0273-2289/03/105-108/0515/$20.00

Characterization of Molecular Weight Distribution of Oligomers from Autocatalyzed Batch Hydrolysis of Xylan

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Abstract

Oat spelt xylan was treated with water in a batch reactor at temperatures of 180 and 200°C. Ion-moderated partition (IMP) chromatography was then applied to separate oligomers in solution according to their molecular size. Calibration of the IMP measurements based on peak height was found to quantify dissolved monomer and oligomer yields well. Oligomer concentrations in the liquid hydrolysate were also determined from the difference in xylose monomer concentrations measured by high-performance liquid chromatography before and after posthydrolysis of dissolved xylooligosaccharides to xylose. Delayed formation and then rapid disappearance of oligomers from DP10 to DP2 was observed by IMP, and total oligomer yields measured by IMP and posthydrolysis were very similar at these times. However, while IMP detected virtually no oligomers initially, posthydrolysis measurements gave significant amounts of soluble oligomers at these times, indicating that oligomers with chain lengths >10 were in solution but not detectable by the IMP system used.

Index Entries: Autohydrolysis; hydrolysis; ion-moderated partition chromatography; oligomers; thermochemical; xylan.

Introduction

The fractionation of lignocellulosic materials for production of a variety of marketable chemicals is an attractive approach for biomass utilization, and the hemicellulose portion can be hydrolyzed into sugars for fermentation to a variety of valuable products. Xylooligomers are important intermediates in hemicellulose hydrolysis for pretreatment and sugar recovery. They also have potential applications in many fields including pharmaceuticals, feed formulations, agricultural applications, and func-

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tional foods. However, their comparatively high production costs hinder such uses. A better understanding of the role of xylooligomers in hemicellulose hydrolysis may lower the cost of biomass pretreatment and expand the use of xylooligomers.

Production of xylooligomers from lignocellulosic materials or xylan can be carried out in a single step by reaction with steam or water, often termed *autohydrolysis*, producing solubles consisting of primarily xylooligomers, xylose, furfural, and other degradation products, and leaving a solid residue made up of primarily cellulose and lignin*(1–3)*. The present study characterizes the molecular weight distribution of oligomers released during the batch autohydrolysis of xylan at 180 and 200°C and follows xylooligomer time profiles for xylan autohydrolysis at 200°C for a 5% solids concentration.

Materials and Methods

Substrates

Oat spelt xylan was obtained from ICN (Aurora, OH). Its composition was measured and found to contain about 82.7% xylan. Monomeric xylose was obtained from Sigma (St. Louis, MO). Xylobiose, xylotriose, xylotetraose, and xylopentaose were obtained from Magazyme International Ireland (Bray, County Wicklow, Ireland).

Reaction System

Tubes made of made of 316 stainless steel with a 0.5-in. OD, 5-in. length, and 0.035-in. wall thickness were obtained from Swagelok (Bangor, ME) and used for the reaction system shown in Fig. 1. Xylan at a 5% solids concentration was added to the tubes, which were then sealed with 0.5-in. diameter Swagelok caps. The tubes were dropped into boiling water for 1 min before being transferred to a 4kW sand bath (Cole-Parmer, Vernon Hills, IL) controlled at a target temperature of 180 or 200°C. After reaction for a specified time of between 3.5 and 30 min, the tubes were removed and quenched in an ice bath. The liquid and solid contents were then transferred to microcentrifuge tubes and separated by centrifuging at 6022*g* for 10 min.

Measurement of Xylose Monomer and Oligomer Yields

To measure the yield of monomeric xylose, the liquid decanted from the centrifuge tubes was passed through a 0.2-µm syringe filter before being analyzed by a Waters (Milford, MA) high-performance liquid chromatography (HPLC) separation module 2695, equipped with a Waters refractive index (RI) detector model 2414 and a Bio-Rad (Hercules, CA) Aminex HPX-87P column. The HPLC pump was set at a flow rate of 0.6 mL/min, and the HPLC heater was operated at 85°C.

