BIODEGRADATION OF RED 40 DYE BY THE MUSHROOM *Pleurotus sp florida*

BIODEGRADAÇÃO DO CORANTE VERMELHO-40 PELO COGUMELO Pleurotus sp *florida*

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ABSTRACT: A study was made of the capacity of the mushroom Pleurotus florida to biodegrade red 40, a dye used in foods. Mycelia grown in nutrient-rich and poor liquid mediums were used in different concentrations of dye and with various pH. Spectrophotometric readings indicated that the dye had biodegraded in concentrations of 1.10^{-5} and 2.10^{-5} Mol.dm^{-3}, with the best results obtained at a pH of 4.5. It was also found that the mycelia grown in both nutrient-rich and nutrient-poor media presented red 40 biodegrading activity.

KEYWORDS: Biodegradation. *Pleurotu.* Red 40

INTRODUCTION

A genus of the fungi of the Basidiomycetous group belonging to the order *Aphylopholares* and the family *Polyporaceae*, the edible mushroom *Pleurotus* is widely distributed and is cultivated in Japan, China and Europe (SENYAH et al., 1989). *Pleurotus* is used in biotechnological processes of bioconversion and bioremediation, such as the fungal degradation of chlorinated monoaromatics and BTEX compounds (BUSWELL, 2001), as well as in the biodegradation of xenobiotic compounds (MORGAN et al., 1991), in the purification of air, water and soil, in the cleanup of contaminated soils and in the treatment of industrial effluents (MILES; CHANG, 1997, REID et al., 2002). Studies have also shown the use of fungi in processes such as the biodegradation of Tussah (*Antheraea pernyi*) silk fibroin films by a proteolytic enzyme and the oxidation of domestic (*Bombyx mori*) silk fibroin by mushroom tyrosinase (MONTI et al., 2004). These processes involve the action of important biodegrading enzymes, e.g., ligninase, hemicellulase, cellulase, xylanase and β-glucosidase, which are produced by *Pleurotus sajor-caju* (SINGH et al., 2003).

Another application is the biodegradation of industrial effluents such as dyes. A large number of dyes are released for consumption, including red 40. Although the risks posed by red 40 are unknown and its use is forbidden in some countries, this dye is widely utilized. The possible risk to humans that ingest food containing dyes can be extended to the environment, for factories discharge their residues into nearby rivers and streams (BONTEMPO, 1985). This work involved an investigation of the use of the mushroom *Pleurotus florida* in the degradation of red 40 dye, a possible alternative in the treatment of industrial effluents.

MATERIALS AND METHODS

Red 40 dye belongs to the Monoazo class and its principal compound is disodium salt of 6-hydroxi-5-[(2-methoxi-5-methyl-4-sulfophenyl)azo]-2-nophthal enesulfonic acid. The concentration allowed in food products is 2 to 6.10^{-5} Mol.dm^{-3} (ANVISA). In this investigation, we used a concentration equal to or greater than 10^{-5} Mol.dm^{-3}.

The nutrient-rich medium (1 liter) was prepared with glucose 10g, ammonium tartarate 2g, KH_2PO_4 1g, yeast extract 1g, $MgSO_4 . 7H_2O$ 0.5g, KCl 5g and 1.0ml of solution containing trace elements (in 100ml: $Na_2B_4O_7 . 10H_2O$ 10mg, $ZnSO_4 . 7H_2O$ 7mg, $FeSO_4 . 7H_2O$ 5mg, $CuSO_4 . 5H_2O$ 1mg, $MnSO_4 . 4H_2O$ 1mg, $(NH_4)_6Mo_7O_{24} . 4H_2O$ 1mg). The nitrogen-poor medium (1 liter) contained glucose 10g, KH_2PO_4 2g, 1.0ml of solution containing trace elements, yeast extract 0.2g, and peptone 0.1g. The dye's degradation was analyzed using a spectrophotometer with a 500 nanometer light wavelength.

