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Severity Function Describing the Hydrolysis of Xylan Using Carbonic Acid

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Abstract

Beech wood derived xylan to hydrolyzed to predominantly xylose monomer units after exposure to hot, compressed liquid water saturated with carbon dioxide. Similar treatment without CO_2 saturation resulted in only minor hydrolysis and a smaller fraction of monomers among the hydrolysis products. Severity of the hydrolysis reaction was correlated to reaction time, temperature, and carbon dioxide partial pressure and followed a function similar to those used to characterize mineral acid systems. Results from parallel hydrolysis experiments with an aqueous system and a very dilute sulfuric acid system allowed an approximation of the dissociation constant of carbonic acid in the temperature range of 170–230°C. Results suggest that carbonic acid may be a viable reagent for promoting hydrolysis without mineral acids, especially in the case of a bioprocessing plant that produces carbon dioxide.

Index Entries: Xylan; carbonic acid; severity; hydrolysis; biomass conversion; pretreatment; carbon dioxide.

Introduction

Rapid use of the world's oil reserves has prompted much research into finding alternative sources for fuels and chemicals. The most likely renewable resource is biomass (i.e., plant materials such as agriculture and forestry wastes). The limiting factor in using biomass is hydrolyzing the raw material into fermentable sugars, which can then be biologically converted into a myriad of fuels and chemicals *(1)*.

Two hydrolysis approaches are commonly used: acid and enzymatically catalyzed. In the current state of the art, both methods require some acid, although the enzymatic method requires far less *(2–4)*. Eliminating all need for mineral acids (such as sulfuric) from the hydrolysis process would be highly valuable to reduce the operating, construction, and environmental costs associated with a commercial biomass conversion *(5,6)*.

The aim of the present study was to evaluate the potential of carbonic acid (dissolved carbon dioxide) as a hydrolysis agent for xylan, which may be present as a residue in biomass-derived hydrolysates *(7)*. There is increasing interest in the use of carbonic acid in industrial processes *(8,9)*; however, little is known about its fundamental behavior in the range of 150–250°C. Whether carbonic acid can serve the function of a xylan hydrolysis agent is not clear because of its weak acidity and poorly understood behavior at elevated temperatures and pressures. Puri and Mamers *(10)* reported that the addition of carbon dioxide to a steam explosion reactor increased the degree of "in vitro organic matter digestibility." There is at least one report of carbonic acid being capable of hydrolyzing cellulose, but to my knowledge this result has not been replicated *(11,12)*. If carbonic acid is effective, it could prove preferable to stronger acids because of its reduced corrosion and neutralization requirements. In addition, carbonic acid is likely to be available at no cost, because carbon dioxide is a byproduct of the fermentation processes used for conversion of biomass to other products *(10–12)*.

The objectives of the present study were to characterize the hydrolyzing effect of carbonic acid on xylan and to determine a severity function to describe the action of carbonic acid under the conditions tested. Severity was investigated by quantifying the extent of hydrolysis to monomer sugars.

Materials and Methods

Materials

Xylan was derived from beech wood (X-4252; Sigma-Aldrich). This xylan is alkali extracted, which effectively strips it of its acetyl groups (Jeffries, T., personal communication). Sulfuric acid was standard reagent grade, and sodium hydroxide was high-performance liquid chromatography quality (Fisher). Gasses used were regular grade CO_2 and ultra-high-purity He for the high-performance anion-exchange chromatography (HPAE).

Hydrolysis

Hydrolysis experiments were performed either in a 50-mL glass serum vial with crimp seal or in a simple reactor constructed of 1/2-in. 316 stainless steel tubing. The volume of the stainless steel reactor was 15 mL and it was filled and emptied by removing a swage connection on one end. For experiments involving pressurization with CO_2, a 1/8-in. stainless steel tubing connection and valve were fitted to the 15-mL reactor to allow for charging the CO_2 from a gas cylinder (Fig. 1).

