PROPERTIES OF FIBER MADE WITH BIOPULPED WOOD

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ABSTRACT

Biopulping, the process of pretreating chips with fungus before mechanical pulping, results in significant energy savings and improved sheet strength. It has been assumed that biopulping fungi cause these changes by enzymatic depolymerization of lignin. This work presents constituent sugar analyses and titrations that suggest hydroxyl radicals may be used by *Ceriporiopsis subvermispora* to accomplish biopulping. Increases in acid groups within the fiber account for some of the property improvements observed in biopulped fiber.

INTRODUCTION

Among wood decay fungi there are two broad classifications, white-rot and brown-rot. These classifications are generally based on observations of wood after extensive decay. Most white-rot species have the ability to degrade and mineralize lignin. In contrast, most brown-rot species only modify lignin and do not mineralize it. The traditional view is that brown-rot fungi use non-specific oxidants like hydroxyl radical while white-rot fungi use enzymes to degrade lignin.

By using phylogenetic analysis of representative DNA sequences, researchers have begun to provide an outline of the relationships among wood decay fungi[1]. Interestingly, white and brown-rot species are often intermixed on the phylogenetic tree. Furthermore, the data suggest that brown-rot species evolved from white-rot species. Although genetic changes that differentiate a white-rot species from a brown-rot species are not known, one can conclude that the enzyme systems that are genetically available to both kinds of fungi are very similar. The differences are more likely found in the regulation of gene expression.

Although simple classification of white versus brown-rot does not completely describe the diversity of action of all fungi[1, p121], it has provided guidance in selection of species for application in the pulp and paper industry. Since some species of white-rot fungi selectively remove lignin and leave cellulose intact, they and their enzymatic systems have been the subject of research directed at producing pulp and paper[2, p259].

During biopulping, a white-rot fungus, *Ceriporiopsis subvermispora,* is allowed to grow on wood chips for 10-15 days. When these chips are used to produce mechanical pulp, the refining requires 30-40% less energy and the sheet strength properties are improved. Lignin depolymerization by oxidative enzymes has been assumed to be responsible for biopulping. Since lignin degrading enzymes are typically 40-80kDa[2, p290], they are too large to penetrate the secondary wall due to limited pore size, even after a two-week biopulping treatment[3]. Thus, direct enzymatic action is unlikely. An alternate mechanism would be the production of low molecular weight diffusible oxidants [4, 5] as well as hydroxyl radicals [6, 7]. This alternate mechanism is consistent with cellulose depolymerization and lignin modification during the first 15 days of biopulping [8, 9].

Phylogenetic analysis has placed *C. subvermispora* among the polypore clade of the basidiomycetes[10]. As suggested above, this clade includes many closely related white and brown-rot species. Since brown-rot and white-rot fungi are genetically very similar, the initial white-rot mechanism may be similar to that identified in a brown-rot fungus where a low molecular weight diffusible hydroquinone reacts with iron(III) ions to create hydroxyl radicals [11]. This theory has been previously proposed based on chemiluminesent identification of hydroxyl radicals in the early stages of both white and brown-rot action[7, 12, 13]. If hydroxyl radicals are responsible for the biopulping effect, one would expect hydroxylation of the aromatic

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structures in lignin and depolymerization of carbohydrates [14, 15]. Both of these chemical modifications of the wood biopolymers would be expected to increase fiber saturation point (FSP). Indeed, previous work suggested that the FSP of wood was increased during biopulping.

The beneficial impact of acid groups on FSP and paper strength properties is well documented [16, 17]. Acid groups swell pulps because the acid counter ion is bound to the fiber and increases osmotic pressure inside the fiber. This draws water into the fiber. The production of acid groups and phenolic structures during hydroxyl radical attack may be similar to the chemical modification seen during cold soda treatments. In cold soda treatment, NaOH is added to chips before mechanical refining. The resulting deesterification increases acid group content and results in refiner energy savings and better sheet properties. Acid groups produced in the lignin, for instance by sulphonation, increase the plasticity of the lignin. This results in better bonding but not necessarily refiner energy savings.

The purpose of this study is to determine if hydroxyl radicals are responsible for the chemical and physical changes observed during biopulping. This paper presents data on acid group generation and carbohydrate depolymerization during biopulping.

EXPERIMENTAL

Treatment. Fresh chips of white spruce (*Picea glauca*), aspen (*Populus tremuloides*) and eucalyptus (50/50 mix of *E. grandis* and *E. salignia*) were placed in 20L bioreactors and decontaminated with atmospheric steam for 10 minutes. After cooling, water (final moisture of 50%), corn steep liquor (.05% on dry wood) and innoculum were added and incubated for 2 weeks. *C. subvermispora* (L14807-SS3) was added as blended mycelia (0.0005%) and grown at 27°. *Phanerochaete chysosporium* (RP-78) was inoculated with spores ($3x10^8$/kg) and grown at 39°. Samples were stored frozen.