The yield of oligomers was determined by adding 72% H_2SO_4 to the centrifuged liquid from autohydrolysis to achieve a final acid concentra-

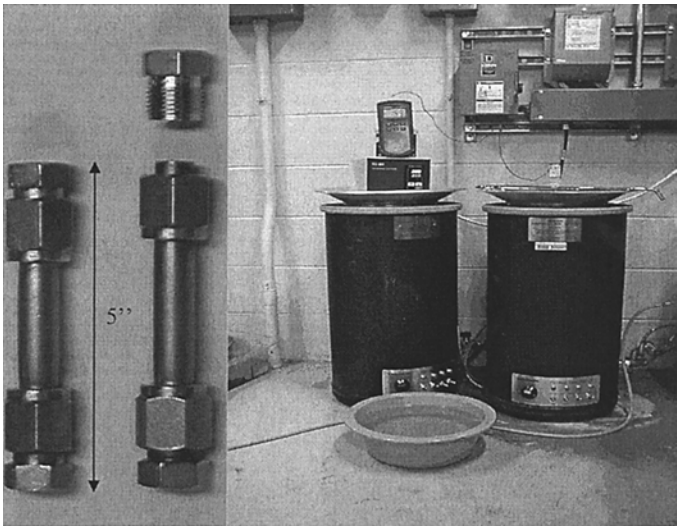

Fig. 1. Tubular reactor system used to hydrolyze xylan.

tion of 4%. These samples were then autoclaved for 1 h at 121°C in sealed autoclave bottles. Next, the hydrolysate was neutralized with calcium carbonate to a pH of 5.0–6.0 before being analyzed by HPLC. A set of sugar standards of known concentration was taken through the same analytical procedure in parallel to determine losses owing to the destruction of sugars and to provide a correction factor for adjusting the sugar concentrations following posthydrolysis*(4)*. The increase in monomer concentration resulting from the posthydrolysis procedure provided a measure of the fraction of the total dissolved xylose that is oligomeric*(5,6)*.

The solid residues from the centrifuge tubes were vacuum dried and subjected to quantitative saccharification to measure the remaining xylose content *(7)*.

Molecular Weight Determination for Oligomers

A Waters model 717 chromatography system, equipped with a Waters RI detector 410 and a Bio-Rad Aminex HPX-42A ion-moderated partition (IMP) column was connected to a Waters Fraction Collector II. Centrifuged samples were filtered and analyzed by IMP at a flow rate of 0.2 mL/min and a column temperature of 85°C. Concentrations were converted to yields on a weight basis.

Results and Discussion

Table 1 and Fig. 2 show the sugar recovery for autohydrolysis of xylan at 180 and 200°C for a 5% solids concentration. As expected, xylan and xylooligomers were hydrolyzed faster at the higher temperature: the maximum xylose monomer yield was about 11% at 200°C but <3% at

Table 1
Sugar Recovery for Autohydrolysis of Xylan
at 180 and 200°C with 5% Solids Concentration

	Time (min)				
Yield (% of potential xylose)	0.00	5	10	20	30
Temperature = 180°C					
Xylan in solid residue	100.00	43.96	36.96	26.47	16.53
Xylose in liquor	0.00	0.00	0.32	1.43	2.39
Xylooligomers in liquor	0.00	37.04	41.53	42.92	50.17
Total	100.00	81.00	78.81	70.82	69.09
Temperature = 200°C					
Xylan in solid residue	100.00	35.08	12.55	7.67	11.12
Xylose in liquor	0.00	0.61	2.59	11.40	10.52
Xylooligomers in liquor	0.00	41.06	48.52	25.12	2.76
Total	100.00	76.75	63.66	44.19	24.40

