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Comparison of Aspen Wood Hydrolysates Produced by Pretreatment with Liquid Hot Water and Carbonic Acid

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Abstract

Hydrolysates produced by the pretreatment of aspen wood with liquid hot water were compared with hydrolysates produced using carbonic acid pretreatment. Pretreatment temperatures tested ranged from 180° to 220°C; reaction times were varied between 2.5 and 30.5 min. Under most conditions tested, it was found that the presence of carbonic acid had no discernible effect on the amount of xylose released or concentration of furan compounds, as indicated by ultraviolet-visual absorbance between 270 and 280 nm. Thus, there appears to be little difference in the severity of the pretreatment conditions with or without the presence of carbonic acid. The presence of carbonic acid did, however, result in a hydrolysate with a higher final pH. It is hypothesized that the presence of the carbonic acid during the reaction may have the effect of reducing the accumulation of organic acids in the hydrolysate.

Index Entries: Carbonic acid; pretreatment; liquid hot water pretreatment; autohydrolysis; aspen wood; pH.

Introduction

Conversion of lignocellulosic material to ethanol requires hydrolysis of carbohydrate polymers to their constituent sugars *(1–3)*. Enzymatic hydrolysis is a common approach to hydrolysis and offers the benefits of mild reaction conditions and selective hydrolysis. To achieve useful rates of enzymatic hydrolysis, the lignocellulose must first be pretreated to reduce the recalcitrance of the substrate to hydrolysis. Pretreatment accomplishes many alterations of the biomass. Depending on the technology chosen, these effects typically include, to varying degrees; hydrolysis of the

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hemicellulose, solubilization of lignin and carbohydrate oligomers, and increased accessibility of the cellulose to cellulase enzymes *(4)*. Several pretreatment methods have been explored. The most commonly reported technologies include dilute-acid pretreatment, in which sulfuric acid is used in low concentrations (on the order of 1%) and at temperatures usually <200°C *(5)*, and steam explosion, which exposes the substrate to steam at elevated temperature and then explosive decompression to physically break apart the plant fibers *(6)*. Often, steam explosion is coupled with acid catalysis by impregnating the substrate with sulfur dioxide prior to steam treatment. Other techniques include ammonia fiber explosion, which breaks down the lignin using ammonia and explosive decompression; treatment with organic solvents, and treatment with liquid hot water *(7–9)*. Some methods that have been examined less thoroughly include treatment with super-critical fluids and carbonic acid *(10,11)*.

Steam explosion and dilute-acid pretreatment have undergone research and development for many years. Much of this research has been devoted to fuel production from biomass. Dilute-acid pretreatment offers good performance in terms of recovering hemicellulosic sugars, but a drawback is its use of sulfuric acid. Sulfuric acid is highly corrosive and its neutralization results in copious production of solid wastes *(12)*. For applications in which unconverted biomass can be used to generate power, the disposal of gypsum is sufficiently problematic that a reduced ethanol yield is considered by some to be preferable to the increased cost of waste disposal associated with dilute-acid pretreatment (W. de Laut, personal communications). The calcium sulfate resulting from neutralization also has problematic solubility characteristics in that it becomes less soluble at higher temperatures, such as those encountered in a reboiler *(11)*. Compared to dilute-acid pretreatment, steam explosion does not use sulfuric acid and is less corrosive both to equipment and as a recovery product. However, steam explosion yields lower amounts of hemicellulosic sugars. Studies suggest an 80% recovery of 5-carbon sugars with dilute-acid pretreatment compared with 65% using steam explosion *(13,14)*. SO_2-catalyzed steam explosion achieves higher recovery of hemicellulosic sugars, but this again introduces a reliance on sulfur compounds and the requisite neutralization.

One process that may offer benefits of acid catalysis without the drawbacks of sulfuric acid is the use of carbonic acid. The pH of carbonic acid is determined by the partial pressure of carbon dioxide in contact with water, and thus it can be neutralized by releasing the reactor pressure. Carbonic acid is relatively mild and hence does not offer the same hydrolytic capability of sulfuric acid. However, van Walsum *(11)* has demonstrated that at temperatures on the order of 200°C, carbonic acid does exhibit a catalytic effect on hydrolysis of xylan. van Walsum *(11)* observed enhanced release of xylose and low degree of polymerization xylan oligomers compared to pretreatment using hot water alone. Puri and Mamers *(15)* compared steam explosion of biomass with and without carbon dioxide pressurization and reported enhanced enzymatic degradation with the carbonic acid–

enhanced steam explosion. Thus, carbonic acid pretreatment may be a viable alternative to water-only and sulfur catalyzed pretreatment.