In all experiments reported here, the initial concentration of xylan was 1.0 g/L. Temperature control of the hydrolysis reactor was achieved by quickly immersing the reactor in a fluidized sand bath (model SBL 2D;

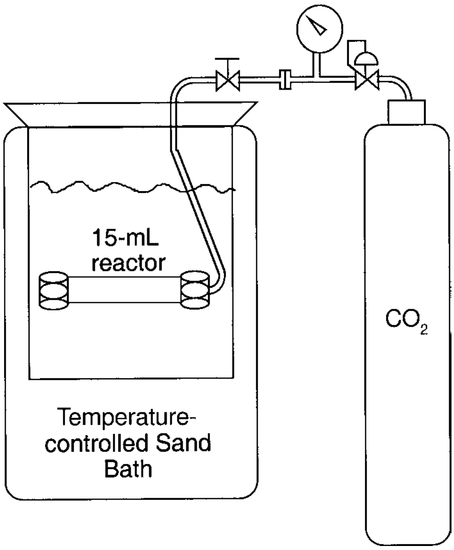

Fig. 1. Schematic diagram of hydrolysis apparatus. Construction of reactor components is 316 stainless steel.

Techhne, Oxford UK) with temperature controller (Techne model TC-8D) that maintained temperature in the bath to ±1°C. Reaction temperatures were set to values ranging from 121 to 270°C. Reactions were allowed to continue for between 2 and 30 min before removing the reactor from the sand bath and either air cooling (glass vials) or quenching in a cold water bath (stainless steel reactor).

Carbon dioxide pressure in the reactor was initially set at room temperature using the gas cylinder regulator (0–800 psig). A standard correlation between reactor pressures at room temperature and pressures at elevated temperature was determined by fitting a high-temperature stainless steel Bourdon tube pressure gage onto the reactor and immersing the entire assembly into the sand bath. To simplify the experimental apparatus, most hydrolysis experiments did not use the attached pressure gage, and their reaction pressure was inferred from this standard correlation.

Analysis

The hydrolysate was analyzed using HPAE (models GP50 and AS3500; Dionex, Sunnyvale CA). A sodium hydroxide eluent was run at a concentration of 25 m*M*/L for 10 min prior to injection, a gradient from 25 to 500 m*M*/L NaOH over 30 min, and a final hold at 500 m*M*/L for a final 15 min. The column used was the Dionex Carbopac PAX-100 column. Detection was done with pulsed amperometric detection (Dionex model ED40). Data were collected using the Dionex Peak One software package running on a Pentium-quality PC.

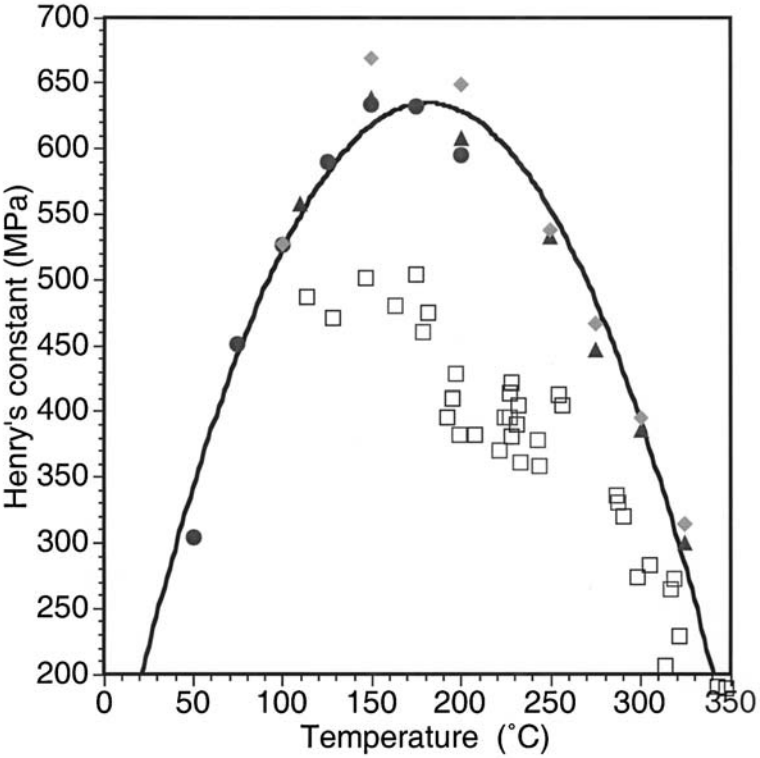

Fig. 2. Literature values for Henry's constant describing CO_2 solubility in water at elevated temperatures. □, *(13)*; ▲, *(14)*; ◆, *(21)*; ●, *(22)*. Curve fit to all data except ref. *13*.

Theory

Solubility and Dissociation Correlations

The chemical engineering literature has relatively little to report on the behavior of carbonic acid in the vicinity of 200°C and elevated pressures. Data are available from studies reported in the geochemistry literature, however, and these are reported here.

