HIGH YIELD BIOKRAFT PULPING OF *EUCALYPTUS GRANDIS*: EVALUATION OF THE BIOPROCESS USING WOOD CHIPS BIOTREATED BY *CERIPORIOPSIS SUBVERMISPORA* IN DIFFERENT SUPPLEMENTING CONDITIONS

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ABSTRACT

Biopulping comprises the biodegradation of wood by lignin-degrading fungi prior to conventional pulping processes. This bioprocess allows energy or active alkali savings and increases mechanical properties of the pulps. In this work, *Eucalyptus grandis* wood chips were biotreated by *Ceriporiopsis subvermispora* during 15 days and submitted to high yield kraft pulping. Biotreatment was carried out in three culture conditions: (**A**) unsupplemented; (**B**) supplemented with corn steep liquor (CSL); and (**C**) supplemented with CSL plus glucose. Condition **C** induced the highest manganese peroxidase (MnP) activity (1102 $\pm$ 79 IU/kg) and oxalic acid production (1381 $\pm$ 161 mg/kg) by the fungus, which provided the highest wood weight loss (4.1 $\pm$ 0.6%). Condition **B** induced lipid peroxidation reactions, estimated by the quantification of thiobarbituric reactive substances (TBARS, 359 $\pm$ 16 μg/kg) and consequently provoked the highest lignin loss (9.9 $\pm$ 0.4%). Mycelium grew approximately 3 times, from the starting biomass (500 mg/kg), in all conditions. Biotreated wood chips underwent high yield kraft pulping, wherein conditions **A** and **B** allowed reduction of active alkali charge (AA) from 15 to 14,3% and increased pulp yield from 62 to 63%. Conversely, condition **C** did not provide any difference concerning alkali consumption and moreover decreased the pulp yield by 6.5%. In fact, the supplementation herein applied to the chips satisfactorily induced distinct fungal metabolism and biodegradation, but the wood alterations range herein obtained, especially concerning lignin loss, seem not to be enough to increase even more the traditional benefits of biokraft pulping.

INTRODUCTION

Biopulping process is defined as the biotreatment of wood chips by lignin-degrading fungi prior to conventional pulping processes [1]. Removal and structure changes caused by these organisms on the wood components, mainly to lignin and extractives, allow the production of pulps in less drastic conditions, providing chemical reagents or energy economy and besides, the low drastic conditions provide pulps with better mechanical properties than conventionally. The white-rot basidiomycete *Ceriporiopsis subvermispora* has been proposed as the most suitable specie for biopulping, due to the rapid wood colonization and high specificity to lignin degradation, remaining cellulose practically untouched in early stage cultures [1-8].

C. subvermispora degrades lignin using enzymatic and chemical mechanisms. The ligninolytic enzymes concern mainly manganese-peroxidase (MnP), which is produced constitutively. Low laccases activity has been detected during the first week of culturing [9]. MnP must be oxidized (activated) by H_2O_2 (or some organic peroxide) and than it is prompted for oxidizing a phenolic portion of lignin (or other phenolic compound), reaction that drives to an aril-ether linkage breakdown. The enzyme can act directly on its substrates or throughout the reduction of a Mn^{3+} ion, which is produced by the enzyme. Mn^{2+} ion is oxidized by MnP to Mn^{3+} and then it is taken off from its enzyme site by oxalic acid. Oxalate-Mn^{3+} is a low molecular mass compound that, beyond helping the enzyme cycle, can penetrate the wood-cell-wall, reaching regions where MnP can not do in early stages of biotreatment. When the ion is released by the oxalate, it can oxidize phenolic portions of lignin [10,11].

MnP can also oxidize non-phenolic regions of lignin through a lipid peroxidation intermediate system. Linoleic acid, found in sound wood and produced by the fungus [12], has been proposed to be oxidized by Mn^{3+}, forming a carbon centered radical, which reacts with oxygen, giving rise to a peroxyl radical. This radical can abstract one electron from non-phenolic substrates [13]. Moreover lignin oxidation, this reaction produces H_2O_2 [10], which can be used by MnP for its activation, and either produces aldehydes, like hexanal, pentanal and glyoxal [14]. Quantification of these aldehydes has been proposed by Enoki *et al.* (1999) [15] for estimating lipid peroxidation reactions that took place during culturing.

