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ORIGINAL PAPER

Evaluation of a combined brown rot decay–chemical delignification process as a pretreatment for bioethanol production from *Pinus radiata* wood chips

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Abstract Wood chips of *Pinus radiata* softwood were biotreated with the brown rot fungus (BRF) *Gloeophyllum trabeum* for periods from 4 and 12 weeks. Biodegradation by BRF leads to an increase in cellulose depolymerization with increasing incubation time. As a result, the intrinsic viscosity of holocellulose decreased from 1,487 cm^3/g in control samples to 783 and 600 cm^3/g in 4- and 12-week decayed wood chips, respectively. Wood weight and glucan losses varied from 6 to 14% and 9 to 21%, respectively. Undecayed and 4-week decayed wood chips were delignified by alkaline (NaOH solution) or organosolv (ethanol/water) processes to produced cellulosic pulps. For both process, pulp yield was 5–10% lower for decayed samples than for control pulps. However, organosolv bio-pulps presented low residual lignin amount and high glucan retention. Chemical pulps and milled wood from undecayed and 4-week decayed wood chips were pre-saccharified with cellulases for 24 h at 50°C followed by simultaneous saccharification and fermentation (SSF) with the yeast *Saccharomyces cerevisiae* IR2-9a at 40°C for 96 h for bioethanol production. Considering glucan losses during wood decay and conversion yields from chemical pulping and SSF processes, no gains in ethanol production were obtained from the combination of BRF with alkaline delignification; however, the combination of BRF and organosolv processes resulted in a calculated production of 210 mL ethanol/kg wood or 72% of the maximum theoretically possible from that pretreatment, which was the best result obtained in the present study.

Keywords Brown rot decay · *Pinus radiata* · *Gloeophyllum trabeum* · Bioethanol · Alkaline pulping · Organosolv pulping

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Introduction

Lignocellulosic materials are the most promising and potential source of carbohydrates for fuel ethanol production. Nevertheless lignocellulosic biomass is roughly 70% polysaccharides (cellulose and hemicelluloses) and its saccharification to monomeric sugars is difficult owing to the ultrastructure's resistance to breakdown and the presence of lignin, which covers the fibers and protects carbohydrates from enzymatic attack [2, 5]. When wood or other lignocellulosic feedstock is used as raw material, its structure should be first disrupted in a way that cellulose and hemicelluloses become more accessible to enzymes and the monosaccharides released can be further fermented to ethanol. Enzymatic hydrolysis is attractive because it produces better monosaccharide yields than acid-catalyzed hydrolysis and lower formation of inhibitors which may severely decrease the ethanol production from the hydrolyzate [12, 17]. Several pretreatment processes are available and have been evaluated in order to remove lignin, reduce cellulose crystallinity, and increase the accessibility of the pretreated material to enzymes and further fermentation of sugars to ethanol. These processes include steam explosion, organosolv

delignification, ammonia fiber expansion (AFEX), hydrolysis with concentrated or diluted acid, among others [5, 8, 18, 19]. Biological pretreatment of wood with lignin-degrading white rot fungi combined with organosolv or kraft delignification was developed as an environmentally friendly process to produce cellulosic pulp for papermaking in a process known as bio-pulping [3, 9]. Recently, white rot decay associated with ethanol–water pulping was evaluated as a way to improve wood delignification to produce pulps that were further submitted to separated hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) for bioethanol production [11]. This previous work showed that the combination of a biological and chemical delignification process may act synergistically, reducing the severity of the pretreatment and improving cellulose saccharification. On the basis on this precedent, we hypothesize that the biotreatment of wood with brown rot fungi (BRF) could also be a feasible alternative to combine with chemical or mechanical processes to generate cellulosic substrates for bioethanol production. The former is based on the particular mechanism of wood decay by BRF. These organisms use mainly non-enzymatic mechanisms to produce activated oxygen species, such as hydroxyl radical (·OH), through the Fenton reaction to degrade wood in the initial steps of decay [6, 7]. Suzuki et al. [16] showed that the biodegradation of softwoods with BRF leads to an increase in cellulose depolymerization with increasing incubation time. A brown-rotted wood with partially depolymerized cellulose submitted to a chemical delignification process could be more easily hydrolyzed by enzymes and fermented to ethanol by yeasts. In this work, wood chips of *Pinus radiata* were biotreated by the brown rot fungus *Gloeophyllum trabeum* for different periods. Milled wood and pulps from alkaline or ethanol/water processes were submitted to enzymatic hydrolysis with cellulases and fermented with the yeast *Saccharomyces cerevisiae* for bioethanol production.

