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## Kraft pulping and ECF bleaching of *Eucalyptus globulus* pretreated by the white-rot fungus *Ceriporiopsis subvermispora*

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**ABSTRACT.** *Eucalyptus globulus* wood chips were decayed by the lignin-degrading fungus *Ceriporiopsis subvermispora* as a pretreatment step before kraft pulping. Weight and component losses of wood after the biotreatment were the following: weight (5%), glucans (1.5%), xylans (4.3%), lignin (5.7%) and extractives (57.5%). The residual amount of lignin (expressed by the kappa number) in pulps from biotreated wood chips was lower than that of pulps from the undecayed control. Depending on the delignification degree, kraft biopulps presented similar or up to 4% increase in pulp yield and 20% less hexenuronic acids (HexA) than control pulps. The extended delignification with O<sub>2</sub> decreases approximately 50% of the kappa number of the pulps and increases brightness, but had no effect in HexA reduction. The bleaching steps with chlorine dioxide (D<sub>0</sub>ED<sub>1</sub> sequence) decreased the kappa number up to 97%, increased pulp brightness up to 84% ISO and decreased HexA amount up to 91%. The use of *C. subvermispora* in biopulping of *E. globulus* generated important benefits during the production of kraft pulps that are reflected in a high pulp yield, low residual lignin content, low HexA amount, high brightness and viscosity of the biopulps as compared with pulps produced from untreated wood chips.

**Keywords:** biopulping, *Ceriporiopsis subvermispora*, *Eucalyptus globulus*, kraft pulping.

## Polpação kraft e branqueamento ECF de *Eucalyptus globulus* pretratado pelo fungo de degradação branca *Ceriporiopsis subvermispora*

**RESUMO.** Cavacos de madeira de *Eucalyptus globulus* foram degradados pelo fungo degradador de lignina *Ceriporiopsis subvermispora* como uma etapa de pré-tratamento antes da polpação kraft. As perdas de massa e de componentes da madeira depois do biotratamento foram as seguintes: massa (5%), glicanas (1,5%), xilanas (4,3%), lignina (5,7%) e extrativos (57,5%). A quantidade de lignina residual (expressada como número kappa) nas polpas de madeira biotratada foi menor que nas polpas de madeira controle não degradada. Dependendo do grau de deslignificação, as bio-polpas kraft apresentaram rendimento de polpa similar ou até 4% maior e com 20% menos ácidos hexenurônicos (HexA) que as polpas controle. A deslignificação estendida com O<sub>2</sub> reduz em aproximadamente 50% o número kappa das polpas e aumenta a alvura, mas não tem efeito na redução de HexA. As etapas de branqueamento com dióxido de cloro (sequência D<sub>0</sub>ED<sub>1</sub>) diminuem o número kappa em até 97%, aumentam a alvura das polpas até 84% ISO e diminuem o conteúdo de HexA em até 91%. O uso de *C. subvermispora* na biopolpação de *E. globulus* gera benefícios importantes durante a produção de polpas kraft que se traduzem em alto rendimento de polpa, baixo conteúdo de lignina residual, baixo conteúdo de HexA, alta alvura e viscosidade das bio-polpas quando comparadas com polpas produzidas desde cavacos de madeira não tratada.

**Palavras-chave:** biopolpação, *Ceriporiopsis subvermispora*, *Eucalyptus globulus*, polpação kraft.

### Introduction

Biopulping is the pretreatment of wood chips with lignin-degrading white-rot fungi (WRF). The so-called "biotreated wood chips" were further used in mechanical or chemical pulping processes where several benefits of this biological pretreatment have been reported for both softwoods and hardwoods (AKHTAR et al., 1998; MENDONÇA et al., 2002, 2004; FERRAZ et al., 2008). Biopulping can be

carried out in closed bioreactors or in open Wood chip piles, depending on the requirements of the particular microorganism would have for optimal results (AKHTAR et al., 1998; FERRAZ et al., 2008). The partial degradation and structural modifications of native lignin are considered the main characteristics that facilitate its removal and wood softening in the subsequent pulping processes. Mendonça et al. (2002) showed that high-yield kraft pulps from *Pinus taeda* wood chips pretreated with

*Ceriporiopsis subvermispora* were obtained with less active alkali in cooking liquor than the needed to produce pulps from untreated wood at same kappa number. Wood extractives removal and lignin depolymerization observed during wood biotreatment were related with the benefits observed in the biokraft pulping.