The solubility of CO_2 in water achieves a minimum near 160–170°C. Typically, literature reports of solubility relate Henry's constant to temperature. Figure 2 presents values taken from several studies. A curve was fit through the data from the three more recent studies. Points from an earlier study by Ellis *(13)* were not included in this fit because the data do not appear to have been replicated, even by his own later work with Golding *(14)*. The equation was found to be

$$H(T) = -0.017037 \times T^2 + 6.1553 \times T + 78.227 \tag{1}$$

in which T is expressed in °C and H in MPa, which defines the partial pressure of gaseous CO_2 in equilibrium with a mole fraction of dissolved CO_2.

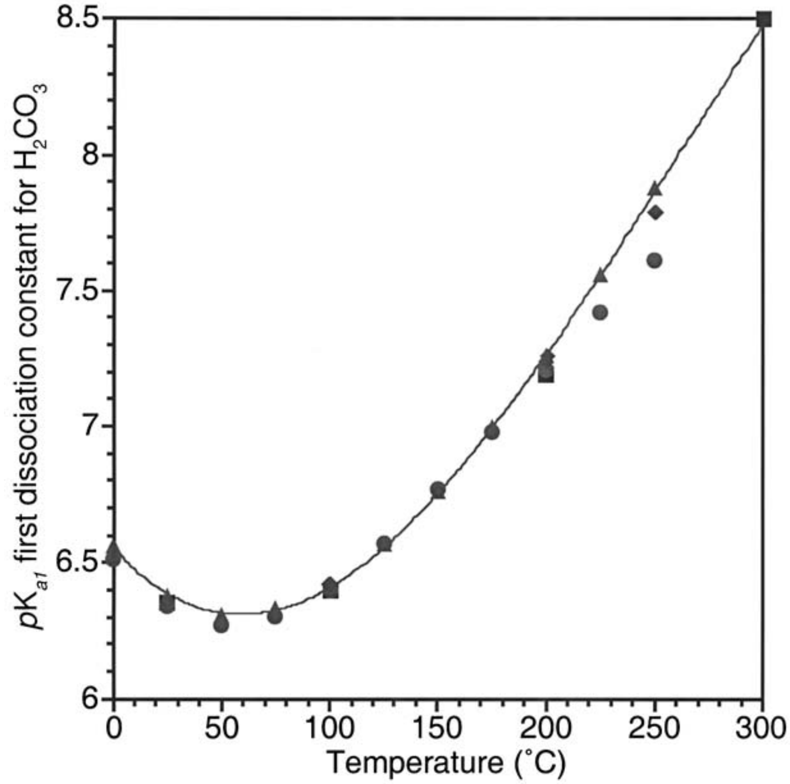

Fig. 3. Literature values for the first dissociation constant of H_2CO_3 in water at elevated temperatures. ■, CRC handbook; ▲, *(15)*; ◆, *(23)*; ●, *(24)*.

The dissociation of H_2CO_3 in water is also highly temperature dependent. Literature values for the pK_{a1} of H_2CO_3 are given in Fig. 3. The correlation of pK_{a1} to temperature proposed by Ryzhenko *(15)* closely matched those of several other investigators and has been adopted for this study:

$$pK_{a1} = (2382.3/T) - 8.153 + 0.02194 \times T \tag{2}$$

in which T is expressed in degrees kelvin.

Prediction of pH of Carbonic Acid

The pH of carbonic acid was estimated from the dissociation equation for CO_2 in water:

$$K_{a1} = \frac{[H^+]\,[HCO_3^-]}{[CO_{2(aq)}]} \tag{3}$$

The H^+ ions present originate almost entirely from the dissociation of carbonic acid, because at the temperatures considered, the dissociation constant of water is still relatively low (neutral pH ≅ 5.65 *[16]*). Thus, as a first

