Solubilities of Oligomer Mixtures Produced by the Hydrolysis of Xylans and Corn Stover in Water at 180 °C

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This paper investigates the solubility limitations of oligomers in solutions produced by the hydrolysis of commercial xylans and corn stover in just water at 180 °C. These experiments show that higher degree of polymerization (higher-DP) oligomers precipitate when hydrolyzates of birchwood and oat spelt xylan are cooled from 80 to 26 °C. Furthermore, even dilution by a factor of 10 at 80 °C is not sufficient to prevent precipitation upon cooling to 26 °C. Fewer high-DP oligomers precipitate from corn stover hydrolyzate solutions than from commercial xylans at the same solids concentrations, likely because of its lower fraction of hemicellulose. Overall, solubility limits do not seem to be significant during reaction up to the highest solids concentration investigated (18% at 180 °C), although these limitations are very important as the liquid hydrolyzate is cooled from 80 °C to 26 °C. These results indicate that oligomer yields from batch xylan hydrolysis could be considerably greater if the oligomers were separated from the solids while hot, rather than allowing the hydrolyzates to cool before analysis, as typically reported.

Introduction

Pretreatment of biomass with just water in a batch/co-current reactor offers some important advantages. Unlike acid pretreatment, neutralization and expensive materials of construction are avoided, and, contrary to flow systems, large volumes of water are not needed. However, in batch water-only systems, few researchers have reported hemicellulose sugar yields in excess of 80%,[1] with most yields being <65%.[2] On the other hand, yields as high as 95% have been attained in flow systems.[3] In addition, it has been observed that xylose yields increase at low solids loadings for batch pretreatment of corn stover in just water.[1,4] These differences imply that large volumes of water are necessary to obtain high yields, possibly because mass-transfer and/or solubility limitations are important.

During pretreatment in water at high temperatures, it is postulated that hemicellulose, which is insoluble at room temperature, is hydrolyzed to shorter chain length oligomers that have greater solubility and dissolve as the chains decrease in length with reaction.[5] However, the total amount of a particular oligomer that can dissolve is controlled by the saturation limit for that species. This effect is augmented by the fact that the presence of other oligomers, sugar monomers, and other components in solution reduces the saturation point, relative to what it would be for a pure compound.[6−8] Thus, the upper limit of oligomer dissolution in batch systems is controlled by their solubility limit and competition with other species for a given amount of water used. The greater amounts of water generally applied to flow systems can dissolve far more oligomers than possible in a batch and thereby recover more of them, increasing yields. However, at the time this model was proposed, very little was understood about the factors that actually control the release of sugar monomers and oligomers during hydrolysis.

This study was undertaken to better understand how solubility limitations affect oligomer behavior for hemicellulose hydrolysis

in just hot water, based on three main variables that affect dissolution: reaction time, solids loading, and initial substrate type. Because long-chain oligomers predominate at short reaction times and solubility is expected to decrease with increasing chain length, oligomer concentrations were measured as a function of reaction time. Also, because solubility limitations should be approached at increasing solids loadings, the effect of solids loading was included. The solubility of hemicellulose and its oligomers is also dependent on their linkages to other compounds. For example, if the presence of lignin reduces solubility as expected,[3,9,10] hemicellulose oligomer−lignin complexes that are released from unmodified agricultural residues such as corn stover should be less soluble than those from delignified substrates. Accordingly, three different substrates were tested: purchased birchwood xylan, purchased oat spelt xylan, and corn stover. A reaction temperature of 180 °C was used for hemicellulose hydrolysis, to facilitate comparisons with results by other researchers.[1,11]

To evaluate whether solubility is important at temperatures typical of biomass pretreatment in just water, direct measurements of sugar concentrations at those temperatures would be desirable. However, such measurements are challenging, in that soluble and insoluble species must be separated quickly at reaction conditions without addition of water. An alternative approach is to determine whether solubility limitations are reached at lower temperatures, because, if not, solubility limitations should not be a factor at higher temperatures at which oligomers should be more soluble.[12] On the other hand, if solubility limitations are evident, then it would be necessary to apply higher temperatures. Consistent with this reasoning, oligomer concentrations were determined for hemicellulose hydrolyzates that were cooled to temperatures of 80 and 26 °C. However, because chemical analysis must be conducted at room temperature, oligomer concentrations at 80 °C were determined by adding dilution water at 80 °C to reduce precipitation when the mixture was cooled to room temperature.

Materials and Methods

Feedstocks. Corn stover was graciously provided by the National Renewable Energy Laboratory (NREL) from a con-

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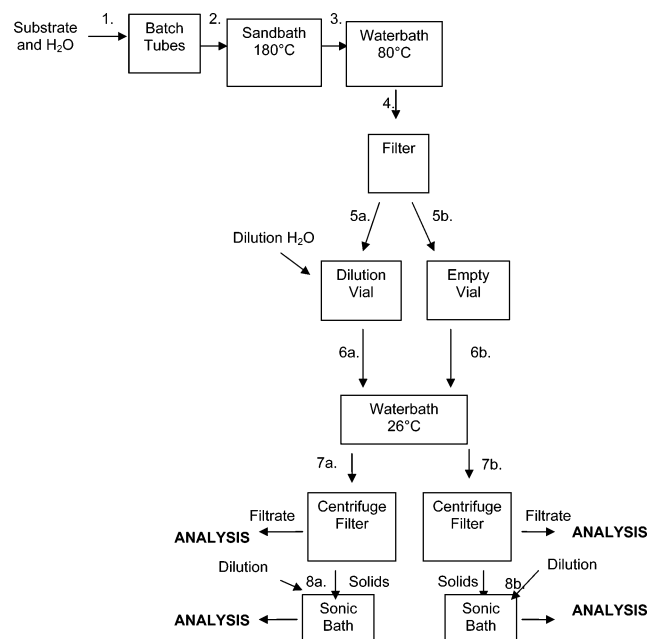
Figure 1. Sequence of steps used to prepare and recover dissolved components released during hydrolysis of birchwood xylan, oat spelt xylan, and corn stover.

trolled source they obtained from Harland, IA, and ground to a particle size of 2 mm. Birchwood xylan was obtained from Sigma−Aldrich (St. Louis, MO) and oat spelt xylan was obtained from ICN Biotech (Aurora, OH). All three substrates were stored in a vacuum oven at room temperature. A solids analysis revealed dry weight concentrations of 97%, 93%, and 92%, respectively. Compositions of xylan, glucan, arabinan, mannan, galactan, Klason lignin, and ash for commercial birchwood xylan and oat spelt xylan were measured at Celunol Louisiana LCC, according to standard analytical procedures published by NREL;[13] the uronic acid content was measured by Dr. Johnson at NREL. The corn stover used for this work was part of the supply used for the CAFI I project, and its composition was measured by NREL.[14] Table 1 lists details about the compositions used in this experiment.