The benefits of biopulping observed in the different works found in literature varied according to the species, pulping process and conditions used, therefore, its impact for a given combination of wood, fungus and degree of delignification should be evaluated. Usually, for bleachable-grade pulps (pulps with low kappa number), the reduction in alkali charge and the increase in pulp properties were less expressive when compared with high-yield chemical pulping or thermomechanical processes (MENDONÇA et al., 2002; FRANCO et al., 2006; VICENTIM et al., 2009). However, additional benefits not yet evaluated could be found and also be important for the overall pulping process. These benefits could be related with some specific characteristic of the pulp after cooking that could have some impact in the further bleaching process, i.e., reduction in chemicals consumption and/or increase of optical properties of pulp, pulp viscosity and reduction of hexenuronic acids content. Some of these benefits were not yet evaluated and are reported in this paper.

In the present work, we reported the evaluation of the biotreatment of *Eucalyptus globulus*, the main hardwood species used by the Chilean pulp and paper industry, with the biopulping fungus *Ceriporiopsis subvermispora* and its effects in the kraft delignification and elementary chlorine free (ECF) bleaching of pulps.

## Material and methods

### Chemicals

All chemicals used were of analytical grade, except where indicated, and used as received without further purification. Malt extract (Fluka, Germany), soybean peptone (Fluka, Germany), ethanol (Merck, Germany), toluene (Merck, Germany), sulfuric acid (Merck, Germany), sodium tetraborate (Sigma-Aldrich, Germany), sodium hydroxide (Merck, Germany), galacturonic acid (Fluka, Germany), mercuric chloride (Sigma-Aldrich, Germany), sodium acetate (Sigma-Aldrich, Germany), magnesium sulfate (Sigma-Aldrich, Germany), m-hydroxydiphenyl (Sigma-Aldrich, Germany), sodium sulfite technical grade (Merck, Germany).

### Wood preparation and biodegradation

Wood chips from 10-12 years old *E. globulus* were obtained from a pulp mill located in the Bío-Bío Province, Chile. Wood chips were air-dried in a room acclimatized at 27°C until 12% moisture and stored in dry conditions. Before fungus inoculation, wood chips were immersed in tap water for a 16h period. Residual water was drained and 2.5 kg (dry basis) of moist wood chips were sterilized in 20-L bioreactors at 121°C for 20 min. Liquid culture medium (200 mL) previously sterilized (121°C for 20 min.) and composed by 2% (w v<sup>-1</sup>) malt extract and 0.5% (w v<sup>-1</sup>) soybean peptone was inoculated with 20 discs (8 mm in diameter) of *C. subvermispora*-pre-cultured solid medium. These liquid cultures were maintained unshaken for 10 days at 27°C. The grown mycelium mat was filtered and washed with 300 mL of sterilized water. Washed mycelium obtained from several cultures was blended with sterilized water in 3 cycles of 15 seg. The mycelium suspension was used to inoculate the sterilized wood chips with a volume of suspension corresponding to 500 mg of fungal mycelium kg<sup>-1</sup> of dry wood. The inoculated wood chips were incubated at 27°C and 55% of relative air humidity in an acclimatized room and maintained unshaken for 15 days. After the biodegradation, the bioreactors were opened and the wood chips were washed with water to remove the superficial mycelium. Wood chips were air-dried and the initial and final dry weights were used to determine weight loss due to fungal biotreatment. Undecayed wood chips were prepared in a similar form to be used as a control sample.

