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Pretreatment of Corn Fiber by Pressure Cooking in Water

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ABSTRACT

The pretreatment of corn fiber using liquid water at temperatures between 220 and 260°C enhances enzymatic hydrolysis. This paper describes the laboratory reactor system currently in use for cooking of corn fiber at temperatures ranging from 200 to 260°C. The corn fiber at approx 4.4% solid/liquid slurry was treated in a 2-L, 304 SS, Parr reactor with three turbine propeller agitators and a Proportional-Integral-Derivative (PID), controller that controlled temperature within ± 1°C. Heat-up times to the final temperatures of 220, 240, or 260°C were achieved in 50 to 60 min. Hold time at the final temperature was less than 10 s. A serpentine cooling coil, through which tap water was circulated at the completion of the run, cooled the reactor's contents to 180°C within 2 min after the maximum temperature was attained. Ports in the reactor's head plate facilitated sampling of the slurry and monitoring the pH. A continuous pH monitoring system was developed to help observe trends in pH during pretreatment and to assist in the development of a base (2.0 *M* KOH) addition profile to help keep the pH within the range of 5.0 to 7.0. Enzymatic hydrolysis gave 33 to 84% conversion of cellulose in the pretreated fiber to glucose compared to 17% for untreated fiber.

Index Entries: Corn fiber; water pretreatment; enzyme; hydrolysis; cellulose; glucose.

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INTRODUCTION

Aqueous pretreatments have been used to pretreat lignocellulosic material for more effective enzymatic conversion of the cellulose to glucose. Bobleter et al. *(1)* evaluated liquid water as a pretreatment medium to enhance susceptibility of lignocellulosic material for enzymatic hydrolysis. Hydrothermolysis studies, such as those by Haw et al. *(2)*, Hormeyer et al. *(3)*, and Walch et al. *(4)*, have shown that hot water removes and solubilizes hemicellulose, particularly when its hydrolysis is catalyzed by small quantities of acid. Solubilization of some of the lignin also occurs at temperatures above 180°C. Mok and Antal *(5)* found that hemicellulose will dissolve at 200 to 230°C. Amorphous cellulose was also removed from the lignocellulose at this temperature range. van Walsum et al. *(6)* pretreated fresh sugarcane bagasse, aspen chips, and mixed hardwood flour using liquid hot water at 220°C. Whereas hemicellulose solubilization was nearly complete, solubilization of cellulose was less than 10%.

High temperature steam, sometimes combined with pressurized inert gas, and acid injection, is another hydrothermal pretreatment *(7)*. Steam penetrates the lignin, hemicellulose, and cellulose. The mixture is explosively decompressed, steam explosion, and the resulting expansion increases cellulose accessibility *(8)*. The operating conditions promote acid formation and result in degradation of cellulose by autohydrolysis. Hydrolysis is considered to be an important and necessary aspect of the steam pretreatment *(8,9)* and acid addition is recommended to promote this effect *(7)*. Brownell and Saddler *(10)* reported that steam pretreatment of lignocellulosic material without the explosive decompression was at least as effective for pretreatment of aspen chips as steam explosion.

Our approach to water pretreatment, by comparison, has the goal of maintaining a liquid phase (under pressure) during pretreatment, while keeping the pH between 5.0 and 7.0 in order to minimize, and preferably, to avoid cellulose hydrolysis and sugar degradation reactions *(11–15)*.

MATERIALS AND METHODS

Materials

Standard chemical and analysis procedures were used to analyze the compositions of the various materials *(16)*. The (coarse) corn fiber was provided by A. E. Staley Manufacturing, (Lafayette, IN) in 1 x 5 mm particle size, and was stored in a freezer until use. The procedures used in this work are summarized below.

Analytical Methods

The determination of total solids, carbohydrates, acid insoluble/soluble lignin, and ash in the pretreated and untreated samples of biomass were determined using the standard analysis and testing procedures 001, 003, 004, 008, and 009 provided by the National Renewable Energy Laboratory (NREL, Golden, CO).

Total Solids/Moisture

NREL standard procedure 001 was followed. The large particles (1 x 5 mm) of biomass sample were milled in a Thomas-Wiley (Philadelphia, PA) mill using a 35-mesh screen. A 1 to 5 g sample of the biomass was weighed in an aluminum foil weighing dish, placed in a convection oven at 105 ± 3°C and dried to constant weight (± 0.1%). The oven-dried sample was cooled in a desiccator and weighed to obtain the difference in weight due to moisture.

