

Characterization of the degree of polymerization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water

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Abstract

Mechanisms that control xylan removal during pretreatment of lignocellulosic biomass are not well understood. For example, although hemicellulose hydrolysis is virtually always assumed to follow first-order homogeneous kinetics, the increase in xylan removal with flow rate for flowthrough pretreatment systems is inconsistent with the predictions for such models, and better information is needed to understand the causes of such discrepancies. Thus, new methods were developed to follow the fate of xylooligomers with degrees of polymerization of up to 30, a range not possible before, for water-only flowthrough pretreatment of oat spelt xylan and corn stover for temperatures of 200–240 °C. Material balances based on the oligomer release profiles produced by batch and flowthrough operations could be closed, suggesting the methods were quite accurate. However, the results also showed that increasing the flow rate from 0 to 2 and then 25 mL/min affected the size distribution of the xylan oligomers (DP < 30) released from corn stover but not from oat spelt xylan and also increased overall hemicellulose sugar solubilization. One explanation for these difference is that lignin and lignin–xylan compounds in particular play an important role in the hydrolysis of lignocellulosic biomass.

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1. Introduction

Autohydrolysis, as a pretreatment for enhancing cellulose digestion in lignocellulosic biomass, is based on the addition of just water at high temperatures and pressures in a batch or co-current flow mode, with the release of acetic acid postulated to catalyze the removal of hemicellulose and enhance cellulose digestibility (Allen et al., 2001a; Bobleter, 1994; Grohmann et al., 1985; Hsu, 1996; Torget et al., 1990; von Sivers, 1995). Important advantages of this approach compared to use of acid are that chemical and materials of construction costs are reduced significantly and cellulose degradation is low. However, autohydrolysis

in batch systems suffers from low hemicellulose sugar yields of about 65% or less (Heitz et al., 1991). A number of groups have shown that passing just water in a flowthrough mode through lignocellulosic biomass can improve hemicellulose sugar yields to over 90% and also achieve nearly theoretical cellulose digestibility (Allen et al., 2001b; Bobleter et al., 1981; Bonn et al., 1983; Liu and Wyman, 2003; Torget, 2000; Torget et al., 1998). Furthermore, while batch pretreatment with just water removes little of the lignin, flowthrough pretreatment with hot water removes up to 75% (Bobleter and Concinn, 1979; Liu and Wyman, 2003; Mok and Antal, 1992). The nearly linear relationship observed between lignin and xylan removal in flowthrough systems suggests that complexes of lignin and xylose oligomers dissolve during hemicellulose hydrolysis. The complexes are then removed from the reactor before the lignin compounds can break away and repolymerize as

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precipitates as observed for batch operations (Liu and Wyman, 2003). The apparent coupling of lignin and hemicellulose release during hemicellulose hydrolysis also suggests that disruption of lignin plays a key role in realizing highly digestible cellulose (Yang et al., 2004; Yang and Wyman, 2004).

The mechanism responsible for increasing xylan removal with flow rate is not fully understood. In particular, this observation is inconsistent with the predictions of the first-order homogeneous kinetic models widely applied to describe hemicellulose hydrolysis, as these can only account for the effects of time, acid concentration, and temperature (Liu and Wyman, 2003). Recent research suggested that increased flow rate may augment mass transfer, and thus the heterogeneous nature of the substrate may account for some of these differences (Brennan, 2003; Liu and Wyman, 2003). Greater solubilization of xylan oligomers with large amounts of water could also account for the change in reaction rate with flow rate and solids concentration (Gray et al., 2003; Jacobsen, 2000; Li et al., 2003). However, none of these possible explanations are yet proven, and the current study focused on gaining new insight into the release of xylan oligomers during hemicellulose hydrolysis for batch versus flow systems. In particular, we developed techniques to characterize a wider range of oligomer chain lengths than possible before to provide more in depth information on the yield of short and long-chain xylose oligomers, the ratio of short- to long-chain oligomers, and the distribution profile of short chain oligomers in addition to total xylan yields. When we tested this new method on batch and flow-through hydrolysis of corn stover and pure oat spelt xylan at 200, 220, and 240 °C, we found significant differences in the oligomer profiles for hydrolysis of these two test substrates.

