermolecular hydrogen bonding). The intermolecular hydrogen bonds are believed to be the primary factor holding the cellulose chains together forming the fibrous structure. The state of hydrogen bonding in cellulose also determines other physical properties of cellulose, such as the extent of crystallinity.

Among all possible nonreaction factors (e.g., physical conformation, diffusion, crystallinity, chemical composition), the state of hydrogen bonding stands out as the primary factor controlling the main resistance in acid hydrolysis of cellulose. There is yet another support for this contention. Of all the physical factors, only hydrogen bonding can undergo such an abrupt change in reaction rate and structure in response to temperature and concentration of acid as seen in our experiments.

The state of hydrogen bonding is the primary factor determining the molecular level structure of cellulose. Kinetics of acid hydrolysis of cellulose is therefore strongly dependent on the state of hydrogen bonding . A better understanding of hydrogen bonding as to how it relates to the molecular structure of cellulose and finding an effective means to disrupt the hydrogen bonding may prove to be a fruitful way to establish acid hydrolysis as a viable biomass saccharification process.

Acknowledgments

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