Using Simons stain to evaluate fiber characteristics of biomechanical pulps

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ABSTRACT: Aspen wood chips were treated with the white-rot fungus Ceriporiopsis subvermispora for four weeks and then refined through a single-disc mechanical refiner. Pulp obtained from the treated wood chips contained extensively fibrillated fibers. In contrast, pulp obtained untreated wood chips exhibited little fibrillation. The extensive fibrillation of the biomechanical pulp fibers appeared to be associated with improved paper strength properties and decreased electrical energy requirements during refining. Simons stain—a differential stain used to evaluate the degree of fibrillation in beaten fibers—was applied to fibers from both pulps. Fibers from the fungus-treated chips stained to a deep orange, indicating extensive fibrillation, while pulp from untreated control chips stained to a deep blue, indicating little fibrillation. The results demonstrate that Simons stain can serve as a rapid screening tool for evaluating the biopulping efficiency of different species and stains of fungi. The stain also can be used to determine the best pretreatment conditions for fungal strains that show promise as candidates for use in biomechanical pulping processes.

KEYWORDS: Biotechnology, Evaluation, Fiber analysis, Fibrillation, Mechanical pulping, Populus, Stain, White rot fungi

Pretreating hardwood or softwood chips with fungi prior to mechanical pulping results in significant improvements in paper strength properties and energy savings (*1-5*). Pretreatment of aspen wood chips with *Phanerochaete chrysosporium* reduced energy requirements for mechanical pulping by more than 40% while producing a pulp with superior strength properties (*3, 6*). For loblolly pine wood chips, pretreatment with *Ceriporiopsis subvermispora* improved burst and tear indices 30-60% over the untreated control while saving 21-37% electrical energy during refining (*2, 7*). Handsheets from these treated pulps had lower brightness but no change in opacity. However, the pulps responded well to peroxide bleaching and readily bleached to the same or higher brightness than untreated control pulps (*6, 8*).

Handsheet properties of biomechanical pulp are superior to those of thermomechanical pulp and are about equivalent to those of chemithermomechanical pulp (CTMP) (*9*). Biomechanically pulped wood fibers display unique characteristics when observed under electron microscopy. Such fibers tend to be more uniform in length, with greater fibrillation, and have a woolly appearance (*10*). These distinguishing characteristics reflect the superior strength properties and lower energy requirements of biomechanical pulps.

Simons stain is used in fiber microscopy to demonstrate the degree of fibrillation in beaten fibers (*11, 12*). Fibrillated fibers take on an orange color, while intact fibers are tinted blue. This report describes the use of Simons stain as a visual aid in identifying the degree of fibrillation of biomechanically pulped fibers. The differential stain also was tested for use as a rapid screening tool to evaluate the effectiveness of different species and strains of fungi as biomechanical pulping agents.

Results and discussion

Strength properties

Handsheets made from aspen wood chips pretreated with *C. subvermispora* were stronger than handsheets prepared from untreated control chips. **Table I** shows that burst index for the fungus-treated chips increased by 51%, while tear index increased by 67%. Electrical energy required to refine the pulps to 100 mL CSF was 40% lower for the treated chips. The superior strength is in agreement with results obtained in other studies (*9, 10*), where biomechanical pulps were judged to be comparable with CTMP, a high-yield pulp that produces paper with excellent strength characteristics.

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The superior strength of handsheets prepared from biomechanical pulp appears to be the result of the greater bonding capacity of the fungus-treated fibers. The increased fibrillation and other physical modifications of the fiber cell wall contribute to the superior bonding qualities. Previous work based on mechanical pulps pretreated with *P. chrysosporium* reported a softening of the fiber walls, ribbons of fibrils at fiber edges, a woolly appearance, and loose, less compact fibers (*10*). The results presented here show similar characteristics for wood after pretreatment with *C. subvermispora*. Such fiber characteristics appear to be unique to biomechanically pulped wood.

I. Comparison of 60-g/m² handsheets from unbleached refiner mechanical pulps obtained from untreated control chips and from fungus-treated chips

	Strength properties		Total refining energy,[a] W·h/kg
	Burst index, kPa·m²/g	Tear index, mN·m²/g	
Untreated control chips	1.03	2.54	1861
Fungus-treated chips[b]	1.56	4.25	1122

[a]Electrical energy required to produce pulp with freeness of 100 mL CSF.
[b]Treated with FP-90031-sp strain of *C. subvermispora*.

