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The enhancement of xylose monomer and xylotriose degradation by inorganic salts in aqueous solutions at 180 °C

Chaogang Liu and Charles E. Wyman*

Thayer School of Engineering, Dartmouth College, Hanover, NH 03755, USA

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Abstract—The inorganic salts KCl, NaCl, $CaCl_2$, $MgCl_2$, and $FeCl_3$, and especially the latter, significantly increased xylose monomer and xylotriose degradation in water heated to 180 °C with unaccountable losses of xylose amounting to as high as 65% and 78% for xylose and xylotriose, respectively, after 20 min incubation with 0.8% $FeCl_3$. Furthermore, losses of both xylose and xylotriose were well described by first order homogeneous kinetics, and the rate constants for xylose and xylotriose disappearance increased 6- and 49-fold, respectively, when treated with 0.8% $FeCl_3$ solution compared to treatment with just pressurized hot water at the same temperature. Although the addition of these inorganic salts produced a significant drop in pH, the degradation rates with salts were much faster than could be accounted for by a pH change. For example, the rate constants for the disappearance of xylose and xylotriose with 0.8% $FeCl_3$ were 3-fold and 7-fold greater, respectively, than for treatment with very dilute sulfuric acid at the same pH. In addition, xylose losses were greater than could be accounted for by just furfural production, suggesting that other degradation products were also formed, and xylose losses to unidentified compounds increased significantly with the addition of $FeCl_3$. The unidentified compounds could be formed through aqueous furfural resinification and condensation reactions that are accelerated by $FeCl_3$, but the actual mechanisms are still not clear.

Keywords: Sugar degradation; Xylose; Xylotriose; Inorganic salts; Ferric chloride

1. Introduction

Biomass pretreatment is one of the most important but expensive unit operations in the processing of cellulosic biomass to ethanol via biological methods.[1] Biomass pretreatment with just hot water or steam (180–230 °C), or autohydrolysis, is a relatively straightforward process that has been widely applied in biomass fractionation, pulp and paper making, fiberboard production, and the animal feed industry.[2,3] Autohydrolysis can solubilize most of the hemicellulose,[4] remove a large fraction of lignin,[5,6] and produce a very digestible fiber.[7–9] For biomass pretreatment with just hot water or very dilute acid, a large fraction of the solubilized hemicellulose is in the form of oligomers.[6,10] However, the total yield of xylose monomer and oligomers is only about 65% for batch pretreatment of corn stover,[2] and xylan losses of about 15–20% were reported for the pretreatment of poplar and agricultural residues by uncatalyzed steam explosion.[11] Furthermore, for biomass pretreatment with liquid hot water, the total xylose yield decreased with increasing solid concentration.[12] These low hemicellulose sugar yields are apparently due to the heterogeneous nature of the hemicellulose hydrolysis reactions and the rapid decomposition of sugars derived from hemicellulose.[2,11] Temperature, acid concentration, and residence time all contribute to the degradation of monomeric sugars including xylose, glucose, galactose, arabinose, and mannose.[13] Degradation of monomeric sugars in dilute acids at a low pH (<1) has been extensively studied, and these reactions are normally considered to be general acid–base catalyzed reactions with monosaccharide degradation following

* Corresponding author at present address: Bourns College of Engineering, Center for Environmental Research and Technology, University of California, 1084 Columbia Avenue, Riverside, CA 92507, USA. Tel.: +1 951 781 5703; e-mail: cewyman@engr.ucr.edu

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first-order kinetics over a pH range of 1–4 and temperatures of 170–230 °C.[14] Reaction rates are a minimum over a pH range of 2–2.5, and above pH 2.5, monosaccharide degradation becomes more rapid due to the base-catalyzed reaction.[15] However, few papers have reported degradation reactions in aqueous solution at a high temperature and a near neutral pH, although there is evidence that sugar oligomers directly degrade in just hot water at neutral pH.[16]

Sugar loss is also observed in sucrose processing, and inorganic salts have been shown to increase its thermal degradation, even at moderate conditions (pH 7.0, ⩽90 °C).[17,18] Many forms of lignocellulosic biomass, including agricultural residues, have a high ash content (~6%), with the major cations being calcium, potassium, magnesium, sodium, alumina, and iron, and most of the cations are considered to be bound to inorganic anions (SO_4^{2-}, PO_4^{3-}, Cl^{1-}, OH^{1-}). A number of researchers have reported the impact of inorganic salts on thermal degradation of cellulose and hemicelluose for biomass gasification.[19,20] It has been shown that minerals in lignocellulosic biomass neutralize a portion of the added acid during acid-catalyzed biomass pretreatment, thus impacting hemicellulose hydrolysis and enzymatic digestion of pretreated substrates.[21–23] However, little has been reported on how such inorganic salts affect the degradation of hemicellulose sugars released during biomass pretreatment.