Carbohydrates

NREL standard procedure 002 was used to determine the quantity of cellulose and hemicellulose in the solid corn fiber. An HPX-87C liquid-chromatography column was used for this procedure. Cellobiose was detected in addition to glucose, xylose, and arabinose. The biomass sample was treated at 30°C with 72% sulfuric acid for 2 h and then autoclaved at 121°C, after diluting the acid to 4% with deionized water. The hydrolysate was neutralized with calcium carbonate, vacuum filtered, and run in the HPLC column. A blank was run in the HPLC column (operated at 60°C) to determine the retention time of the eluting acid peak. Standardization curves were developed for the major components of the pretreatment supernatant.

Klason Lignin

NREL standard procedure 003 was followed in which the biomass sample was thoroughly mixed in 72% sulfuric acid and then allowed to stand for 2 h at 20°C. The hydrolysate was diluted to 3% acid and boiled for 4 h in a distilling column in a hood. The solution was vacuum filtered through a filtering crucible. The residue was dried at 105 ± 3°C for 2 h. The resulting weight was corrected for the acid-insoluble ash (determined by igniting the contents at 575 ± 25°C) in order to obtain the ash-free dry weights.

Acid Soluble Lignin

NREL standard procedure 004 was used. The filtrate saved in procedure 003 was analyzed directly using a spectrophotometer. The

absorbance was measured at 205 nm, using the 10-mm light path cuvet. When the absorbance was not in the range 0.2–0.7, the filtrate was diluted accordingly with 3% sulfuric acid.

Ash

A 0.5 to 1.0 g sample of biomass was placed in a crucible, ignited in a muffle furnace at 575 ± 25°C, cooled in a desiccator, and then weighed.

Enzymatic Hydrolysis

The enzymatic hydrolysis was carried out using NREL procedure 009. The enzyme hydrolyses were performed on a laboratory scale using a 10-mL working volume that included deionized water, 0.1 *M* citrate buffer (pH 4.8), 1 g wet substrate (treated and untreated), or 1 mL of pretreatment supernatant and cellulase enzyme (67 μL), Spezyme CP. The hydrolyses were carried for 48 h with 50-μL samples being removed at 0, 24, and 48 h. These samples were analyzed for their glucose content using a Beckman (Fullerton, CA) glucose analyzer 2. Sodium azide at 200 mg/L was added to the buffer to prevent microbial growth.

Acid Hydrolysis

Acid hydrolysis was performed to determine the quantity of xylan and araban oligosaccharides that were solubilized into the pretreatment supernatant. The hydrolyses were performed on a laboratory scale by hydrolyzing 250 mL liquid supernatant with 1.33 mL sulfuric acid (0.1 *M* sulfuric acid solution) in a 500-mL Pyrex round-bottomed flask with a 24 | 40 ground glass opening. A Kimax glass condenser with a 24 | 40 connection was mounted on top of the round-bottomed flask and cooling water was circulated through the condenser to allow for reflux. The top of the condenser was open to the atmosphere. The round-bottomed flask was set into a heating mantle (Glas-Col Apparatus, Terre Haute, IN) with the heat being controlled by a variable autotransformer (Superior Electric, Bristol, CT). The autotransformer was set to "70" and the hydrolysate refluxed at atmospheric conditions for 4 h. At the end of the 4 h the hydrolysate was cooled, a 3-mL sample was taken, and the rest was stored in a refrigerator.

Pretreatment Reactor

A Parr, 304 SS reactor (Model 4843; Moline, IL) was used to pretreat corn fiber in water at selected temperatures and pH values (Fig. 1). The reactor has a total volume of 2 L, with three turbine propeller agitators and a PID temperature controller (± 1.0°C). Cooling water was circulated

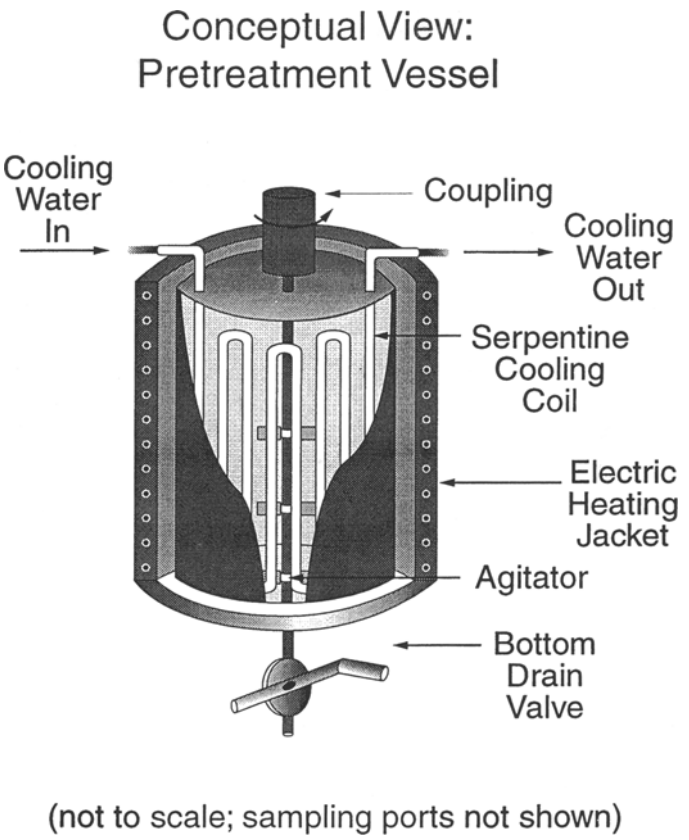

Fig. 1. Schematic diagram of the pretreatment vessel.

