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Xylan Hydrolysis in Zinc Chloride Solution

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ABSTRACT

Xylan is the major component of hemicellulose, which consists of up to one-third of the lignocellulosic biomass. When the zinc chloride solution was used as a pretreatment agent to facilitate cellulose hydrolysis, hemicellulose was hydrolyzed during the pretreatment stage. In this study, xylan was used as a model to study the hydrolysis of hemicellulose in zinc chloride solution. The degradation of xylose that is released from xylan was reduced by the formation of zinc- xylose complex. The xylose yield was >90% (w/w) at 70°C. The yield and rate of hydrolysis were a function of temperature and the concentration of zinc chloride. The ratio of zinc chloride can be decreased from 9 to 1.3 (w/w). At this ratio, 76% of xylose yield was obtained. When wheat straw was pretreated with a concentrated zinc chloride solution, the hemicellulose hydrolysate contained only xylose and trace amounts of arabinose and oligosaccharides. With this approach, the hemicellulose hydrolysate can be separated from cellulose residue, which would be hydrolyzed subsequently to glucose by acid or enzymes to produce glucose. This production scheme provided a method to produce glucose and xylose in different streams, which can be fermented in separated fermenters.

Index Entries: Xylan; xylose; hydrolysis; zinc chloride; zinc-xylose complex.

INTRODUCTION

About 70% of the dry mass in lignocellulosic biomass are cellulose and hemicellulose (*1*). The utilization of these two carbohydrate fractions would affect the economical feasibility of any biomass conversion process.

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If these two carbohydrates are to be utilized by a fermentation process efficiently, hemicellulose should be hydrolyzed completely to D-xylose (50–70% w/w) and L-arabinose (5–15%, w/w), and cellulose should be converted to glucose. The subsequent fermentation of these sugars can be carried out separately or simultaneously with a coculture process using both glucose-fermenting yeast and xylose-fermenting yeast (2). However, it has been shown that the glucose inhibits the uptake of xylose (3–5). For this reason, it is preferable to ferment xylose separately, especially, if the fermentation is carried out in a continuous fermentor (6).

It has been shown that the nonglucose carbohydrate portion of the cellulosic biomass is much more sensitive to acid hydrolysis than the glucose portion (7,8). Although hemicellulosic sugars, especially xylose, can be easily obtained by acid hydrolysis in a reasonable yields (over 70%), the treatments were usually performed between 100 and 160°C. The hydrolysis of hemicellulose is accelerated at elevated temperatures owing to a relatively high-activation energy in the solid–liquid phase reaction (7–10). At a high temperature, part of xylose released from hemicellulose can be degraded rapidly, and cellulose in the amorphous region can yield glucose, which complicates the utilization of xylose (11).

Cellulosic biomass hydrolysis proceeds faster in a liquid state than in the solid or colloid state. The liquefaction of cellulosic materials can be carried out in zinc chloride solution. Zinc chloride is a salt that is not highly toxic to living cells and is easily recyclable (12). At a high concentration, the Hammit acidity of this salt solution ranges from −2 to −6, and cellulose and hemicellulose can be easily dissolved. At an elevated temperature, zinc ions can react with carbohydrates to form zinc–cellulose complexes, which are more susceptible to acid hydrolysis (13). Also, the zinc ions form complexes with monosaccharides and retard degradation of the mono-sugars during hydrolysis, but at a low reaction temperature, without the presence of acids, zinc chloride can hydrolyze cellulose only to low-DP celludextrin (14), and hemicellulose completely to monosaccharidesh. In this article, we tried to determine the effect of the zinc chloride solution on the hydrolysis rate of hemicellulose.

MATERIALS AND METHODS

Materials

Xylan (Sigma Chemical Company, St. Louis, MO) was used as a model compound of hemicellulose. Wheat straw was obtained from local farm in West Lafayette, which was ground to 60 mesh powder by dry milling before pretreatment of zinc chloride. The moisture content of the powder was 8% (w/w) after milling. Technical-grade zinc chloride was purchased from Ashland Chemical Company.