Because xylose monomer and oligomers are the primary products in hemicellulose hydrolysis and biomass pretreatment with just hot water, the fates of xylose and xylotriose were followed at various concentrations of inorganic salts (KCl, NaCl, $CaCl_2$, $MgCl_2$, and $FeCl_3$) in this study to shed light on how the latter affect reactions of sugar oligomers and monomers that are derived during hemicellulose hydrolysis in hot water at 180 °C. In addition, degradation rate constants were determined from the xylose and xylotriose data to better understand how salts alter reaction pathways.

2. Results and discussion

2.1. Impact of inorganic salts on xylose and xylotriose disappearance

Figure 1 summarizes some of our data on the treatment of xylose with hot water at 180 °C with and without addition of KCl, NaCl, $CaCl_2$, $MgCl_2$, or $FeCl_3$. As expected, the xylose concentration decreased with increasing reaction times because of the decomposition of xylose at high temperatures, but all of the inorganic salts increased the rate of xylose decomposition. Furthermore, $FeCl_3$ had a particularly strong effect, with 90% of the initial xylose disappearing after 20 min versus only about 40% for addition of just hot water at the same temperature and same reaction time (see Fig. 5). Overall, the impact of the different salts increased in the following order: NaCl, KCl, $CaCl_2$, $MgCl_2$, and $FeCl_3$.

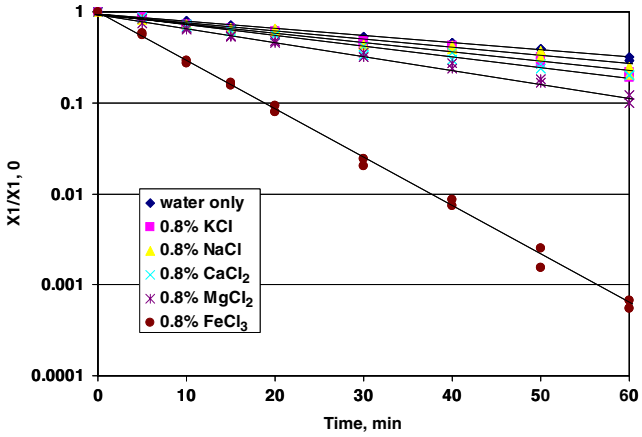

Figure 1. The effect of addition of inorganic salts on degradation of xylose in hot water at 180 °C.

A similar trend was observed for treatment of xylotriose with hot water at 180 °C, as illustrated in Figure 2. However, although the order of impact was the same as for xylose, all the salts had a greater impact on xylotriose than on xylose. Furthermore, addition of 0.8% $FeCl_3$ at 180 °C resulted in an almost 100% reaction of xylotriose after 1 min while it took more than 20 min to react 60% of the xylotriose when only water was added (see Fig. 6).

2.2. Determination of reaction rate constants

As shown in Figures 1 and 2, a plot of $\ln(X_1/X_{1,0})$ versus time and $\ln(X_3/X_{3,0})$ versus time, where $X_{1,0}$ and $X_{3,0}$ are the initial xylose and xylotriose concentrations, respectively, and X_1, X_3 represent xylose and xylotriose concentrations at any time, respectively, followed a linear relationship. Thus, xylose and xylotriose degradation in hot water with and without added inorganic salts can be described as first-order homogeneous reactions. Accordingly, reaction rate constants were determined by minimizing the sum of the squares of the differences between predictions of first-order homogeneous kinetic models and the experimental data using the Solver routine in Microsoft Excel. As shown in Table 1, the rate constant for treatment of xylose with just hot water at 180 °C was 0.0200 min^{-1}. However, addition of 0.8% KCl, NaCl, $CaCl_2$, $MgCl_2$, and $FeCl_3$ increased the rate constants by factors of 1.3, 1.2, 1.4, 1.8, and 6.1, respectively. A similar trend was observed for the addition of these salts to xylotriose, with the enhancement ratios now being even greater at 1.5, 1.4, 2.2, 2.4, and 48.7, respectively. Thus, we see that all of these salts promoted the reaction of xylose and xylotriose and that

