Enzymatic preparation of nanocrystalline and microcrystalline cellulose

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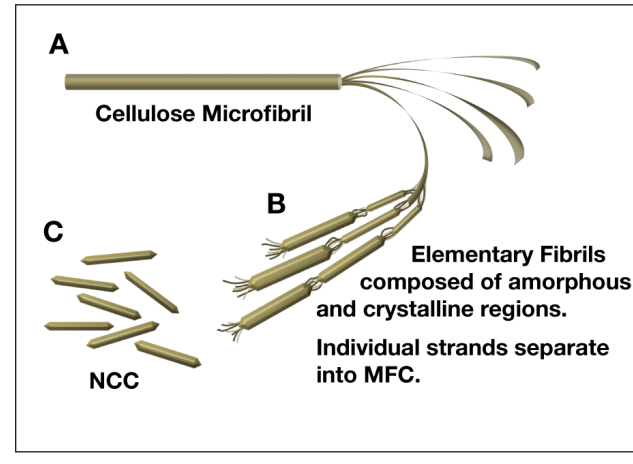
ABSTRACT: Traditional cellulose nanocrystal (CNC) production methods use harsh chemicals, are energetically expensive, and result in a hydrophilic sulfate surface chemistry with limited utility. Enzymatic production of CNCs is a less expensive alternative production method that eliminates the need for harsh chemicals and requires much less energy for mechanical fibrillation and heating. Furthermore, enzymes that selectively degrade the amorphous regions of cellulose fibers, and do not significantly digest the crystalline areas, result in CNCs that retain a hydroxyl group surface chemistry. Retention of hydroxyl groups allows for easier chemical manipulation, and thus an expanded commercial potential. Here we show that cellulase from *Aspergillus niger* is capable of producing CNC and microfibrillated cellulose (MFC) from well-solubilized kraft pulp feedstock with minimal processing, and that a chimeric cellulase partially digests kraft pulp and live wood feedstock. Additionally, we show that as a feedstock source, milled pulp from bug-killed dead and downed trees has significantly reduced energy requirements to process the feedstock into elementary fibers and MFCs when compared to live wood feedstock sources.

Application: Cellulase from *A. niger* can be used to reduce the energy and chemical costs of converting wood feedstocks into CNCs and MFCs.

Cellulose nanocrystals (CNCs) and microfibrillated cellulose (MFC) are renewable biopolymer nanomaterials with novel applications. Many of these unique attributes arise as a result of their nano-scale morphology. Depending on the composition, cellulose nanomaterials can be biodegradable and provide a more environmentally friendly alternative to plastics. Furthermore, because of the low density and high tensile strength, nanocellulose has the potential to lighten and strengthen packaging, and ultimately to reduce the reliance on petroleum-based plastics [1,2]. CNCs also have the ability to form a sol-gel and have been incorporated into scores of polymers, resins, and biopolymers to better improve strength over other composite materials [3,4]. CNCs also exhibit liquid crystal behavior when dried to a film because of the twisted chiral nature of individual CNCs [5]. This affords the potential for developing films and sensors. MFCs are also an ideal component in medical films and gels, largely because of its ability to absorb water and act as a prophylactic barrier. In fact, such MFC-based films are being explored for controlled drug delivery [6,7].

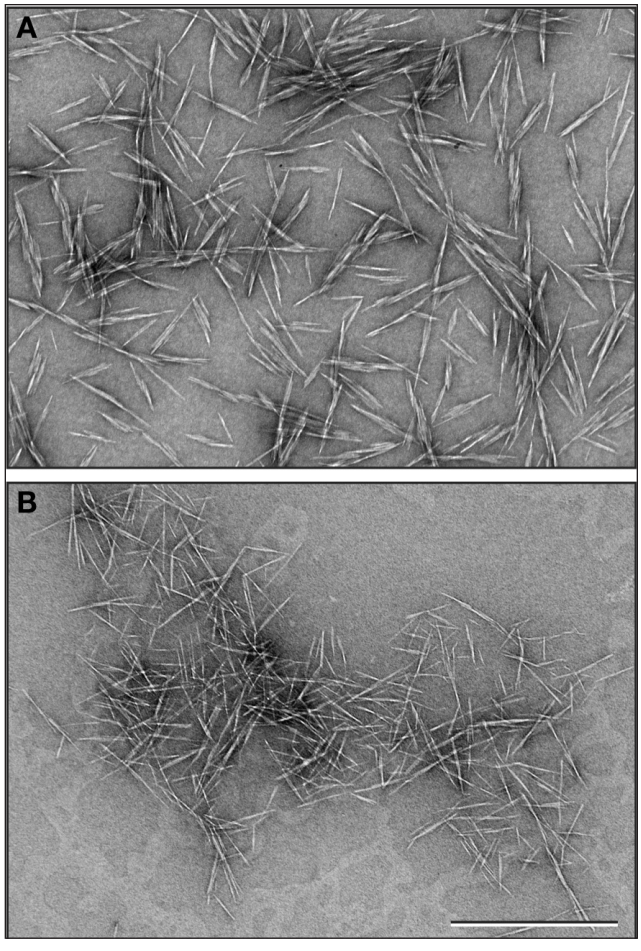
Cellulose elementary fibrils are composed of β-1,4-glucose linked chains that adopt a twisted morphology, where some regions pack tightly into crystals and alternate with amorphous regions distorted by the twisting fibril [8]. MFCs result when elementary fibrils are separated into long fibers of alternating crystalline and amorphous regions (**Fig. 1**). MFCs are generally about 10-100 nm in width and up to several microns in length [8,9]. The amorphous regions of elementary fibrils



