Surface and Pore Structure Modification of Cellulose Fibers Through Cellulase Treatment

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ABSTRACT: The surface and pore structure of cellulose fibers have a significant impact on the properties and performance in applications. Cellulase enzymatic hydrolysis of cellulose fibers can result in changes to the surface and pore structure, thus providing a useful tool for fiber modification. This research characterizes these changes using various test methods such as fiber dimension, water retention value (WRV), hard-to-remove (HR) water content, freezing and nonfreezing bound water content, polymer adsorption, and crystallinity index. For a high-dosage cellulase treatment (600 U/g dry solid), the fiber length was significantly decreased and the fibers were ''cut'' in the cross direction, not in the axial direction. The swelling capacities as measured by the WRV and HR water content increased for the high-dosage treatment. Three independent measurements (nonfreezing bound water, polymer adsorption, and crystallinity index) are in good agreement with the statement that the amorphous regions of cellulose fibers are a more readily available substrate relative to crystalline regions. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 3833–3839, 2007

Key words: cellulose fibers; cellulase; pore structure; surface structure

INTRODUCTION

The enzymatic modification of cellulose has been an important research topic over the last several decades. In the pulp and paper industry, various applications that have been investigated include the deinking of recycled fibers,[1] the pretreatment of wood to accelerate the pulping process,[2,3] the drainage improvement of pulps,[4,5] the reduction of refining energy,[6] and the strength and smoothness improvement of handsheets.[7] In the textile industry, enzymes have been used to achieve the stone-washed look of denim garments.[8] Most of all, enzymes for cellulose hydrolysis as the first step in converting plant biomass (lignocellulosic fibers) to fuels and chemicals are also of prospective importance for the next generation of energy sources.[9]

Even though the enzymatic modification of cellulose has significant potential benefits in all industries using cellulose-based fibers, there exist many difficulties in implementation, such as expensive enzyme cost and the high sensitivity of enzymes to environmental variables. However, with advances in fermentation technology, enzymes are becoming less expensive to produce. Further, rising energy costs have prompted an explosive demand for the research of cellulose modification by a multi-enzyme system. The mechanisms and the effects of cellulase on cellulose fibers have been extensively reviewed.[9–12]

Cellulase is a general term for a group of enzymes that hydrolyze the β-(1,4)-linkages in cellulose. Cellulase consists of three different enzymes that act synergistically in the hydrolysis of cellulose. Endoglucanase (EG) randomly hydrolyzes the β-(1,4)-linkages within the water-insoluble cellulose chain. Cellobiohydrolase (CBH) hydrolyzes the linkages at the reducing ends of cellulose chains to form cellobiose. Cellobiase or β-glucosidase converts the water soluble cellobiose into two glucose residues.

Cellulase hydrolyzes cellulose fibers by cutting cellulose molecular chains into shorter segments and cleaving glucose units from the molecular chains. As a result of enzymatic hydrolysis, the surface and pore structure of the cellulose fibers are expected to change.

An increase in the crystallinity index as measured by X-ray diffraction was reported for cotton fiber[13] and dissolving pulp and cotton linters,[14] indicating degradation of the amorphous regions. However, it was also reported that there was no discernible difference in the crystallinity index for hemp fibers[15] and unbleached kraft pulp.[16] It was found that the specific surface area of regenerated bead celluloses increased with treatment time as measured by nitrogen adsorption.[17] A gradual increase in surface roughness at the nanoscale was detected for cotton fibers based on SEM and AFM observations.[13,18] In addition, different effects on surfaces were observed by AFM when different types of enzyme were utilized on cotton fibers

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as a substrate.[19] Moisture regain of cotton fibers was enhanced after the enzyme treatment.[18]

Mercury porosimetry was applied to investigate the structural changes with enzyme treatment time and it was found that the porosity of the bead celluloses increased with treatment time[17] and the porosity of hemp fibers increased for the first 4 h, but decreased after that.[15] It was reported that the concentration of pores smaller than 6 nm in cotton fabric decreased after enzyme treatment as measured by size exclusion liquid chromatography, but no significant changes were observed for the concentration of pores larger than 6 nm.[20]

In this study, the surface and pore structure of cellulose fibers after treatment by a commercially available cellulase enzyme were examined. Various test methods were performed to investigate the enzymatic effects on cellulose fibers such as fiber dimension, water retention value (WRV), hard-to-remove (HR) water content, freezing and nonfreezing bound water content, polymer adsorption, and crystallinity index.