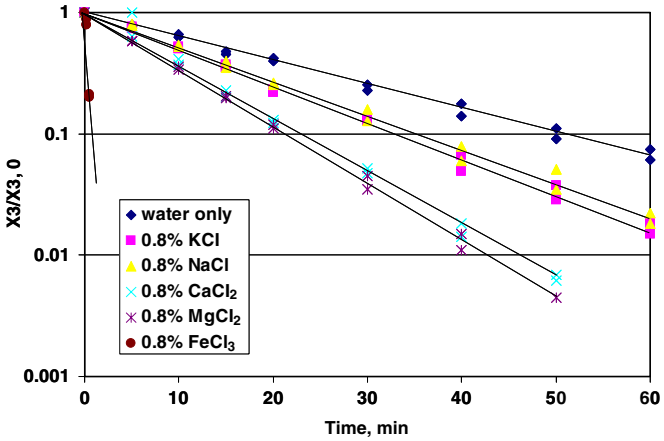

Figure 2. The effect of addition of inorganic salts on degradation of xylotriose in hot water at 180 °C.

Table 1. Rate constants for the disappearance of xylose and xylotriose when reacted in hot water containing inorganic salts at 180 °C

Solvent	pH	Xylose		Xylotriose	
		Rate constant (min^{-1})	Ratio to control	Rate constant (min^{-1})	Ratio to control
Water only (control)	6.98	0.0200	—	0.0458	—
0.8% KCl	5.68	0.0251	1.3	0.0690	1.5
0.8% NaCl	5.72	0.0239	1.2	0.0650	1.4
0.8% $CaCl_2$	5.59	0.0281	1.4	0.1012	2.2
0.8% $MgCl_2$	5.87	0.0360	1.8	0.1082	2.4
0.8% $FeCl_3$	1.86	0.1229	6.1	2.2304	48.7

addition of $FeCl_3$ significantly increased the rate of disappearance of xylotriose.

2.3. Enhancement of reactions by $FeCl_3$

As reported in Table 1, all of the solutions containing inorganic salts were acidic, but the pH of 0.8% $FeCl_3$ solution was particularly low at 1.86. Thus, we might expect that the low pH could account for the enhanced degradation of xylose and xylotriose. To test this hypothesis, the pH of HPLC grade water (pH 6.98) was first adjusted to a value of 1.86 by adding dilute sulfuric acid and then used to treat xylose and xylotriose at the same temperature as used previously. The rate constants were determined for the reaction of xylose and xylotriose in this water without the addition of any salts and compared to the rate constants for just the water at pH 6.98 and for the addition of 0.8% $FeCl_3$ (Fig. 3). As expected, xylose degraded more rapidly in water with its pH adjusted to 1.86 by the addition of sulfuric acid, and the rate constant of disappearance of initial xylose increased to 0.0332 min^{-1} versus 0.0200 min^{-1} for treatment of xylose with just HPLC grade water (pH 6.98) at the same temperature. However, the rate constant was even higher (0.1229 min^{-1}) when xylose was treated with 0.8% $FeCl_3$ at the same temperature. The results for treatment of xylotriose with stock HPLC grade water, HPLC grade water adjusted to a low pH with sulfuric acid, and the $FeCl_3$ solution shown in Figure 4 also revealed that $FeCl_3$ has a larger influence on reaction than does the use of low pH water alone. However, the addition of sulfuric acid had a greater relative effect on the disappearance of xylotriose compared to xylose, and the rate constants for xylotriose disappearance were 0.0458, 0.3159, and 2.2304 min^{-1} for treatment with just water, pH-adjusted water, and 0.8% $FeCl_3$, respectively, at 180 °C.

