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Fed-batch Decolorization of Poly R-478 by *Trametes versicolor*

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ABSTRACT

Physiological aspects were evaluated to determine optimal conditions for the decolorization of a synthetic dye, Poly R-478, by white-rot fungus Trametes versicolor # 52J. The decolorization experiments were carried out in semicontinuous operation during three cycles to improve the process efficiency. The best decolorization efficiencies (65% to 80%) were obtained in fungal cultures performed in nitrogen limited conditions under aerobic conditions.

Key words: Decolorization, *Trametes versicolor*, Poly R-478, ligninolytic enzymes

INTRODUCTION

There is currently considerable environmental interest in the problem of color removal for a wide range of wastewaters. In the textile manufacturing industry, up to 50% of the dyes are lost after the dyeing process and therefore, they are disposed out in large quantities in the effluents. Moreover, most of the dyes are of environmental concern because of their toxic properties on living ecosystems (Dawson, 1981). These effluents cause a significant loss in luminosity and increase in temperature, which greatly diminishes the dissolved oxygen concentration in wastewaters, with the subsequent alteration of the aquatic life (Young and Yu, 1997). Before their disposal, they are to be treated to reduce their levels of toxicity and thus, to minimize pollution impact. Physic chemical treatments have high operational costs associated and limited applicability, which render these techniques unattractive. Other conventional processes based on biological treatment (aerobic-anaerobic) are relatively ineffective in effluent decolorization, because high molecular weight compounds are not easily degraded by bacteria, and colored compounds pass through the treatment system largely undegraded (Lema et al., 2002).

Ligninolytic peroxidases secreted by white-rot fungi may comprise a biological treatment proposal since they possess an extracellular degradation system, which enables the degradation of lignin (Bumpus et al., 1985). This degrading ability has opened new prospects for the development of biotechnological processes aimed at the degradation of complex polymers, such as xenobiotic (polyaromatics, polyphenolics, etc.) compounds (Field et al., 1993), effluent decolorization (Lema et al., 2002) and biobleaching of kraft pulp (Moreira et al., 1997).

Several reports have described bioreactor designs for industrial effluent decolorization from white-rot fungi in submerged and immobilized liquid

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cultures (Pallerla and Chambers, 1997; Royer et al., 1991; Mielgo et al., 2002). The aim of the work was focused on the knowledge of the degradative capability of a complex compound with the final objective of facilitating the development of decolorization systems. The decolorization of a polymeric dye Poly R-478, with structural similarity to lignin, has been selected as a model compound.

MATERIALS AND METHODS

Microorganism and culture conditions

Trametes versicolor #52J was kindly provided by M. Paice, Paprican (Pulp and Paper Institute of Canada). The fungus was maintained at 4ºC on peptone yeast extract slants from which it was transferred to glucose malt extract (ME) plates (Mester et al., 1996). The ME plates were incubated at 30ºC for 7 days. Five agar plugs (5-mm diameter) were punched from the leading edge and cultured in Fernsbach flasks with 100 mL of N-limited culture medium (C/N ratio 62.5/1) containing 10 g/L glucose, 2.2 mM NH_4^+-N as ammonium tartrate and BIII mineral medium (Tien and Kirk, 1988) for 6 days at 30ºC. After this time, the mycelium was crushed in a Blender for 1 min before its use as the inoculum.

Erlenmeyer flasks (250 mL) containing 0.1 g/L dye, 90 mL of the N-limited medium was inoculated with 10% (v/v) homogenised mycelium in triplicate assays. Abiotic controls were run in parallel. Decolorization experiments were performed in an orbital shaker (150 rpm) at 30°C under an air or oxygen atmosphere by the periodical flushing of O_2 gas (0.8 bar manometric pressure). The culture broth was periodically monitored for MnP activity and glucose. After glucose depletion conditions and peroxidase peaks, two fed-batch additions of the dye (0.1 g/L) and glucose (5 g/L) was introduced to perform decolorization.