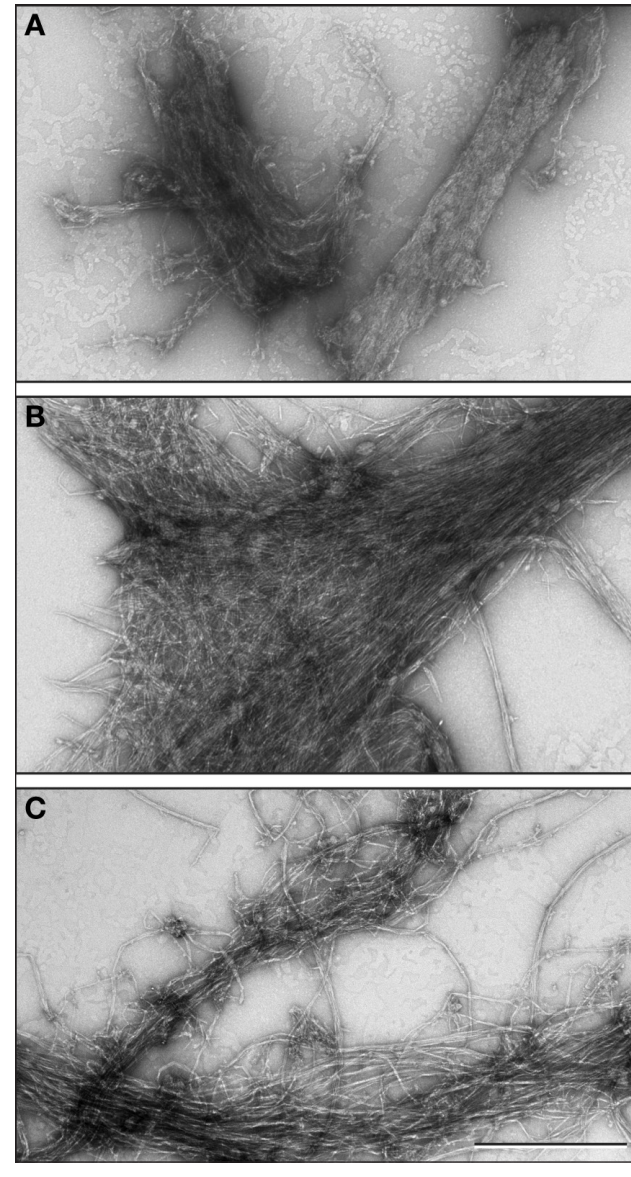
1. (a) Schematic representation of cellulose microfibrils composed of several elementary fibrils; (b) elementary fibrils consisting of individual strands of cellulose packed into crystalline and amorphous regions, forming microfibrillated celluloses; (c) digestion occurs preferentially in the amorphous regions leaving cellulose nanocrystals.

are more susceptible to acid hydrolysis, leaving the nanocrystalline regions intact as needle-like crystals [10]. The specific morphology of CNCs is dictated by the cellulose source, with wood feedstock producing CNCs with widths of about 5-10 nm and lengths of 100-200 nm [11,12].

The traditional method of producing MFCs begins with mechanical processing, which separates elementary fibrils from the larger cellulose microfibrils. The cellulose source



2. Transmission electron microscopy images of nanocrystalline cellulose products when fully bleached kraft pulp is treated using (a) sulfuric acid digestion and (b) ammonium persulfate digestion. Scale bar is 500 nm for all images in this figure.