Mendonça *et al.* (2002) [5] showed that biopulping can be more advantageous when producing high yield kraft pulps, which contain elevated residual lignin. In that work, *Pinus taeda* wood chips were biotreated during 15 days and submitted to kraft pulping. Biotreatment caused 9.6% lignin loss and allowed economizing 16% of active alkali charge or 17 minutes of cooking time, for Kappa 80 pulp production.

Attempting to improve the efficiency of the bioprocess, a previous work of our group [9] evaluated supplementing conditions of *E. grandis* wood chips that induced different metabolic levels in *C. subvermispora*, and consequently provided distinct biodelignification during a 15 days culturing. Conditions that provided low and high levels of biodelignification and MnP production were chosen to be repeated at a larger scale, in order to supply enough amount of wood chips for pulping processes. In the present work, Kappa 80 kraft pulps were prepared from wood chips that suffered different

levels of biodegradation and the response to kraft pulping process was evaluated.

EXPERIMENTAL

Fungus, inoculum preparation and wood biodegradation

Ceriporiopsis. subvermispora (Pilat) Gilbn. & Ryv. (strain SS-3) cultures were maintained on 20 g/L malt extract (Acumedia, Maryland), 2 g/L yeast extract (Vetec, Brazil) agar slants at 4 ◦C. 200 mL of liquid medium containing potato extract broth (24 g/L, DIFCO, Maryland) and yeast extract (7 g/L) was inoculated with 20 discs (8mm diameter) of *C. subvermispora* precultured solid medium. This liquid culture was maintained unshaken for 12 days at 27 ◦C. The grown mycelium mat was filtered and washed with 300 mL of sterile water. Mycelium obtained from several cultures was blended with 100 mL of sterile water in three cycles of 15 seconds. The mycelium suspension was used to inoculate the wood chips in 20-L bioreactors. *E. grandis* wood chips measuring approximately 2.5 cm × 1.5 cm × 0.2 cm were obtained from 8-year-old trees with a local pulp mill. Prior to the biodegradation experiments, 2 kg (dry basis) of wood chips were immersed in water for 16 h. The surplus water was drained, the bioreactors were sealed and the system was at 121 ◦C for 15 minutes. The moisture of autoclaved wood chips was approximately 55%. Three different culture conditions were used according to Vicentim and Ferraz (2006) [9]. Cultures were supplemented with previously sterilized solutions as follows: (A) unsupplemented; (B) addition of 0,5% corn steep liquor (CSL); and (C) addition of 0.5% glucose and 0.5% CSL. The concentrations are referred as supplement mass per wood mass, all in dry basis. Before supplement addition, they were dissolved in a final volume of 11 mL water and transferred to the bioreactors containing sterilized wood chips. 11 mL sterile water was added to unsupplemented culture. After addition of culture supplements, each bioreactor was loaded with 10 mL mycelium suspension corresponding to a fungal mycelium/wood ratio of 500 mg/kg, shaken by hand and stationary stored at 27 ◦C for 15 days. The bioreactors were supplied with 2.3 L/h of air, during all the biotreatment period. The air was humidified and sterilized passing through $KMnO_4$ solution, sterilized water and 0.22 μm filter membranes. Three bioreactors were incubated for each culture condition, in order to have three replicates for enzymes and wood weight loss determinations. For other chemical and wood properties analyses and pulping process, biotreated wood chips from a same condition were mixed, and three portions were taken off for analyses, in order to have three replicates. One set of bioreactors were sterilized but non-inoculated and represented the control for all experiments. For wood weight loss determination, chips were air dried for 7 days, weighed and moisture content was determined in an OHAUS infrared heating balance.

