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Removal of Erionyl blue A-R and Solophenyl Black FR Textile Dyes using Enzymatic Extracts of Laccases of *Pleurotus ostreatus* and *Pleurotus djamor*

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In this research, extracellular fungal extracts containing *Pleurotus ostreatus* and *Pleurotus djamor* were used for the removal of erionyl blue A-R (anthraquinone) and solophenyl black FR (azo) textile dyes in artificially contaminated water at 200 ppm. For erionyl blue A-R, removals of up to 93.6 and 42.85% were achieved, and for solophenyl black FR of 27 and 31.14%, using the enzymatic extracts of *P. ostreatus* and *P. djamor* respectively. Enzymatic activity values of 888.41 IU and 152.22 IU were reached for the laccases obtained from the submerged culture extract of *Pleurotus ostreatus* and *Pleurotus djamor* respectively. The extract obtained from *P. ostreatus* was partially purified using dialysis and anion exchange chromatography (DEAE), by polyacrylamide gel electrophoresis. A molecular mass of 67 kDa was determined.

Keywords*:* *Pleurotus*, dye, laccase, enzyme.

The textile industry is one of the main sources of pollution, it discharges effluents containing a wide variety of dyes, the most important ones are the azoids and the anthraquinones and therefore constitute the largest group of all organic dyes on the market[1, 2].

The toxicity of azo acids is because they produce aromatic amines during natural processes of degradation[3-6]. In addition, azo dyes affect water quality by preventing the passage of light and by increasing the demand of chemical oxygen. In contrast, there is little information on the biodegradation of anthraquinone dyes[7].

The "maquilas" or laundries, as they are called locally in the State of Puebla, are responsible for the discoloration, bleaching or dyeing of the denim. They are mainly located in community dwellings such as San Rafael Tenanyecac, Villa Alta, San Mateo Ayecac and Santa Ana Xalmimilulco,[8] which requires a large amount of water use, which at the end of the process contains, in addition to the dyes, chemical additives used to treat the garments and give them different finishes. Therefore, the wastewater they produce contains various chemical pollutants that are discharged

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to municipal drainage or irrigation canals most of the time, without treatment or with inadequate treatments, which eventually flow into the Atoyac River. In order to contribute to this problem, the use of ligninolytic fungi such as those of the genus *Pleurotus* capable of synthesizing a complex of extracellular enzymes, mainly peroxidases and Laccases (p-diphenol: dioxygen: oxide-reductase) these are extracellular glycoproteins containing copper and a molecular weight between 60 and 80 kDa, have the capacity to reduce molecular oxygen to two molecules of water and simultaneously work in the oxidation of many aromatic substrates[9]. The range of oxidizable substrates is broad and includes pentachlorophenol, 2,6-dimethoxyphenol aromatic amines and other easily oxidizable aromatic compounds, as well as azo dyes[10, 11].

MATERIALS AND METHODS

The production of laccase enzymes was carried out by submerged culture, using a mineral medium consisting of magnesium sulfate heptahydrate (J.T.Baker ®) ferrous sulfate heptahydrate (J.T.Baker ®) manganese sulfate (J.T.Baker ®), sodium chloride (J.T.Baker ®), ammonium sulfate (J.T.Baker ®), potassium phosphate dibasic (J.T.Baker ®) and as carbon source wheat bran at a pH of 6.2 and inoculated with 4 units of 1 cm^2 of PDA (BD Bioxon ®) with the strains of *P. ostreatus* and *P. djamor* of the ceparium of the Department of Research in Agricultural Sciences of the Institute of Sciences of the Benemérita Universidad Autónoma de Puebla, it was incubated at 28 ° C and a stirring speed of 120 rpm. Monitoring of laccase activity was performed on the seventh day using 2,22 -Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich ®) ABTS as an oxidizable substrate and measuring at a wavelength (λ) of 420 nm for 1 min. using a molar extinction coefficient (ε) 36000 $M^{-1}cm^{-1}$ (12). The enzymatic activity was expressed as international units as a function of volume (IU / ml).