The large differences in the effects of 0.8% $FeCl_3$ and water adjusted to the same pH suggested that a different mechanism may exist for the reaction in the presence of $FeCl_3$ versus reaction at a low pH without adding this salt. Two mechanisms seemed possible: (1) $FeCl_3$ impacts the structure of water[17,24] and (2) $FeCl_3$ catalyzes the dehydration of carbohydrates. However, the fact that xylotriose disappears much faster than xylose under the same conditions and that the relative effects of acid are different for xylose and xylotriose suggests that the mechanisms for $FeCl_3$ action could be different for the two carbohydrates. We plan a more in depth investigation to try to explain these differences in the future.

2.4. Xylose losses and degradation products

The xylose remaining, furfural recovered, and unaccounted for xylose losses were determined as a percent of the initial xylose added over time for treatment of xy-

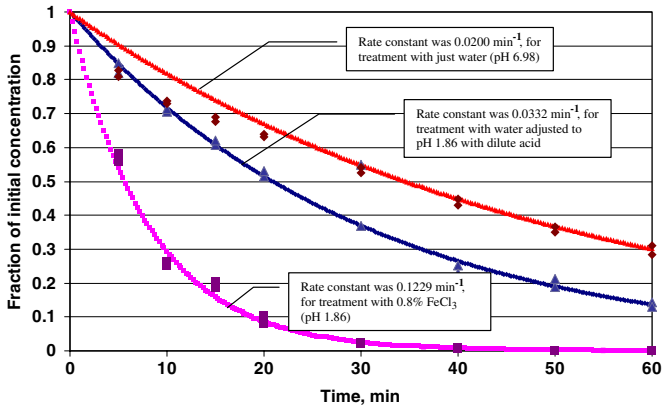

Figure 3. The enhancement of xylose disappearance by 0.8% $FeCl_3$ in hot water at 180 °C.

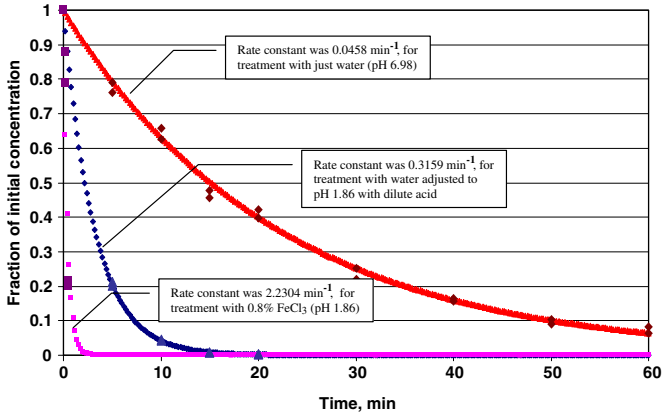

Figure 4. The enhancement of xylotriose disappearance by 0.8% $FeCl_3$ in hot water at 180 °C.

lose with hot water at 180 °C, with and without added salts, as shown in Figure 5. As expected, the yield of furfural, a key degradation product, increased with reaction time. However, a large fraction of xylose losses was not accounted for by the increase in furfural concentration, and these unidentified xylose losses increased significantly with $FeCl_3$ addition. For example, when xylose was treated with 180 °C water containing 0.8% $FeCl_3$ for 20 min, the loss of xylose increased to 65% versus about 32% for treatment of xylose with just hot water at the same temperature and residence time (Fig. 5b and a).

This enhancement in the unaccountable xylose loss was also observed for xylotriose treatment with 0.8% $FeCl_3$, as shown in Figure 6, in terms of percent of the xylotriose present initially. In this case, xylotriose degradation at this temperature produces xylobiose, xylose, and furfural, with the relative amounts depending on the reaction time. For example, when xylotriose was treated with hot water at 180 °C for less than 5 min, primarily xylotriose and xylobiose could be measured, but almost 15% of the material added initially could not be accounted for. After 5 min, xylose and furfural were also produced, and both increased with an increase in the reaction time after that (Fig. 6a). This result is consistent with the traditional concept that a higher degree of polymerization (DP) oligomers first degrade to lower DP oligomers and then to monomer and furfural.[25] However when xylotriose was treated with hot water containing 0.8% $FeCl_3$ at the same temperature, xylotriose disappeared completely in only 1 min, and xylose, the only degradation product measurable at that time, could not nearly account for all of the xylotriose added initially (Fig. 6b). This result showed that $FeCl_3$ addition significantly increased direct degradation of xylotriose.