Enzyme extraction and determination

Extracellular enzymes were extracted with 50 mM sodium acetate buffer (pH 5.5) containing Tween 60 (0.1 g/L). 50 g (dry basis) of wood chips were extracted with 200 mL of extracting solution for 5 h at 10±1 ◦C [16]. The crude extracts were recovered by filtration through porous glass filter number 4 and further through 0.45 μm membranes. MnP activity was measured by the phenol red oxidation. The reactions were performed in 30 mL assay tubes containing 3.0 mL 50 mM sodium succinate buffer at pH 4.5, 3.0 mL 50 mM sodium lactate, 1.0 mL 1.0 mM $MnSO_4$, 1.0 mL 1 g/L phenol red, 0.5 mL 10 g/L serum bovine albumin, 1.0 mL enzymatic extract and 0.5 mL 2.0 mM H_2O_2. At 1-min intervals, 2 mL of this reaction mixture was added to 60 μl of a 6.5 M NaOH solution, and absorbance was measured at 610 nm (22 $mM^{-1}.cm^{-1}$ molar absorptivity for oxidized phenol red) [17]. Laccase activity was determined with 1 mM ABTS [2,2’-azinobis-(3-ethylbenzenthiazoline-6-sulfonic acid)] as substrate [18]. Reactions were carried out in 3 mL cuvettes containing 0.6 ml 200 mM sodium phosphate and 100 mM citric acid buffer at pH 5.0, 0.2 mL water, 1.0 mL enzyme extract and 0.2 mL 1.0 mM ABTS. Reactions were monitored at 420 nm up to 5 min with 10-s intervals (36 $mM^{-1}.cm^{-1}$ absorptivity for oxidized ABTS). Total cellulases (filter paper activity) and xylanases were assayed with filter paper Whatman no. 1 [19] and birchwood xylan [20], respectively. Released reducing sugars were determined with dinitrosalicylic acid [21].

Determination of ergosterol contents in biotreated wood samples

Ergosterol contents of biotreated wood were determined as an estimate of mycelial biomass grown on wood chips. Approximately 10 closed test tubes containing 250 mg of milled wood chips, 0.5 mL 2.0 M NaOH and 2.0 mL methanol were heated in a domestic microwave oven operating at 2450 MHz and 620 W for 20 s. After cooling for 15 min, another 10-s heating cycle was performed. The reaction mixture was neutralized with 1.0 mL 1.0 M HCl, treated with additional 2mL methanol, vortexed and then three times extracted with 2 mL aliquots of hexane [22]. Hexane fractions were combined, dried over anhydrous magnesium sulfate, filtrated and evaporated under nitrogen stream. Residual material was dissolved in 1.0 mL methanol and analyzed by HPLC for ergosterol contents. The HPLC equipment (Shimadzu LC10AD, Japan) was fitted with a 5-μm ODS2 Spherisorb 150 mm × 4.6 mm column (Phase Separation Ltd., UK) eluted with methanol at a flow rate of 1.0 mL/min. Ergosterol was detected at 282 nm in a Shimadzu SPD-10AV detector.

pH and oxalate determinations

Biotreated wood chips (10 g on dry basis) were soaked with 60 mL of bidistilled water (pH previously adjusted to 7.0) for 48 h. After this period, the pH was measured in the resulting aqueous extract. The extracts were recovered by filtration through filter paper and used for oxalate determinations. Water extracted from wood chips were further extracted with 0.1 M HCl. For this purpose, 10 g of wood chips were extracted with 60 mL of 0.1 M HCl at 27 ◦C and 120 rpm for 48 h. Acid extracts were recovered by filtration through filter paper. Oxalate was determined in aqueous and acid extracts by HPLC. The extracts were cleaned-up by treatment with active charcoal (200 mg for 20 mL of extract) at 120 rpm for 2 h. After treatment with active charcoal, the extracts were filtered through a 0.45 μm membrane (Millipore JBR 610163) and injected into a 300 mm × 7.8 mm HPX-87H BioRad column (BioRad Laboratories Ltd., CA) eluted at 0.6 mL/min with 8 mM H_2SO_4. Oxalic acid was detected at 210 nm in a SPD10AV-Shimadzu detector at 6.9 min of elution, which was confirmed by co-elution with an authentic standard. Pure oxalic acid was used for calibration of the HPLC-chromatographic system. For this, a wood extract from undecayed *E. grandis* was contaminated with known concentrations of oxalic acid and treated as previously described. This procedure was useful to compute differentiated adsorption of oxalate in presence of phenols in wood extracts. Peak areas were measured after baseline correction by tailing.

TBARS determination

5 g of wood chips were washed 5 times with 60 mL of distilled water for 1 h in orbital shaker at 120 rpm. After air drying, the chips were extracted with 60 mL chloroform/methanol (2:1) solution, for 1 h, 120 rpm. Organic extracts were concentrated by evaporation to dryness and resuspended in 100 μL of chloroform. Concentrated extracts were treated with 3.0 mL of thiobarbituric acid (TBA) reagent for 15 min in a boiling water bath. TBA solution consisted of 0,335% (w/v) of TBA and 10% (w/v) trichloroacetic acid was reacted. After cooling, the flocculent precipitates were removed by centrifugation at 1000 *g* for 10 min. The amount of TBARS was determined in the soluble fraction by measuring their absorbance at 532 nm considering an absorption coefficient of 1.56 x 105 $M^{-1}.cm^{-1}$ [15,23].