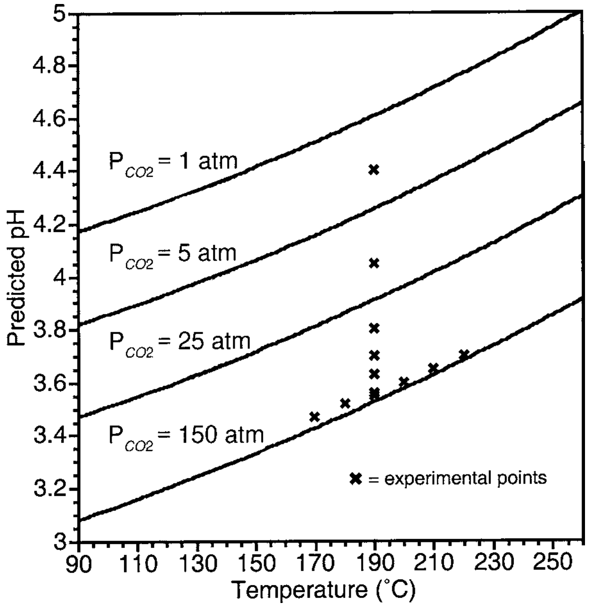

Fig. 4. Calculated values of temperature sensitivity of pH of carbonic acid at different partial pressures of CO_2. P_{CO_2} = 1, 5, 25, or 150 atm as indicated. ×, Experimental conditions of points investigated[2].

approximation, it is assumed that the concentrations of bicarbonate and hydronium ions are roughly the same:

$$[H^+] \approx [HCO_3^-] \tag{4}$$

At higher temperatures, this assumption no longer holds. Because the solubility of CO_2 in water is affected by the dissociation of carbonic acid in water, the total CO_2 dissolved is determined as a molar balance on carbon:

$$CO_{2(\mathrm{solubilized})} = CO_{2(\mathrm{aq})} + H_2CO_3 + HCO_3^- \tag{5}$$

By assuming a partial pressure of CO_2 in contact with water at a given temperature, the H^+ ion concentration can be determined using Eqs. 1–5 and the steam tables. This involves making an assumption about the contribution of dissolved CO_2 to the liquid volume. In this case, because of the low solubility of CO_2 at the temperatures considered, it was simply assumed that dissolved CO_2 contributed equally, on a molar basis, to the volume of the liquid phase. Figure 4 shows predicted pH values vs temperature and partial pressures of CO_2 (P_{CO_2}). It is clearly seen that pH is a strong function of both temperature and pressure. Eq. 6 was fit to these generated points and expresses the expected pH of the binary CO_2-H_2O system as a function of temperature and pressure in the range of 100–250°C and up to a CO_2 partial pressure of 150 atm:

$$\mathrm{pH} = 8.00 \times 10^{-6} \times T^2 + 0.00209 \times T - 0.216 \times \ln(P_{CO_2}) + 3.92 \tag{6}$$

in which T is expressed in °C and P_{CO_2} is in atms. Figure 4 also illustrates the approximate conditions of experiments conducted for this study.

Severity Function

The effectiveness of biomass pretreatment is often correlated using a severity function, which combines the effects of time and temperature into one function. Overend and Chornet *(17)* have defined the following function to quantify the severity of a biomass hydrolysis system in batch or plug-flow reaction modes:

$$R_o = t \times \exp\left[\frac{(T - 100)}{14.75}\right] \tag{7}$$

in which t is time in minutes and T is expressed in °C. The effect of acid concentration can also be included by using a combined severity function, which has been shown by Chum et al. *(18)* to be effectively represented as

$$\text{combined severity} = \log R_o - \text{pH} \tag{8}$$

Thus, Eqs. 6 and 7 can be inserted into Eq. 8 to give the combined severity of the carbonic acid system as a function of reaction time, temperature, and P_{CO_2}:

$$\begin{aligned} \text{combined severity} = \log \left\{ t \times \exp\left[\frac{(T - 100)}{14.75}\right]\right\} - \\ 8.00 \times 10^{-6} \times T^2 + 0.00209 \times T - 0.216 \times \ln(P_{CO_2}) + 3.92 \end{aligned} \tag{9}$$

Quantifying Severity

For studies conducted on raw biomass, severity is often correlated to solubilization of substrate *(18–20)*. However, because purified xylan was completely solubilized under conditions of even the mildest severity, in this study the severity is correlated to the accumulation of xylose monomers, which offered response to severity over a greater range of reaction times.

