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Research Article

Use of Endoglucanase and Accessory Enzymes to Facilitate Mechanical Pulp Nanofibrillation

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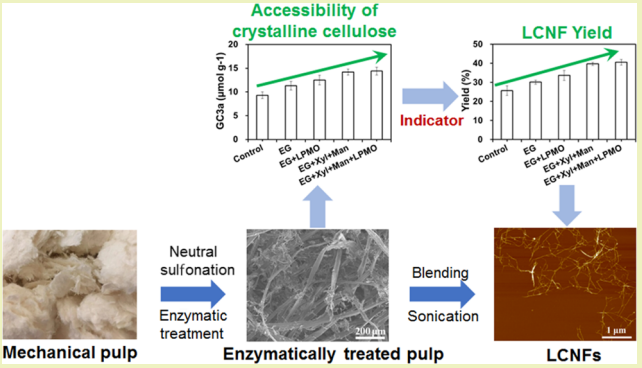
ABSTRACT: Although selective enzyme treatments have been used to successfully fibrillate chemical pulps, high lignin-containing mechanical pulps have proven to be more recalcitrant. When a bleached chemi-thermomechanical pulp (BCTMP) was sulfonated prior to enzymatic treatment, relatively good fibrillation was achieved, although some pulp hydrolysis occurred after 6 h hydrolysis when using a commercial cellulase enzyme preparation (Cellic CTec 3). To try to minimize pulp losses, various enzyme cocktails, including endoglucanase (EG), xylanase, mannanase, and lytic polysaccharide monooxygenase (LPMO), were assessed for their ability to enhance fibrillation while minimizing cellulose hydrolysis. It was apparent that the yield as well as the zeta potential of the lignin-containing cellulose nanofibrils increased with enzyme treatment. This was likely due to an increase in surface charge and a decrease in particle size after LPMO and hemicellulase treatments, respectively. When carbohydrate-binding modules (CBMs) were used to quantify fiber changes, it was apparent that sulfonation had increased the accessibility of enzymes, while the combined action of the hemicellulases and LPMO increased EG accessibility to the less-ordered regions of the mechanical pulp, resulting in enhanced fibrillation. This work described, for the first time, the synergistic action of EG and various accessory enzymes enhancing mechanical pulp nanofibrillation.



KEYWORDS: *lignin-containing cellulose nanofibrils (LCNFs), softwood mechanical pulp, carbohydrate-binding modules (CBMs), endoglucanase, accessory enzymes*

INTRODUCTION

The markets of products using cellulose nanofibrils (CNFs) have been growing steadily for uses in packaging, paperboard, nanocomposites, and films.[1−3] To date, most nanofibrils have been produced by defibrillating chemical pulps such as Kraft and sulfite pulps with little work assessing the potential of enhancing the fibrillation of high lignin-containing, high-yield mechanical pulps.[1,4] Similarly, the potential of enzyme-mediated fibrillation of mechanical pulps has not received much attention to date, primarily focusing on the potential modification of chemical pulps.[1,5,6]

High-yield mechanical pulps have been traditionally used to produce newsprint, resulting in high yields, containing much of the original woody biomass, and the advantages of lower production costs and a significantly lower environmental impact compared to chemical pulps.[7,8] Bleached chemi-thermomechanical pulp (BCTMP) is one of the highest-volume products derived from Canadian softwoods. However, primarily due to the decline of newspapers, the demand for BCTMP has dramatically reduced. It is therefore of pressing urgency to expand the portfolio of products that can be derived from mechanical pulps. Recent work has shown that lignin-containing cellulose nanofibrils (LCNFs) are less polar and are more hydrophobic.[9−15] However, although the lignin component is key to these beneficial properties, it has also been shown to restrict enzyme accessibility, limiting enzyme-mediated nanofibrillation.[16,17] Similarly, although the presence of hemicellulose has been shown to limit microfibril coalescence,[18] it also enhances the close association of the lignocellulosic matrix within mechanical pulp and can restrict enzyme-mediated nanofibrillation.[17]

Earlier work has shown that neutral sulfonation of mechanical pulps enhances fiber swelling, resulting in increased cellulose accessibility while minimizing lignin and hemicellulose removal.[17] Although effective fibrillation of the