Methods

Wood biodegradation with *Gloeophyllum trabeum*

Wood chips of approximately 2.5 × 2.0 × 0.3 cm from *P. radiata* D. Don were obtained from a local pulp mill. Before fungus inoculation, wood chips were immersed in water for 16 h. Residual water was drained and 2,500 g (dry basis) of moist wood chips (55–60% moisture) were sterilized inside 20-L bioreactors at 121°C for 30 min.

Sterilized liquid culture medium (200 mL) containing 2% malt extract and 0.5% soybean peptone was inoculated with 20 discs (5 mm in diameter) of *G. trabeum* (ATCC 11539) pre-cultured in solid medium and incubated under stationary conditions for 10 days at 27°C. The grown mycelium from several culture flasks was filtered, washed, and blended in a laboratory blender with 300 mL of sterilized water. The fungal suspension was used to inoculate the sterilized wood chips with a volume of suspension corresponding to 1 g of fungal mycelium per kg dry wood. Bioreactors were incubated at 27°C and 55% of relative humidity for 4, 8, and 12 weeks. Bioreactors with sterilized but non-inoculated wood samples were also prepared and used as controls to compare with decayed wood chips. After each biodegradation period, bioreactors were opened; wood chips were washed with tap water to remove superficial mycelium and dried to determine weight loss and chemical composition.

Chemical delignification of *P. radiata* wood chips

The undecayed control and the 4-week decayed wood chips were delignified by alkaline and organosolv processes to produce cellulosic pulps. Alkaline pulping was performed in an 1-L Parr reactor loaded with 50 g (dry basis) of wood chips and 500 mL of 25% NaOH solution (w/w, on wood basis). Reactor was heated at 1.6°C/min until 180°C and maintained at this temperature for 300 min. Organosolv pulping was also performed in the 1-L Parr reactor which was loaded with 50 g (dry basis) of wood chips and 300 mL of ethanol–water solution (60:40, v/v). Reactor was heated at 1.6°C/min until 200°C and maintained at this temperature for 60 min [11]. After each pulping, the residue was filtered over a filter paper in a Buchner funnel and the solids were disaggregated with 2 L of water in a TAPPI laboratory blender for 10 min at 2,800 rpm. Pulps were washed abundantly with tap water and centrifuged until 30–35% consistency. Pulps were weighed and the exact moisture content was determined in a portion of the pulp to calculate the pulp yield. Pulps were stored in plastic bags at 4°C until use. Never-dried pulps were used for enzymatic saccharification and fermentation assays.

Chemical characterization of wood and pulps

Undecayed and decayed wood chips were milled in a knife mill and sieved with a 40/60 mesh screen. Milled wood was extracted with ethanol/toluene according to TAPPI standard method T204 cm-97. Milled wood samples and pulps (also milled but not extracted) were characterized for carbohydrates and lignin content [10]. A sample (300 mg) was weighed in a test tube and 3 mL of 72% (w/w) H_2SO_4 was added. The hydrolysis was carried out in a water bath at 30°C for 1 h with stirring every 10 min. Later, the acid was diluted to 4% (w/w) with 79 mL of distilled water; the mixture was transferred to a 250-mL Erlenmeyer flask and autoclaved for 1 h at 121°C as a post-hydrolysis treatment.

The residual material was cooled and filtered through a number 4 sintered-glass filter. Solids were dried to constant weight at 105°C and determined as insoluble lignin. Soluble lignin was determined by measuring the absorbance of the solution at 205 nm. The concentration of monomeric sugars in the soluble fraction was determined in an HPLC equipment (Merck Hitachi) with an AminexHPX-87H column at 45°C, eluted at 0.6 mL/min with 5 mM H_2SO_4 and using a refractive index detector. Calibration curves were prepared with sugar standards. The standard deviation from the average values determined by this technique was equal to or lower than 3%. Component losses were calculated on the basis of wood composition of undecayed or decayed wood chips.

Holocellulose was prepared by weighing 250 mg of extractive-free milled wood in a 50-mL round-bottom flask, adding 5 mL of deionized water, 2 mL of glacial acetic acid, and 5 mL of sodium chlorite 80%. The flask was sealed with a glass cap and immersed in a water bath at 90°C for 1 h. After this period, a further 2 mL of glacial acetic acid and 5 mL of sodium chlorite 80% were added to the flask and the reaction were carried out for another 1 h at 90°C. The reaction was stopped by immersion of the flask in a water bath at 10°C. Solids were filtered through a porous glass filter number 2, washed with 500 mL of deionized water, dried at 105°C until constant weight, and determined as holocellulose [20].

