

# Understanding Wood Chemistry Changes During Biopulping

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## ABSTRACT

Biopulping is the process of pretreating chips with fungus before mechanical pulping, resulting in significant energy savings and sheet strength improvements. This work presents sugar analysis, methylene blue adsorption, and titration data suggesting an increase in acid group content in wood is common with biopulping treatment. Some discussion of possible mechanisms of acid generation and fungal self-protection is presented.

## INTRODUCTION

The main advantages of biopulping are similar to those of cold soda treatment: reduced refiner energy and increased mechanical properties in the resulting pulp. The effects of cold soda are due to acid group generation from the deesterification of hemicellulose (1). This study investigated whether increases in acid group content may be responsible for the chemical and physical changes in wood during biopulping as well.

Enzymes are often identified as the decay agents in white rot fungi, which includes biopulpers, while hydroxyl radical is the primary degradation tool of brown rot fungi (2). Since enzymes are too large to penetrate sound wood (3), fungi must use small diffusible agents during initial decay to open up the wood structure. Brown rot fungi appear to have evolved from white rot fungi (4), suggesting that white rot fungi may have a competent system for production of extracellular hydroxyl radical. This has been supported by evidence of extracellular hydroxyl radical production in white rot fungi (5-7). If biopulping fungi produced hydroxyl radical, one would expect hydroxylation of the aromatic lignin structures and depolymerization of carbohydrates (8). Both of these chemical reactions would generate acidic sites in the wood, which increases the fiber saturation point (FSP) of the wood and sheet properties of the pulp.

There are many other possible routes to acid generation on pulp as well. Oxalic acid, known to crystallize on hypha as calcium oxalate, provides several possibilities. Oxalate radical decays to formate radical, which could add to wood forming a terminal acid group. Alternatively, esterification of one end of oxalate ion (or other dicarboxylic acid) onto any alcohol in the wood would leave the free acid end bound to wood substrate.

Acid groups produced by oxidative depolymerization of carbohydrates or oxidation of aldehydes could be accomplished with manganese peroxidase, laccase with a mediator, or other oxidants such as peroxy radical.

We suspect that biopulping fungi might use a mechanism of hydroxyl radical production as has been shown in the closely related brown rot fungus *Postia placenta* (2). Constant acid production might curtail radical production immediately adjacent to the hypha, protecting the fungus, and still allow the reaction to proceed at a distance. Calculations in this paper show that a pH gradient to accomplish this is very unlikely.

## METHODS AND MATERIALS

### Treatment

Fresh chips of white spruce (*Picea glauca*), aspen (*Populus tremuloides*), white pine (*Pinus taeda*), and eucalyptus (50/50 mix of *E. grandis* and *E. salignia*) were placed in 20-L incubators and decontaminated with atmospheric steam for 10 min. After cooling, water (to make final 50% solids), corn steep liquor (0.05% on dry wood), and inoculum were added and incubated for 2 weeks. SS3 (*Ceriporiopsis subvermispora* L14807-SS3) was added as blended mycelia (0.0005% w/w) and grown at 27°C. BKM and RP78 (*Phanerochaete chrysosporium* BKM and the monokaryot daughter strain RP-78) were inoculated as spores ( $5 \times 10^8$ /kg) and grown at 39°C. Pulp was atmospheric refined to 750 freeness (CSF) and stored frozen. All of these fungi produce consistent energy savings and strength improvement during biopulping.

### Sugar analysis

Samples of control and SS3 treated white pine chips were milled to pass a 1-mm screen and ca. 100 mg subsamples were treated with 5.0 mL of water at 121°C for 1, 2, or 4 h. All treatment solutions also contained 0.11% ethylene glycol. Aliquots of treatment extracts were filtered, acidified with H<sub>2</sub>SO<sub>4</sub>, and hydrolyzed for carbohydrate analyses. Residues were collected by filtration and analyzed for carbohydrate content. The hydrolysis and chromatographic conditions used are described elsewhere (9), except that the primary hydrolysis step was omitted in the case of the extracts. Standard deviation in sugar % of dry weight was 0.02%.