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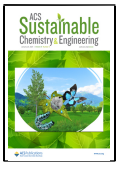

sulfonated mechanical pulp could be achieved after the addition of low loadings of a cellulase cocktail, it was at the expense of too high a substrate loss.[17] Thus, to decrease deconstruction and maximize fibrillation, the incubation time or the enzyme loading could be decreased, or cocktail composition could be varied. In earlier work, low loadings of endoglucanase (EG) have been shown to randomly cleave the cellulose β-1,4 linkages at the less organized region, consequently improving chemical pulp nanofibrillation while minimizing cellulose hydrolysis.[6,19−22] In related work, so-called accessory enzymes such as hemicellulases[5,23−28] and lytic polysaccharide monooxygenases (LPMOs)[25,29−32] have also been used to work synergistically with EGs, facilitating chemical pulp fibrillation. It has been suggested that the synergistic action of the accessory enzymes improves cellulase accessibility by removing the hemicellulose barrier, consequently loosening the fibers and enhancing hydrophilicity.[5,25]

In the work described here, a softwood-derived BCTMP was neutrally sulfonated to see if cellulase accessibility could be enhanced. As the exact mechanism involved in the synergistic action of EG and the various accessory enzymes had not been fully resolved, we also wanted to assess how the selective addition of enzymes might enhance mechanical pulp fibrillation. When different combinations of enzymes were added to a BCTMP, followed by mechanical disintegration and sonication, various degrees of fibrillation were observed. To try to further elucidate fiber changes, four distinct fluorescent protein-tagged carbohydrate-binding modules (FP-CBMs) were used to assess overall fiber accessibility and the relative location of crystalline/paracrystalline cellulose, xylan, and mannan in various enzymatically treated pulps. Previous work had shown that CBM-based probes, which are the non-catalytic polysaccharide-recognition modules of carbohydrate-active enzymes, showed good specificity toward particular fiber sites.[33−36] When the CBM observations were supplemented with morphological, chemical, and thermal analysis using transmission electron microscopy (TEM), atomic force microscopy (AFM), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and thermogravimetric analysis (TGA), it was apparent that enzyme cocktails could be selected to facilitate mechanical pulp fibrillation while minimizing cellulose hydrolysis.

EXPERIMENTAL SECTION

Pulp and Chemicals. Bleached softwood chemi-thermomechanical pulp (softwood BCTMP) prepared from spruce, pine, and fir was provided by Quesnel River pulp Ltd., Canada. It was washed with large amounts of deionized water and disintegrated using a disintegrator. It was subsequently vacuum-filtered to an 80% moisture content and stored at 4 °C. Other reagents were of analytical grade and purchased from Fisher Scientific, US. The EG, xylanase, mannanase, and LPMO enzymes were kindly provided by Novozymes (Novozymes, Bagsvaerd, Denmark).

Neutral Sulfonation Treatment. The disintegrated BCTMP was neutral-sulfonated according to Chandra et al.[16] Briefly, the sulfonation treatment was performed in a Parr high-pressure batch reactor (1 L capacity, T 316 stainless steel, Parr Instrument Company, Moline, IL) at a 10% (w/w) consistency, 0.16 g of Na_2SO_3/g dry pulp, and 160 °C for 3 h. The treated slurry was subsequently filter-washed using deionized water. The wet solid fraction was stored at 4 °C.

Enzymatic Treatment and Mechanical Treatment. The sulfonated BCTMP (S-BCTMP) was suspended in acetate buffer (50 mM, pH 5.0) at 50 °C and 180 rpm for 2 h. It was subsequently treated by either individual or cocktails of EG, xylanase, mannanase, and LPMO at a 1% consistency, 50 °C, and 180 rpm for 6 h. Oxygen was injected for 2 min per hour to facilitate LPMO's function.[30] The enzyme dosages of EG, xylanase, mannanase, and LMPO were 1, 1, 1, and 5 mg protein/g dry matter (DM), respectively. The hydrolysis yield of glucan, xylan, arabinan, mannan, and galactan was calculated based on the corresponding original polysaccharide weight.[37−41] The enzymatic treatments were terminated by heating at 85 °C for 20 min.[17] The solid residue was washed and filtrated until the conductivity approached that of deionized water.

