Integrated Production of Cellulose Nanofibrils and Cellulosic Biofuel by Enzymatic Hydrolysis of Wood Fibers

Abstract. *One key barrier to converting woody biomass to biofuel through the sugar platform is the low efficiency of enzymatic cellulose saccharification due to the strong recalcitrance of the crystalline cellulose. Significant past research efforts in cellulosic biofuels have focused on overcoming the recalcitrance of lignocelluloses to enhance the saccharification of the recalcitrant cellulose. However, in this work [1,2], we demonstrate the conversion of recalcitrant cellulose into cellulosic nanomaterials, which have attracted a great deal of attention recently due to their unique properties and remarkable strength. Significant levels of sugars can be readily hydrolyzed from wood pulp, leaving yields of cellulosic solid substrate of 60%–90%. The crystallinity index of the recalcitrant cellulose was found to be higher than that of the starting pulp, verifying that enzymatic hydrolysis occurs preferentially on amorphous cellulose. Cellulose nanofibrils (CNFs) were produced from the recalcitrant cellulose using various mechanical refining approaches, and the morphology of the CNFs varied drastically depending on the level and method of refining. Extensive hydrolysis and mechanical disintegration resulted in CNFs with morphology similar to cellulose nanocrystals, which are discrete rod-like nanoparticles of highly crystalline cellulose. CNF films created in this research show good mechanical properties and transparency, and their use as composite reinforcements is being investigated.*

Keywords. cellulose nanofibrils, cellulosic biofuel, enzymatic hydrolysis.

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Introduction. The production of cellulosic biofuels can mitigate climate change through the reduction of greenhouse gas emissions for sustainable development [3]. However, cellulosic biofuel production is not economical using current technologies. One of the key technical barriers to economical cellulosic biofuel production through biochemical conversion is the high cost and low efficiency of enzymatic cellulose saccharification of lignocellulosic biomass because of its strong recalcitrance [4]. Significant past research efforts in cellulosic biofuels have focused on overcoming the recalcitrance of cellulose and enhancing the saccharification of lignocelluloses [5]. Instead, we have focused on converting the recalcitrant cellulose into cellulosic nanomaterials, which are potentially a high-value co-product for the biorefinery process and may improve the economics of producing chemicals and fuels from lignocelluloses.

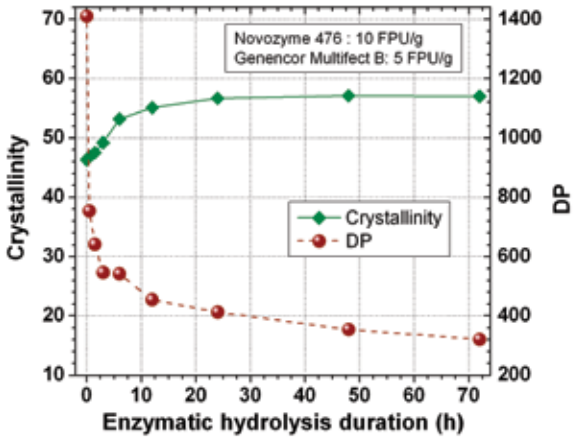

Figure 1. *Effect of enzymatic treatment duration on DP and crystallinity of the resulting cellulosic solids.*

Methodology. The starting cellulose for most of this work has thus far been commercial fully bleached kraft eucalyptus pulp, and the enzymes have been commercial cocktails, such as Novozym 476 (Novozymes, Franklinton NC) or Genencor Multifect B (Genencor, Palo Alto CA). However, future plans for this work include using other pulps and customized enzyme systems. Enzyme loading levels have been in the range of 1 to 10 FPU/g fibers with cellulose concentrations of about 10% in water. Enzyme fractionation experiments were typically done at 50°C for durations ranging from 1 to 72 h, although future experiments are planned at elevated temperatures using thermally stable enzymes. At the end of each hydrolysis, the solid and liquid fractions are separated for further conversion into biofuels or cellulose nanofibrils. The solid cellulose fraction was converted into cellulose nanofibrils using either a Microfluidizer (M-110EH-30, Microfluidics, Newton MA) or a SuperMassColloider