Degradation of the dye was investigated in nonincubated, incubated, and incubated and centrifuged material. The mycelium in the nutrient-rich liquid medium was grown according to the method of Heinfling et al. (1998). The mycelium

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was grown in a rotary shaker at 120 rpm for six days at 28°C, with an average of 1.58 grams (wet weight) of mycelium inoculated for every 250ml of culture medium, to which 1.10^{-5} Mol.dm^{-3} of red 40 dye was added. An evaluation was also made of the degradation by mycelium cultivated in a nutrient-poor liquid medium, to which 1.10^{-5} Mol. of red 40 dye was likewise added.

The degradation of red 40 dye was also investigated in a nutrient-poor medium, at pH 4.5 (buffer ammonium acetate) and 7.2 (buffer potassium fosfato), in red 40 dye concentrations of 1.10^{-5} Mol.dm^{-3} and 2.10^{-5} Mol.dm^{-3}. The only control used here was culture medium (pH 4.5). The absorbancies before and after incubation were observed in mediums with and without mycelia.

RESULTS AND DISCUSSION

The liquid nutrient-rich culture showed the following results (Figure 1): The values (in nm) for the experiment using culture medium, mycelium and red 40 dye were 0.241 for the control (nonincubated) material, 0.153 for the incubated material and 0.145 for the incubated and centrifuged material. In this experiment, carried out in nutrient-rich culture, the mycelium was found to degrade the red 40 dye.

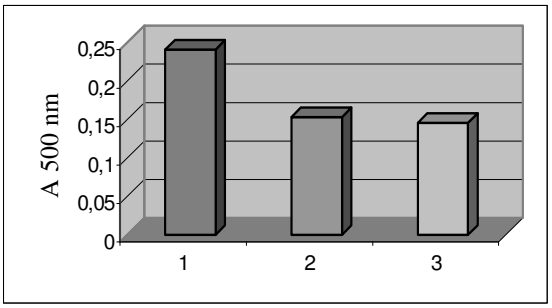

Figure 1. Degradation measured in absorbance (nm) of dye in a liquid nutrient-rich culture medium.
1- Nonincubated material; 2- Incubated but noncentrifuged material;3- Incubated and centrifuged material

The results of the degradation experiment with the liquid nutrient-poor culture (nitrogen deficient) showed values (Figure 2) of 0.193 for the control (nonincubated) and 0.018 for the incubated material (noncentrifuged and centrifuged). In this experiment, the difference between nonincubated and incubated material demonstrated the degrading action of *Pleurotus florida* even in a nutrient-poor medium. It should be kept in mind that the use of a liquid medium may facilitate biodegradation, since the degradation process begins with the release of extracellular enzymes into the medium in which the degradation of the substrate's molecules will occur, a process that has already been observed with lignin and cellulose (FIELD et al., 1993). The ability of *P. florida* to biodegrade gossypol has already been demonstrated (RAJARATHNAM et al, 2001*),* and the cultivation of mushrooms in liquid medium has also been found to increase biodegradation, for it favors hexosamine and laccase activity.

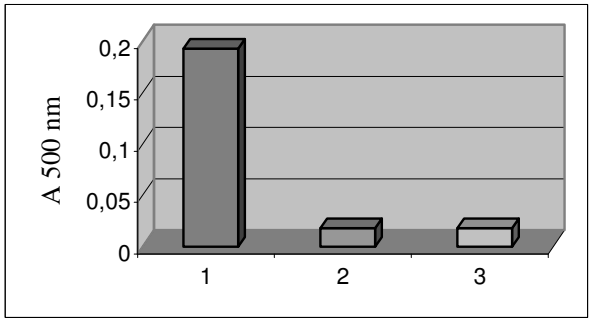

Figure 2. Degradation measured in absorbance (nm) of dye in a liquid nutrient-poor culture medium.
1- Nonincubated material; 2- Incubated but noncentrifuged material; 3- Incubated and centrifuged material