3. Transmission electron microscopy images of feedstocks: (a) dry, fully bleached kraft pulp soaked 24 h and disc milled for five passes; (b) wood pulp from live trees, disc milled 1 h; and (c) wood pulp from bug-killed dead and downed trees (on the ground for several years) and disc milled 1 h. Scale bar is 500 nm for all images in this figure.

material must be milled through several passes, a process that uses much energy [9]. Alternative processing can include cryo-crushing, high pressure homogenization, or ultrasonication [13,14]. The processing requirements, and therefore energy costs, can be reduced by combining mechanical processing techniques with additional treatments such as 2,2,6,6-tetramethylpiperidine-l-oxyl (TEMPO) oxidation, chemical treatment with sodium hypochlorite or sodium bromide, or enzymatic pretreatment [9,15,16]. These methods result in the separation of microfibrils into MFC fibers. Costs can be further reduced by selecting inexpensive or byproduct feedstock sources.

Acid hydrolysis is the main process in the traditional manufacture of CNCs. Mechanical methods such as those discussed previously precede the acid digestion and improve solubility and dispersion of the fibers [12]. Removal of the amorphous regions can be achieved with a variety of acids, including sulfuric acid, hydrochloric acid, phosphoric acid, or ammonium persulfate. Sulfuric acid hydrolysis methods are fairly variable, but the general method involves heating feedstock material to 45°C-60°C in 55%-65% sulfuric acid for 2 h [12,17]. Acid hydrolysis using sulfuric acid results in highly soluble crystals with a charged surface of sulfate esters (**Fig. 2a**). CNCs with sulfate surface chemistry are useful in applications that require high solubility in aqueous solution; however, the surface chemistry is not conducive to covalent functionalization or incorporation into hydrophobic solvents, polymers, and resins. Acid hydrolysis using hydrochloric acid or ammonium persulfate treatment results in CNCs with poor aqueous dispersion with hydroxyl or carboxyl surface groups, respectively (Fig. 2b) [12]. However, the hydroxyl surface groups can be expanded using traditional organic

Enzyme	Source	Activity
Fibercare (*Trichoderma reesei* cellulase)	Novozyme	*Multi-enzymatic complex able to digest all states of cellulose into glucose.
Cellulase from *Aspergillus niger*	Sigma	*Hydrolysis of endo-1,4-β-D-glycosidic linkages in cellulose, lichenin, barley glucan, and the cellooligosaccharides cellotriose to cellohexaose, does not cleave cellobiose or p-nitrophenyl-β-D-glucoside. Cleaves glycosaminoglycan from a core peptide by hydrolyzing xylosyl serine linkage.
Chimeric EG A18 Cellulase from Termite	Protein Expression Laboratory, FNLCR	Endo-β-1,4-glucanase activity.
Thermostable β-Glucanase	Sigma	*Endo-xylanase, arabinoxylanase, β-xylosidase and β-glucosidase activities.
Thermostable β-Glucanase-2	Sigma	*Cell wall modifications and carbohydrate hydrolysis.
Cellulase from *Trichoderma longibrachiatum*	Sigma	*Xylanase, pectinase, mannanase, xyloglucanase, laminarase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase, and protease activity.
Thermostable cellulase from *Dictyoglomus turgidum*	Sigma	*Endo-cellulase, β-glucanase, β-mannase, exo-cellulase and cellobiohydralase activity.
Thermostable cellulase from *Clostridium thermocellum*	Sigma	*Endo-cellulase, β-glucanase, exo-cellulase and cellobiohydralase activities.

I. Enzymes screened for cellulose nanocrystal and microfibrillated cellulose production.
**** Activities are from the Sigma products pages. Available [Online] http://www.sigmaaldrich.com.***

chemistry techniques and incorporated into resins and plastics, whereas the sulfate esters produced by the sulfuric acid digestion are more difficult to chemically modify. These methods require mechanical grinding pretreatments, corrosive reagents, and heating to high temperatures for several hours, all of which are costly.

Enzyme production of CNCs is desirable because it does not require harsh chemicals, has lower energy requirements, and is environmentally sustainable. Some mechanical grinding is required as a pretreatment to liberate the cellulose into elementary fibrils for enzyme accessibility; the degree of processing depends on the feedstock. However, the energy usage is reduced by the need for less mechanical processing and by heating to lower temperatures. After the initial processing, the energy requirements are reduced to a 35°C incubation and gentle shaking. Enzyme digestion also reduces water consumption and hazardous waste by eliminating the need for concentrated acids, which must be removed with washing and properly disposed of after traditional production. CNCs produced using enzymes with endoglucanase activity have unmodified hydroxyl surface groups similar to CNCs produced using hydrochloric acid digestion.