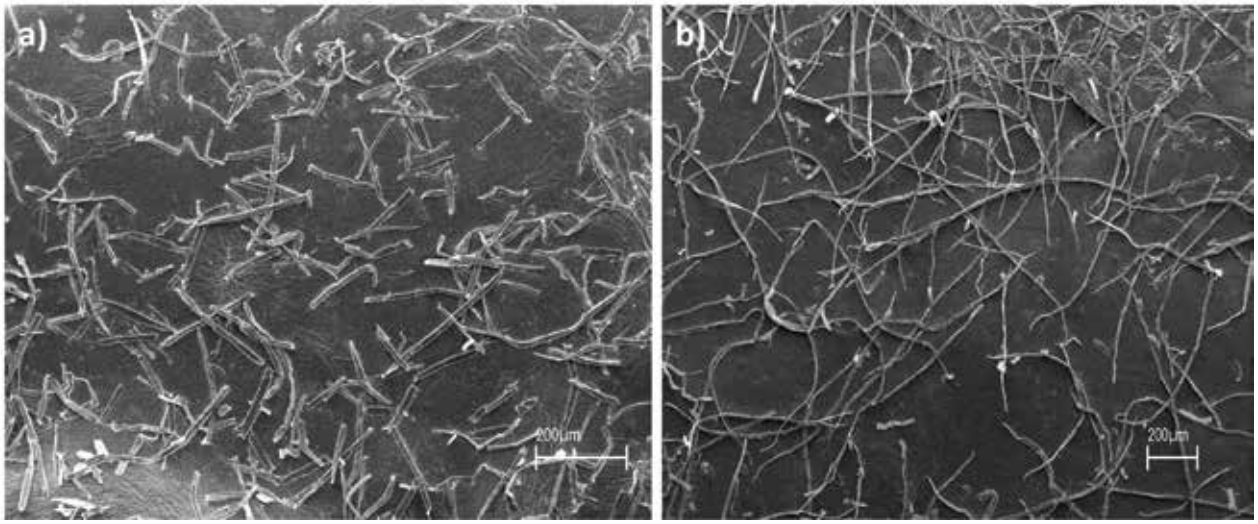

Figure 2. *Scanning electron microscope image of a) fractionated recalcitrant cellulose resulting from 48 h of enzymatic hydrolysis and b) the original control bleached kraft eucalyptus pulp fibers.*

(MKZA6-2, Masuko Sangyo Co., Ltd, Saitama, Japan) or both in combination. The cellulose was typically diluted to 1%–3% in water before mechanical refining. The duration and intensity of refining has also been varied to determine the effect of refining on the morphology and properties of the resulting CNFs [2,6].

The resulting CNFs were typically characterized for various properties, including their crystallinity, morphology, and suspension viscosity. Crystallinity measurements were conducted either by X-ray diffraction (XRD) or by Raman spectroscopy, and the morphology is typically characterized by scanning or transmission electron microscopy. The CNFs were also often formed into films by ultrafiltration, and the mechanical properties of the films were evaluated.

Results. Cellulase fractionation of the cellulose fibers resulted in an initially rapid decrease in the degree of polymerization (DP) of the cellulose for about the first twelve hours, followed by a slow decrease, as shown in Fig. 1. In this case, the DP of the cellulose decreased from 1400 to about 600 in the first four hours and to about 400 after 48 hours. Similarly, the crystallinity of the cellulose increased initially, but leveled off after about the first 24 hours (Fig. 1). The length of the fibers was reduced from over 1 mm before hydrolysis to about 200µm after 48 h of hydrolysis, as can be seen from the electron microscopy images in Fig. 2.

The solid recalcitrant cellulose was converted to CNFs by mechanical refining using varying conditions in a Microfluidizer, a SuperMassColloider, or both. The properties of the materials varied significantly with the number of passes through the refiners. For example, the

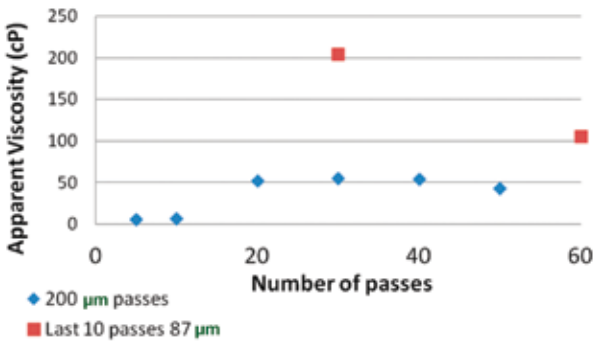

Figure 3. *Viscosity of CNF suspension at various numbers of passes through a Microfluidizer.*

viscosity of the suspension increased dramatically after 10 passes through the 200µm Microfluidizer chamber, but leveled off after 20 passes through the same chamber, as shown in Fig. 3. However, additional passes through the 87µm chamber dramatically increased the viscosity of the suspension beyond what could be achieved with the larger chamber. Such behavior occurred due to changes in the morphology of the cellulose material. Fifty passes through the 200µm followed by 10 passes through the 87µm chamber resulted in completely nanofibrillated cellulose material, as shown in Fig. 4. In one experiment, we found that cellulose fibers treated by enzymatic hydrolysis followed by a combination of grinding in a SuperMassColloider followed by a Microfluidizer resulted in CNFs that closely resembled cellulose nanocrystals (Fig. 5). We also found that the morphology of the CNFs affected the mechanical properties of films. For example, the tensile modulus of CNFs created by extensive hydro-