2. Methods

2.1. Feedstocks

The corn stover used in this study was graciously provided by the National Renewable Energy Laboratory (NREL) in Golden, Colorado from a large lot they obtained from Harlan, Iowa and maintained at controlled conditions. This material was milled to pass through a 2 mm opening and then screened to obtain a -420 to $+250$ μm fraction that was stored in plastic Ziploc bags and kept in a freezer (-20 °C) as a single source for all tests. The composition of the corn stover, as determined through NREL LAP procedures 001, 002, and 012 (NREL, 2004), was found to be 37.8% glucan, 21.3% xylan, 1.6% arabinan, 3.8% mannan, 1.4% galactan, 17.8% lignin, and 7.8% ash by dry weight. Oat spelt xylan was obtained from ICN (Aurora, OH) and had a composition of about 78.9% xylan. Xylose was obtained from Sigma (St. Louis, MO).

2.2. Batch reactor

The 0.5" OD \times 0.035" wall thickness \times 6" long Hastelloy C-276 batch reactors used in this study were cut from tubing (Maine Valve and Fitting Co., Bangor, ME). Although each reactor had a total working volume of 14.3 mL, only 6 mL of feedstock was added to provide enough room for thermal expansion during heat-up. Thermocouple probes (Omega CASS-18U-12, Omega Engineering Co., Stamford, CT) were inserted along the centerline of the tubes and pushed 3/16" and 2" into the reactor to follow the temperature during pretreatment. Reaction temperatures of 200, 220, and 240 °C were applied for water only pretreatment with total initial solids concentrations of 5%.

Because temperature transients can impact biomass hydrolysis in batch tubes, a three bath heat-up procedure based on a prior modeling study was used to minimize these effects (Stuhler and Wyman, 2003). The sequence began by preheating each tube in boiling water for 2 min followed by immediately transferring it to a sand bath (model SBL-2D, Techne Co., Princeton, NJ) set at 20 °C above the target reaction temperature for 60 s. Then, the tube was transferred to a second sand bath set at the target reaction temperature, with the time of this transfer defined as zero. After being subjected to the target reaction temperature for a specified time, the tubes were quickly transferred to an ice water bath to quench the reaction. Next, the tubes were taken from the ice water and dried, and the end caps and plugs were removed. The contents were pushed out and separated into liquid hydrolysate and solid fractions by filtration for analysis (Lloyd and Wyman, 2003; Stuhler, 2002).

2.3. Flowthrough reactor

A 0.5" OD \times 6" long Hastelloy C-276 tubing with a 0.035" wall thickness and an internal volume of 14.3 mL was employed as the flowthrough reactor. All reactor parts were obtained from Maine Valve and Fitting Co. (Bangor, ME). A 0.25" stainless steel thermocouple (Omega CASS-18U-12, Omega Engineering Co., Stamford, CT) was installed at the outlet of the reactor to monitor temperature. A section of 316 stainless steel tubing (0.25" OD \times 0.35" wall thickness \times 50" long) was used to preheat the water flowing into reactor, and another section of 316 stainless steel tubing (0.25" OD \times 0.35" wall thickness \times 50" long) was installed as a cooling coil for the effluent. Two grams of corn stover or oat spelt xylan was loaded into the reactor, and the inlet and outlet tubes were then connected to the reactor. Distilled water at room temperature was pumped through the system first to completely wet the feedstock and bring the system to the desired pressure and then the flow was stopped. Next, the reactor and preheating coil were submerged for 2 min in a 4-kW fluidized sand bath (model SBL-2D, Techne Co., Princeton, NJ) set at a temperature of 100 °C and then moved to a second sand bath set at the target reaction temperatures of 200,

220, or 240 °C. Flow was started at that time and maintained until the desired reaction time, at which point flow was terminated, and the reactor and preheating coil were transferred to an ice water bath to stop the reaction (Liu and Wyman, 2003; Yang and Wyman, 2004). The liquid hydrolysate and residual solid were removed and separated by filtration.