EXPERIMENTAL

Sample preparation

Fully bleached softwood kraft pulp was obtained directly after the bleaching stage at Weyerhaeuser (Plymouth, NC). This was a mixture of loblolly pine and southern pine. Never dried pulp was used for all experiments. The average dimensions of the nontreated fibers were 30.0 μm of fiber width (arithmetic average), 2.56 mm of fiber length (length weighted average, 0.070–10.00 mm range), and 3.05% of fines content (length weighted, 0.070–0.200 mm range) as measured by FQA (Fiber Quality Analyzer, OpTest Equipment, Ontario, Canada), Table I.

Cellulase from *Trichoderma reesei* was purchased from Sigma-Aldrich (Fluka, 22173) and stored at 4°C before use. Cellulase treatments were done in one day to avoid the complication that the cellulase activity might decrease overtime. Cellulase activity was 6 U/mg based on manufacturer, but not measured. Two cellulase charges, a high dosage (600 U/g dry solids) and a low dosage (60 U/g), were used to determine the effect of dosage. Cellulase hydrolysis was performed for 0 (untreated), 30, 60, 120, and 240 min in a gyratory water bath (New Brunswick Scientific, G76) at a consistency of 3.0% and temperature of (50 ± 2)°C to maximize cellulase activity. Deionized water was used and the pH was around 7, but not controlled (optimum value of 4.8 for *Trichoderma reesei*), to simulate the paper-making environment, which might be a potential application. At the selected hydrolysis time, the treated pulps were washed extensively with deionized water to remove the cellulases and dissolved sugars by filtration (Whatman No. 4). The samples were stored in a refrigerator at about 10% consistency. The percent degradation was calculated by determining the consistencies of samples before and after treatment.

To investigate the effect of small solutes on freezing point depression, sodium chloride (Fisher Scientific, Certified ACS), α-D glucose (Sigma-Aldrich, 15896-8), and dextran (Sigma-Aldrich, D4626) were used.

For the 240-min high-dosage treated sample, the sample was fractionated using a 100-mesh wire (openings of 0.15 mm). Fines and long fraction were collected to measure the HR water and bound water contents.

Swelling capacity measurements

The water retention value (WRV)[21] is the ratio of water to dry fiber mass after centrifugation (Centra CL3R with 958 swing horizontal rotor, International Equipment Company) and was measured following SCAN test method (SCAN C102XE: 3000 g, 15 min, and 1700 g/m^2). The HR water content[22] is a measure of the amount of water that is hard to evaporate from cellulose fibers during isothermal TGA experiments (TGA Q500, TA Instruments). The HR water content

TABLE I
Characteristics of Cellulase-Treated Fibers

	Untreated	Low dosage		High dosage	
Hydrolysis time (min)	0	60	240	60	240
Fiber length (mm)	2.56	2.49	2.54	1.76	0.32
Fiber width (μm)	30.0	30.1	30.4	31.2	33.4
Fines content (%)	3.02	2.90	3.13	4.83	33.4
Degradation[a] (%)	0.00	0.92	1.78	25.7	46.3
Polymer adsorption[a] (μeq/g)	40.6	36.2	40.12	35.8	23.8
Crystallinity index[b] (%)	52.8	–	–	54.8	54.3

[a] Samples for 30 and 120 min treatment were not measured.
[b] Samples for low dosage treatment and 30-and 120-min high-dosage treatment were not measured.

is determined by measuring the moisture ratio of the fibers at the transition between the constant rate zone and the falling rate zone. An isothermal temperature of 90°C was utilized and the nitrogen gas flows were 40 (balance gas) and 60 mL/min (sample gas). Detailed experimental procedures can be found in previous experiments by Park et al.[22]