Chemical analysis of wood chips

Untreated and biotreated wood chips were milled to pass through a 0.5 mm screen. Approximately 1.5 g of milled samples were extracted with 95% ethanol for 6 h in a Soxhlet apparatus. Ethanol-extracted wood samples were hydrolyzed with 72% (w/w) sulfuric acid at 30 ◦C for 1 h (300 mg of sample and 3 mL of acid) as described by Ferraz *et al.* (2000) [24]. The acid was diluted to a final concentration of 3% (w/w) with the addition of 79 mL water, and the mixture was autoclaved at 121 ◦C for 1 h. The residual material was cooled and filtered through porous glass filter number 3. Solids were dried until constant weight at 105 ◦C and determined as insoluble lignin. Soluble lignin concentration in the aqueous fraction was determined by measuring the absorbance at 205 nm using the value of 105 L/g.cm as the absorptivity of soluble lignin. The concentrations of monomeric sugars in the soluble fraction were determined by HPLC using a BIORAD HPX-87H column at 45 ◦C, eluted at 0.6 mL/min with 5 mM sulfuric acid [24]. The chemical composition values were the averages obtained from duplicate hydrolysis experiments. The relative deviations from the average values were lower than 3%.

Kraft pulping

Kraft cooking of the wood chips was carried out in 85 mL stainless-steel reactors. Kraft liquor was composed of 25% sulfidity 11 to 25% active alkali (AA) concentrations (expressed as g of NaOH equivalents/100 mL of liquor). Cooking liquor concentrations were determined by titration with 0.5 M HC1, as reported elsewhere [25]. The liquor/wood ratio used for all the kraft cooking was 60 mL of cooking liquor for 10 g of wood chips. Each cooking reaction started with the immersion of the reactor in a silicone oil bath preheated at 25 ^oC, which was heated until during 25 min to reach 170°C, and the reactions were conducted for 180 min. After that, the reactors were air cooled for 2 h, the residual unscreened pulp was filtered and washed with fresh water until the filtrate became colorless. The final fibrous material was dried at 60°C to constant weight, and stored for analysis of residual lignin, as describe in the previous topic. Initial and final dry weights were used to determine the unscreened pulp yield.

RESULTS AND DISCUSSION

High yield kraft pulps are those which contain elevated residual lignin, presenting Kappa number between 60 and 120, and generally they are not bleached [26]. In previous studies of our group, it was demonstrated that modifications caused in *Pinus taeda* by the biotreatment step allow the wood chips to be more easily delignified by the kraft liquor, and the high yield kraft process seems to be more advantageous than low yield one [5]. Thus, the experiments accomplished in this work were designed to obtain kraft pulps from *E. grandis* with Kappa number around 80.

E. grandis wood chips were biotreated in some supplementation conditions reported in a previous work [9]. Those conditions induced different metabolism of *C. subvermispora* (concerning especially fungal growth and MnP production) and different extent of wood biodegradation. Thus, three conditions from that work were chosen to be carried out in this work, in 20 L bioreactors containing 2 kg of wood chips: **A** – unsupplemented: condition that provided low fungal metabolism and lignin

biodegradation; **B** – addition of corn steep liquor (CSL): it provided intermediary fungal metabolism but caused high lignin biodegradation; and **C** – addition of CSL and glucose: highest fungal metabolism and intermediary wood biodegradation. CSL is a complex byproduct from the corn-processing industry. It is composed of peptides, sugars, lactic acid, vitamins and metallic ions [1].

The enzyme activities and oxalic acid production recovered after the 15 days of wood biotreatment (Tables 1 and 2) were very close to those previously reported [27,9]. Laccases were not detected, and total cellulolytic activities (filter paper activity method – FPA) were not high enough to be considerable. Cellulolytic values found did not represent 4% of filter paper conversion to reducing sugars [19], so the FPA activity detected might represent mainly new reducing ends released by the cleavage of amorphous regions of cellulose due to the action of endoglucanases, once *C. subvermispora* does not secrete cellobiohydrolases [28].

Table 1: Fungal growth and enzymatic activities on *E. grandis* wood chips biotreated by *C. subvermispora* for 15 days.