Dye decolorization rate

The decolorization of Poly R-478 dye (polyanthraquinone) was monitored by the percentage reduction in the absorbance ratio at 520 and 350 nm. Before the absorbance readings, 0.2 mL of centrifuged sample (8,000 rpm, 10 min) was diluted with 0.8 mL of distilled water and measured at 30ºC with a 1-cm cuvette in a spectrophotometer (Hitachi U-2000, USA).

Enzyme and analytical assays

Enzyme activities were spectrophotometrically determined at 30ºC. MnP and Laccase activities were measured by the oxidation of 2,6-dimethoxyphenol (DMP) at 468 nm (Field et al., 1993). The extinction coefficients used for the dimeric product of dimethoxyphenol (DMP) oxidation was 49,600 $M^{-1}cm^{-1}$. The reaction was initiated by addition of 0.4 mM H_2O_2. One unit of enzyme activity (U) is equivalent to 1μmol of product formed per min. Reducing sugars were determined by the dinitrosalicilyc acid method using D-glucose as standard according to Ghose (1987). Residual hydrogen peroxide was measured by analytical indicators (Merckoquant peroxid-test) (Mielgo et al., 2002).

RESULTS AND DISCUSSION

Attempts to induce decolorizing activity by the extracellular ligninolytic system of *Tramete versicolor* were carried by considering different nutritional regimes (N-limitation or N-sufficiency expressed as C/N ratio 62.5/1 or 12.5/1, respectively) with passive aeration or periodical flushing of oxygen. Decolorization in repeated cycles was planned to improve the process efficiency.

According to the results obtained by the combination of the different nitrogen regimes and aeration supply, the best decolorization percentages corresponded to the assays carried out in nitrogen limitation and under an air atmosphere (Table 1). These culture conditions led to secondary metabolism, which favoured the decolorization of the dye. In contrast to results reported for *Phanerochaete chrysosporium* (Glenn and Gold, 1983), an oxygen atmosphere did not have a beneficial influence on decolorization, as it is evident for the comparison of decolorization percentages under air or oxygen.

Table 1 - Maximum decolorization percentages of the dye Poly R-478.

Assay	Assay		
	First	Second	Third
Oxygen/N-sufficiency	38.7	39.4	38.6
Air/N-sufficiency	53.1	51.5	53.3
Oxygen/N-limitation	59.5	61.3	61.5
Air/N-limitation	72.3	82.0	66.3

A higher concentration of nitrogen (C/N ratio 12.5/1) greatly diminished the final decolorization percentage with average reductions of 30%. On the other hand, the supply of oxygen also negatively affected the decolorization efficiency to values around 60% in comparison with the best results of 82%, obtained in the second cycle of the assay performed in N-limitation and air supply.

The decolorization of the dye could be attributed to the presence of peroxidases and oxidases as MnP and Laccase. Therefore, we investigated the physiological regulations of the enzymatic activity for these two enzymes under the mentioned culture conditions. Figs. 1 and 2 show the enzymatic activities obtained in the presence of different nutritional regimes and passive aeration or periodical flushing of oxygen.

Considering the first cycle, the level of enzymatic activity as MnP and Laccase did not significantly vary on the nitrogen regime. However, this tendency was different in the following cycles.