Sugar analysis. Samples of control and *C. subvermispora.* biopulped *Pinus taeda* chips were milled to pass a 1 mm screen, and *ca.* 100 mg subsamples treated with 5.0 mL of water at 121°C for 1, 2, or 4 hr. All treatment solutions also contained 0.1 1% ethylene glycol. Aliquots of treatment extracts were filtered and acidified with H_2SO_4 for carbohydrate analyses. Residues were collected by filtration and analyzed for carbohydrate and lignin content. The hydrolysis and chromatographic conditions used are described elsewhere[18], except that the primary hydrolysis step was omitted in the case of the extracts. Standard deviation in sugar % of dry weight is 0.02%.

Acid group analysis. Chips were mechanically refined, then extracted following TAPPI T-264-88 with the substitution of toluene/ethanol(19:81 molar ratio) for benzene/ethanol. The extracted pulp was alternately soaked and rinsed in 0.1N HCl (total time 18 hours). After rinsing with millipore water, samples were freeze dried.

Conductivity titrations and analysis generally followed the method of Katz[19]. Titration of 1.4g pulp in 1L of 0.0015N KCl with 0.1N KOH (3ml/hr from syringe pump) was followed with pH and conductivity measurements. 20 µM of HCl was added at the start of the titration to extend the initial strong acid portion of the conductivity curve. The endpoints for strong and total acid groups are the intersections of the horizontal minimum value line with the initial downward slope line and the final upward slope line as demonstrated in Figure 1. Two or more runs were performed for each condition.

OBSERVATIONS

Sugar Analysis

Sugar analyses shown in Table I indicate that the biopulping fungus consumes mannose, glucose, and xylose from the wood and makes galactose. This suggests that the fungus selectively solubilizes glucomannans, glucouronoxylans, and arabinoxylans during initial colonization of the wood. Table 2 reinforces this pattern, showing that hemicelluloses, especially xylose, are more easily extracted after

biopulping treatment. The data shown are for a 4 hour extraction; land 2 hour extractions followed the same pattern.

The hemicelluloses could be solubilized by depolymerization or by an opening of the cell wall matrix. Depolymerization would be a significant product of fungal hydroxyl radical production, and due to the high solubility of hemicelluloses, relatively few chain scissions would be needed. Opening the cell wall matrix would require releasing crosslinks or increasing the swelling force in the cell wall. Generation of acid groups would swell the cell wall and facilitate solubilization.

Table I: Original P. taeda wood composition (% dry wt)

	Arab	Gal	Rham	Glc	Xyl	Man	Tot Carbos
control	1.24%	2.06%	0.16%	41.54%	6.49%	11.01%	62.5%
C.s.	1.20%	2.14%	0.17%	41.31%	6.29%	10.71%	61.8%

	Arab	Gal	Rham	Glc	Xyl	Man	Tot Carbos
control	69.6%	20.9%	33%	0.59%	4.94%	5.88%	4.09%
C.s.	89.1%	43.4%	39%	1.61%	19.3%	17.7%	9.45%
ratio	1.3	2.1	1.2	2.7	3.9	3.0	2.3

The significant extraction of arabinose and galactose is likely due to acid hydrolysis of these groups from the polymer backbone during the water extraction. These are typically very acid labile.

Titrations

Table 111 shows that the biopulping treatment clearly increased the number of acid groups bound to the cell wall. Published data[16] indicate an increase in breaking length of 17% to 20% for spruce and aspen, respectively, with a 25 meq/Kg increase in acid groups. Though high electrolyte concentrations diminish the swelling effect of acid groups, it is still strong. For instance, at 0.5M NaCl, the swelling effect is still at least 50% of that observed in deionized water[20, 21]. Thus it appears that this increase in acid groups could be a significant contributor to the improvement in paper strength observed in biopulping.

The refiner energy savings is better understood by looking at FSP. In one study[16], the increase in FSP of black spruce and aspen was 12% and 6% respectively for a 25 meq/Kg increase in acid groups. Refiner energy savings should be expected from the fraction of those acid groups present in carbohydrates.

