The Effect of Cellulase on the Pore Structure of Cellulose Fibers

SUNKYU PARK*, RICHARD A. VENDITTI†, DAVID G. ABRECHT**,

HASAN JAMEEL‡, JOEL J. PAWLAK∫, and JUNG MYOUNG LEE*

*Graduate Student, **Undergraduate Student,
†Associate Professor, ‡Professor, ∫Assistant Professor
Department of Wood and Paper Science, North Carolina State University, Raleigh, NC 27695, USA
†Corresponding Author: richard_venditti@ncsu.edu

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, hard-to-remove water content, freezing and non-freezing bound water content, polymer adsorption, and crystallinity index. For a high-dosage enzyme treatment (0.10 g/g), 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 (non-freezing 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. Based on the experimental results obtained herein, a model was proposed to explain surface and pore structure modification of cellulose fibers via enzymatic treatment.

INTRODUCTION

The enzymatic modification of cellulose has been an important research topic over the last several decades. In the pulp and paper industry, the various applications that have been investigated include the deinking of recycled fibers [Heise et al. 1996], the pretreatment of wood to accelerate the pulping process [Jacobs et al. 1998], the drainage improvement of pulps [Pommier et al. 1989], the reduction of refining energy [Freiermuth et al. 1994], and the strength and smoothness improvement of handsheets [Mansfield and Saddler 1999]. Most of all, enzymes for cellulose hydrolysis as the first step in converting plant biomass (lignocellulosic fibers) to fuels and chemicals is also of prospective importance for the next generation of energy sources [Lynd et al. 2005].

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. Exoglucanase or cellobiohydrolase (CBH) hydrolyzes the linkages at either the reducing or non-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, cutting cellulose molecular chains into short 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 [Wang et al. 2006] and dissolving pulp and cotton linters [Cao and Tan 2005], indicating degradation of the amorphous regions. However, it was also reported that there was no discernible difference in the crystallinity index for hemp fibers [Buschle-Diller et al. 1999]. It was found that the specific surface area of bead celluloses increased with treatment time as measured by nitrogen adsorption [Buschle-Diller et al. 1995]. A gradual increase in surface roughness at the nano-scale was detected for cotton fibers based on SEM and AFM observations [Wang et al. 2006].

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 [Buschle-Diller et al. 1995] and the porosity of hemp fibers increased for the first four hours, but decreased after that [Buschle-Diller et al. 1999]. 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 [Li

et al. 2001].

In this study, the surface and pore structure of cellulose fibers were examined after treatment by a commercially available cellulase enzyme. Various test methods were performed to investigate the enzymatic effects on cellulose fibers such as fiber dimension, water retention value, hard-to-remove water content, freezing and non-freezing bound water content, polymer adsorption, and crystallinity index. Based on the observations herein, a model was proposed to describe the enzymatic modification of cellulose fibers.

MATERIALS AND METHODS

Sample Preparation

Fully bleached softwood kraft pulp was obtained directly after the bleaching stage at Weyerhaeuser (Plymouth, NC). The average dimensions of the untreated fibers were measured by FQA (Fiber Quality Analyzer, OpTest Equipment Inc.), Table 1.

Enzyme (cellulase from Trichoderma reesei) was purchased from Sigma-Aldrich (Fluka). Two enzyme charges, a high dosage (0.1 g/g dry solids) and a low dosage (0.01 g/g), were used to determine the effect of dosage. Enzyme hydrolysis was performed for 0 (untreated), 30, 60, 120, and 240 minutes in a gyratory water bath at a consistency of 3.0% and temperature of 50±2°C. Deionized water was used and the pH was around 7. At the selected hydrolysis time, the treated pulps were washed extensively with deionized water to remove the enzymes and soluble sugars by filtration (Whatman No. 4).

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

Table 1. Characteristics of enzyme treated fibers.