The solid fraction of the enzymatic treated S-BCTMP was suspended into a suspension at a 0.125% (w/w) consistency and mechanically sheared (~20,000 rpm) using a blender (Vitamix 6300, US) for 20 min, followed by ultrasonication (Fisher model 700) at a 90% amplitude for 60 min. The slurry was centrifuged at 5000 rpm for 30 min, and the supernatant was defined as the LCNF suspension. The concentrations of the LCNFs were assessed by oven-drying a defined volume of the LCNF suspension with the blank of distilled water.[42] The yield of LCNFs was calculated as follows: (concentration of LCNFs × volume of the LCNF suspension)/mass of S-BCTMP before enzymatic hydrolysis.

Characterization. *Compositional Analysis.* The chemical composition of the samples was assayed according to TAPPI Standard Method T-222. The sugar contents were assayed using a Dionex DX-3000 HPLC (Sunnyvale, CA) equipped with an anion-exchange column (Dionex CarboPac PA1).

Fiber Quality Analysis. The length and width of fibers were assayed using a fiber quality analyzer (LDA02; OpTest Equipment, Inc., Hawkesbury, ON, Canada) according to previous procedure.[43]

Conductometric Titration. The amount of acid groups in enzyme-treated pulps was assayed by the conductometric titration method according to Katz et al.[44] Briefly, 0.15 g of pulp (dry matter) was re-suspended and soaked in 15 mL of 0.1 M HCl overnight. The samples were then washed with 250 mL of distilled water through a small Buchner funnel. 50 mL of 0.001 M NaCl and 200 μL of 0.05 M HCl were added to each sample and the suspensions consequently titrated using 0.05 M NaOH. The conductivity was assayed by a conductivity meter (Fisher Scientific accuMET XL200).

Fiber Surface Accessibility Assessment Using the CBM Adsorption Assay. Selective CBMs were used to analyze the relative accessibility of fibers treated by different enzymes. Four highly specific FP-CBMs, namely, GC3a, CC17, OC15, and CC27 (for the detection of highly ordered cellulose, paracrystalline cellulose, xylan, and mannan, respectively), were constructed and utilized according to previous work.[36,45−49] The detailed procedures are shown in Text S1.

Morphology Observation. The morphology of LCNFs was observed by AFM and TEM, and the morphology of pulps was observed by SEM. The detailed procedures are shown in Text S2.

UV−Visible Spectroscopy. The light transmittance of the LCNF suspensions (0.1 wt %, w/v) was assessed at wavelengths of 200−800 nm using a Cary 50 UV−visible (UV−vis) spectrophotometer.

Zeta Potential Value. The zeta potential of LCNF suspensions (0.1 wt %, w/v) was assayed using Zetasizer nano-ZS (Malvern, CA).

Attenuated Total Reflectance−FTIR Spectra Analysis. The FTIR spectra of freeze-dried LCNFs were recorded using an FTIR spectrometer (PerkinElmer, Wellesley, MA) equipped with a universal attenuated total reflectance (ATR) accessory. The wavenumber was from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and a total of 80 scans.

X-ray Diffraction Analysis. The XRD patterns of freeze-dried LCNFs were assayed according to the previous study.[17,50] The detailed procedures are shown in Text S3.

Thermogravimetric Analysis. TGA of freeze-dried LCNFs was performed using a thermogravimetric analyzer (NETZSCH, TG 209F1, Germany). Approximately 10 mg of the sample was heated from room temperature to 600 °C, with a rate of 20 °C/min under a nitrogen stream of 20 mL/min.