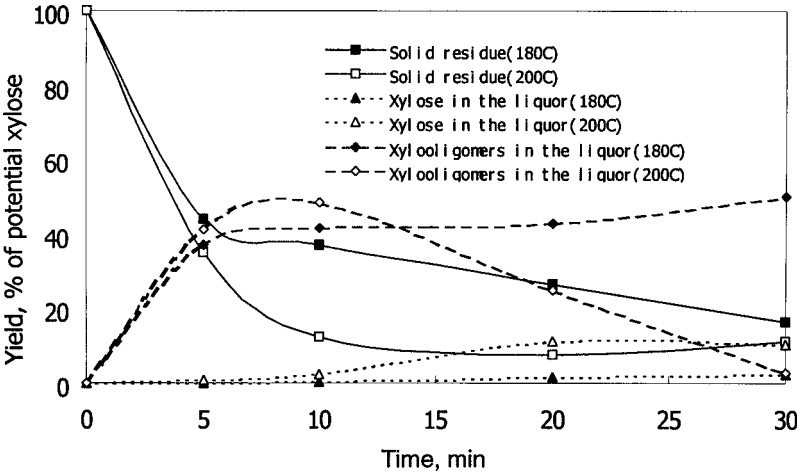

Fig. 2. Sugar recovery for uncatalyzed xylan hydrolysis at 180 and 200°C with 5% solids concentration.

180°C over the 30-min run time. At 200°C, the yield of xylooligomers reached a maximum of about 50% of potential xylose at 10 min and then dropped. At 180°C, the xylooligomer yield reached about 50% of potential xylose at 30 min, the end of our run time, but appeared to be still increasing. On the other hand, the amount of xylose in the solid residue decreased with increasing reaction time, reaching a nearly constant value after 10 min at 200°C but still continuing to drop after 30 min at 180°C.

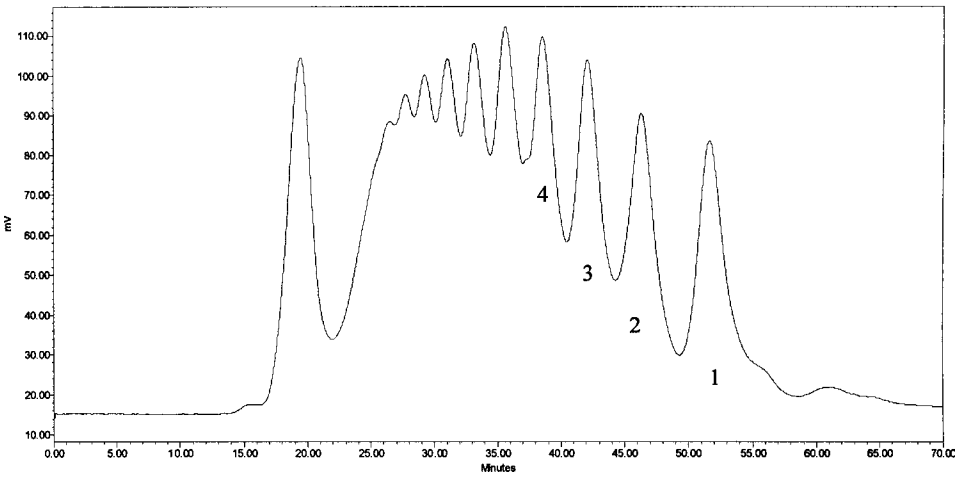

Fig. 3. IMP chromatogram of hydrolysate from uncatalyzed hydrolysis of oat xylan for a 5% solids concentration at 200°C for 10 min. 1, DP1; 2, DP2; 3, DP3; 4, DP4.

