*Ind. Eng. Chem. Res.* **2004,** *43,* 2781−2788

# The Effect of Flow Rate of Very Dilute Sulfuric Acid on Xylan, Lignin, and Total Mass Removal from Corn Stover

**Chaogang Liu and Charles E. Wyman***

*Thayer School of Engineering, Dartmouth College, Hanover, New Hampshire 03755*

Flowing compressed hot water through cellulosic biomass enhances removal of total mass, xylan, and lignin and increases cellulose digestibility compared to batch operations at otherwise identical conditions. Staged countercurrent operation with very dilute sulfuric acid has been shown to provide similar benefits. This study focused on developing comprehensive information on the combined effects of flow and very low concentrations of sulfuric acid (0.05−0.1 wt %) on hemicellulose hydrolysis in corn stover at 180 °C. A flow rate of 10 mL/min in a 3.8-mL reactor enhanced xylan removal by about 25% compared to batch operation at the same conditions. More than half of the dissolved xylan was oligomeric, and the oligomer fraction increased with flow rate but decreased with acid concentration. Increasing fluid velocity at a constant residence time also significantly accelerated xylan solubilization. Although lignin removal was limited to about 12% without flow, up to half of the lignin was swept out with flow, and lignin removal could be related to hemicellulose dissolution at high flow rates. Enhancement of xylan removal at higher flow rates could be attributed to replenishment of sulfuric acid to compensate for neutralization by biomass minerals, but this possibility does not account for similar observations without acid addition. Thus, it is postulated that both mass transfer and chemical reaction influence hemicellulose solubilization. Furthermore, it appears that hemicellulose oligomer−lignin compounds dissolve initially but release lignin fractions that are less soluble or form low-solubility condensation compounds when held for extended times.

## Introduction

Cellulosic biomass provides an inexpensive, renewable resource for production of ethanol as a liquid fuel for transportation and commodity chemicals that can have environmental, economic, and strategic benefits.[1−4] Potential feedstocks for large-scale production of such products include agricultural and forestry residues, municipal paper waste, and herbaceous energy crops. Generally, five primary operations are needed for enzymatic conversion of such cellulosic biomass sources to ethanol: pretreatment, cellulase enzyme production, enzymatic hydrolysis, fermentation, and distillation.[2] The first of these, pretreatment, is vital to breakdown the complex hemicellulose−lignin structure that shields cellulose and achieve the high yields of sugars from cellulose critical to commercial success. Moreover, pretreatment can affect other features of cellulose such as surface area and crystallinity that enhance performance and reduce the amount of lignin that can interfere with enzyme action and nonproductively bind enzyme.[5,6]

In the past half century, many pretreatment approaches have been investigated, including physical (e.g., ball milling and grinding, high-energy radiation, steam explosion), chemical (acid, base, and organic solvent as catalysts), and biological methods.[7,8] Dilute acid pretreatment, particularly using 0.7−3.0 wt % sulfuric acid, has proven to be very effective for realizing high hemicellulose solubilization and recovery and for achieving high yields in subsequent cellulose hydrolysis by enzymes.[9,10] Moreover, most of the soluble sugars from dilute acid pretreatment are monomers (primarily xylose) that can easily be fermented to ethanol by recombinant organisms.[11,12] As a result, dilute sulfuric acid is often favored for pretreatment.[13,14] However, hemicellulose sugar yields, while good, are still limited to about 90%, and more importantly, high cellulase loadings are needed to convert cellulose to glucose with high yields.[15] Fermentation inhibitors are also released or produced during dilute acid pretreatment that must be removed prior to subsequent hydrolyzate fermentation. Other limitations include expensive materials of construction and costs for acid and its neutralization. Consequently, dilute acid pretreatment is still among the most expensive steps in biomass conversion to fuels and commodity chemicals, and lower cost advanced technologies must be developed for cellulosic ethanol to compete as a pure transportation fuel.[1,16]

Flow of compressed hot water or hot very dilute sulfuric acid (~0.07 wt %) past cellulosic biomass displays a number of favorable pretreatment features including high hemicellulose sugars yields, high lignin removal, high cellulose digestibility, less chemical costs, and limited fermentation inhibitors.[17−22] For example, dilute sulfuric acid (0.07 wt %) pretreatment of poplar wood in a shrinking-bed countercurrent flowthrough reactor achieved almost theoretical yields of hemicellulose sugars and high cellulose digestibility by simultaneous saccharification and fermentation (SSF).[23] However, the large amounts of water consumed for flow systems result in very high energy use for both pretreatment and product recovery, and their equipment configurations are complex to implement.

Improving our knowledge of these flow-based systems could reveal new paths to enhancing pretreatment performance relative to dilute acid systems while also reducing costs. Some have reported that sugar recovery

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* To whom correspondence should be addressed. Tel.: (603) 646-3193. Fax: (603) 646-2277. E-mail: charles.e.wyman@dartmouth.edu.