2.4. Analytical procedures

Sugar and acid insoluble lignin content of solids were determined using the Klason lignin procedures published as NREL LAP 003 and 014 (Ruiz and Ehrman, 1996; Templeton and Ehrman, 1995). Sugar concentrations were measured by a high performance liquid chromatography (HPLC) system (Waters 2695, Milford, MA) equipped with a pulsed refractive index detector (Waters 2410, Milford, MA). The column was equilibrated with deionized water at a flow rate of 0.6 mL/min. An Aminex HPX-87P (Bio-Rad, Sunnyvale, CA) column was used for quantification of xylose concentrations.

2.5. Determination of total oligomers

Liquid hydrolysate samples were autoclaved in 4% sulfuric acid for 1 h at 121 °C to breakdown oligomers into monomeric sugars as described in the NREL methods (NREL, 2004). Sugar standards containing known sugar concentrations were also autoclaved for the same time and at the same acid concentration to estimate hydrolysis loss factors. The total amount of oligomers in the liquid sample was then calculated as

$$\text{Oligomers(g)} = \text{Total xylose(g) in the hydrolysate corrected for degradation} - \text{Monomers(g) in the hydrolysate liquid before autoclaving.}$$

This quantity was then divided by the sugar content of the initial biomass material to calculate the yield of oligomers.

2.6. Degree of polymerization determination for xylooligomers

The liquid hydrolysate described above was passed through a 0.2 µm syringe filter (Gelman Sciences, Ann Arbor, MI) and then analyzed by the Dionex DX-600 Ion Chromatograph (IC) system described above to quantify xylan oligomer chain lengths over a degree of polymerization (DP) range of 1–30, the maximum the system could isolate. The IC system consisted of a BioLC[®] GP50, ED50 with Cell Au electrode, AS50 with TC and 1.5 mL tray (Dionex, Sunnyvale, CA) equipped with a CarboPac[™] PA-100 column set (4 mm, Dionex, Sunnyvale, CA). The column was run at 30 °C with an eluent flow rate of 1 mL/min. The gradient method consisted of eluting 20–150 mM NaOAc over 30 min according to Curve 6 (150 mM NaOH throughout) based on the detection wave-

form from Dionex Technical Note 21 (Dionex, Sunnyvale, CA). Xylan oligomers concentrations with DPs from 1 to 5 could be calculated by comparison to results with known amounts of standards (xylobiose, xylotriose, xylo-tetraose, and xylopentaose) obtained from Magazyme International Ireland (Bray, County Wicklow, Ireland). However, because such materials were not available for DPs greater than 5, we calculated the concentration of each of these species by taking the ratio of each peak height to the peak height for xylobiose and multiplying this ratio by the measured concentration of the latter according to a procedure we developed previously (Li et al., 2003).

3. Results

To test the new analytical method and gain new insight into the effect of flow versus batch operation on xylose removal during biomass hydrolysis with just water, the HPLC system described above was used to follow the release of xylose monomer and oligomers into solution over time for hydrolysis of corn stover and oat spelt xylan for batch operation and selected flow rates with the flow-through system. Fig. 1 presents an example chromatogram for the liquid hydrolysate that resulted following water-only hydrolysis of corn stover with a 5% solids concentration at 200 °C for 10 min in the batch system. As shown, the CarboPac[™] PA-100 column separated oligomers up to DP30. The degree of polymerization distribution data for application of this approach to hydrolysis of corn stover and xylan for batch operation and also for flow with 200 °C water at 2 and 25 ml/min is summarized in Fig. 2. We see that a large fraction of the solubilized xylan was as monomers and lower DP oligomers for corn stover (Fig. 2a), with the distribution shifting toward higher DP oligomers as the operation was changed from batch to flow of 2 and then 25 ml/min of water. On the other hand, although the balance is predominately lower DP oligomers for hydrolysis of pure xylan in batch operation, it shifted dramatically to very high DP values for both flow rates (Fig. 2b). In addition, although there is a slight shift toward higher DP oligomers with increased flow, the yields are quite similar for both low and high flow rates.

