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Biopulping of wood chips with *Phlebia brevispora* BAFC 633 reduces lignin content and improves pulp quality

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ABSTRACT

The white-rot fungus *Phlebia brevispora* BAFC 633 produces laccases in large proportions. In this work *P. brevispora* BAFC 633 was grown on *Pinus taeda* wood chips in 10-L bioreactors. To select the biopulping experimental conditions, we analyzed the variables affecting enzymatic laccase activity in the culture supernatants, indicating that the suitable incubation temperature was 30 °C in order to promote enzyme stability. *Phlebia brevispora* BAFC 633 secreted 744 U/g of laccase, selectively removing lignin during biotreatment of wood chips, causing a reduction in Kappa number and 10% weight loss, and creating a more open structure and better access to the pulping liquor, which would require less chemical consumption, thus diminishing the environmental impact of the chemical pulping process.

These results support the biotechnological potential of *P. brevispora* BAFC 633 for biopulping processes and enhance the potential for bioprospecting native isolates of the microflora of our country's natural environment.

1. Introduction

Pulp and paper constitute one of the major manufacturing activities in Misiones, Argentina. The strong demand for paper as a commodity leads to a steady expansion of paper industries in our country. The main objective of the paper manufacturing processes is the removal of lignin from wood; this is the first step in chemical pulping. Kraft pulping is the most widespread process (Da Re et al., 2010). Kraft pulping and bleaching stages use large amounts of chlorine and chloride chemicals (Polaina and MacCabe, 2007; Selvam et al., 2011). The derived products of these chemicals are chlorinated organic substances, some of which are toxic, mutagenic, persistent and can cause numerous harmful disturbances in biological systems (Bajpai and Bajpai, 1997).

In this sense filamentous fungi of the phylum Basidiomycota have been widely studied for their ability to degrade wood (Kirk and Cullen, 1998); these fungi are unique in their ability to degrade most components of wood due to their ability to synthesize the relevant hydrolytic and oxidative extracellular enzymes (Poojary et al., 2012). Within the basidiomycetes, white-rot fungi (WRF) have received special attention because they are the only organisms capable of mineralizing lignin to CO_2 and H_2O (Martinez, 2002) by secreting oxidative enzymes, such as peroxidases and laccases, which have a broad range of substrates (Field et al., 1993). Some white-rot fungi have the ability to selectively remove extensive amounts of lignin with only insignificant losses of cellulose and moderate to low losses of hemicellulose (Blanchette et al., 1985; Otjen and Blanchette, 1986). One of the less harmful and more promising alternatives to improve conventional pulp and paper processes is the use of microorganisms (such as WRF) and enzymes as a treatment for wood chips to reduce lignin content. This alternative process is known as biopulping (Otjen and Blanchette, 1987; Blanchette and Burnes, 1988; Villalba et al., 2006). This process can save substantial amounts of energy,

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improves paper quality, reduces the environmental impact of pulping, and enhances economic competitiveness.

The objective of this study was to evaluate the WRF *Phlebia brevispora* BAFC 633 for biotechnological application in the biopulping of *Pinus taeda* chips.

2. Materials and methods

2.1. Fungal strain and wood chips

Phlebia brevispora BAFC 633 was provided by the Mycological Culture Collection of the Department of Biological Sciences, Faculty of Exact and Natural Sciences, University of Buenos Aires, Argentina. Stock cultures were maintained on malt agar (12.7 g l^{-1}), agar (2% w/v) (MEA) slants at 4 °C with periodic transfer.

Wood chips of *Pinus taeda* were selected for this study because they have regional importance in the production of cellulose, and they are fast-growing trees. There are previous studies using white-rot fungi for the treatment of this substrate (Otjen and Blanchette, 1987; Blanchette and Burnes, 1988; Villalba et al., 2006).

2.2. Preliminary studies of laccase temperature and pH optimum, and enzyme thermo-stability on culture supernatants

Preliminary tests were performed using culture supernatants from 50 ml of ME medium (12.7 g l^{-1} malt extract and 5 g l^{-1} corn steep liquor) at 10 days of incubation, pH 4.5, in static conditions to establish optimal conditions for laccase activity. The optimum temperature of laccase was determined between 30 and 80 °C. The optimum pH for laccase was determined using sodium acetate buffer 0.1 M at a pH range of 3.6–5.6.