	Untreated	Low dosage (0.01 g/g)		High dosage (0.10 g/g)	
Treat 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[1], %	0.00	0.92	1.78	25.7	46.3
Adsorp.[1], μeq/g	40.6	36.2	40.1	35.8	23.8
Crystallinity[2], %	52.8	·	·	54.8	54.3

1) Samples for 30 min and 120 min treatment were not measured.

2) Samples for low dosage, 30 and 120 min were not measured.

Swelling Capacity Measurements

The water retention value was measured following SCAN test method (SCAN C102XE: 3000 g, 15 min, and 1700 g/m2). The HR water content [Park et al. 2006a] 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 is determined by measuring the moisture ratio of the fibers at the transition between the constant rate zone and the falling rate zone. Detailed experimental procedures can be found in previous experiments by Park et al. [2006a].

Bound Water Measurements: Freezing and Non-freezing Bound Water

Freezing bound water (water that has its freezing/ melting temperature depressed due to the presence of a substrate), was measured to calculate pore size distribution using differential scanning calorimetry (DSC Q100, TA Instruments). Samples of approximately 5.0 mg were sealed in a DSC aluminum hermetic pan. 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, -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 [Maloney et al. 1998]. Detailed experimental procedures for the DSC operation can be found in previous work by Park et al. [2006b].

To measure the non-freezing 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°C/min to 15°C. The amount of non-freezing 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 [Park et al. 2006b].

Polymer Adsorption and Crystallinity Index

In order to estimate the accessible surface area of hydrolyzed fibers, polymer adsorption tests were performed using poly-DADMAC (Sigma-Aldrich) having a molecular weight of 5,000 ~ 20,000 [Gruber et al. 1996]. About 0.5 g (dry solids) of sample was slurried in 100 ml of 0.0010N poly-DADMAC solutions and stirred for 10 minutes 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 0.0030N PVSK (Sigma-Aldrich) 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 was assumed to be proportional to the accessible surface.

The crystallinity index was determined using x-ray diffraction (XRD, Philips XLF, Omni Instruments Inc.) 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. Indexes were calculated by comparison of the height of the 002 peak to the sum of the heights of the amorphous peak at 2θ of 19° and the 002 peak [Clark, Terford 1955]. Background was removed digitally prior to calculation. These values were calibrated using the peak ratio of Avicel PH-101 (Sigma Aldrich) as an external standard [Hermans, Weidinger 1949] with a reported crystallinity index of 58.8% [Pekarovicova *et al.* 1997].

RESULTS AND DISCUSSION

Changes in Fiber Length and Enzyme Degradation

After the enzymatic treatment of cellulose fibers, the fiber length and fines content were measured, Figures 1 and 2 and Table 1. For the low-dosage (0.01 g/g) treatments, the average fiber length and fines content did not change with enzyme treatment, whereas significant changes were observed for the high-dosage (0.10 g/g) 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 1.

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 (Figure 3 (c)) after the 240 min high-dosage treatment relative to the untreated fibers (Figure 3 (a)) due to '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.

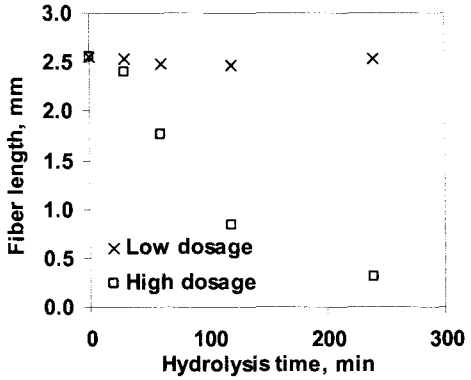

Figure 1. Average fiber length (length weighted) of fibers versus enzyme treatment time.

