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A new alternative process for Kraft E1 effluent treatment

A combination of photochemical and biological methods

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Abstract

Lentinus edodes (UEC-2019 strain) was selected after screening 51 ligninolytic strains of fungi for their ability to decolorize phenolic industrial effluent with high content of lignin peroxidases, Mn-peroxidases and beta-glucosidases. This strain removed 73 % of color in the *Eucalyptus* Kraft E1 effluent in 5 days without any additional carbon sources. A 13% mycelial adsorption was found. Correlation between mass loss, COD, TOC and decolorization was observed. When an effluent pre-irradiated (10 min) in the presence of ZnO was treated with *L. edodes,* a marked enhancement of the decolorization at 48 h was obtained. *L. edodes* is an active fungus in this pre-treatment and biobleaching process. The combined photo-biological decolorization procedure appears to be an efficient decontamination method with great potential in industrial effluent treatment.

Abbreviation: COD – Chemical oxygen demand, TOC – Total organic carbon

Introduction

The pulp and paper industry discharges large volumes of brown colored effluents as a result of different processes applied in wood and pulp bleaching (Sant'Anna 1992). The chlorolignins and chlorophenols originating mainly from lignin degradation products in these effluents are toxic and their elimination is of great importance (Bergbauer & Eggert 1992).

Alternative decontaminating physical/biological methods were recently compared with conventional ones (e.g. ultrafiltration/ anaerobic, aerobic treatment (Eriksson 1992); photodegration/ aerobic treatment (Duran et al. 1991; Duran 1992).

Fungal treatment is the most attractive method for the decolorization and decontamination of bleaching Kraft mill effluents containing chlorolignins (Eriksson, 1992). Recently, the potential use of white-rot fungi for biopulping, biobleaching and treatment of pulp mill waste effluents has been reviewed (Eriksson, 1990; Duran et al. 1990; Bourbonais & Paice 1992). *Phanerochaete chrysosporium, Trictoporia sp., Aspergillus sp. and Coriolus versicolor* were used for this purpose (Duran 1992). A good example of this procedure is the mycelial color removal process (MyCor) for decolorizing E1 bleach plant effluent (Yin et al. 1989).

Since the enzyme expression in the growth culture medium is different than that seen in the effluent (Esposito et al. 1991)(similar in Archibald et al. 1990) it was necessary to carry out a specific fungal selection for effluent treatment. In general, in the effluent, lignin-peroxidase activity decreases

while laccase and peroxidase activities increase in both cases, with beta-glucosidase undergoing slight changes (Esposito et al. 1991). The latter enzyme was previously suggested as being important in lignin degradation (Kondo et al. 1990: Duran et al. 1992).

In a previous screening a succesful treatment of E1 effluent by *L. edodes* (UEC-2019) with 73% decolorization, 60% chemical oxygen demand reduction, 30% of biochemical oxygen demand reduction, 30% total phenolic reduction and only 13% of decolorization due to mycelial adsorption (Esposito et al. 1991) was reported. In these experiments it was found that the decolorization capacity did not exhibit any apparent correlation with the lignin peroxidase production capacity and this conclusion has been recently corroborated by Archibald (1992).

Besides a good fungal selection for effluent treatment, a pre-treatment is necessary, in order to shorten the hydraulic retention time of the effluent in a bioreactor. Previously, it was found that pre-irradiation of the effluent (15 min) at pH 6.5, followed by fungal culture filtrate (from *C. sitophila* (TFB-27441)) treatment, results in efficient decolorization and that decolorization is enhanced in the presence of hydrogen peroxide which acts as an enzymatic cofactor (Duran et al. 1991). Since the total enzymatic content of this fungus was known (Duran et al. 1987; Ferrer et al. 1991; Ferrer et al. 1992), the importance of ligninolytic enzymes was quite evident.

In view of this fungal strain with a high decolorization capacity of E1 Kraft effluent and the fact that pre-irradiation in the presence of a semiconductor also enhances the enzymatic degradation of the effluent lignins, the present study recommends an efficient method for decolorization of Kraft effluent by a combination of a photochemical pretreatment (Duran et al. 1991) and a biological decolorization. Pre-selected fungus, *L. edodes* (UEC-2019) (Esposito et al. 1991) without any additional nitrogen or other carbon sources in the effluent was studied.

Material and methods

Strains

L. edodes (UEC-2019) was obtained from UNICAMP Culture Collection (Biol. Chem. Lab.). Stock cultures were maintained in malt agar medium at 5° C.

Culture conditions

Strain culture were grown as previously described (Duran et al. 1987; Esposito et al. 1991): 1.25% malt extract at 28°C, pH 5.0 at 150 rpm for 5 days. After *L. edodes* growth at standard conditions, the mycelial mass was filtered and added to 25 mL of non sterilized effluent at initial pH of 5.0 and shaken at 150 rpm 28°C.