Solutions of water artificially contaminated with the dyes were prepared at 200 ppm erionyl blue A-R (Ciba Specialty Chemicals Inc. ®) and solophenyl black FR (Ciba Specialty Chemicals Inc. ®). To the water artificially contaminated with the dyes to be studied was gradually added the enzymatic extract of laccase, determining the percentage of removal by UV-Vis spectroscopy (Varian Cary 50) all the determinations were made by triplicate-sample method, one of the extracts was subjected to a dialysis process for 15 h in 20 mM phosphate buffer, at a pH of 7.4 and at a temperature of 4 ° C, once the time was over, the dialysate was centrifuged at 7500 rpm for 20 min, then anion exchange chromatography was performed with the pre-equilibrated Diethylaminoethyl (DE-53 Whatman) resin at a pH of 7.4. The proteins obtained were eluted with 100 ml of 20 mM phosphate buffer - 1 M NaCl, at a pH of 7.4, for determination of the molecular mass, was performed by electrophoresis in 12% concentration polyacrylamide gel, where 15 µl of the extract were added and let it run at 120 V / 2.30 h.

RESULTS AND DISCUSSION

The maximum enzymatic activity obtained for the laccase produced by *P. ostreatus* was 888.41 IU / ml and for *P. djamor* 153.22 IU / ml. With these enzymatic extracts, it was possible to remove the dyes studied in the percentages reported in Table 1.

In figure 1, the UV-Vis spectra of the erionyl blue dye A-R are presented before and after the reactionIn this research using the *P.ostreatus* extract, it was possible to remove 93.77% of the dye erionyl blue A-R (Figure 2), classified as acid blue 260 with CAS number 62168-86-9 and molecular formula $C_{26}H_{23}ClN_3NaO_6S$, molecular

Table 1. Percentage of removal of the studied dyes

Colorant	Wavelength	*P. ostreatus*	*P. djamor*
Erionyl Blue A-R	630	93,67	42.85
Solophenyl Black FR	490	27.00	31.14

mass of 563.96 g / mol and belongs to the family of anthraquinones[13].

On the other hand, Sánchez-López[14] demonstrated the ability of the enzyme extract (laccase and manganese peroxidase) from *Trametes maxima* strain MUCL 44155 to remove acid blue anthraquinone dye 62 by 91% measured at 630 nm. Other fungi such as *Thanatephorus cucumeris* have been used to degrade anthraquinone dyes as the blue reactive dye 5 by DyP peroxidase having percentages above 90% measured at 630 nm[15]. In Japan, Itoh *et. al.* [16] have used the white rot fungus *Coriolus versicolor* to degrade violet dye 12 (4-dihydroxyanthraquinone).

The UV/Vis absorption spectra of the reaction products (Figure 1) showed an increase in absorbance from 520 nm and towards the ultraviolet region behavior very similar to those reported by Sánchez-López[14] and Sugano[15]. With regard to the *Pleurotus djamor* strain, lower removal values were observed with respect to *P. ostreatus* (Table 1).

The solophenyl black dye (direct black 22) is classified as Index 35435, indicating that it is a polyazo dye. (17, 18) its structure is reported in figure 3.With respect to the oxidation of azo dyes like the solophenyl black FR by laccases and peroxidases, several authors report the formation of naphthoquinones[19-22]. In contrast, Mohana et al[23] report that using a microbial consortium applied in the treatment of water contaminated with direct black 22 (solophenyl black), obtained as