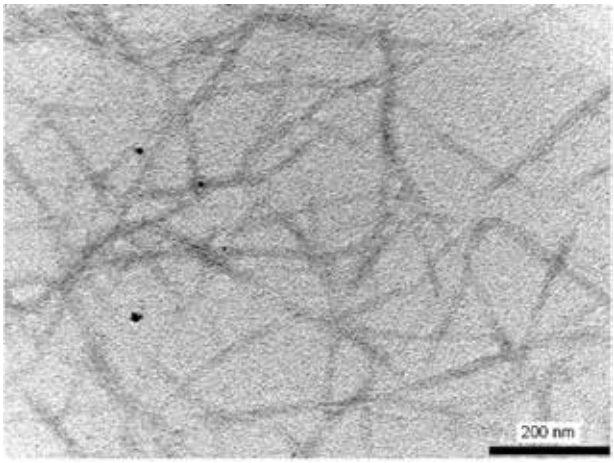

Figure 4. *Transmission electron micrograph of CNFs produced using a Microfluidizer.*

lysis and refining (such as those shown in Fig. 5) was about 14 GPa with a strain at break of about 2%–3%, whereas the modulus and strain at break of CNFs produced without enzymatic hydrolysis were about 7 GPa and 10%–12% respectively. Therefore, CNFs with a wide range of morphologies and properties can be produced from recalcitrant cellulose by enzymatic hydrolysis of wood pulp.

Conclusions. This work has demonstrated a novel concept to integrate the production of cellulose nanofibrils and sugars for conversion biofuels from lignocellulosics. The cellulase fractionation produced a high-quality glucose stream for biofuels and recalcitrant cellulose, which could be readily converted into cellulose nanofibrils by mechanical methods. This work also demonstrates the wide range of morphologies and properties that various enzymatic, chemical, and mechanical treatments can impart to nanofibrillated cellulose.

References

[1] Zhu, J. Y., Sabo, R., and Luo, X. Integrated production of nanofibrillated cellulose and cellulosic biofuel (ethanol) by enzymatic fractionation of wood fibers. Green Chemistry 13(5):1339 (2011).

[2] Qing, Y., Sabo, R., Zhu, J. Y., *et al*. A comparative study of cellulose nanofibrils disintegrated via multiple processing approaches. Submitted to Carbohydrate Polymers, February 2013.

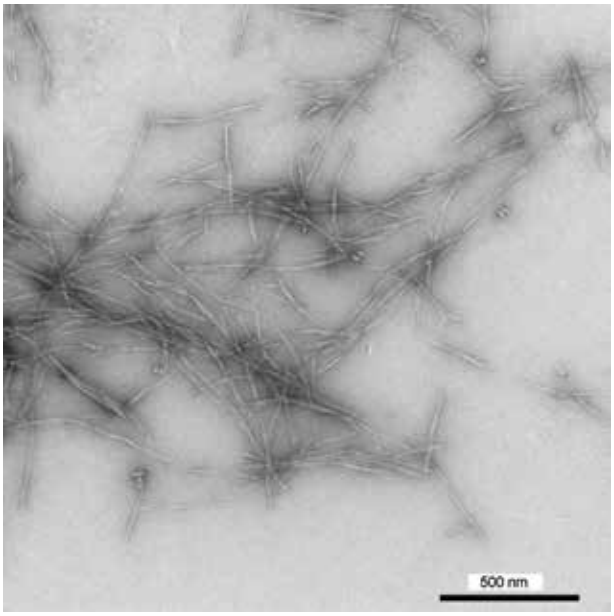

Figure 5. *Transmission electron micrograph of CNFs produced by enzymatic and mechanical means.*

[3] Farrell, A. E., Plevin, R. J., Turner, B. T., *et al*. Ethanol can contribute to energy and environmental goals. Science 311(5760): 506 (2006).

[4] Himmel, M. E., Ding, S.-Y., Johnson, D. K., *et al*. Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315(5813): 804 (2007).

[5] Zhang, Y.-H. P. and Lynd, L. R. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Biotechnology and Bioengineering 88(7): 797 (2004).

[6] Wang, Q. Q., Zhu, J. Y., Gleisner, R., *et al*. Morphological development of cellulose fibrils of a bleached eucalyptus pulp by mechanical fibrillation. Cellulose 19(5): 1631 (2012).

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Production and Applications of Cellulose Nanomaterials

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Cellulose nanocrystals incorporated within the overcoat varnish of the cover!

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