For thermo-stability evaluation during the enzymatic bioprocess, culture supernatants were incubated continuously for 7 h at optimum pH and temperature. The effect of temperature on stability of laccase on culture supernatants was determined for 7 h at 20, 30, 40, 50, and 60 °C.

2.3. Preparation of fungal inocula

One agar plug (0.5 mm^2) of fungus grown on an MEA plate at 29 °C for 5 days was added to 50 mL of ME medium and incubated at 29 °C statically for 10 days. For the inoculation experiments, the mycelium was removed under aseptic conditions, washed in sterile distilled water three times, mashed for 5 s to achieve a homogenous mixture, and finally added to the substrate.

2.4. Wood preparation

Ideal chip size for commercial pulping is 1 × 2.5 × 0.3 cm, so in order to achieve these dimensions, *P. taeda* chips from the sawmill were carefully selected and separated manually. After classification, they were thoroughly mixed and placed in dishes. Three aliquots of the initial chips were taken to determine the moisture content. Wood chips were autoclaved for 30 min at 121 °C to prevent contamination by microorganisms that can antagonize or inhibit the growth of fungus. Subsequently, 400 g (on a dry weight basis) under sterile conditions were poured into the 10-L bioreactors.

2.5. Inoculation procedure

Crushed fungus mycelium was used as inoculum (0.5 mg mycelium/g chips) and poured over the wood chips with extensive mixing. Sodium acetate buffer 0.1 M pH 3.6 was added to the system to reach 65% moisture content. The incubation step was carried out at bioreactors maintained at 29 °C in an environmentally controlled chamber. The experiments were conducted in duplicate.

After 30 days, the incubation period was completed. The chips were air-dried and stored at −20 °C in plastic bags to stop fungal activity before pulping.

2.6. Weight loss measurement

Before incubation, the wood chips were dried to constant weight at 80 °C, and the initial weight was calculated. After the incubation, the wood chips were washed with sterile distilled water and filtered to remove residual mycelium. The washed chips were dried at 80 °C to constant weight, and the weight loss was calculated based on the initial and final dry weights.

2.7. Microscopic characteristics

Optical microscopy was used to visualize morphological changes in the wood as a result of fungal degradation. To obtain thin slices (0.1 mm thickness) for microscopic analysis, the commercial chip samples were taken from each of the treatments and controls, boiled for 3 h with distilled water, and then sliced by hand using a scalpel. The wood sections were stained according to the technique described by Isenberg (1967).

2.8. Enzyme assays

Laccase (EC 1.10.3.2) activity was measured at 30 °C using 5 mM of 2,6-dimethoxyphenol (DMP) in 0.1 M sodium acetate buffer pH 3.6. Absorbance was monitored at 469 nm ($E_{469} = 27.5\ mM^{-1}\ cm^{-1}$) in a Shimadzu UV-3600 spectrophotometer. One laccase activity unit was defined as the amount of enzyme required to oxidize 1 μmol of DMP per minute at 30 °C and expressed as U ml^{-1} (Moreira et al., 2004).

Endo-β-1,4-glucanase (EC 3.2.1.4) activity was determined by measuring the liberation of reducing sugar with the 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959) using 0.5% carboxymethylcellulose (w/v) as substrate in 0.05 M sodium citrate buffer pH 5. Reactions were incubated at 50 °C for 30 min. Absorbance was measured at 540 nm in a Shimadzu UV3600 spectrophotometer. The carbohydrate fraction was extracted from the culture supernatant, and the amount of sugar liberated was calculated using a glucose standard curve. One endo-β-1,4-glucanase activity unit was defined as the amount of enzyme that releases 1 μmol of reducing sugar per minute at 50 °C.

Endoxylanase activity was determined indirectly through reducing sugars released by hydrolysis of soluble xylan beechwood (Sigma–Aldrich, USA) and subsequent detection by the 3,5-dinitrosalicylic acid method (DNS) previously described by Miller (1959).

The enzyme reaction was carried out at 50 °C for 60 min in sodium acetate buffer 0.05 M (pH 4.8) containing 1% beechwood soluble xylan (w/v). One endoxylanase unit was defined as the amount of enzyme that releases 1 μmol of reducing sugar per minute under assay conditions.

Enzyme activities were calculated on a chip weight basis and reported in U g^{-1}.