Intrinsic viscosity of holocellulose and chemical pulps was determined following ISO 5351:2004 standard. Wood solubility in 1% NaOH was determined by the ASTM D1109-84(2007) standard.

Enzymatic saccharification and fermentation of wood and pulps

Enzymatic saccharification of undecayed and 4-week decayed milled wood as well as of alkaline and organosolv pulps from the selected wood samples (control and 4-week decayed) was performed with cellulases under the following conditions: 2 or 4 g of substrate (wood or pulp, on a dry basis) was weighed into a 250-mL Erlenmeyer flask to which was added 40 mL of 0.05 M citrate buffer solution (pH 4.8). Under these conditions the consistency of the medium was 5 or 10%, respectively. Commercial preparations of Celluclast 1.5 L (Novozymes, USA) at 20 FPU/g pulp and β-glucosidase Novozym 188 (Novozymes, USA) at 20 or 40 UI/g pulp were added to the medium and enzymatic hydrolysis was carried out for 24 h at 50°C and 150 rpm. After this period, a 2-mL aliquot was withdrawn for quantification of glucose released during the enzymatic hydrolysis. After that and in the same flask, 3 g/L of the thermotolerant yeast *Saccharomyces cerevisiae* IR2-9a was added to medium which was also supplemented with 5 g/L yeast extract, 5 g/L peptone, 1 g/L NH_4Cl, 1 g/L KH_2PO_4, and 0.5 g/L $MgSO_4$ [1]. The temperature of the shaker was set to 40°C and the flasks were incubated at 150 rpm for 96 h. Aliquots of 2 mL were periodically sampled for ethanol quantification in the time course of fermentation. Ethanol concentration was determined by gas chromatography on a Perkin-Elmer autosystem XL Headspace using a flame ionization detector (FID) and an HPSMS 30-m column. The theoretical ethanol yield was calculated by assuming that all the glucose in the pretreated material (milled wood or pulps) is available for fermentation and that each 1 g of glucose generates 0.51 g ethanol. All experiments described in this section were performed in triplicate.

Results and discussion

Brown rot biodegradation of *P. radiata*

The chemical composition of undecayed *P. radiata* wood chips was 45.1 ± 0.3% glucans, 24.4 ± 0.3% hemicelluloses, 27.2 ± 0.4% lignin, and 2.6 ± 0.2% ethanol/toluene-soluble extractives. *G. trabeum* quickly colonizes pine wood and after 10 days of incubation all wood chips were covered with the yellowish-brown mycelium mats of the fungus. Table 1 summarizes results obtained for wood component losses due to fungal biodegradation for 4–12 weeks period and also shows the viscosity of holocellulose preparations and wood solubility in 1% NaOH. Average wood weight losses due to fungal decay were from 6 to 14%. According to a brown rot biodegradation pattern, carbohydrates (i.e., glucans and hemicelluloses) were extensively decayed as compared with lignin. Hemicelluloses were more affected reaching 31% loss in 4 weeks and up to 47% loss after 12 weeks of incubation. Glucan losses were from 9 to 21% in the same period. Lignin was not severely attacked by brown rot fungi and no losses were observed until 2 months of biodegradation. As reported in several studies, the main effect in lignin during brown rot decay is related to demethylation and dihydroxylation reactions in which the aromatic structure is not severely affected [4, 7]. Both wood weight and glucan losses were important factors to observe when applying this biotreatment when considering further wood saccharification. High weight losses are undesirable as they affect the cost of the raw material and a loss in glucan content represents a lower amount of glucose available for fermentation. As a consequence of carbohydrate degradation during the fungal incubation period, the amount of holocellulose and its intrinsic viscosity in residual wood decrease from 68 to 51–55% and from 1,487 to 600 mL/g, respectively. Suzuki et al. [16] associated the decrease in hollocellulose viscosity with the decrease in the degree of polymerization of cellulose as the viscosity is