through a serpentine coil to cool the reactor contents at the end of each run. A bottoms port and two inlet ports allowed sampling of the pretreated material and addition of reagents to the reactor.

Pretreatment Procedures

The corn fiber was pretreated under six conditions. Three final set-point temperatures were 220, 240, and 260°C. Two runs were made at each set-point temperature, one in which KOH was added to keep the pH above 5.0 and another in which no base was added.

The corn fiber was first soaked in water at room temperature for approx 14–18 h. The amount of wet corn fiber loaded corresponded to a dry weight of 44 g/L. The working volume of the pretreatment vessel was 1.5 L for the 220 and 240°C pretreatments, and 1.0 L for the 260°C pretreatments. The working volume for the 260°C pretreatment was decreased since the volume of water at 260°C will expand to 1.25 times the

volume of water at room temperature. At that volume and temperature, water has the potential to completely fill the vessel creating a possibly explosive and harmful situation. Hence, this measure was taken for safety considerations. If the volume of the slurry was decreased, the ratio of solids to liquids was maintained.

Prior to pretreatment, the pumps for the pH-monitoring system were primed with deionized water (Fig. 2). This was done to calibrate the two pumps to the same flow-rate and to evaluate the system for leaks and for plugging.

The pretreatment agitator was initially set at 115 rpm, and the reactor contents were initially at ambient temperature. The heater set point temperature was set at 220, 240, or 260°C, and heat-up was initiated by turning the heat switch to high. The heat-up rate for the corn fiber was between 3.5 and 4.0°C/min.

Whereas the control of temperature and heat-up was readily achieved using the PID controller supplied by Parr (Moline, IL), a continuous pH-monitoring system was developed to sample the supernatant without loss during the pretreatment. The system consisted of two Milton Roy pumps (Mini-pump, 46/460 mL/h) (Fig. 2). pH monitoring was initiated when the pretreatment temperature reached 150°C, with a vessel pressure of 50 psig (± 3 psig). Pump 1 continually metered liquid supernatant from the reactor through a 0.2-micron filter stone (Alltech, Deerfield, IL), then through 1/8 in od (0.085 id) stainless-steel tubing. The vessel pressure ranged from 0 to 700 psig over the course of the pretreatment and hence, provided the back pressure required for the supernatant to pass through the filter stone. The supernatant passed through an ice-bath then through pump 1. From pump 1, the supernatant went through a water bath, at ambient temperature, and was pumped through a check valve (set at 750 psig) into a pH probe/flow cell at a rate of 8.5 to 9.0 mL/min. The total time for supernatant to travel from the stone in the pretreatment vessel to the pH flow cell was approx 1.5 min.

The flow cell, which housed the pH probe, was fabricated from a 50-mL graduated cylinder with two approx 1 mm id (3/8 in od) glass ports fitted at the top and bottom of the cylinder by the Purdue University Chemistry Glass Shop. The temperature of the supernatant entering the flow cell was 24°C. The fluid entered a port at the bottom of the cylinder and flowed out through a port at the top into a reservoir. The reservoir was a graduated beaker that held approx 40 mL fluid. The volume of the reservoir was checked regularly to calibrate the flow rates of the two pumps in the monitoring line. A second Milton Roy pump (Mini-pump, 46/460 mL/h) then returned the supernatant from the reservoir to the pretreatment vessel at approx the same flow rate as pump 1. The total time for supernatant to flow through this circuit was approx 10 min.

