TARGETED DISRUPTION OF HYDROXYL CHEMISTRY AND CRYSTALLINITY IN NATURAL FIBERS FOR THE ISOLATION OF CELLULOSE NANO-FIBERS VIA ENZYMATIC TREATMENT

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Cellulose is the Earth's most abundant biopolymer. Exploiting its environmentally friendly attributes such as biodegradability, renewability, and high specific strength properties are limited by our inability to isolate them from the secondary cell wall in an economical manner. Intermolecular and intramolecular hydrogen bonding between the cellulose chains is the major force one needs to overcome in order to isolate the cellulose chain in its microfibrillar form. This paper describes how a hydrogen bond-specific enzyme disrupts the crystallinity of the cellulose, bringing about internal defibrillation within the cell wall. Bleached kraft softwood pulp was treated with a fungus (OS1) isolated from elm tree infected with Dutch elm disease. FT-IR spectral analysis indicated a significant reduction in the density of intermolecular and intramolecular hydrogen bonding within the fiber. X-ray spectrometry indicated a reduction in the crystallinity. The isolated nano-cellulose fibers also exhibited better mechanical strength compared to those isolated through conventional methods. The structural disorder created in the crystalline region in the plant cell wall by hydrogen bond-specific enzymes is a key step forward in the isolation of cellulose at its microfibrillar level.

Keywords: Cellulose nano-fibers / microfibrils; Enzyme pre-treatment; Hydrogen bonds; Internal defibrillation; FTIR; X-Ray crystallography

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INTRODUCTION

Isolation of cellulose microfibrils (nano-cellulose) from plant cell walls has gained considerable research attention, especially in the nano-bio-composite field (Tashiro 1991; Berglund, 2004; Nakagaito 2004; Saito 2006). There are numerous other potential applications for nano-cellulose, e.g. (1) paper and paperboard applications (as a dry strength agent or surface strength agent), (2) food applications, (3) cosmetics, (4) pharmaceutical and hygiene, and (5) emulsion and dispersion applications.

Isolation of nano-cellulose is currently achieved through either (1) a purely mechanical process that involves high shearing followed by either micro-fluidizing or cryo-crushing (Chakraborty and Sain 2005) or (2) a bio-mechanical method that involves a combination of enzymatic treatment followed by high shearing (Pääkkö et. al. 2007). The main constraint with the mechanical approach is the high energy consumption associated with high shear refining and microfluidization. The bio-mechanical approach

overcomes the high energy requirement to a certain extent; however, the use of cellulose-hydrolytic enzymes also has a negative impact on the molecular weight and the chain length of the isolated nano-cellulose fibers. Also, the degree of fiber separation achieved in the isolated nano-cellulose is also limited. Although the nano-cellulose isolated through a bio-mechanical process could be used for a variety of applications, its reinforcing potential in nanocomposites will be limited due to extreme entanglement, as the nanofibres generated are not fully individualized and also these isolated nano-fibres normally tend to have lower aspect ratio. The nano-fibres isolated through this technique The treatment of wood fibers with fungus capable of secreting hydrogen bond-specific enzymes is the focus of this research, and some earlier results have shown the nano-cellulose isolated in that work to be distinctive, having lower diameter distribution and high aspect ratio (Sreekumar and Sain 2007).

EXPERIMENTAL

Materials

Wood fibre

Bleached kraft softwood pulp from northern black spruce was used as the starting material for the isolation of microfibrils. The fiber has a cellulose content of 86% and hemicellulose content of 14%.

Fungus

OS1, a fungus isolated in our laboratory from elm tree infected with Dutch elm disease was used as the source of enzyme for the fibre treatment.

Methods

Bio-treatment

Bleached kraft fibre was soaked overnight, thoroughly disintegrated in 2 liters of water, and autoclaved for 20 minutes. OS1 fungal culture was added to this fibre suspension in a sterile flask with an appropriate amount of sucrose and yeast extract to support the fungal growth. The fungus was left to grow on the fibres at room temperature for a period of two to four days with slow agitation at room temperature. The fibres were autoclaved after their respective treatment time, and then washed for further processing to cellulose nano-fibers.

