

harvesting & utilization

Mountain Pine Beetle-Killed Lodgepole Pine for the Production of Submicron Lignocellulose Fibrils

Ingrid Hoeger, Rolland Gleisner, José Negrón, Orlando J. Rojas, and J.Y. Zhu

The elevated levels of tree mortality attributed to mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) in western North American forests create forest management challenges. This investigation introduces the production of submicron or nanometer lignocellulose fibrils for value-added materials from the widely available resource represented by dead pines after an outbreak. Lodgepole pine (*Pinus contorta* Dougl. ex Loud.), trees from two different times since infestation and a noninfested live tree as a control were used for mechanical fibrillation. Fiber deconstruction down to the micro-/nanoscale from infested wood was performed using mechanical fibrillation, without any chemical (pre)treatment. The effects of fibrillation were monitored as a function of processing time, and the respective products were characterized. The changes in fibril morphology, cellulose crystallinity, water retention value, and cellulase adsorption capacity were determined. Interestingly, no significant differences were found between fibrillated samples from the live and the MPB-killed trees. It can be concluded that MPB-killed lodgepole pine is a suitable feedstock for the production of lignocellulose micro-/nanofibrils.

Keywords: cell wall deconstruction, cellulose nanofibrils, lignocellulose fibrils, mechanical fibrillation, mountain pine bark beetle

Mountain pine beetles (MPBs) (*Dendroctonus ponderosae* Hopkins) use a number of pine (*Pinus*) species as hosts, resulting in tree mortality. Approximately 0.53 million hectares of coniferous forests in Colorado have been affected by eruptive populations of MPBs from 1996 through 2011 (Klutsch et al. 2009). Although the MPBs are an integral part of the ecology of these forests, extensive tree mortality caused by the insects poses a severe challenge to land managers and private land owners (Klutsch et al. 2009). For instance, MPB-caused tree mortality alters the arrangement, composition, and moisture content of forest fuels (Jenkins et al. 2008) and may influence fire behavior (Klutsch et al. 2009, Jenkins et al. 2014). The time from MPB infestation to utilization can affect the usefulness of the MPB-killed trees for different wood and fiber products (Feng and Knudson 2007, Dalpke et al. 2009). In addition, the reduction in the moisture content of the wood makes it prone to checking, affecting the value of product recovery at sawmills and chip quality for pulping negatively (Woo et al. 2005).

Large-volume and high-value utilization is important to mitigate the cost of harvesting MPB-killed trees (Zhu et al. 2007). Several studies that used MPB-killed trees for a variety of applications have been conducted. When the MPB successfully attacks a tree it brings

spores of various species of fungi in the genus *Ophiostoma*. The fungus is carried in a mycangium, which is a specialized structure that the insect has on its body surface. The fungus grows into the parenchyma cells of the xylem and blocks water conduction. The moisture reduction caused by the fungus and wood degradation of MPB-killed lodgepole pine have been characterized (Chow and Obermayer 2007, Lewis and Thompson 2011). Sapwood moisture content decreases 100% by the time beetle-killed trees reach the gray stage, which occurs about 3 years after mortality (the gray stage is a commonly used term to indicate that a killed tree has lost all of its foliage). Chow and Obermayer (2007) indicated that the extensive presence of blue stain and subsequent checking and warping of the wood decreases its value; however, blue stain was found to have no effect of wood mechanical properties (Lum et al. 2006). Checks created by releasing drying stress are more frequently observed in MPB-killed standing trees than in live trees (Magnussen and Harrison 2008). As time since death lengthens, secondary attacks by rot- or decay-causing fungi or insect-seeking birds significantly increases the risk of wood decay (Lewis et al. 2006). Spores of decay fungi cannot enter through the bark; therefore, they enter the tree through insect bore holes, weather checks, cracks, or injuries such as broken branches or broken tops and often are carried on the bodies of insects (Lowell et al. 2010). Wood decay caused loss of 12% of the

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tree for producing power poles, and basal decay can increase the rejection rate to 50% (Tegethoff et al. 1977). MPB infestation also affected wood fiber properties (Woo et al. 2005). For example, the infested wood has low specific gravity, low concentrations of extractives, and low hemicellulose content and is more permeable. When MPB-killed trees are used for fiber production, it appears that no significant effects occur in kraft pulping, but sheet and surface structure may be affected when thermomechanical pulps are used (Dalpke et al. 2009). Studies also found that the MPB-killed trees have great potential for wood-plastic composites (Lam and Chang 2010) and cement-bonded particleboard (Chang and Lam 2009).