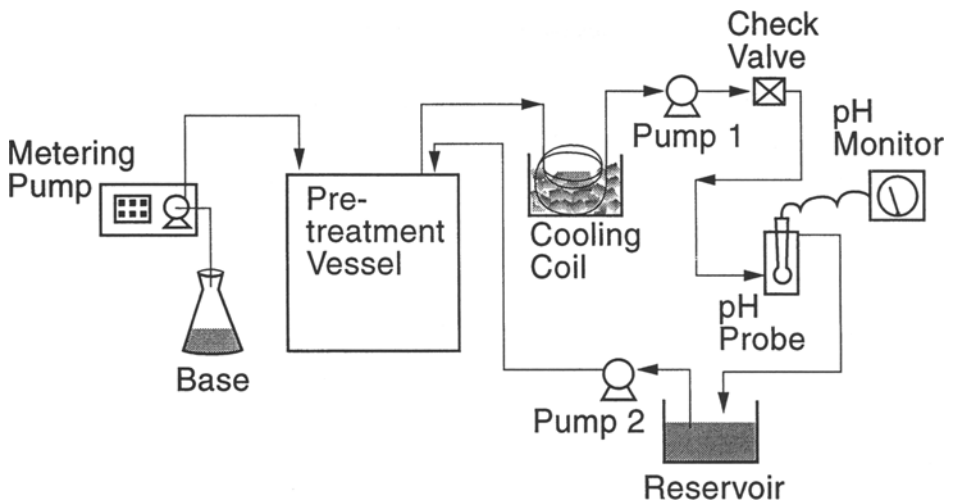

Fig. 2. Schematic diagram of the pH-monitoring system.

A Markson pH meter (Markson Science, Phoenix, AZ) and pH electrode (Markson V-830) were used to determine the pH of the supernatant in the flow cell. The pH electrode was calibrated using standardized buffer solutions (Fischer Scientific, Pittsburgh, PA) of pH 4.0 and 7.0. The electrode was stored in pH 4.0 buffer when it was not being used.

For the pretreatments in which the pH was controlled by the addition of KOH, the base was metered into the reactor by manual control using a third pump, Dynamax SD - 200 (Woburn, MA), pumping at 10 mL/min (Fig. 2). The base was added directly to the pretreatment vessel to reduce the time between reading the pH meter and the physical contact of the base with the supernatant in the pretreatment vessel. This minimized the possibility of alkaline degradation reactions that might otherwise occur if the base were added to the reservoir, where a small portion of the slurry can sit at a high pH (7.0–12.0) at room temperature for a period of 5 min.

For the pretreatments in which KOH (2.0 *M*) was added to control the pH, the target pH range was calculated to be between 5.0 and 7.0 during the pretreatment. The maximum amount of KOH added to the pretreatment vessel to control the pH during the pretreatment was 10% of the working volume.

Once the predetermined set-point temperature was obtained, the heater was turned off and cooling water was charged through the serpentine cooling coil. The contents of the reactor cooled down to 180°C in approx 2 min, and to 150°C in approx 5 min. The reactor was kept sealed, and the slurry agitated until the reactor headplate had cooled to approx 50°C. The agitator drive was then disconnected and the reactor physically removed from the heating jacket. Then the reactor was opened, and the contents were removed for further analysis and testing.

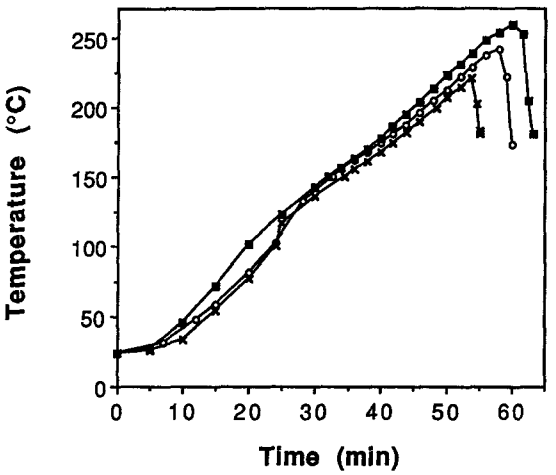

Fig. 3. Typical temperature profile during pretreatment of corn fiber to 200°C (×), 240°C (○), and 260°C (■).

RESULTS AND DISCUSSION

Pretreatment Temperature and pH

There were six pretreatment conditions evaluated for the corn fiber. The target set-point temperatures were 220, 240, and 260°C. For the pretreatment, 2.0 *M* KOH was added to each vessel to control the pH. This pretreatment was compared to a pretreatment at the same temperature in which the pH was not controlled.

The heat up of the corn fiber and water mixture from 25°C to the final set-point temperature took between 50 and 60 min (Fig. 3). Cooling from the final set point temperature to below 180°C took approx 2 min.

The pH range for the pretreatments in which the pH was not controlled ranged from 4.0 at the start of the pretreatment to approx 3.1 at the end of the pretreatment (Fig. 4). This pH was close to the pH, 2.8, at which autohydrolysis occurs *(17)*.

Prior to the pretreatments where the pH was controlled, approx 20 mL 2.0 *M* KOH was added to bring the pH from 4.0 to 7.0 (Fig. 5). During the pretreatment approx 60 to 75 mL KOH solution was added to the vessel to keep the pH above 5.0 and below 7.0.

The material balance for the six pretreatments was closed to within 95% of the original material balance with the exception one pretreatment, 220°C with KOH added to control pH, in which 92% of the original material was recovered after pretreatment, Table 1.