High shear refining

The bio-treated fibres were processed through a PFI high shear refiner (Paperindustriens Forkninginstitutt, Oslo, Norway) to further bring about internal defibrillation. 24 grams of fiber at 10% consistency was charged in to the PFI mill and sheared for 100,000 revolutions to affect internal defibrillation in the fibre.

Crystallinity Determination by FT-IR

The degree of crystallinity of the cellulose samples was determined using FT-IR. Dried cellulose samples over phosphorus pentoxide in a desiccator were made into a

pellet with KBr powder (1:5, cellulose: KBr) and analyzed by FT-IR spectroscopy. The fiber samples were weighed out within ± 0.005 g range to preserve the quantitative aspect of the spectra. The FT-IR spectra (256 scans, 4 cm^{-1}) were determined by the diffuse reflectance method (DRIFT) using a Bruker Tensor 27 spectrometer. The ratio of the peak intensities at 1282 to 1202 cm^{-1} of the deconvoluted spectrum was used to determine the relative crystallinity of the cellulose samples, as described previously (Michell 1990).

Crystallinity Determination by X-Ray Diffraction

The bio-treated and untreated fibres were used as samples for X-Ray diffraction tests. X-ray diffractometry curves were recorded in reflection mode by a Rigaku RU-200 BH with Ni-filtered Cu-K_α radiation (λ = 0.1542 nm) generated at 40 kV and 40 mA. Copper K_β was eliminated by the use of nickel foil filters in the incident beam. The beam and detector slit were set at 1° and 0.25°, respectively. The scanning was made through 2θ = 10° to 60°, and intensity data were recorded with a digital recorder. Separation of peaks was done using a least-squares profile fitting program, Peak Fit, assuming a Gaussian function for each peak. The diffraction angle was calibrated every time with the sodium fluoride diffraction line (d = 0.2319 nm).

Mechanical Strength Determination

The mechanical strength properties of the cellulose were determined using a Micro Tensile Testing Machine.

The cellulose nanofibre sheets for the mechanical test are prepared by vacuum filtration. 0.5% nanofibre suspension was stirred for 45 minutes and then pre-filtered through an 80 mesh filter to separate out any larger fibre tangles that may potentially act as weak spots in the nanofibre sheets. The pre-filtered nanofibre suspension was then filtered on a Buchner funnel using a 200 mesh filter screen to minimize basis weight variation across the surface. After filtration, the wet nanofibre sheets were placed between filter papers and then between two metal plates and dried at 40 ^oC for about 24 hrs. The nanofibre sheets had a targeted basis weight of 60 ± 1 g/m^2. The sheets were then cut to micro tensile test specimens and tested on a Micro Tensile Testing Machine.

RESULTS AND DISCUSSIONS

FT- IR Bands in 1400 – 800 / cm^{-1} Region

Enzymatic fiber treatment can produce some marked differences in the IR absorption characteristics in the 900 to 1100 cm^{-1} region. The IR absorption spectrum of each sample shown here is an average of at least five spectra. The IR absorption near the 900 to 1000 cm^{-1} region is found to be very sensitive to the amount of crystalline versus amorphous structure of cellulose; i.e. broadening of the band reflects a higher degree of disordered structure. Since the disorder of the cellulose structure is caused by the angle changes around β-glycosidic linkage and rearrangement of hydrogen bonds (Blackwell 1971, 1977), examination of the IR absorption charac-teristics of the enzyme treated fibres in this region showed a slight broadening and a marked increase in intensity.