Table III: Acid groups determined from conductometric titrations

	Fungus	Strong Acid	Total Acid		
			Control	Biopulped	Change
Extracted Spruce	*C.s.*	29	96	121	25
Extracted Aspen	*C.s.*	27	80	112	32
Unextracted Eucalyptus	*C.s.*	12 control/21 Treated	52	74	22
Extracted Eucalyptus	*P.c.*	13	56	59	3
Units: meq/Kg pulp, pooled standard deviation =6					

The lack of acid group generation in extracted eucalyptus inoculated with *P. chrysosporium* suggests a different mechanism might be at work in this fungus, even though it accomplishes biopulping just as well as *C. subvermispora.* There is also the possibility that the acid group generation in *C. subvermispora* is contained in the remnants of hyphae left in the fiber.

Figure 1 shows typical pH and conductivity curves under identical conditions. The pH curve for treated pulp is shifted to the right, clearly showing the increase in acid groups. The final pH after addition of 3ml

of KOH was consistently lower for treated spruce and aspen pulps. We attribute this to increased need for neutralization and screening charges in treated pulp.

The conductivity curves show a longer weak acid (flat bottom) region in treated samples than control samples as well as a shallower outgoing slope due to a higher proportion of charges going into the pulp for screening. The flat bottom portion of the curve corresponds to pH values from 5 to 9.5.

The main product of hydroxyl radical attack of wood is expected to be hydroxylation of aromatic rings. Therefore we expect to see deprotonation of phenol type structures in the titration curves. Phenol and guaiacol both have pKa values of 9.8-10, so at first glance their presence is questionable. However, Donnan equilibrium states that all ions, including potassium and hydroxyl, will be disproportionately drawn into the pulp. This will result in an increased effective pH and lower apparent pKa.

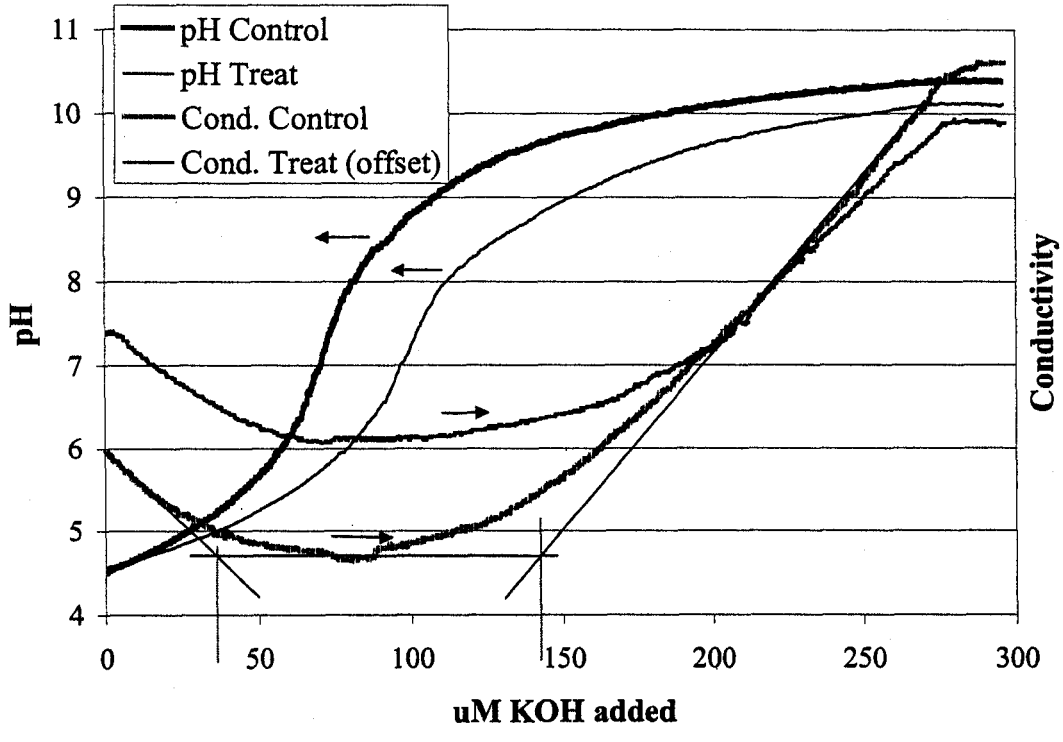

Figure 1. pH and Conductivity curves for two spruce pulps under identical conditions. Initial slope, final slope, and minimum line are drawn on the conductivity curve for control pulp. Strong and weak acid concentration are determined by the intersections on the respective left and right side of the minimum line.

CONCLUSIONS

Fiber from wood treated with *C. subvermispora* has a significant decrease in mannose, glucose, and xylose, as well as a significant increase in solubility of all hemicellulose components. At the same time, acid groups increase by approximately 25meq/Kg. The additional acid groups explain some of the benefits seen in biopulping treatment. Hydroxyl radical production by *C. subvermispora* is a plausible method of generating these changes in wood.

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