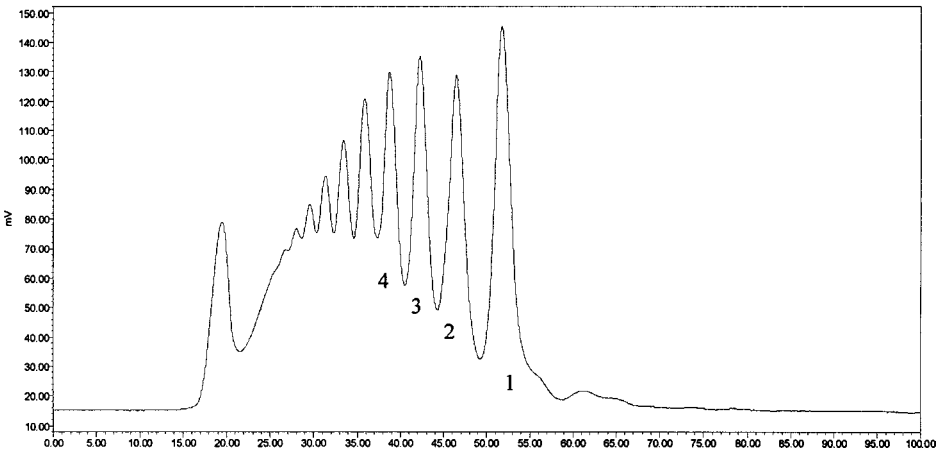

Fig. 4. IMP chromatogram of hydrolysate from uncatalyzed hydrolysis of oat xylan for a 5% solids concentration at 200°C for 20 min. 1, DP1; 2, DP2; 3, DP3; 4, DP4.

Figures 3 and 4 show IMP chromatograms of the liquid from uncatalyzed hydrolysis of xylan with a 5% solids concentration run at 200°C for 10 and 20 min, respectively. As shown, IMP separated oligomers up to DP10, and less monomer and more long-chain oligomers were observed at a 10-min than at 20 min. The average DP decreased as reaction time increased.

Pure xylose, xylobiose, xylotriose, xylotetraose and xylopentaose standards were used to calibrate the IMP system for species of DP1–DP5,

Table 2
Known Concentrations of Xylooligomers from DP1 to DP5 and Concentrations Calculated Based on Peak Height Relative to Peak Height for Measured Concentration of Xylobiose

	Actual Concentration (mg/mL)	Concentration based on peak height ratio (mg/mL)
Xylose	1.00	0.94
Xylobiose	1.02	1.02
Xylotriose	1.13	1.18
Xylotetraose	0.79	0.79
Xylopentaose	0.69	0.71

allowing us to calculate the concentration of DP1–DP5 in the liquors from chromatograms at each sample time of the type shown in Figs. 3 and 4. By testing a variety of samples of known composition, we found we could obtain better accuracy if we based the determination of concentrations on peak height rather than area. We also found that we could determine the concentration of each of these species well 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; this procedure proved more accurate than using xylose monomer as a basis.

An example of this calculation follows. A known sample containing 1.02 mg/mL of xylobiose gave a peak height signal of 33,252 mV, while a known sample of 0.79 mg/mL of xylotetraose gave a peak height signal of 25,894 mV. By a ratio of the peak heights, the xylotetraose concentration is calculated to be $(25{,}894/33{,}252) \times 1.02 = 0.794$ mg/mL, a value that is in good agreement with the known amount in the standard.

Table 2 summarizes a test with known concentrations of DP1–DP5 and the calculated concentration based on the ratios to the height of the xylobiose peak. This approach was extended to quantifying the concentrations of DP6–DP10 based on chromatograms such as in Figs. 3 and 4 for each sample time. However, we were unable to obtain standards to confirm the concentrations of DP6–DP10.

Based on the aforementioned approach, Fig. 5 shows the yield distribution of oligomer chain lengths from DP1–DP10 vs time for uncatalyzed xylan hydrolysis at 200°C with a 5% solids concentration. No oligomers in the DP1–DP10 range were observed until about 7 min into the run, and even then the concentrations were very low. At the 10-min mark, each of the concentrations suddenly rose to about 4% of the total potential sugar, although the monomer, DP2, DP10, and DP9 yields appeared to be somewhat lower than for the other oligomers. The highest oligomer concentration appeared to be for DP5, but the yields of a number of species were very similar.