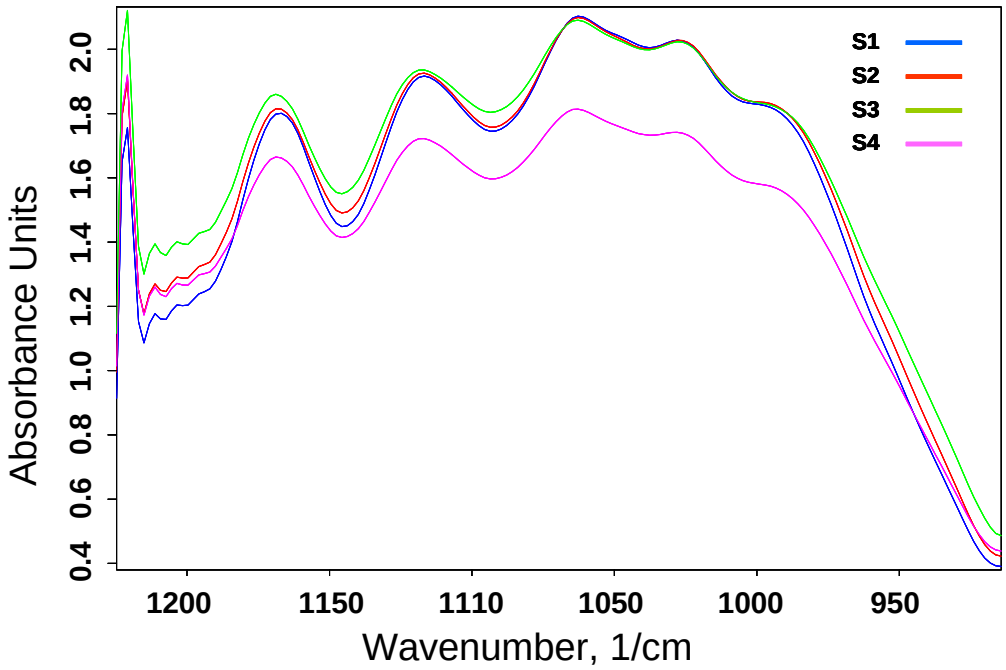

Figure 1. IR absorption characteristics of cellulose fibres in the 800 to 1400 /cm region: S1 = 4-day OS1 treatment, S2 = 4-day treatment with OS1 extract, S3 = 2-day OS1 treatment, S4 = untreated

In the context of the present research, this observation is quite interesting due to the fact that an increase in disorderness in the cellulose structure can be viewed as an increase in the internal defibrillation of the cellulose, and also rearrangement of hydrogen bonds in the treated cellulose structure helps to explain the effects detailed in Fig. 1. Although not conclusive, the evidence presented so far supports the fact that enzyme treatment of cellulose fibres brings about structural disorder in the cellulose and also rearrangement of hydrogen bonds in the structure.

X-Ray Diffraction Crystallography

Cellulose is found in a number of structural variations – cellulose I, II, III, and IV. Cellulose I, which also is called native cellulose, is the most abundant in nature and has two polymorphs - I_α and I_β. Cellulose II is the variety found in regenerated cellulose. The various celluloses have distinct and well-defined X-Ray diffraction patterns as a result of the crystalline nature of the cellulose structure. The crystallinity, therefore, can provide insight into the nano-structure of the cellulose. For a given cellulose source, the crystallinity of the cellulose fibres has been found to have a significant dependence on the treatment history. Physical treatments such as grinding, beating, or bio-treatments that can interfere with the cellulose structure have been found to have a distinct effect on the X-Ray diffraction pattern (Ellefsen et al. 1971; Blackwell 1971; Segal and Conrad 1957).

Cellulose Crystallinity

There have been numerous attempts to examine and quantify the crystallinity of cellulose. Although an acid hydrolysis approach (Philips et al. 1947) and infrared

measurements (O'Connor 1958; and Basch 1958) were used for early qualitative studies of crystallinity, X-ray diffraction has been extensively used for both the qualitative and qualitative estimate of cellulose crystallinity.

In this study, a quantitative study of the cellulose crystallinity has been carried out to understand the effects of bio-treatment on the structure of the cellulose and also as tool to validate the FT-IR spectral study done to correlate angle changes around the β-glycosidic linkage and rearrangement of hydrogen bonds.

As can be seen from Fig. 2, the X-ray diffraction pattern of untreated cellulose, S4, showed a well-defined primary peak at the diffraction angle $2\theta = 22.5^{\circ}$ and two secondary peaks at $2\theta = 15^{\circ}$ and 16.8°. The X-ray diffraction pattern of bio-treated cellulose showed a marked decrease in the intensity of the primary peak and also the secondary peaks at $2\theta = 15^{\circ}$ and 16.8° tended to merge. This crystallographic pattern behavior in cellulose was only observed due to degradation in the crystallite structure and hence decreased crystallinity.

