SCREENING OF WHITE-ROT FUNGI FOR EFFICIENT DECOLOURIZATION OF BLEACH PULP EFFLUENTS

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SUMMARY. Several white-rot fungi collected in the South of Chile were evaluated for their ability to decolourize kraft pulp bleached effluents. Strain 158, recently identified as a Ramaria sp., showed the highest potential for the biological treatment of E1 effluent: 90% of the colour was removed by this strain grown under air, with a similar rate and extent of decolourization as Phanerochaete chrysosporium, under oxygen.

INTRODUCTION

The first alkaline extraction stage (E1) bleach plant effluent is the major source of colour in the effluents from pulp and paper industry. The main contributors to the colour are polymeric, chlorinated, heavily oxidized degradation fragments of lignin (Kirk et al., 1983, Royer et al., 1983). Moreover their high chlorine content, 3 kg of lignin bound chlorine/ton of bleached paper (Wainwright, 1990), is highly toxic (Livernoche et al., 1983). Most microorganisms cannot attack lignin or its polymeric degradation products and, as a result, the coloured and toxic materials from bleach plants are largely released in an almost unmodified form. Even though some physico-chemical processes, such as ultrafiltration, carbon adsorption and lime precipitation are able to effectively remove colour, all have severe drawbacks, such as high cost per unit volume of wastewater treated or unreliability in operation (Joyce et al., 1984).

White-rot fungi, a limited group of basidiomycetes, possess an active ligninolytic system (Paice et al., 1989, Kirk et al., 1978), which is able to degrade protolignin as well as heavily modified lignins, such as Kraft lignin and chlorinated lignins (Chang et al., 1983). The latter suggests the potential of these fungi for decolourization and degradation of chlorolignins in the E1 effluent. Most of the work on fungal effluent treatment has been carried out with the ligninolytic white-rot fungus Phanerochaete chrysosporium (Bumpus et al., 1987; Chang et al., 1983). This fungus is able to decolourize this type of effluent, but only in the presence of high oxygen partial pressures, and an external carbon source is usually required (Kirk et al., 1978, Huynh et al., 1985). As much as 80% of the colour can be removed by treating bleached pulp effluent in film reactors inoculated with P. chrysosporium. (Eaton et al., 1980).

Silva et al. (1990) have recently reported the isolation and characterization of two ligninolytic basidiomycetous fungi of Southern Chile, that rapidly degrade modified lignins under air and at low temperatures (25°C). Moreover, these strains showed a high ligninase activity. The aim of this work was to evaluate the ability of these, and several other white-rot fungi isolated from Southern Chile, to efficiently decolourize bleached pulp effluents.

MATERIALS AND METHODS

Microorganisms. The strains used were: Phanerochaete chrysosporium (BKM-F-1767), Coriolus versicolor (INTA 9A), Poria placenta (MAD-698), Gloeophyllum trabeum (MAD 6M-R) and several unidentified local ligninolytic basidiomycetous fungi collected in the South of Chile (strains 5, 14, 27, 58, 157, 158, 184, 207, 268, 310 and 441). Isolation and characterization of these fungi has been described elsewhere (Silva et al., 1990). All strains were stored on malt agar slants at 4°C. Strain 158 was recently identified as a Ramaria sp.

Effluent. E1 effluent, kindly provided by CMPC-Laja, was used throughout this work. This effluent was kept at 4°C and the pH was adjusted to 4.5 and filter-sterilized through a 0.45 um Millipore filter before fungal treatment.

Culture medium. The medium used was that reported by Kirk et al. (1986). Incubation was conducted in 100mL flasks containing 10mL culture medium.

Inoculum. P. chrysosporium was grown on malt agar (2%) medium in Petri dishes for 72 hours at 39°C and then spores were suspended in sterile water, as described elsewhere (Silva et al., 1990). Each flask was inoculated with the equivalent of 2x10⁶ spores/mL culture medium. The other strains were grown in agitated cultures (200 rpm) in 100mL flasks containing a C-limited medium, for 3 days at 30°C (Silva et al., 1990). Then, the cultures were centrifuged and the mycelium was ground in 20mL sterile water. Each flask was inoculated with the equivalent to 48 mg dry weight of mycelium/L culture medium.
**Incubation.** *P. chrysosporium* was incubated at 39°C either in an atmosphere of 100% oxygen or under air. The other strains were incubated at 25°C and under air. After 3 days of fungal growth, 0.4 mM veratryl alcohol and 5mL of the E1 effluent were added. Hence, this incubation time corresponds to the onset of degradation. The cultures were stopped 200 hours later.

**Colour Measurement.** The evolution of colour effluent during incubation was determined according to the CPPA method (CPPA Std., H.SP, 1974). Every 12 hours, 2 flasks/strain were stopped and the mycelium was removed by filtration through Whatman Nº 3 filter paper. The clear supernatant was employed for measurement of absorbance at 465 nm against distilled water.

**Molecular weight distribution of chlorolignins.** 1 mL of the clear supernatant was applied to a 100 x 1cm Sephadex G-50 column equilibrated with NaOH-LiCl 0.1N. 2.0 mL fractions were collected and chlorolignins were followed at 280 nm. The column was calibrated with veratryl alcohol (MW 168), aprotinin (MW 6500) and cytochrome C oxidase (MW 12400).

**RESULTS AND DISCUSSION**

The kinetics of effluent decolourization by strains 58, 158, 441 and *P. chrysosporium* is shown in Figure 1. The initial colour concentration was 14500 CU. All four strains decolourized the brownish effluent to a clear yellow solution and no apparent adsorption of chromophores on the surface of the mycelium was observed. This suggests that the coloured components of the effluent were biochemically decomposed and not simply adsorbed.

*P. chrysosporium* under 100% oxygen and the strain 158, under air, showed the highest rate of decolourization (Fig. 1). About 90% of the colour was removed after 190 hours and 140 hours of cultivation, respectively. However, when *P. chrysosporium* was grown under air, only 70% of the colour was removed after a similar time of incubation. Other strains studied showed a limited colour removal of the effluent after 190 hours of incubation (Table 1), except for *C. versicolor* that decolourizes about 80% of the original effluent colour, in accordance with previous published results (Livernoche et al., 1983). As expected, the brown-rot fungi *G. trabeum* and *P. placenta* showed the lowest ability for effluent decolourization.

The highest specific activity for decolourization was obtained with the fungi *P. chrysosporium* and *C. versicolor*, even though only a limited growth on the E1 effluent was found for both fungi (Table 1). On the other hand, the significant decolourization observed for strain 158 is apparently related to its capacity to grow efficiently even in the presence of chlorolignins.

![Figure 1. Kinetics of effluent decolourization by strains 58 ( ), 158 ( ), 441 ( ), *P. chrysosporium* under 21% O2 ( ), and 100% O2 ( ).](image-url)

Strains 5, 157, 207, and 310, although allowing only limited colour removal, showed a specific decolourization activity similar to strain 158. Their poor growth could arise from growth inhibition caused by some aromatic compounds present in the E1 effluent. Finally the brown-rot fungi *G. trabeum* and *P. placenta* showed a low specific activity for decolourization, too.