Because no standards were available to calibrate the measurements of concentrations of DP greater than 5, it was important to test the validity of quantifying the concentrations of xylooligomers with a DP greater than 5 based on the ratio of their peak heights to that for xylobiose. The total xylooligomers released into the liquid for both corn stover and oat spelt xylan were added to that left in the solid for each run condition to determine how well material balances could be closed. To facilitate following the plots of these results, the fate of xylan was grouped into five categories: (1) xylooligomers that were measured based on direct IC calibration (DP 1 through 5), (2) xylooligomers with a lower DP range from 6 to 10 that were quantified based on their peak height compared to xylobiose, (3) xylooligomers with a higher DP range from 11 to 30 that

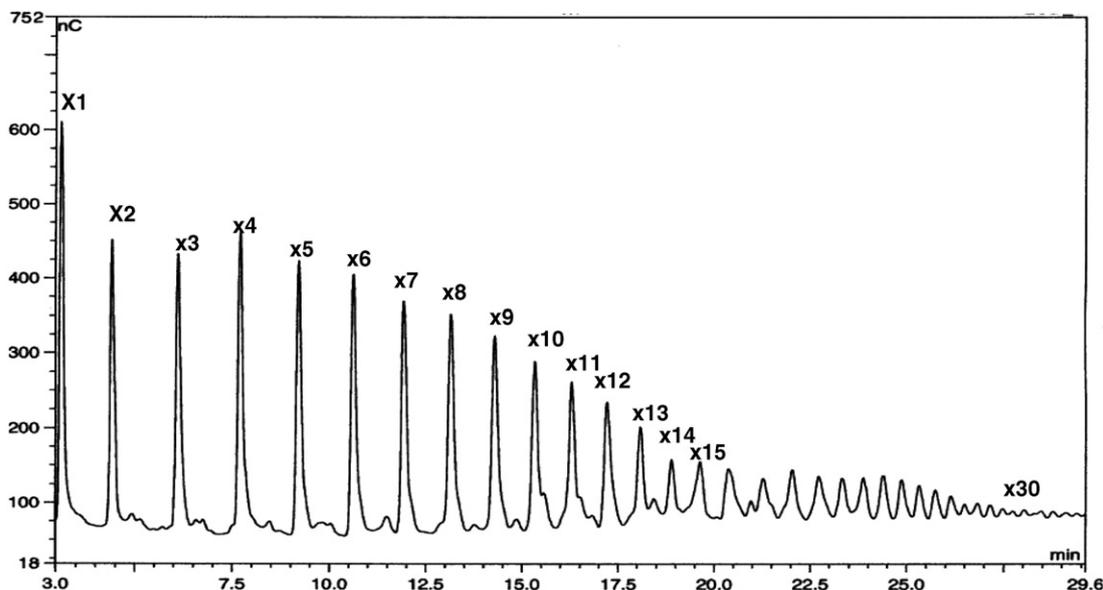


Fig. 1. IC chromatogram for hydrolysate from water-only hydrolysis of corn stover for a 5% solids concentration at 200 °C for 10 min showing degree of polymerization range from 1 to 30.

were also quantified based on the ratio to the xylobiose concentration, (4) dissolved xylooligomers with DPs greater than 30 that were determined by post hydrolysis, and (5) xylan left in the solids measured by post saccharification. As shown in Fig. 3 and summarized in Table 1, the total amount of xylooligomers accounted for in this way was nearly equal to the total originally available for the flowthrough system for both corn stover and xylan, consistent with flowthrough systems removing oligomers so rapidly that they have limited opportunity to degrade. On the other hand, the total xylose accountability was somewhat less for batch hydrolysis of both substrates, no doubt due to some degradation resulting from longer exposure to high temperatures even at residence times far short of those required to realize maximum yields. Thus, although not conclusive, the results in Fig. 3 support the accuracy of the oligomer characterization method.