10.1021/ie030754x CCC: $27.50

increases with water content in batch systems, suggesting that flowthrough performance may benefit from the large amounts of water used.[24−26] Our recent study showed that increasing flow rate significantly enhanced xylan solubilization for pretreatment of corn stover in just hot water.[21] Mechanisms such as mass transfer effects,[25] structured water,[27] the heterogeneous nature of the reaction,[28] and interactions due to molecular forces[29] have been proposed to explain the effects of flow on reaction rates, but the mechanisms responsible for enhanced performance when water passes biomass are still not clearly defined. We hypothesize that oligomer reactions and mass transfer play important roles in explaining the effects of flow on hemicellulose hydrolysis and that introducing acid can shift the balance between these factors. To evaluate this hypothesis, this study focused on gathering extensive new data on the effect of flow rate and fluid velocity of very dilute sulfuric acid solutions on removing xylan and lignin from corn stover.

## Materials and Methods

**Substrate.** The National Renewable Energy Laboratory (NREL) graciously provided corn stover from a large supply obtained from Harlan, IA, and maintained by NREL in Golden, CO. Material for this study was ground to a particle size of 250−420 $\mu$m using a laboratory knife mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) and passed through 35-mesh sieves to obtain small particles appropriate for the small reactors used. Samples prepared in this manner were kept in a freezer at −4 °C for all tests. The composition of a representative sample was 38.6% glucan, 20.6% xylan, and 17.6% Klason lignin as determined by standard NREL methods.[30]

**Flowthrough Apparatus and Experiments.** The flowthrough system was as described elsewhere.[21] The tubular reactor was constructed of 316 stainless steel VCR fitting parts obtained from Maine Valve and Fitting Company (Bangor, ME) and had an internal volume of about 3.8 mL. Two 316 stainless steel gasket filters with an average pore size of 5 $\mu$M were used at each end of the reactor to contain the biomass. A 316 stainless steel tubing was used for a $^1/_4$-in. o.d. × 0.035-in. wall thickness preheating coil and a $^1/_8$-in. o.d. × 0.028-in. wall thickness cooling coil and to connect the reactor to other system components. The preheating coil was sufficiently long for incoming water to reach the desired temperature before it entered in the reactor, as measured experimentally. A $^1/_8$-in. stainless steel thermocouple (Omega Engineering Co., Stamford, CT) was installed at the outlet of the reactor to monitor temperature. A high-pressure pump (Acuflow Series III Pumps, Fisher, Puerto Rico) with a flow rate range from 0 to 40 mL/min, a pressure gauge (Cole-Parmer Instrument Co., Vernon Hill, IL) with a range of 0−10.3 MPa, and a back-pressure regulator (Maine Valve and Fitting Co., Bangor, ME) were used to control water pressure and flow through the system.

To operate the flowthrough unit, about 0.5 g of corn stover was loaded into the reactor, and the reactor was then connected to the preheating and outlet coils. Distilled water at room temperature was pumped through the reactor for a few minutes to purge air, completely wet the biomass in the reactor, and pressurize the system to the set pressure of 2.1−2.4 MPa by controlling the back-pressure regulator. Next, the reactor and preheating coil were submerged in a 4-kW model SBL-2D fluidized sand bath (Techne Co., Princeton, NJ) set at 100 °C for 2 min and then quickly transferred to a second sand bath set at the desired reaction temperature. At the same time, defined as time zero, the pump was turned on. When the target reaction time was reached, the pump was turned off and the reactor and preheating coil were immediately transferred to an ice water bath to quench the reaction. Once the temperature in the reactor dropped to about 50 °C, cold water was pumped through the system to purge liquid remaining in the reactor and exit tube until about 10 mL of additional effluent was collected.

**Sample Analysis.** The pH of each liquid sample was promptly measured upon cooling to room temperature with a pH meter (model 8000, VWR Scientific). Then, a portion of the liquid was dried under vacuum at 60 °C until weight loss ceased to determine the fraction of the total mass dissolved in the liquid fraction. The rest of the sample was washed into a glass vial, dried at 105 °C, and weighed for sugar analysis and determination of Klason lignin as described below. The amount of lignin removed was calculated as the difference between the dry mass of Klason lignin in the feed material and that in the solid residue and expressed as the percentage of the dry mass in the feed material.

Compositional analyses of all solid and liquid samples were performed by standard methods.[30] Sugar monomers in the liquid portion were analyzed quantitatively by a Waters HPLC model number 2695 system equipped with a 2414 refractive index detector and a Waters 2695 autosampler using Millenium[32] chromatography manager 3.2 software (Waters Co., Milford, MA). A Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories, Hercules, CA) was employed for sugar analysis. Total xylose as both monomers and oligomers in the liquid fraction was determined after posthydrolysis with 4 wt % sulfuric acid at 121 °C for 1 h.[31] Xylo-oligomers in the liquid were calculated as the difference between the total xylose and monomeric xylose measurements and expressed as a percent of the original total xylose in the feed solids. All the experiments were performed in duplicate, with the average value reported.