Table 1 Characterization of *P. radiata* wood chips decayed by *G. trabeum*

	Control	4-week decayed	8-week decayed	12-week decayed
Weight loss (%)	–	6 ± 2	10 ± 2	14 ± 1
Glucan loss (%)	–	9 ± 1	12 ± 1	21 ± 2
Hemicellulose loss (%)	–	31 ± 1	37 ± 2	47 ± 2
Lignin loss (%)	–	0	0	3 ± 1
Holocellulose (%)	67.7 ± 0.3	54.9 ± 0.5	51.5 ± 0.4	51.4 ± 0.7
Holocellulose viscosity (mL/g)	1,487 ± 6	783 ± 8	653 ± 5	600 ± 6
1% NaOH solubility (%)	17.7 ± 0.1	37.9 ± 0.6	41.1 ± 0.3	43.3 ± 0.3

more affected by the fragmentation of long-chain polymers. Same authors also reported a rapid decrease in holocellulose viscosity of approximately 50% in the first week of decay of wood blocks followed by a less intense reduction in this property thereafter. In the present work, 50% of reduction in holocellulose's viscosity was observed at the end of the 4th week of incubation of wood chips (no shorter-term assays were performed at this time). Wood solubility in 1% NaOH showed a trend to increase up to the first 4 weeks of decay (from 17 to 38%) indicating the removal of easily soluble substances generated during wood biodegradation.

Chemical delignification of brown-rotted *P. radiata*

Chemical delignification by alkaline or ethanol–water liquors were performed in the undecayed and 4-week decayed *P. radiata* wood chips. Wood chips obtained after 8 and 12 weeks of decay were not used due to the high weight and carbohydrates losses. Moreover, holocellulose viscosities and wood solubilization in 1% NaOH values were similar to the 4-week decayed sample. The aim of the delignification processes was to remove lignin and recover the cellulosic residue (pulp) for further enzymatic hydrolysis and fermentation to ethanol. During the alkaline process, the 4-week decayed wood chips were solubilized to a higher extent than the control samples and only 32% of solids were recovered as pulp versus 42% of pulp yield obtained with the undecayed control. The chemical composition of alkaline pulps indicated that carbohydrates were solubilized in higher amounts than lignin, and glucan amount is lower in pulps from decayed wood (77% glucan) than in control pulps (83% glucan). The higher solubilization of carbohydrates in decayed wood resulted also in pulps with higher amount of residual lignin (17%). Probably, the low degree of polymerization of cellulose chains in decayed wood favored the peeling and hydrolysis reactions of the carbohydrates in alkaline medium. The intrinsic viscosities of pulps also indicate that the short cellulose and hemicellulose chains were generated during cooking with values that are 45% lower than control pulps.

Organosolv cooking of *P. radiata* wood chips with ethanol/water was more selective for delignification than the alkaline process. Low residual lignin amount and higher glucan content were obtained for pulps of 4-week decayed wood as compared with control pulps. However, wood solubilization was higher in decayed wood and resulted in lower pulp yield (41%) than the obtained for control wood chips (46%). As the amount of glucose on a wood basis is the same for both pulps (~34%) the reduction in pulp yield for pulp from decayed wood was associated with greater removal of lignin and hemicelluloses in this sample than in control pulps. Moreover, ethanol and solubilized lignin may act as a scavenger for the free radicals formed during pulping, reducing the extent of lignin condensation which can contribute to the higher pulp yield obtained as compared with the alkaline pulping [13, 14]. The viscosity of organosolv pulps from both control and decayed wood chips is similar or even higher than the viscosity of alkaline pulps. The reduction in viscosity of organosolv pulp from decayed wood chips was 18% lower as compared with the control pulp.

Enzymatic saccharification and fermentation of *P. radiata* wood and chemical pulps

The effect of brown rot decay in enzymatic hydrolysis and fermentation was evaluated in milled wood samples, alkaline and organosolv pulps of undecayed and 4-week decayed wood samples. Assays were performed at two levels of substrate consistency (5 and 10% of solids) and two enzyme loads (20:20 and 20:40 Celluclast to β-glucosidase ratio). The biological process was divided into two steps: in the first, the enzymatic hydrolysis of cellulose in wood and pulps was performed at 50°C for 24 h; in the second, the yeast was added to the same flask and the temperature was lowered down to 40°C for fermentation in a simultaneous saccharification and fermentation process (SSF).

Figure 1 shows the results for the pre-enzymatic hydrolysis of wood and pulps. As expected, wood without any kind of pretreatment is not hydrolyzed by enzymes and only 5–7% of glucans were converted into glucose. However,