Methods

Xylan Hydrolysis in Zinc Chloride Solution

Solid zinc chloride was dissolved in 20 mL deionized water to make a concentrated zinc chloride solution (ranging from 64 to 78% w/w). Ten grams of xylan were mixed with 20 mL water to form a slurry. The xylan slurry was mixed with a zinc chloride solution to form zinc–xylan complex. The ratio of zinc chloride to xylan ranges from 4.0 to 9.0. After xylan was liquefied, a certain amount of water was added to adjust xylan concentration to 10% (w/v). The hydrolysis is then carried out in a water batch at various temperatures. The end of hydrolysis is indicated by the absence of precipitation when hydrolysate is dropped into ethanol. The samples were drawn periodically and added to a solution of sodium carbonate to remove zinc as precipitate of zinc carbonate. After centrifugation, the supernatant were analyzed by HPLC.

Acid Hydrolysis of Xylan

Ten grams of xylan were mixed with 100 mL 3% HCl solution and were heated in a 100°C water bath to begin the hydrolysis process. The samples that were taken periodically were treated with sodium carbonate solution and analyzed by HPLC as shown above.

Pretreatment of Wheat Straw and Hemicellulose Hydrolysis

Twenty grams of wheat straw powder (60 mesh) was dissolved in 64% zinc chloride solution with stirring at 60°C to form 100 mL of solution. After 3 h, 50 mL of water was dropped into the solution to adjust zinc chloride concentration below 35% (w/w). In this condition, cellulose and lignin were precipitated and separated from hemicellulose hydrolysate. The hydrolysate sample was treated and analyzed by HPLC after zinc ion was precipitated by the addition of saturated sodium carbonate solution.

Analytical Methods

Soluble sugars were measured by HPLC (Waters Associate 712 WISP system) with column HPX-87H (Bio-Rad; column size: 300 × 7.8 mm; mobile phase: 0.05 mol H_2SO_4; flow rate: 0.8 mL/min; temperature: 60°C). Zinc content was determined with an atomic absorption spectrophotometer (2380, Perkin-Elmer).

RESULTS AND DISCUSSION

Procedures of Xylan Hydrolysis in Zinc Chloride Solution

Four experimental procedures were set to determine the effectiveness of zinc chloride in the hydrolysis of xylan and cellulose (Fig. 1). Although

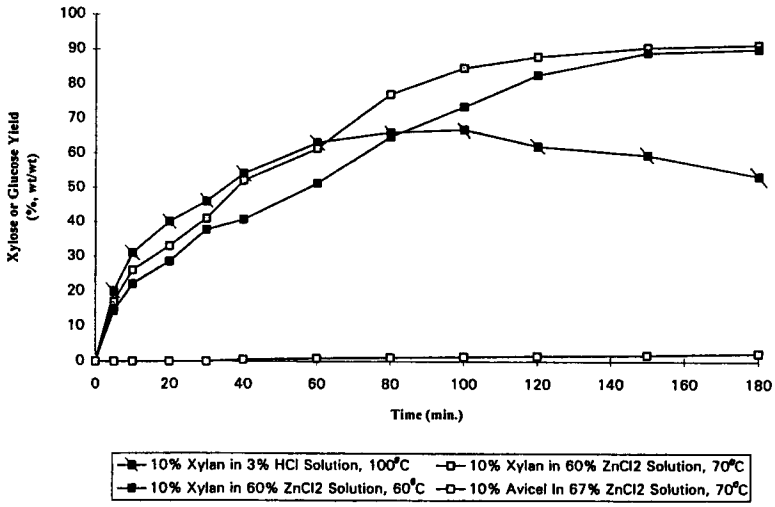

Fig. 1. Time curve for xylan hydrolysis in zinc chloride solution.

two strong mineral acids, hydrochloric and sulfuric, had always been contenders for industrial development (*13,14*), only diluted hydrochloric acid could be added into the zinc chloride pretreatment process without precipitation of zinc salt. The temperature of hydrolysis process was set below 75°C to control the hydrolysis of cellulose.