Comparing Figures 5 and 6 reveals that less total xylose including monomers and oligomers was lost for treatment of xylotriose than for treatment of xylose with just hot water at 180 °C (24% vs 32% after 20 min). However, for treatment with water containing 0.8% $FeCl_3$, total xylose losses were greater for the reaction of xylotriose than of xylose. For example, xylose losses were 78% when xylotriose was treated with hot water containing 0.8% $FeCl_3$ for 20 min versus losses of 68% for treatment of xylose at otherwise identical conditions.

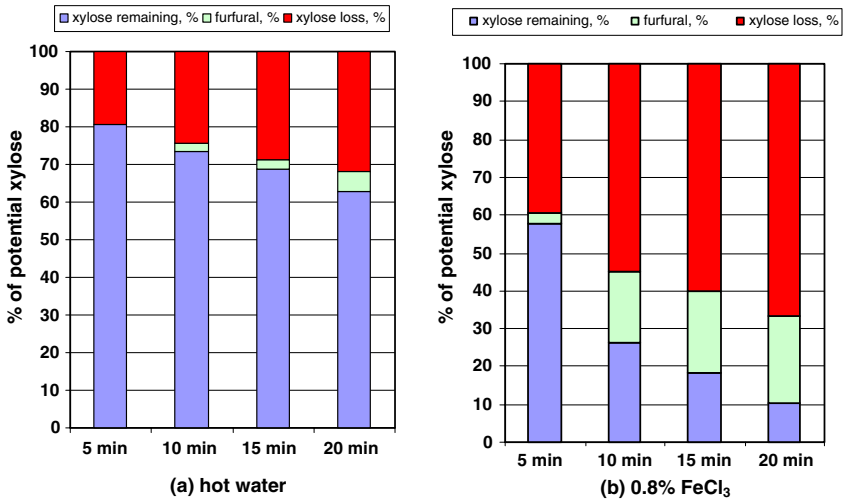

Figure 5. The effect of 0.8% $FeCl_3$ on xylose remaining, furfural formation, and unaccountable xylose losses for treatment of xylose with hot water at 180 °C.

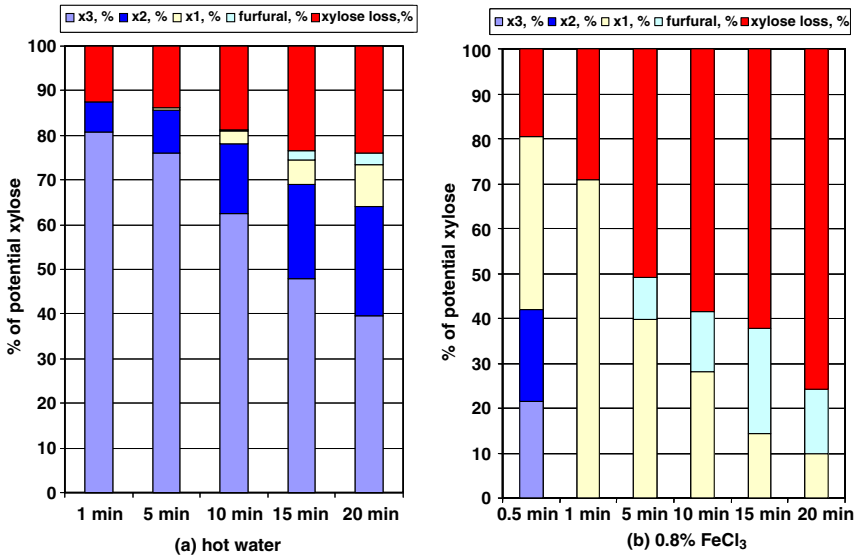

Figure 6. The effect of 0.8% $FeCl_3$ on xylobiose (X_3) remaining, xylobiose (X_2) formation, xylose (X_1) release, furfural production, and unaccountable xylose losses for treatment of xylotriose with hot water at 180 °C.