Although the closure of xylan material balances was very good for both corn stover and xylan, the oligomer release patterns in Figs 2 and 3 and Table 1 differed significantly for the two substrates. Similar to previous results, increasing flow rate enhanced xylan removal for corn stover hydrolysis at 200 °C: at a flow rate of 2 mL/min, for example, about 57% of the original xylan dissolved after 10 min, whereas at a flow rate 25 mL/min, about 78% dissolved in that time. In addition, a wide range of DPs were dissolved during corn stover hydrolysis, with a substantial fraction being monomers and low DP oligomers, and the distribution shifted significantly toward higher DP oligomers as the flow rate increased. On the other hand, batch hydrolysis of oat spelt xylan resulted in dissolution of a much greater fraction of the total and gave a much higher fraction of high DP oligomers under all conditions than corn stover. This trend is shown in Table 1 in which batch hydrolysis of corn stover for 10 min at 200 °C led to 38.1%

of the total original xylan being recovered as dissolved monomers and oligomers and 28.1% as short chain xylan oligomers (DP1 ~ 30), whereas the same operation with oat spelt xylan resulted in dissolution of 73.1% of the total xylan with 30.1% being short chain oligomers. The DP also shifted to all oligomers having a DP over 30 at both flow rates for oat spelt xylan, and xylan removal was almost the same (around 92% at 10 min) for each flow rate. Thus, overall xylan solubilization was very sensitive to flow rate for corn stover, while yields with oat spelt xylan increased dramatically as operation was first shifted from batch to flow but changed little with flow rate beyond that.

Ratios of shorter (DP 30 or less) to longer chained xylooligomers (DP > 30) were also calculated, as shown in Table 1. Batch hydrolysis of corn stover resulted in a 2.8:1 ratio of shorter to longer chain xylooligomers, but the ratio dropped to 0.7:1 and 0.1:1 when the flow rate was increased to 2 mL/min and 25 mL/min, respectively. Remarkably, an increase in flow rate from 2 mL/min to 25 mL/min did not affect the ratio of shorter to longer chain oligomers much for oat spelt xylan hydrolysis, with the value remaining at about 0.003. On the other hand, batch hydrolysis of oat spelt xylan gave a much higher ratio of shorter to longer chain oligomers (about 0.7:1) than did flowthrough (about 0.003:1), because batch operation gave the oligomers a longer time to break down into shorter fragments than flowthrough operation (Liu and Wyman, 2003; Stuhler, 2002).

Data were also generated for hydrolysis of corn stover and oat spelt xylan solids for reaction times of 2 and 5 minutes, as summarized in Fig. 4. Consistent with Fig. 3, yields of dissolved xylan increased with flow rate for corn stover at all times and also continually increased with time. Interestingly, although yields of oligomers in solution increased continually with time for oat spelt xylan, flow rate had little