Recently, MPB-killed trees were evaluated as feedstock for the production of biofuel. SO_2 -catalyzed steam explosion and organosolv pretreatments were applied to overcome the known strong recalcitrance of lodgepole pine to enzymatic saccharification for biofuel production (Ewanick et al. 2007, Pan et al. 2008, Del Rio et al. 2010). In a previous study, we found that fungi decay enriched the glucan content in MPB-killed lodgepole pine by as much as 3 percentage points (Luo et al. 2010). The enrichment was noted to increase with the time since tree death. Furthermore, it was found that fungi decay produced wood that was more susceptible to SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose) pretreatment and facilitated enzymatic saccharification of cellulose (Luo et al. 2010). These observations are in agreement with studies using the organosolv pretreatment (Pan et al. 2008). Enzymatic saccharification of the pretreated solid substrates from MPB-killed lodgepole pine and combined fermentation of the enzymatic hydrolysate produced slightly higher ethanol yields compared with those measured for equivalent live trees harvested from the same site (Luo et al. 2010, Zhu et al. 2011a). For example, ethanol yields ≥ 270 L/ton were achieved from MPB-killed lodgepole pine (Luo et al. 2010, Tian et al. 2010) even at a very high titer of >40 g/liter (Lan et al. 2013). Deployment of operations taking advantage of these findings are, however, undermined by the fact that water is a scarce commodity in the mountainous areas and biofuel production uses water-intensive processes such as those discussed above. Transport of MPB-killed pines to water resource sites may not be economically sustainable because biofuels are low-value commodities. Production of higher value-added materials, however, can make it affordable to transport wood to water resource sites, which is at the center of interest in the present effort. In particular, we investigate the utilization of MPB-killed pine to produce lignocellulose micro-/nano-sized fibrils (LCNFs), which could be used in rapidly advancing areas of cellulose-based material (composites, higher performance additives, and others).

Thus, the objective of the present study is to evaluate the feasibility of producing LCNFs directly from MPB-killed lodgepole pine without using any chemical (pre)treatment. We pay particular attention to the utilization of trees with advanced decay and with no value for lumber or wood products. Nanocellulosic materials have attracted great attention in the material research community recently (Síró and Plackett 2010, Klemm et al. 2011, Ten et al. 2013). The outstanding mechanical properties of cellulose nanofibrils, i.e., tensile modulus reaching 135 GPa levels and tensile strength of $>5,000$ kN $\cdot$ m/kg (Šturcová et al. 2005), make them suitable as reinforcements in a variety of polymer composites. Therefore, nanocellulose from MPB-killed pines has the potential to be a high-value product.

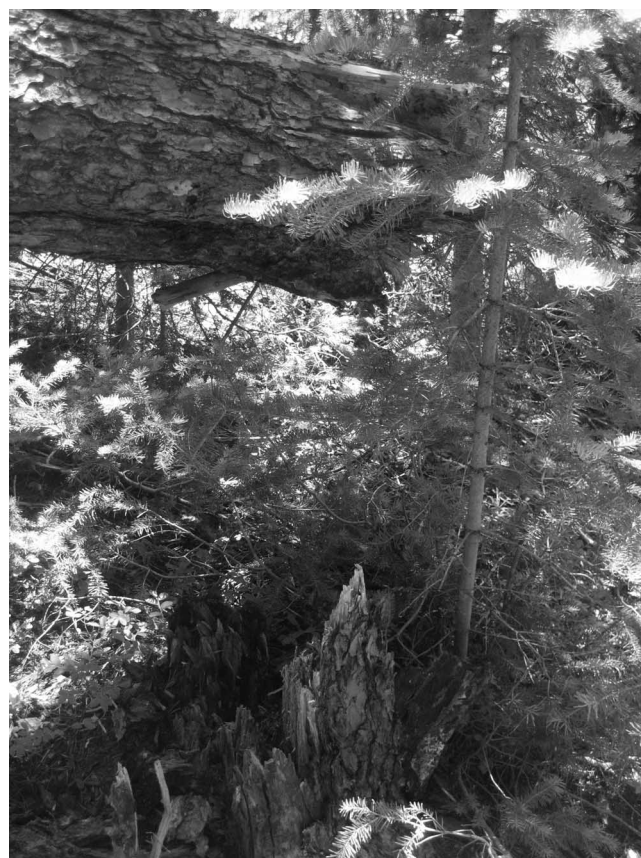


Figure 1. Image of the decayed FDD8 tree broken from the stem due to wind fall. Beetle-killed trees typically break at the lower bole as a result of decay from saprophytic fungi.

Table 1. Tree identification and general description of samples used in this investigation.

Tree label	GPS coordinates	Infestation age and tree condition	Moisture (%)
FL	13S04255004415649	Live	43.3
FD5	13S04255004415649	~5 years, dead	15.6
FDD8	13S04253294417171	~8 years, dead, wind fall on ground	19.2

Data from Luo et al. (2010).

The proposed potential utilization of MPB-killed lodgepole pine is important to mitigate the high harvesting cost in mountain areas. Unlike many early studies that used bleached pulp to produce cellulose nanofibrils (Iwamoto et al. 2007, Henriksson et al. 2008, Stelte and Sanadi 2009, Zhu et al. 2011b, Wang et al. 2012b), in this study we directly used MPB-killed lodgepole pine wood chips, with no chemical pretreatment, to produce LCNFs via mechanical fibrillation. This approach poses significant challenges because of the presence of lignin. Previous studies suggested that LCNF films produced from thermomechanical and chemical pulps containing lignin and residual cell wall polysaccharides presented toughness similar to and in some cases greater than that obtained from fully bleached fibers (Spence et al. 2010, Ferrer et al. 2012a, 2012b). Thus, the utilization of untreated MPB-killed tree is expected to add a dimension to efforts to understand the processability of this important and widely available source of fiber.



Figure 2. Images of the three wood chip samples used in this study. A. FL. B. FD5. C. FDD8. (From Luo et al. 2010.)

Table 2. Chemical composition of the different trees.