In this work, we sought to achieve enzymatic production of uncharged nanowhiskers that were morphologically equivalent to CNCs produced via acid hydrolysis. We also wanted to minimize the use of harsh chemicals and energy to develop a more cost effective process. Enzymes with endoglucanase activity were examined for their ability to produce CNCs and MFCs from various feedstocks. Analysis of enzymatic function was monitored by a colorimetric assay for reducing ends produced by the enzymes, and the resulting cellulose morphology was monitored using transmission electron microscopy (TEM).

Kraft pulp and live and dead wood thermomechanically processed pulp feedstocks were examined and optimized for small and well-dispersed fibers before enzyme introduction. Several commercially available cellulase enzymes were explored for selective digestion of amorphous cellulose and the ability to produce the desired morphologies of 5-10 nm width and approximately 200 nm length for CNCs and several microns for MFCs (**Table I**). We focused on cellulases with endo-β-glucanase activity, which catalyzes the cleavage of internal bonds of cellulose chains. Fully bleached kraft pulp (FBK) (**Fig. 3a**) is a highly processed product treated with sodium hydroxide and sodium sulfide at high temperature. Heavy washing is required after treatment to remove sulfur-containing byproducts and to neutralize the pulp. Additional feedstocks were provided by the U.S. Department of Agriculture's Forest Products Laboratory as water suspensions. Feedstocks were thermomechanically processed pulps from either live source fibers (Fig. 3b) or dead source fibers (Fig. 3c) from bug-killed dead and downed trees that had fallen several years ago [18]. Dead wood is an inexpensive source of cellulose and achieves a looser fiber morphology with significantly less mechanical pre-processing than does live wood.

Trichoderma reesei fungus has been used to digest cotton

fibers into nanowhiskers [19]. This digestion using the whole fungus requires nutrient additives and results in significant protein contamination, both of which must be removed during the final processing. Additionally, an endoglucanase clone from *Aspergillus oryzae* has been used to digest filter paper fibers subjected to several pretreatments to successfully produce CNCs [20]. As with our experiments, highly processed feedstocks lead to better yields of CNCs. The methods of Xu et al. effectively remove the need for acids to produce CNCs, but the mechanical pretreatment is still costly, considering the processing required to produce filter paper and the pre-processing performed before enzyme digestion [20]. Henriksson et al., extensively studied the synergistic nature of mold cellulases and concluded that several cellulases are required to reduce cellulose fibers to glucose [16]. The role of the endoglucanases family of cellulases is to digest the less ordered regions of cellulose, making the crystalline regions more accessible to cellobiohydrolases, which are then able to digest the crystalline regions. Because of the interest in ethanol production from cellulose, cellulases and multicellulase systems that are able to reduce cellulose to glucose have received more attention. The ability to make CNCs or MFCs using enzymes could give new life to enzymes previously "rejected" by the biofuels industry because of limited activity [21]. We surveyed the activity of isolated proteins, cell extracts, and expressed termite cellulases for the ability to selectively digest amorphous and less ordered regions of cellulose to produce CNCs or MFCs using TEM to examine morphology (Table I) [22].

MATERIALS AND METHODS

Feedstock preparation

Fully bleached kraft pulp (FBK) was prepared by pre-soaking in double distilled water for 24 h, disc milled for five passes, and allowed to settle by gravity. Only the top milky fraction was used.

Thermomechanically processed pulps from live and bug-killed dead and downed trees that had fallen several years ago (dead wood) [18] were allowed to settle and the top fraction was used. The live wood feedstock was milled for 11 h, and the dead wood was milled for 1 h. The pulps contained all of the original components of the source material (hemicellulose, lignin, etc.).

All feedstocks were centrifuged at 5 × *g* for 5 min. The supernatant was centrifuged again at 30 × *g* for 10 min and the resulting supernatant was centrifuged at 100 × *g* for 10 min to yield the final supernatant used in the enzyme digestion.

Ammonium persulfate digested CNCs

CNC digestion was performed according to the method described by Leung et al. using FBK as the feedstock [23]. The resulting CNCs were spun down and washed with ultrapure water five times, and lyophilized into a white powder. The powder was reconstituted in ultrapure water to approximately 0.5 mg/mL for TEM imaging.

Enzyme preparation

The gene for chimeric termite EG-A18 cellulase [20] was synthesized in a form optimized for expression in *Spodoptera frugiperda* insect cells (DNA2.0; Menlo Park, CA, USA). The clone contained a C-terminal 6×His tag, and an N-terminal honeybee melittin signal peptide for secretion. The final verified clone was transferred to a Gateway-modified pFastBac vector with a baculovirus polyhedrin promoter. Bacmid DNA was generated by transposition using the Bac-to-Bac kit and the manufacturer's recommended protocols (Life Technologies; Carlsbad, CA, USA). Bacmid DNA was transfected into Sf9 cells to generate recombinant baculovirus, which was titered and used to infect *Trichoplusia ni* (Hi5) cells to produce recombinant protein. Infected cells were grown at 21°C for 72 h and media were collected for protein purification.