Simons stain as a visual aid

These structural characteristics can be easily observed and distinguished using the simple staining procedures described by Simons (*11*). Although Simons stain has not been used previously for this purpose, it has been used successfully to evaluate the condition of fibers that have been beaten for various time periods. Unbeaten fibers stain to a relatively uniform blue coloration, whereas beaten fibers are more fibrillated and stain orange (*11*). Fiber ends that are "broomed" stain an intense orange, with the color fading to blue within the interior of the fiber cell wall.

Refined pulps. Pulp fibers were refined to 100 mL CSF in a disc refiner and observed microscopically after application of Simons stain. **Figure 1** shows that the untreated fibers stained blue. The edges of these fibers exhibit only a few orange-stained regions, and fiber edges are distinct, showing very little fibrillation. The treated fibers, however, absorbed large concentrations of orange stain, as seen in **Fig. 2**. The ends of fibers and the edges of the fiber walls stained an intense orange-yellow, with the inner regions of the fiber staining orange or blue-green. Figure 2B shows the extensive fibrillation that is characteristic of biomechanically pulped fibers. Fungal pretreatment affects the woody cell wall in such a way that when the fiber is refined, the cut edges are frayed with fibrils that have been ripped from the wall. Fiber cell walls also appear modified and absorb increased quantities of orange stain.

Coarse pulps. Wood fragments were collected after one pass through a disc refiner and stained in an effort to determine whether the effects of biomechanical pulping could be observed early in the refining process. Untreated wood stained only a deep blue, as seen in **Fig. 3**. The results for *C. subvermispora*-treated wood chips were quite different, as seen in **Fig. 4**. After one pass through the refiner, the edges and cut ends of fibers from fungus-treated wood chips showed the same orange coloration that was apparent in the fibers of the highly refined pulp (Fig. 2).

Fungal pretreatment initiated changes in wood cell walls, resulting in a frayed fiber surface after only one pass through the disc refiner. The extraordinary amount of fibrillation is readily detectable with Simons stain and microscopic observation.

Simons stain as a screening tool

The Simons stain procedure is well suited for use as a simple, rapid technique for evaluating the effectiveness of different species and strains of fungi in biomechanical pulping. Since this method directly depicts the condition of the fiber, it should prove to be an effective screening method for choosing superior fungal isolates or for evaluating growth conditions and other variables that can be modified to optimize the biopulping process.

Several other strains of *C. subvermispora* were grown on aspen wood chips. These strains were evaluated to further establish the relationship between the degree of fibrillation and the strength properties and energy savings for biomechanical pulps. All strains provided similar amounts of energy savings and similar improvements in strength properties (*13*). Fibers from these biomechanical pulps were treated with Simons stain, and all showed the intense orange color characteristic of highly fibrillated fibers.

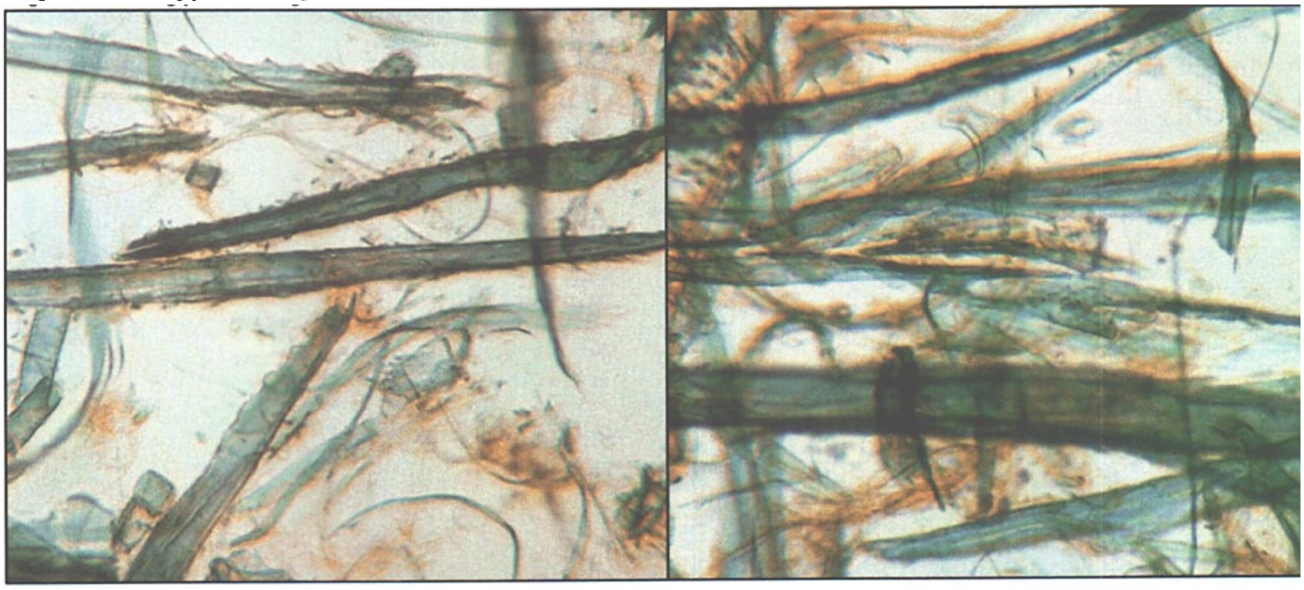
Many of the fungi that have been successful in biopulping are selective, lignin-degrading, white-rot fungi (*14–16*). Fungal pretreatments during biomechanical pulping are relatively short, and weight losses of only 4–7% are common (*2*). At these early stages of white-rot degradation, lignin degradation is relatively low. Previous investigation has shown no correlation between loss of Klason lignin and improvements in burst, tensile, and tear or with energy savings. At present, the mechanism that causes "softening" of the cell wall is unknown. Investigations focusing on the early stages of white-rot colonization and subsequent cell-wall modification are needed to identify the processes involved. Simons stain should prove to be a valuable tool for observing the sequential changes that occur within treated wood chips and the resulting biomechanical pulp.