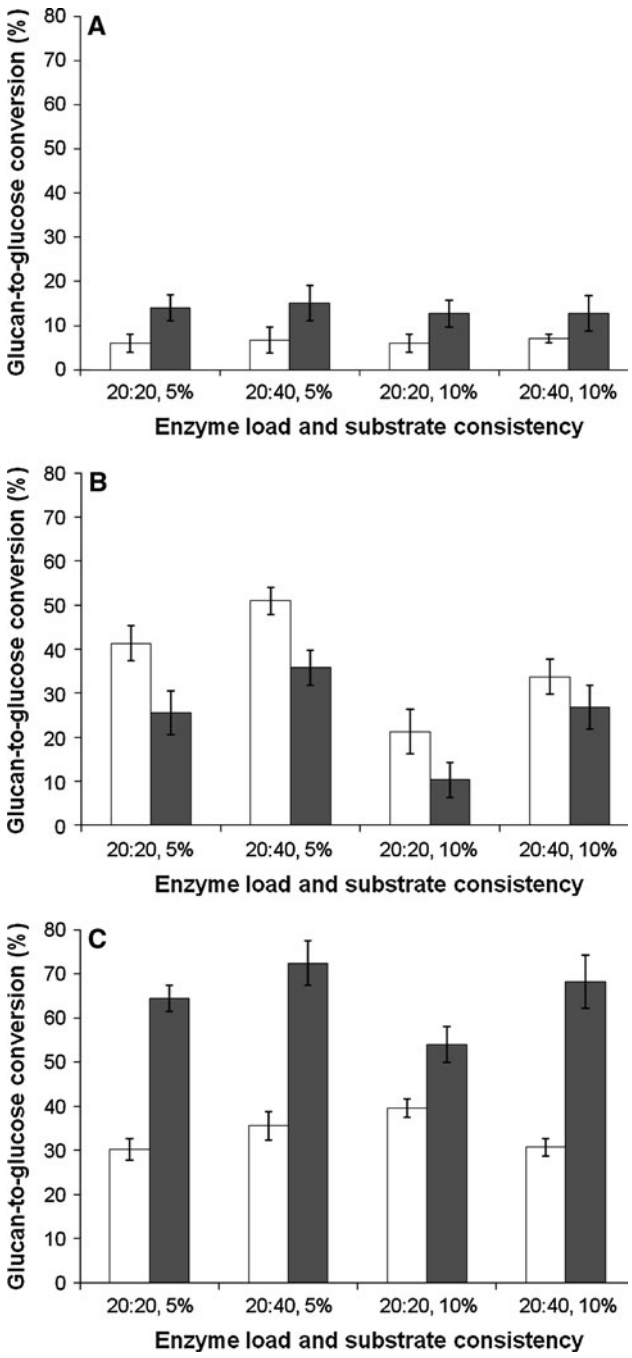

Fig. 1 Enzymatic hydrolysis of *P. radiata* wood and chemical pulps. *White bars* represent control samples and *gray bars* represent 4-week decayed samples

biotreatment with BRF and the partial depolymerization of cellulose helps to increase glucose liberation by cellulases in decayed milled wood and a twofold higher glucan-to-glucose conversion was observed (Fig. 1a). Enzymatic hydrolysis of alkaline pulps showed no difference between glucose released from control and pulp from decayed wood in assays carried out under the same conditions (Fig. 1b). Experiments performed at low consistency and high β-glucosidase load presented approximately 50% of glucan-to-glucose conversion which is the highest result obtained as compared with the other conditions. High substrate concentrations can cause inhibition of enzymes which decreases the hydrolysis rate which also depends on the ratio of total enzyme load to the amount of substrate added [15]. For alkaline pulps, the amount of residual lignin in pulp can also be a drawback in the process and slow down or inhibit the enzymatic hydrolysis. Lowering consistency and increasing enzyme load can increase the yield of hydrolysis but could also increase the cost of pulp processing.

The enzymatic step applied to organosolv pulps showed that pulps from brown rot decayed wood were more easily hydrolyzed than control pulps (Fig. 1c). A glucan-to-glucose conversion of 55–70% was obtained for bio-pulps while for control pulps the same conversion was between 30 and 40%. Enzyme loads and substrate consistency seem to play a minor role in this case since similar results were obtained for each condition and the main difference is related to the origin of the pulp.

When the partially hydrolyzed wood is submitted to simultaneous saccharification and fermentation a low yield of ethanol is obtained (Fig. 2). Approximately a 30% ethanol yield (from the maximum theoretically possible considering that all glucose is fermented to ethanol with a yield of 51%) is obtained from decayed wood and only 10% from control milled wood which reflects what was previously observed during the enzymatic hydrolysis step and could also be associated with the depolymerization of cellulose due to fungal decay.

When alkaline pulps were submitted to SSF similar ethanol yields were obtained from pulps from undecayed and decayed wood (Fig. 3). In this case, the amount of solids and the enzyme loads had a significant effect on ethanol production. Experiments performed at 5% pulp consistency and with 20:40 Celluclast to β-glucosidase ratio (Fig. 3a) presented the highest ethanol yield (70%) as compared with experiments performed with high amounts of solids in suspension (Fig. 3b) in which part of the problem seemed to be associated with mixing and mass transfer in the fermentation process or even the increase in inhibitory compounds.