Cells from 2 L of culture supernatant were harvested by centrifugation at 1775 × g for 20 min. The supernatant was collected and filtered using a Sartobran P (Sartorius; Göttingen, Germany) sterile capsule (0.65 +0.45 µm). The filtrate was concentrated to 200 mL using two 0.1 m^2 Hydrosart (cellulose), 30 kDa cutoff ultrafiltration slice cassettes in a Sartocon Slice stainless steel holder (Sartorius). To maintain appropriate sample flow, PharMed BPT tubing (size 73, Masterflex; Gelsenkirchen, Germany) was used in a high-flow peristaltic pump (Masterflex). After concentrating, the protein solution was buffer exchanged with 2.5 L of 1× phosphate buffered saline (PBS), pH 7.2, amended to 50 mM imidazole, and applied at 1 mL/min onto a 5 mL immobilized metal ion affinity chromatography (IMAC) column (HisTrap, GE Healthcare; Little Chalfont, UK) equilibrated with equilibration buffer (EB) (1× PBS, pH 7.2, 50 mM imidazole). The column was washed to baseline with EB and proteins eluted with a 20 column-volume (CV) gradient from 50 mM to 500 mM imidazole in 1x PBS, pH 7.2. Elution fractions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie-staining. Protein-containing fractions were pooled, the pool dialyzed to 1× PBS, pH 7.2, and stored at -80°C.

Enzyme activity assay

Carboxymethyl cellulose was used as an analog to assay enzyme activity for the ability to cleave β-1,4-glucose linkages and produce reducing ends via the colorimetric tetrazolium blue assay [24]. Enzyme activity was assayed using 1% carboxymethyl cellulose or 0.05% feedstock solutions at 1, 5, 10, 15, and 20 min time points. Commercial enzymes (Table I) were diluted in water and termite cellulase was diluted in 0.1 M sodium acetate (NaOAc) buffer (pH 5.5), all to 20 ng/mL.

Enzyme digestion

Whole and optimized feedstocks were mixed to a final concentration of 1 mg/mL of commercial enzymes or termite cellulase and incubated at intervals from 6-72 h at 35°C before imaging. Samples were stored whole at 4°C after incubation.

TEM imaging

All samples were prepared on 200 mesh carbon film grids via drop casting and washed twice with double distilled water in the absence of buffer, or washed once with dilute acetic acid and once with double distilled water in the presence of buffer. Sample grids were then negative stained using 0.5% uranyl acetate and imaged using an H-7650 TEM (Hitachi; Tokyo, Japan) and Technai-12 TEM (FEI; Hillsboro, OR, USA).