The present study seeks to characterize the qualities of hydrolysates produced by liquid hot water and carbonic acid–catalyzed pretreatment of aspen wood. Metrics of pretreatment effectiveness will include recovery of xylose monomers, production of microbial inhibitors, and the final pH of the hydrolysate after pretreatment.

Materials and Methods

Materials

Aspen wood chips were kindly supplied by the USDA Forest Products Laboratory in Madison, WI. Prior to pretreatment, the chips were ground in a domestic brand coffee grinder and sifted to a particle size of between 0.5 and 1 mm. Dry weight of the wood was determined by oven drying. CO_2 was of standard laboratory grade, and H_2O was of standard laboratory deionized quality.

Hydrolysis

Reactions were performed in a simple reactor constructed of 316 stainless steel tubing, 1/2-in. diameter. The reactor had a liquid volumetric capacity of 15 mL. It was filled and emptied by removing a swage connection on one end. For reactions using CO_2, a 1/8-in. stainless steel tubing connection and valve was fitted to the 15-mL reactor to allow introduction of CO_2 from a gas cylinder. CO_2 pressure was regulated using a high-pressure regulator on the CO_2 cylinder. The reaction temperature was controlled by quickly immersing the reactor in a fluidized sand bath (model SBL 2D; Techne, Oxford, UK) with temperature controller (Techne model TC-8D) that maintained temperatures in the bath to ± 1°C. Reaction temperatures ranged from 180 to 220°C. Reactions were carried out for durations ranging from 2.5 to 30.5 min before removing the reactor from the sand bath and quenching the reaction in a cold-water bath. In most cases, 90 s was added to the reaction time to allow heating of the reactor up to reaction temperature. In some later experiments, when a second sand bath became available, the reactors were heated in a separate bath maintained at the reaction temperature plus 40°C. This allowed for the transient heating time to be reduced to 45 s. Further description of the hydrolysis procedure has been reported previously *(11)*.

Experimental Procedure

In each of the reactions, 0.100 g of ground aspen wood and 8.0 mL of H_2O were used. In the samples reacted with carbonic acid, the reactor was also charged with 800 psi of CO_2 at room temperature. The experimental conditions included time intervals of 2.5, 4.0, 6.5, 14.5, and 30.5 min and temperatures of 180, 200, and 220°C. For most conditions, triplicate experiments were conducted, although some points had only one experimental datum while others were repeated more than three times (see Table 1).

Table 1
Experimental Data for Pretreatment Reaction Conditions.