Supplem. condition	**A** *unsupplem.*	**B** CSL	**C** CSL+glu
Ergosterol[a]	19.7±1.1	23.4±1.5	18.8±1.3
MnP[b]	297±87	534±152	1102±79
Xylanases[b]	3262±857	4188±302	4089±577
Cellulases[b]	37±9	31±7	51±6

- [a] Ergosterol (mg/kg of wood) is a lipid present on the fungal cell membrane, which concentration on *C. subvermispora* corresponds to approximately 1% (w/w) [7];
- [b] Enzymatic activities unit is IU/kg of wood;
- The numbers after the average values are standard deviations from 3 replicates.

MnP and xylanases reached the highest activity (1102 ± 79 and 4089 ± 577 IU/kg, respectively) in condition **C**. Fungal growth was estimated by ergosterol, which is a lipid present in fungal cell membrane that can be directly correlated to the biomass. Table 1 presents hydrolytic and oxidative enzyme activities and fungal growth.

Oxalic acid was also quantified in sound and biotreated wood chips, seen that it is a very important low molecular mass metabolite that takes part in MnP cycle and wood degradation [10,29,30]. Beyond these functions, it was suggested by Hunt *et al.* (2004) [31] that this acid could also esterificate to cellulose during biotreatment of wood, which in turn would make easier the pulp refining process and enhance mechanical properties of the paper sheets. Thus, according to the methodology proposed by Hunt *et al.* (2004) [31], it was quantified free oxalic acid by extraction with bi-distilled water, insoluble oxalate by extraction with 0.1 M HCl solution, and the fraction linked to fibers was extracted with 0.1 M NaOH solution.

The concentration of free oxalic acid found in sound wood was 64 mg/kg of wood (dry basis) and increased to an average of 724 mg/kg in biotreated ones. The amount of insoluble oxalate, which is supposed to be linked mainly to calcium ions, increased specially in supplemented conditions, from 47 mg/kg to an average of 350 mg/kg. Oxalate esterified to fibers was also increased by the biotreatment, from 83 mg/kg to an average of 220 mg/kg. The pH value was found to diminish from 5.0 in sound wood to around 3.8 in biotreated ones, as shown in Table 2. The pH decreasing is a consequence of acids production by the fungus, which oxalic acid is the main one [32], and wood biomodifications, like acetic acid release from hemicellulose and formation of some lignin degradation products like vanillic and syringic acids [33].

The supplemented conditions induced higher fungal growth and enzymatic activities, but did not result in significative increase in oxalic acid concentration. This can be attributed to oxalate concentration control of white-rotters, comprised by enzymatic systems, like oxalate oxidase and oxalate decarboxylase, that degrade oxalate thus controlling pH of culture media. The acid degradation can also be raised by the action of MnP or lignin-peroxidase (LiP) intermediates. As *C. subvermispora* has been described to produce only MnP, Mn^{3+} is the only intermediate to be considered. This ion can be chelated and stabilized by oxalate to be diffused through the wood fiber cell wall and promote lignin degradation in regions where the enzymes can not penetrate in early stage cultures, due to their large volume. However, the acid itself can also be oxidized and degraded by Mn^{3+}. So, the acid production could have been induced in supplemented conditions, like the enzymes were, but the higher MnP activity may have contributed for controlling of acid concentration, due to the higher Mn^{3+} generation [30,34-37]. Likewise, the amount of esterified oxalate was found to be practically the same in all biotreated samples. Only insoluble oxalate was observed to increase even more in supplemented conditions, fact that can be attributed to the higher concentration of some metals, especially Ca^{2+}, introduced into these systems by CSL.

Table 2: Oxalic acid and pH of *E. grandis* wood chips biotreated by *C. subvermispora*.

Suppl. cond.	**Control**	**A** *unsuppl.*	**B** CSL	**C** CSL+glu
Free [a]	64±4	750±127	672±11	751±63
Insol. [a]	47±11	203±53	342±14	358±14
Ester. [a]	83±33	216±31	217±30	271±85
Total [a]	194±48	1169±211	1232±55	1380±161
pH	5.0	4.1	3.8	3.6

- [a] Oxalic acid fractions (mg/kg of wood);
- The numbers after the average values are standard deviations from 3 replicates.