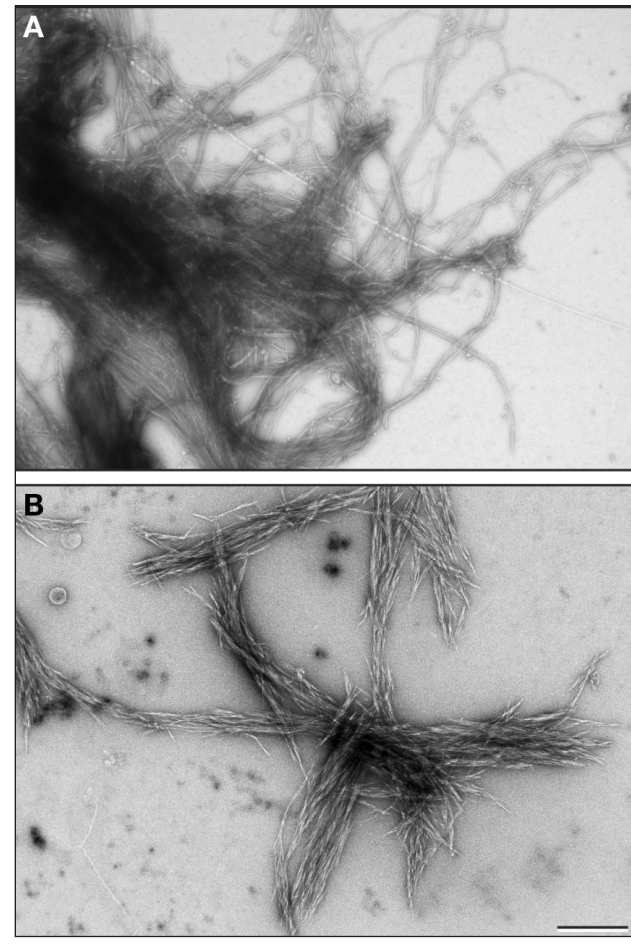
Yield estimate

Lyophilized FBK was reconstituted at 10 mg/mL in ultra-pure water and well suspended. *A. niger* cellulase was added to a concentration of 1 mg/mL and the solution was incubated at 35°C for 62 h. The solution was filtered through double-ply gauze and washed with an equal volume of double-distilled water. The eluent was then centrifuged for 1 h at 30000 × g at 25°C. The pellet (nanowhiskers) was lyophilized and weighed for yield estimation. The pellet and supernatant were both imaged by TEM as described above to reaffirm sample identity.

RESULTS AND DISCUSSION

Of the enzymes surveyed, *A. niger* cellulase most closely exhibited the desired activity, being able to produce MFCs (**Fig. 4a**) and nanowhiskers (Fig. 4b) upon treatment of FBK. On the live wood feedstock, *A. niger* cellulase resulted in a morphological change, though it had an incomplete digestion to nanowhiskers or MFC fibers (**Fig. 5a**). We were not able to observe any apparent change in the morphology of the dead wood feedstock after enzyme treatment (data not shown). This might have been because of other non-cellulose components present in the feedstock or because the dead wood feedstock has a much looser fiber structure, which would make enzyme effects more subtle when observed using TEM. The FBK and live wood feedstocks are much more compact in structure before enzyme treatment, making the morphological changes much more apparent. By varying the time of the incubation of *A. niger* cellulase with FBK, MFCs were produced and found at 6 h incubation (Fig. 5b) and CNCs were produced and found at 24 h and longer (Fig. 5c). Unlike CNCs produced by sulfate digestion, which are individually dispersed in aqueous solution, enzymatically produced nanowhiskers are poorly soluble and agglomerate, similar to CNCs produced by the hydrochloric acid method. The poor solubility is because cellulases simply cleave the internal glycosidic bonds of cellulase and do not chemically alter the surface hydroxyl groups. The resulting enzyme-digested fibers have the same surface chemistry as the source fibers.

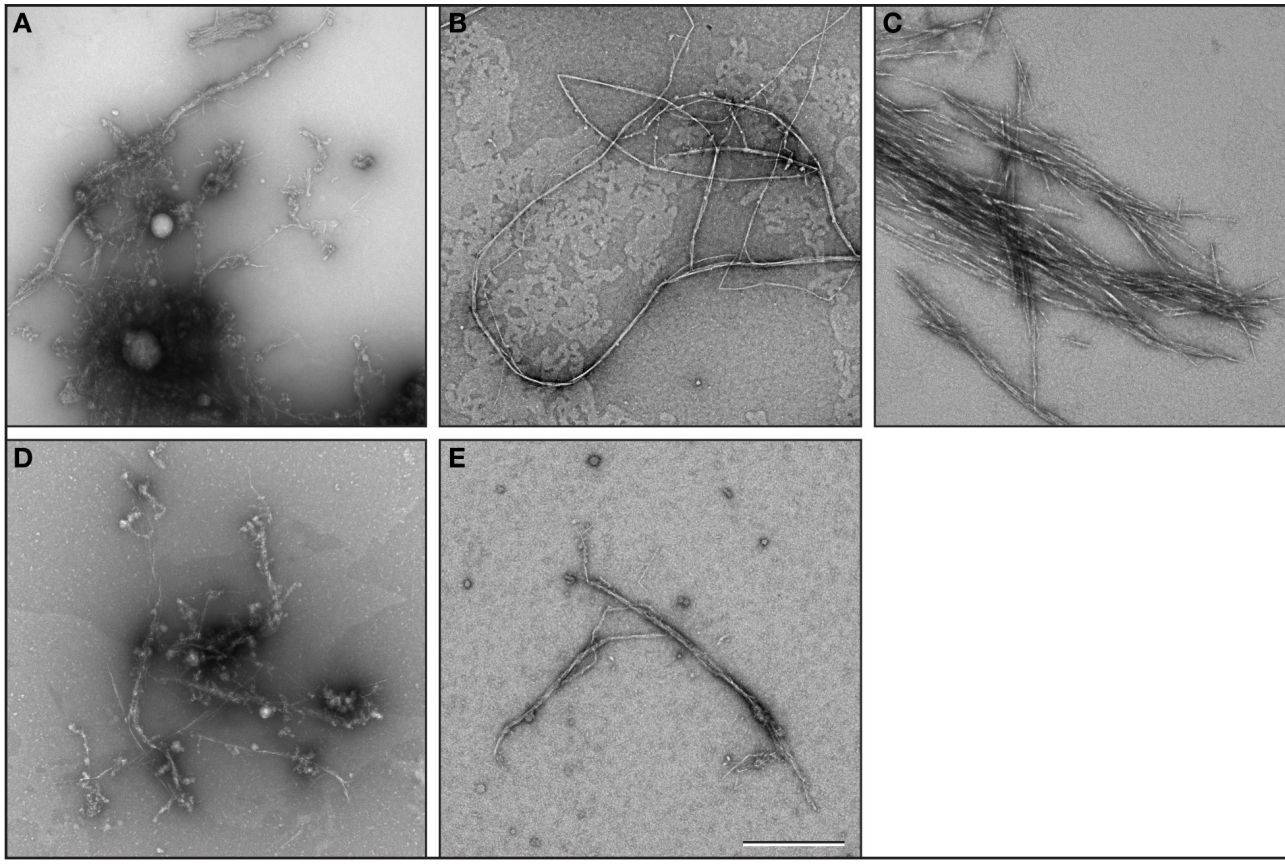
Chimeric termite A18 partially digested live wood feedstock (Fig. 5d), and caused a morphological change in FBK (Fig. 5e). Although some MFC-like fibers were present, the bulk of the feedstocks incubated with chimeric termite A18 were unreacted. This low activity might be because of poor accessibility to the substrate, as chimeric termite A18 does not have a cellulose binding domain.