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RESULTS AND DISCUSSION

Increasing Enzyme Accessibility to Softwood BCTMP by Neutral Sulfonation. As discussed earlier, BCTMP was used as the biomass substrate due to its high yield (over 90% in general) and minimum chemical and refining energy input. The BCTMP contained 24.6% lignin, 44.0% glucan, 5.4% xylan, 1.3% arabinan, 2.0% galactan, and 9.5% mannan, which was close to the chemical composition of the original softwood chips.[51] The relatively high lignin content of the BCTMP was anticipated as the mild thermal and mechanical refining of the chips was primarily used to soften the lignin and reduce the refining energy required.[7,8] Previous work had also shown that bleaching of BCTMP did not remove lignin, only modifying the chromophore structure to reduce the color intensity of the pulp.[7,8]

Although past work had shown that BCTMP was poorly hydrolyzed (28%) due to the lignin component restricting enzyme accessibility to the cellulose,[17,51] subsequent work indicated that neutral sulfonation could cost-effectively increase enzyme accessibility and facilitate nanofibrillation and hydrolysis.[17] It was also apparent that the fiber length and width were relatively unchanged before (2119, 32.6 μm) and after (2168, 33.2 μm) neutral sulfonation, while the sulfonic group content of the pulp increased from 55 to 156 mmol/kg, consequently increasing fiber swelling.[51] Thus, this increase in cellulase accessibility combined with the good retention of lignin encouraged us to assess the potential of producing enzyme-mediated LCNFs.

Potential Enzyme-Mediated Nanofibrillation of S-BCTMP. To try to enhance fibrillation while minimizing deconstruction, EG and accessory enzymes including xylanase, mannanase, and LPMO were used to treat the S-BCTMP (control). It was apparent that the addition of EG alone resulted in minimum hydrolysis as more than 99% of the original pulp was recovered (Figures 1 and S1) and essentially the same composition of S-BCTMP was achieved (Table 1) with very little change in the fiber length of the pulp observed, from 2168 to 2144 μm (Figure 1).

Previous work had shown that LPMO acts by oxidative cleavage of accessible cellulose regions, disrupting and opening up the highly organized cellulose structure by adding carboxylic acid groups to the fiber surface.[25,52] When LPMO was combined with EGs, little deconstruction was again observed with more than 99% of the original pulp and S-BCTMP recovered (Figures 1 and S1 and Table 1). However, the fiber length of the S-BCTMP decreased to 2015 μm, likely due to the synergistic actions of LPMO and EG (Figure 1), while the amount of weak acid groups on the fibers increased from 218.6 (S-BCTMP treated with only EGs) to 237.5 mmol/kg (S-BCTMP treated with a combination of EGs and LPMO). An LPMO addition of 5 mg/g was used as lower dosages (1 mg/g) showed minimum changes to the surface charge of the pulp or enhanced nanofibrillation. As described in more detail below, it was hoped that the enhanced negative charge associated with the fibers would facilitate fibrillation due to an increase in repulsive forces while reducing fiber aggregation.[25,31]

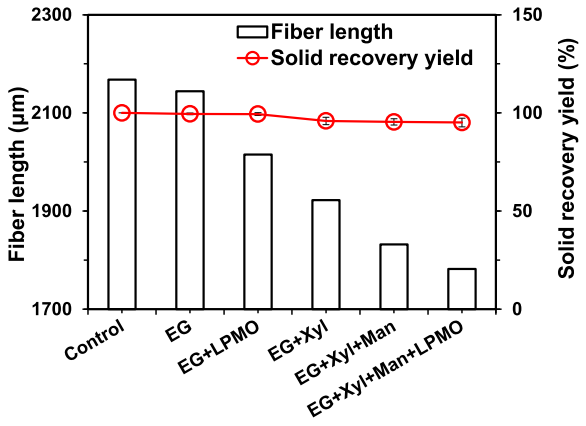

Figure 1. Fiber length and solid recovery of S-BCTMP after enzyme treatments. Control, no enzyme added. EG, endoglucanase; Xyl, xylanase; Man, mannanase; LPMO, lytic polysaccharide monooxygenase.

As previous work had shown that the synergism action of EG and xylanases could open up the cellulosic fibers present in Kraft pulps with a minimum loss in yield,[5] a similar enzyme cocktail was added to the S-BCTMP and a good solid recovery was observed (96%), although the fiber length decreased to 1922 μm (Figure 1). When an EG−xylanase−mannanase was assessed, still little mannan hydrolysis was observed (Table 1), likely due to its close association with xylan and lignin, as suggested previously.[5,17] When a complete cocktail of EG, xylanase, mannanase, and LPMO were added to S-BCTMP, although there was a further decrease in fiber length (1782 μm), a good solid recovery was observed (95%) (Figure 1). It was also apparent that the fibrous nature of the S-BCTMP was retained after treatment with the complete enzyme cocktail (Figure S2).