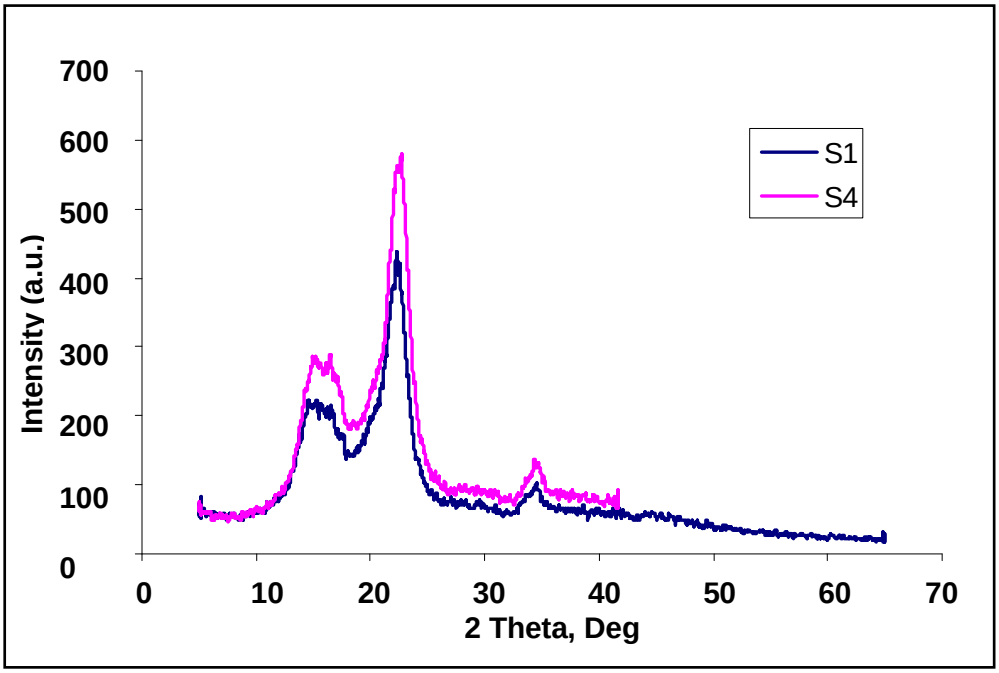

Figure 2. X-Ray diffraction patterns of cellulose. S1: bio-treated cellulose, S4: untreated cellulose

The crystallinity Index was used to quantify the crystallinity of the cellulose. The crystallinity Index (*CI*) is defined as,

$$CI = (I_{max} - I_{min}) / I_{max} \quad (1)$$

where, I_{max} is the height of the peak at $2\theta = 22.5^{\circ}$ and I_{min} is the height of the minimum at $2\theta = 19^{\circ}$.

Table 1. Crystallinity Index of Bio-treated and Untreated Cellulose

Cellulose	I_{max}	I_{min}	*CI*
Untreated(S4)	576	182	0.69
Bio-treated(S1)	420	161	0.61

Table 1 provides an estimate of the degree of reduction in the crystallinity index of the bio-treated and untreated cellulose. An 8 to 10% reduction in the crystallinity index, as calculated from the X-ray diffraction pattern is consistent with the FT-IR absorbance intensity (*A*), $(A_{untreated} - A_{treated})/A_{treated}$ value calculated from Fig. 1, at peak absorbance intensity in the 800 to 1400 cm^{-1} region.

Mechanical Strength Properties of Cellulose Nano-fibres

Cellulose nano-fibre films were prepared from their suspension via vacuum filtration. During filtration the nano-fibres were made to deposit on a 200 mesh filter. In suspension, the interaction between the cellulose nano-fibres is overcome by the fiber-water interaction, but when the fibers are deposited on the filter, the fiber-fiber interaction becomes more prominent. As the nano-fibre films are dried, a strong nanofibre network is formed with very high specific properties.