Bound water measurements: Freezing and nonfreezing bound water

Freezing bound water, which has its freezing/melting temperature depressed due to the presence of a substrate, was measured using differential scanning calorimetry (DSC Q100, TA Instruments). Samples of ~5.0 mg were sealed in a DSC aluminum hermetic pan (TA Instruments, Part #900793.901 for bottom and Part #900794.901 for lid). The sample pan was cooled to −30°C and maintained for 5 min. The temperature was then raised to −20°C at a heating rate of 1°C/min, and the sample was maintained isothermally until the heat flow returned to the baseline value. Subsequent heating steps to slightly higher temperatures (−15, −10, −6, −4, −2, −1.5, −1.1, −0.8, −0.5, −0.2, and −0.1°C) were then performed in succession. Each endothermic peak represents the melting of water. It is assumed that the water is contained in cylindrical pores and the size of the pores can be estimated using the Gibbs-Thomson equation.[23,24] Thus, the freezing bound water content was calculated by the summation of the peak areas. Detailed experimental procedures for the DSC operation can be found in a previous work by Park et al.[25]

To measure the nonfreezing bound water (water that does not display a freezing/melting transition due to the association with the substrate), a sample was cooled to −30°C and continuously scanned at 1 to 15°C/min. The amount of nonfreezing bound water was calculated by subtracting the total freezable water (both freezing bound water and unbound water) in the sample, determined from integration, from the moisture ratio in the sample.[26] The moisture ratio was determined gravimetrically using a TGA microbalance (drying at 110°C) after the DSC run on the same sample with a hole pierced in the top of the sample pan.

Polymer adsorption and crystallinity index

To estimate the accessible surface area of hydrolyzed fibers, polymer adsorption tests were performed using poly(diallyldimethylammonium chloride) (poly-DADMAC, Sigma-Aldrich, 522376), having a molecular weight of 5000–20,000.[27,28] About 0.5 g (dry solids) of sample was slurried in 100 mL of 0.0010*N* poly-DADMAC solutions and stirred for 10 min using a small magnetic stirring bar. Samples were then filtered through a 100 mesh stainless steel screen. A filtrate sample of 5 mL was titrated with a 0.0030*N* poly(vinyl sulfate) potassium salt (PVSK, Sigma-Aldrich, 271969) using a particle charge detector (PCD-03, Mütek) to determine the concentration of residual poly-DADMAC. The amount of poly-DADMAC adsorbed initially to the fiber surfaces, assumed to be proportional to the accessible surface, was calculated as the difference of the initial charged amount minus the amount in the filtrate.

The crystallinity index was determined using X-ray diffraction (XRD, Philips XLF, Omni Instruments) with Cu tube. Handsheets were formed for the untreated and 60 min treated samples and air-dried, while 240-min high-dosage samples were measured as dry powders. A sheet could not be formed from 240-min high-dosage samples as the treatment rendered the fiber length of the pulp too small. Indexes were calculated using a standard cellulose (microcrystalline cellulose, Avicel PH-101, Sigma-Aldrich, 11365) by comparing the height at the 002 peak to the sum of the heights of the amorphous region at 2θ of 19° and the 002 peak.[29]

RESULTS AND DISCUSSION

Changes in fiber length and cellulase degradation

After the cellulose treatment of cellulose fibers, the fiber length and fines content were measured (Figs. 1 and 2, Table I). For the low-dosage treatments, the average fiber length and fines content did not change with cellulase treatment, whereas significant changes were observed for the high-dosage treatment. After 240 min of the high-dosage treatment, the average fiber length decreased from 2.56 to 0.32 mm and the fines content increased from 3.02 to 33.4%. The average fiber width was not reduced after the 240-min high-dosage treatment, but actually increased as shown in Table I.

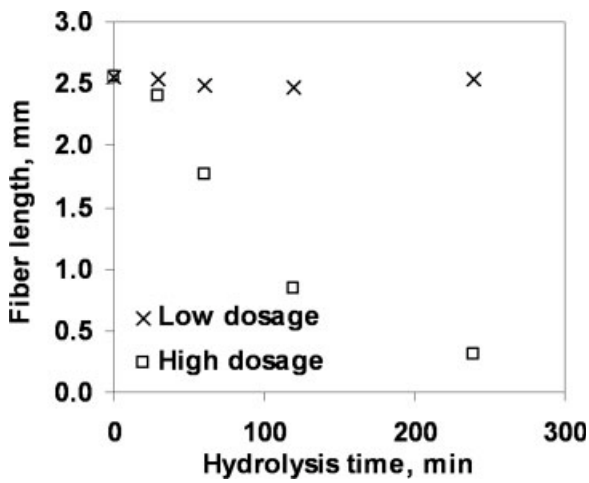

Figure 1 Average fiber length of fibers versus cellulase treatment time.