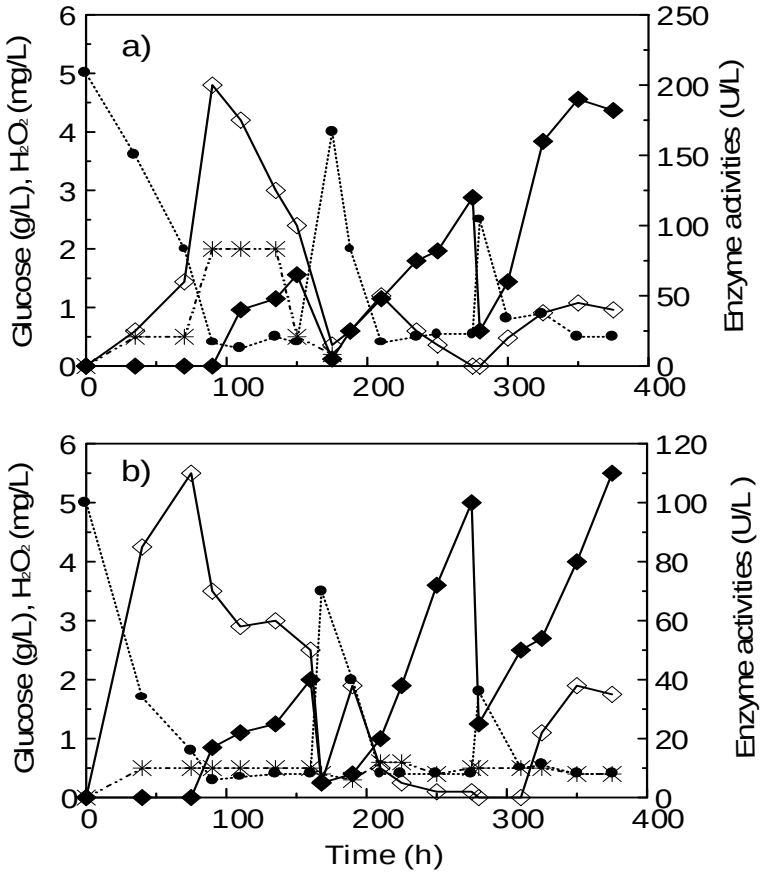

Figure 1 - Trends of glucose and ligninolytic activity during Poly R-478 decolorization in experiments with sufficiency of nitrogen and passive aeration (a) or oxygen periodical flushing (b) Enzymes activities: MnP (◇), Laccase (◆), H_2O_2 (∗) and Substrate: Glucose (●).