### Chemical composition of wood

Approximately 1.5 g of milled wood (40/60 mesh) was extracted in a Soxhlet with ethanol:toluene 1:2 for 8h, followed by extraction with ethanol 95% for another 8h. The ethanol:toluene-soluble extractives were determined based on dry weight of extracted and unextracted wood samples. Extracted wood samples were hydrolyzed with 72% (w w<sup>-1</sup>) sulfuric acid at 30°C for 1h (300 mg of sample and 3 mL of acid). The acid was diluted with the addition of 79 mL of water and the mixture was autoclaved at 121°C for 1h. The residual material was cooled and filtered through porous glass filter number 3. Solids were dried at 105°C until constant weight and determined as insoluble lignin. Soluble lignin in the aqueous fraction was determined by measuring the absorbance at 205 nm, using 110 L g<sup>-1</sup>cm as the absorptivity of acid-soluble lignin. The concentration of monomeric sugars in the

hydrolyzate was determined by high-pressure liquid chromatography (HPLC) using a BIORAD HPX-87H column at 45°C, eluted with 5 mol L<sup>-1</sup> sulfuric acid at 0.6 mL min.<sup>-1</sup> (MENDONÇA et al., 2008).

Methylglucuronic acids were quantified in the wood hydrolyzates by using the colorimetric method proposed by Blumenkrantz and Asboe-Hansen (1973). One mL of acid hydrolyzate and 6 mL of 12.5 mmol L<sup>-1</sup> sodium tetraborate in concentrated sulfuric acid were added to a test tube immersed in an ice-water bath. The tube was shaken in a vortex and placed in a water bath at 95°C for 15 min. The solution was cooled in an ice-water bath and 200 µL of m-hydroxydiphenyl solution (0.15 in 0.5% NaOH) was added into the tube. After 5 min. of reaction, the absorbance was read at 520 nm. To prepare the blank solution, the m-hydroxydiphenyl was replaced by 200 µL of 0.5% NaOH. A calibration curve was prepared using galacturonic acid as standard in concentrations between 4 and 24 mg L<sup>-1</sup>.

#### Kraft pulping

*E. globulus* kraft pulps were produced by cooking the untreated and biotreated wood chips in a 1-L Parr reactor. Each cooking was carried out with 50 g of wood chips (dry basis) and 5:1 liquor:wood ratio. White liquor composition was 15% active alkali (AA) and 25% of sulfidity (expressed as NaOH). Cooking time at maximum temperature (165°C) was varied to achieve different H-factors (500 to 3000) and pulps with different kappa numbers and hexenuronic acid amount. Pulps were disintegrated in a TAPPI laboratory blender, thoroughly washed with tap water and centrifuged. Total pulp yield was determined based on the weight of the pulp divided by the weight of the wood chips (both in dry basis) multiplied by 100%. Kappa number was determined following the standard procedure described in TAPPI test method T-236 om-99.

Hexenuronic acids were quantified by the method proposed by Chai et al. (2001). Approximately 50 mg of air-dried pulp with known moisture content was placed in a 20 mL vial containing 10 mL of the hydrolysis solution (0.6% of mercuric chloride and 0.7% of sodium acetate). The sealed vial was hand shaken for mixing and heated at 65°C in a water bath for 45 min. The solution was cooled to room temperature, filtered by a nitrocellulose filter (0.22 µm pore) and the UV absorption was measured in a 10 mm path length silica cell at two wavelengths, 260 and 290 nm. The fresh hydrolysis solution was used as the blank for the UV absorption measurements. The HexA content in pulp was calculated using the equation:

$C_{\text{HexA}} = 0.287[(A_{260} - 1.2A_{290})V/w]$ ; where  $C_{\text{HexA}}$  is the content of hexenuronic acid groups in pulp (µmol g<sup>-1</sup>); 0.287 is the calibration factor for the method;  $A_{260}$  and  $A_{290}$  are the absorption intensities at 260 and 290 nm, respectively; 1.2 is the relationship between the lignin absorption at the two wavelengths;  $V$  is the volume of the hydrolysis solution (mL) and  $w$  is the oven-dried weight of the pulp sample (g).