Wood	Klason lignin	Arabinan	Galactan	Glucan	Xylan	Mannan
FL	29.2 ± 0.2	2.1 ± 0.1	4.2 ± 0.1	39.1 ± 0.6	6.0 ± 0.3	10.0 ± 0.6
FD5	28.6 ± 0.3	1.5 ± 0.1	2.8 ± 0.2	41.7 ± 0.6	5.9 ± 0.4	11.3 ± 0.6
FDD8	28.2 ± 0.3	1.1 ± 0.1	2.4 ± 0.1	42.0 ± 0.0	4.6 ± 0.1	9.5 ± 0.1

Data from Luo et al. (2010).

Materials and Methods

Materials

Three lodgepole pine trees were harvested from the Fraser Experimental Forest (F), Sulfur Ranger District (Arapaho-Roosevelt National Forest), located west of the continental divide, as described previously (Luo et al. 2010, Zhu et al. 2011a). A live uninfested tree (FL) was used as a control sample and two MPB-killed trees were used in the study. The two MPB-killed trees were infested approximately 5 and 8 years before sampling as determined by crown condition (Klutsch et al. 2009) and are labeled as FD5 and FDD8 (Figure 1), respectively. The system presented by Klutsch et al. (2009) estimates postmortality time up to 6 years, when large twigs are no longer present in the crown, but the tree is still standing. The FDD8 tree was a recent tree fall, broken at the base of the tree with large twigs absent from the crown. Based on this, we estimated time since mortality at about 8 years. We acknowledge that this represents a small sample; however, the small sample size is due to logis-

tical limitations associated with the laborious process of collecting and the cost of shipping and sample processing. Sample trees were randomly selected after a walk-through assessment of MPB-killed trees in the stands. The locations of the trees are provided using global positioning systems (GPS) coordinates together with their general condition and moisture content (Table 1). All trees were about 100 years old, with a dbh of approximately 25 cm. Logs were hand-debarked at the harvesting site, wrapped in plastic bags, and shipped to the US Forest Service Forest Products Laboratory in Madison, Wisconsin. The moisture content of the trees was noted to decrease after the MPB attack (Table 1). The wood logs were chipped and screened to remove all particles >38 mm and <6 mm in length. The accepted chips have typical thicknesses ranging from 3 to 8 mm. Evidence of the blue stain fungus and other fungal decays in the MPB-killed trees was clearly observed in the wood chips (Figure 2). Advanced decay was more obvious in the case of FDD8 tree (note that this tree was broken at the base, which is characteristic

of MPB-killed trees) (Figures 1 and 2C). The chemical compositions of the three wood chip samples are listed in Table 2.

Endoglucanase (Fibercare), was kindly provided by Novozymes North America (Franklinton, NC). This enzyme was used in adsorption experiments to determine the accessible surface area. All other chemicals used were ACS reagent grade from Sigma-Aldrich (St. Louis, MO).

Mechanical Predefibrillation of Lodgepole Pine Wood Chips

The lodgepole pine wood chips were presteamed (atmospheric refiner; Andritz Sprout-Bauer, Springfield, OH) for 10 minutes to increase their moisture. The chips were then processed in an atmospheric mechanical disk refiner (Sprout-Waldron Operation; Koppers Company, Muncy, PA) using water at less than 50° C to avoid any degradation of lignin. Disk-plates with pattern D2-B505 were used. The gap of the disk plates was set at 0.51 mm in the first pass and 0.18 mm in the second pass. The energy used during this mechanical pretreatment was recorded. A vibratory screen (Cooper Crouse-Hinds, Houston, TX) was used to remove shives (fiber bundles) with a mesh opening size of 0.15 mm.

Table 3. Energy consumed during mechanical fiberization of woodchips along with resultant characteristic fiber length and freeness (CSF).

Sample	Fiberization energy (kWh/kg)	Average fiber length (mm)	CSF (ml)	Fibrillation energy at 12 h (kWh/kg)
FL	3.9	1.2 ± 0.5	660 ± 14	29.2 ± 0.4
FD5	3.6	1.3 ± 0.7	685 ± 11	31.4 ± 1.5
FDD8	2.7	1.2 ± 0.6	390 ± 17	38.9 ± 2.8

The energy consumed upon subsequent fibrillation (via grinding after 12 hours) is also included.

Characterization of the Mechanical Fibers

The average fiber length of the fibers obtained after disk refining was determined using an optical fiber analyzer (Kajaani FS-100; Metro, Kajaani, Finland). The Canadian Standard Freeness (CSF) of the fiber suspensions after screening was determined in accordance with Technical Association of the Pulp and Paper Industry (TAPPI) Standard T 227 om-99 (TAPPI 2009).

Production of LCNFs via Grinding

Each lodgepole pine mechanical fiber sample was soaked in de-ionized water for 24 hours (2% in oven-dry solids) before fibrillation using stone grinding with a Supramasscolloider (model MKZA6-2, DISK model: MKGA6-80#; Masuko Sangyo Company, Ltd., Honcho, Japan), which consisted of two stone disks. As described previously (Wang et al. 2012b, Hoeger et al. 2013), the fiber suspension was continuously fed by gravity into the grinder rotating at 1,500 rpm. The zero gap between the two disks was determined right at the contact position before loading of fibers, in a dry state. The operating gap was set at −100 μm. The presence of fibers in the gap ensured that there was no direct contact between the two disks even at the negative setting during grinding. The resultant suspension of LCNFs was sampled periodically and the time-dependent energy consumption was recorded using a power meter (model KWH-3 energy meter; Load Control, Inc., Sturbridge, MA). To avoid mold growth, the samples were treated with Kathon CP/ICP II (Rohm and Haas Company, Bellefonte, PA) at a dose of 10 ml/liter fiber suspension and stored at 4° C until use.