2.9. Analysis of wood composition

The chemical composition of non-treated and biotreated wood chips was determined according to the laboratory analytical procedure (LAP) and biomass analysis of the National Renewable Energy Laboratory (NREL). Sample preparation for compositional analysis was done according to NREL/TP-510-42620 (2008). Ethanol

extractives (42619; 2008), ash (NREL/TP-510-42622, 2008), and lignin (NREL/TP-510-42618, 2008) were also determined.

Carbohydrates determination by HPLC was performed with neutralized samples on an SHODEX SP810 column under the following conditions: pure water as eluent at a flow rate of 0.6 ml/min, 85 °C, and refractive index detector. The acetyl content was determined by HPLC using an AMINEX-HPX87H column under the following conditions: 4 mM·H_2SO_4 as eluent, flow rate 0.6 ml/min, 35 °C, and refractive index detector. All results are expressed on a dry wood basis (OD).

2.10. Kraft cooking: yield and kappa number

Kraft cooking runs were conducted using 200 g of dry chips. The chips were previously classified by square mesh sieves through the fraction passing the 25-mm sieve and retained on the 5-mm sieve. The kraft cooking was conducted in an M/K digester of 7-L capacity, with liquor recirculation under conditions shown in Table 1.

At the end of the cooking period, the pulp was washed in the digester by recirculation of water at 70 °C for 10 min. Defibration was then performed using a standard disintegrator to a consistency of 2%. The pulp obtained was discharged onto a 270 mesh sieve and washed thoroughly with water. The pulp was refined using a Somerville equipment with slots of 0.15 mm. Total, refined (accepts), and rejects content were determined. The percentage of accepts and rejects fractions provides the total yield. The kappa number determinations were performed according to TAPPI T236 om-99.

2.11. Physical and mechanical properties of the pulp

Physical and mechanical properties of refined pulps were determined at 4000 PFI revolutions. The standards used were: refining, TAPPI T 248 sp-00; drain resistance (Schopper-Riegler method), ISO 5267-1:1979; formation sheets for physical testing, TAPPI T 205 sp-95; physical testing, TAPPI T 220 sp-96.

2.12. Statistics analysis

Two-way ANOVA with Bonferroni post test was performed using GraphPad Prism 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Preliminary studies of laccase temperature and pH optimal, and enzyme thermo-stability in culture supernatants of P. brevispora BAFC 633

Many variables affect enzymatic activity during the bioprocess. Preliminary studies were carried out on culture supernatant to determine the best conditions to adapt fungal growth on wood chips and laccase activity during the biopulping process.

Table 1
Kraft cooking conditions.

Parameter	Condition
AA/wood (%)	24
Sulfidity (%)	30
Liquor/wood rate	5
Max. temperature. (°C)	170
Impregnation time (min)	60
H Factor	2000

Estimated temperature and pH optimum for laccase activity on culture supernatants were 55 °C and 3.6, respectively (Fig. 1A and B).

Regarding enzyme stability at different temperatures, lower temperatures (20 and 30 °C) did not affect the enzyme activity, while higher temperatures caused a reduction of activity (Fig. 1C). At 50 °C the half-life was approximately 2 h on culture supernatants, while at 60 °C the half-life was less than 1 h.

3.2. Effect of P. brevispora BAFC 633 growth on Pinus taeda wood chips in bioreactors

The experiments at the bioreactors were conducted at 30 °C and a pH of 3.6. This temperature did not match the optimum temperature found in the preliminary experiments; nevertheless, 70% of the maximum laccase activity was achieved. The enzyme activity at the bioreactor temperature (30 °C) showed a half-life of 50 h on culture supernatants (Fig. 1D).

At the end of the incubation step, the fungus secreted 744 U/g of laccase and 0.55 U/g of endo-β-1,4-glucanase, while endoxylanase activity was not detected. After 4 wk of colonization, the fungus grew in the form of a thin layer of velvety appearance leaving a reddish color on the chips' surface.

At the end of the fungal treatment the weight of chips decreased 10% compared with the control without treatment ($p < 0.05$).

Longitudinal sections of wood chips inoculated with *P. brevispora* BAFC 633 clearly showed fungal hyphae penetrating the lumens, vessels, and cells through pits (Fig. 2).