Experimental System. Figure 1 outlines the experimental procedure used. In all cases, 0.5-in.-diameter by 6-in.-long cylindrical Hastelloy C276 tubes with a 0.035-in.-thick wall, obtained from Maine Valve & Fitting (Bangor, ME), were used as pretreatment batch reactors. For all xylans and for corn stover with ≤10% solids, a known amount of deionized water at a pH of 6.5−7.0 was first pipetted into the tubes. An amount of solid substrate appropriate to achieve the target solids loading then was added to each tube, based on its weight, as determined on a Ohaus AS120 balance (Pinebrook, NJ) (with a repeatability of 0.1 mg), and the tube contents were stirred. For solids loadings of >10%, corn stover had to be soaked in excess water overnight, poured into a Buchner funnel with a 0.2-μm filter,

and vacuum-dried for approximately a minute, while stirring the slurry to yield a solids loading (as measured by drying) of 19%.[1] This mixture was then loaded into each tube for reaction, as shown in step 2 of Figure 1.

Each tube was suspended by wires in a fluidized sand bath (Techne, Cambridge, U.K.) controlled at 180 °C. At the completion of reaction (step 3), the tubes were immediately transferred to an 80 °C water bath outfitted with an Isotemp 2100 Immersion Circulator (Fisher Scientific) that maintained a stable temperature of ±0.1 °C at a pumping rate of 14 L/min. Glass stir rods, 10 mL of Luer-Lock, syringes (from Beckon Dickinson, Franklin Lakes, NJ), and 25-mm-diameter 0.5-μm filters (from Millipore Corp., Bedford, MA) were placed in a plastic container and added to the bath. After a period of 1−1.5 h, each tube was removed individually and quickly uncapped, and the contents were poured into a preheated syringe with a filter attached and the plunger removed (step 4 of Figure 1). For corn stover, a preheated glass stir rod was used to squeeze liquid from the solids. For corn stover samples at 19% solids loading, both ends were uncapped, and a glass stir rod was used to push all the solids into the syringe. In steps 5a and 5b, the syringe plunger was quickly reinserted, and the contents were filtered into two preweighed 20 mL scintillation vials (one containing ∼10 mL of water, and the other being empty). In steps 6a and 6b, the vials were capped and placed in a circulating water bath that was thermostatically controlled at 26 °C.

After a few hours, the vials were removed from the water bath, dried, and weighed. In steps 7a and 7b, solids were separated from the liquid with a 0.2-μm centrifuge filter, and the filter and tube were weighed separately. Following thorough shaking, 700 μL was pipetted from each scintillation vial into the filter, and the filter was weighed. The centrifuge tubes were next centrifuged at 13 500 rpm for at least 15 min, with some of the solutions taking as long as an hour for all the liquid to be removed. The tube and filters then were weighed separately again. In steps 8a and 8b, the solids on the filters were each placed in a 20-mL scintillation vial containing 5 mL of water that was, in turn, added dropwise into an ultrasonic water bath for at least 5 min. At this point, there were four different liquid samples from each batch reaction tube filtered at 80 °C: (1) material dissolved in dilution water at 80 °C and still in solution at 26 °C, (2) material dissolved in dilution water at 80 °C but insoluble at 26 °C, (3) material dissolved without dilution water at 80 °C and still in solution at 26 °C, and (4) material dissolved without dilution water at 80 °C but insoluble at 26 °C.

Analytical Methods. Each of the four liquid samples was analyzed using three methods: direct high-performance liquid chromatography (HPLC), post-hydrolysis, and dry weight. Sugar and oligomer concentrations were measured by HPLC, using a Waters 2695 separations module (Milford, MA) equipped with a Waters 2414 refractometer and employing either an Aminex HPX-87P column to measure sugar monomers or a HPX-42A column to measure low-molecular-weight sugar oligomers over

Table 1. Compositions of Birchwood Xylan, Oat Spelt Xylan, and Corn Stover, and the Information Sources Used

| | Composition (g/100 g of Dry Substrate) | | | | | | | |
substrate	xylan	glucan	arabinan	Klason lignin	ash	uronic acids	acetic acid	source
birchwood xylan	86.8	0.4	0.3	1.3	6.4	5.7		this work
oat spelt xylan	81.7	0.4	0.4	2.3	9.6	5.2		this work
corn stover	20.5	35.9	3.1	23.8	7.2		1.9	Mosier[14]

| | Composition of Uronic Acids (g/100 g of Dry Substrate) | | | | |
substrate	MeGA	MeGAX1	galacturonic acid	glucuronic acid	GlucX
birchwood xylan	1.9	3.6	0.1	0.1	0.1
oat spelt xylan	1.6	3.4	0.0	0.1	0.1

a degree-of-polymerization (DP) range of 1−6 (Bio-Rad, Hercules, CA). Sugar standards were used to calibrate the first column, and for the second, oligomers were quantified based on the refractive index response of xylose.[11] Low-DP oligomers were expressed as xylose equivalents by accounting for the water of hydration. The 26 °C samples were diluted 5-fold, to ensure that they did not precipitate. All concentrations were expressed as xylose equivalents.

Post-hydrolysis was used to measure the total amount of xylose in the form of oligomers or monomer in each sample, with the approach based on NREL's Laboratory Analytical Procedure Number 13.[15] In this operation, 5-mL sample solutions and 174 μL of 72 wt % sulfuric acid from Sigma−Aldrich were added to a 10-mL glass test tube to produce a combined solution with an acid concentration of 4 wt %. Solutions filtered at 26 °C without added water were diluted, because <5 mL was available in most cases. Also, only 4 mL of the filtered samples was used to leave enough solution for analysis by the other two methods, and 1 mL of water was added. These dilutions were carefully noted, and samples were held at 121 °C for an hour. Because some monomer degradation is unavoidable, sugar recovery standard solutions were taken through the process in parallel and used to correct for monomer degradation as noted in the NREL procedure. After heating, the samples were neutralized with $CaCO_3$, filtered in 45-μm syringe filters obtained from Gelman Laboratory (East Hills, NY), and analyzed by HPLC on an Aminex 87P column.

Calculations. The concentration of xylose monomer was calculated from

$$C_{\text{monomer}} = \text{PH}_{\text{monomer}}\left(\frac{C_{\text{xylose,standard}}}{\text{PH}_{\text{xylose,standard}}}\right)\left(\frac{M_{\text{diluted\_sol'n}}}{M_{\text{sol'n}}}\right) \quad (1)$$

in which C_{monomer} is the concentration of xylose monomer in the sample, $\text{PH}_{\text{monomer}}$ the xylose peak height in the sample measured by HPLC, $C_{\text{xylose,standard}}$ the concentration of xylose in a standard solution, $\text{PH}_{\text{xylose,standard}}$ the xylose peak height for the standard solution measured via HPLC, $M_{\text{diluted\_sol'n}}$ the mass of solution after dilution in step 5, and $M_{\text{sol'n}}$ the mass of solution before dilution in step 5.