Characterization of the Fibrillated Material

The morphology of the LCNF material was analyzed by a field emission scanning electron microscope (FE-SEM, 6400F; JEOL, Peabody, MA) operating at 10 kV. A few drops of LCNF suspension at approximately 0.1% solid content were air-dried onto clean silicon wafers and then fixed on carbon tape and coated with a layer of

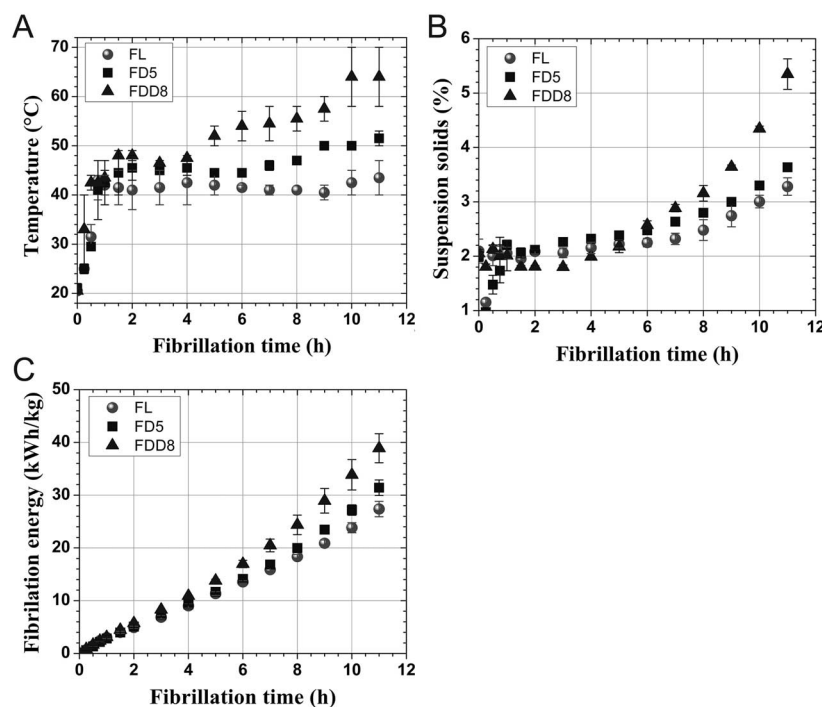


Figure 3. Relations among different mechanical fibrillation process variables. A. Time-dependent fibril suspension temperature. B. Time-dependent fibril suspension solids. C. Time-dependent mechanical fibrillation energy consumption.