Sample	Temperature C (°C)	Time (min)	pH out	UV absorbance	Xylose concentration (mg/L)
H_20/wood	180	2.5	4.96, 4.89, 4.98, 5.16	0.046, 0.061, 0.052, 0.037	0, 0, 0
H_20/wood/CO_2	180	2.5	5.35, 5.16, 6.00, 5.26	0.059, 0.057, 0.039, 0.037	0, 0, 0
H_20/wood	180	6.5	4.29, 3.29, 4.25, 4.02	0.087, 0.135, 0.076, 0.169	19.7, 4.3, 0
H_20/wood/CO_2	180	6.5	4.70, 3.62, 4.23, 4.29	0.091, 0.456, 0.099, 0.092	19.3, 10.0, 28.1
H_20/wood	180	14.5	3.95, 3.22, 3.59, 3.78	0.153, 0.185, 0.168, 0.139	34.8, 21.2, 54.2
H_20/wood/CO_2	180	14.5	4.89, 3.54, 3.89, 4.04	0.134, 0.243, 0.133, 0.132	39.8, 55.8, 77.1
H_20/wood	180	30.5	3.85, 3.34, 3.56, 3.65	0.193, 0.443, 0.190, 0.179	62.0, 143.3, 189.8
H_20/wood/CO_2	180	30.5	4.34, 3.75, 3.90, 4.42	0.197, 0.325, 0.144, 0.122	172, 115.4, 71.7, 159.8
H_20/wood	200	2.5	4.18	0.131	
H_20/wood/CO_2	200	2.5	4.63	0.129	
H_20/wood	200	4.0	3.86, 3.91, 3.80, 3.56, 3.66, 3.66	0.185, 0.136, 0.186, 0.213, 0.202, 0.195	43.5, 59.3, 78.2, 43.5, 28.1, 61.3
H_20/wood/CO_2	200	4.0	4.29, 4.1, 4.1, 4.26,	0.172, 0.145, 0.16, 0.186,	77.7, 136.2, 125.9, 61.0
H_20/wood	200	6.5	3.91, 3.73, 3.88	0.193, 0.335, 0.233	36.5, 39.9
H_20/wood/CO_2	200	6.5	4.58, 3.88, 4.47	0.170, 0.291, 0.138	33.7, 58.6
H_20/wood	200	14.5	3.88, 3.63, 3.60	0.300, 0.596, 0.435	400, 333
H_20/wood/CO_2	200	14.5	5.13, 3.78, 4.01	0.240, 0.451, 0.304	445, 314
H_20/wood	200	30.5	3.55, 3.14, 3.47	0.511, 1.395, 0.728	649, 1396
H_20/wood/CO_2	200	30.5	3.96, 3.62, 3.98	0.438, 0.885, 0.502	693, 648
H_20/wood	220	2.5	3.93, 3.65, 3.91, 3.87	0.285, 0.259, 0.202, 0.189	158.5, 114.2, 77.1
H_20/wood/CO_2	220	2.5	4.13, 3.96	0.271, 0.196	120.3
H_20/wood	220	4.0	3.75, 3.66, 3.66	0.319, 0.297, 0.315	234.9, 309.8, 376.8
H_20/wood/CO_2	220	4.0	4.04, 3.95, 3.92	0.261, 0.246, 0.277	467.2, 303.1, 342.9
H_20/wood	220	6.5	3.62, 3.29, 3.79, 3.55	0.444, 0.404, 0.338, 0.337	46.6, 292.6, 442.3
H_20/wood/CO_2	220	6.5	3.89, 3.47, 3.92, 3.55	0.468, 0.394, 0.247, 0.347	91.9, 276, 452.1
H_20/wood	220	14.5	3.42, 3.18, 3.04, 3.34, 3.36	1.035, 1.359, 0.789, 0.988, 0.942	234, 397, 268.3, 366.9
H_20/wood/CO_2	220	14.5	3.69, 3.35, 3.21, 3.43, 3.46	0.684, 0.035, 1.359, 0.702, 0.767	612, 300, 273.6, 508.4
H_20/wood	220	30.5	3.45, 3.06, 3.37, 3.32	0.764, 1.335, 1079, 1.247	94.7, 61.4, 76.1
H_20/wood/CO_2	220	30.5	3.87, 3.29, 3.37, 3.31	0.722, 2.819, 1.107, 1.232	172, 52, 79.6

[a] Multiple entries represent results from repeat experiments.

Analysis

Hydrolysate absorbance was analyzed using an ultraviolet-visual (UV-VIS) spectrophotometer (model DU 500; Beckman, Fullerton, CA). The hydrolysates were diluted to give the greatest spectral resolution. Spectrophotometric analyses were conducted at wavelengths ranging from 190 (UV) to 1100 nm (VIS) at 5-nm increments. Spectrophotometric analysis focused on wavelengths between 190 and 300 nm. This was the range of wavelength that gave the most significant absorbance peaks during previous experimentation. Absorbance at 270–280 nm correlates to furan concentration, which, in turn, has been correlated to microbial toxicity *(17)*. pH was analyzed using a pH meter (Acumet research model AR15, Fisher Scientific) with the samples depressurized and at room temperature. Soluble carbohydrate concentration was measured using high-performance anion-exchange chromatography with pulsed amperometric detection, a Dionex Carbopac PAX-100 column, and a DS3 conductivity cell (Dionex, Sunnyvale, CA).

Results

The data presented herein are summarized in Table 1.

Xylose Yield

Figure 1 plots the concentration of xylose measured in the hydrolysates produced under different reaction conditions. Error bars on the graph represent ± 1 standard deviation (SD). Comparing the aspen and carbonic acid system to the aspen and water system, there was no apparent difference in the trend of xylose production at the three temperatures tested. Mean xylose concentrations ranged between 0 mg/L for the least severe conditions tested (180°C, 2.5 min) to 1011 mg/L for intermediate severity conditions (200°C, 32 min, 800 initial psig CO_2). This high concentration has a large degree of error associated with it (see Fig. 1). With an initial hemicellulose concentration of approx 1.6 g/L (assuming 20% hemicellulose content in aspen [S. Mansfield, personal communication]), the high xylose value represents about 64% of the initial hemicellulose. Oligomer production was also pronounced but has not yet been quantified. Higher-severity conditions at 220°C resulted in lower xylose concentrations, presumably owing to degradation of the xylose product.