### Methylene blue analysis

Known weights of pulp were placed in 50-mL centrifuge tubes with 5 mL 1M borate buffer (pH 8.5), five clean steel balls, and mixed to break clumps. Aqueous methylene blue (35 mL, 1mM) was added and tubes tumbled for 1 h. Samples (1.5 mL) were removed and centrifuged at 20,000 × g for 5 min. Two dilutions of 0.5 mL into 5 mL H<sub>2</sub>O and 0.125 mL 1N HCl were made and vortexed. Two (0.2-mL) samples from each dilution were analyzed at 610 nm in a UV/VIS microtiter plate reader. Micromoles of dye adsorbed by pulp equalled  $35 \cdot (1 - A_{\text{sample}}/A_{\text{blank}})$ .

## Acid group titration

Refined chips from a single pass (750 mL CSF) were aused. Extracted samples followed Tappi T-264-88 with the substitution of toluene/ethanol (19:81 molar ratio) for benzene/ethanol. All pulps for titration were then alternately soaked and rinsed in 0.1N HCl (total of 18 h). After rinsing with millipore water, samples were freeze dried.

Conductivity titrations and analysis generally followed the method of Katz (10). Titration of 1.4 g pulp in 1 L of 0.0015 N KCl with 0.1 N KOH (3 mL/h from syringe pump) was followed with pH and conductivity measurements. HCl (20 mmol) 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 (see Fig 2). Two or more runs were performed for each condition.

## Wood pH

Wood blocks approximately 3 cm on a side were removed from incubators at various intervals and frozen. When all blocks were collected, they were thawed and the end grain smoothed with a microtome knife. The blocks were then squeezed in a vise until water pooled on the end grain surface. The pH of this solution was measured with a flat pH probe.

## RESULTS AND DISCUSSION

### Sugar analysis

Data in Table 1 indicate that the biopulping fungus SS3 decreased mannose, glucose, and xylose content in the wood but increased galactose. This suggests that the fungus degrades glucomannans, glucuronoxylans, and xylans during initial colonization of the wood. Data in Table 2 reinforce this pattern, showing that hemicelluloses, especially xylose, are more easily extracted after biopulping treatment.

	Arab	Gal	Rham	Gluc	Xyl	Man	sum
control	1.24	2.06	0.16	41.54	6.49	11.01	62.5
SS3	1.20	2.14	0.17	41.31	6.29	10.71	61.8

**Table 1.** Wood composition, *P. taeda* (% dry wt)

	Arab	Gal	Rham	Gluc	Xyl	Man	sum
control	69.6	20.9	33	0.59	4.94	5.88	4.09
SS3	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	2.3

**Table 2.** % of total wood sugar as analyzed in table 1 extracted with 121° water, 4hrs

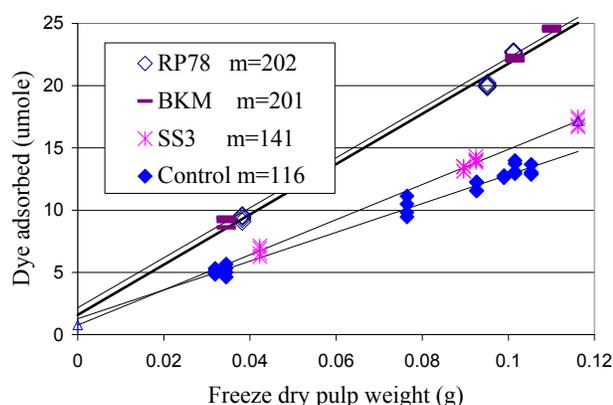
The hemicelluloses could be solubilized by depolymerization or by an opening of the cell wall matrix. Depolymerization would be a significant product of fungal hydroxyl radical production, and due to the high solubility of hemicelluloses, relatively few chain scissions would be needed. Opening the cell wall matrix

would require releasing crosslinks or increasing the swelling force in the cell wall. Generation or deposition of acid groups would swell the cell wall and facilitate solubilization.

The significant extraction of arabinose and galactose is likely due to hydrolysis of these groups from the polymer backbone during water extraction. These are typically labile. All the patterns observed in 4 h extraction (Table 2) are present in the 1- and 2- h extractions (not shown).

### Methylene blue adsorption

Methylene blue dye has a positive charge that associates with acid groups on pulp. The slope, *m*, represents micromoles of acid groups per gram of pulp. According to this analysis, *Ceriporiopsis SS3* increased acid groups by 25  $\mu\text{mol/g}$ , and the two *Phanerochaete* strains BKM and RP78 increased acid groups by 85  $\mu\text{mol/g}$  (Fig. 1).