Results

Effect of Pressure

The effect of varying P_{CO_2} on the hydrolysis system was tested by subjecting a suspension of xylan to varying CO_2 pressures at constant temperature (190°C) and reaction time (16 min). Figure 5 shows HPAE traces from several runs conducted with different CO_2 pressures. The marked pressures refer to the initial pressure of the CO_2 when the reactor was charged at room temperature. It can be seen that at higher pressures, more xylose is released and the low-DP (degree of polymerization) oligomers are also more plentiful. This shows clearly that adding carbonic acid hydro-

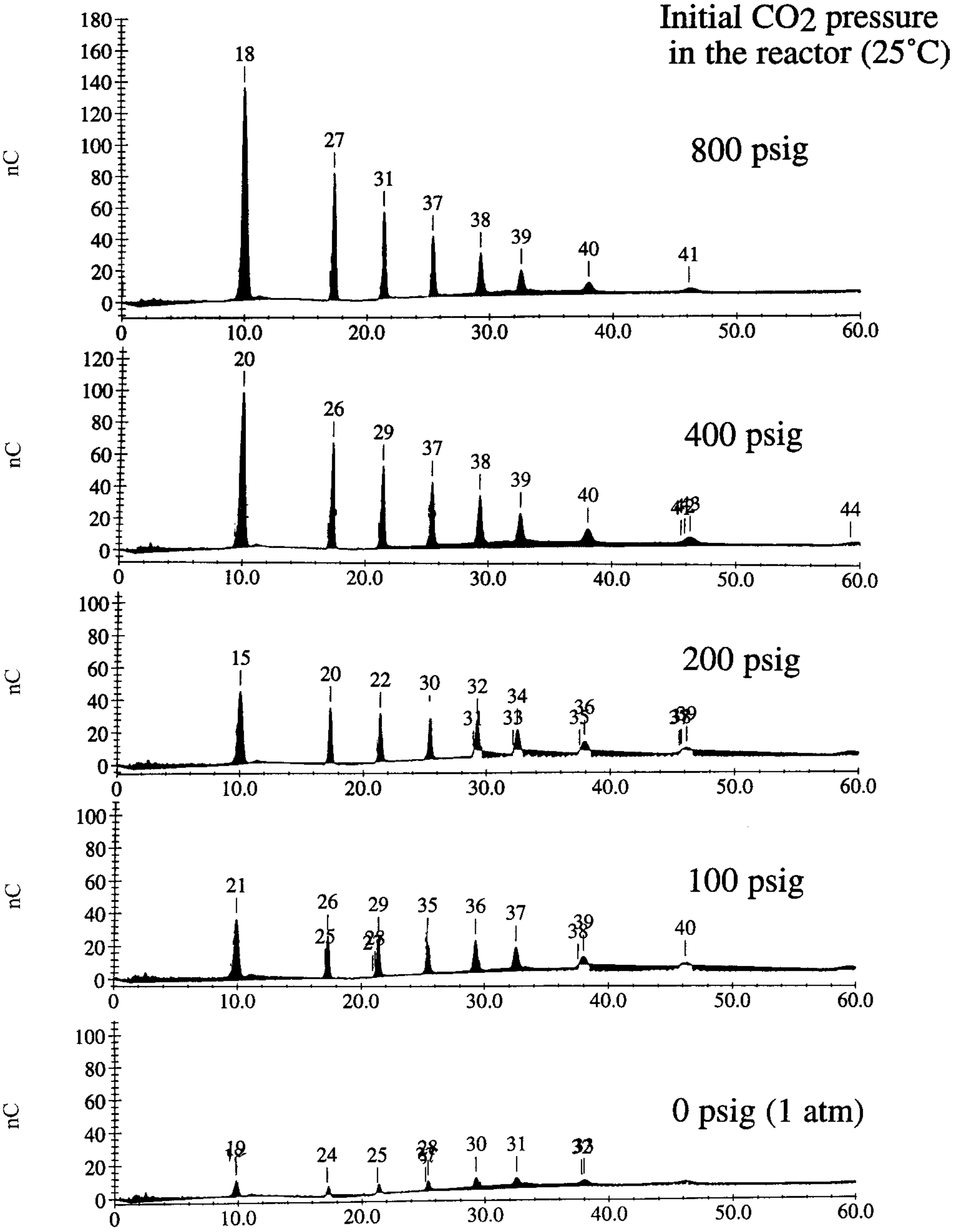

Fig. 5. Output from HPAE-PAD indicating varying degrees of hydrolysis with differing initial reactor pressures of CO_2. Sequential peaks represent oligomers of increasing degree of polymerization.

lyzes xylan. Figure 6 plots the amount of xylose released vs the estimated P_{CO_2} in the reactor at reaction temperature. The trend of increased xylose release with increased pressure is clearly discernible.