Absorption at 270–280 nm

Figure 2 shows two example absorbance spectra for hydrolysates produced with aspen wood using carbonic acid or liquid hot water pretreatment. Absorption measurements were taken at wavelengths from 190 to 300 nm. Previous experimentation discovered no significant absorption phenomenon beyond a wavelength of 300 nm. In this wavelength region, two predominant peaks emerged; the first at 205 nm and a second at 275 nm.

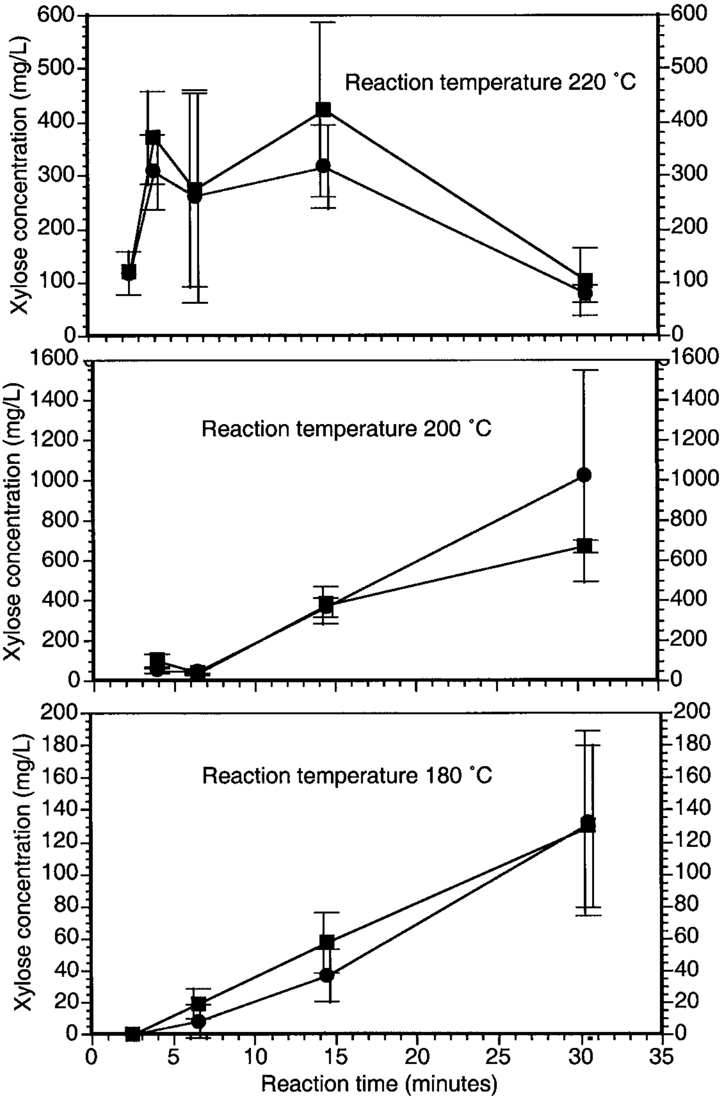

Fig. 1. Xylose concentration (mg/L) vs reaction time at 180, 200, and 220°C. (■), Aspen wood with carbonic acid; (●), aspen wood with water. Error bars represent ±1 SD.

The cause of the 205 nm peak is unclear, but as reactions increased in severity, the absorption at this wavelength became less and less dominant compared with the peak at 275 nm. As stated, the UV absorption between 270 and 280 nm is an indicator of furfural and hydroxymethylfurfural concentrations. Figure 3 illustrates that the observed absorption generally increased with increasing reaction time and temperature. This follows the logical assumption that the degradation products are proportional to the severity of the reaction. As with the xylose measurements, there was no clear trend indicating any difference between reactions carried out with or without the addition of CO_2.