3.3. Chemical analysis

Wood extractives are those components soluble in neutral organic solvents consisting of wax, fats, resins, phytosterols, and volatile hydrocarbons. The extractives content of wood chips treated with *P. brevispora* BAFC 633 increased significantly ($p < 0.001$) (Table 2).

One of the main objectives of chemical pulping processes is to remove lignin selectively. The goal of wood chip treatment with the fungus previous to chemical pulping is to reduce the lignin content, thus obtaining a higher delignification in the pulping process. It was possible to achieve a loss of 4.22% of the total lignin in treated wood chips ($p < 0.01$) (Table 2).

The carbohydrate and lignin analysis provides information about the selectivity of the wood-degrading fungus. Arabans decreased significantly only in the treated samples ($p < 0.01$), whereas the other carbohydrates did not show significant differences ($p > 0.05$). These data support the fungus selectivity toward lignin selected degradation, preserving polysaccharides, a fundamental feature required for pulping processes.

3.4. Pulp yield, kappa number, and optical properties

The yield in treated samples decreased 1.73 points, but there was also a decrease of 6.3% kappa number, which indicates significant lignin reduction ($p < 0.01$). Usually it is desirable to achieve the highest degree of delignification with the production preservation, with the bonus that lignin removal facilitates the accessibility of the pulping liquor. It is convenient to evaluate the benefit of the reduction in the lignin due to fungal treatment against loss of carbohydrate yield, since it is important to keep this content as high as possible.

Fungal treatment caused a 7.1% increase in paper brightness ($p < 0.05$). This property is critical, since it affects the amount of bleaching chemicals used. Reducing use of these chemicals is beneficial for both economic and environmental reasons (Table 3).

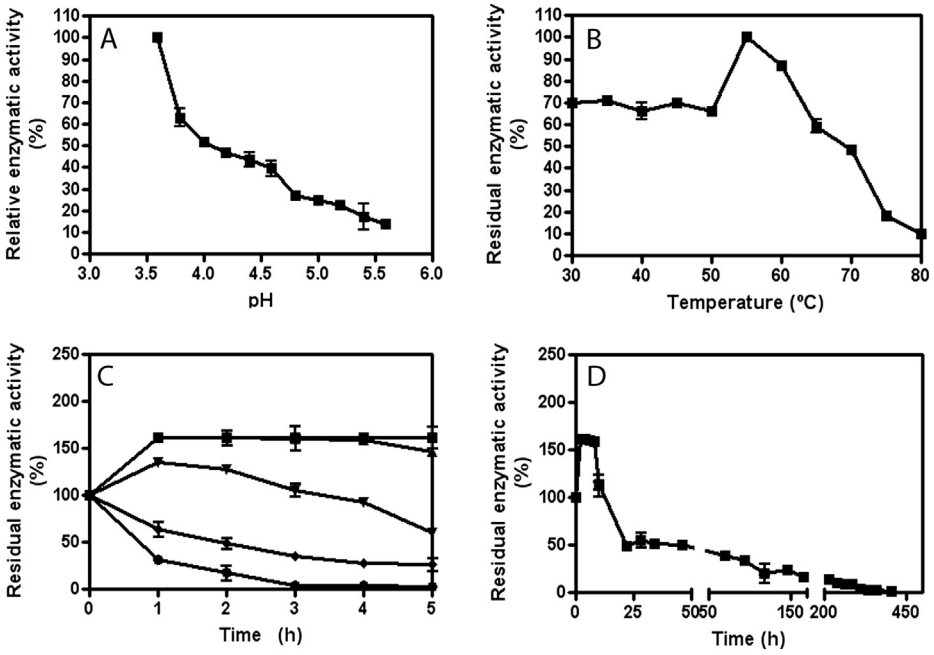

Fig. 1. Laccase activity in culture supernatants of *P. brevispora* BAFC 633, depending on pH (A) and temperature (B). Enzymatic stability of laccase activity at different temperatures (C): 20 °C (■), 30 °C (▲), 40 °C (▼), 50 °C (◆), and 60 °C (●), and after prolonged incubation at 30 °C (D).