Concluding remarks

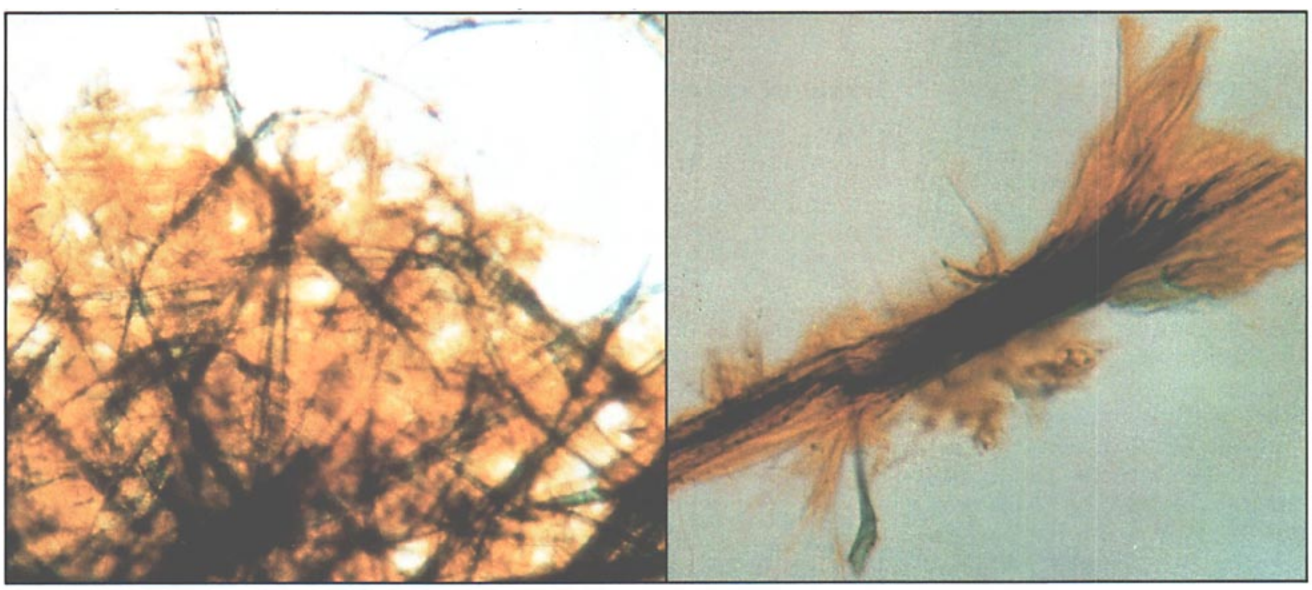
Biological pretreatments of wood chips with *C. subvermispora* can reduce the electrical energy required to produce refiner mechanical pulp. The resulting paper has greater strength and appears equivalent to paper made from the CTMP process.

Biomechanical pulp fibers have distinctive characteristics that promote interfiber bonding. These can be readily observed microscopically by treating fibers with Simons stain. The fungus appears to "soften" the wood so that the cut cell walls are frayed and ripped when they are cut by the disc refiner, resulting in extensively fibrillated fibers that stain orange. The cell wall also appears to be modified, as evidenced by the absorp-

1. Refined pulp fibers (100 mL CSF) from untreated wood chips after application of Simons stain. Fibers stained blue, with only a few fiber fragments showing yellow-orange.