The concentration of low-DP oligomers was calculated as the sum of the concentrations of oligomers that have a DP value of 2−6, according to the following relationship:

$$C_{\text{Low−DP}} = \sum_{2}^{6} C_{\text{Oligo\_}n} \quad (2)$$

in which

$$C_{\text{Oligo\_}n} = \text{PH}_{\text{Oligo\_}n}\left(\frac{C_{\text{xylose,standard}}}{\text{PH}_{\text{xylose,standard}}}\right)\left(\frac{M_{\text{diluted\_sol'n}}}{M_{\text{sol'n}}}\right)\left(\frac{n \times 150}{\text{MW}_n}\right) \quad (3)$$

for which n is the chain length of the xylo-oligomer of interest, $C_{\text{Oligo\_}n}$ the concentration of xylooligomer of chain length n, $\text{PH}_{\text{Oligo\_}n}$ the xylo-oligomer peak height of chain length n from HPLC, MW_n the molecular weight of a xylo-oligomer of chain length n, $C_{\text{Low−DP}}$ the concentration of xylo-oligomers with a chain length of 2−6, and $C_{\text{High−DP}}$ the concentration of xylo-oligomers with a chain length of >6.

Because the amount of high-DP oligomers could not be measured directly by HPLC, they were estimated as the post-hydrolysis total minus the total amounts of low-DP oligomers and monomer, as follows:

$$C_{\text{High−DP}} = C_{\text{Total\_xylose}} - C_{\text{Xylose\_monomer}} - C_{\text{Low−DP}} \quad (4)$$

$$C_{\text{Total\_Xylose}} = \text{PH}_{\text{xylose,sample}}\left(\frac{C_{\text{xylose,standard}}}{\text{PH}_{\text{xylose,standard}}}\right)\left(\frac{1}{\%\text{Rec}}\right)$$
$$\left(\frac{V_{\text{sol'n \& acid}}}{M_{\text{diluted\_sol'n}}}\right)\left(\frac{M_{\text{diluted\_sol'n}}}{M_{\text{sol'n}}}\right)(\rho_{\text{H}_2\text{O}}) \quad (5)$$

in which $C_{\text{Total\_Xylose}}$ is the concentration of xylose after post-hydrolysis, $\text{PH}_{\text{xylose,sample}}$ the xylose peak height from HPLC in the sample after post-hydrolysis, $C_{\text{xylosestandard}}$ the concentration of xylose in a standard solution, $\text{PH}_{\text{xylose,standard}}$ the xylose peak height from HPLC from the standard solution, %Rec is the percent recovery of xylose standard solution after post-hydrolysis, $V_{\text{sol'n \& acid}}$ the volume of solution and acid from the posthydrolysis procedure, $M_{\text{diluted\_sol'n}}$ the mass of solution after dilution in step 5, $M_{\text{sol'n}}$ is the mass of solution before dilution in step 5, and $\rho_{\text{H}_2\text{O}}$ is the density of water.

To quantify the amount of xylose monomer, low-DP oligomer, and high-DP oligomer in the precipitates, dilution in steps 7a and 7b had to be considered for each. On this basis, the concentration of xylose monomer was calculated from

$$C_{\text{monomer}} = \text{PH}_{\text{monomer}}\left(\frac{C_{\text{xylose,standard}}}{\text{PH}_{\text{xylose,standard}}}\right)\left(\frac{M_{\text{diluted\_sol'n}}}{M_{\text{sol'n}}}\right)\left(\frac{5 \text{ mL}}{V_{\text{liquid}}}\right) \quad (6)$$

with V_{liquid} being the volume of liquid centrifuged in step 7. The concentration of low-DP oligomers was determined by

$$C_{\text{LowDP}} = \sum_{2}^{6} C_{\text{Oligo\_}n} \quad (7)$$

in which

$$C_{\text{Oligo\_}n} = \text{PH}_{\text{Oligo\_}n}\left(\frac{C_{\text{xylosestandard}}}{\text{PH}_{\text{xylose,standard}}}\right)\left(\frac{M_{\text{dilutedsol'n}}}{M_{\text{sol'n}}}\right)\left(\frac{n \times 150}{\text{MW}_n}\right)$$
$$\left(\frac{5 \text{ mL}}{V_{\text{liquid}}}\right) \quad (8)$$

Finally, the concentration of high-DP oligomers was calculated from

$$C_{\text{High−DP}} = C_{\text{Total\_xylose}} - C_{\text{Xylose\_monomer}} - C_{\text{Low−DP}} \quad (9)$$

$$C_{\text{High−DP}} = C_{\text{Total\_xylose}} - C_{\text{Xylose\_monomer}} - C_{\text{Low−DP}} \quad (10)$$

The yields of monomer, low-DP oligomers, and high-DP oligomers were added to give an overall total solubilized xylose balance. The yield of monomer, low-DP oligomers, or high-DP oligomers was taken to be the amount soluble at that temperature divided by the initial amount of xylan times a factor of 1.136 (this factor accounts for the water of hydrolysis).