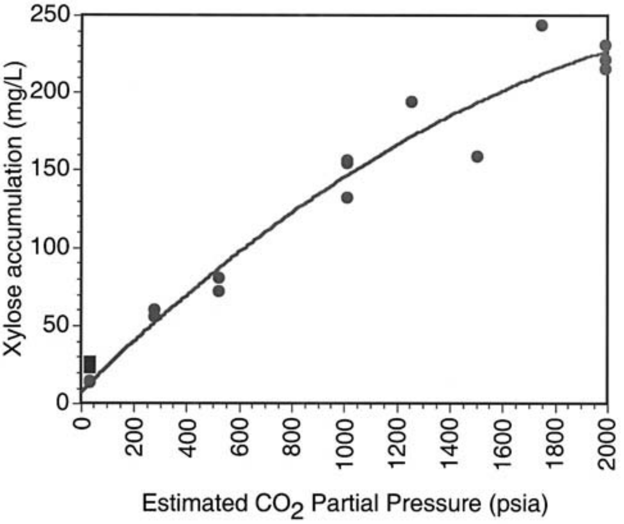

Fig. 6. Xylose accumulation vs P_{CO_2}. Reaction temperature, 190°C; duration, 16 min; ■, no supplemental CO_2; ●, CO_2 added.

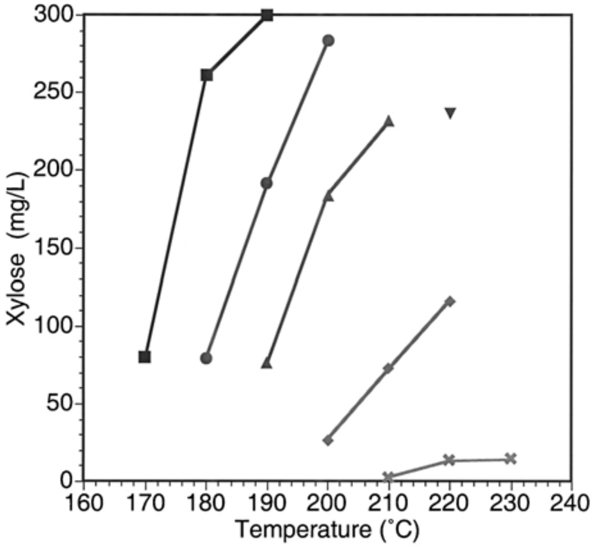

Fig. 7. Xylose release resulting from varying reaction temperatures and durations. Initial CO_2 pressure for all points was 800 psig. ■, 28.5 min; ●, 14.5 min; ▲, 6.5 min; ▼, 5.5 min; ◆, 2.5 min; ×, 0.5 min.

Effect of Temperature and Reaction Time

Figure 7 shows the results of a series of experiments that varied the duration and temperature of the hydrolysis reaction. Immersion times for the reactor varied from 2 to 30 min. Actual reaction times were taken as 1.5 min shorter duration than the immersion time to allow the reactor to

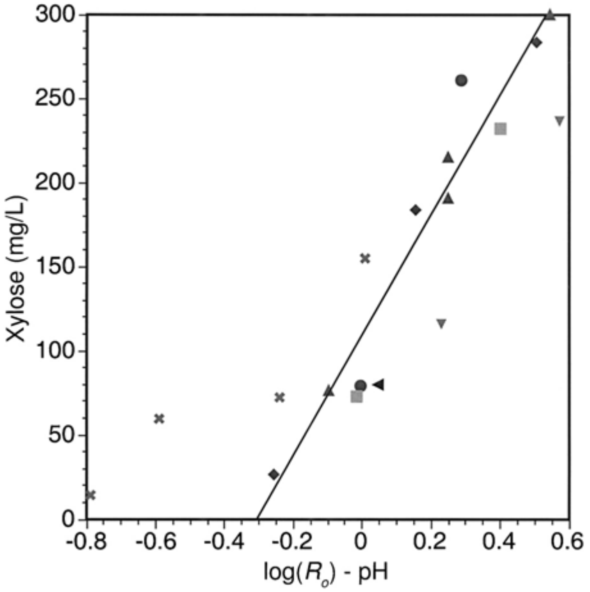

Fig. 8. Collected data plotted vs combined severity factor log(R_o) – pH. For solid symbols, initial CO_2 pressure 800 psig. ◀, 170°C at 28.5 min; ●, 180°C at 28.5 and 14.5 min; ▲, 190°C at 6.5, 14.5, and 28.5 min; ◆, 200°C at 14.5, 6.5, and 2.5 min; ■, 210°C at 6.5 and 2.5 min; ▼, 220°C at 5.5 and 2.5 min. For × symbols, initial CO_2 pressure varies: × = 190°C, 14.5 min at 400, 200, 100, and 0 psig initial CO_2 pressure.

heat up. All reactions were performed with an initial CO_2 pressure of 800 psig. The effect of higher temperature is clearly visible, as is the effect of increased reaction duration. The reduction in xylose production at higher temperatures suggests that temperature may have a less potent effect on reaction rate than would normally be expected from a system using strong acids.