The experiment conducted to assess the mycelium's efficiency in nutrient-poor medium showed satisfactory results (Figure 3). The absorbance value in the medium without mycelium, with pH 4.5 and a dye concentration of $1.10^{-5}.dm^{-3}$, was 0.251, while the medium with mycelium showed an absorbance of 0.030. Again, without mycelium in a culture medium with pH 7.2 and the same concentration, the value was 0.245, while the medium with mycelium showed 0.222. In the medium at pH 4.5 and a dye concentration of $2.10^{-5}.dm^{-3}$, the results were 0.454 for the medium without mycelium and 0.064 with mycelium. In other words, even with double the concentration of dye, the fungus displayed efficient biodegrading activity. In the flasks containing only medium (with and without mycelium), the values were 0.007 and 0.005 nm, respectively. In this experiment, the results confirmed that pH 4.5 is optimal for the mycelium's action. This result was found earlier with the fungus *Aspergillus niger* (ANTIER et al., 1993) and is similar to that found for *Pleurotus sajor-caju*, whose optimal pH for the extraction of β-glucosidase was 4 (SINGH et al., 2003). Therefore, the present study focused on the ability of *Pleurotus florida* to degrade red 40 dye, probably as a result of the activity of the mushroom's enzymatic complex. Although let us not find other works on the biodegradation of red 40, the enzymatic activity in biodegradation was ascertained by Fan et al. (1981), as was the biodegrading ability of fungi, i.e., their use as depollutants in studies involving *Phanerochaete chrysosporium*, which was used in the degradation of organic (AUST, 1990) and inorganic (BUMPUS; BROCK, 1987) pollutant compounds. Atrazine, a herbicide containing a triazine ring, has also already been biotransformed using *Pleurotus pulmonarius* (MASAPHY et al., 1996). Another example of this application is the evidence that the fungus *Pleurotus sordida* is able to degrade substances such as DDT, TDDD, benzo(a)pyrene, lindane and pentachlorophenyl (TAKADA et al., 1996).

In summary, the results reported here document indicated the capacity of the mushroom *Pleurotus florida* to biodegrade red 40 dye in nutrient poor medium, in a dye concentrations of 1.10^{-5} and 2.10^{-5} $Mol.dm^{-3}$, with the best results obtained at a pH of 4.*5*.

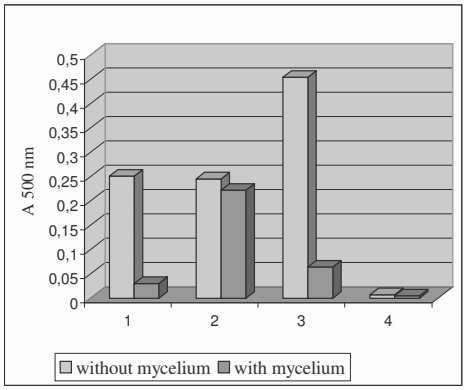

Figure 3. Absorbance readings (nm) of the solutions with and without mycelium in deficient culture medium, at difference concentrations of red 40 dye and various pH.
1- Culture medium and dye (1.10^{-5} $Mol.dm^{-3}$; pH 4.5); 2- Culture medium and dye (1.10^{-5} $Mol.dm^{-3}$; pH 7.2); 3- Culture medium and dye (2.10^{-5} $Mol.dm^{-3}$; pH 4.5); 4- Only culture medium (pH 4.5)

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RESUMO: Nesse trabalho foi verificada a capacidade do fungo *Pleurotus sp florida* em biodegradar o red-40, um corante utilizado em alimentos. Foram utilizados micélios cultivados em meio líquido rico e em meio pobre em nutrientes, em diferentes concentrações de corante e diferentes pH. Os resultados obtidos com leitura em espectofotômetro indicaram a biodegradação do corante em concentrações de 1.10^{-5} e 2.10^{-5} $Mol.dm^{-3}$, sendo que os melhores resultados foram obtidos em pH 4,5. Foi observado ainda que tanto o micelio cultivado em meio rico quanto o micelio cultivado em meio deficiente apresentaram atividade de biodegradação do red-40.

PALAVRAS-CHAVE: Biodegradação. *Pleurotus*. Vermelho 40.

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