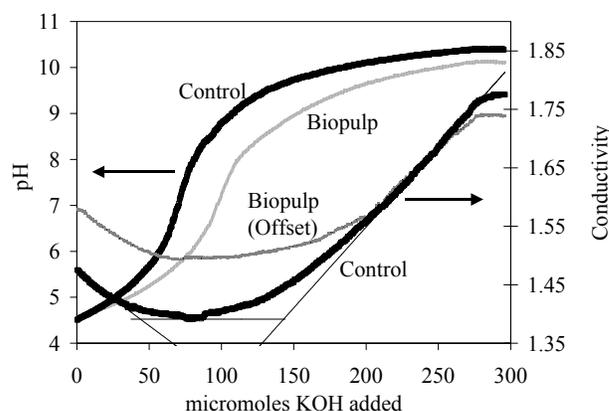


**Fig. 1.** Methylene blue (acid groups) vs. pulp weight for aspen with three different biopulping fungi.

An increase of 25  $\mu\text{mol/g}$  has been shown to increase breaking length 17% to 20% for spruce and aspen, respectively (1). Tensile strength is often increased 25% in biopulping (11-13), but this is variable depending on fungal and wood species. In those cases where tensile strength is improved, acid group generation would be a reasonable explanation of the cause.

### Titration

Figure 2 shows typical pH and conductivity curves for control and biopulped wood fiber under identical conditions. The pH curve for treated pulp clearly shows an increase in acid groups relative to the control. The final pH after addition of 3 mL of KOH was consistently lower for treated pulp than for control in both spruce and aspen. We attribute this to increased acid groups and the subsequent need for more ions from solution to form an electric double layer. The number of ions needed to screen acid groups is unknown, making these electrostatic effects difficult to quantify.



**Fig 2.** 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 concentrations are determined by the intersections on the respective left and right side of the minimum line. Biopulp = SS3 2 weeks.

The conductivity curves show a longer weak acid (flat bottom) region in treated samples than control samples as well as a shallower outgoing slope. The straight line extrapolations show how acid groups are quantified in conductometric analysis. The theory of the analysis is that in the initial stages, added KOH has the result of replacing strong acid protons in water with potassium ions of lower mobility, thus reducing conductivity. In the flat zone, weak acid groups in the pulp trade a proton for a  $K^+$ , resulting in no net change in conductivity. Finally, in the 3<sup>rd</sup> region the acid groups are consumed and added base goes into solution. The slope of the conductivity curve at high pH is not equal for control and treated pulps. This indicates that there may be acid groups in the pulp with pKa values in the 9–10 range that are not estimated in this method. Hydroxyl radical addition on aromatic lignin should yield phenols with a pKa of approximately 9.9. A recent comparison of acid group analyses (14) also suggests that the conductometric titration fails to detect phenolic groups. An increased electric double layer effect would also result in a lower outgoing slope.

Table 3 shows the number of acid groups determined by conductometric titration. As with methylene blue, there is usually an increase in number of acid groups with biopulping. Methylene blue results on aspen with SS3 showed an increase from 116 to 141  $\mu\text{mol/g}$ , a difference of 25. Though the absolute values from conductometric titrations are about 30% less, the trends agree. The effect of RP78 is clearly not the same. Acid groups might have been generated and then blocked by termination. We hope to find the cause of this discrepancy through further measurements. The mechanical properties were not tested

	Fungus	Strong Acid	Total Acid		
			Control	Bio-pulp	Change
Extracted Spruce	SS3	29	96	121	25
Extracted Aspen	SS3	27	80	112	32
Unextracted Eucalyptus	SS3	12 control/ 21 Treated	52	74	22
Extracted Eucalyptus	RP78	13	56	59	3

**Table 3:** Acid groups determined from conductometric titrations, ( $\mu\text{mole/g}$  pulp), pooled standard deviation =6 for this material, and lower than normal improvement could correlate with reduced acids.

### Self-protection mechanisms

Since the fungus is likely producing small diffusible oxidants, one obvious question is: How does the fungus avoid degrading itself along with the wood? According to the mechanism for Fenton hydroxyl radical production described by Jensen (15), hydroquinone (produced by the fungus) reacts with  $\text{Fe}^{3+}$  to make  $\text{Fe}^{2+} + \text{H}^+$ . Subsequent semiquinone reaction with  $\text{O}_2$  leads to  $\text{H}_2\text{O}_2$  and a quinone that is reduced by fungal enzymes. The peroxide and ferrous iron make hydroxyl radical.