Results

Precipitation. Figures 2a−c show the partitioning of the total solubilized xylose (the sum of high-DP xylo-oligomers, low-DP xylo-oligomers, and xylose concentrations) for hydrolysis solutions from the two commercial xylans and from corn stover diluted at 80 °C before cooling and for solutions that were not diluted before cooling. For the two xylans, 10 mL of dilution water was insufficient to prevent precipitation of components that were soluble at 80 °C for the shorter reaction times. For birchwood xylan at 4.5% solids, and 10 min of reaction time, the vial in which the solution was diluted at 80 °C had a total
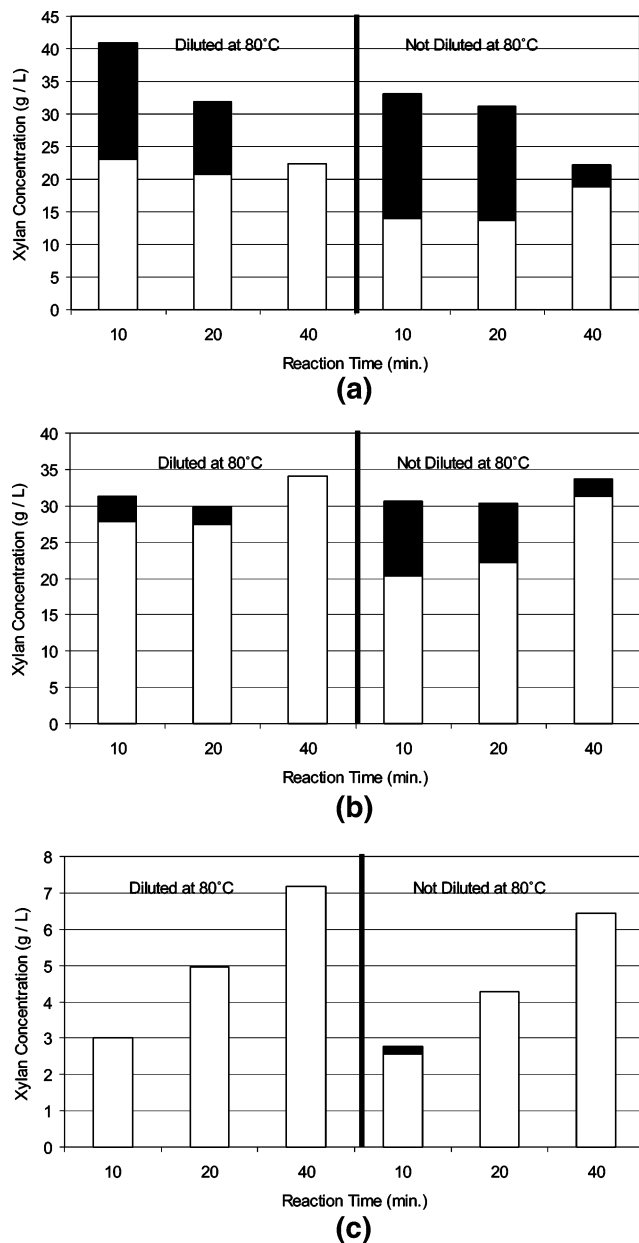
(a)

(b)

(c)

Figure 2. Concentrations expressed in xylose equivalents for hydrolyzates produced by reacting (a) a 4.5% solids concentration of birchwood xylan, (b) a 4.4% solids concentration of oat spelt xylan, and (c) a 4.6% solids concentration of corn stover, each at 180 °C for 10, 20, and 40 min and filtering the resulting product into water (left panel) or an empty tube (right panel) at 80 °C. The upper shaded area of each bar represents the fraction that precipitated when the sample was cooled to room temperature for analysis, and the clear lower portion corresponds to the material remaining in solution after cooling.

dissolved concentration of xylose oligomers and monomers of 23 g/L and a precipitate that added another 18 g/L. As shown in Figure 2a at 20 min, there was less precipitate in the diluted solution, and at 40 min, there was no precipitate. For this reason, the total soluble xylose at 80 °C was determined by including both the precipitate and the components soluble at room temperature, because both were soluble when the hydrolyzate was filtered at 80 °C (step 5a in Figure 1).

Figure 2b shows that precipitation upon cooling was less pronounced for a 4.4% concentration of oat spelt xylan than for birchwood but it was still significant. For a 4.6% concentration of corn stover, Figure 2c shows that virtually all the solubilized xylose at 80 °C remained dissolved upon cooling to 26 °C, probably because the corn stover contained only 21%
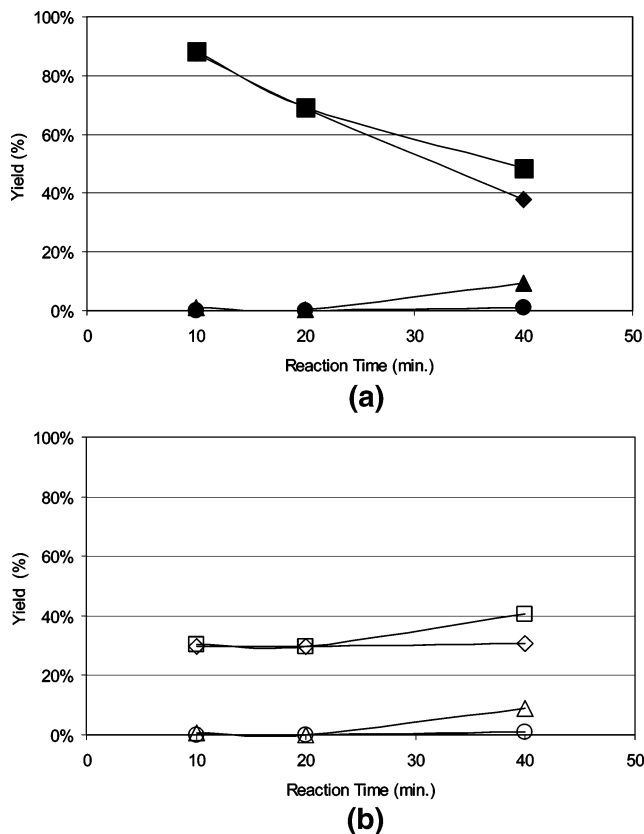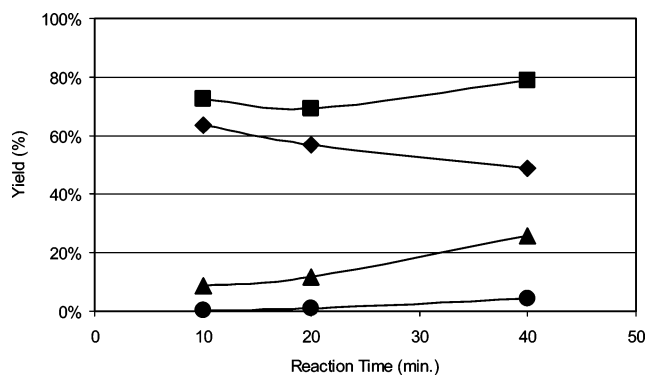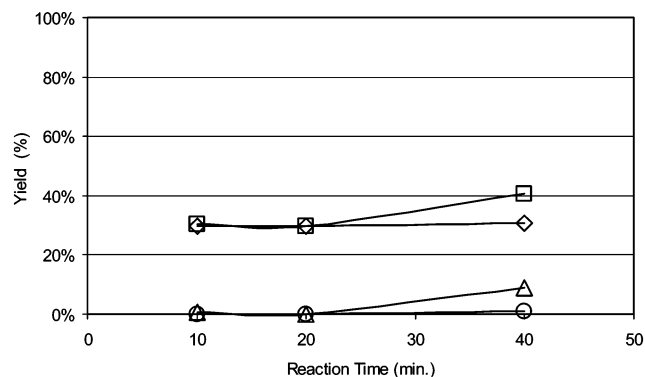

(a)

(b)

Figure 3. Yields versus time for water-only hydrolysis of birchwood xylan at 180 °C and 4.4% solids loading, recovered at (a) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (b) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose).

xylan, as compared to 86.8% and 81.7% xylan in commercial birchwood xylan and oat spelt xylan, respectively. The three figures also show that, with the exception of the first data point in Figure 2a, the total amount of xylose accounted for in solution and the precipitate at each reaction time was the same, within ± 1 g/L, for both diluted and undiluted solutions. This result supports the accuracy of this procedure in that, while the partition between dissolved xylan and precipitated xylan should change, depending on whether or not dilution water is added, the total amount should remain constant.