The stress-strain behavior of the of the cellulose nano-fibre films, made with cellulose nano-fibres isolated from original fibers and those isolated from bio-treated fibers are shown in Fig. 3. The curve exhibits two relatively linear regions, one up to a displacement of 0.5mm and the other after the yield stress of approximately 0.012 kN.

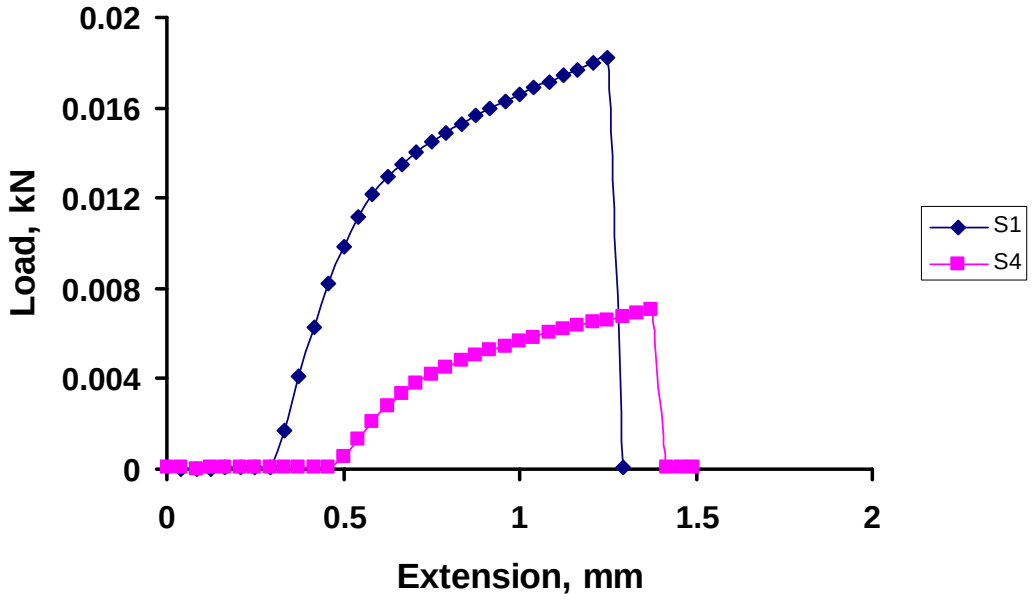

Figure 3. Stress-strain curves of cellulose nano-fibre films prepared with nano-fibres from S4 - untreated fibers and those isolated from S1 - bio-treated fibers

The films prepared nano-fibres from original fibers and those isolated from bio-treated fibers roughly followed a similar stress-strain curve. The strain to failure ratio and hence the tensile strength of the films from nano-fibres isolated from bio-treated fibers showed a higher valve compared to nano-fibres original fibers.

The strength properties of the cellulose nano-fibres depend on three key parameters, namely aspect ratio, degree of polymerization, and the degree of crystallinity. The bio-treatment of the fibers leads to a decrease in the cellulose content, probably a decrease in the degree of polymerization, and also a decrease in the degree of crystallinity of the fibers. However, the cellulose nano-fibres isolated through bio-treatment had two distinct characteristics compared to the cellulose nano-fibres isolated through the conventional technique, (a) a very narrow diameter distribution and hence high aspect ratio, and (b) fibers that were more distinct and non-segregated. TEM images of the cellulose nano-fibers isolated from bio-treated fibers and untreated fibers are shown in Fig. 4. Greater than 90% of the cellulose nano-fibres isolated from bio-treated fibres had diameter < 50 nm, while only 55% of the nano-fibers isolated without treatment were below the 50 nm range. A detailed comparison of diameter distribution is reported elsewhere (Sreekumar and Sain 2007).