2.5. Mechanism for 'xylose loss'

It was hypothesized that intermediates or sugar–salt complexes could be produced before the formation of furfural that may account for the unaccounted xylose losses. However, our HPLC detected no other products before any furfural for short residence times. In addition, the peak heights were the same for sugars dissolved with salt addition as with just deionized water, suggesting no sugar–salt complexes were formed. We did observe at least two unidentified peaks before the furfural peak for treatment of xylose or xylotriose in hot water with or without the addition of organic salts, for increased residence times (Fig. 7). The unidentified compounds could be furfural resins formed by mechanisms consistent with resinification and condensation reactions that occur for furfural formation in aqueous systems.[26] In any event, it is clear that inorganic salts, and particularly $FeCl_3$, significantly increased production of unidentified compounds (Fig. 7) suggesting that inorganic salts not only catalyze furfural formation but also accelerate furfural resinification and condensation. It was reported that ash removal could reduce char formation during biomass pyrolysis,[27] indicating that inorganic salts may also catalyze the formation of chars at high temperatures. Furthermore, the chars produced may also account for some of the xylose disappearance. In the future, we plan to seek other ways to characterize the unidentified degradation products and develop a mechanism that could explain why inorganic salts and particularly $FeCl_3$ accelerate sugar losses.

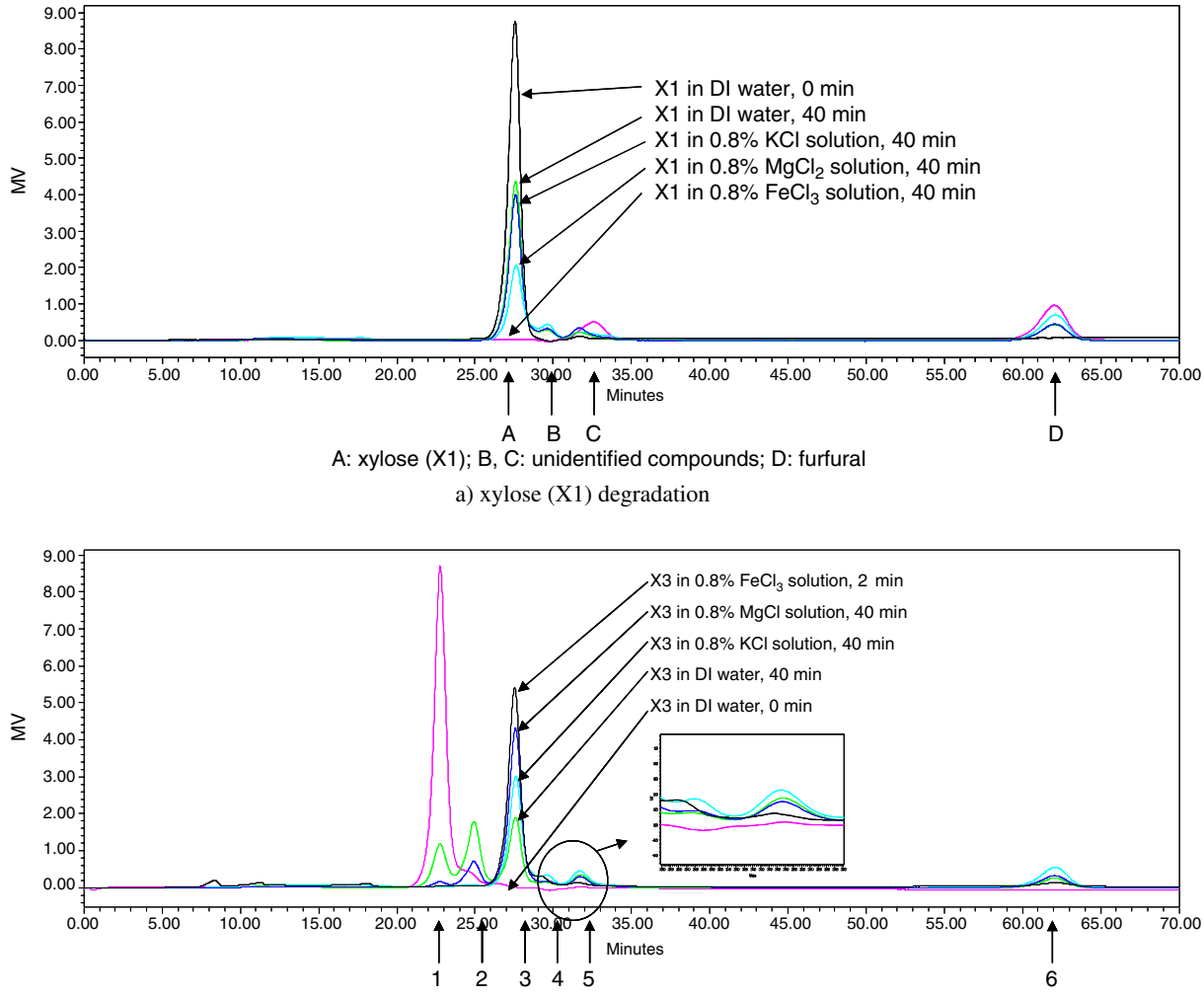

Figure 7. Chromatographs for degradation of xylose (X_1) and xylotriose (X_3) in hot water at 180 °C with or without the addition of inorganic salts.