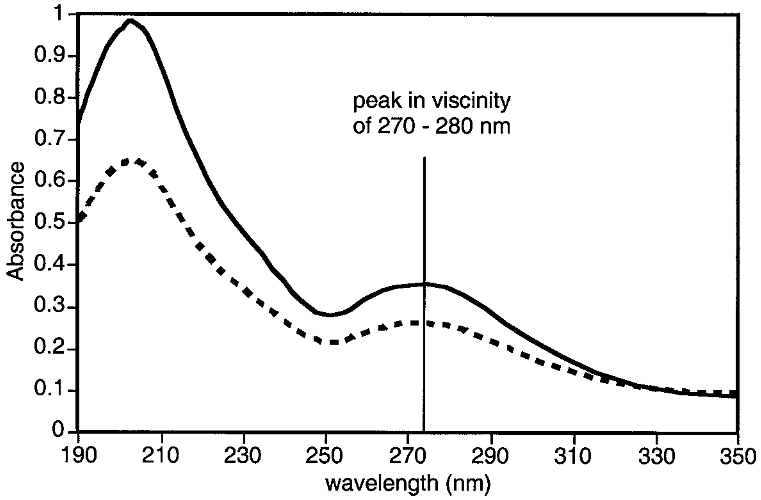

Fig. 2. Sample absorption spectra for hydrolysates of pretreatment with and without carbonic acid. Reaction temperature 220°C, duration 4 min. (—), Aspen and carbonic acid; (– – –), aspen with water.

Hydrolysate pH

Contrary to the xylose and absorbance results, the final (depressurized, room temperature) pH of the hydrolysate did indicate a difference between reactions carried out with and without carbonic acid in the reactor. Figure 4 illustrates that the final pH of the hydrolysate of the carbonic acid reactions was consistently higher than the water-only reactions across the full range of reaction conditions. At a given temperature, the increase in the mean final pH with the use of carbonic acid was relatively consistent. At 180°C the increase in pH ranged from 0.4 to 0.5, at 200°C the range was from 0.45 to 0.61, and at 220°C it varied within the range of 0.15–0.28. Figure 5 illustrates the differences between water and carbonic acid results calculated for each set of paired experimental data (data that were generated by the same researcher on the same day). It can be seen that even for these nonaveraged data, the trends for xylose and absorbance data are random and relatively evenly distributed above and below the zero axis, whereas the trend in the pH data clearly shows that the vast majority of experiments produced a higher final pH when carbonic acid was present.

Response to Reaction Severity

Figure 6 shows collected average data for the three reaction temperatures plotted vs the log of reaction severity, defined as follows *(17)*:

$$Ro = t \times \exp[(T - 100)/14.75)] \qquad (1)$$

in which t is the reaction (min), and T is the temperature (°C). It can be seen that absorbance at 270–280 nm increases while pH decreases with increasing reaction severity. Xylose concentration increases up to a point and then