Figure 4 shows the results for SSF of organosolv pulps under the different conditions of consistency and enzyme loads. The most suitable pulp for bioethanol production was that from 4-week decayed wood and fermented at 5% consistency with 20:40 enzyme load (Fig. 4a) in which 95% of ethanol yield was obtained after 96 h of fermentation. In this process a clear benefit of the biotreatment can be seen especially when comparing the ethanol yield of the control pulp with 5% consistency and 20:40 enzyme load and the bio-pulp processed at 10% consistency and 20:20 enzyme load which produced the same yield of ethanol.

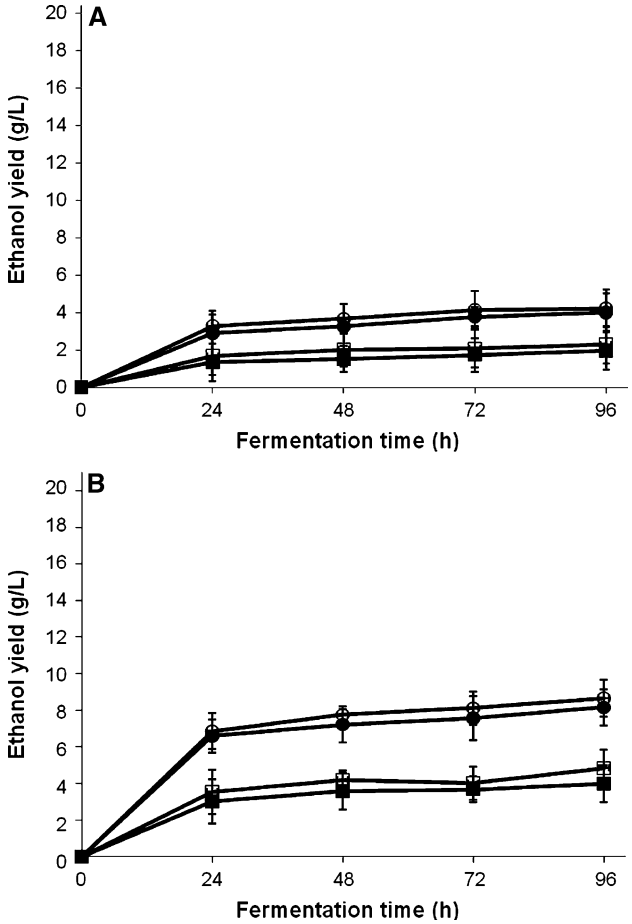

Fig. 2 Ethanol production from milled wood of control and 4-week decayed samples at different substrate consistency (**a** 5% and **b** 10%), and enzyme load (*filled squares*) control, 20:20; (*open squares*) control, 20:40; (*filled circles*) decayed, 20:20; (*open circles*) decayed, 20:40