4. Enzymatically produced (a) microfibrillated cellulose and (b) nanowhiskers. **Aspergillus niger** ***cellulose was used to treat FBK for (a) 6 hours, and (b) 24 hours. Scale bar is 500 nm and is the same for all images in this figure.***

Enzyme activity was determined using the tetrazolium blue assay because of its sensitivity into the nanomolar range. Commercial *A. niger* cellulase activity was calculated at 7.85 units/mg and chimeric termite A18 was 1.98 units/mg. When dilute feedstock solution was assayed, no activity was apparent. Pulps are opaque and could not be examined at a concentration higher than 0.05% because of interference in the colorimetric assay.

The other enzymes either digested the FBK and live wood feedstocks completely (Fibercare, *Trichoderma longibrachiatum*) or had no activity under experimental conditions (Thermostable β-Glucanase, Thermostable β-Glucanase-2, *Dictyoglomus turgidum*, *Clostridium thermocellum*). The dead wood feedstock appeared unreactive with all of the enzymes, including *A. niger* cellulose and chimeric termite A18 cellulase. As mentioned, the thermomechanically processed dead wood feedstock has an open fiber structure, and changes in morphology were not obvious by TEM when compared to the very apparent morphological changes observed in live wood and FBK digestions. The dead wood feedstock was milled for only 1 h before enzyme digestion and had a much



5.* Aspergillus niger *cellulase digestion on (a) live wood feedstock incubated for 24 h; (b) fully bleached kraft pulp (FBK) for 6 h; and (c) FBK for 24 h. Chimeric termite enzyme on (d) live wood feedstock for 24 h; and on (e) FBK for 24 h. Scale bar is 500 nm for all images in this figure.

more open fiber structure than the live wood feedstock processed for 11 h (Fig. 3b and c). The reduced milling time represents significant energy savings, and makes bug-killed dead and downed trees a promising feedstock source for MFC production. The apparent lack of enzyme activity for nanowhisker production might be because of the irreversible changes in wood cellulose structure after drying that make the cellulose fibers less accessible to enzymes [25]. However, this feedstock is the natural food source for termites and molds, and the lack of desired activity in these experiments highlights the complexity of natural multi-cellulase systems with different enzymes, possibly provided by different organisms, fulfilling different stages of cellulose digestion.

Pure mechanical fibrillation can also produce CNC-like cellulose nanowhiskers [26]. However, the energy costs are very high. It is clear from these experiments that increased feedstock pre-processing and hydration affect enzyme digestibility. Our experiments show proof of principle in the ability of cellulase from *A. niger* to produce nanowhiskers. The yield was estimated to be 10% weight nanowhiskers from a 62 h digestion at a ratio of 10:1 FBK to *A. niger* cellulase. Centrifugation of a gauze-filtered digested solution leads to a loose pellet, and TEM imaging confirms that some nanowhiskers and the majority of the enzyme remain in the supernatant. Although current yields are only about 10%, many areas are yet to be explored for further optimization of the enzyme digestion, including enzyme concentration, reaction times, etc. Further experiments should investigate enzymatic activity on other grades of pulp, such as semi-bleached and unbleached kraft pulp, and alternative pre-processing techniques to increase nanowhisker yields. Because pre-processing is the chemically and energetically expensive step, determining the least processed feedstock that is able to be enzymatically digested to CNCs could lower production costs. Furthermore, the crystallinity of the nanowhiskers should be verified by x-ray diffraction to ascertain whether they are true CNCs. Methods for post-digestion removal or deactivation of enzymes would also be needed, with final purity depending on the intended commercial use of the CNCs.

