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Action of White-Rot Fungus *Panus tigrinus* on Sugarcane Bagasse

Evaluation of Selectivity

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Abstract

Biological pretreatments with three selected strains of *Panus tigrinus* were used for delignification of sugarcane bagasse. The fungi with potential for delignification were analyzed by determining the chemical composition of the decayed bagasse samples, and the selectivity in terms of weight loss of the different components was evaluated. All the strains grow abundantly on bagasse as unique carbon source. After determining the chemical composition of degraded bagasse, *P. tigrinus* FTPT-4745 was selected as the most efficient strain on a 6-g scale, since the carbohydrates were preserved. *P. tigrinus* FTPT-4741 and FTPT-4742 were the most efficient strains on a large scale (100 g).

Index Entries: *Panus tigrinus*; selective biodegradation; sugarcane bagasse; white-rot fungus.

Introduction

Biodegradation of lignocellulosics is one of the most important processes in nature. Sugarcane bagasse is a lignocellulosic residue with a complex structure and can be efficiently decayed, especially by white-rot fungi *(1–5)*. The introduction of this natural process into the pulp and paper industry has great economic potential and contributes to environmental

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preservation. White-rot fungi are able to degrade cellulose, hemicelluloses, and lignin at similar rates. Some strains belonging to this group of fungi degrade lignin selectively. Akhmedova et al. *(6)* reported on an example of selective degradation by white-rot fungi. They utilized 34 strains of xylophagous fungi and screened 15 that were able to oxidize gallic and tannic acids, presenting also the high potential of these strains to degrade lignin. *Panus tigrinus* UzBi-013 stood out as a producer of peroxidase, laccase, cellobiase, endoglucanase, and xylanase. The same researchers also observed an increase in biopolymer degradation during the first 10 d of incubation *(6)*.

The production of ligninolytic and hemicellulolytic enzymes has been studied in depth in recent years *(7–10)*. Prasad et al. *(7)* investigated the influence of enzymatic preparations from raw extracts on the bleachability of thermomechanical pulps obtained from sugarcane bagasse and found that the brightness of the pulps increased by two to three points in relation to a control not treated with enzymes.

Gonçalves et al. *(11)* evaluated the action of *P. tigrinus* strains on the pretreatment of sugarcane bagasse, using Fourier transform infrafred techniques together with enzymatic activities and pulp properties to screen the strains. In the present work, three strains of the white-rot fungus *P. tigrinus* previously screened *(3,11)* were utilized (FTPT-4741, FTPT-4742, FTPT-4745) for biologic delignification of sugarcane bagasse to be employed in the production of pulp. The reason for choosing this kind of treatment is that it gives rise to a most efficient and economic pulping process, without damaging the environment.

Materials and Methods

Figure 1 illustrates the experiment.

Fungi

Three strains of *P. tigrinus* (FTPT-4741, FTPT-4742, FTPT-4745) from a collection belonging to Fundação Tropical de Pesquisa e Tecnologia André Tosello (Campinas, SP, Brazil) were grown from a vegetative inoculum on malt extract agar (4%) at 28°C for 5 d. The three strains had been previously screened by Esposito et al. *(3)* and Gonçalves et al. *(11)*.

Sugarcane Bagasse

The sugarcane bagasse used in our experiments contained glucan (51%), pentoses (23%), acetyl groups (2%), Klason lignin (21%), H_2SO_4-soluble lignin (1%), and ash (3%). Its particles averaged 2.1 cm in length and 0.12 cm in width.

Culture Conditions

Three semi-solid-state fermentation cultures were carried out at 28°C with no additional carbon source in 250 mL-Erlenmeyer flasks with and