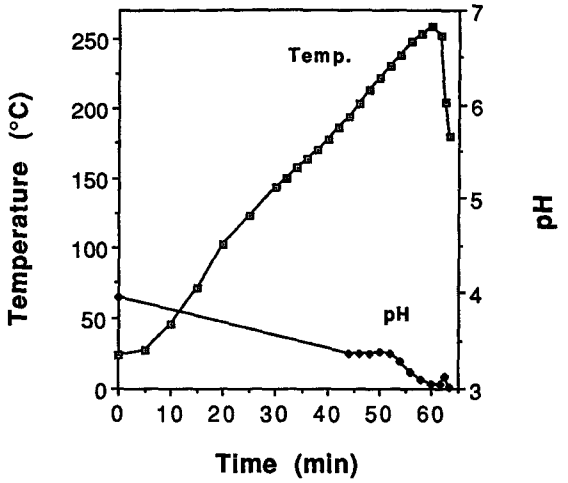

Fig. 4. Typical pH profile for corn fiber pretreatment without pH control.

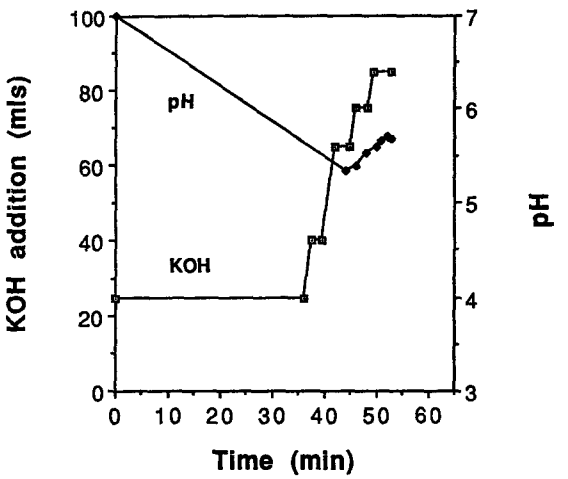

Fig. 5. Typical pH profile for corn fiber pretreatment with KOH added to control pH.

Effect of Pretreatment on Corn Fiber

In excess of 70% of the total corn fiber material was solubilized during the pretreatment (Fig. 6). There does not appear to be a correlation between the final set-point pretreatment temperature and the quantity of solid material solubilized.

There is significant modification to the composition of the solid portion of the pretreated corn fiber compared to the original corn fiber, Table 2. Aqueous pretreatment of the corn fiber caused an increase in the cellulose content of the remaining solids ranging from 21 to 47% of the total pretreated solids compared to 17.5% cellulose for the untreated fiber. There was a positive effect of increased solid cellulose content when the pH was

Table 1
Material Balances for Each Pretreatment Condition

	220°C	220°C KOH	240°C	240°C KOH	260°C	260°C KOH
Material In:						
Dry (g)	67.6	70.6	68.2	68.4	46.1	46.3
Liquid (g)	1582.4	1579.4	1581.8	1581.8	1053.9	1053.7
KOH (g)	0.0	86.2	0.0	92.8	0.0	68.0
Total In (g)	1650.0	1736.2	1650.0	1743.0	1100.0	1168.0
Material Out:						
Dry (g)	18.9	17.5	17.5	12.9	11.4	7.4
Liquid (g)	1537.0	1516.5	1518.6	1580.7	1016.4	1075.8
Samples (g)	20.2	20.2	25.3	25.3	30.2	30.4
Losses (g)	10.1	40.4	20.2	40.4	10.1	10.1
Total Out (g)	1586.2	1594.6	1581.6	1659.3	1068.1	1123.7
% Recovery	96.1	91.9	95.9	95.2	97.1	96.2

controlled using KOH during the pretreatment. The increase in the solid cellulose content was not as pronounced for the pretreatments in which KOH was not added.

Starch and hemicellulose were dissolved completely at the pretreatment conditions studied, Table 2. The only pretreatment in which hemicellulose remained in the solid was the pretreatment to 220°C with KOH added to control pH. For the pretreatment to 220°C with KOH addition to control pH, there was 12.98 g xylose and 6.18 g arabinose in the hemicellulose in the original corn fiber. After the pretreatment, the remaining solids contained 3.25 g xylose and no detectable quantity of arabinose. The liquid supernatant for this pretreatment contained 0.2 g xylose and no detectable amount of arabinose. The remaining 9.5 g xylose and 6.18 g arabinose can be accounted for as solubilized hemicellulosic oligosaccharides as confirmed by acid hydrolysis.

Cellulose solubilization ranged from 50 to 70% of the total cellulose originally in the solid corn fiber for the pretreatment conditions evaluated (Fig. 7). There was a linear correlation that as the final set-point pretreatment temperature increased so did the quantity of cellulose solubilized for the pretreatments in which the pH was not controlled. The quantity of cellulose solubilized for corn fiber pretreated to 220°C was 50% and this value increased to 70% for the pretreatment to 260°C.