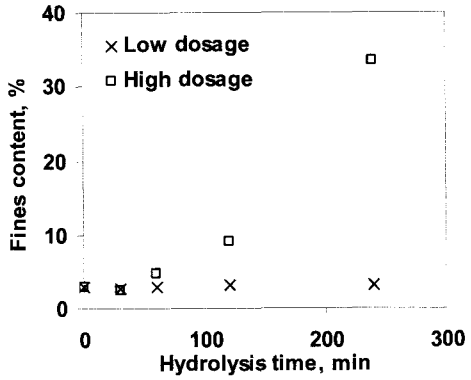

Figure 2. Fines content (length weighted) of fibers versus enzyme treatment time.

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 1. 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 enzyme concentration based on the results obtained in this study. The high-dosage treatment utilized ten times greater dosage of enzyme and the degradation was much greater than ten times (1.78% versus 46.3%).

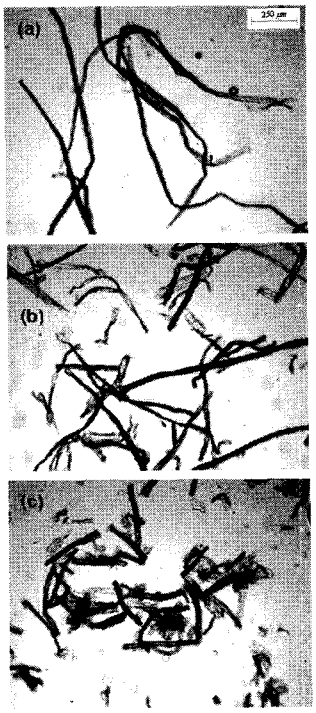

Figure 3. Microscopic images for (a) no treatment, (b) 60 min, and (c) 240 min high-dosage (0.1 g/g) enzyme treatments. Scale bar shows 250 μm in (a).

Swelling Capacity

The water retention value (WRV) was measured to evaluate the effect of enzyme treatment on the swelling capacity, Figure 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 increases fines content as shown in Figure 2. It has been reported that the swelling of fines is approximately double that of the fiber fraction [Laivins and Scallan 1996]. Increases in the WRV after enzyme hydrolysis were reported for bead cellulose [Buschle-Diller et al. 1995], but Eremeeva et al. [2001] reported no changes in the WRV for bleached hardwood pulp. However, the fines content and fiber length were not measured in these studies. An alternative explanation is that the fiber has been modified by the enzyme 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).

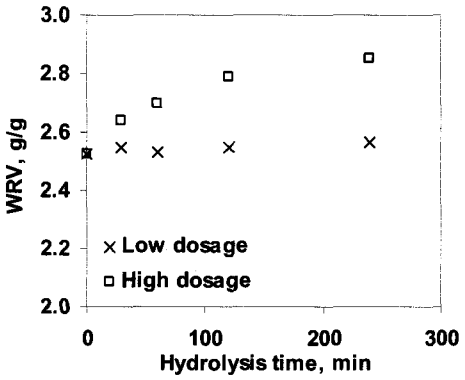

Figure 4. Water retention value of fibers versus enzyme treatment time.

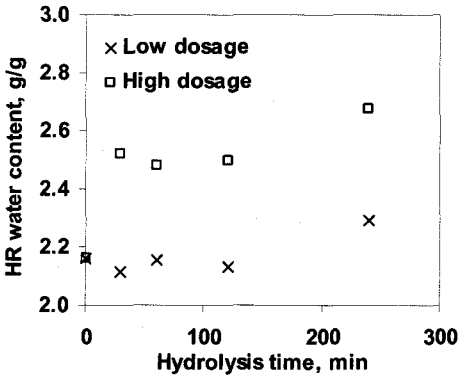

Figure 5. HR water content of fibers versus enzyme treatment time.

The HR water content displayed similar trends as the WRV with enzyme treatments, Figure 5. The HR water content was previously shown to have a one-to-one relationship with the WRV for a given fiber type [Park et al. 2006a]. 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).

Based on these results, it was considered that the changes in swelling capacity as measured by the WRV and HR water content were insignificant when the effect of the increased fines content was excluded.