## Results

**Impact of the Flow Rate on Removal of Total Mass and Xylan.** Initially, a range of flow rates was applied to our flowthrough reactor system for very dilute sulfuric acid (0.05 and 0.10 wt %) pretreatment at 180 °C, and performance only changed significantly up to a flow rate of 10 mL/min. Thus, more extensive investigations were conducted at flow rates of 0, 1, and 10 mL/min, as reported here. To facilitate comparisons between batch and flowthrough reactors, all experiments were conducted in the same experimental equipment so that flow rate was the only feature differentiating the experiments.

Increasing the flow rate over the targeted range enhanced total mass removal at 0.05 and 0.10 wt % sulfuric acid concentrations, as shown in Figure 1. For example, when flow rate was increased from 0 to 10 mL/min, total mass removal increased from 20% to 40% after 16 min at 180 °C with 0.05 wt % sulfuric acid. As expected, more total mass was removed as the acid concentration was increased, especially at the highest flow rate.

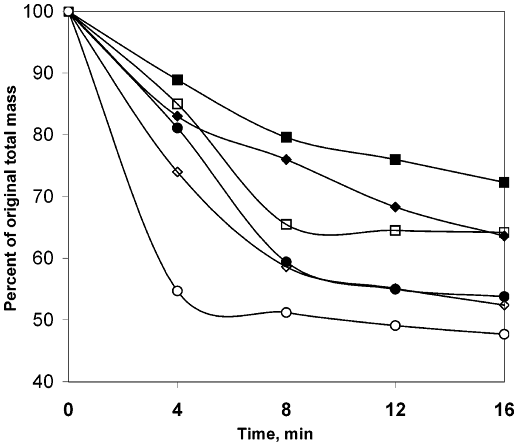

**Figure 1.** Effect of the flow rate and acid concentration on total mass remaining as solids for a very dilute acid pretreatment of corn stover at 180 °C: (■) 0.05 wt % acid, 0 mL/min; (◆) 0.05 wt % acid, 1 mL/min; (●) 0.05 wt % acid, 10 mL/min; (□) 0.1 wt % acid, 0 mL/min; (◇) 0.1 wt % acid, 1 mL/min; (○) 0.1 wt % acid, 10 mL/min.

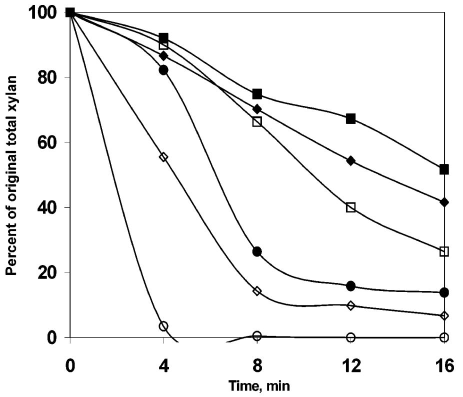

**Figure 2.** Effect of the flow rate and acid concentration on residual xylan for a very dilute acid pretreatment of corn stover at 180 °C: (■) 0.05 wt % acid, 0 mL/min; (◆) 0.05 wt % acid, 1 mL/min; (●) 0.05 wt % acid, 10 mL/min; (□) 0.1 wt % acid, 0 mL/min; (◇) 0.1 wt % acid, 1 mL/min; (○) 0.1 wt % acid, 10 mL/min.

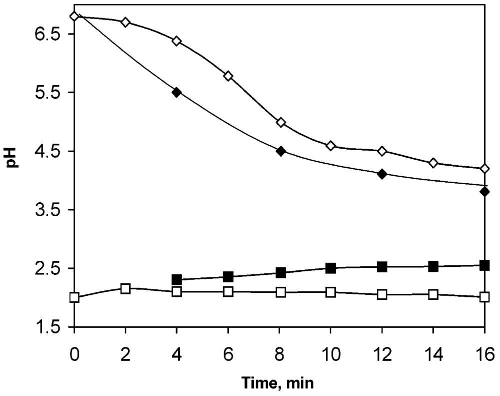

**Figure 3.** Change of pH in liquid fractions for hot water only and 0.05 wt % sulfuric acid pretreatment of corn stover at 180 °C: (■) 0.05 wt % acid, 0 mL/min; (□) 0.05 wt % acid, 10 mL/min; (◆) water only, 0 mL/min; (◇) water only, 10 mL/min.

Figure 2 shows that xylan removal was enhanced by increasing the flow rate for both 0.05 and 0.10 wt % sulfuric acid concentrations, especially early in the reactions for the highest flow rate runs. For example, when the flow rate was increased from 0 to 10 mL/min, xylan removal increased from about 40% to 95% after 16 min for 0.05 wt % sulfuric acid pretreatment of corn stover at 180 °C. As expected, the rate of xylan solubilization increased significantly with acid concentration.