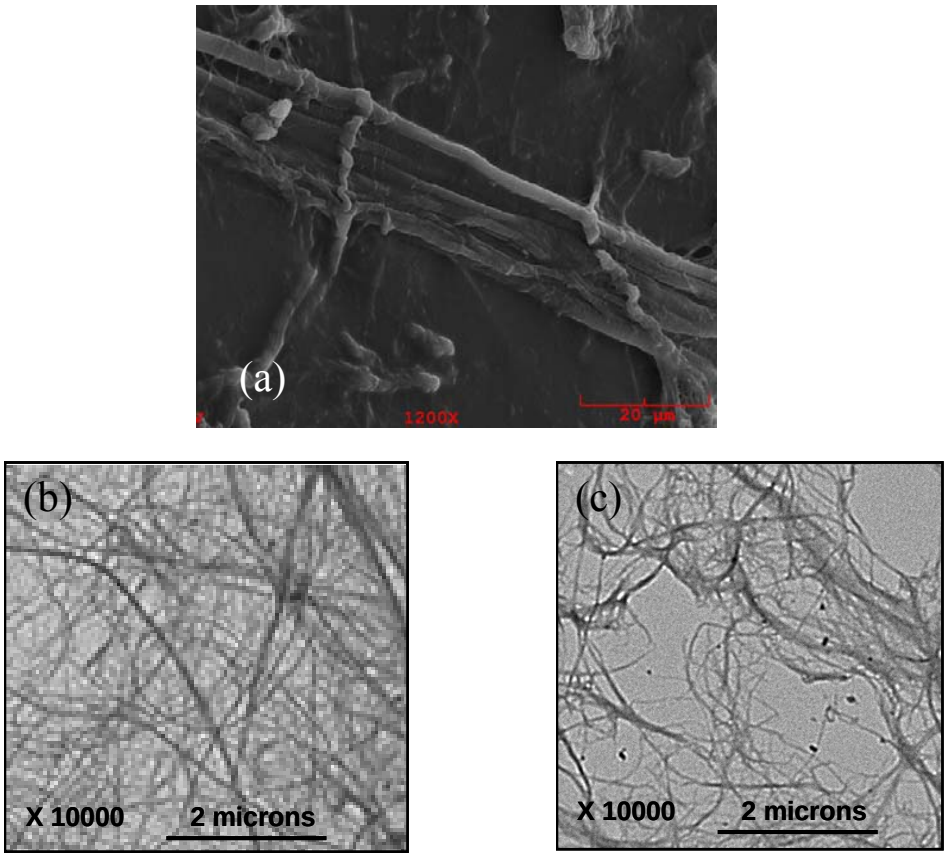

Figure. 4. (a). SEM Images fungal growth on fibers, (b) TEM of cellulose microfibrils isolated from Bio-treated and (c) TEM of cellulose microfibrils isolated from untreated wood fibres

The average tensile strength of the nanofibre film made from nanofibre isolated through bio-treatment showed a higher value compared to that isolated from the untreated fiber, 243 MPa compared to 222 MPa, respectively. This result is not surprising based on the above points (a) and (b).

Table 2. Average Mechanical Properties of Films Prepared from Cellulose Nanofibres Isolated from Bio-treated Fibers and those Isolated from Untreated Fibers

Nanofibre film from	Tensile Strength, MPa	Modulus, GPa
Bio-treated fibers (S1)	244	18
Untreated fibers (S4)	222	16

CONCLUSIONS

1. Analysis of the FT-IR absorption characteristic spectra of the enzyme-treated nano-cellulose fibres showed a marked decrease in the intermolecular hydrogen bonded OH functionality, while only a marginal change was observed in intramolecular hydrogen bonded OH groups and free OH groups.

2. FT-IR and X-ray crystallinity studies indicated a decrease in the crystallinity index in the bio-treated fibers and an increase in the degree of disorderness in the macro-molecular structure. This is an indication of the increased internal defibrillation caused by bio-treatment.

3. The mechanical strength of the nano-cellulose fibres isolated via a bio-mechanical process was marginally higher to that of those isolated via a conventional mechanical process. This is anticipated because the nano-cellulose fibres isolated through fiber bio-pretreatment have higher aspect ratio and are more distinct.

ACKNOWLEDGEMENT

The authors are grateful for the support of Natural Science and Engineering Research Council of Canada.

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Article submitted: November 23, 2010; Peer review completed: January 24, 2011; Revised version received: February 18, 2011; Second revision received and accepted: February 25, 2011; Article published: February 28, 2011.