Biotoxicity

A standard method with *Spirillum volutans* was used (Goetcher et al. 1983).

Enzyme activities

All the enzymes activities were measured at least in duplicated.

- *Ligninase.* Enzyme activities were measured as published (Tien & Kirk, 1984). One unit corresponds to 1 umol veratryl alcohol oxidized per min per liter.
- *Mn-peroxidase.* Enzyme activity was determined by oxidizing phenol red in the presence of hydrogen peroxide and Mn(II) (Kuwahara et al. 1984).The extinction coefficient of oxidized phenol red (E= 4460 M-l cm-l) was used (Michel et al. 1991). One unit corresponds to 1 umol of phenol red oxidized per min per liter.
- *Phenoloxidase.* Laccase and peroxidase activities (U/L) were measured as published (Szklarz et al. 1989). One unit corresponds to 1 umol of syringaldazine oxidized per min per liter.
- *Beta-glucosidase.* Enzyme activity was measured using p-nitrophenyl-beta-D-glucopyranoside as substrate (Tan et al. 1987). One unit corresponds to 1 umol of p-nitro phenol formed per min per liter (E= 18.5 mL $umol^{-1}$ cm^{-1}).

Total phenols
The phenol analyses were carried out using the standard procedure (APHA, 1989a).

Effluent
The effluent (RIGESA S.C. Brazil) used in this study was the first alkaline extraction stage (pH 9.5–13.0) from a bleached Kraft pulp *(Eucalyptus)* paper mill. This effluent has 0.05% of reductant sugar, around 0.3 mg/L phosphorous and 6 mg/L nitrogen. The effluent was stored at 0°C, under nitrogen, pH 2.0.

Measurement of color in treated Kraft effluent
Color removal by *L. edodes* was measured in duplicate by a standard method (CPPA, 1975). The nature of the color before and after decolorization was characterized by a published method (Momohara et al. 1989).To 3 mL of E1 (initial absorbance at 465 nm of 0.74, pH 7.6) 85 mg of sodium dithionite was added and the solution was left at pH 5.5 for 24 h. After the pH was adjusted to 7.6, the solution was centrifuged and the absorption measured at 465 nm (Quinone-like color). To 3 mL of E1 (initial absorbance at 465 nm of 0.74, pH 7.6) 100 mg of sodium borohydride was added and the solution was left at pH 10.5 for 24 h. After adjusting the pH to 7.6,the solution was centrifuged and the absorption measured at 465 nm.

Adsorption experiments with dead mycelium
In the experiment with dead fungi, the wet mycelium was autoclaved for 30 minutes at 120°C, and the experiment was carried out as described above for live mycelium.

Sugar determination
The carbohydrate determination of Kraft effluent was carried out by the DNS procedure (Miller, 1959).

Cod determination
The chemical oxygen demand (COD) was determinated by standard methods (APHA, 1989b).

Total organic carbon
TOC was measured with a TOC-5000 total organic carbon analyser (Shimadzu, Japan) according to ISO standard 8245 (ISO, 1987).

Photochemical pre-treatment
The reaction was carried out in a beaker containing 25 mL of effluent, with an initial pH value of 5.0 and 50 mg of the ZnO photocatalyst from Aldrich. The solution was irradiated from the top using a 250 W Phillips lamp (without glass cover) at a distance of 12 cm (fluence rate 108 W m-2 at > 254 nm) for 10 min and then this pre-irradiated effluent was divided in two parts, one being the blank and the other was treated with a mycelial pellet (2 g of wet mass, 97% humidity) (initial abs. 0.44 at 465 nm). During the illumination, the solution was stirred while bubbling oxygen through it (Duran et al. 1991). The following control experiments were carried out: Effluent/ light/oxygen; effluent/ZnO/oxygen/dark; effluent/ZnO/ nitrogen/light.

Results and discussion

Effluent treatment

Since in a previous screening (Esposito et al. 1991), *Lentinus edodes* (UEC-2019) was found to be the most efficient fungus for effluent decontamination, the kinetics of decolorization by this fungi was studied (Fig. 1). A color increase in the effluent was observed during the first 24 h. This is probably due to a high laccase activity in the culture previous to the effluent addition (Table 1). The same effect with laccase-producing fungi, as *Phlebia radiata,* was recently published (Lankinen et al. 1991). Although laccase activity was high after 120 h, the decolorization process was efficient due to lignin peroxidase, Mn-peroxidase and beta-glucosidase by the effluent induction (e.g from 4.2 to 10.3 U/L in lignin peroxidase, from 0 to 11.2 U/L in Mn-peroxidase and from 8.0 to 14 U/L in beta-glucosidase).