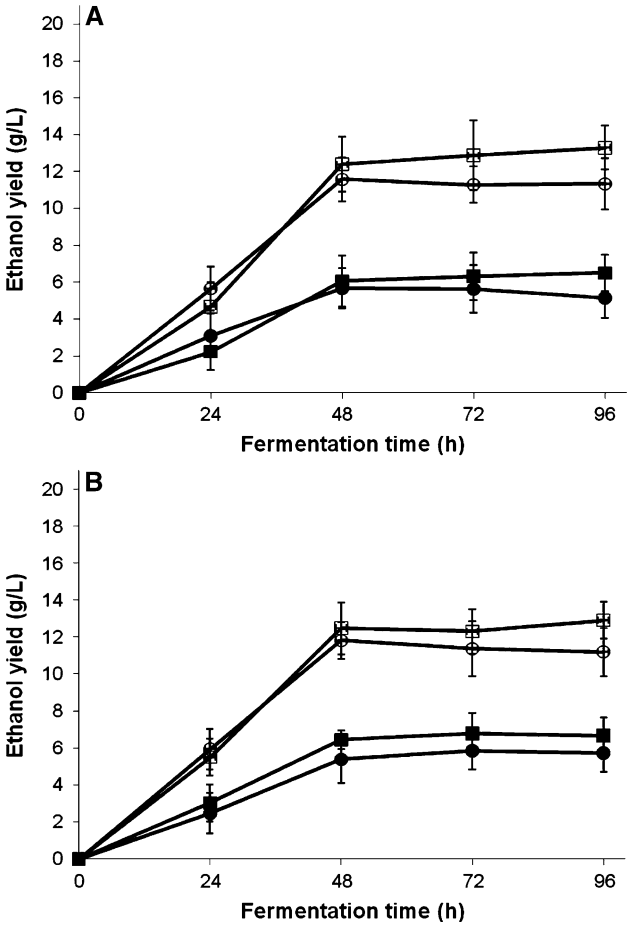

Fig. 3 Ethanol production from alkaline pulps from control and 4-week decayed wood at different substrate consistency (**a** 5% and **b** 10%), and enzyme load (*filled squares*) control, 20:20; (*open squares*) control, 20:40; (*filled circles*) decayed, 20:20; (*open circles*) decayed, 20:40

Since more mass is present in the bio-pulp at 10% consistency, in absolute values, more ethanol is produced from this pulp than from the control pulp at 5% consistency.

Despite similar or even superior performance of bio-pulps in generating ethanol from both alkaline and organosolv processes, one important parameter to consider is the integrated ethanol yield taking into account both glucose losses due to fungal decay and delignification processes. This approach can indicate the real effect of biotreatment and the delignification steps in the bioethanol production. Calculations were done considering that the maximum amount of ethanol obtainable from *P. radiata* undecayed wood is 322 mL/kg wood and from 4-week decayed wood is 293 mL/kg wood (calculated considering the 9% of glucose loss by fungal treatment). This calculation assumed that all the glucose available in wood is converted into ethanol with 51% yield (ethanol density of 790 kg/m^3). Results obtained for the combinations of 5 and 10% wood or pulp consistency and 20:40 enzyme load are shown in Fig. 5, which clearly shows that fermentations at 5% consistency provided higher ethanol yields from chemical pulps as compared with 10% consistency assays. No clear differences were observed from milled wood regarding consistency, and ethanol production is around 45 and 85 mL/kg wood for control and 4-week decayed wood, respectively.

A significant effect of the different delignification processes in the wood samples is noted. In the alkaline process, control pulps generated more ethanol than decayed samples, which can be associated directly to the low pulp yield and low carbohydrate retention in pulp from decayed wood (Table 2). When 5% consistency and 20:40 enzyme load were used for fermentation, alkaline pulps generated almost 160 mL ethanol/kg wood or roughly 50% of the maximum possible from wood (Fig. 5a). Organosolv pulping was a more efficient process for bioethanol production which is also enhanced by the use of 4-week biotreated wood. A yield of 210 mL ethanol/kg wood was obtained from pulp of 4-week decayed wood or 72% of the maximum possible

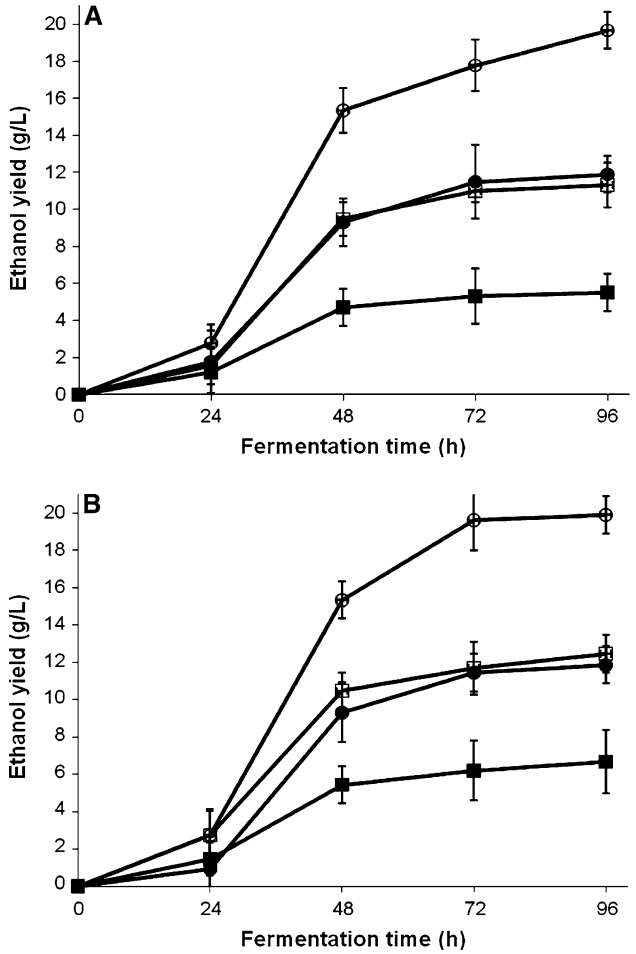

Fig. 4 Ethanol production from organosolv pulps from control and 4-week decayed wood at different substrate consistency (**a** 5% and **b** 10%), and enzyme load (*filled squares*) control, 20:20; (*open squares*) control, 20:40; (*filled circles*) decayed, 20:20; (*open circles*) decayed, 20:40