**Possible Role of Acid Neutralization.** Flow rate appears to act somewhat like acid concentration and temperature in terms of its effect on xylan removal, a behavior that appears to be inconsistent with the first-order mechanisms traditionally applied to describe hemicellulose hydrolysis in terms of only temperature and acid concentration. However, as reported in the literature,[32,33] protein and ash in cellulosic biomass consume a significant portion of the acid added for pretreatment. For example, Maloney et al.[28]calculated that the sulfuric acid concentration for batch pulping at 170 °C decreased from 0.01 to 0.004 M due to neutralization by ash components in poplar wood, and biomass species differ significantly in their ability to neutralize acids.[32] Acid neutralization in this way might explain why flow rate affects xylan removal via a mechanism that is consistent with the effect first-order kinetic models.

To evaluate whether acid neutralization could account for the differences between batch and flowthrough results, the pH was measured for liquid effluent samples collected over intervals of a few minutes each from both batch runs and flowthrough runs. As shown in Figure 3 for pretreatment of corn stover with 0.05 wt % sulfuric acid at 180 °C, the pH increased with time for batch runs, supporting the idea that acid was neutralized. For example, after 16 min, the pH increased from about 2.00 to 2.55 as the sulfuric acid concentration dropped from 0.05 to 0.014 wt %, and the neutralization capability of corn stover is calculated to be 2.0−2.5 g of sulfuric acid/kg of corn stover at this temperature. On the other hand, the pH at the highest flow rate with 0.05 wt % acid stayed quite constant at about 2.0 throughout the run because of the continuous supply of acid.

When considering the effect of acid neutralization, it is instructive to consider that our previous studies showed that a limited portion of the total xylan was removed after 16 min of batch operation with compressed hot water at 180 °C, but more than half of original xylan could be removed at a flow rate of 10 mL/min at otherwise identical conditions.[21] In fact, the rate of xylan solubilization was faster and more xylan was removed for flowthrough operation with compressed hot water at a flow rate of 10 mL/min than for batch pretreatment with 0.05 wt % sulfuric acid, as illustrated in Figure 4. On the other hand, as shown in Figure 3, the pH decreased during compressed hot water pretreatment because of the release of acetyl groups from hemicellulose while the lowest pH for flowthrough operation at 10 mL/min over 16 min was around 4.5, higher than for either hot water batch (about 4.0) or 0.05 wt % acid batch (about 2.0) runs over that time period. Thus, although acid neutralization could potentially explain why flowthrough pretreatment removes xylan more rapidly than the batch system for very dilute acid pretreatment of biomass, neutralization cannot explain why flow rate enhances xylan removal when just compressed hot water is used. Thus, it is reasonable to conclude that other factors such as mass transfer likely play an important role in hemicellulose hydrolysis.

**Effect of the Velocity on Xylan Removal.** To further evaluate whether mass transfer effects could account for the change in performance with flow rate, we examined the impact of fluid velocity on very dilute acid pretreatment of corn stover at a constant residence

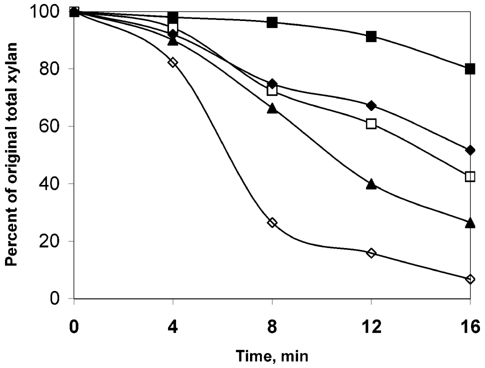

**Figure 4.** Effect of the flow rate and acid concentration on xylan removal for pretreatment of corn stover at 180 °C: (■) hot water only, 0 mL/min; (□) hot water only, 10 mL/min; (◆) 0.05 wt % acid, 0 mL/min; (◇) 0.05 wt % acid, 10 mL/min; (▲) 0.1 wt %, 0 mL/min.

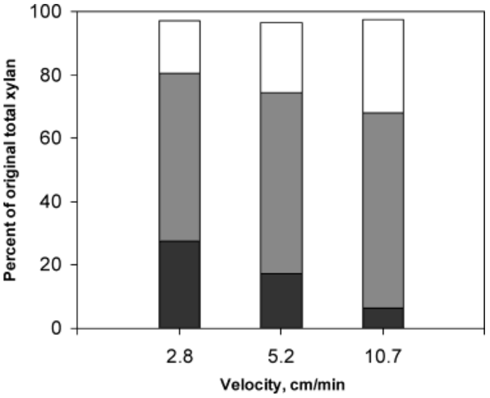

**Figure 5.** Effect of the fluid velocity on xylan remaining and dissolution after 12 min of pretreatment of corn stover in 0.05 wt % sulfuric acid at 180 °C: (black □) xylan remaining; (gray □) oligomers; (□) monomer.