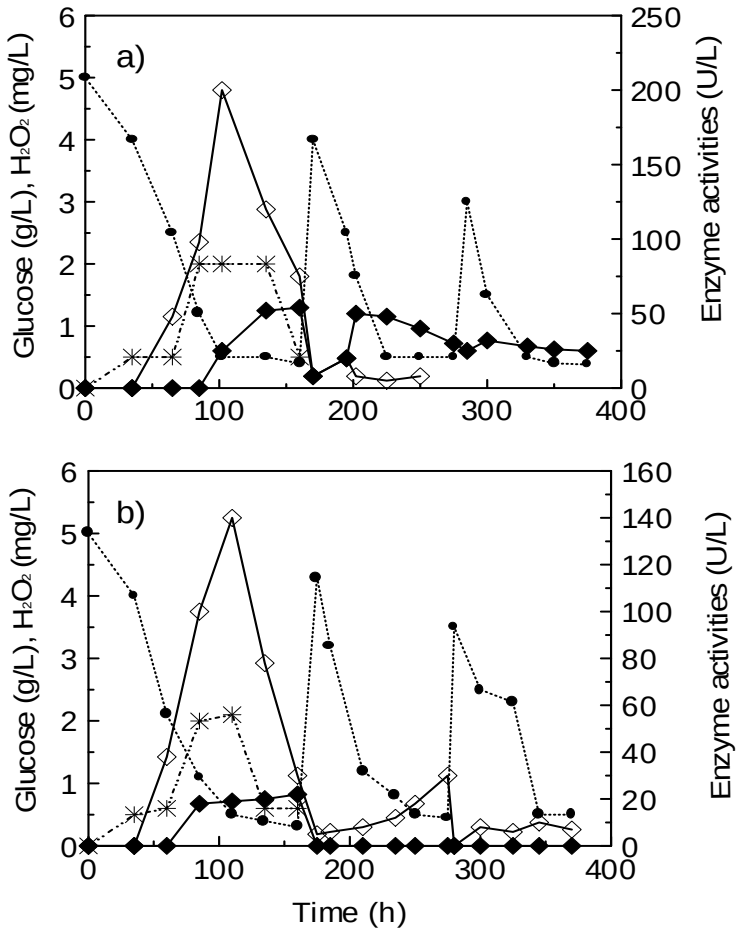

Figure 2 - Trends of glucose and ligninolytic activity during Poly R-478 decolorization in experiments with limitation of nitrogen and passive aeration (a) or oxygen periodical flushing (b) Enzymes activities: MnP (◇), Laccase (◆), H_2O_2 (∗) and Substrate: Glucose (●).

According to results corresponding to decolorization and enzymatic titers, both processes are not necessarily closely interconnected as would be expected in principle. The more favourable nitrogen regime for decolorization corresponded to nitrogen limitation, in contrast to the enhanced ligninolytic activity found in nitrogen sufficient conditions, which was especially evidenced for Laccase in the subsequent cycles of operation. Based on the attributed decolorization role of MnP and Laccase, this apparent contradiction can be explained by two facts: i) decolorization should be attributed to an event occurring in substrate limited cultures, which is typically associated to the secretion of ligninolytic enzymes and ii) the enhanced ligninolytic activity is possibly due to the higher biomass concentration under enriched nitrogen conditions; the supplementation of glucose pulses with residual nitrogen still permitted the growth of microorganism and new production of enzymatic activity, especially Lacasse. The mycelium remained uncolored after the treatment with no apparent adsorption of the Poly R-478 on its surface.

After decolorization assays, the absorbance profiles of the dye before and after treatment were compared. As it can be deduced from, a significant breakdown of the molecule associated to the chromophore group was obtained (Fig. 3); the major removal of the absorbance peak at 520 nm was obtained as a result of the enzymatic treatment.

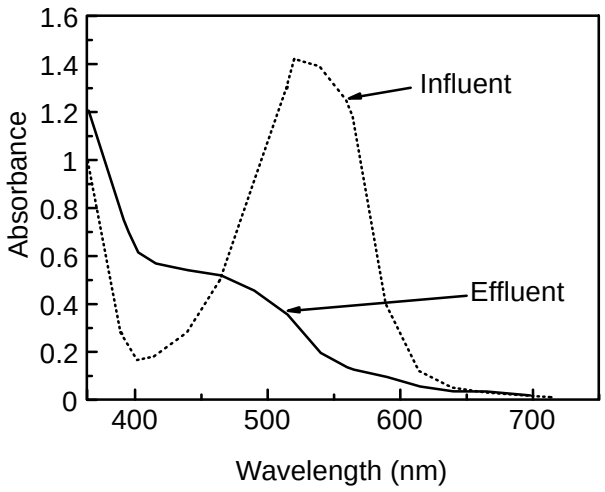

Figure 3 - Scan Poly R-478 dye before and after *Trametes versicolor* treatment.

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RESUMO

Efluentes industriais altamente coloridos são difíceis de degradar utilizando as tecnologias fisico-químicas e bacterianas existentes atualmente. O sistema enzimático extracelular secretado pelo fungo de podridão branca apresenta um grande potencial oxidativo, capaz de dar início a decomposição das estruturas residuais dos compostos responsáveis pela cor destes efluentes. Aspectos fisiológicos foram avaliados para optimizar as condições de descoloramento de um corante sintético, Poly R-478, pelo fungo de podridão branca *Trametes versicolor* # 52J. Os experimentos de descoloramento foram realizados em operação semi-contínua durante três ciclos para melhorar a eficiência do processo. As melhores eficiências de descoloramento (65 % a 80%) foram obtidas em culturas de fungos tratadas em condições limitadas de nitrogênio em atmosfera de ar.

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