The free oxalic acid, fraction that can take part in MnP cicle, was found in all biotreated conditions to be higher than 1 mM, concentration

enough to support MnP activity, as experimented by our group (Aguiar, A., personal communication). The free acid concentration detected in sound and biotreated wood chips represents 0.71 and around 8 mM, respectively, based on the moisture content retained in the wood during biotreatment, 55%.

The high extent of lignin biodegradation normally observed for *C. subvermispora* cultures, can not be attributed only to the action of MnP, because it can attack only phenolic regions of the macromolecule, which represents less than 10% of it [33]. Despite LiP has not been detected in *C. subvermispora* cultures, non phenolic regions of lignin can be broken down by this fungus throughout MnP intermediate systems, and the most suitable one that has been proposed concerns the lipid peroxidation reactions [15]. These reactions comprise the oxidation of an unsaturated fatty acid (by Mn^{3+} ion formed by MnP), giving rise to a radical compound that reacts with dioxygen, forming a peroxyl radical, which can in turn take an electron off a non-phenolic portion of lignin. In order to estimate lipid peroxidation reactions that took place during biotreatment, Enoki *et al.* (1999) [15] proposed quantifying aldehydes that are formed after the reaction of organic-peroxides with lignin. It is possible to extract those aldehydes with an appropriate organic solvent and react them with thiobarbituric acid, giving rise to compounds termed TBARS, which can be easily analyzed by UV/VIS spectroscopy.

In this work, it was found that TBARS increased from 145 μg/kg of wood in control sample to an average of 350 μg/kg in supplemented conditions (Table 3). Likewise general fungal metabolism, especially concerning MnP, lipid peroxidation reactions were induced by CSL. However, condition **B** presented a little higher concentration of TBARS than **C** (which provided the highest MnP activity). It seems that lipid peroxidation reactions also depend on linoleic acid production by the fungus, and probably condition **B** was more effective to induce its production than condition **C**.

Supplementation applied to the cultures, meaningfully influenced fungal metabolism, and consequently increased the extension of wood biodegradation, especially concerning lignin. Wood mass loss was 105% higher in culture supplemented by CSL and glucose (condition **C**), and lignin loss was increased by 83% in culture added by CSL (condition **B**), as shows Table 3. Conversely, glucan and xylan losses were found to be fewer than 3% (w/w), the same range of standard deviation of the analytical methods employed for their determinations. This fact was expected since polysaccharide losses atrats to be significant only when wood mass losses are higher than 9% [27,4].

Lignin biodegradation was observed to be more pronounced in condition **B**, where higher TBARS concentration was detected. These data are according to a recent study of our group (data not reported yet), in which a direct correlation between TBARS concentration and the breakdown of aril-ether linkages of lignin was found. Concerning wood weight loss, it was found to be higher in condition **C**, which induced more intense fungal metabolism. Although lignin was the only wood component which losses were detected, extractives (not determined) and even cellulose and hemicelluloses must have suffered some degree of biodegradation in condition **C**.

Table 3: Quantification of TBARS and properties of *E. grandis* wood chips biotreated by *C. subvermispora*.

Supplem. condition	**A** *unsupplem*	**B** CSL	**C** CSL+glu
TBARS[a]	188±10	359±16	340±19
Lignin loss[b]	5.4±0.2	9.9±0.4	6.8±0.4
Wood loss[c]	2.0±0.7	3.0±0.4	4.1±0.6

- [a] TBARS concentration in sound wood was 145 ± 12 μg/kg (a).
- [b] Lignin loss unit = % (w/w). Lignin Concentration in sound wood was 28.1 ± 0.2% (w/w, lignin/wood)
- [c] Total wood weight loss unit = % (w/w)
- The numbers after the average values are standard deviations from 3 replicates.

After biodegradation experiments, biotreated and sound wood chips were submitted to kraft pulping, intending the production of pulps with Kappa number around 80. Pulping process was evaluated concerning active alkali (AA) charge and pulping yield.

The AA charges applied in this work, from 11 to 25%, provided a wide delignification pattern, which allowed the evaluation of biopulping in a large Kappa number range, from 155.7 ± 0.5 to 22.9 ± 0.4 as shows Figure 1. AA required to produce Kappa 80 kraft pulp from control wood was found to be 15%, and it could be reduced to 14.3% using biotreated wood in conditions **A** and **B**. The delignification profile presented in Figure 1 shows that these biotreated samples could provide approximately the same economy to produce pulps with Kappa number from 57 to 116.