2. Refined pulp fibers (100 mL CSF) from wood chips pretreated with *C. subvermispora* after application of Simons stain. Fibers show extensive yellow-orange stain, especially in fibrillated ends and edges. Inner regions of fibers are green to blue.



tion of the orange stain component within Simons stain. Untreated control wood chips (same incubation period and refining process) contain fibers that are not fibrillated, and these stain blue.

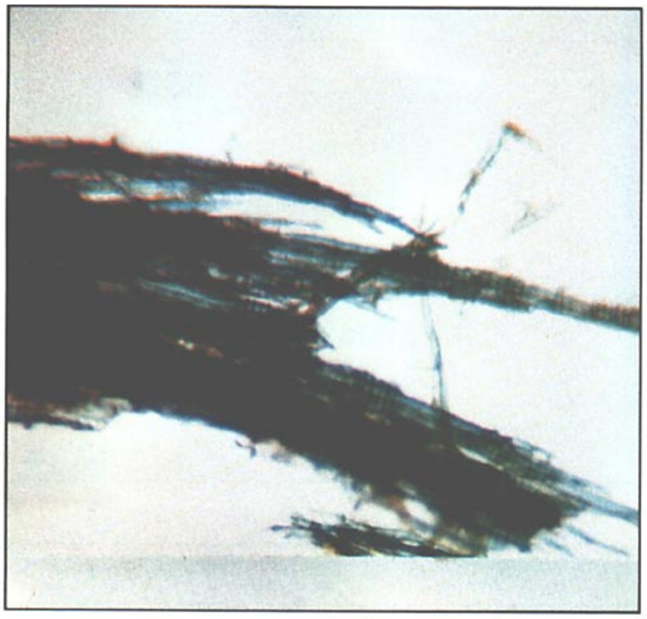
The distinguishing characteristics of biomechanical pulp fibers can be detected in the very early stages of refining. Samples of biopulped wood were passed only once through the disc refiner, and all cut edges stained a deep orange-yellow.

The results indicate that this simple and rapid staining procedure can be used to screen the effects of various fungi and treatments. The Simons stain method thus should prove to be a useful research tool in efforts to optimize the biomechanical pulping process.

Experimental procedures

Fungal pretreatment

Ceriporiopsis subvermispora (Pil.) Gilbn. et Ryv. was selected for pretreatment because of its superior ability to grow on aspen (*Populus tremuloides* Michx.) wood chips and its selectivity for lignin degradation as compared with other white-rot fungi (*15*). Strain FP-90031-sp of *C. subvermispora* was chosen because it was found to be superior to other strains evaluated during biomechanical pulping (*1*). This strain was obtained from the Center for Forest Mycology Research, Forest Products Laboratory (Madison, WI). The details about wood chips, seed inoculum preparation, chip preparation and bioreactor inoculation, chip fiberization, pulp refining, and handsheet production and testing have been described previously (*1, 3, 6*).



3. Coarse pulp fibers (>700 mL CSF) from untreated wood chips after one pass through a single-disc mechanical refiner and subsequent application of Simons stain. Dark blue stain of wood cells is evident.

4. Coarse pulp fibers (>700 mL CSF) from wood chips pretreated with *C. subvermispora* after one pass through a single-disc mechanical refiner and subsequent application of Simons stain. The edges and ends of the fibers show a deep yellow-orange stain, while the inner regions of large wood fragments are blue.

Pulp fiber analyses

Untreated control chips and *C. subvermispora*-treated chips were passed once through a single-disc mechanical refiner. Freeness for both pulps was over 700 mL CSF. The pulps were then further refined until freeness dropped below 100 mL CSF.

Fibers from the coarse pulp (one pass through the refiner) and the refined pulp were placed on microscope slides and covered with 1-2 mL of Simons stain. When the fibers were flooded with the stain, slides were heated at 60°C on an electrical slide warmer until the solution evaporated. A cover glass was placed over the fibers, which were then rinsed with distilled water until all excess stain had been removed. Fibers were microscopically examined and photographed immediately after preparation.

The stain consisted of 1% aqueous solution of Pontamine Sky Blue 6BX and 1% aqueous solution of Pontamine Fast Orange 6RN (Pylam Products, Garden City, NY) mixed in a 1:1 ratio. The mixture functions as a differential stain, with fibrillated or modified fibers turning orange, and nonfibrillated or undamaged fibers turning blue (*11, 12*).□

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