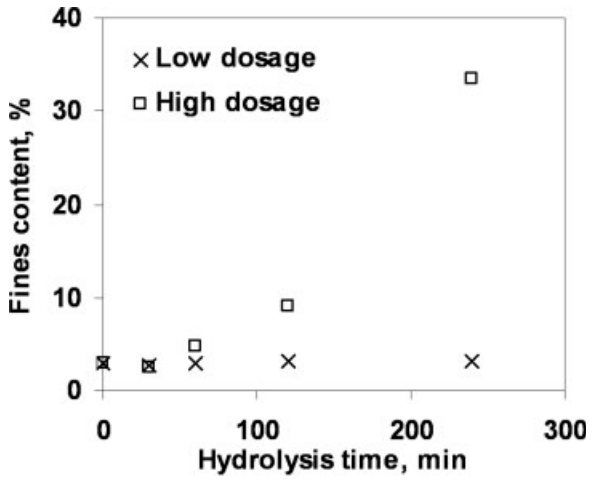

Figure 2 Fines content of fibers versus cellulase treatment time.

The significant decrease in fiber length, but not decrease in fiber width, indicates that enzymatic degradation does not cause cleavage in the fiber axial direction. This is demonstrated by the microscopic images in Figure 3. The fiber length significantly decreased [Fig. 3(c)] after the 240-min high-dosage treatment relative to the untreated fibers [Fig. 3(a)] due to the ''cuts'' in the cross direction of the fibers, not the axial direction. The slight increase of fiber width may be due to the enzymatic degradation that solubilized fine materials, making the average diameter larger.

When the cellulose fibers were hydrolyzed, the soluble fraction was produced by cleaving the linkages of the molecular chains. Degradation increased with hydrolysis time as summarized in Table I. For the high-dosage treatment, 46.3% of the original mass was solubilized for the 240-min treatment. It was observed that the degradation was not proportional to the cellulase concentration based on the results obtained in this study. The high-dosage treatment utilized 10 times greater dosage of cellulase, and the degradation was much greater than 10 times (1.78 vs. 46.3%).

Swelling capacity

The WRV was measured to evaluate the effect of cellulase treatment on the swelling capacity (Fig. 4). For the low-dosage treatment, the WRV remained constant with hydrolysis time, but the WRV increased with treatment time for the high-dosage treatment. This may be caused by the increase in the fines content as shown in Figure 2. It has been reported that the swelling of fines is approximately double that of the fiber fraction.[30] Increase in the WRV after the hydrolysis were reported for bead cellulose,[17] but Eremeeva et al.[31] reported no change in the WRV for bleached hardwood pulp. However, the fines content and fiber length were not reported in these studies. An alternative explanation is that the fiber has been modified by the cellulase such that the fiber swells more and this contributes to the increased WRV for the high-dosage treatment. However, the WRV of the long fiber fraction for the 240-min high-dosage treatment (2.77 g/g) was slightly lower than the whole pulp (2.86 g/g).

The HR water content displayed similar trends as the WRV with cellulase treatments (Fig. 5). The HR water content was previously shown to have a one-to-one relationship with the WRV for a given fiber type.[22] In addition, the HR water content of the fines for 240-min high-dosage treatment (3.36 g/g) was greater than both the long fraction (2.41 g/g) and whole fibers (2.67 g/g).

On the basis of these results, it is considered that the changes in swelling capacity as measured by the WRV and HR water content are insignificant when the effect of the increased fines content is excluded.

Bound water content

Both freezing and nonfreezing bound water contents were examined using DSC. The DSC experiment requires a pure water system to estimate pore sizes. If there are solutes present, such as salts, glucose, or dextran, the freezing bound water may be overestimated.[32] The freezing point depression due to small