Hydrolysis of xylan at 60 and 70°C was compared with hydrolysis of avicel at 70°C (Fig. 1). In 60% zinc chloride solution (the ratio of zinc chloride to xylan is 9.0), xylan was liquefied to form hemicellulose solution immediately; by 3 h of hydrolysis, similar xylose yields of about 91% at both 60 and 70°C were obtained, although the hydrolysis rate at 60°C was slower than that at 70°C. In same condition, avicel (crystalline cellulose) only was hydrolyzed at <2% glucose yield. Therefore, using zinc chloride as solvent and catalyst, a xylose solution with high purity can be obtained at 60°C according to the difference in sensibility of xylan and cellulose to acid hydrolysis. This process provided a method to produce xylose and glucose in different streams.

When xylan was treated by diluted acid at 100°C, its hydrolysis rate in 3% hydrochloric acid was faster than that in zinc chloride solution at 70°C, but the xylose produced was degraded during the hydrolysis, and the maximal xylose yield was only about 66%. These results, compared with 91% xylose yield in zinc chloride case, indicate that zinc–xylose complex is stable until 70°C. The lower reaction temperature and higher xylose yield in zinc chloride could result from the phase transformation of xylan from solid to liquid and the function of zinc ion forming complexes with carbohydrates. The formation of zinc–xylose complex would catalyze the acid hydrolysis of xylan.

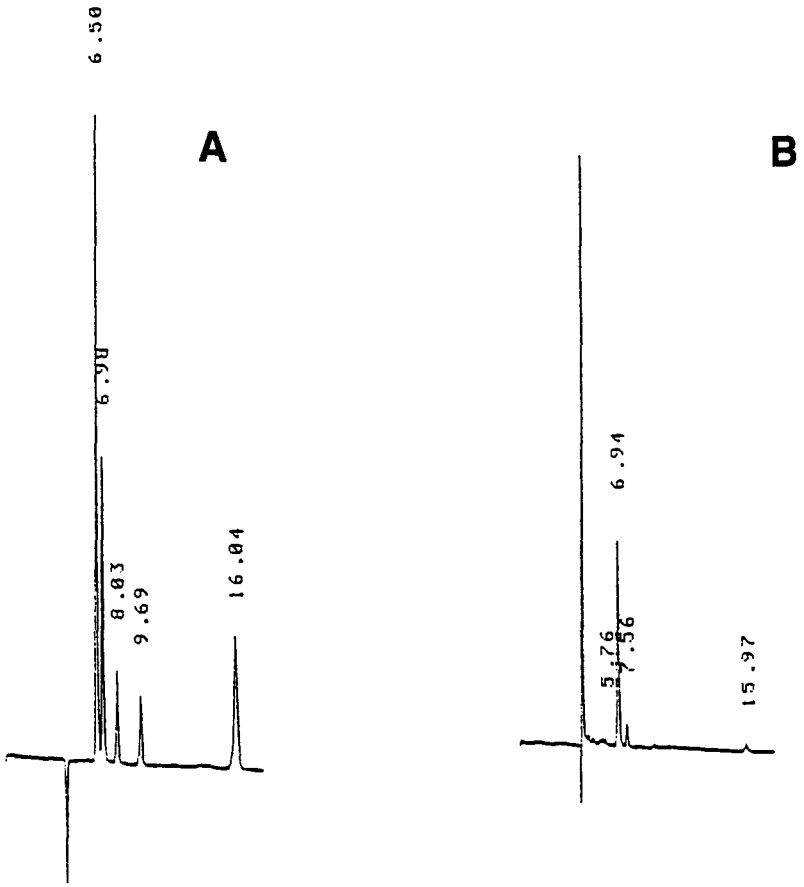

Fig. 2. HPLC result of hemicellulose hydrolysate of wheat straw pretreated by $ZnCl_2$. A: Standard HPLC chromatogram of glucose and xylose; glucose RT: 6.50, xylose RT: 6.98. (column: HPX-87H; column size: 300 × 7.8 mm; mobile phase: 0.05 mol H_2SO_4; flow rate: 0.8 mL/min; temperature: 60°C). B: Hemicellulose hydrolysate.