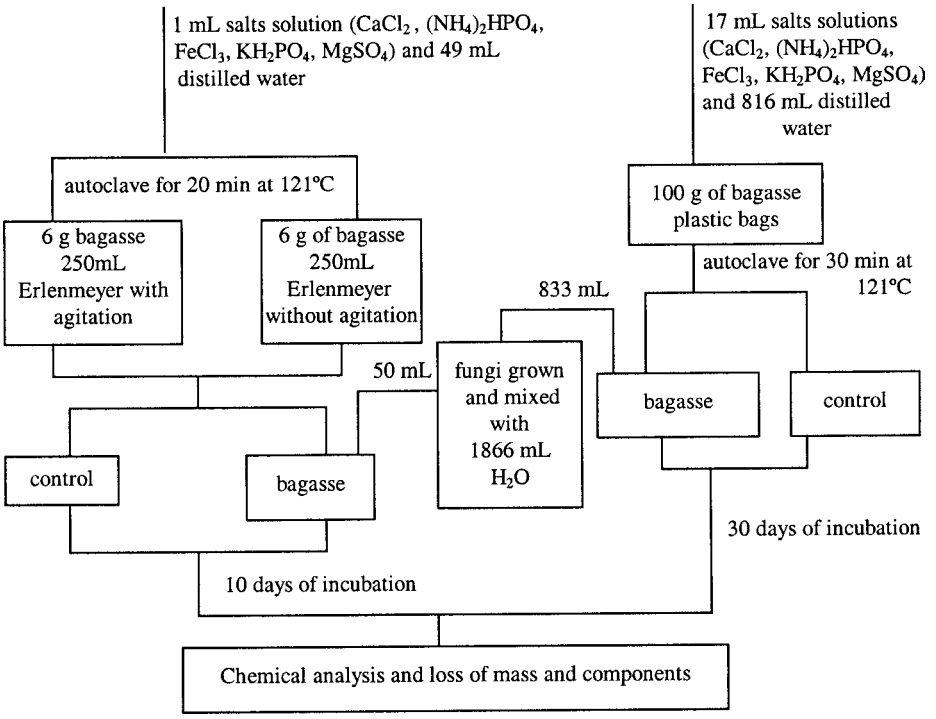

Fig. 1 Illustration of the experiment.

without shaking for 10 d. Samples (6 g) of depithed and sterilized sugarcane bagasse were supplemented only with 1 mL of salts solution (0.3 g/L of $CaCl_2$, 2 g/L of $[NH_4]_2HPO_4$, 0.2 g/L of $FeCl_3$, 1 g/L of KH_2PO_4, 0.5 g/L of $MgSO_4$) and 49 mL of distilled water. Another set of experiments was carried out for 30 d, using plastic bags containing 100 g of sterilized sugarcane bagasse supplemented with 17 mL of the same salts solution and 816 mL of distilled water. The experiments were realized in duplicate and the volume of 1866 mL was divided into 50 mL for each flask and 833 mL for each plastic bag. The inoculum/bagasse ratio was maintained at 5 to 6% (v/w).

Chemical Analysis of Decayed Sugarcane Bagasse

The modified Klason method was utilized *(12)*. Samples (1 g) of decayed and undecayed sugarcane bagasse were washed with water and treated with 5 mL of 72% H_2SO_4. After a 7-min stirring at 45°C, 25 mL of water was added to the mixture, which was posthydrolyzed under 1.05 bar for 30 min. The product was filtered and the insoluble portion (Klason lignin) quantified. The pH of the hydrolysate was adjusted to 1.0–3.0 with 1 mol/L of NaOH, filtered in a Sep-Pak C_{18} cartridge, and analyzed by high-performance liquid chromatography in a Shimadzu LC10 chromatograph with an Aminex HPX-87H column at 45°C. Mobile phase was 0.005 mol/L of H_2SO_4 at 0.6 mL/min. The hydrolysis products were determined by refraction

index and quantified by using calibration curves *(13,14)*. Soluble lignin was determined as described by Rocha et al. *(15)* using absorption at 280 nm of alkaline solutions obtained from the hydrolysate.

The mass loss of sugarcane bagasse and the loss of each sugarcane bagasse component were calculated by using Eqs. 1 and 2, respectively. For Eq. 1,

$$L_m = \left[\frac{m_i - m_f}{m_i}\right] \times 100\% \qquad \text{(Eq. 1)}$$

in which L_m is mass loss; m_i is initial mass of bagasse (dry base); and m_f is final mass of bagasse, after treatment (dry base). For Eq. 2,

$$L_c = \left[\frac{m_{ci} - m_{cf}}{m_{ci}}\right] \times 100\% \qquad \text{(Eq. 2)}$$

in which L_c is component loss; m_{ci} is the amount of component in the original bagasse (%) × initial mass of bagasse; and m_{cf} is the amount of the component in the bagasse after treatment (%) × final mass of bagasse.

Statistical Analyses

The experiments on fungal growth were performed in duplicate and the chemical analyses were performed in triplicate. The standard deviation was calculated using the mean values of the data. Statistical validation of the data was performed by using the STATGRAPHICS software at 95% confidence level.

Results and Discussion

Chemical analysis

Chemical analysis of decayed samples of sugarcane bagasse is useful for the determination of the loss of mass and components after treatment with fungal strains. Selectivity values were calculated from these parameters. Quantification procedures were based on hydrolysis of the samples adopting standard methods.