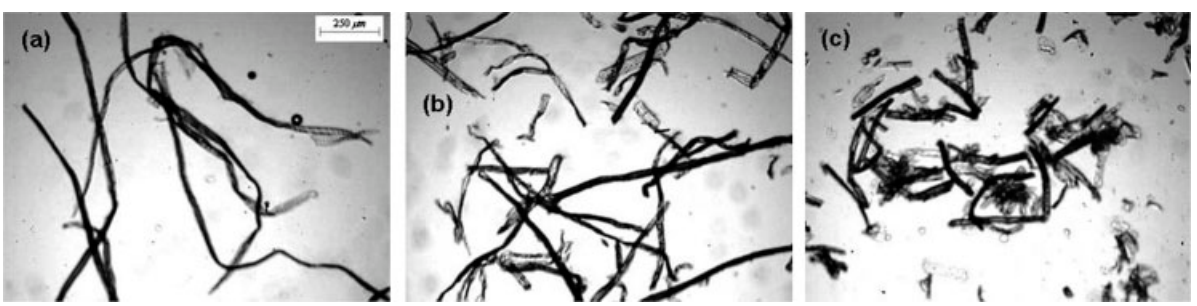

Figure 3 Microscopic images for (a) no treatment, (b) 60 min, and (c) 240-min high-dosage cellulase treatments. Scale bar shows 250 μm in (a).

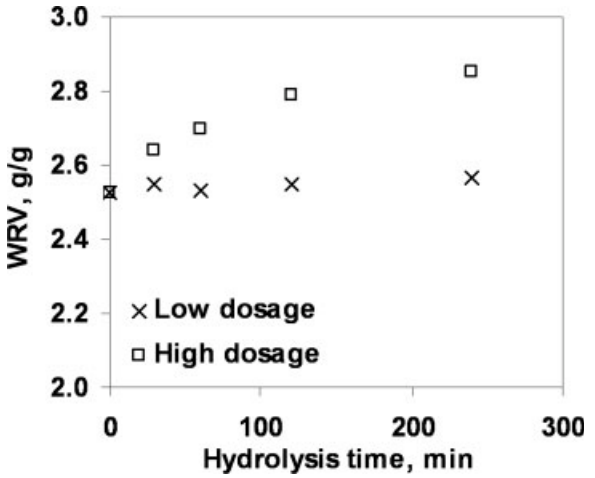

Figure 4 Water retention value of fibers versus cellulase treatment time.

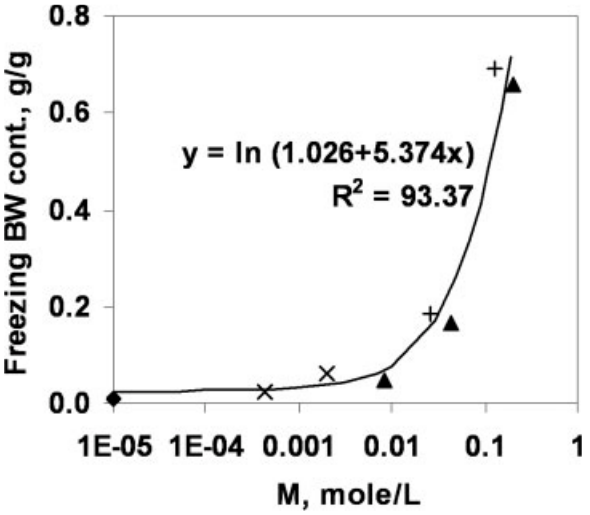

Figure 6 Freezing bound water content versus molar concentration for solutes: NaCl (+), glucose (▲), and dextran (×). Pure water (◆) was plotted at $10^{-5}M$ instead of 0.0*M*. Unit (g/g) represents the ratio of freezing bound water and total water.

molecular weight solutes is demonstrated in Figure 6. No fibers were present in these experiments. The water content having a depressed freezing point increased with the molar concentration regardless of the solute type. To eliminate this phenomenon, fiber samples were washed extensively on filter paper after the cellulase treatment.

The cumulative bound water content versus pore diameter for low- and high-dosage treatment is shown in Figures 7 and 8. The amount of water plotted at 2 nm indicates the amount of nonfreezing bound water. It was observed that the concentration of large pores decreased more than that of small pores. This becomes clear when freezing bound water is plotted with hydrolysis time (Fig. 9). Freezing bound water content decreased with hydrolysis time for both low and high dosages. Freezing bound water can be interpreted as the amount of pore water in cellulose fibers based on the Gibbs-Thomson equation.[25] However, it should be noted that the DSC equipment used in this experiment could measure only up to 400 nm in a diameter, which corresponds to a depression temperature of −0.1°C. Based on the finding that cellulase could attack the cellulose surface to enlarge the pore size and roughen the surface,[13] it is speculated that the pore size becomes larger than 400 nm practically, which is out of the detection range of the DSC experiment. Other methods such as mercury porosimetry and nitrogen adsorption were not tested since these measurements require dried samples and pore structure is expected to be altered during even for a freeze drying.