3. Conclusions

A number of inorganic salts significantly accelerated degradation of xylose monomer and xylotriose in hot water at 180 °C. The addition of 0.8% $FeCl_3$ had a particularly strong effect, with the rate constants for the disappearance of xylose and xylotriose increasing 6.1- and 49-fold, respectively. In addition, $FeCl_3$ addition resulted in significantly greater losses of xylose to unidentifiable compounds for treatment of xylose monomer and xylotriose at 180 °C. Furthermore, the addition of $FeCl_3$ increased losses of xylotriose to unknown compounds even more than xylose. The unidentified compounds could be furfural resins formed by aqueous furfural resinification and condensation reactions that are accelerated by salts. However, the mechanisms are still not clear, and work is ongoing to define a pathway that could account for the enhancement of xylose monomer and xylotriose degradation by inorganic salts.

4. Experimental

4.1. Materials

Pure monomeric xylose (purity $\geqslant$ 99.9%) was obtained from Sigma Chemical Co. (St. Louis, MO). Furfural (purity > 99.9%) was obtained from Fisher Scientific (Chicago, IL). Xylobiose and xylotriose (purity ~ 99.5%) were purchased from Magazyme International Ireland, Limited (Bray, County Wicklow, Ireland). All inorganic salts (KCl, NaCl, $CaCl_2{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, $FeCl_3{\cdot}7H_2O$) were obtained from Sigma Chemical Co. (St. Louis, MO). HPLC grade water was purchased from Fisher Scientific Co. (Chicago, IL).

4.2. Experimental operation

HPLC grade water was added to pure xylose and xylotriose to make solutions with concentrations of 0.0266

and 0.0228 mol/L, respectively. Then, additional HPLC grade water containing 1.6 wt % KCl, NaCl, $CaCl_2$, $MgCl_2$ or $FeCl_3$ was added to each sugar solution to reduce the sugar concentration to half its initial value. Additional solutions were prepared at the same dilutions but using just HPLC grade water without added salts to provide controls with the same xylose and xylobiose concentrations.

Aliquots (600 μL) of the xylose or xylotriose solutions described above were added to flat bottom clear glass crimp vials and sealed with aluminum crimp tops with TFE/silicone liners (7 mm × 40 mm, 800 μL, Alltech Associates Inc.; Deerfield, IL). These tiny reactors were then rapidly heated to the target temperature of 180 °C by immersing them in a 22.8 cm ID × 35 cm deep 4 kW model SBL-2D fluidized sand bath (Techne Corporation, Princeton, NJ). After the appropriate reaction time, the vials were immediately removed from the sand bath and submerged in ice water to quench the reaction. The contents of the vials were removed with a syringe and transferred to a centrifuge vial fitted with a 0.2 μm nylon filter. Enough calcium carbonate was added to each vial to adjust the pH to a range of 5–6. After centrifugation supernatants were transferred to 0.5 mL polyethylene HPLC vials for HPLC analysis.

4.3. Sugar analysis

Xylotriose, xylobiose, xylose, and furfural were all analyzed using a Waters (Milford, MA) high performance liquid chromatography (HPLC) system (module 2695) equipped with a Waters refractive index (RI) model 2414 detector and a Bio-Rad (Hercules, CA) Aminex HPX-42A ion-moderated partition (IMP) column. Deionized water was used as the eluent at a flow rate of 0.4 mL/min with a column temperature of 85 °C and a run time of 60 min. All sugars, including xylose, xylobiose, and xylotriose, were calibrated with pure xylose (⩾99.9%) as a standard.[28] A known concentration of dilute furfural solution was used for calibrating furfural concentration in the samples. All experiments were performed at least in duplicate.