Bound Water Content

Both freezing and non-freezing bound water contents were examined using DSC. The cumulative bound water content versus pore diameter for low- and high-dosage treatment is shown in Figures 6 and 7. The amount of water plotted at 2 nm indicates the amount of non-freezing 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, Figure 8. 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 [Park et al. 2006b]. 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 enzyme could attack the cellulose surface to enlarge the pore size and roughen the surface [Wang et al. 2005], it is speculated that the pore size becomes larger than 400 nm, which is out of the detection range of the DSC experiment.

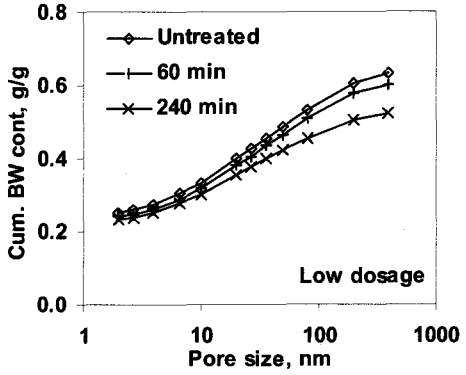

Figure 6. Cumulative bound water content versus pore size for the low-dosage treatment.

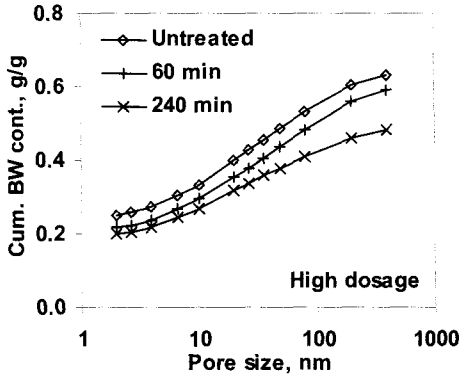

Figure 7. Cumulative bound water content versus pore size for the high-dosage treatment.

Non-freezing bound water also decreased somewhat with hydrolysis time, Figure 9. Larger decreases in non-freezing bound water were observed for the high-dosage treatment. Non-freezing bound water is considered to be proportional to the accessible surface area [Maloney et al. 1998] and thus the amorphous fraction of cellulose fibers [Jeffries 1964]. For the fines generated from the 240 min high-dosage treated sample, it was found that the non-freezing 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 7. This indicates that the fines have a higher crystalline fraction than unfractionated fibers.

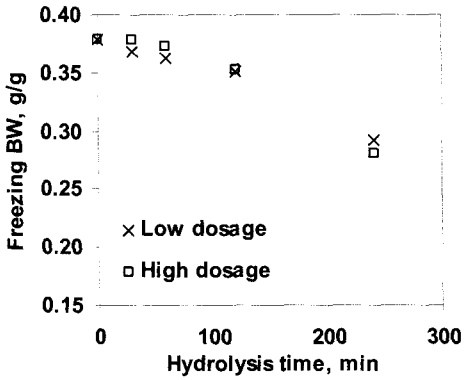

Figure 8. Freezing bound water of fibers versus enzyme treatment time.

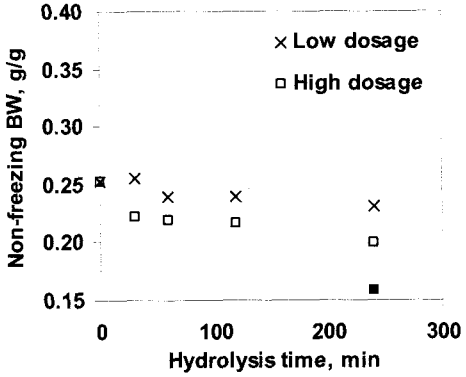

Figure 9. Non-freezing bound water of fibers versus enzyme treatment time. The filled square (■) represents fines from the 240 min high-dosage treatment.