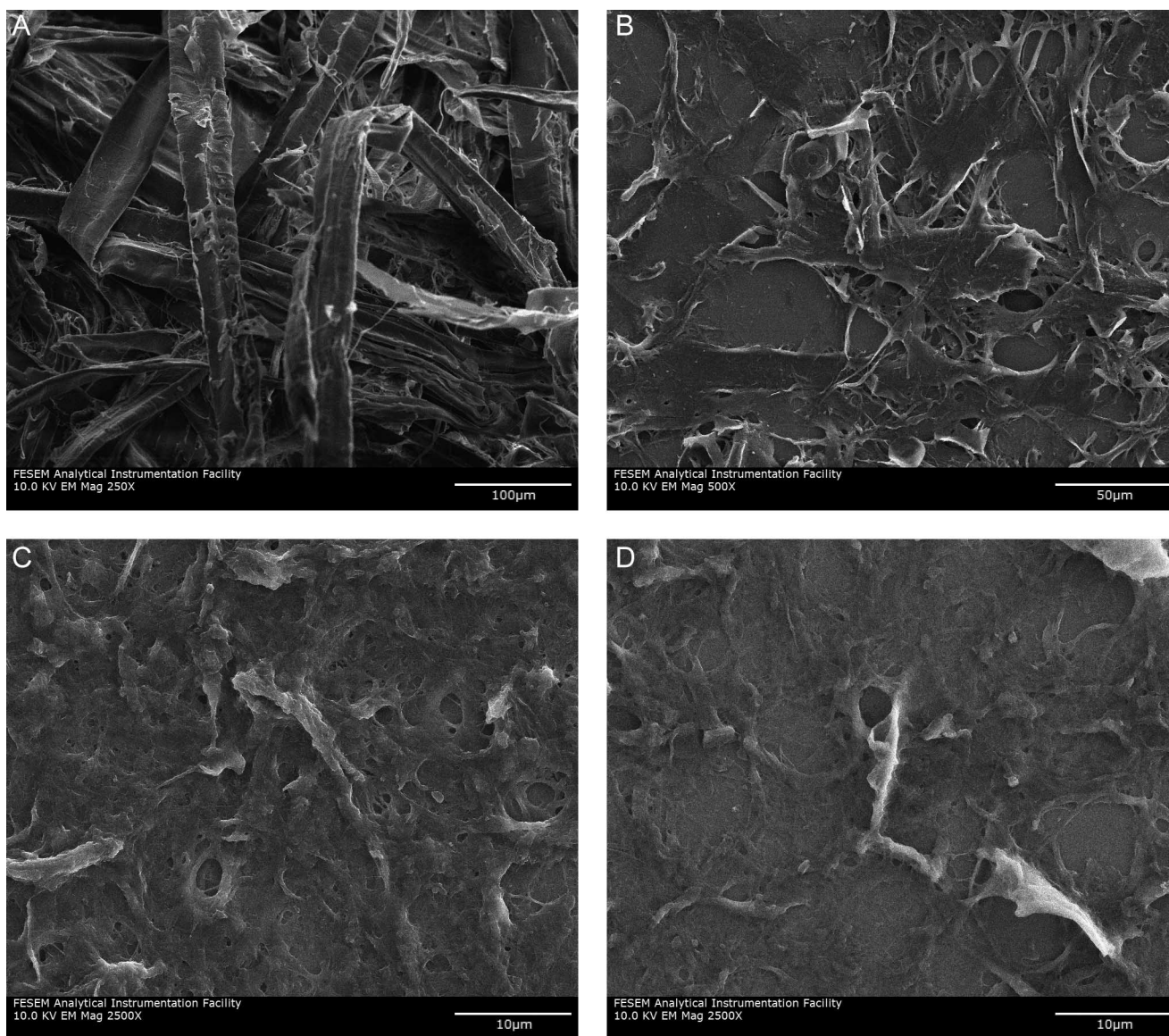


Figure 4. Scanning electron microscope images of fibrils from wood sample FL at different fibrillation times: $t = 0$ hours (A), scale bar = $100\ \mu\text{m}$; $t = 0.25$ hour (B), scale bar = $50\ \mu\text{m}$; $t = 6$ (C) and 9 (D) hours, scale bars = $10\ \mu\text{m}$.

gold/platinum. The diameter distribution was obtained from at least 300 fibrils randomly selected and analyzed using commercial image processing software (Revolution software, 4pi Analysis, Inc., Durham, NC).

The crystallinity of the LCNF samples were determined with an X-ray diffractometer (Rigaku SmartLab, Tokyo, Japan), equipped with a monochromator using copper $K\alpha$ radiation at 40 kV and 44 mA. The scans were performed at $5\text{--}50^\circ$ (2θ) with a step size of 0.05° and a count time of 15 seconds in each step. The crystallinity was calculated using the Segal method (Segal et al. 1959) with the intensity corresponding to the (002) crystal plane at 22.5° (2θ) and subtraction of the intensity of the amorphous contribution at 21° (2θ):

$$\text{Crystallinity} = [1 - (I_{\text{amorphous}}/I_{(002)})] \cdot 100 \quad (1)$$

Water Retention Value (WRV) of LCNFs

The WRV is an indirect measure of the total internal pore surface area including the interfibril surfaces, relevant to the substrate ac-

cessibility to cellulases (Luo and Zhu 2011). Suspension samples (4% solids) corresponding to 0.25 g of oven-dry mass were centrifuged at 900g force for 30 minutes (interactional centrifuge model EXD; International Equipment, Boston, MA). The centrifuged samples were weighed before and after oven drying (105°C) until they reached constant weight. The WRV was calculated as the % amount of water held by the centrifuged fibrils per unit oven-dry weight.

Cellulase Adsorption

The accessibility of each of the LCNF samples to cellulase enzymes was measured by the amount of enzyme adsorbed and was taken as indicative of the degree of cell wall deconstruction after mechanical fibrillation. A 0.1 g sample (oven-dry weight) of each LCNF sample was mixed with a commercial grade endoglucanase (Fibercare) at a loading of 50 mg of protein/liter in 110 ml of acetate buffer (pH 4.8) at 4°C . The amount of free cellulase in the fibril suspension was quantified by a UV-visible spectrometric method