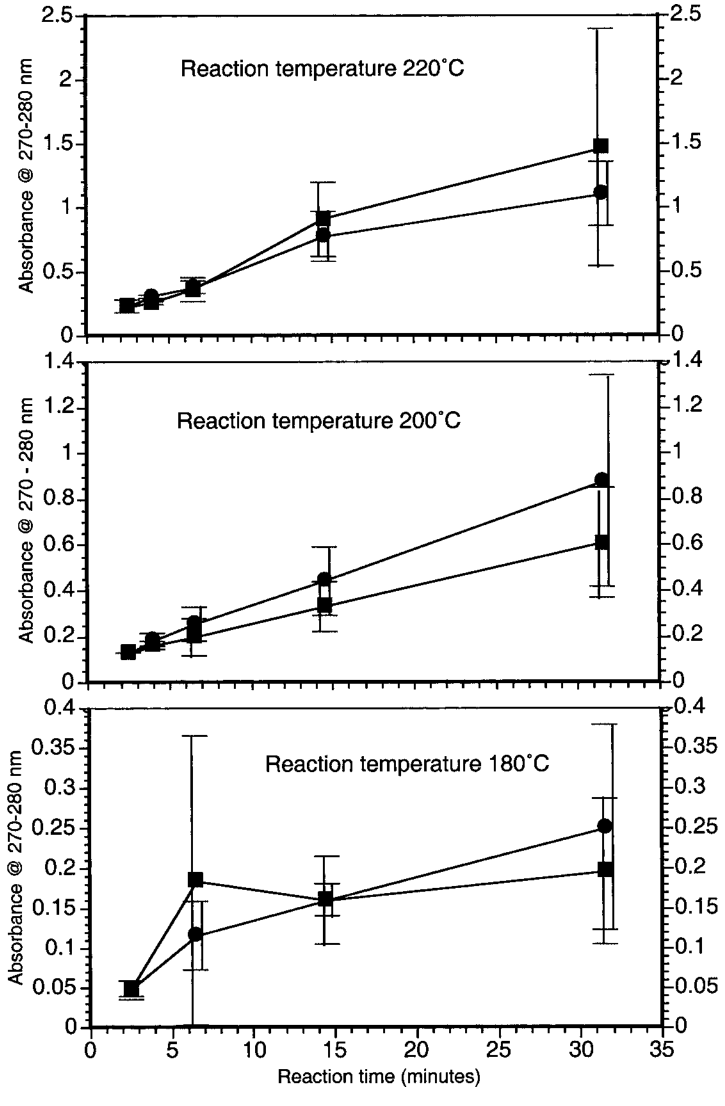

Fig. 3. UV-VIS absorbance at 270–280 nm vs reaction time at 180, 200, and 220°C. (■), Aspen wood with carbonic acid; (●), aspen wood with water. Error bars represent ±1 SD.

decreases as the severity increases. These plots again show the relative similarity between the water and carbonic acid systems for absorbance and xylose measurements and the consistent difference observed in the final pH for these two systems.

Discussion

Xylose Recovery

Hydrolysis of hemicellulosic fractions in aspen wood appeared to be little influenced by the presence or absence of carbonic acid. Release of xylose increased with reaction severity, as expected, and also decreased as

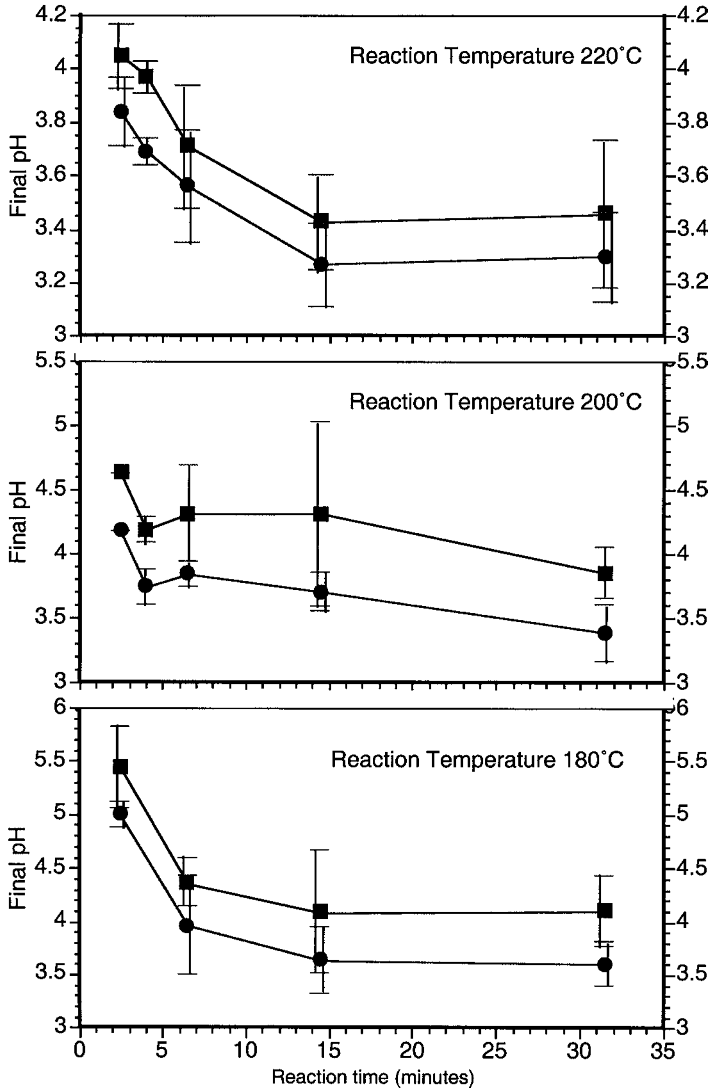

Fig. 4. Final (depressurized, room temperature) pH vs reaction time at 180, 200, and 220°C. (■), Aspen wood with carbonic acid; (●), aspen wood with water. Error bars represent ±1 SD.