Nonfreezing bound water also decreased somewhat with hydrolysis time, Figure 10. Larger decrease in nonfreezing bound water was observed for the high-

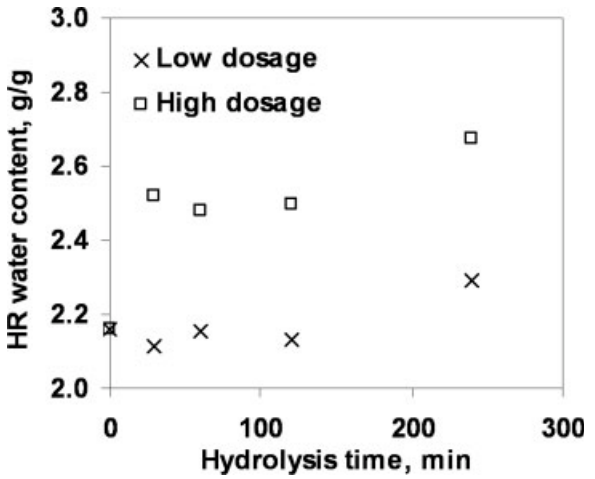

Figure 5 HR water content of fibers versus cellulase treatment time.

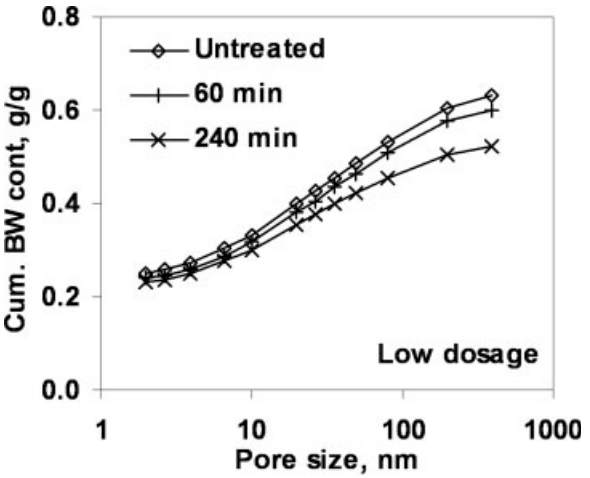

Figure 7 Cumulative bound water content versus pore size for the low-dosage treatment.

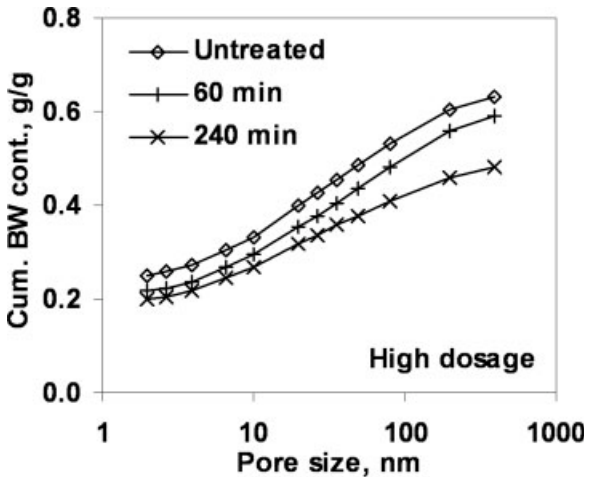

Figure 8 Cumulative bound water content versus pore size for the high-dosage treatment.

dosage treatment. Nonfreezing bound water is considered to be proportional to the accessible surface area,[23] and thus the amorphous fraction of cellulose fibers.[33] For the fines generated from the 240-min high-dosage treated sample, it was found that the nonfreezing water content of fines (0.160 g/g) was lower than the whole fraction of the 240-min high-dosage treated sample (0.199 g/g), plotted as a filled square (■) in Figure 10. This indicates that the fines have a higher crystalline fraction than unfractionated fibers.