time. In this case, flowthrough pretreatment with 0.05 wt % sulfuric acid was performed in three different reactors at a constant flow rate of 10 mL/min at 180 °C. These reactors each had the same internal volume (16.8 mL) but different diameters and lengths (10.9 mm i.d. × 180 mm L, 15.7 mm i.d. × 87 mm L, and 21.2 mm i.d. × 47 mm L) to give the same liquid residence time of 1.68 min in each but with different velocities of 10.7, 5.2, and 2.8 cm/min, respectively. The biomass loading for each reactor was about 3 g of dry corn stover.

Figure 5 shows that the xylan remaining in the solid residues dropped significantly with increasing velocity for this system after 12 min of 0.05 wt % sulfuric acid pretreatment of corn stover at 180 °C. For example, when the fluid velocity was increased from 2.8 to 10.7 cm/min, xylan removal increased from about 72% to 94% at this condition. These results also show that the yield of xylose monomers and oligomers in the hydrolyzate increase with velocity. A similar trend was seen for pretreatment of corn stover in hot water, as we reported previously.[34] This change in performance with flow rate strongly supports the concept that mass transfer is important in hemicellulose hydrolysis and suggests that increasing mass transfer can significantly accelerate the solubilization of hemicellulose for hot water or very dilute acid pretreatment of biomass.

**Monomer and Oligomer Yields.** Figure 6 illustrates how the highest flow rate in the flowthrough mode impacts the total yield of xylose monomer and oligomers over time for two acid concentrations compared to batch operation, and we can see that flow rate significantly enhanced the total xylose yield at each acid concentration. For example, parts A and B of Figure 6 show that the total xylose yield increased from 35% for batch operation to 95% for the flowthrough reactor at 10 mL/min when both were conducted in 0.05 wt % sulfuric acid for 16 min. Moreover, these results reveal that most of the dissolved xylan for these runs was as oligomers and that flow substantially increased xylose oligomer yields. Increasing the acid concentration increased the total yield of dissolved xylan for all of the times covered, as shown in Figure 6C,D. For example, when 0.1 wt % sulfuric acid was applied at a flow rate of 10 mL/min, the total xylose yield reached about 97% after only 8 min. Furthermore, the xylose monomer yield increased with increasing acid concentration for all runs at 180 °C in both the batch and flowthrough modes, but more than half of the dissolved xylan was still as oligomers for all but the 8-min sample for batch operation. These results suggest that increasing acid concentration enhances the dissolution of xylan and the reaction of oligomers to monomers. The total xylose yield increased with time for all runs, showing that the hold time was not sufficient for significant degradation to occur for the batch operation.

As shown in Figure 5, the total yield of xylose monomers and oligomers increased significantly with fluid velocity. For example, when the fluid velocity was increased from 2.8 to 10.7 cm/min, the total xylose yield increased from about 70% to 90% after 12 min of pretreatment with 0.05 wt % sulfuric acid. As before, most of the dissolved xylan was as oligomers for all velocities, and the oligomer yield increased with fluid velocity. A particularly interesting result was that the xylose monomer yield increased significantly with fluid velocity. For example, when the fluid velocity was increased from 2.8 to 10.7 cm/min, the xylose monomer yield nearly doubled from about 16% to 30% after 12 min even though the residence time was the same. Therefore, increasing fluid velocity not only accelerates the solubilization of hemicellulose as oligomers, but also enhances the formation of monomers.

**Lignin Removal.** As presented in Figure 7, only a small fraction of the original Klason lignin (less than 12%) was removed for the batch operations (flow rate = 0 mL/min) for both acid concentrations. However, when flow was introduced, lignin removal increased significantly with time and flow rate. For example, when 0.05 wt % sulfuric acid was passed through corn stover at a flow rate of 10 mL/min for 16 min, about half of the original Klason lignin was removed, and the amount removed increased slightly to about 54% when the acid concentration was increased to 0.1 wt % at the same flow rate. At the lower flow rate of 1 mL/min, however, lignin removal decreased with increasing acid concentration. For example, lignin removal dropped from 27.8% to 11.8% after 16 min when the acid concentration was increased from 0.05 to 0.1 wt % at a flow rate of 1 mL/min. Thus, lignin removal is enhanced by both flow rates but decreased by acid concentration.