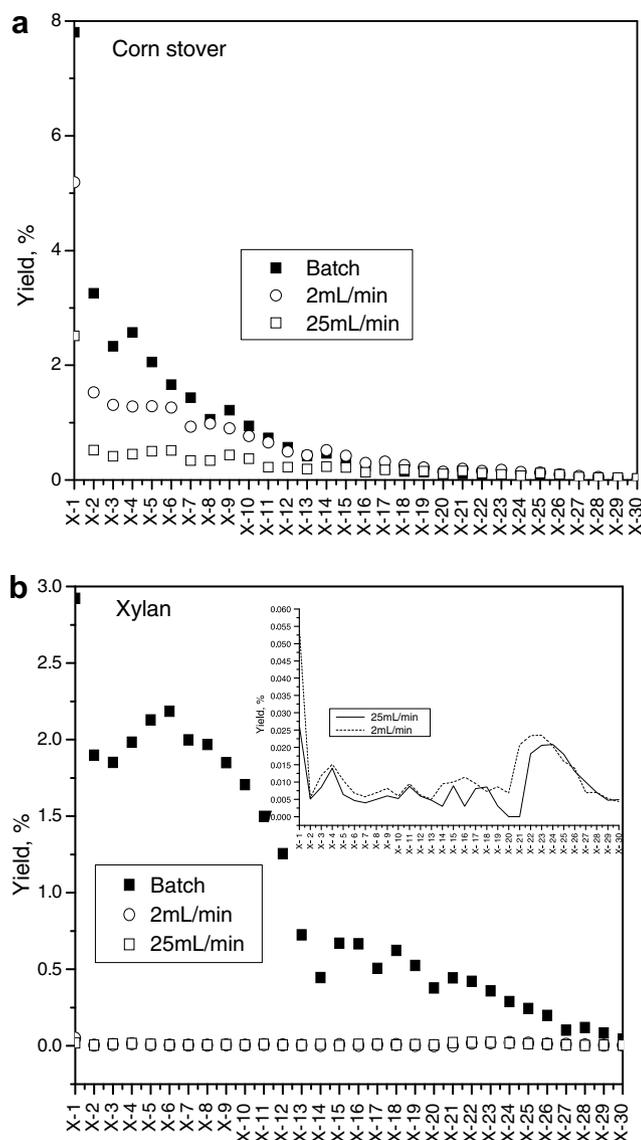


Fig. 2. Effect of flow rate on the distribution of DP 1 through 30 xylan oligomers after hydrolysis of corn stover (a) and oat spelt xylan (b) for 10 min at 200 °C.

effect on the yields of xylan in solution once flow was initiated. Furthermore, although a wide range of oligomer DP values were observed for corn stover at each flow rate, only high DP oligomers were measured for oat spelt xylan (data not shown).

We also studied the effect of temperatures of 200, 220, and 240 °C on total xylan removal and degree polymerization of the solubilized oligomers released during corn stover hydrolysis in batch and flowthrough operations. As shown in Fig. 5 for the oligomers formed with DPs from 1 to 10 after 10 min of reaction, the yields of lower DP oligomers increased with temperatures at all times and for all modes of operation. Furthermore, higher temperature operation under batch conditions increased monomer yields somewhat as higher DP species were hydrolyzed. In addition, more low DP xylooligomers were formed at

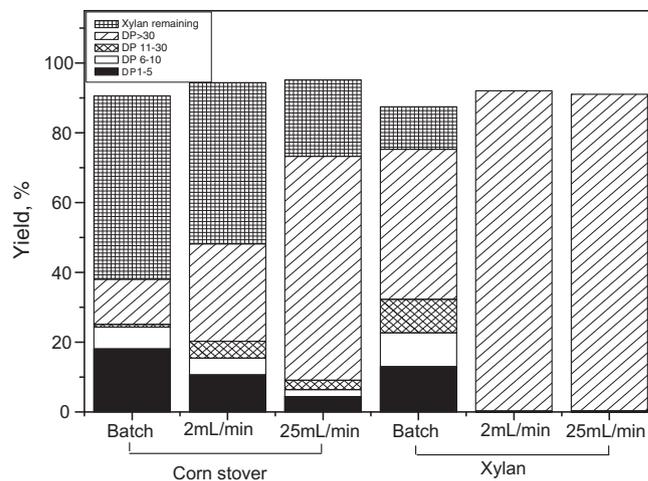


Fig. 3. Distribution of degree of polymerization and xylan mass balance for water-only hydrolysis of corn stover and oat spelt xylan at 200 °C for 10 min.