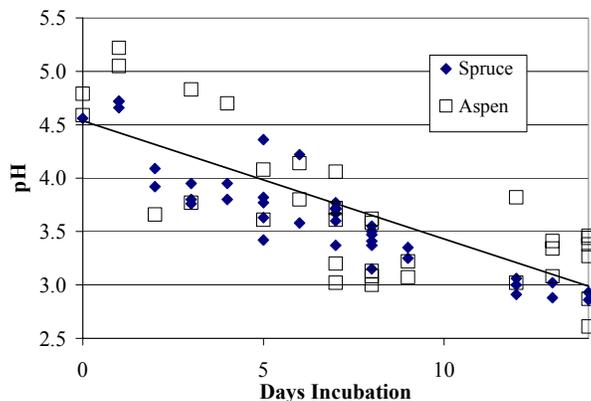
Because a proton is produced at the iron reduction stage, a high proton concentration would reduce the rate of this reaction. We surmise that a fungus that maintained a lower pH near the hypha would then be protected relative to the higher pH wood. There is, however, a competing shift of  $\text{Fe}^{3+}$  species towards a less chelated state at high acid that favors reduction. A second protection mechanism would be an oxygen gradient, which would slow the formation of  $\text{H}_2\text{O}_2$  at the lowest oxygen concentrations, presumably at the fungus.

Let us consider what would be needed to maintain a pH gradient sufficient to protect the hypha at the 1-week time point. In the first week, pH drops (roughly) from 4.5 to 3.8 (Fig. 2). Given 50% solids and a specific gravity of 0.4, this translates to proton production of  $10^{-14}$  mol/cm<sup>3</sup> of wood/min. Microscopic cross sections revealed hypha in 75% of the cells at this time. Assuming 10% of the visible hypha are active and 30- $\mu\text{m}$  wide cells, there is 1 active hypha per 12,000  $\mu\text{m}^2$  of cross section. A 1  $\mu\text{m}$  long  $\times$  1  $\mu\text{m}$  wide segment of this hypha would then produce  $10^{-14}/12000 = 1.2 \times 10^{-22}$  mol/min of protons.

The diffusion constant of  $3 \times 10^{-5}$  cm<sup>2</sup>/min at 300 K (16) for sodium in 10% NaOH traveling tangentially through saturated wood is a gross overestimation, but it serves as a starting point for discussion. Using Fick's law of diffusion,  $J/D = dc/dz$ , we have  
 Flux,  $J = 1.2 \times 10^{-22}$  mol/min/ $\mu\text{m}^2$   
 Diffusion constant,  $D = 3 \times 10^{11}$   $\mu\text{m}^2$ /min  
 Gradient,  $dc/dz = 4 \times 10^{-34}$  mol/ $\mu\text{m}^4 = 4 \times 10^{-19}$  mol/ $\mu\text{m}$ .

We assume that the hypha has cell wall degradation within 10  $\mu\text{m}$  of the growing tip, and would need to be at least 0.3 pH units lower to be effective in slowing the reaction. At pH 4 this translates to a gradient of  $10^{-5}$  mol/ $\mu\text{m}$ .

Clearly the diffusion constant for acid in the conditions of fungal decay is far lower than for  $\text{Na}^+$  as measured, but there are 14 orders of magnitude between the gradient in this rough calculation and the minimum necessary to produce the desired effect. We find it very unlikely the fungus produces a proton gradient strong enough to provide such a self-protective mechanism.



**Figure 2.** Change in pH with *Ceriporiopsis subvermispota* SS3 treatment

Oxygen must diffuse through a myriad of tunnels and cracks of the wood to get to the living fungus. The fungus consumes oxygen in order to conserve sufficient energy to grow. A mucilaginous layer is often observed in cultures and biopulped wood, which will likely slow the diffusion of oxygen to the fungus. These observations taken together would provide a potential gradient of oxygen where it would be lowest adjacent to the fungus. This would provide a better environment for quinone reduction and lessen the potential for immediate reoxidation.

## CONCLUSION

The evidence in this paper suggests that the acid group content of wood is often increased during biopulping, which is consistent with increased mechanical properties of the resulting paper. There are conditions, however, when tensile strength of biopulped paper is no different from that of the control, and in one case we observed no change in acid group content with biopulping. It would appear that acid group generation is a common but not universal effect of colonization by biopulping fungi. We expect that those conditions that produce large quantities of acid groups will also produce the best strength enhancements in the resulting paper.

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