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References

1. Lynd, L. R. *Annu. Rev. Energy Environ.* **1996**, *21*, 403–465.
2. Heitz, M.; Capek-Menard, E.; Korberle, P. G.; Gange, J.; Chornet, E.; Overend, R. P.; Taylor, J. D.; Yu, E. *Bioresour. Technol.* **1991**, *25*, 23–32.
3. Lora, J. H.; Wayman, M. *Tappi J.* **1978**, *61*, 47–50.
4. Mok, W.; Antal, M. J. *Ind. Eng. Chem. Res.* **1992**, *31*, 1157–1161.
5. Bobleter, O. *Prog. Polym. Sci.* **1994**, *19*, 797–841.
6. Liu, C.; Wyman, C. E. *Ind. Eng. Chem. Res.* **2003**, *42*, 5409–5416.
7. Allen, S. G.; Kam, L. C.; Zemann, A. J.; Antal, M. J. *Ind. Eng. Chem. Res.* **1996**, *35*, 2709–2715.
8. Laser, M.; Schulman, D.; Allen, S. G.; Lichwa, J.; Antal, M. J.; Lynd, L. R. *Bioresour. Technol.* **2002**, *81*, 33–44.
9. Liu, C.; Wyman, C. E. *Bioresour. Technol.* **2005**, *96*, 1978–1985.
10. Liu, C.; Wyman, C. E. *Ind. Eng. Chem. Res.* **2004**, *43*, 2781–2788.
11. Saddler, J. N.; Ramos, L. P.; Breuil, C. Steam Pretreatment of Lignocellulosic Residues. In *Bioconversion of Forest and Agricultural Plant Residues*; Saddler, J. N., Ed.; Biotechnology in Agriculture Series; CAB International: Oxford, UK, 1993; Vol. 9, pp 73–92.
12. Stuhler, S. L. MS Thesis, Dartmouth College, 2002.
13. Baugh, K. D.; McCarty, P. L. *Biotechnol. Bioeng.* **1988**, *31*, 50–61.
14. Root, D. F.; Saeman, J. F.; Harris, J. F.; Neill, W. K. *Forest Prod. J.* **1959**, *9*, 158.
15. Baugh, K.; McCarty, P. L. *Biotechnol. Bioeng.* **1988**, *31*, 50–61.
16. Li, X. Master Thesis, Dartmouth College, 2004.
17. Eggleston, G.; Vercellotti, J. R.; Edye, L. A.; Clarke, M. A. *J. Carbohydr. Chem.* **1996**, *15*, 81–94.
18. Clarke, M. A.; Brannan, M. A.; Carpenter, F. G. In 1976 Technical Session on Cane Sugar Refining Research, New Orleans, LA, 1977; p 46.
19. Varhegyi, G.; Antal, M. J., Jr.; Szekely, T.; Till, F.; Jakab, E.; Szabo, P. *Energy Fuels* **1988**, *2*, 273–277.
20. Antal, M. J., Jr. In *Advances in Solar Energy*; Boer, K. W., Duffie, J. A., Eds.; American Solar Energy Society: Newark, DE, 1982; Vol. 1, pp 61–111.
21. Maloney, M. T.; Chapman, T. W. *Biotechnol. Bioeng.* **1985**, *27*, 355–361.
22. Kim, S. B.; Lee, Y. Y. *Biotechnol. Bioeng. Symp.* **1987**, *17*, 71–84.
23. Lloyd, T.; Wyman, C. E. *Appl. Biochem. Biotechnol.* **2004**, *113–116*, 1013–1022.
24. Febbema, O. In *Principles of Food Science, Part 1*; Fennema, O., Ed.; M. Dekker: New York, 1976; pp 13–39.
25. Garrote, G.; Dominguez, H.; Parajo, J. C. *J. Chem. Technol. Biotechnol.* **1999**, *74*, 1101–1109.
26. Zeitsch, K. J. *The Chemistry and Technology of Furfural and Its Many By-products*; Elsevier: New York, 2000; pp 19–22.
27. Blasi, C. D.; Branca, C.; Derrica, G. *Thermochim. Acta* **2000**, *364*, 133–142.
28. Li, X.; Converse, A. O.; Wyman, E. C. *Appl. Biochem. Biotechnol.* **2003**, *105–108*, 515–522.