Time Series. Figure 3a shows xylose yields for the reaction of a 4.5% solids concentration of birchwood xylan at three different times. After the first 10 min, 88% of the birchwood xylan was dissolved at 80 °C as high molecular weight oligomers that could be measured by post-hydrolysis but could not be accounted for as xylose monomers or low-DP oligomers with DP = 2−8. After 20 min, the xylan was still all in the form of high-DP oligomers, but the total solubilized xylose balance decreased to 70%, although no low molecular weight oligomers or monomers were found, implying that the high molecular weight oligomers were degrading to non-xylose-based compounds. This result is consistent with findings of Li et al., which projected parallel kinetic pathways of degradation and depolymerization for individual short-chain xylo-oligomers in water at 180 °C.[11] At 40 min, Figure 3a shows a substantial concentration of low-DP oligomers and xylose; however, losses limited the total solubilized xylose balance to <50%. Figure 3b shows that much lower yields of dissolved species were observed for birchwood xylan at 26 °C, compared to the 80 °C results in Figure 3a, particularly at 10 and 20 min, because of

(a)

(b)

Figure 4. Yields versus time for water-only hydrolysis of oat spelt xylan at 180 °C and 4.4% solids loading, recovered at (a) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (b) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose).
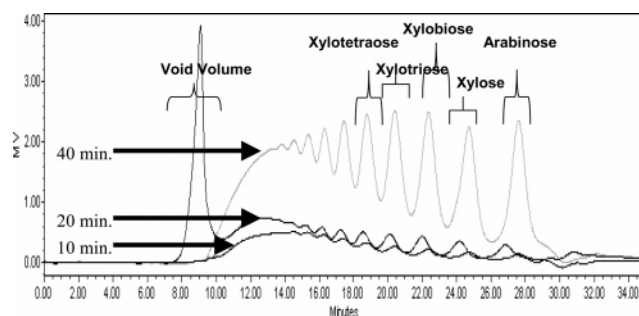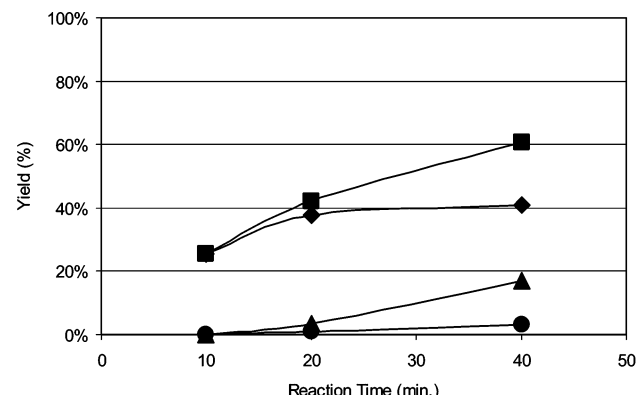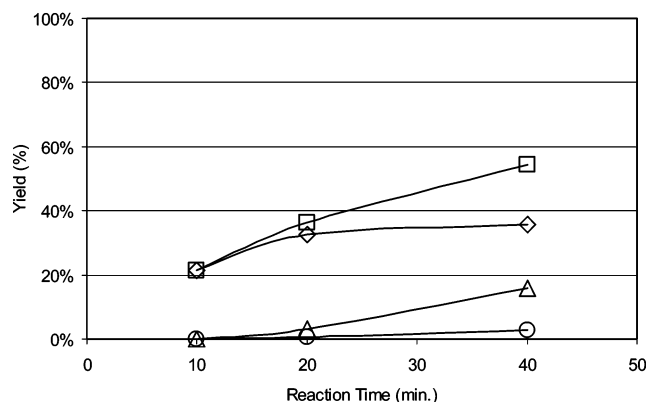

Figure 5. IMP chromatograms of the hydrolyzates from water-only hydrolysis of oat spelt xylan at 180 °C, recovered at 80 °C, and 4.4% solids loading at 10, 20, and 40 min of reaction time.

(a)

(b)

Figure 6. Yields versus time for water-only hydrolysis of corn stover at 180 °C and 4.4% solids loading, recovered at (a) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (b) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose).

precipitation. However, this difference was reduced at longer reaction times when the average DP was lower; for example, at 40 min, only a small amount of xylan soluble at 80 °C precipitated when the solution was cooled to 26 °C. Also, only a small fraction of the low-molecular-weight oligomers and xylose precipitated when cooled to 26 °C, compared to 20% of the high molecular weight oligomers, showing that larger oligomers precipitate preferentially.

As shown in Figure 4a, a 4.4% solids concentration of oat spelt xylan followed the same general trends as birchwood xylan, in that the amount of low-DP oligomers and xylose increased and that of high-DP oligomers decreased with hydrolysis time. However, over the entire time followed, a larger proportion of the oat spelt xylan was in the form of low-DP oligomers than that for birchwood xylan, with half the xylan in the form of low-DP oligomers at 40 min. Thus, either depolymerization was more rapid and/or the initial DP was less. The amount of high-DP oligomers that precipitated was at a maximum at 10 min and subsequently decreased with reaction time. In contrast to birchwood xylan, the total solubilized xylose balance remained fairly constant (between 69% and 79%), which suggests less degradation. By comparison, Li et al. also measured the DP distribution of oat spelt xylan in water at 180 °C by performing the reaction at temperature, quenching in an ice bath, and separating the precipitated solids from the liquid by centrifuging at room temperature. They reported an oligomer yield of 42% and a total solubilized xylose balance of 79% at 10 min, and values of 44% and 71%, respectively, at 20 min.[11] These oligomer yields are similar to values obtained in this study at 26 °C (shown in Figure 4b), and their total solubilized xylose

balances are similar to the total yields obtained here at 80 °C. The IMP chromatograms in Figure 5 further illustrate the depolymerization reactions that reduce the DP value over time. At 10 and 20 min, only small peaks were observed for the low-DP oligomers, but at 40 min, arabinose, xylose, and xylo-oligomers of DP = 2−9 all have large peaks.