Polymer adsorption and crystallinity index

The results of polymer adsorption and crystallinity index for the low- and high-dosage treatments on cellulose fibers are shown in Table I. The decrease in polymer adsorption and the increase in the crystallinity index are strong evidences to support that the amorphous portion of the cellulose is more readily

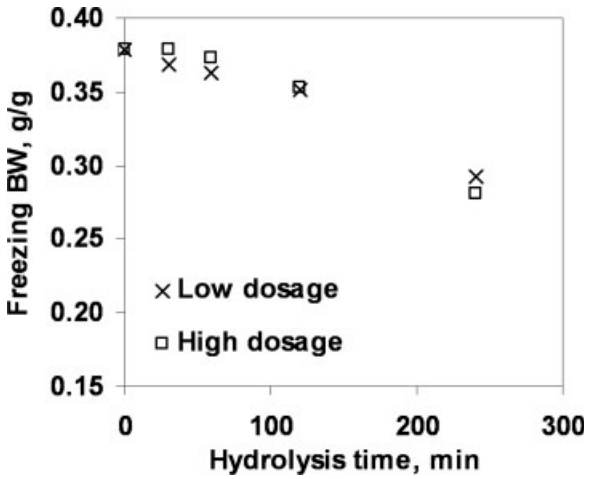

Figure 9 Freezing bound water of fibers versus cellulase treatment time.

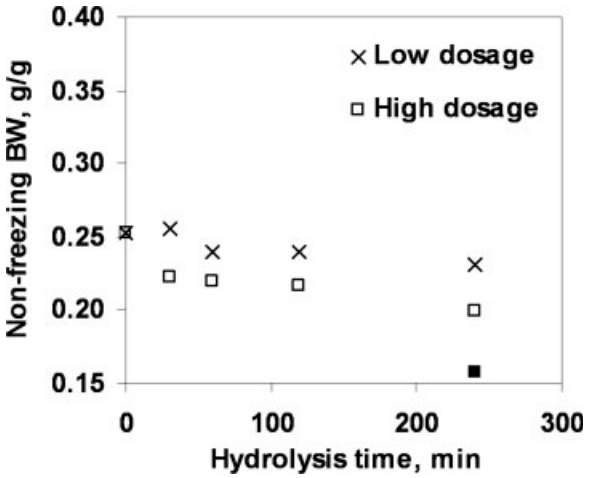

Figure 10 Nonfreezing bound water of fibers versus cellulase treatment time. The filled square (■) represents fines from the 240-min high-dosage treatment.

hydrolyzed than the crystalline region, which has been presented in several other studies.[12,34] Also, the increase in crystallinity index relative to untreated fiber is in agreement with other studies using softwood kraft pulp,[35] cotton fiber,[13] and dissolving pulp and cotton linters.[14] With these results in mind, decrease in nonfreezing bound water content with cellulase treatment could be understood, confirming that the amorphous regions of cellulose fibers are the preferable substrate relative to crystalline regions.

CONCLUSIONS

Effects of cellulase treatment on cellulose fibers were shown in this study for the low- (60 U/g) and high-dosage (600 U/g) treatments. The average fiber length and fines content did not change for the low-dosage treated fibers, whereas significant changes were observed for the high-dosage treatment. Fiber length was significantly decreased from 2.56 to 0.32 mm. It was found that the fibers were ''cut'' in the cross direction, not the axial direction, based on microscopic images and fiber width data. Fines content also significantly increased for the high-dosage treated fibers and this might be the reason for the increased swelling capacity measured by the WRV and HR water content.

Pore size distribution was plotted for low- and high-dosage treatment. It was found that the concentration of large pores decreased more than that of small pores. However, it might be due to an instrument limitation that could measure up to 400 nm in a diameter based on Gibbs-Thomson equation. Thus, there is a possibility that pores larger than 400 nm are formed, which is out of the detection range of the DSC experiment.

Nonfreezing bound water was measured using DSC and showed a decrease with enzymatic treatment for

both treatment levels with hydrolysis time, indicating that the amorphous regions of the cellulose fibers are the preferable substrate relative to crystalline regions. A decrease in polymer adsorption and an increase in the crystallinity index were also observed. These three independent measurements all indicate that the amorphous portion of the cellulose is more readily hydrolyzed than the crystalline region. It was also found that the nonfreezing water content of fines generated from cellulase treatment followed by fractionation was lower than the whole fiber fraction.

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