The addition of KOH to control the pH during pretreatment did little to reduce the extent of cellulose solubilization (Fig. 7). The amount of cellulose that was solubilized during pretreatment with KOH added to con-

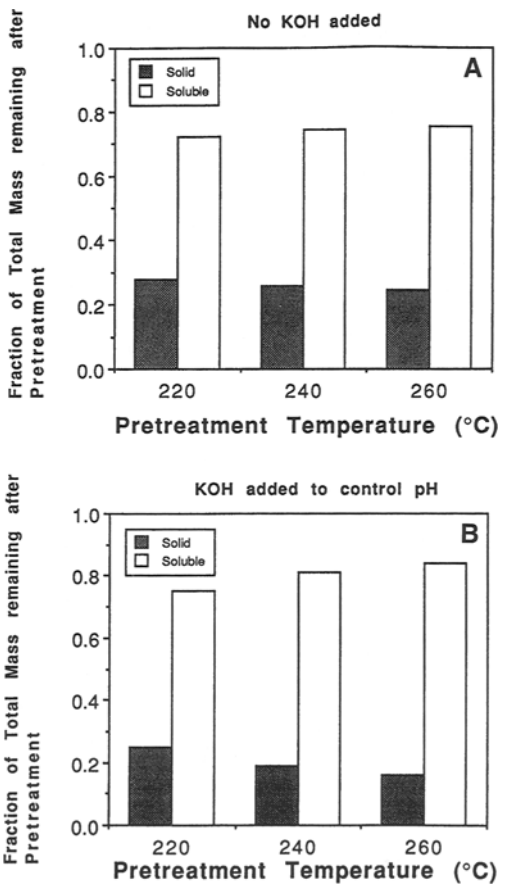

Fig. 6. Fraction of the corn fiber in the remaining solids compared to the amount that was solubilized for **(A)** the pretreatments in which the pH was not controlled and for **(B)** the pretreatments in which the pH was controlled with KOH.

trol pH ranged from 49% for the 240°C pretreatment to 71% for the 260°C pretreatment. The quantity of cellulose solubilized during the pretreatments with KOH added to control pH may indicate that the cellulose was in a reactive state.

Enzymatic Hydrolysis

The use of KOH for pH control does not greatly affect the solubilization phenomena. This may be explained by the high hemicellulose content of the initial material and the already reactive state of the cellulose in the original corn fiber.

The benefit of adding KOH to the pretreatment supernatant prevents the degradation of solubilized cellulosic oligomers to products that will not produce glucose during enzymatic hydrolysis. The pretreatments in

Table 2
Composition of Treated and Untreated Solid Corn Fiber (% Basis)

	220°C	220°C KOH	240°C	240°C KOH	260°C	260°C KOH	Untreated
Starch	0.0	0.0	0.0	0.0	0.0	0.0	3.5
Cell	30.4 (±1.4)	31.8 (±1.3)	27.0 (±1.3)	47.3 (±2.0)	20.8 (±0.7)	31.9 (±1.4)	17.5 (±1.0)
Xylans and Arabans	0.0	16.3 (±0.6)	0.0	0.0	0.0	0.0	23.8 (±0.8)
Klason Lignin[a]	31.4 (±0.7)	17.1 (±0.1)	54.6 (±1.0)	29.2 (±0.2)	59.6 (±0.4)	44.6	4.5 (±0.1)
Acid Lignin	7.8 (±0.0)	4.9 (±0.1)	6.4	4.2 (±0.1)	5.6 (±0.1)	6.3	8.3
Ash	8.2 (±0.0)	15.7 (±0.8)	6.6 (±0.01)	11.2 (±0.2)	6.7 (±0.1)	8.8	8.0 (±0.1)
Total	77.8	85.8	94.6	91.9	92.7	91.6	65.6

[a]These values contains components other than Klason lignin.

which the pH was controlled by the addition of KOH showed higher enzymatic conversions at 48 h than the pretreatment in which no base was added for all three pretreatment temperatures. This increase in conversion is most noticeable at the pretreatment temperatures of 240°C at which enzymatic conversion of the supernatant increased 10-fold with the use of KOH and 260°C at which KOH addition increased the enzymatic conversion of the supernatant by 16%, Table 3. The pretreatment to 220°C with KOH added to control pH gave the highest enzymatic conversion to glucose of approx 75% of the theoretical limit. The majority of glucose production during enzymatic hydrolysis of the pretreatment supernatant occurred during the first 24 h (Fig. 8).