Table 1

Yield of xylan oligomers (DP < 30) and total xylan recovery in the hydrolysate after treatment of corn stover and oat spelt xylan at 200 °C for 10 min

Feedstock	Flow rate (mL/min)	Yield (%)			Ratio of shorter chain to longer chain oligomer
		Total xylan recovery ^a	DP 1–30	Long chain oligomer ^b	
Corn stover	0 (Batch)	38.1	28.1	10.0	2.8
	2	48.2	20.3	27.9	0.7
	25	73.3	9.1	64.2	0.1
Oat spelt xylan	0 (Batch)	73.1	30.1	43.0	0.7
	2	92.1	0.3	91.8	0.003
	25	91.1	0.4	90.8	0.004

^a Total xylan recovery = yield of xylose in hydrolysate + yield of oligomers in hydrolysate (xylose equivalent).

^b Yield of long chain oligomer (DP > 30) = total xylan recovery – yield of DP1 ~ 30.

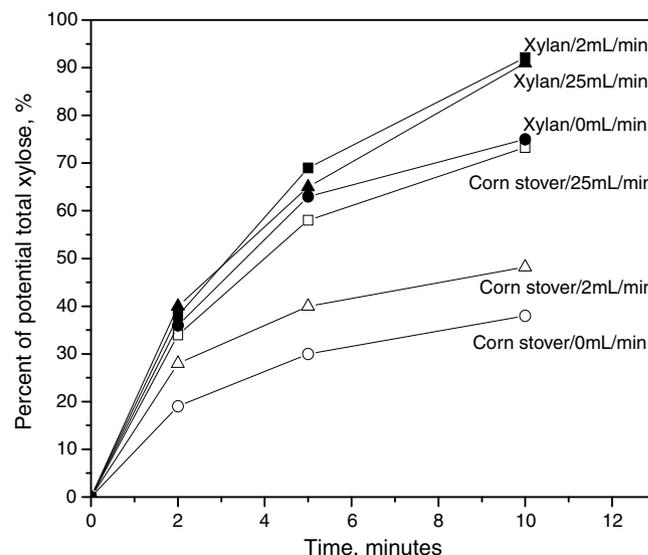


Fig. 4. Effect of flow rate on xylan removal from corn stover and oat spelt xylan at 200 °C.

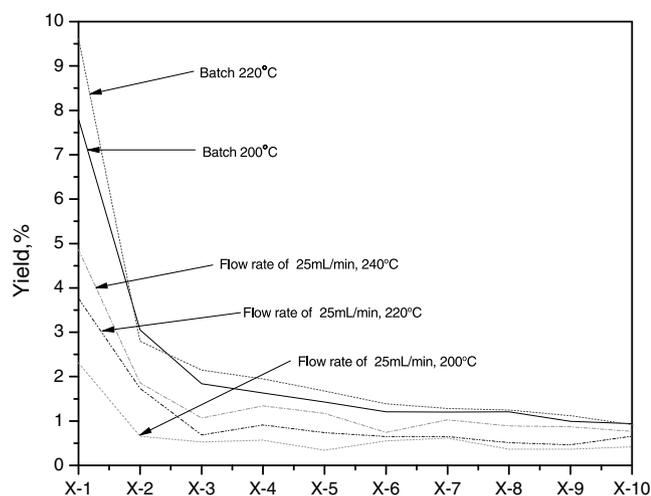


Fig. 5. Effect of flow rate and temperature on the distribution of xylan oligomer chain lengths in hydrolysates collected for hydrolysis of corn stover for 10 min.

higher temperatures in flowthrough operation even at the higher flow rate of 25 mL/min.

4. Discussion

Xylooligomers produced by hydrolysis of corn stover and xylan in just water with degree of polymerizations as high as 30 were separated using a new IC procedure. The sum of the yields of all of the oligomers determined in this way, the yield of oligomers with DPs greater than 30 determined by post hydrolysis, and the amount of xylan left in the solids was about equal to the total amount of xylan originally available for the corn stover and xylan materials used in the flowthrough reactor, suggesting that calibration based on xylobiose was reasonably accurate. The somewhat lower overall recovery of xylan for batch pretreatment is expected because of the greater time for degradation of dissolved species (Allen et al., 2001a). Thus, the IC method appears to quantify the fate of xylooligomers well.