Combined Severity of Reaction

The applicability of the combined severity function defined in Eq. 9 is shown in Fig. 8, which combines the data presented in Figs. 6 and 7. The effects of pressure and temperature on the reaction severity seem to be adequately expressed by the severity correlation at higher pressures, but the low-pressure data stray from the correlation.

Comparison to Sulfuric Acid and pH Confirmation

Because the determination of pH through Eq. 6 is entirely theoretical and involves a number of simplifying assumptions, an experiment was undertaken to estimate the effective pH of the carbonic acid system by comparing it to a very dilute sulfuric acid system. Hydrolysis of 1.0 g/L of xylan was performed with various concentrations of sulfuric acid, giving a spectrum of pH conditions from 2.9 to 3.7. The pH of the sulfuric acid was

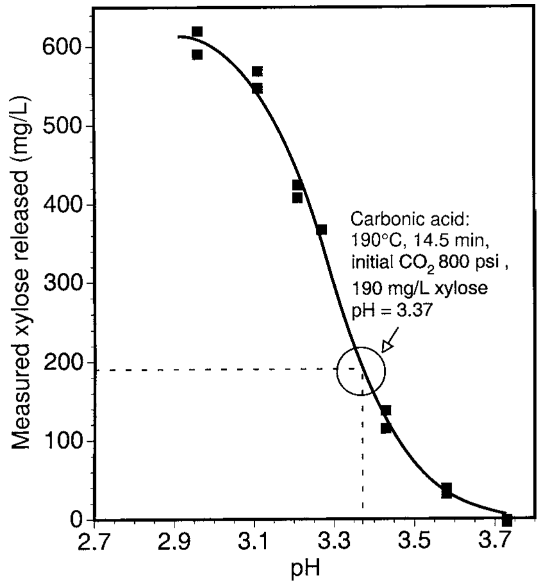

Fig. 9. Comparison of carbonic acid performance to a sulfuric acid system. Data points represent concentration of xylose released from 1.0 g/L xylan slurry reacted for 14.5 min at 190°C in dilute sulfuric acid at the pH indicated.

estimated by assuming complete first dissociation and a formation constant of K° = 25617.5 for the second dissociation *(25)*. Under reaction conditions of 190°C and 16-min immersion (14.5-min reaction) time, conversion of xylan to xylose ranged from 0 to 60%. Figure 9 shows the results of this experiment. Also shown is the performance of the corresponding carbonic acid system (1.0 g/L of xylan, 16-min immersion time, 190°C), operating at a P_{CO_2} of 2000 psia. It is apparent that even with the high pressure of CO_2, carbonic acid under the severity conditions tested achieved a lower conversion of xylan to xylose than was possible with dilute sulfuric acid. For the carbonic acid system, the theoretically estimated pH was 3.56; however, it appears that the hydrolysis performance achieved is comparable to a sulfuric acid system with a pH on the order ~3.37. Thus, there appears to be a discrepancy between the theoretical prediction and observed reactivity of the carbonic acid system.

Discussion

In the chemical engineering literature, there is relatively little information about the characteristics of carbonic acid under the conditions tested in the present study. Much of the fundamental work undergirding the theory presented herein originated in the geochemistry field. Yet, it appears that carbonic acid may have utility for some applications requiring mildly acidic conditions under high temperature and pressure. From this investigation, it is clear that xylan hydrolysis is promoted by CO_2 in the temperature range of 170–230°C, provided that the CO_2 partial pressure is sufficiently high. This may mean that carbonic acid is useful for enhancing aqueous pretreatment systems, particularly for improving recovery of monomeric xylose from aqueous pretreatment hydrolysates.

The possible use of CO_2 effluent from a fermentor to assist catalytic pretreatment has many advantages. If carbonic acid could replace sulfuric acid for biomass pretreatment, the many disadvantages of sulfuric acid (purchase cost, high corrosion activity, need for neutralization, separation of calcium sulfate) could be eliminated. Fermentation-derived CO_2 would provide a low-cost source of catalyst, and although it is not sequestered by the process *per se*, it does use an otherwise valueless waste stream.