The yield versus time profile for a 4.6% concentration of corn stover were much different than those for the purchased xylans, as shown in Figure 6a and b. The total amount of solubilized xylose recovered in solution increased steadily for corn stover with time, from 25% at 10 min to 61% at 40 min, whereas the total solubilized xylose balance for the purchased xylans either decreased or remained relatively constant. Thus, hemicellulose solubilized more slowly for corn stover than xylan for the purchased xylans. This is not surprising, in that the
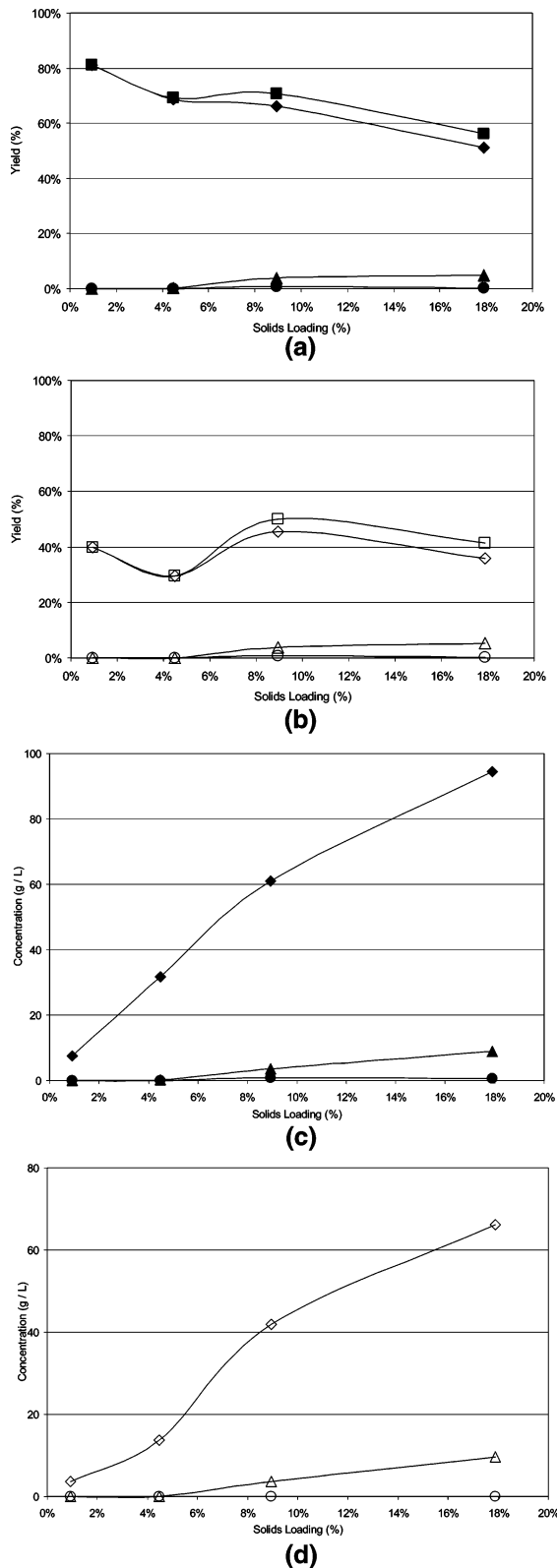
Figure 7. Yields versus solids concentration for water-only hydrolysis of birchwood xylan at 20 min of reaction time, recovered at (a) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose), and (b) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose). Concentrations expressed in xylose equivalents versus solids concentration for water-only hydrolysis of birchwood xylan at 20 min of reaction time, recovered at (c) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, (■) total solubilized xylose) and (d) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose).

attachment of lignin to corn stover hemicellulose through benzylic ether, benzylic ester, and glycosidic ether linkages mediated by ferulic acid side groups, is expected to decrease solubility and delay the release of xylose into solution.[3,9,10,16] For the hydrothermal treatment of maize bran, these linkages have been shown to remain largely unchanged at temperatures as high as 210 °C.[17] Linkages to lignin are expected to decrease solubility, and, for corn stover, a substantial decrease in molecular weight occurs before these linkages are broken. For this reason, and because corn stover contains roughly one-fourth as much xylan as the commercial xylans, only slightly more high-DP oligomers are recovered at 80 °C than at 26 °C. At 40 min, the 80 °C total xylose yield was ∼61% and at 26 °C it was ∼54%, which is approximately the same as the maximum yield reported by some researchers for batch pretreatment of corn stover.[2]

These results were compared to data from Stuhler at the same temperature and 5% solid concentration.[1] In that study, oligomer yields measured by washing the solids after reaction with large amounts of water and then quantifying oligomer concentrations in the liquid by post-hydrolysis were ∼27% after 20 min.[1] This result compares to our total yields of solubilized xylose at 20 min of ∼41% at 80 °C and ∼36% at 26 °C. Thus, it seems that separating the solid and the liquid at 80 °C recovers a substantial fraction of soluble xylose oligomers that washing the solid at room temperature would not redissolve.

Solids Loading. The effect of initial solids loadings from as low as 1% to as high as 18% was also investigated for the three substrates. If the reaction is homogeneous and first-order at reaction temperatures, the yield should be independent of solids loadings, and concentrations in solution should increase linearly with increasing solids. For a biomass component i, the yield (Yield$_i$) is a function of its concentration (C_i), the solution volume ($V_{sol'n}$), the mass of solid (M_{solids}), and the mass fraction of component i in the solids (X_i):

$$\text{Yield}_i = \frac{C_i V_{sol'n}}{M_{solids} X_i} \qquad (11)$$

Furthermore, the solids loading (%Solids) is related to the mass of solids and the mass of water (M_{water}) in the system as follows:

$$\%\text{Solids} = \frac{M_{solids}}{M_{solids} + M_{water}} \qquad (12)$$

By direct substitution, the yield can be expressed as a function of solids loading, and the result can be further simplified in terms of the solution density ($\rho_{i,sol'n}$) to give

$$\text{Yield}_i = \frac{C_i V_{sol'n}}{\%\text{Solids}(M_{solids} + M_{water}) X_i} = \frac{C_i}{\%\text{Solids}(\rho_{i,sol'n}) X_i} \qquad (13)$$

in which

$$\rho_{i,sol'n} = \frac{M_{solids} + M_{water}}{V_{sol'n}} \qquad (14)$$

The rates and, therefore, yields are independent of solids loading for homogeneous first-order kinetics;[18] thus, the value of Yield$_i$ should be a constant value, defined here as K:

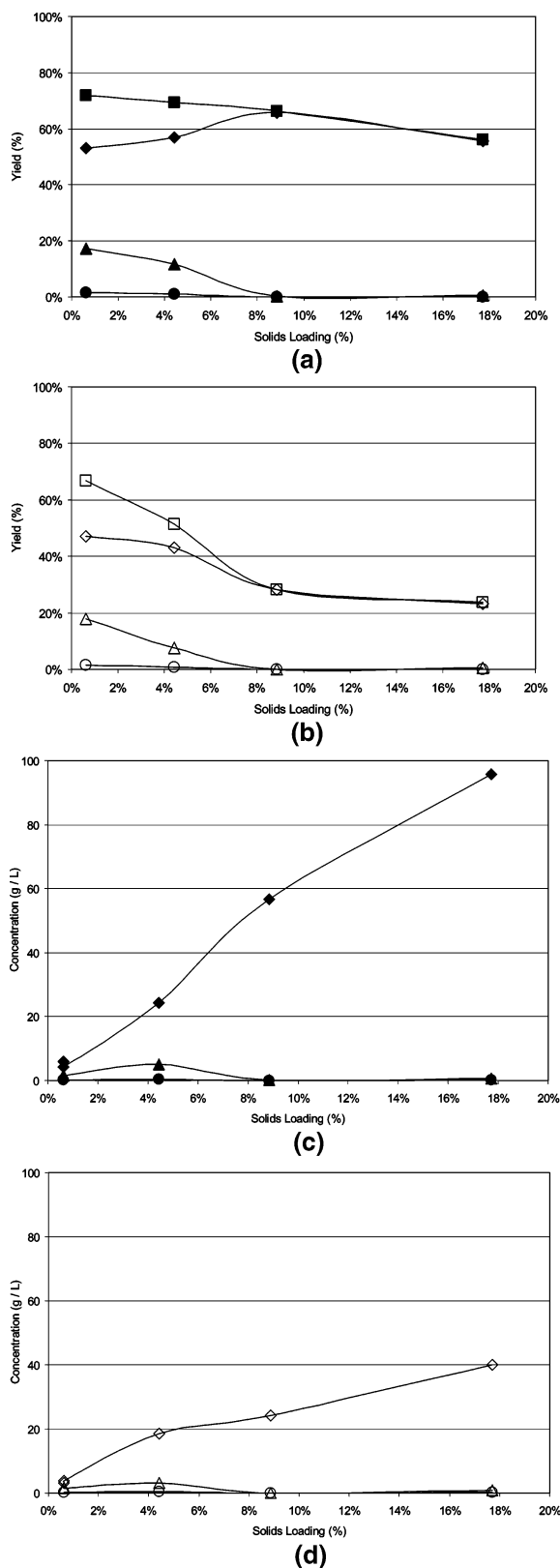$$K = \frac{C_i}{\%\text{Solids}_i}(\rho_{i,sol'n}) \qquad (15)$$

Figure 8. Yields versus solids concentration for water-only hydrolysis of oat spelt xylan at 20 min reaction time, recovered at (a) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (b) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose). Concentrations expressed in xylose equivalents versus solids concentration for water-only hydrolysis of oat spelt xylan at 20 min reaction time, recovered at (c) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (d) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose).

With this substitution, the aforementioned equation can be rearranged to read

$$C_i = K(\%\text{Solids}_i)(\rho_{i,\text{sol'n}}) \qquad (16)$$

Thus, the concentration of any species formed by a first-order reaction is predicted to be a linear function of the solids loadings if the component fully dissolves and the density changes are small.

Figure 7a and b presents yields of xylose monomers and oligomers for hydrolysis of birchwood xylan at 0.9%, 4.4%, 9.0%, and 17.9% initial solids. The overall xylose balance at 80 °C decreased from 81% at 0.9% solids to 56% at 17.8% solids, and the extent of precipitation upon cooling (indicated by the difference in the high-DP yield at 80 and 26 °C) decreased at high solids loading. A comparison of these figures to the time series for birchwood xylan at 4.4% solids (Figure 3a and b) shows that the total solubilized xylose balance at 0.9% solids is similar to that of a time earlier than 20 min (~15%), and the total solubilized xylose balance at 17.9% solids is similar to that of one observed later (~30%).

The change in results with solids concentration could be explained in terms of the dependence of the autohydrolysis reaction rates on both the autoionization of water and the concentration of organic acids released from the substrate. Because commercial xylan is deacetylated, uronic acid groups are the dominant acid that would catalyze hydrolysis rates. However, some of the acid is neutralized by the ash in the substrate, and the remainder will stay active. Birchwood xylan contains 5.7% uronic acids and 6.4% ash. At higher solids concentrations, more acid is available and reaction rates are increased.

The previous equations predict that, for first-order kinetics, the concentration of each component should be proportional to the initial solids loading, unless a solubility limitation was reached. In that event, the concentration should remain at a limiting value with increasing solids. Replotting the data from Figures 7a and 7b as Figures 7c and 7d, with the concentration as the ordinate, shows that concentrations of all components at both 80 °C and 26 °C increased linearly at least up to a solids concentration of 9%. At 17.9% solids, however, the concentration of high-DP oligomers was significantly less than that projected by extrapolation of the previous data, apparently because of precipitation between 180 °C and 80 °C or solubility limitations at reaction temperatures. Concentrations of all of the other major components were linearly related to solids concentrations over the range tested. The data for high-DP oligomers measured at 26 °C at 9.0% solids is somewhat higher than that observed for the other three measurements, likely because of experimental variability. In any event, concentrations of low-DP oligomers and monomers measured at both temperatures were linearly related to solids concentration, but the ratio of high-DP oligomers to low-DP oligomers at 80 and 26 °C decreased as the solids loadings increased, likely because of the increased uronic acid concentration. Thus, the average DP at low solids loadings in Figure 7d is greater, resulting in more precipitation of high-DP species. At high solids, precipitation is reduced and the divergence is less. This behavior is evident in the yield plots of Figures 7a and 7b, in which the yield of high-DP oligomers measured at 80 °C that remained soluble at 26 °C increased from 50% of the high-DP yield at 80 °C for 0.9% solids to 64% at 17.9% solids.