The data also showed that the yield of dissolved xylose increased with flow rate for both corn stover and xylan, consistent with past results (Bobleter, 1994; Bobleter et al., 1983; Hoermeyer et al., 1988; Liu and Wyman, 2003). However, new insight from this analytical method showed that the balance in xylooligomer degree of polymerization increased with flow rate for both corn stover and oat spelt xylan. This result could be attributed to the greater amount of water enabling more of the longer oligomers to dissolve at higher flow rates so they do not continue to react in the solid phase. On the other hand, the higher flow rate could also remove dissolved oligomers from the reactor before they can hydrolyze as far.

An increased flow rate during corn stover hydrolysis increased the total xylose yield and the recovery of long-chain xylan oligomers but decreased the yield of short

chain oligomers. However, an increase in flow rate did not similarly affect yields and solubility for oat spelt xylan hydrolysis. These differences would not be expected for the typical first-order kinetic models applied to describe hemicellulose hydrolysis, giving rise to an important question: why did different substrates show different responses to the same change in flow rate at the same conditions? The release of more high DP xylooligomers into solution would be expected with more water based on oligomer solubility studies (Gray et al., 2007), but other factors would appear to cause the differences in distribution of DP profiles between corn stover and xylan. One possibility could be corn stover contains more pores that could trap higher DP oligomers alternatively, because corn stover contains a significant amount of lignin that is covalently bonded to hemicellulose, hemicellulose could be anchored to the solid until short enough fragments are formed that are free to be released into solution while release of oligomers from pure xylan would not be impeded in this fashion. Thus lignin is in effect holding onto long chain xylooligomers for some time before being cleaved by degradation to short chain oligomers. In addition, because lignin has a much lower solubility than xylan, it may be necessary to form lower DP xylan fragments with a high enough solubility to compensate for the limited solubility of the attached lignin, as proposed previously (Matsushita et al., 2004; Vazquez et al., 1997). Such a mechanism is consistent with results from studies in which more lignin was removed in flowthrough reaction of corn stover than in batch or co-current flow systems (Jacobsen and Wyman, 2002; Liu and Wyman, 2003; Yang et al., 2004). Once in solution, these fragments continue to react, releasing lignin for precipitation back on the solids either as is or as condensation products. Thus, while most of the lignin appears to simply remain in the solid during batch pretreatment, we believe that it forms soluble products for a short time and then precipitates out of solution. This results is even more likely in batch reactions because the lignin–xylooligomer bonds have more time to break (Liu and Wyman, 2003; Yang and Wyman, 2004). But because more lignin–xylooligomers will be dissolved when flushed with a large amount of hot liquid, it is proposed that in flowthrough reactions a larger fraction of oligomers are swept out of the reactor. This concept is supported by the higher ratio of long-chain to short-chain oligomers as higher flow rates. It is further supported by the longer residence time for the low flow rate with corn stover reaction resulting in an increase in short-chain oligomer recovery and a simultaneous decrease in lignin–xylan oligomer solubility and total xylan recovery. The drop in DP with temperature at high flow rates with corn stover suggests that solubility is not limiting, consistent with the idea that xylooligomers could not be released into solution until they reached a low enough DP to either break free from the lignin or to compensate for the lower solubility of attached lignin fragments. Overall, these results indicate that lignin–xylan oligomers and their solubility could have a large effect on the rates and yields of

lignocellulosic biomass pretreatment, although little attention has been paid to their interactions (Shigematsu et al., 1994, 1995). The nature of lignin should also be considered, as it affects the solubility of lignin–xylan-oligomers. (Shevchenko et al., 1999; Shigematsu et al., 1994, 1995).

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