The enzymatic conversion of pretreated cellulose in the remaining solids was between 86 and 100% of the theoretical limit compared to 17% enzymatic conversion for the untreated corn fiber, Table 3. The highest enzymatic conversion, 100%, was achieved for corn fiber pretreated to 260°C without pH control. Corn fiber pretreated to 220°C with KOH added to control the pH followed with 95% conversion. Whereas a significant fraction of the glucose production from enzymatic hydrolysis of the solid material occurred during the first 24 h, there was significant glucose production occurring during the 24–48-h period, most notably, for the corn fiber pretreated to 260°C without pH control (Fig. 9).

The overall conversion was determined as the total production of glucose from cellulose via enzymatic hydrolysis from both the remaining solids and the liquid supernatant based on the quantity of cellulose or cel-

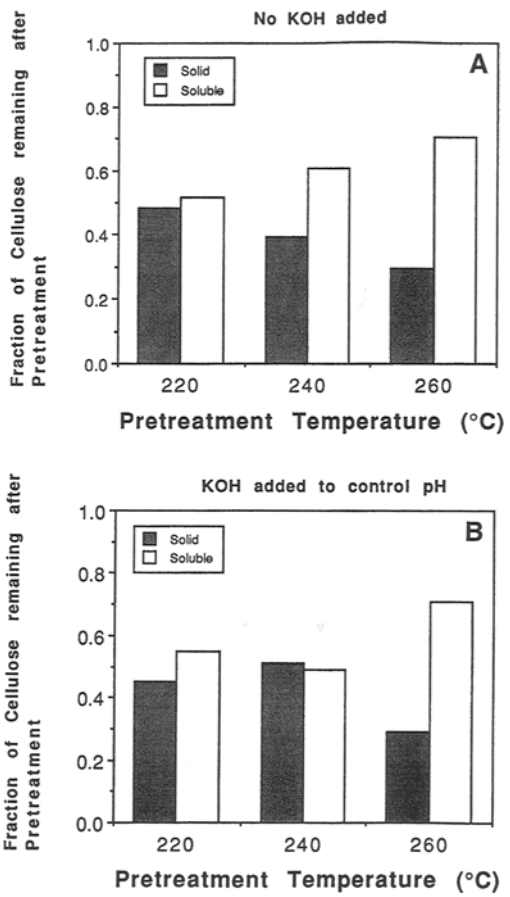

Fig. 7. Fraction of the cellulose in the remaining solids compared to the amount that was solubilized for **(A)** the pretreatments in which the pH was not controlled and for **(B)** the pretreatments in which the pH was controlled with KOH.

Table 3
Enzymatic Conversion to Glucose after 48 H from both the Solid Portion and the Supernatant of Pretreated Corn Fiber

	220°C	220°C KOH	240°C	240°C KOH	260°C	260°C KOH	Untreated
Solid	0.86	0.95	0.88	0.86	1.00	0.89	0.17
Liquid	0.71	0.75	0.05	0.49	0.00	0.16	—
Total	0.78	0.84	0.38	0.68	0.33	0.37	0.17

(g glucose produced/g glucose available from cellulose)

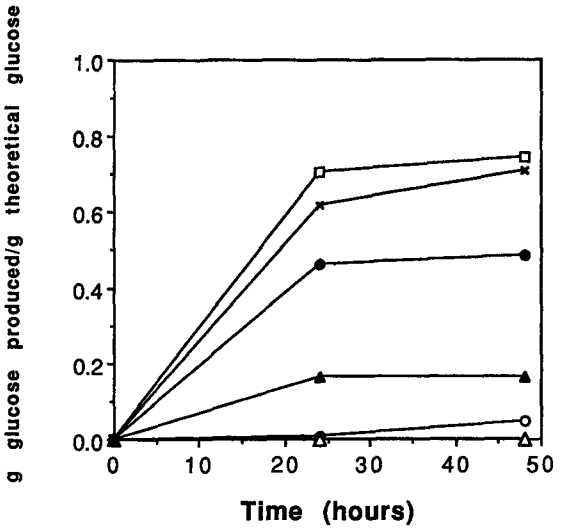

Fig. 8. Enzymatic conversion of cellulose in the pretreatment supernatant to glucose for corn fiber pretreated to 220°C (×), 220°C with KOH added (□), 240°C (○), 240°C with KOH added (●), 260°C (△), and 260°C with KOH added (△). The enzymatic hydrolysis consisted of 1.0 mL pretreatment supernatant, 67 μL cellulase enzyme (Spezyme CP), 0.1 *M* citrate buffer, and deionized water to 10 mL working volume. The enzymatic hydrolysis was held at 50°C and pH 4.8 for 48 h.