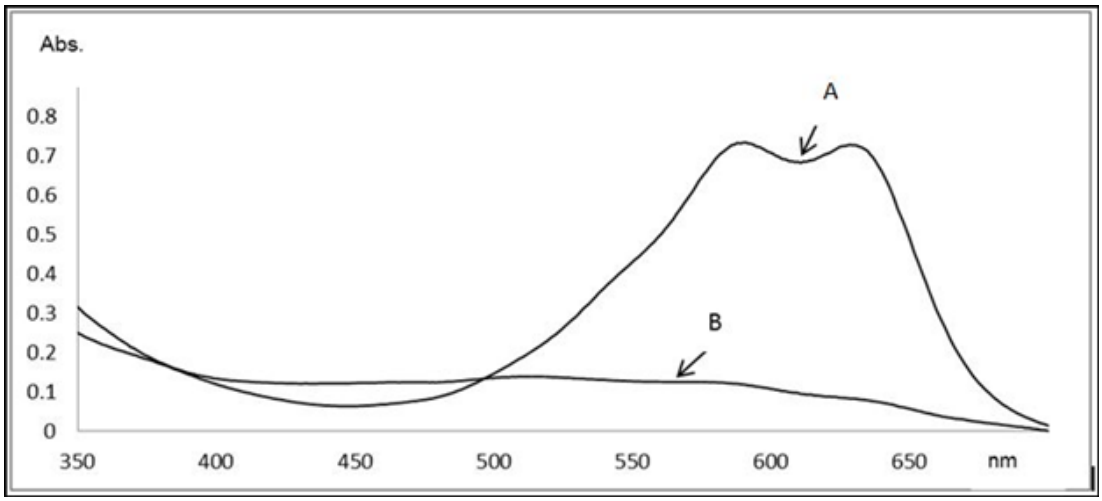

Fig. 1. Spectrum of UV-Vis absorption of water artificially contaminated with erionyl blue A-R dye at 200 ppm and treated with *Pleurotus ostreatus*. A) Untreated water b) Treated water

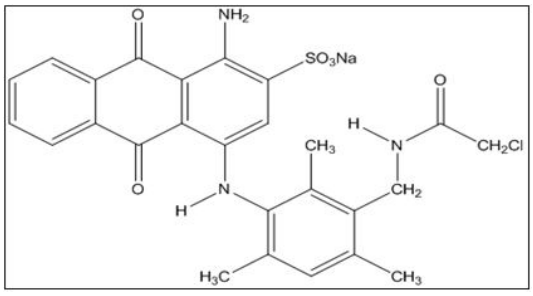

Fig. 2. Erionyl Blue A-R

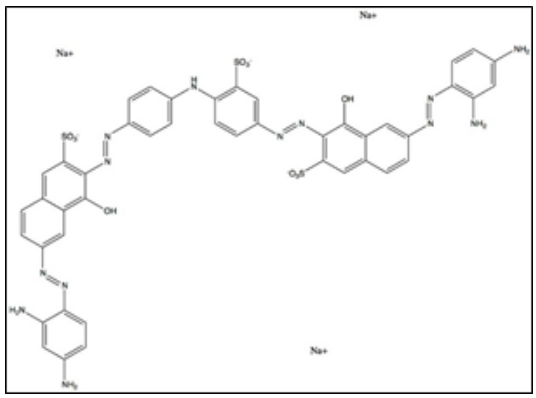

Fig. 3. Solophenyl Black FR

degradation products 1-naphthol and diphenylamine as a product of anaerobic reduction, similar results obtained by Chávez[24], but using microbial consortia isolated from the Alseseca River of the City of Puebla, Mexico, these products are toxic so it would be convenient to use oxidation mechanisms with laccases or peroxidases and not chemical reduction. Although the removal rates for this azo-type dye were low (Table 1), it is important to emphasize that in this case, the laccases of the strain *Pleurotus djamor* were more efficient than those of *Pleurotus ostreatus*. In addition, for wastewater treatment it is convenient to use a treatment train for the process to be efficient. It has been reported that ligninolytic enzymes produced by Basidiomycetes of the genus *Trametes* remove better the dyes of an anthraquinone nature than the azo[25, 26], as found in this research.

The partially purified enzyme had a molecular mass of 67 kDa as determined by polyacrylamide gel electrophoresis. It has been reported that most of these enzymes range from 50-70 kDa[27, 28].

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