Figures 8a and 8b show yield data for the xylose monomer and oligomers formed by reaction of oat spelt xylan under the same conditions as those for birchwood. Although a substantial
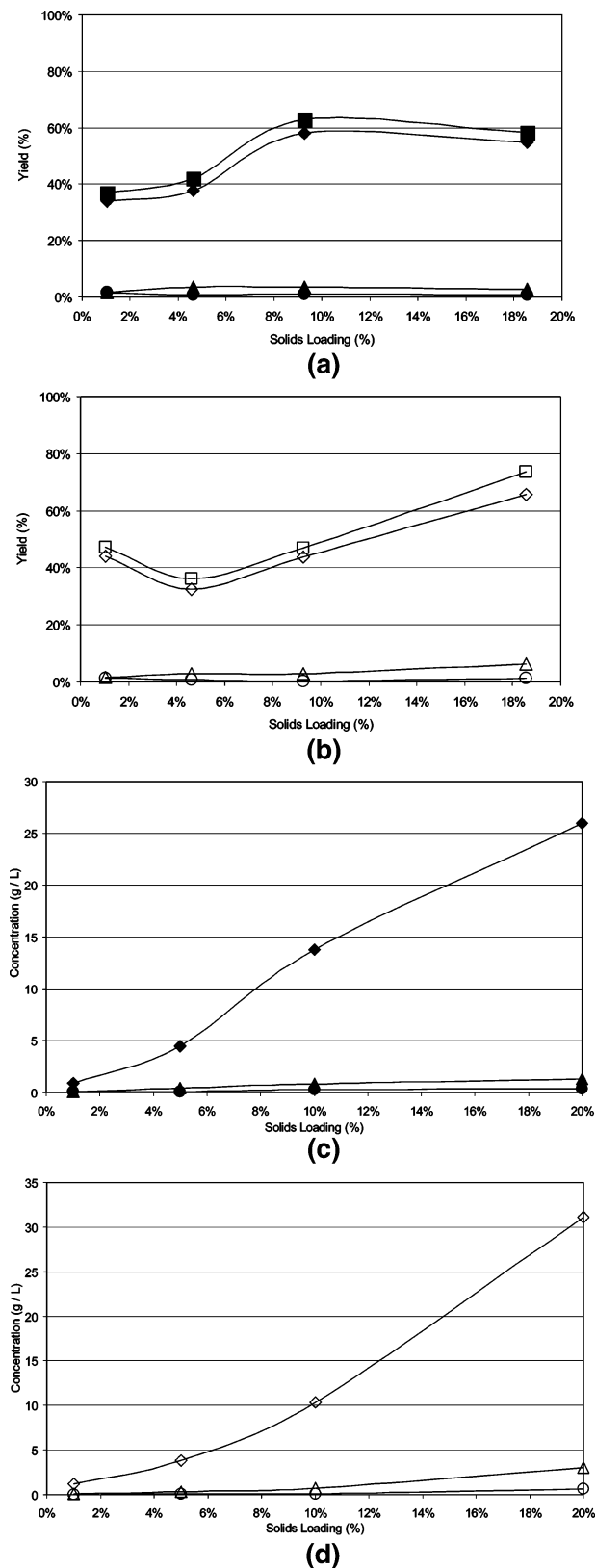
Figure 9. Yields versus solids concentration for water-only hydrolysis of corn stover at 20 min reaction time, recovered at (a) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (b) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose). Concentrations expressed in xylose equivalents versus solids concentration for water-only hydrolysis of corn stover at 20 min reaction time, recovered at (c) 80 °C ((●) xylose monomer, (▲) xylose low-DP oligomer, (◆) xylose high-DP oligomer, and (■) total solubilized xylose) and (d) 26 °C ((○) xylose monomer, (△) xylose low-DP oligomer, (◇) xylose high-DP oligomer, and (□) total solubilized xylose).

quantity of low-DP oligomers were measured at low solids loadings, none were observed at high solids loadings, contrary to the hypothesis used to explain the ratio of high-DP oligomers at 80 and 26 °C in the hydrolysis of birchwood xylan. This difference can be explained by noting the difference in composition between the two commercial xylans: oat spelt has almost the same amount of uronic acid as birchwood but contains more ash (9.6% compared to 6.4%). Esteghlalian indicated that a higher ash content made corn stover less reactive in dilute acid than poplar and switchgrass.[19] If the neutralizing activity of ash is greater than the acidifying activity of uronic acid side chains in oat spelt xylan, increasing solids loading would produce a net increase in neutralizing capacity, and the reaction would be slowed. The concentration data at different initial solids loadings in Figures 8c and 8d are similar to those for birchwood xylan and again indicate that solubility limitations did not seem to have been reached in the reactor or upon cooling from 180 °C to 80 °C.

Figures 9a and 9b present results for treatment of corn stover under the same conditions as those used for birchwood and oat spelt xylan. In this case, the experimental error was somewhat greater, because of challenges in using HPLC to measure the less-concentrated solutions that resulted from the lower xylan content of corn stover. For example, at 1.0% solids, the yield of oligomers was greater for the 26 °C test in Figure 9b than at 80 °C in Figure 9a. However, because of the dilution steps, the concentration difference between these two samples by HPLC was <0.01 g/L, and a slight change in the baseline during analysis could account for this difference. Nevertheless, the key trend remained discernible: Figures 9c and 9d show that the product concentrations continued to increase with solids loading, indicating no solubility limitations. Figures 9a and 9b show slight yield increases with increasing solids loading, which is expected because of the organic acid groups in corn stover. Also, a somewhat greater concentration of lower-DP oligomers was observed at higher solids loadings, implying that the average DP decreased at higher solids loading.

Conclusions

These experiments have shown that, in a hydrolyzate containing mixtures of oligomers created by water-only hydrolysis of birchwood and oat spelt xylan, oligomers with a higher degree of polymerization (DP) precipitated as the solution was cooled from 80 °C to 26 °C. Thus, oligomer yields were likely underestimated in many reports of batch pretreatment in water, because the products were cooled before analysis, unless the sample was washed excessively to redissolve high-DP oligomers. However, even dilution by a factor of 10 at 80 °C was not sufficient to prevent precipitation upon cooling to 26 °C. Fewer high-DP oligomers precipitated in the hydrolysis of corn stover, because of the lower fraction of hemicellulose in this feedstock. Comparison of the ratio of low-DP oligomers to high-DP oligomers indicates that the average DP of soluble oligomers decreased as the reaction time increased.

Solids concentration had a significant effect on xylose recovery. For birchwood xylan that was reacted at 180 °C for 20 min, the ratio of low-DP oligomers to high-DP oligomers increased as the solids loadings increased. However, for oat spelt xylan that was reacted under the same conditions, this ratio decreased with the initial solids loading. These conflicting results may be due to differences in ash content. For birchwood xylan, which has less ash to neutralize its uronic acid, at higher solids concentrations, the effective uronic acid concentration is higher, resulting in faster hydrolysis. For oat spelt, the ash content may

be great enough to neutralize all the organic acids, which would mean that hydrolysis proceeds only via the autohydrolysis of water. At higher solids concentrations, more ash is available to neutralize the hydronium groups, leading to slower hydrolysis. For corn stover, similar yields of low-DP oligomers were recorded at all solids loadings. The concentration of high-DP oligomers, low-DP oligomers, and xylose were linearly proportional to the initial solids loading, except for birchwood xylan at 17.9% solids. This result suggests that, for solids loadings of ≤9%, solubility limitations were not approached at 20 min of reaction time for all three substrates. Further testing is required to determine if solubility limitations are encountered at a higher temperature for commercial birchwood xylan at 17.9% solids.

The solubility limitations observed for the water-only hydrolysis of commercial xylan and corn stover have important implications for the way in which biomass is processed. For example, biomass should be washed with adequate amounts of hot water after pretreatment to remove oligomers that were liberated in pretreatment but precipitated upon cooling.

Acknowledgment

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