## Discussion

**Proposed Mechanism for Hemicellulose Hydrolysis.** Traditionally, the reaction pathway for hemicellulose hydrolysis is pictured as the following sequence of reactions analogous to those applied by Saeman[35] to

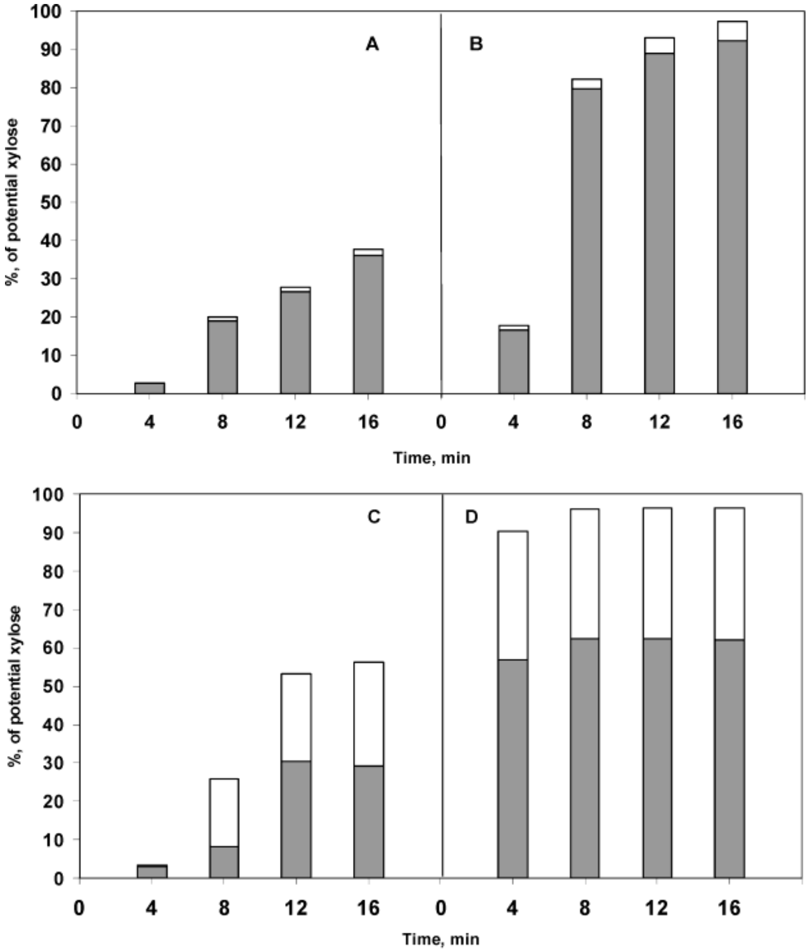

**Figure 6.** Yields of dissolved xylose as monomers and oligomers for pretreatment of corn stover at 180 °C with the following combination of sulfuric acid concentration and flow rates, respectively: (A) 0.05 wt %, 0 mL/min; (B) 0.05 wt %, 10 mL/min; (C) 0.1 wt %, 0 mL/min; (D) 0.1 wt %, 10 mL/min; (■) xylose oligomers; (□) monomeric xylose.

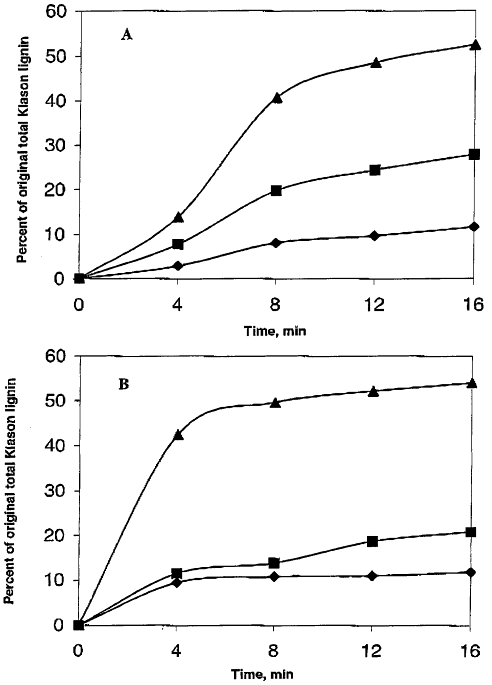

**Figure 7.** Effect of the flow rate on lignin removal for a dilute sulfuric acid pretreatment of corn stover at 180 °C at 0.05 wt % (top) and 0.10 wt % (bottom) sulfuric acid concentrations: (◆) flow rate of 0 mL/min; (■) flow rate of 1 mL/min; (▲) flow rate of 10 mL/min.

describe cellulose hydrolysis

$$\text{hemicellulose} \rightarrow \text{oligomers} \rightarrow \text{monomer} \rightarrow \text{degradation products}$$

Furthermore, the kinetics for this pathway are modeled as homogeneous first-order reactions, and according to this traditional approach, hemicellulose hydrolysis should only be impacted by temperature and acid concentration.[33] However, this model originally developed from batch experimental data cannot explain flowthrough performance satisfactorily. First, the change in hemicellulose removal with flow rate is inconsistent with this traditional kinetic model, and the impact of fluid velocity on release of oligomers suggests that mass transfer effects are important. Second, our experimental data showed that oligomers are important intermediates for very dilute acid or no acid hydrolysis of hemicellulose, and oligomers are typically not included in existing kinetic models. On the basis of these results, we postulated that the oligomer mass transfer may also play an important role in hemicellulose hydrolysis, especially at very low acid or no acid conditions.