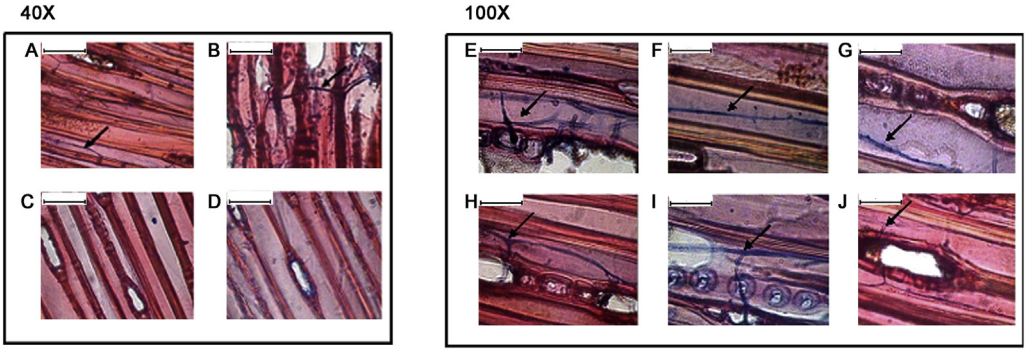

Fig. 2. Microscopic longitudinal sections (40X bar markers = 50 μm, and 100X bar markers = 15 μm) from *Pinus taeda* wood chips treated (A, B, E, F, G, H, I, J) and untreated (C, D) with *P. brevispora* BAFC 633. Arrows indicate the blue-stained hyphae and the red-dyed wood.

3.5. Physical and mechanical properties

The changes introduced by fungal treatment of wood chips and subsequent pulping process generally have an impact on the structural features of the paper, which in turn will affect the paper's properties (Table 4).

Table 2
Chemical analysis of wood chips with and without *P. brevispora* BAFC 633 treatment.

% Oven dry wood	Treated chips	Control chips
Glucan	42.15 ± 0.9	42.66 ± 0.52
Xylan	6.10 ± 0.03	6.19 ± 0.09
Araban	0.98 ± 0.00**	1.01 ± 0.04
Galactan	1.59 ± 0.07	2 ± 0.09
Mannan	7.44 ± 0.19	8.57 ± 0.39
Acetyl	1.60 ± 0.08	1.79 ± 0.15
Insoluble acid lignin	26.72 ± 0.08	28.79 ± 0.01**
Soluble acid lignin	2.73 ± 0.19**	1.96 ± 0.15
Total lignin	29.45	30.75
Alcohol extractives	3.26 ± 0.11***	1.29 ± 0.03
Ash	0.27	0.36

(*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

Measurement of the drainability of the refined pulp, an index of the degree of refinement, was conducted in the Schopper-Riegler apparatus and expressed in °SR. Determining the value of this index is one of the most important stages in the paper production process and strongly influences the conformation of the sheet and its physical properties. A slight increase of 1°SR of the refined pulps is considered an improvement of the pulp characteristics compared to unrefined samples. For the correct formation of the pulp in the paper machine, higher °SR is required.

The grammage (g m^{-2}) of the treated samples increased 0.49 points compared to the control samples, while the thickness, the

Table 3
Pulping of treated and untreated wood chips.

Pulps	Refined yield %	Rejects %	Total yield %	Kappa N°	Brightness % ISO
With fungal treatment	43.64	0	43.64	20.8 ± 0.21**	31.0*
Without fungal treatment	45.37	0,02	45.39	22.2 ± 0.16	28.8

(*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$.

Table 4
Physico-mechanical properties of kraft pulps refined at 4000 PFI revolutions, with and without *P. brevispora* BAFC 633 treatment.

Properties	Treated pulps	Control pulps
Drain resistance (°SR)	36	35
Paper weight [g/m^2]	64.89*	64.40
Thickness [mm]	0.08 ± 0.001	0.08 ± 0.001
Bulk [cm^3/g]	1.28*	1.27
Density [g/cm^3]	0.78	0.79
Burst [kPa m^2/g]	7.76 ± 0.28	7.61 ± 0.3
Tear [mN m^2/g]	10.16 ± 0.61	10.91 ± 0.41
Tensile [N m/gr]	100.56 ± 5.3	96.52 ± 5.1
Elongation %	3.30*	3.45
TEA [J/m^2]	146.03 ± 17.7	145.87 ± 18.4
Index TEA [J/g]	2.25 ± 0.273	2.27 ± 0.258
Airflow resistance [s]	357.91*	333.48
Permeability [μm/Pa s]	0.36 ± 0*	0.39 ± 0.1

(*) $p < 0.05$.

distance between both sides of the paper, remained constant. The density of the paper decreased in 0.01 points. Bulk and percentage elongation did not show significant differences ($p > 0.05$).