At the same hydrolysis condition, wheat straw was totally dissolved in zinc chloride solution, and was hydrolyzed (the ratio of zinc chloride to cellulosic biomass was 4). After 3 h, a certain amount of water was dropped into the solution to adjust zinc chloride concentration below 40% (w/w). In this condition, cellulose and lignin was precipitated and separated from hemicellulose hydrolysate. At 60°C, a 86% yield of xylose was obtained and the HPLC analysis (Fig. 2) showed that the hydrolysate contained xylose and arabinose without detectable glucose. This preliminary work implied that essentially all of the nonglucose part of the lignocellulose was accounted for in the xylose, and the part with the most glucose was not hydrolyzed. Compared with the result of normal diluted acid

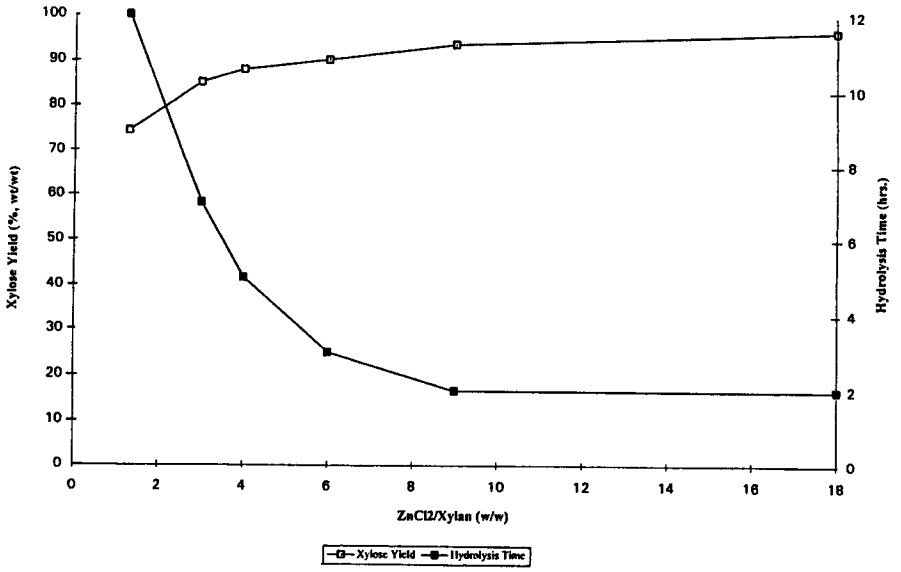

Fig. 3. Effect of ratio of zinc chloride to xylan on hydrolysis yield. Different ratios of zinc chloride to xylan were applied for the hydrolysis at 60°C.

pretreatment (*7,15*), this method provides a possibility to produce xylose free from glucose.

Effect of Zinc Chloride:Xylan Ratio on the Xylose Yield

The yield and rate of xylan hydrolysis in zinc chloride were a function of the concentration of zinc chloride (Fig. 3). The ratio of zinc chloride to xylan did not affect the hydrolytic rate if the ratio was above 9.0. When this ratio was lower than 9, the rate of xylan liquefaction and hydrolysis was in proportion to the concentration of the zinc chloride solution. This phenomenon is reflected by the rapid reduction of the acidity in the zinc chloride solution when zinc chloride solution is diluted. Figure 3 shows that when the zinc chloride:xylan ratio ranges from 18.0 to 4.0, the xylose yield at 60°C was maintained constant at about 90%, but the hydrolysis time increased from 2 to 5 h. When the ratio of zinc chloride to xylan was further decreased to <4, xylan was liquefied slowly, and the xylose yield started to reduce rapidly. At a ratio of 1.3 (w/w), it only gave 76% xylose yield after a prolonged hydrolysis time.