Photochemical pre-treatment

Experiments with *L. edodes* cultures were carried out with pre-irradiation of the Kraft E1 effluent in

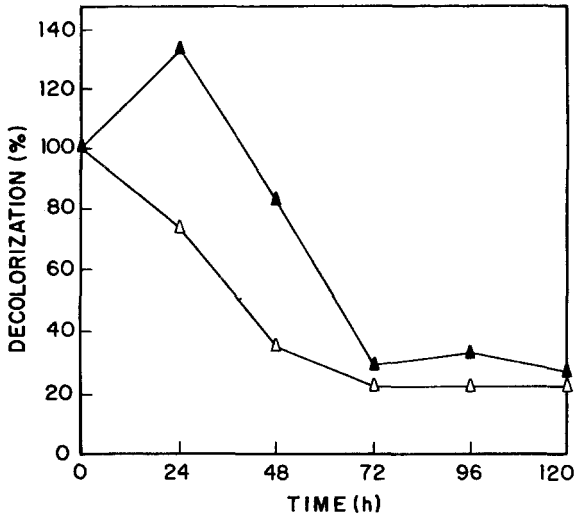

Fig. 1. Decolorization kinetics for biological treatment (-▲-) and for the pre-irradiated/biological treatment system (-△-).

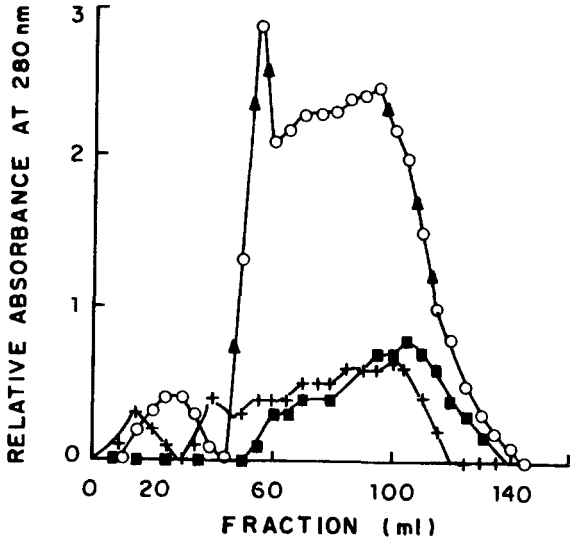

Fig. 2. Changes in the molecular weight distribution of Kraft effluent (E1) (at 280 nm) after 120 h of incubation. Untreated (-○-), pre-irradiated (-▲-), biological treatment (-+-) and pre-irradiated/biological treatment system (-■-).

order to asses the importance of this pre-treatment for decolorization. All the control experiments (see Materials and Methods) showed that the only catalytic effect by the ZnO was in the presence of light (>254 nm) and oxygen. Effluent was colored only when oxygen in the absence of ZnO was used.

The results for the kinetics of decolorization in both systems (Fig. 1) (biological decolorization and pre-irradiated-biological decolorization system) showed that at 24 h the biological decolorization exhibited an increase of the color value and that only after 48 h an effective decolorization occurred. On the other hand, no color increase and an efficient decolorization in the pre-irradiated-biological system were observed. Hence, decolorization in the pre-irradiated system reduces the time taken for biological decolorization by ca. 36 h. At 120 h, the decolorization in the biological system and the pre-irradiated-biological system were 73% and 78%, respectively. This is a good indication that decolorization in the pre-irradiated effluent was kinetically more efficient than that in the non-irradiated one. Under pre-irradiation conditions, the enzyme profile was different (Table 1). Laccase and peroxidase were drastically diminished in the irradiated effluent compared to the non-irradiated one. Beta-glucosidase decreased to half its original value, but lignin peroxidase and Mn-peroxidase increased significantly, about 4 folds, in both cases. It is noteworthy that the presence of lignin peroxidase in *L. edodes* (UEC-2019) under the studied conditions has also been detected by other authors (Leatham et al. 1991; Bonnarme & Jeffries, 1990). The molecular mass distribution of the treated effluent in both systems after 120 h is shown in Fig. 2. Although both systems showed significant decreases in the to-

Table 1. Enzyme present in the fungal culture, and fungi acting on E1 effluent and in photochemical pre-treatment of effluent after 120 h (U/L).