Polymer Adsorption and Crystallinity Index

The results of polymer adsorption and crystallinity index for the low- and high-dosages treatments on cellulose fibers are shown in Table 1. The decrease in polymer adsorption and the increase in the crystallinity index are strong evidence to support that the amorphous portion of the cellulose is more readily hydrolyzed than the crystalline region, which has been presented [Henriksson et al. 2005]. Also the increase in crystallinity index relative to untreated fiber is in agreement with other studies using cotton fiber [Wang et al. 2006] and dissolving pulp and cotton linters [Cao and Tan 2005]. With these results in mind, decreases in non-freezing bound water content with enzyme treatment could be understood, confirming that the amorphous regions of cellulose fibers are the preferable substrate relative to crystalline regions.

Enzymatic Hydrolysis Model

A model is proposed based on the experimental results obtained from this study. An untreated fiber consists of crystalline and amorphous regions with surface fibrils, Figure 10 (a). In nature cellulose fibers are highly crystalline with a crystallinity index of 60-70% [Bertran and Dale 1986] and this structure may be destroyed by dissolving and swelling treatments such as pulping and bleaching. When enzyme is introduced to wet fibers, the enzyme could preferably attack and hydrolyze amorphous regions. By enzyme activity, new pores are generated in the mostly amorphous rich regions as shown in Figure 10 (b). Enzymes further attack these regions and a surface fibril is eventually cleaved from a main chain, creating a fine in the system as shown in Figure 10 (c). Enzymes can penetrate into the newly created pore and pores could be enlarged. A fiber cutting in the cross direction is also demonstrated in Figure 10 (c).

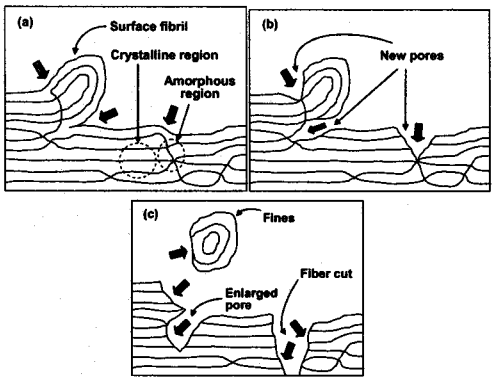

Figure 10. A model of enzymatic hydrolysis on a cellulose fiber.

Parallel lines indicate crystalline regions. (a) An untreated fiber, which consists of crystalline and amorphous regions. Arrows indicate possible attack sites on cellulose surface by enzyme. (b) Amorphous regions are hydrolyzed and new pores are generated. (c) A surface fibril is cleaved as a fine and pores are enlarged. A fiber is cut in the cross direction.

CONCLUSIONS

Effects of enzyme treatment on cellulose fibers were shown in this study for the low- (0.01 g/g) and high-dosage (0.10 g/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 (0.10 g/g) 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 an instrument limitation, which could measure only up to 400 nm in a diameter based on Gibbs-Thomson equation. Thus, there is a possibility that the pore size becomes larger than 400 nm, which is out of the detection range of the DSC experiment.

Non-freezing bound water was measured using DSC and decreases with 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. The decrease in polymer adsorption and the increase in the crystallinity index were also observed. These three different measurements all indicate that the amorphous portion of the cellulose is more readily hydrolyzed than the crystalline region. It was also found that the non-freezing water content of fines generated from enzyme treatment followed by fractionation was lower than the whole fiber fraction.

A model was proposed to explain surface and pore structure modification of cellulose fibers.

REFERENCES

Bertran MS and Dale BE, Determination of cellulose accessibility by differential scanning calorimetry, J. Appl. Polymer Sci., 32 (3): 4241-4253 (1986).

Buschle-Diller G, Fanter C, and Loth F, Effect of cellulase on the pore structure of bead cellulose, Cellulose, 2 (3): 179-203 (1995).

Buschle-Diller G, Fanter C, and Loth F, Structural changes in hemp fibers as a result of enzymatic hydrolysis with mixed enzyme systems, Textile Res. J., 69 (4): 244-251 (1999).