Previous results of degradation of sugarcane bagasse by the three strains show that all the samples had about the same chemical composition after degradation *(3)*. FTPT-4741 preserved carbohydrates and lowered the lignin content, but only FTPT-4742 preserved the xylan/glucan ratio. Esposito et al. *(3)* also showed that FTPT-4745 has the highest enzyme production, and this fact was correlated to the highest amount of soluble lignin.

Tables 1–3 show the chemical composition of the decayed and undecayed (control) bagasse samples in the three systems utilized. Unlike Esposito et al. *(3)*, we increased the agitation during hydrolysis to favour the reaction and measured the ash content.The composition of control samples was a little different because the samples are subject to slight

Table 1
Chemical Composition of Decayed and Undecayed (Control) Sugarcane Bagasse After Shaking Flasks

	FTPT-4741	FTPT-4742	FTPT-4745	Control
Glucan (%)	39.76 ± 0.05	37.87 ± 0.03	40.58 ± 0.02	41.10 ± 0.20
Acetyl (%)	1.87 ± 0.02	1.97 ± 0.02	2.21 ± 0.03	2.32 ± 0.05
Xylan (%)	22.55 ± 0.02	21.50 ± 0.20	23.34 ± 0.02	23.57 ± 0.05
Arabinan (%)	1.62 ± 0.01	1.68 ± 0.03	0.24 ± 0.01	1.89 ± 0.05
Total carbohydrates (%)	65.80 ± 0.10	63.00 ± 0.30	66.37 ± 0.08	68.80 ± 0.40
Klason lignin (%)	18.82	18.59	19.90	19.66
Soluble lignin (%)	5.14 ± 0.04	7.50 ± 0.70	2.50 ± 0.20	3.30 ± 0.10
Total lignin (%)	24.00	26.10	22.40	22.90
Ash (%)	0.64	0.32	0.70	0.57
Mass balance (%)	90.40	89.40	89.50	92.30
Xylan/glucan ratio	0.567	0.567	0.575	0.574
Pentoses/glucan ratio	0.655	0.664	0.636	0.677

Table 2
Chemical Composition of Decayed and Undecayed (Control) Sugarcane Bagasse In Stationary Flasks

	FTPT-4741	FTPT-4742	FTPT-4745	Control
Glucan (%)	40.54 ± 0.07	40.80 ± 0.50	43.1 ± 0.50	40.48 ± 0.02
Acetyl (%)	1.56 ± 0.05	2.19 ± 0.02	2.34 ± 0.04	2.27 ± 0.03
Xylan (%)	24.03 ± 0.04	25.01 ± 0.51	26.07 ± 0.03	23.20 ± 0.20
Arabinan (%)	1.29 ± 0.03	1.27 ± 0.01	1.33 ± 0.02	1.84 ± 0.01
Total carbohydrates (%)	67.40 ± 0.20	69.00 ± 1.00	72.7 ± 0.60	67.8 ± 0.30
Klason lignin (%)	19.59	18.26	18.17	19.68
Soluble lignin (%)	4.19 ± 0.02	6.00 ± 1.00	4.79 ± 0.05	2.59 ± 0.01
Total lignin (%)	23.80	24.10	23.00	22.30
Ash (%)	0.49	0.37	0.29	0.45
Mass balance (%)	91.70	93.80	96.00	90.60
Xylan/glucan ratio	0.593	0.613	0.606	0.574
Pentoses/glucan ratio	0.663	0.698	0.692	0.676

degradation, especially chemical hydrolysis, during sterilization. In some decayed samples, the xylan/glucan ratio did not change whereas in others it increased (in comparison with the control), showing that the fungus does not attack xylan very strongly. Results from Table 1 (shaken flasks) show that FTPT-4742 produced samples with a low glucan content but with a soluble lignin content (7.5%) 2.3 times higher than that of the control.

In the stationary system (Table 2), the amounts of glucan were very similar in the samples decayed by FTPT-4741 and FTPT-4742 strains, and higher in the samples decayed by FTPT-4745. Acetyl content of in natura bagasse was 2%, and only sample decayed by FTPT-4741 showed a smaller value, owing to its deacetylation. This sample also showed a lower xylan/

Table 3
Chemical Composition of Decayed and Undecayed (Control) Sugarcane Bagasse in Plastic Bags