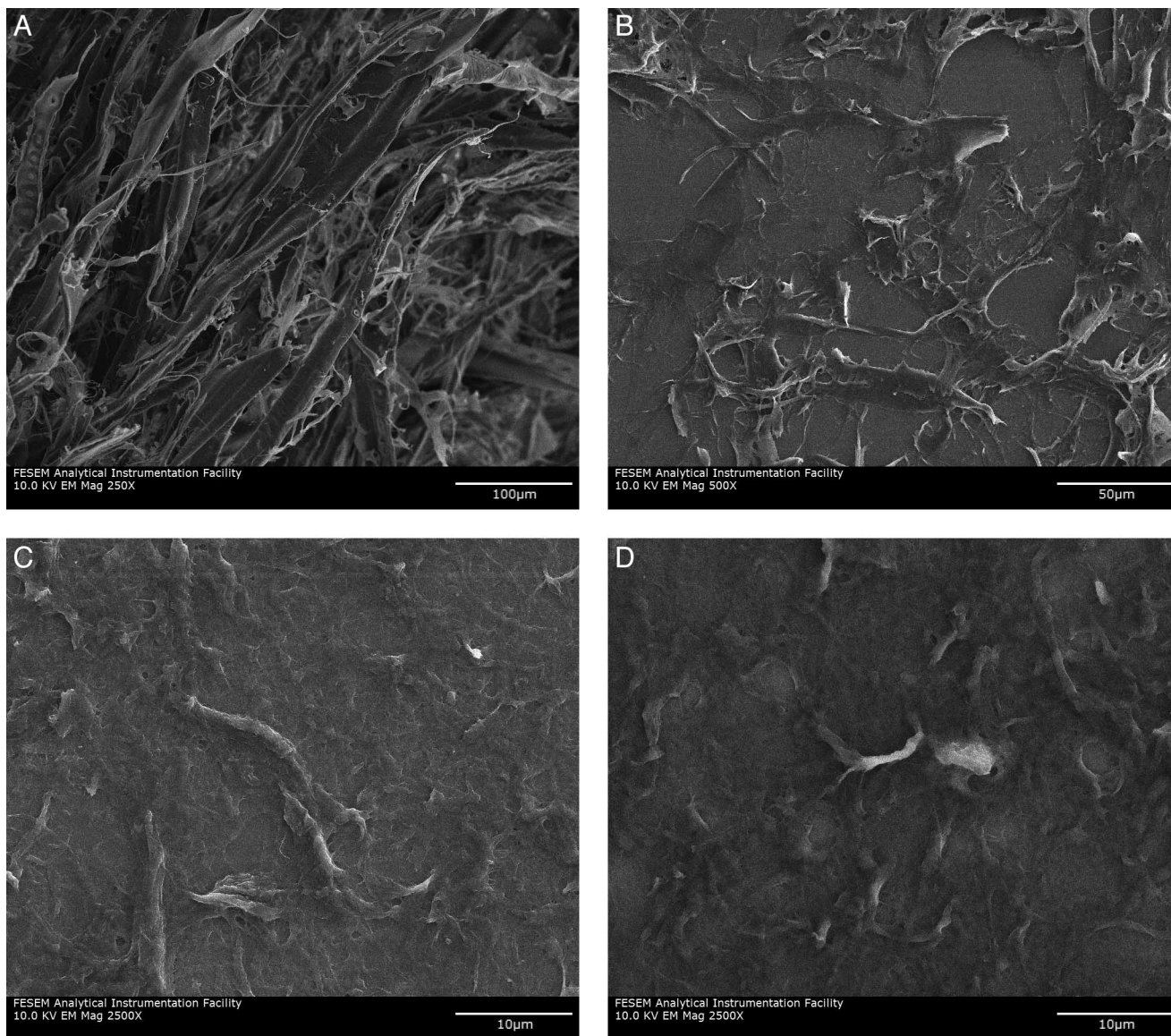


Figure 4 (Continued). Scanning electron microscope images of fibrils from wood sample FD5 at different fibrillation times: $t = 0$ hours (A); $t = 0.25$ hour (B); $t = 6$ (C) and 9 (D) hours.

(Liu et al. 2011, Wang et al. 2012a). The free enzyme concentration in the solution was continuously monitored by a UV-visible spectrometer (model 8453; Agilent Technologies, Palo Alto, CA) at 291 nm wavelength. The second derivate method (Liu et al. 2011) was applied to correct for spectral interferences from light scattering by small particles and absorption by lignin leached during experiments (Chai et al. 2001).

Results and Discussion

Characterization of the Prefibrillated Samples

The dead tree samples FD5 and FDD8 had a lower moisture content than the live tree, FL (Table 1). A higher gas permeability (Cai and Oliveira 2008) and higher moisture sorption capacity (Todoruk and Hartley 2011) have been reported for MPB-killed lodgepole pine compared with those for uninfested, stain-free wood. The higher permeability and higher sorption capacity may contribute to the low moisture content of the killed trees. Along with the

weaker structure of the dead wood chips, the high permeability and sorption capacity also facilitated mechanical refining for fiber production. This is clearly observed in the lower energy consumption measured for fiberization through atmospheric mechanical refining (Table 3).

No significant difference in the average length of the fibers after fiberization was observed; however, a significantly lower freeness (as measured by the CSF) of the FDD8 fiber suspension was apparent. This indicates that FDD8 fibers are capable of retaining more water, in accordance with the reported higher sorption capacity of wood obtained from MPB-killed trees than from live trees (Todoruk and Hartley 2011). The higher water sorption was attributed to the presence of the fungi (Todoruk and Hartley 2011). The higher fine contents (fiber fragments with length < 0.2 mm, not reported here) of the FDD8 sample may also be a factor contributing to the low CSF. The chemical composition can also affect fiber swelling capacity (Hill 2008), albeit our measurements indicated only a small difference in the chemical makeup of the chips compared with those