Characterization of the LCNFs after Enzyme-Mediated Treatment of the Sulfonated Mechanical Pulps. *Lignin-Containing Cellulose Nanofibril Yield.* As past work had shown that mechanical refining and ultrasonication enhanced fibrillation,[5,53,54] the enzyme-treated S-BCTM pulps were mechanically defibrillated (~20,000 rpm, 20 min), followed by ultrasonication (90% amplitude, 60 min) to try to enhance the production of LCNFs. Although EG treatment increased the yield of LCNFs from 25.7% to 30.1% (Figure 2), unlike the previously observed significant increase in fibrillation resulting from EG treatments of chemical pulps, there was only a marginal increase in fibrillation of the mechanical pulps. It was likely that the compact structure of the mechanical pulps hinders enzyme accessibility since no further increase was observed in the LCNF yield (30.5%) when bovine serum albumin (BSA) was added to block enzyme adsorption to the lignin.

The combined addition of the EG and the LPMO increased the LCNF yield by about 12% (Figure 2), likely due to the LPMO opening up the cellulose structure while adding carboxylic acid groups to the fiber surface.[25,29,30,32] Although xylanase addition increased the LCNF yield from 30% to 37%, further mannanase supplementation resulted in only a marginal increase in yield. The highest LCNF yields (40.5%) were obtained when the combined EG, xylanase, mannanase, and LPMO cocktail was added to the S-BCTMP.

Stability and Characteristics of the LCNF Suspensions. It was apparent that the transmittance of enzymatically treated S-BCTMP-derived LCNF suspensions (Figure S3) increased with the addition of the enzymes, with the highest transmittance achieved when the EGs were combined with the hemicellulases and LPMO (Figures 3a and S4). All the zeta

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Table 1. Chemical Composition of Enzymatically Treated S-BCTMP[a]

pulp type	Klason lignin (%)	glucan (%)	xylan (%)	arabinan (%)	galactan (%)	mannan (%)
control	18 ± 1	49 ± 1	5 ± 0	1 ± 0	2 ± 0	10 ± 0
EG	18 ± 1	49 ± 2	5 ± 0	1 ± 0	2 ± 0	10 ± 1
EG + LPMO	19 ± 1	49 ± 1	5 ± 0	1 ± 0	2 ± 0	10 ± 0
EG + Xyl + Man	19 ± 1	50 ± 1	3 ± 0	1 ± 0	2 ± 0	10 ± 1
EG + Xyl + Man + LPMO	20 ± 1	51 ± 1	3 ± 0	1 ± 0	2 ± 0	10 ± 0

[a]S-BCTMP was enzymatically pretreated with different enzymes. Control, no enzyme added. EG, endoglucanase; Xyl, xylanase; Man, mannanase; LPMO, lytic polysaccharide monooxygenase.

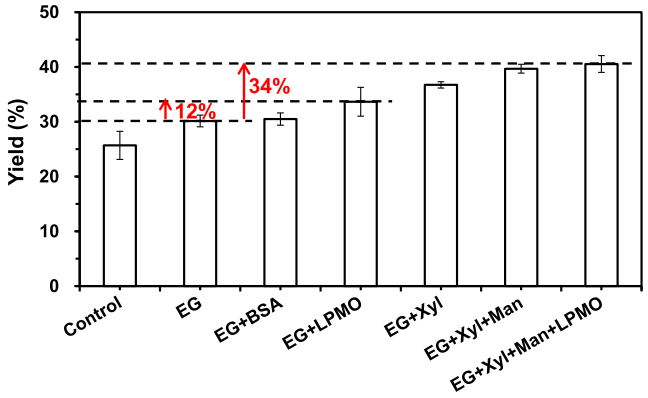

Figure 2. LCNF yield of various enzymatic treated S-BCTMP. Control, no enzyme added. EG, endoglucanase; BSA, bovine serum albumin; Xyl, xylanase; Man, mannanase; LPMO, lytic polysaccharide monooxygenase.