A much greater separation in yields of the various chain lengths was measured at the 15-min mark, as shown in Fig. 5. Furthermore, the yields decreased in the exact order of the degree of polymerization with the

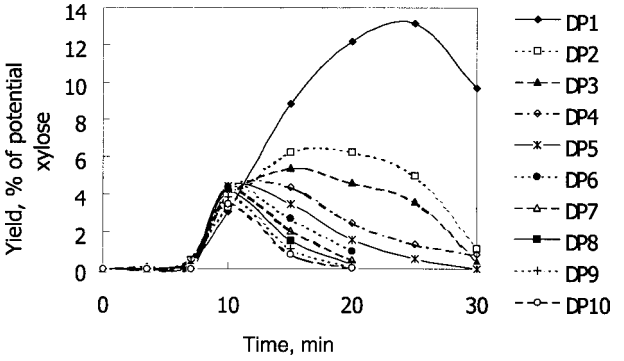

Fig. 5. Oligomer time profiles for uncatalyzed xylan hydrolysis at 200°C with a 5% solids concentration.

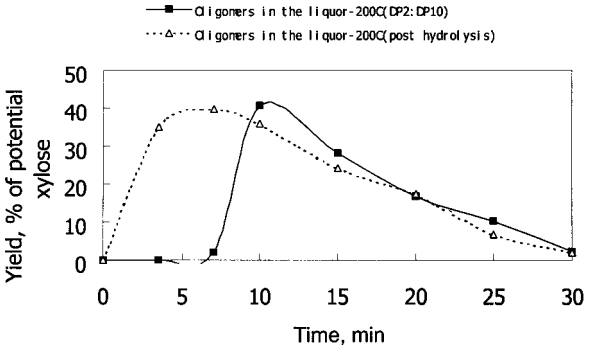

Fig. 6. Total oligomer yields determined by IMP and posthydrolysis.

highest yields being for monomers and the lowest for the DP10 oligomers. We also observed that those species from DP4–DP10 dropped from the peak seen at the previous time while xylose monomer yields continued to increase until the 25-min time. DP2 and DP3 reached their maximum levels at the 15-min sample time.

Because no significant yields of oligomers were observed via IMP until about 10 min into the run, we wished to determine whether very little solubilization occurred in these earlier times or whether the chains lengths were too long for our IMP system to measure them. Accordingly, we measured overall oligomer yields at each time by the posthydrolysis procedure and compared the results to the total oligomer yields measured by adding the yields of DP2–DP10, as shown in Fig. 6. Very good agreement was found between the total oligomer yield measured by both approaches from about 10 min on, suggesting that the IMP did a good job of capturing most of the oligomers in solution at longer run times and that use of the peak height ratios is reasonable. On the other hand, posthydrolysis revealed levels of oligomers in solution at the 3- and 7-min marks similar to at 10 min while almost no oligomers were measured via IMP for these earlier times.

These differences suggest that the chain length of the oligomers in solution is too long for the IMP column used to measure.

Conclusion

The oligomer yield profiles measured by IMP follow the expectation that longer chains depolymerize to form shorter chains that ultimately result in release of soluble oligomers and monomer. However, it is somewhat surprising how rapidly oligomers and monomer from DP10 to DP1 appeared and then mostly disappeared. Virtually no oligomers or monomers were observed with IMP until several min into the reaction, and at that time, the total yields measured by IMP and posthydrolysis of oligomers were very similar, suggesting that the peak height method of determining oligomer concentrations is reasonably accurate. However, while IMP provided no evidence of significant yields of oligomers at early times, posthydrolysis indicated that significant amounts of soluble oligomers with degrees of polymerization >10 were in solution but not detectable by the IMP system used. Accordingly, we plan to try other systems in an attempt to find columns that can measure longer soluble oligomers.

Acknowledgment

We are very grateful to the National Science Foundation Division of Bioengineering and Environmental System for supporting this research through contract BES-9985351.

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