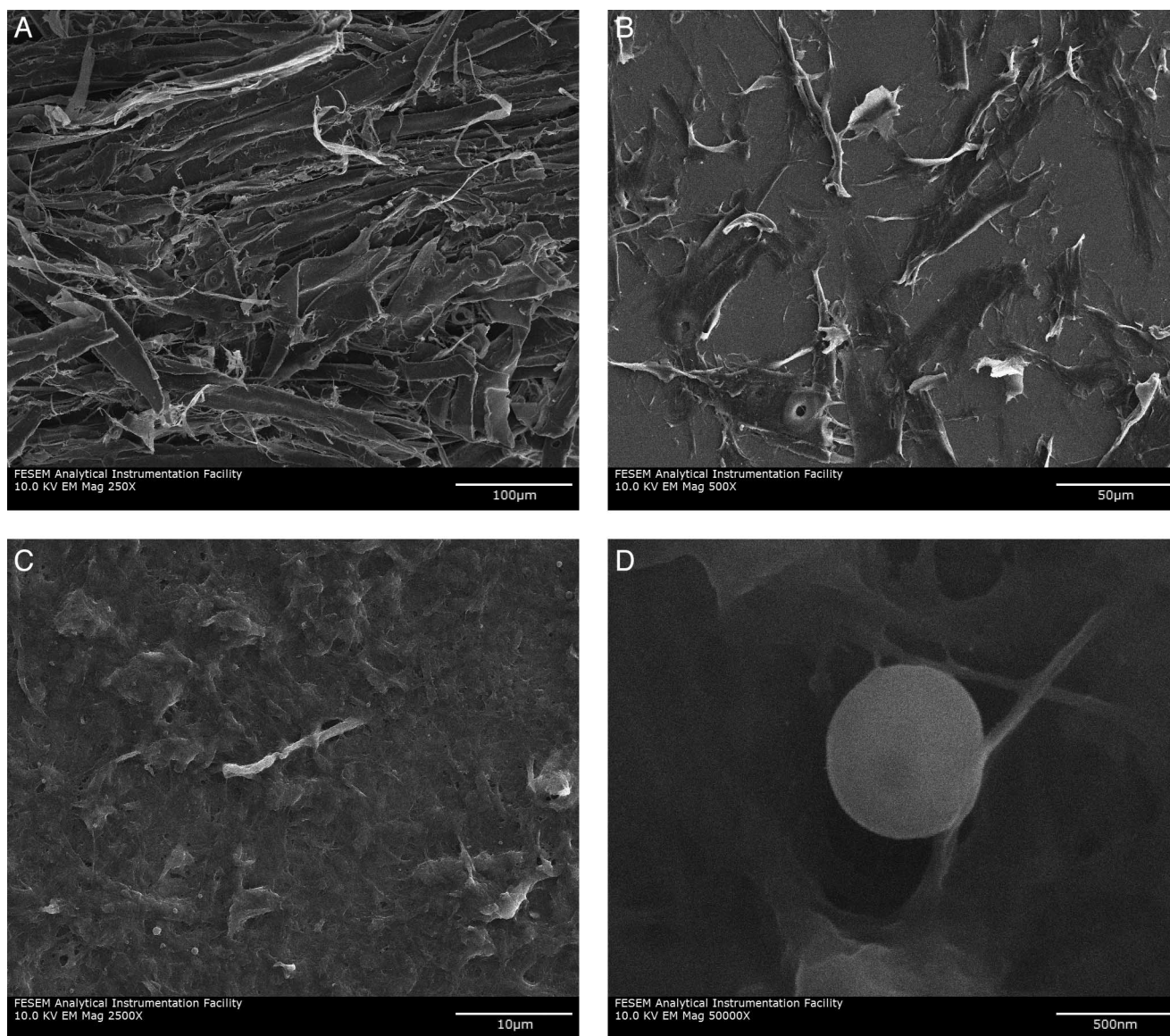


Figure 4 (Continued). Scanning electron microscope images of fibrils from wood sample FDD8 at different fibrillation times: $t = 0$ (A); 0.25 (B); 6 (C) and 9 (D) hours.

from the live (FL) and the dead (FDD8) trees (Table 2). It is interesting to note that the three chip samples have different color (Figure 2A, B, and C), which can be ascribed to the discoloration from fungi attack.

From the properties investigated so far, we concluded that the apparent difference among the samples is the higher swelling capacity of FDD8 fibers. More importantly, compared with FL samples, less energy is required to fiberize FDD8 through mechanical refining.

Energy Consumption during Mechanical Fibrillation (LCNF Production)

The energy consumed during mechanical fibrillation via grinding of the three different fiber suspensions follows a linear correlation with the fibrillation time (Figure 3A). The energy used to fibrillate FDD8 fibers was substantially higher than that used during processing of the FL fibers. It was noted that the temperature of the suspension during fibrillation increased to a larger extent using the MPB-killed fibers, especially FDD8, compared with that of the FL

fibers (Figure 3B). As a result, more water was evaporated during fibrillation, leading to higher effective solids content in the suspensions (Figure 3C). It appears that the measured consumption of mechanical energy during fibrillation closely correlates with the solids content, independent of the type of fibers in the suspension.