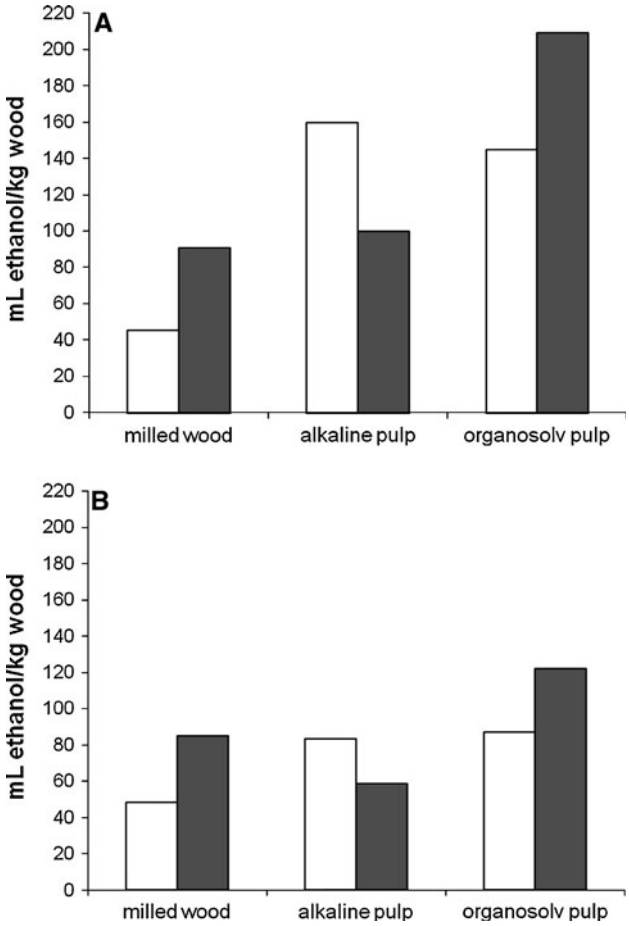

Fig. 5 General yield of the different processes, in mL ethanol/kg wood, at different substrate consistency (**a** 5% and **b** 10%) and at enzyme load of 20:40 Celluclast to β-glucosidade ratio. *White bars* represent control samples and *gray bars* represent 4-week decayed samples. Maximum theoretical ethanol yield from glucose of *P. radiata* control and decayed wood chips is 322 and 293 mL/kg wood, respectively

from decayed wood (Fig. 5a). This was the best result obtained from this work and indicates the benefit of pretreating wood with brown rot fungi combined with the ethanol/water process.

Wood treatment with BRF increases ethanol production from both milled wood and organosolv pulps. The increase in ethanol production could be associated to both depolymerization of cellulose chains in wood and the selective delignification of organosolv pulp which also retained more carbohydrates in the pulp. Alkaline delignification presented a different mechanism which favored the removal of short cellulose chains from decayed wood and does not improve ethanol production from decayed wood. The high amount of residual lignin in this pulp could also decrease enzymatic hydrolysis and fermentation efficiency of the wood. When comparing only the ethanol produced from control pulps from alkaline and organosolv processes, results were similar and 145–160 mL ethanol/kg wood was produced at 5% consistency and 20:40 enzyme load. To make the process more feasible, a shorter treatment period should be evaluated (only 1 or 2 weeks of decay) and also fermentation strategies should be improved to allow the use of high amounts of solids in both enzymatic hydrolysis and fermentation which can lead to more ethanol production per batch.

Table 2 Characteristics of alkaline and organosolv pulps produced from *P. radiata* wood chips

	Alkaline pulps		Organosolv pulps	
	Control	4-week decayed	Control	4-week decayed
Pulp yield (%)	42 ± 2	32 ± 2	46 ± 1	41 ± 1
Glucose (%)	83 ± 2	77 ± 2	75 ± 1	82.4 ± 0.5
Hemicellulose (%)	9 ± 1	6 ± 1	8.8 ± 0.3	4.7 ± 0.2
Lignin (%)	11.1 ± 0.3	17.0 ± 0.5	13 ± 1	7.0 ± 0.2
Pulp viscosity (mL/g)	569 ± 4	309 ± 3	688 ± 3	565 ± 5

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