Cao Y and Tan H, Study on crystal structures of enzyme-hydrolyzed cellulosic materials by X-ray diffraction, Enzyme and Mocrobial Technol., 36 (2-3): 314-317 (2005).

Clark GC, Terford HC, Quantitative X-ray determination of amorphous phase in wood pulps as related to physical and chemical properties, Anal. Chemistry, 27 (4): 888 (1955).

Eremeeva T, Bikova T, Eisimonte M, Viesturs U, and Trimanis A, Fractionation and molecular characteristics of cellulose during enzymatic hydrolysis, Cellulose, 8 (1): 69-79 (2001).

Freiermuth B, Garrett M, and Jokinen O, The use of enzymes in the production of release paper, Paper Technol., 35 (3): 21-23 (1994).

Gruber E, Grossmann K, and Schempp W, Interactions of synthetic cationic polymers with fibres and fillers. Part 1., Wochenblatt Für Papierfabrikation, 124 (1): 4-11 (1996).

Heise OU, Unwin JP, Klungness JH, Fineran WG Jr, Industrial scale-up of enzyme enhanced deinking of non-impact printed toners, Tappi J., 79 (3): 207-212 (1996).

Henriksson G, Christiernin M, and Agnemo R, Monocomponent endoglucanase treatment increases the reactivity of softwood sulphite dissolving pulp, J. Ind. Microbiol. Biotechnol., 32 (5): 211-214 (2005).

Hermans PH, and Weidinger A, X-ray studies on the crystallinity of cellulose, J. Polymer Science, 4 (1): 135 (1949).

Jacobs CJ, Venditti RA and Joyce TW, Effects of enzyme pre-treatments on conventional kraft pulping, Tappi J., 81 (2): 143-147 (1998).

Jeffries R, The amorphous fraction of cellulose and its relation to moisture sorption, J. Appl. Polymer Sci., 8 (3): 1213-1220 (1964).

Laivins GV and Scallan AM, The influence of drying and beating on the swelling of fines, J Pulp Paper Sci., 22 (5): 178-184 (1996).

Li C, Ladisch CM, and Ladisch MR, Pore characterization of cellulase enzyme treated cotton fabric, Textile Res. J., 71 (5): 407-414 (2001).

Lynd LR, van Zyl WH, McBride JE, and Laser M,

Consolidated bioprocessing of cellulosic biomass: An update, Current Opinion in Biotechnol., 16 (5): 577-583 (2005).

Maloney TC, Paulapuro H, and Stenius P, Hydration and swelling of pulp fibers measured with differential scanning calorimetry, Nordic Pulp and Paper Res. J., 13 (1): 31-36 (1998).

Mansfield SD and Saddler JN, Sheet properties of Douglas fir kraft pulp after selective treatments of different fiber length fractions with cellulases, J. Pulp Paper Sci., 25 (3): 84-89 (1999).

Park S, Venditti RA, Jameel H, and Pawlak JJ, Hard to remove water in cellulose fibers characterized by high resolution thermogravimetric analysis – Methods development, Cellulose, Accepted and online available (2006a).

Park S, Venditti RA, Jameel H, and Pawlak JJ, Changes in pore size distribution during the drying of cellulose fibers as measured by differential scanning calorimetry, Carbohydrate Polymers, Accepted on February 2006b.

Pekarovicova A, Venditti RA, Cao H, Lou HM and Jean YC, Cellulose Free Volume Characterization Using Positron Annihilation Lifetime Spectroscopy, J. Pulp Paper Sci., 23 (3): 101-107 (1997).

Pommier J-C, Fuentes J-L, and Goma G, Using enzymes to improve the process and the product quality in the recycled paper industry. Part 1: the basis laboratory work, Tappi J., 72 (6): 187-191 (1989).

Wang L, Zhang Y, Gao P, Shi D, Liu H, and Gao H, Changes in the structural properties and rate of hydrolysis of cotton fibers during extended enzymatic hydrolysis, Biotechnol. Bioeng., 93 (3): 443-456 (2006).