Figure 8 presents a simplified representation of a possible pathway for hemicellulose hydrolysis that includes both chemical reaction and oligomer mass transfer. For this proposed approach, solid hemicellulose is assumed to break down to long-chain oligomers that can either pass into solution by mass transfer or break down further into shorter, more soluble short-chain

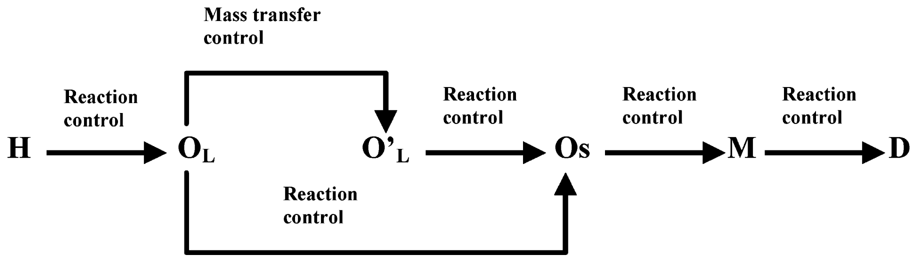

**Figure 8.** Possible pathways for hemicellulose hydrolysis: H, hemicellulose; $O_L$, undissolved long-chain oligomers; $O'_L$, dissolved long-chain oligomers; $O_S$, dissolved short-chain oligomers; M, monomers; D, degradation products.

oligomers that dissolve very rapidly, with these long-chain and short-chain designations being lumped parameters that represent a mixture of chain lengths. The short-chain oligomers rapidly break down to release sugar monomers that can subsequently degrade into furfural, tars, and other products. All of the steps involving a change in degree of polymerization are controlled by temperature and reactant concentrations as typical for chemical reactions. However, the dissolution of long-chain and short-chain oligomers are also controlled by mass transfer effects including the difference between the concentration in solid surface and bulk solution, the thickness of the diffusion layer, the viscosity of the solution, and the molecular size of the diffusing species. Furthermore, mass transfer is assumed to be far more important for long-chain than short-chain oligomers because the former are much less soluble and would diffuse much more slowly. However, the solubility and mobility of long-chain oligomers should increase with temperature.

According to this pathway, solubilization of solid hemicellulose would be controlled by both mass transfer and chemical reaction with their relative roles influenced by the reaction conditions. At relatively high acid concentrations, the importance of mass transfer could be significantly reduced because the high penetration of hydronium ions would rapidly hydrolyze long-chain oligomers to more rapidly dissolving short-chain oligomers and then to monomers. For such a case, the mass transfer step could be neglected and the pathway could be simplified to the traditional Saeman-like reaction model described above. On the basis of this interpretation, the performance of batch and flowthrough reactors should become quite similar at higher acid concentrations. For pretreatment without acid or with very low acid concentrations, however, the degradation of long-chain oligomers is expected to be very slow because of the very low hydronium ion concentrations. At the same time, the solubilization of long-chain oligomers is slowed by formation of an ice-like layer of water on the surface.[21,27] Therefore hemicellulose solubilization should be affected by both mass transfer and chemical reaction.

According to this postulate mechanism, any approaches such as flowthrough operation that increase mass transfer would significantly accelerate hemicellulose solubilization during no acid or very dilute acid pretreatment. Thus, hydrolyzate from flowthrough operations with compressed hot water should be rich in long-chain oligomers, especially at high flow rates because of the limited reaction of such species at these conditions. For flowthrough pretreatment with very dilute acid, however, the dissolved hemicellulose should contain both long-chain and short-chain oligomers, with their ratio depending on the flow rate, acid concentration, and temperature.

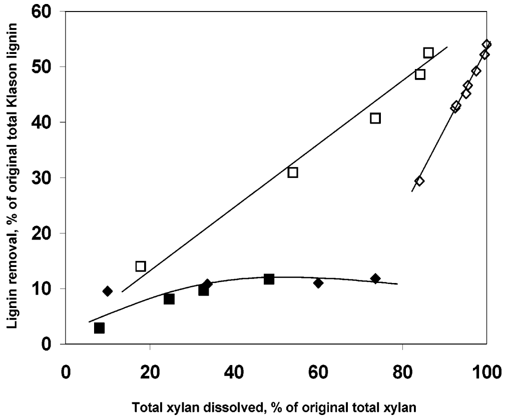

**Figure 9.** Lignin removal vs total xylan dissolved for batch and high flow rate pretreatment of corn stover with 0.05 and 0.10 wt % sulfuric acid at 180 °C: (■) 0.05 wt % acid, 0 mL/min; (□) 0.05 wt % acid, 10 mL/min; (◆) 0.1 wt % acid, 0 mL/min; (◇) 0.1 wt % acid, 10 mL/min.