Properties of LCNFs

The three fiber samples experienced similar morphological changes throughout the fibrillation process (Figure 4). The fiber cell walls were deconstructed by the mechanical fibrillation, releasing substructural fibrils and nanofibrils. The diameter distribution of fibers and the nanofibrillated material after 11 hours indicates a significant reduction in characteristic size, down to the submicron scales (Figure 5A and B). The fibrillation of the lignin-containing fibers yielded coarser fibrils than those reported from bleached fibers, with no or very low residual lignin content (Hoeger et al. 2013). This finding is in contrast with results reported recently indicating that unbleached kraft fibers produced nanofibers of smaller width than those from bleached fibers (Spence et al. 2010,

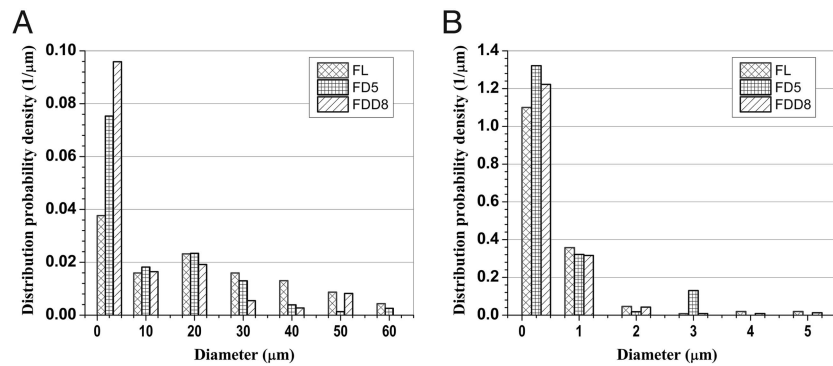


Figure 5. Diameter distribution probability densities of the initial pulp (A) and fibrillated samples (B).

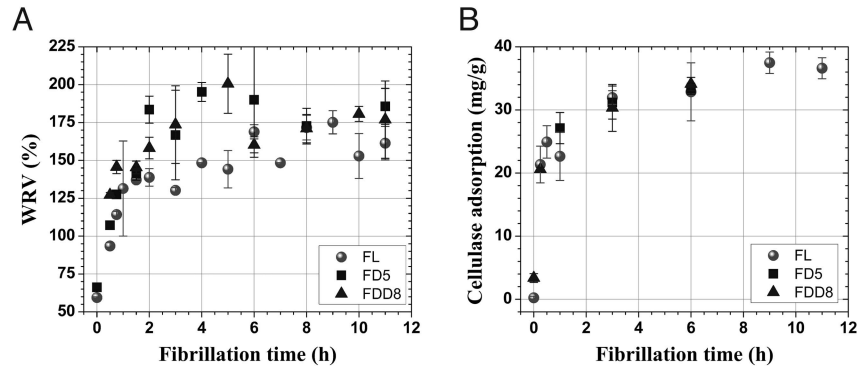


Figure 6. Effects of fibrillation time on the WRV (A) of the fibrils and the amount of cellulase adsorption onto the fibrils (B).

Ferrer et al. 2012b, Solala et al. 2012). However, the total lignin content of the fibers used in these reported investigations was very low (<3%), which is in contrast with the present study involving lignin contents as high as 30%.

FDD8 fibers contained more small-diameter fibers or fiber elements than the other fiber samples (Figures 4 and 5A); however, no significant differences were noted in the characteristic diameter distribution of the three LCNF samples after extensive fibrillation, yielding average fiber diameters in the submicron range (Figures 4 and 5B).

WRV was used to evaluate the degree of fibrillation. As the time of mechanical fibrillation was increased, the water absorption capability, as measured by the WRV also increased (Figure 6A). This result is in agreement with findings of a previous study (Iwamoto et al. 2005). Interestingly, FDD8 fibrils yielded a higher WRV during the first 4 hours of fibrillation. It appears that there was no difference between the fibrils from the live tree and those from MPB-killed trees after 11 hours of fibrillation. The observed differences are within the WRV experimental error. This finding is further corroborated by the maximum amount of cellulase that binds to the fibrils (Figure 6B), which indicated similar accessibility to cellulase enzymes or similar available surface area (Liu et al. 2011, Wang et al. 2012a). The WRV increased rapidly during the first 2 hours of fibrillation but then reached a plateau value of approximately 175%, probably because the extent of fibrillation reached a limiting condition for the given grain size and gap distance used in the process. Mechanical fibrillation also reduced the crystallinity of the fibrils by about 10% after approximately 10 hours of processing (Figure 7). This effect is in agreement with previously reported observations (Iwamoto et al. 2007). Again, no differences in crystallinity were

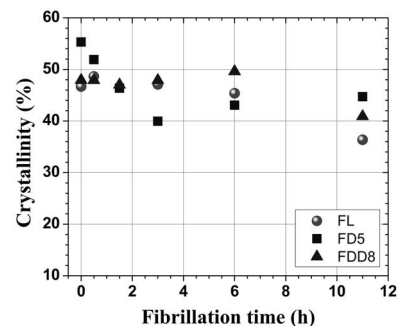


Figure 7. Effect of fibrillation time on the crystallinity of the fibrils.

observed between the sample produced from the live tree and those from the MPB-killed trees.

Conclusion

This investigation demonstrates that MPB-killed pine is a suitable raw material for the production of LCNFs. It is possible to obtain LCNFs with morphological and basic properties similar to those produced from other fiber sources but without any chemical, mechanical, or enzymatic pretreatment. The energy consumed during processing of MPB-killed trees was different from that for a live uninfested tree, but this did not translate into any substantial effect in the measured LCNF properties, such as fiber morphology, water retention, crystallinity, and available surface area. More importantly, the tree with advanced decay (FDD8), which has no value for lumber, produced lignocellulose nanofibrils similar to those from

the live tree. Further evaluation of the mechanical strength properties of the nanofibrils from the advanced decay tree will be conducted in a future study.

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