Effect of Temperature on Xylose Yield

Temperature was an important factor controlling xylan hydrolysis and degradation of released xylose. Both reactions were accelerated by the increase of the temperature. Above 70°C, the xylose degradation rate becomes significant, thus lowering the xylose yield (Fig. 4). Considering

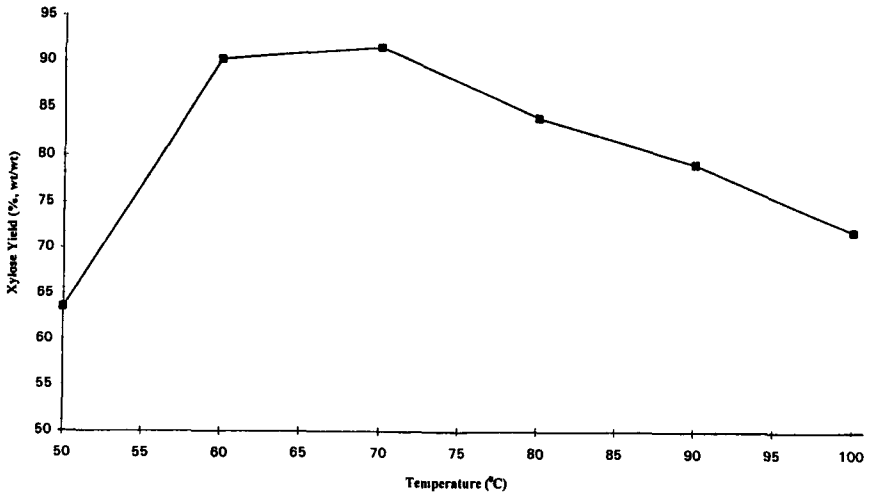

Fig. 4. Effect of temperature on xylan hydrolysis yield. Xylan was hydrolyzed in 60% zinc chloride solution for 3 h at different temperatures.

the fact that cellulose hydrolysis also was accelerated by temperature, a lower hydrolysis temperature (60°C) was chosen to minimize the production of glucose during the process of xylan hydrolysis.

CONCLUSION

Hemicellulose can be hydrolyzed during the pretreatment process of lignocellulose with zinc chloride at low temperature. The hydrolysate did not contain a detectable amount of glucose. This approach provides a method to produce individual streams of five-carbon sugars and glucose that can be separately fermented.

REFERENCES

1. Ladisch, M. R. (1989), in *Biomass Handbook,* Edited by Kitani, O., Hall, C. W., Gordon and Breach Science Publishers, New York, p. 435.
2. Du Preez, J. C., Bosch, M., and Prior, B. A. (1986), *Appl. Microbiol. Technol.* **23,** 228.
3. Rizzi, M., et al. (1988), *Biotechnol. Bioeng.* **34,** 509.
4. Laplace, J. M., Delgenes, J. P., Moletta, R., and Navarro, J. M. (1993), *Appl. Microbiol. Biotechnol.* **39,** 760.
5. Slininger, P. J., et al. (1991), *Biotechnol. Bioeng.* **37,** 973.
6. Panchal, C. J. (1988), *Can. J. Microbiol.* **34,** 1316.
7. Wayman, M. (1986), in *Cellulose,* Young, R. A. and Rowell, R. M., eds., Wiley-Interscience, New York, p. 265.

8. Fontana, J. D., et al. (1984), *Biotechnol. Bioeng. Symp.* **175.**
9. Hormeyer, H. F., Bonn, G., Kim, D. W., and Bobleter, O. (1987), *J. Wood Chem. Technol.* **7,** 269.
10. Bobleter, O., Schwald, W., Bonn, G., and Esterbauer, H. (1985), Austrian Patent No. A 102/85.
11. Banerjee, M. (1989), *Appl. Environ. Microbiol.* **55,** 1169.
12. Nikitin, N. I. (1966), *The Chemistry of Cellulose and Wood,* Israel Program for Scientific Translation, p. 74.
13. Cao, N. J., Xu, Q., Chen, C. S., Gong, C. S., and Chen, L. F. (1994), *Appl. Biochem. Biotechnol.* **45/46, 521.**
14. Cao, N. J., Xu, Q., and Chen, L. F. (1994), Sixteen Symposium on Biotechnology for Fuels and Chemicals, in Gatlinburg, TN.
15. Wenda, L. (1990), *J. Wood Chem. Technol.* **10,** 123.