#### ECF bleaching

ECF bleaching was performed in control and biokraft pulps using an OD<sub>0</sub>ED<sub>1</sub> sequence. Chlorine dioxide solution was kindly provided by a local pulp mill. Oxygen delignification (O step) was carried out in the 1-L Parr reactor with 10% pulp consistency, 2% NaOH (pulp basis), 0.5% MgSO<sub>4</sub> (pulp basis), 6 kgf cm<sup>-2</sup> O<sub>2</sub> pressure, temperature of 90°C and 60 min. of reaction. Each of the chlorine dioxide steps (D<sub>0</sub> and D<sub>1</sub>) was performed in a plastic bag with 10 g pulp (dry basis), 100 mL of 1% ClO<sub>2</sub> solution and 3 mL H<sub>2</sub>SO<sub>4</sub> (98%). Bleaching was carried out in a water bath at 60°C for 60 min. After D<sub>0</sub> stage, pulp was washed with 100 mL of 2% NaOH for 60 min. at 60°C (E step). After D<sub>1</sub> stage, pulp was washed with 100 mL of nanopure water for 60 min. at 60°C, followed by abundant washing with distilled water. After each bleaching step, kappa number (TAPPI Standard T236 om-99), HexA (CHAI et al., 2001), viscosity (TAPPI Standard T230 om-04) and brightness (TAPPI Standard T525 om-86) of pulps were determined.

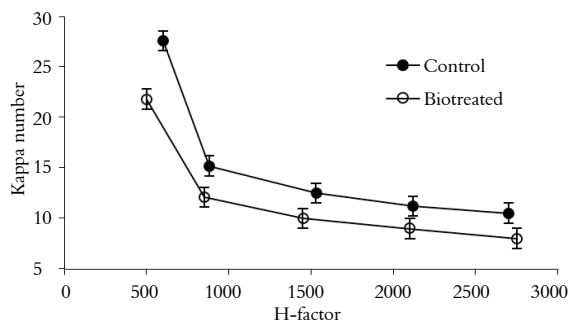
All experiments reported in this section were run, at least, in triplicate. Figures and tables showed the average values.

#### Results and discussion

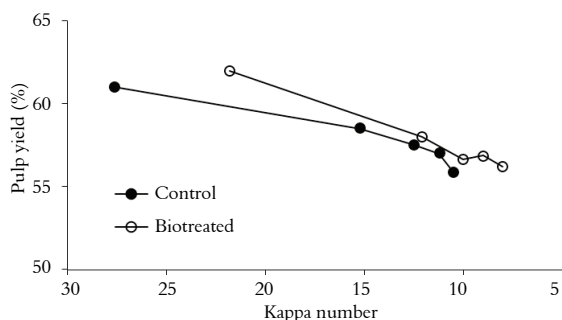
Chemical composition of undecayed *E. globulus* wood chips was 51.9% glucans, 15.1% xylans, 25.6% lignin, 3.6% methylglucuronic acid (MeGlcA) and 3.8% extractives. Weight and wood component losses after 15 days of biotreatment with *C. subvermispora* were: 5% weight, 1.5% glucans, 4.3% xylans, 5.7% lignin, 5% MeGlcA and 57.5% extractives. The high degradation of lignin and extractives over glucan showed a preferential pattern of decay of this fungus that make *C. subvermispora* the main white-rot fungi used for biopulping studies (FRANCO et al., 2006; GUERRA et al., 2003; MARDONES et al., 2006; MENDONÇA et al., 2002; VICENTIM et al., 2009).

Kraft pulping of 15-day biotreated *E. globulus* showed that pulps at given kappa number were obtained with a lower H-factor than the needed for undecayed control indicating that delignification is

faster for biotreated than for control wood chips (Figure 1). This is an effect of the increase in wood cell porosity that allows fast penetration and impregnation of liquor associate with lignin degradation and modification due to the oxidative system of the fungus. At the same time, pulps from biotreated wood present higher pulp yield than control pulps when compared at the same kappa number (Figure 2). Increase in pulp yield varied from 1% in low kappa pulps up to 4% in pulps with kappa number higher than 20.



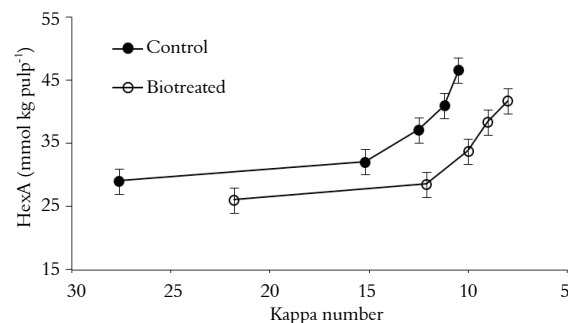
**Figure 1.** Kappa number in *E. globulus* kraft pulps from undecayed control and 15-day biotreated wood chips.