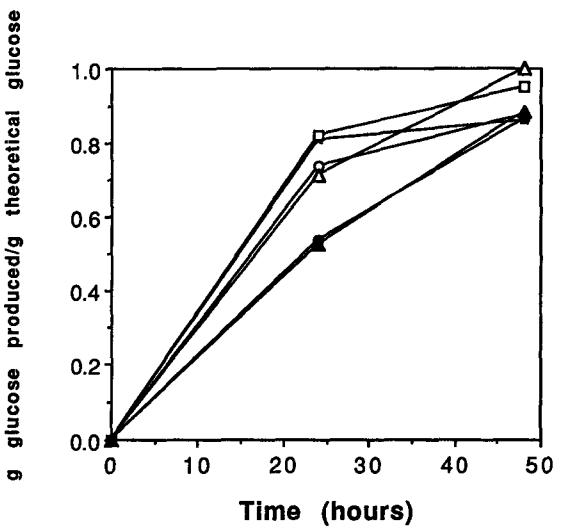

Fig. 9. Enzymatic conversion of cellulose in the pretreated solids to glucose for corn fiber pretreated to 220°C (×), 220°C with KOH added (□), 240°C (○), 240°C with KOH added (●), 260°C (△), and 260°C with KOH added (▲). The enzymatic hydrolysis consisted of 1.0 g pretreated corn fiber, 67 μL cellulase enzyme (Spezyme CP), 0.1 *M* citrate buffer, and deionized water to 10 mL working volume. The enzymatic hydrolysis was held at 50°C and pH 4.8 for 48 h.

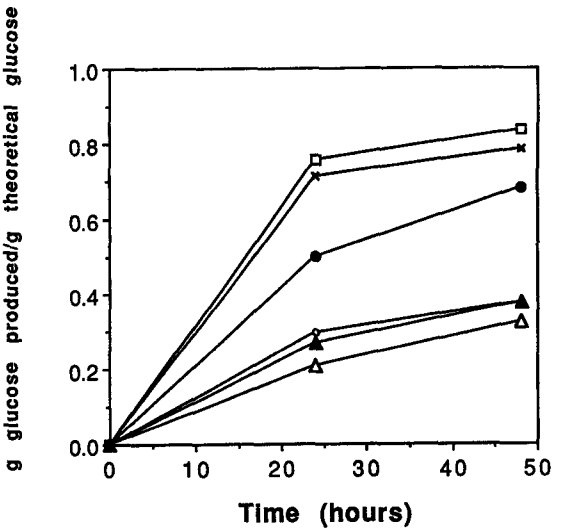

Fig. 10. Overall enzymatic conversion of cellulose to glucose for corn fiber pretreated to 220°C (×), 220°C with KOH added (□), 240°C (○), 240°C with KOH added (●), 260°C (△), and 260°C with KOH added (▲). The enzymatic hydrolysis consisted of 1.0 mL pretreatment supernatant, 67 μL cellulase enzyme (Spezyme CP), 0.1 *M* citrate buffer, and deionized water to 10 mL working volume. The enzymatic hydrolysis was held at 50°C and pH 4.8 for 48 h.

lulosic oligosaccharides in each phase, Table 3. The highest overall conversion achieved was from the treatment 220°C with KOH, which gave an 84% conversion of glucose from cellulose. The pretreatment to 220°C gave a 78% overall conversion of glucose from cellulose overall. The overall enzymatic conversion was highest at the lower final pretreatment temperatures. Overall enzymatic conversion is also enhanced by the addition of KOH during pretreatment. This is because of the large solubilization of cellulose into the supernatant and the benefit of KOH preventing degradation of the solubilized cellulosic oligomers. Overall glucose production occurs mainly within the first 24 h, with some production occurring between 24 and 48 h (Fig. 10).

CONCLUSIONS

Based on the results of this work it appears that the best conditions for pretreating corn fiber would be a pretreatment temperature of 220°C and the addition of KOH to control the pH in the range from 5.0 to 7.0. The cellulose in the corn fiber is probably in an amorphous form, judging from the quantities of cellulose that were solubilized.

From the large quantities of solid materials and cellulose that were solubilized during the pretreatment, it appears that temperatures greater than 220°C are too harsh for the treatment of corn fiber to achieve high and

effective yields of glucose from cellulose. This is also supported by the enzymatic hydrolysis results in which very little glucose was obtained from the supernatant despite the large amount of cellulose being solubilized and degraded at 240 and 260°C. These higher pretreatment temperatures had a stronger effect on the degradation of soluble polysaccharides than addition of KOH to control the pH could prevent.

The addition of KOH is necessary to help neutralize the pH of the supernatant and prevent the acid-catalyzed degradation of the soluble cellulosic oligosaccharides. This benefit can be observed by looking at the results of the enzymatic hydrolysis of the supernatant that show an increase in enzymatic conversion over the pretreatment supernatants in which the pH was not controlled. The production of glucose from soluble cellulosic oligomers for the liquid portion of the pretreatment strongly favors the use of KOH to control pH for all three pretreatment temperatures.

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