potentials were greater than −25 mV (Figure 3a), indicating relatively good fiber stabilization.[55]

Chemical and Physical Properties of LCNFs. The FTIR spectra of LCNF (control), LCNF (EG), LCNF (EG + LPMO), LCNF (EG + Xyl + Man), and LCNF (EG + Xyl + Man + LPMO) indicated a relatively unchanged cellulose and lignin structure (Figure 3b). Absorbance peaks at around 3340, 2896, 1455, 1369, 1159, 1055, and 898 cm^{-1} all indicated a stable cellulose structure.[53,54,56] The characteristic lignin peaks at 1595, 1506, and 1269 cm^{-1} confirmed the presence of lignin.[57,58] The peak at 1700 cm^{-1} associated with carboxyl groups after LPMO treatment (Figure 3c) showed the oxidative cleavage of cellulose by LPMO resulting in the oxidation of either the C_1 or C_4 carbon, producing a carboxylic acid or ketone structure, respectively.[25,31] The FTIR analysis of the carboxyl groups in the LCNFs was also consistent with the increased zeta potential values (Figure 3a) and the increased weak acids indicated by conductometric titration when LPMO was included in the enzyme treatment. All the self-assembled LCNF solids had the same XRD pattern, showing cellulose Iβ characteristic peaks (Figure 3d), although the CrI of LCNF was increased after enzyme treatment. The complete cocktail (EG + Xyl + Man + LPMO)-treated LCNF showed the highest CrI, likely due to the synergistic cooperation of the LPMO and hemicellulases with the EG.

When TGA was conducted to compare the differences in thermal stability of LCNFs prepared by different enzymes (Figure 3e,f), the T_{max} for the LCNF (control) was 283 °C, which agreed with the LCNF derived from hardwood of a