The air permeability of the paper is of great importance in the material specifications for the packaging industry and printing. Permeability is the airflow through the unit area of unity under the pressure difference in the unit of time under standard conditions of the test. In our case the paper permeability decreased slightly (0.03 μm/Pa s, $p < 0.05$), so that there was a minor effect of compaction of the fiber structure. The air resistance, which is the resistance to the passage of air offered by the paper structure, increased 24.45 points, indicating that the resulting fibrous sheet is more closed.

Regarding the remaining mechanical properties, no significant difference was found in strength, explosion, or tear resistance ($p > 0.05$), nor in the tensile energy absorption (TEA) ($p > 0.05$).

4. Discussion

The enzymatic activity and the lignin degradation are influenced by a number of factors, including the fungal strain, the nutrient composition (e.g., Mn^{2+} and Cu^{2+}), moisture content, pH, and temperature (Fonseca et al., 2010; Kaal et al., 1995; Zhao et al., 1996; Fu et al., 1997; Dorado et al., 2001; Snajdr and Baldrian, 2007; Patel et al., 2009). To control these factors, optimal conditions for the pretreatment process should be achieved, which would result in an optimal growth of white-rot fungi on the selected substrates. Knowing the biochemical characteristics of laccase in the supernatant also could serve to reduce the cost of enzyme purification. The literature discloses that the fungal laccase have higher activities at more acidic pH levels (Xu, 1997) and that the optimal temperature is between 50 and 60 °C (Baldrian, 2006). In our case, the optimum pH for laccase activity estimated in the supernatant was 3.6, while optimum temperature was estimated at 55 °C. However, conditions for fungal growth and enzyme production could be different, and these must be considered during bioprocessing with living microorganisms such as fungi. Moreover, enzyme stability is crucial during the biotechnological process. The supernatants containing laccase activity produced by *P. brevispora* BAFC 633 reached a half-life of ~50 h at 30 °C, being further extended to lower temperatures, which would be very important for production in bioreactors without the necessity of refrigeration. Addressing these fundamentals, we decided to conduct the biopulping experiments at 29 °C and pH 3.6 to ensure fungal growth and higher laccase stability.

White-rot fungi are capable of degrading all components of the cell wall, but there is wide variation in the types of degradation produced: Cellulose, hemicellulose, and lignin may be degraded simultaneously or each component may be degraded at different rates, and also the preferential attack to lignin and hemicellulose might occur (Blanchette et al., 1987). In this study, the levels of ligninolytic enzymes indicated a predominance of laccase activity associated with very low endocellulase activity and no evidence of endoxylanase activity.

The reddish pigmentation caused by *P. brevispora* BAFC 633 growing on chips could have resulted in the biosynthesis of melanin, powered by phenol oxidase activity (Ferraz et al., 2003). This pigmentation may be an indication of free radicals nearb hyphae, indicating the increment of oxidative potential more than hydrolytic potential, which was observed in quantitative studies. The reddish appearance due to the production of cinnabarinic acid produced by the fungus has also been described for *Pycnoporus cinnabarinus* (Temp and Eggert, 1999).

It is known that fungi at early stages of inoculation generally consume the nutrients stored in the rays (parenchyma cells), decreasing the sugar content of these cells and causing an initial weight loss (Talaeipour et al., 2010). Our results did not reveal a remarkable decrease of carbohydrates (except for the arabans), indicating a selective degradation of lignin.

Blanchette and Burnes (1988) reported that wood chips lost 38% of their weight after 12 wk of treatment with the fungus *P. chrysosporium* BKM-F1767. In their report, 46% of the weight loss occurred after exposure to the fungus HHB-6251, 17% was due to FPL-V-1706, and 38% was due to the fungus ME-PC-8. The major changes in the cell wall caused by the fungus are broken cell walls, loosening of the tubular structure of the fibers, separation of fiber, and disappearance of the middle lamella. In this sense, the optical microscope observations showed how the hyphae of *P. brevispora* BAFC 633 were introduced into the lumens cell through pits (Fig. 2). However, ultrastructural degradation characteristics could be observed only with the electron microscope. According to the chemical analysis results, the weight loss that occurred in this study could be attributed mainly to the loss of lignin in the wood chips treated since the carbohydrate content remained unchanged.