**Figure 2.** Selectivity of the delignification for *E. globulus* during kraft pulping of undecayed control and 15-day biotreated wood chips.

During fungal treatment and kraft pulping, hemicelluloses were also decayed at some extent. In hardwoods, the glucuronoxylan is more stable toward degradation in alkaline conditions but hexenuronic acids (HexA) are formed from the 4-O-methylglucuronic acids (MeGlcA) ramifications of xylans (BUCHERT et al., 1995). The importance of HexA for the pulp and paper industry is due to its contribution to the kappa number overestimating the real amount of residual lignin in pulp, its reactivity with bleaching chemicals ( $\text{ClO}_2$  and  $\text{O}_3$ ), and the effect in brightness reversion of paper (LI; GELLERSTEDT, 1997; VUORINEN et al., 1999; MONRROY et al., 2008). Franco et al. (2006) showed that the biokraft cooking of a Chilean hardwood, *Drimys winteri* with the white-rot fungus *Ganoderma australe* produces biopulps with a

reduction of 25% in kappa number and up to 12% less HexA as compared with pulps from untreated wood chips. The amount of HexA generated during kraft pulping of *E. globulus* increases with the delignification and cooking severity (high H-factor). HexA concentration was in the range of 26 to 47  $\text{mmol kg}^{-1}$  pulp for cooking with 15% AA, with the highest concentration being found in bleachable grade pulps (kappa number below 15) (Figure 3). At a same kappa number, biopulps presented 10 to 40% lower amount of HexA than pulps from untreated wood chips. The 5% loss of MeGlcA during fungal biotreatment could be responsible for part of the decrease of the HexA amount in biopulps, as also observed in a previous work (FRANCO et al., 2006). The decrease in HexA content is additional benefit of the fungal biotreatment, once HexA are known to contribute to increase kappa number values and consumption of bleaching reagents.



**Figure 3.** Hexenuronic acids (HexA) amount in *E. globulus* kraft pulps from undecayed control and 15-day biotreated wood chips.

Bleachable-grade kraft pulps with kappa number  $16 \pm 1$  were submitted to ECF bleaching sequence in order to determine the effect of fungal biotreatment in lignin removal, brightness increase, viscosity and amount of HexA in pulps. Together with the expected reduction in lignin and HexA amount during bleaching, the main benefit of the fungal pretreatment in comparison with non-decayed wood was observed in a high final brightness of pulp (84 v/s 80% ISO, respectively) with a good retention in the viscosity of pulp (Table 1). As compared with pulps from undecayed wood, biokraft pulps with similar kappa number can be obtained with lower H-factor (representing less cooking time or temperature), high pulp yield, high brightness and viscosity. The results of this work showed are benefits of the biopulping, which were also observed in similar studies (MENDONÇA et al., 2008; VICENTIM et al., 2009), carried out previously and corroborated the potential of fungal pretreatment as a biotechnological tool to enhance pulp production.

**Table 1.** Characteristics of pulps from control and 15-day biotreated wood chips after cooking and ECF bleaching steps.\*

	Control				Biotreated			
	Cooking	O	D <sub>0</sub>	D <sub>1</sub>	Cooking	O	D <sub>0</sub>	D <sub>1</sub>
Kappa number	16	10.5	4.7	1.1	16	7.3	3.4	0.9
HexA (mmol kg <sup>-1</sup> pulp)	33	32	15	6.5	26	27	12	5.5
Viscosity (mPa.s)	26	24	23	18	30	28	25	20
Brightness (% ISO)	35	44	72	80	32	39	77	84

\*O = extended delignification with oxygen; D<sub>0</sub> and D<sub>1</sub> = first and second step of bleaching with chlorine dioxide, respectively.

## Conclusion

Kraft pulping of *E. globulus* wood chips biotreated by *C. subvermispora* produces pulps with lower kappa number and similar or higher pulp yield than the obtained for pulps from undecayed wood chips. According to the cooking conditions used, all biopulps presented a reduction in HexA formation as compared with control pulps which is an additional benefit of the wood biotreatment with white-rot fungi. Biopulps bleached by an ECF sequence presented higher final brightness and viscosity than pulps from undecayed wood. Benefits obtained showed fungal pretreatment as a potential biotechnological tool to enhance pulp production.

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