**Proposed Mechanism for Lignin Solubilization and Removal.** A possible explanation for the change in lignin removal with flow rate and acid concentration is that lignin condensation reactions increase with temperature, acid concentration, and residence time, terms that can be combined as the pretreatment severity.[36,37] In batch operations, lignin is first released into solution during hemicellulose degradation as soluble species, likely in combination with hemicellulose oligomers, but with extended exposure to acid and heat, most of the dissolved lignin will react to condensation products that precipitate out of solution due to their lower solubility. On the other hand, as very dilute acid passes through the material, especially at high flow rates, the dissolved lignin will be removed before it can react and precipitate on the solids, thereby increasing lignin removal.

Although lignin condensation could explain why more lignin is removed for flowthrough than for batch reactors, it cannot explain the nearly linear relationship between the removal of lignin and xylan shown in Figure 9. At the highest flow rate, it appears that lignin solubilization is coupled to xylan release. Moreover, increasing the acid concentration reduces lignin removal at the same level of xylan removal. On the other hand, only very limited lignin was removed for batch operation and low flow rates no matter how much xylan was removed.

It is known that hemicellulose and lignin are covalently linked in native cellulosics,[2] and the relationship in Figure 9 could be explained by the release of hemicellulose oligomer−lignin compounds released during breakdown of hemicellulose. The high solubility of the hemicellulose oligomers in these compounds can facilitate their dissolution, and for operations at high flow rates, these soluble compounds can be removed before any further reactions occur. On the other hand, the dissolved compounds will break down further for batch or low flow rate runs to separate sugar oligomers and lignin fragments, and the latter can degrade to lower molecular weight species and form condensation products. Because lignin fragments and lignin condensation products have limited solubility, they are expected to drop out of solution. Thus, lignin removal would be low for batch and low flow rate systems, especially at comparatively high acid concentration and temperature.

## Conclusions

Increasing flow rate significantly enhanced the removal of total mass, xylan, and lignin for pretreatment of corn stover with very dilute sulfuric acid. In addition, more than half of the dissolved xylan was as oligomers, and their yield increased with flow rate, showing that oligomers are important intermediates during very dilute acid hemicellulose hydrolysis. The change in xylan removal with flow rate is inconsistent with first-order homogeneous kinetic models traditionally applied to describe hemicellulose hydrolysis even if consideration is given to acid consumption by neutralization with biomass minerals during pretreatment. On the other hand, increasing fluid velocity at a fixed residence time significantly accelerated removal of hemicellulose and increased degradation of oligomers to monomers. It is postulated that hemicellulose solubilization is influenced by parallel paths for mass transfer and chemical reaction of oligomers during compressed hot water or very dilute acid pretreatment of biomass. Thus, any approaches that can improve mass transfer such as flowthrough operations can significantly enhance hemicellulose solubilization at low acid concentrations.

For flowthrough pretreatment at high flow rates, lignin removal was as high as 54% and could be directly correlated with xylan solubilization. However, lignin removal for batch operations never exceeded about 12%. These results lead us to believe that lignin is initially solubilized and released together with hemicellulose, possibly as complex hemicellulose oligomer−lignin compounds, and that further degradation of these compounds impacts hemicellulose sugar yields and lignin removal. For flowthrough operation at high flow rates, these compounds are removed before subsequent reactions such as sugar decomposition and lignin condensation have time to occur to a significant extent, increasing lignin removal and sugars yields. For batch or low flow rate flowthrough runs, the released compounds further break down to separate sugar oligomers and lignin fragments that can further degrade to their monomers. Because the solubility of the lignin fragments and lignin condensation products is low, lignin removal drops at low flow rates and suffer more as acid concentration or temperature is increased.

## Acknowledgment

This work was funded by the Initiative for Future Agriculture and Food Systems (IFAFS) Program of the U.S. Department of Agriculture through Contract 00-52104-9663, and we are grateful for that support. The authors also thank the National Renewable Laboratory for kindly providing the corn stover used in this work. In addition, we appreciate the interactions with our partners in this IFAFS-funded project: Y. Y. Lee from Auburn University, Bruce Dale from Michigan State University, Richard Elander from the National Renewable Energy Laboratory, Michael Ladisch from Purdue University, Mark Holtzapple from Texas A&M University, and students and others from these organizations participating in this project. Finally, we acknowledge the Thayer School of Engineering at Dartmouth College for providing facilities and equipment to perform our research.

**Note Added after ASAP Posting.** This article was originally released ASAP on 4/8/04. A slight change to the title was made, and the new version was posted on 4/20/04.

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*Received for review* October 8, 2003
*Revised manuscript received* February 4, 2004
*Accepted* February 23, 2004

IE030754X