Some studies have shown that during biotreatment, the removal of lignin fractions allows better diffusion of enzymes that cause the attack on polysaccharides (Machuca and Ferraz, 2001). This study showed that *P. brevispora* BAFC 633 selectively removed the lignin without causing a decrease of carbohydrates, except for arabans. This null effect on carbohydrates in the treated samples was also observed by Villalba et al. (2006), and Ates et al. (2008). This is because lignin serves as a physical barrier and to some extent a chemical one for enzymatic degradation of these polysaccharides (Pew and Weyna, 1962). The low or null loss of glucans is related to the selective removal of lignin during the biopulping experiment (Akhtar et al., 1993; Ferraz et al., 2000). Other researchers have shown that one of the polysaccharides of the wood was simultaneously degraded with lignin (Eriksson, 1978; Setliff and Eudy, 1980). Ferraz et al. (2003) found that cellulose remained intact and that the carbon and energy sources originate from hemicellulose. These features of wood degradation are important in the process of biopulping. Our results showed that although total lignin decreased, the fraction corresponding to the acid-soluble lignin showed an increase in the samples treated with the fungus, which could be due to degradation products of low molecular weight and hydrophilic derivatives of lignin (Yasuda et al., 1998). However, the high amount of extractives present in the wood during biopulping with *P. brevispora* BAFC 633 could be a disadvantage in the pulping process since its presence is associated with pulp yellowing (Fengel and Wegener, 1984).

A small decrease in yield in the samples treated with the fungus was observed. Fungal pretreatment have been reported that

produce insignificant changes in the yield of chemical pulp (Giovannozzi et al., 1994; Atik et al., 2006; Villalba et al., 2006; Imamoglu and Atik, 2007; Ates et al., 2008). Wood and pulp lignin content was reduced as a result of *P. brevispora* BAFC 633 pretreatment, and this is consistent with results obtained by other authors (Akhtar et al., 1997; Bajpai et al., 2004; Villalba et al., 2006). These results indicate that the fungus causes structural changes in the fiber that would facilitate the penetration of the cooking liquor, which in turn enhances and accelerates the delignification.

The physical properties may be useful in predicting the potential of pulp in paper manufacture, as well as in examining the efficiency of the process under controlled conditions, so as to describe the performance of the final paper (Waterhouse, 1992). Although the fungus acts to open and soften the cell wall structure (Kirk et al., 1994), our results showed no benefits in terms of physical properties. Franco et al. (2006) found that the strength properties of pulps were similar in biotreated and control pulps. These quality parameters are very important, so additional research must be conducted to find the conditions to improve these properties. Moreover, fungal pretreatment improved optical properties, allowing whiter pulp, and these results are similar to those obtained by other authors in wheat straw pulp (Ates et al., 2008) and bagasse (Bajpai et al., 2004), although we achieved higher brightness with a raw material with higher lignin content and more closed structure. These increases are a result of the lower lignin content, which also leads to shorter cooking times, ease of bleaching, and consequently lower consumption of bleaching chemicals. Removing the maximum amount of lignin during the cooking process is very important because bleaching chemicals are much more expensive than cooking chemicals.

5. Conclusions

The inoculation of wood chips with the white-rot fungus *P. brevispora* BAFC 633 selectively removed the lignin during biotreatment, causing a reduction in kappa number associated with decreased levels of lignin in the pulp. The modification introduced by the biological action on the wood chips left a more open structure, which allowed better access of the pulping liquor to the cell wall components. The effectiveness of kraft pulping was improved, as demonstrated by the increased dissolution of lignin in pulps from wood chips treated with *P. brevispora* BAFC 633. The chemically modified lignin was more readily solubilized by the cooking liquor. Considering that brightness is one of the most important parameters defining pulp, and that fungal pretreatment with *P. brevispora* BAFC 633 improved pulp brightness, this work made an important contribution to end-product quality. While the effects of biological treatment (lignin depolymerization and enlarged pores) improved the kraft pulping process, more study is needed on biopulping with *P. brevispora* BAFC 633 so that processing conditions can be optimized.

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