However, before a clear application can be detailed, some areas of question remain to be answered. First, although the proposed combined severity function did incorporate temperature and pressure as pH-moderating influences, and the function could predict the general reactivity trend, the theory of pH prediction should be verifiable with experimental results—which has not yet been shown. Second, the question of how carbonic acid will behave in the presence of actual lignocellulosic feedstock is not yet understood. For example, will the carbonate system act as an acidifying or buffering system in the presence of organic acids liberated from the biomass? Thus, further investigation is warranted.

Conclusion

Experiments with alkali-extracted xylan show that carbonic acid is able to catalyze hydrolysis at temperatures from 170 to 230°C. A proposed combined severity function incorporates the influence of temperature and P_{CO_2} on the pH of the system, and thus the overall severity of reaction. However, the pH predictions based on theory have yet to be validated experimentally. Should it prove a viable catalyst on a realistic substrate, carbonic acid would offer many process and design advantages over sulfuric acid.

Acknowledgments

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References

1. Lynd, L. R., Cushman, J. H., Nichols, R. J., and Wyman, C. E. (1991), *Science* **251(15),** 1318–1323.
2. Torget, R. and Hsu, T. (1994), *Appl. Biochem. Biotechnol.* **45/46,** 5–22.
3. Torget, R., Hatzis, C., Hayward, T. K., Hsu, T., and Phillipidis, G. P. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 85–101.
4. Lee, Y. Y., Wu, Z., and Torget, R. W. (2000), *Bioresour. Technol.* **71(1),** 29–38.
5. Lynd, L. R., Elander, R. T., and Wyman, C. E. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 741–761.

6. van Walsum, G. P., Allen, S. G., Spencer, M. J., Laser, M. S., Antal, M. J., and Lynd, L. R. (1996), *Appl. Biochem. Biotechnol.* **54/55,** 157–170.
7. Shevchenko, S. M., Chang, K., Robinson, J., and Saddler, J. N. (2000), *Bioresour. Technol.* **72,** 207–211.
8. Duranceau, S. J., Anderson, R. K., and Teegarden, R. D. (1999), *J. Am. Water Works Assoc.* **91(5),** 85–96.
9. Minkova, V., Marinov, S. P., Zanzi, R., et al. (2000), *Fuel Process. Technol.* **62,** 45–52.
10. Puri, V. P. and Mamers, H. (1983), *Biotechnol. Bioeng.* **25,** 3149–3161.
11. Pavilon, S. J. (1990), US Patent no. 4952504.
12. Pavilon, S. J. (1992), US Patent no. 5135861.
13. Ellis, A. J. (1959), *Am. J. Sci.* **257,** 217–234.
14. Ellis, A. J. and Golding, R. M. (1963), *Am. J. Sci.* **261,** 47–60.
15. Ryzhenko, B. N. (1963), *Geochemistry* **2,** 151–164.
16. Sweeton, F. H., Mesmer, R. E., and Baes, C. F. Jr. (1974), *J. Solut. Chem.* **3(3),** 191–214.
17. Overend, R. P. and Chornet, E. (1987), *Phil. Trans. R. Soc. Lond.* **A321,** 523–536.
18. Chum, H. L., Johnson, D. K., Black, S. K., and Overend, R. P. (1990), *Appl. Biochem. Biotechnol.* **24/25,** 1–14.
19. Abatzoglou, N., Chornet, E., and Overend, R. P. (1992), *Chem. Eng. Sci.* **47(5),** 1109–1122.
20. Bouchard, J., Nguyen, T. S., Chornet, E., and Overend, R. P. (1991), *Bioresour. Technol.* **36,** 121–131.
21. Takenouchi, S. and Kennedy, G. C. (1964), *Am. J. Sci.* **262,** 1055–1074.
22. Zawasza, A. and Malesinska, B. Y. (1981), *J. Chem. Eng. Data* **26,** 388–391.
23. Read, A. J. (1975), *J. Solut. Chem.* **4(1),** 53–70.
24. Park, S. N., Kim, C. S., Kim, M. H., Lee, I.-J., and Kim, K. (1998), *J. Chem. Soc. Faraday Trans.* **94,** 1421–1425.
25. Bilal, B. A. and Müller, E. (1993), *Z. Naturforsch.* **48a,** 1073–1080.