CONCLUSIONS

We have shown that cellulase from *A. niger* can produce MFC and nanowhiskers from well-solubilized kraft pulp feedstock with minimal processing and is able to produce MFC-like fibers from live wood feedstock with an estimated yield of

10% nanowhisker product. We have also shown that chimeric termite A18 cellulase digests kraft pulp and live wood feedstocks to create a morphological change of the fibers, but was not able to completely produce MFC or nanowhiskers. This might be because of the lack of a cellulase binding domain or because it is a single endoglucanase with activity that is secondary to another cellulase that first acts to open the fibers. Several variables contribute to the success of enzymatic digestion, and enzymes have the potential to be used synergistically, such as in the production of biofuels, to open and then selectively digest fibers. The selection of feedstock is nearly unlimited, as it is the most abundant natural polymer on the planet, produced in varying forms by plants, bacteria, and tunicates, and ideally, a byproduct feedstock such as bug-killed dead and downed trees would make the most sustainable candidate. In our experiments, we show that the enzymes surveyed do not appear to produce significant, observable changes to the dead wood feedstock fiber morphology. Mechanical pre-processing requirements depend on the cellulose source and present the biggest challenge in terms of manufacturing cost. Dead wood from bug-killed dead and downed trees is a promising feedstock to reduce production costs of mechanical MFC manufacture since it is inexpensive and can be milled into a much looser and open fiber structure with significantly less processing than live wood. **TJ**

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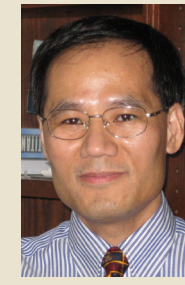

Zhu

The microfibrillated and nanocrystalline cellulose markets are rapidly expanding and poised to greatly affect the future of packaging and manufacturing for a range of consumer products. At the Nanotechnology Characterization Laboratory (NCL) we are interested in all things "nano" and their potential medical uses as prophylactic barriers and cancer therapeutic delivery, along with environmental health impacts, as microfibrillated and nanocrystalline cellulose makes its way into more consumer products. The Forest Products Laboratory's collaboration with NCL began in part to characterize the differences and potential uses of thermomechanically processed pulps from either live trees or from bug-killed dead and downed trees that had fallen several years ago that otherwise have no commercial use.

Previous research has explored enzymatic pretreatment of cellulose feedstocks as a precursor to mechanical and chemical manufacture of CNCs and the digestive activity of other mold-derived cellulases. In our research, we show that *Aspergillus niger* cellulase with endoglucanase activity can selectively degrade the amorphous regions of cellulose fibers in fully bleached kraft pulp and create nanowhiskers with little additional mechanical processing. Our exploration of bug-killed dead and downed tree feedstocks also showed that what is considered a waste product has potential to lower manufacturing costs of microfibrillated and nanocrystalline products.

Transmission electron microscopy (TEM) imaging is often biased and not a quantitative method, and TEM survey of cellulase products is very time intensive. Although we were able to see the desired products, we also had to support our observations of the desired nanowhisker morphology with an additional quantitative method. To do so, we separated the nanowhiskers from the undigested fibers and enzymes to confirm that our results were significant and to identify the yield, which we were pleased to find was near 10% by weight.

The most interesting finding was that *A. niger* cellulase is capable of producing nanowhiskers that are morphologically equivalent to CNCs produced by sulfuric acid digestion, and it is able to produce microfibrillated cellulose (MFC) from well-solubilized kraft pulp feedstock with minimal processing. Additionally, we show that as a feedstock source, milled pulp from bug-killed dead and downed trees has significantly reduced energy requirements to process the feedstock into elementary fibers and MFCs when compared to a live wood feedstock source.

The goal of our research was to find a method to reduce the energy and chemical costs of converting wood feedstocks into CNCs. Although enzymatic production methods are already used in pretreatment, we found that even though milled pulp from bug-killed dead and downed trees does not react with the surveyed cellulases, it presents a very cheap alternative feedstock source that requires much less mechanical processing and would reduce energy requirements in CNC production.

The next steps are to scale up digestion and perform x-ray diffraction to determine crystallinity index and confirm that the nanowhiskers are CNCs and to explore MFC production from bug-killed dead and downed tree feedstocks.

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