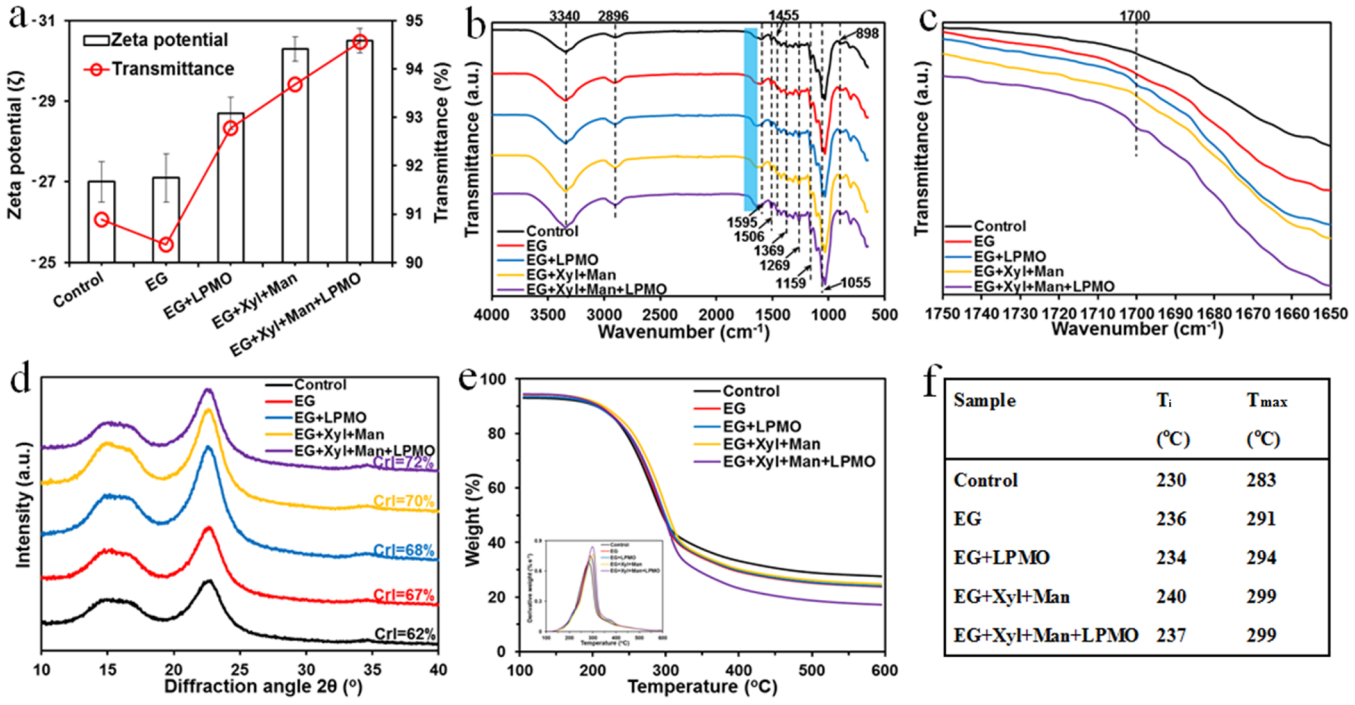

Figure 3. Physical and structural characterization of LCNFs. (a) Zeta potential and UV−vis transmittance (600 nm) of 0.1 wt % LCNF suspensions; (b,c) ATR−FTIR spectra; (d) XRD spectra; (e) TGA curves with embedded DTGA curves; (f) degradation temperature.

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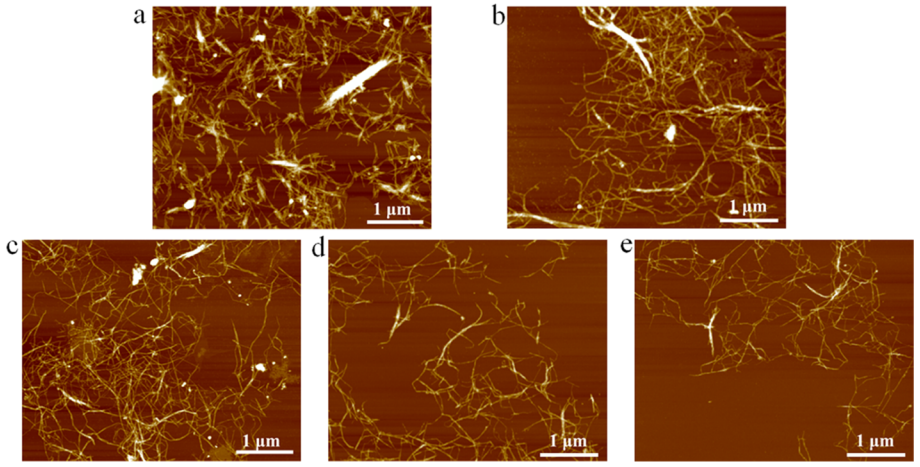

Figure 4. AFM images of LCNFs. (a) LCNF (control); (b) LCNF (EG); (c) LCNF (EG + LPMO); (d) LCNF (EG + Xyl + Man); (e) LCNF (EG + Xyl + Man + LPMO). EG, endoglucanase; Xyl, xylanase; Man, mannanase; LPMO, lytic polysaccharide monooxygenase.

similar chemical composition.[59] After enzymatic treatment, the increase in crystallinity and the partial removal of hemicellulose resulted in an increase in the T_i and T_{max} (Figure 3d, Tables 1 and S1).

Morphologies of the LCNFs. When the morphology of the various LCNFs was assessed by AFM, the non-enzyme-treated LCNFs were found to be composed of both individual nanofibrils and large, integrated fibrils (Figure 4a). The proportion of individual nanofibrils increased after both EG treatment (Figure 4b) and EGs combined with LPMOs (Figure 4c) and hemicellulases (Figure 4d,e), and the average thicknesses decreased from 9.4 to 6.1, 5.2, 3.4, and 3.3 nm, respectively (Figure S5), which have the same trends shown in TEM images (Figure S6). All the AFM images showed the occurrence of spherical lignin particles, likely due to lignin aggregation resulting from its aromatic structure and amphiphilic nature.[17,42]