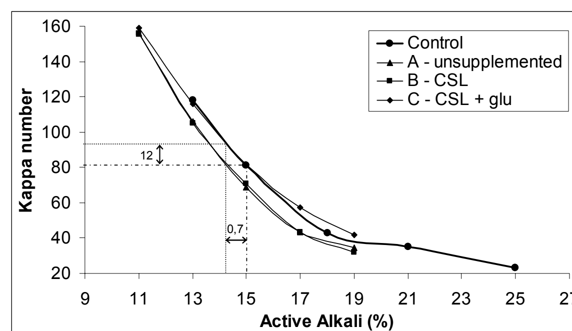

Figure 1: Delignification profile of *E. grandis* wood chips as a function of active alkali applied to produce kraft pulps.

From another point of view, data from Figure 1 show that biotreatment lowered the Kappa number

of the pulps. The same AA that produced Kappa 80 pulp from wood **A** and **B** (14.3%) would produce Kappa 92 pulp from control wood, i.e., a pulp containing 15% more residual lignin. Approximately the same Kappa lowering could be obtained when applied AA ranging from 13 to 17%, as presented in Figure 1.

Although condition **C** had induced the highest fungal metabolism, which consequently caused the highest wood weight loss and intermediate lignin loss, it did no provide any benefit concerning AA economy. Figure 1 shows that the delignification profile of chips biotreated in condition **C** was practically the same of untreated ones.

Biokraft pulps produced from biodegraded wood in conditions **A** and **B** not only were more easily delignified, which resulted in alkali economy or Kappa number lowering, but also provided higher pulp yield. While control pulp yielded 62% (w/w, pulp/wood) for Kappa 80 pulp production, biotreated wood in conditions **A** and **B** yielded 63%. Figure 2 shows that these conditions could provide the same average yield increasing for pulps with Kappa number from 40 to 110. The higher pulp yield observed for these samples can be mainly imputed to the less drastic conditions applied for the cooking. Furthermore, according to Guerra *et al.* (2003) [4], in circumstances that biotreatment provides low wood weight loss (less than 3%), cellulose depolymerization is negligible, situation herein observed in samples **A** and **B** (Table 3). Conversely, condition **C** once more presented an unexpected result, seen that its yield was found to be only 58% for Kappa 80 pulp production. The same average decreasing was observed for pulps with Kappa number ranging from 60 to 110, as shows Figure 2.

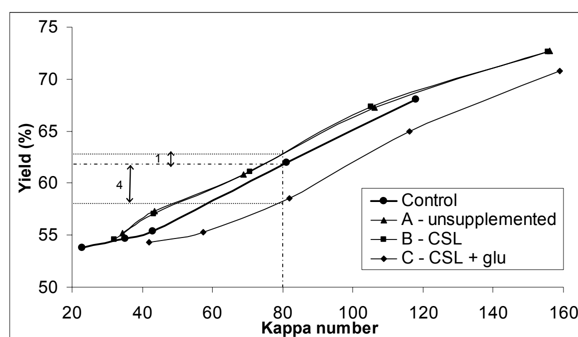

Figure 2: Pulping yield profile of *E. grandis* wood chips as a function of residual lignin concentration in pulps (Kappa number).

The loss of pulp yield observed in condition **C** can be attributed to higher depolymerization of polysacharidic fraction of wood during biotreatment, allowing them to be more easily solubilized by the kraft liquor. Guerra *et al.* (2003) [4] demonstrated, analyzing biodegraded *P. taeda*, that some depolymerization of cellulose starts to be significant when wood mass losses are higher than 3% (situation observed in the condition **C,** reported here). Xylan weight loss was greatly increased in that work when wood weight loss was higher than 3%, so xylan depolymerization probably follows the same trend as cellulose.

Interestingly, despite wood samples **A** and **B** had presented very different lignin and wood weight losses, they provided exactly the same benefits for high yield kraft pulping, concerning the three parameters herein evaluated: active alkali charge, Kappa number and pulp yield.

CONCLUSIONS

All these data taken together show that lignin biodegradation indubitably is important for biopulping benefits. However, at least in the range of lignin biodegradation herein evaluated, it seems not to be the limiting parameter for further benefits. Besides, polysaccharide depolymerization must be avoided, otherwise pulp yield is inevitable affected.

In conclusion, the highly complex fungal mechanisms involved in wood biodegradation, turns difficult to find or distinguish the parameters relevant for biopulping benefits.

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