reaction severity became too high, likely the result of product degradation. Previous work with pure xylan *(11)* had shown that carbonic acid substantially increased hydrolysis activity in comparison to water. Results from the current study on aspen wood suggest that there is no increased hydrolysis activity with the addition of carbonic acid. A possible explanation for these different results is that autocatalysis of the aspen wood is taking place, obscuring the action of the carbonic acid. Autocatalysis is thought to result primarily from the release of acetyl groups and appears to result in activity comparable with that of carbonic acid. Further work should be done to quantify the amount of acetate present in the hydrolysate in order

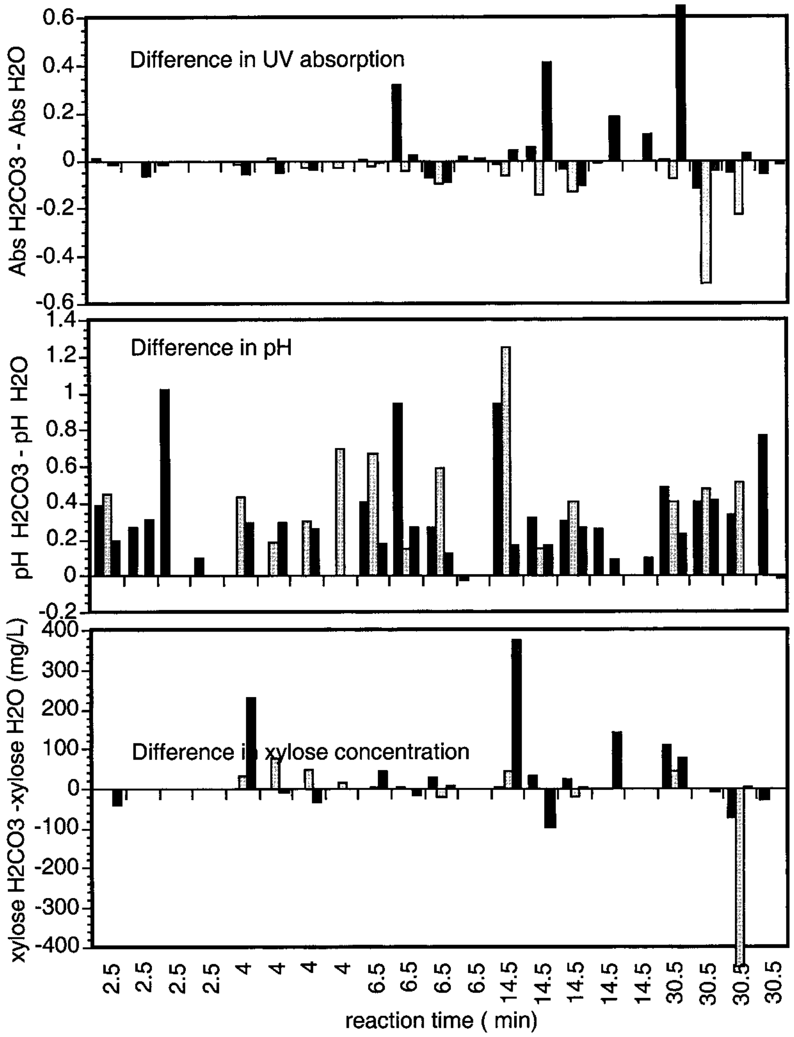

Fig. 5. Differences in results between reactions done with and without carbonic acid. Bars represent differences calculated for paired experiments, i.e., experiments done by the same researcher on the same day. (■) 180°C; (▨) 200°C; (■) 220°C.

to offer an explanation for the comparable extents of hydrolysis. Aspen wood has a relatively high concentration of acetyl groups, and it is possible that application of carbonic acid to a less acidic substrate may show more of an effect attributable to the carbonic acid.