	Laccase	Peroxid.	Lignin.	Mn-peroxid.	B-Glucosid.
Culture	11.0 ± 0.2	0.3 ± 0.04	4.2 ± 0.21	0.0	8.0 ± 0.10
Effluent	11.0 ± 0.2	0.5 ± 0.02	19.3 ± 1.61	11.2 ± 0.10	14.0 ± 0.10
Photo/effluent	1.0 ± 0.1	0.0	78.3 ± 1.90	37.3 ± 1.50	8.0 ± 0.10

The values correspond to the fungal culture after 120 h, and after the mycellium was filtered and added for other 120 h to the E1 effluent. In the pre-irradiated sample the same procedure was followed.

tal effluent lignin masses, the high molecular weight lignins disappeared only with the associated photochemical/biological treatment (Fig. 2).

Table 2 presents biomass losses (this corresponds to mineralization, CO2 and water are not shown) which were 70% in the biobleaching system and 80% in the pre-irradiated-biobleaching system. The chemical oxygen demand reduction in both systems was 60% and 70%, respectively. In the control sample and in the biological treatment gave a 60% reduction (Table 2), indicating a good correlation between mass loss, COD and TOC. The total biodegraded phenol was around 49% in both cases. In an independent process (unirradiated sample) in which a 47% of decolorization was reached a significative decrease of the biotoxicity in the presence of *S. volutans* was found (Table 2).

Decolorization mechanism of spent bleaching liquor by L. edodes *(UEC-2019)*

The color was divided into three types, depending upon its reactivity with dithionite (quinone type color) or with borohydride (total quinone and carbonyl type color) or upon their lack of reactivity with both reagents (denominated as another type of color by Momohara et al. (1989)).

The quinone type color from E1 effluent from RIGESA represents 60% of the color. Therefore, the removal of the quinone type color in this effluent is very important for total removal. Results for the biological decolorization, pre-irradiated, and pre-irradiated-biological decolorization systems are shown in Fig.3.

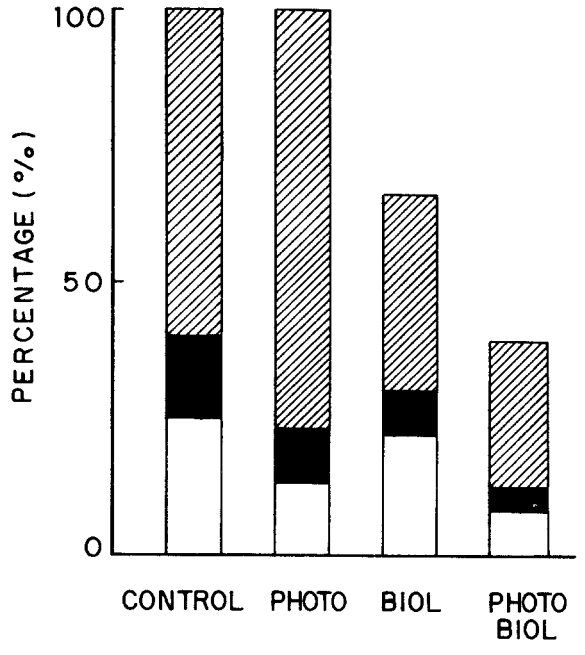

Fig. 3. Distribution of color within the different treatments: Quinone type color (-▨-), carbonyl type color (-■-) and other type color (-□-).

Among the three color types, the quinone type and carbonyl type colors were removed in the biological decolorization more efficiently than the other types of color. After the pre-irradiated effluent and biological treatment for 120 h, no chromophore specificity was observed. Although no change in the initial absorption was observed for the pre-irradiated effluent, a redistribution of the

Table 2. Effluent treated for 120 h with *Lentinus edodes.*

E1 Effl.	Decolor. (%)	COD (mg O_2/L)	COD red. (%)	Total phenol (mg/L)	Total phenol red. (%)	Mass loss (%)	TOC ppm	Red (%)	Toxicity MEC_{90} (b) (c)
Control	0	259	0	90	0	0	902	0	20
Photochem. pre-treat. (a)	0	238	9	83	8	0	ND(d)	ND	ND
Biological treat.	73	105	60	46	49	70	356	60	30
Photochem. (a) pre-treat. + Biol. treat.	78	82	70	45	50	80	ND	ND	ND

a) Photocatalysed with ZnO for 10 min at / > 254 nm. b) Minimum effective concentration (%) of effluent required to eliminate reversing mobility in greater than 90% of viable cell (MEC_{90}). This result corresponds to a sample of effluent treated by *L. edodes* for around 60 h with a 47% decolorization reduction. d) ND not determined.

color types occurred (an increase of quinone type color was observed). This pre-irradiated sample was largely changed in the associated biological process.

In summary, the combined photo-biological decolorization procedure appears to be an efficient decontamination process with no color specificity and with a great potential in effluent treatment. We believe that in combination with other physical method this should be a feasible commercial procedure.

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