	FTPT-4741	FTPT-4742	FTPT-4745	Control
Glucan (%)	43.96 ± 0.07	41.60 ± 0.50	40.60 ± 0.20	44.19 ± 0.20
Acetyl (%)	2.420 ± 0.01	2.32 ± 0.07	2.22 ± 0.03	2.37 ± 0.01
Xylan (%)	24.30 ± 0.20	23.70 ± 0.60	23.80 ± 0.80	25.41 ± 0.01
Arabinan (%)	1.70 ± 0.10	1.78 ± 0.02	1.67 ± 0.01	1.80 ± 0.30
Total carbohydrates (%)	72.30 ± 0.40	69.00 ± 1.00	68.00 ± 1.00	73.80 ± 0.50
Klason lignin (%)	19.80 ± 0.50	19.00 ± 1.00	19.60 ± 0.03	21.90 ± 0.01
Soluble lignin (%)	3.00 ± 2.00	5.00 ± 0.50	4.40 ± 0.60	3.94 ± 0.03
Total lignin (%)	22.00 ± 3.00	24.00 ± 2.00	24.00 ± 0.60	25.84 ± 0.04
Ash (%)	0.26 ± 0.04	0.30 ± 0.20	0.09 ± 0.03	0.38 ± 0.06
Mass balance (%)	94.90	93.70	92.50	100.00
Xylan/glucan ratio	0.552	0.570	0.586	0.562
Pentoses/glucan ratio	0.646	0.668	0.682	0.610

Table 4
Losses of Mass and Components of Bagasse Samples Treated with *P. tigrinus* Strains in Shaken Flasks [a]

Losses (%)/selectivity	FTPT-4741	FTPT-4742	FTPT-4745
Mass	13.32	24.10	7.40
Glucan	16.58	30.00	8.50
Xylan	17.10	30.84	8.32
Lignin	9.42	13.61	9.46
Selectivity	0.60	0.45	1.11

[a]Selectivity is the loss of lignin/loss of glucan; values are related to the control.

glucan ratio. The same low xylan/glucan ratio was provided by FTPT-4741 when using the plastic bag system. The percentage of glucan preservation increased 5.6 and 8.1% in comparison with the values provided by FTPT-4742 and FTPT-4745, respectively. Acetyl contents ranged from 2.2 and 2.4% for both the control and for the decayed samples.

Losses of mass and components were calculated for a better evaluation of the action of the strains on the bagasse, since chemical analysis is restricted to the verification of changes in the composition of the samples. These values together with the selectivity values (loss of lignin/loss of glucan ratio) are given in Tables 4–6. Higher values of glucan and xylan losses occurred in the shaken and stationary flasks (Tables 4 and 5), corresponding to a rapid fungal colonization in the bagasse. On the other hand, the losses of mass and carbohydrates in the plastic bags were lower than in the flasks, and the lignin was efficiently removed.

Table 5
Losses of Mass and Components of Bagasse Samples Treated With *P. tigrinus* Strains in Stationary Flasks[a]

Losses (%)/selectivity	FTPT-4741	FTPT-4742	FTPT-4745
Mass	21.10	16.65	7.76
Glucan	20.97	16.02	2.03
Xylan	18.42	10.13	3.48
Lignin	15.79	9.82	4.98
Selectivity	0.75	0.62	2.43

[a]Selectivity is the loss of lignin/loss of glucan; values are related to the control.

Table 6
Losses of Mass and Components of Bagasse Samples Treated with *P. tigrinus* Strains in Plastic Bags[a]

Losses (%)/selectivity	FTPT-4741	FTPT-4742	FTPT-4745
Mass	5.10	5.15	9.31
Glucan	5.58	10.61	16.60
Xylan	9.37	11.53	15.34
Lignin	18.10	12.00	15.71
Selectivity	3.24	1.13	0.95

[a]Selectivity is the loss of lignin/loss of glucan; values are related to the control.

Lignin should be removed without damage to the carbohydrates. This behavior is reflected by the selectivity, which should be >1.0 for a preferential removal of lignin with respect to the degradation of glucan.

Only the FTPT-4745 strain was selective concerning lignin degradation in flasks, mainly in the stationary flasks. In such a system, the aeration is not effective, decreasing the action of the oxidative laccase enzyme, which also decays carbohydrates. With the plastic bags, the situation was the inverse, but FTPT-4745 showed a selectivity value close to 1.0. The mass losses in plastic bags were very small for FTPT-4741 and FTPT-4742 strains, confirming that the enzymatic activity varies according to the system, as reported in other articles *(3,11)*.

Conclusion

It is possible to evaluate the action of *P. tigrinus* on the sugarcane bagasse fibers by determining the chemical composition of the bagasse and its losses of mass and components. The results show that this fungus is adequate for the pretreatment of sugarcane bagasse, causing delignification and preserving the fibers' properties. Consequently, this process can be

utilized in pulp production. FTPT-4745 was selective for lignin degradation in flasks, mainly in stationary flasks. The mass losses in plastic bags were very small for FTPT-4741 and FTPT-4742 strains.

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