Absorption at 270–280 nm

Results from measurements of absorption were similar to those of xylose recovery in that there was little difference observed in the absorption of the hydrolysates produced with and without carbonic acid. This again points to similar reaction conditions between the two pretreatment

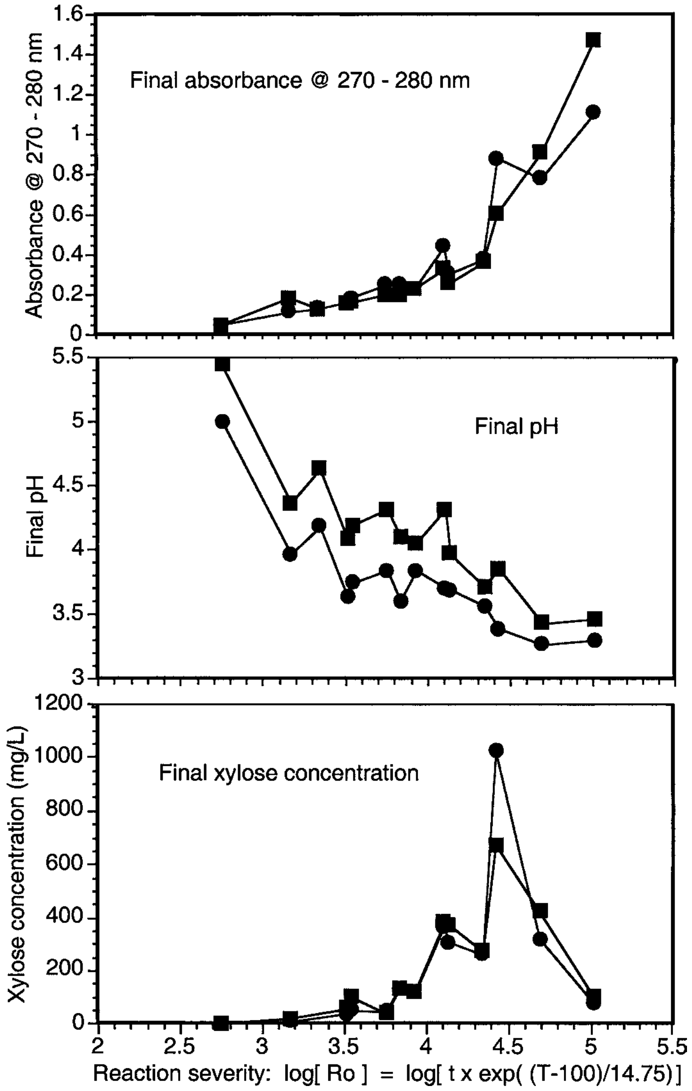

Fig. 6. Average values for absorbance at 270–280 nm, final (depressurized, room temperature) pH, and xylose concentration (mg/L) vs log of reaction severity *Ro*. (■), Aspen wood with carbonic acid; (●), aspen wood with water.

systems. Thus, two indicators of the reaction severity—the xylose concentration and the absorbance—appear to indicate that there is little difference in the reaction severity between the two systems.

Hydrolysate pH

Given that evidence of reaction severity between the two pretreatment systems appears to show little difference in severity, it is surprising that the final pH of the carbonic acid–treated hydrolysate is consistently higher than the liquid hot water hydrolysate, across all reaction conditions.

The average difference in pH is on the order of 0.4, which indicates that the acid concentration in the carbonic acid hydrolysate is less than half that of the liquid hot water hydrolysate. One possible explanation for this result is that the pH at reaction temperature is the same for both reactors—generating similar hydrolysis and degradation results—but that in the case of the carbonic acid reaction, half of the hydronium ion concentration originates from the carbonic acid. As the pressure is released, the carbonate system raises the pH of the solution. If correct, this explanation then implies that acetic acid production at reaction temperature has been diminished by the carbonic acid, but a mechanism to explain this phenomenon is unknown. Accurate determination of the acetic acid concentration in the final hydrolysates would shed light on this hypothesis. Should it prove correct that carbonic acid suppresses the release of acetic acid, this may be a useful result for reducing hydrolysate inhibition caused by acetic acid.

Conclusions

Hydrolysates generated with carbonic acid exhibit a higher final pH than hydrolysates generated under identical severity conditions using water alone. Despite the higher pH, the concentration of xylose monomers and furan degradation products, as measured with UV-VIS absorption, appeared to be maintained compared with those observed in the lower-pH, water-only reactions. This may indicate that the presence of carbonic acid reduces, or at least slows, the release of acetyl groups from hemicellulose. This pH effect appears to decrease with an increase in the temperature of the reaction. Further research will aim to understand the chemical interaction of carbonic acid at reaction temperatures as well as to analyze of the acetic acid concentration in order to explain the pH differences in reactions with and without carbonic acid. Further tests exploring the enzymatic digestibility of pretreated materials and the toxicity of hydrolysates prepared with carbonic acid compared to water alone should further the understanding of downstream effects.

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