Use of Specific CBMs to Characterize LCNF Supramolecular Structure Changes/Nanofibrillation. As described earlier,[36,47,60] CBMs are non-catalytic polysaccharide-recognition modules of carbohydrate-active enzymes that have been successfully used to characterize pulp changes at the fiber and fibril level.[61,62] Four FP-CBM probes, that is, GC3a (well-ordered cellulose), CC17 (less ordered, paracrystalline cellulose), OC15 (xylan), and CC27 (mannan), were used to try to quantify accessibility and any changes to the cellulose and hemicellulose components of the BCTMP before and after neutral sulfonation (Table S2 and Figure S7). It was apparent that the accessibility of the pulps to all the CBMs increased significantly after sulfonation (Table 2). It also appeared that EG and accessory enzyme addition enhanced mechanical pulp nanofibrillation (Table 2) with the N_0 of CC17 (paracrystalline cellulose) decreasing after enzyme treatment. This suggested that the addition of hemicellulases promotes the disruption of the less ordered regions of the fiber (Table 2). In contrast, the N_0 of GC3a (more ordered cellulose) gradually increased with enzymatic treatment, indicating a decrease in the amount of disordered cellulose and a corresponding increase in enzyme accessibility to the more ordered fiber surface. In addition, the increase in the N_0 of GC3a while the unchanged N_0 of CC17 after LPMO treatment suggested that LPMO treatment, rather than elimination of the disordered cellulose region, increased enzyme accessibility by increasing the surface charge and breaking some of the H-bonds within the cellulose.[25,52] It was also apparent that the N_0 of OC15 (xylan) decreased substantially after xylanase treatment due to less xylan being available, while the N_0 of CC27 (mannan) was unchanged due to little mannan hydrolysis (Table 2). These observations were consistent with the compositional analysis which confirmed that the mannan content did not change after enzymatic treatment (Table 1).

Table 2. Accessibility of Pulps after Sulfonation and Enzymatic Treatments, as Indicated by Specific CBM Adsorption[a]

	N_0 (μmol g^{-1})			
pulp type	GC3a	CC17	OC15	CC27
BCTMP	6 ± 0	6 ± 0	2 ± 0	4 ± 0
S-BCTMP (control)	9 ± 1	8 ± 0	3 ± 0	6 ± 0
S-BCTMP (EG)	11 ± 1	5 ± 0	3 ± 0	6 ± 0
S-BCTMP (EG + LPMO)	13 ± 1	6 ± 0	3 ± 0	6 ± 1
S-BCTMP (EG + Xyl + Man)	14 ± 1	3 ± 0	1 ± 0	6 ± 0
S-BCTMP (EG + Xyl + Man + LPMO)	14 ± 1	3 ± 0	1 ± 0	6 ± 0

[a]GC3a, CC17, OC15, and CC27 were specific CBMs for crystalline cellulose, paracrystalline cellulose, xylan, and mannan, respectively.

CONCLUSIONS

EG treatment of sulfonated softwood mechanical pulp was shown to enhance fibrillation with the further addition of LPMO, enhancing the yield of LCNFs due to carboxylic acid group addition. Xylanase showed good synergistic cooperation with the EG by partially solubilizing the hemicellulose and consequently enhancing further enzyme accessibility to the fibers. Previous work had shown that CBMs can specifically bind to different supramolecular structures within lignocellulose substrates. They were successfully used here to track and quantify cellulose and hemicellulose accessibility changes. The CBM data helped better elucidate the likely mechanisms by which selective enzyme addition enhanced pulp accessibility and facilitated the fibrillation of the pretreated mechanical pulps.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.0c08588.

Experimental details of the CBM adsorption assay; experimental details of TEM, AFM, and SEM; experimental details of XRD analysis; chemical composition of LCNFs; enzyme accessibility of the pulps after sulfonation and enzymatic treatment as indicated by selective CBM adsorption; hydrolysis yield of S-BCTMP after enzyme treatments; SEM images of S-BCTMP before and after enzymatic treatment; images of LCNF aqueous suspensions; UV−vis transmittance of LCNF suspensions; height distribution of LCNFs via AFM images; TEM images and width distribution of LCNFs; and binding of CBMs onto the pulps after sulfonation and enzymatic treatment (PDF)

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Notes

The authors declare no competing financial interest.

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