LACCASE FROM SPENT PLEUROTUS OSTREATUS: A PROMISING APPROACH FOR DYE DEGRADATION IN TEXTILE WASTEWATER

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ABSTRACT

The textile industry represents a significant economic driver for Brazil, particularly in the state of Minas Gerais. However, one significant challenge in the textile industry is the treatment of wastewater generated during the dyeing process. This effluent, estimated to be 50-100 liters per kilogram of fabric produced, contains dyes and other chemicals with potent polluting potential. This study investigated the degradation of three dyes: Coomassie brilliant blue (CBB) at 16 mgL^{-1}, Congo red (CR) at 50 mgL^{-1}, and chromotrope 2R (C2R) at 234 mgL^{-1}. The degradation was achieved through the use of unpurified laccase, extracted from spent Pleurotus ostreatus substrate. Approximately 2.1 $mUmL^{-1}$ of laccase effectively degraded 40% of CBB within three hours, while 41% of CR was degraded after 15 hours. Similarly, 48% of C2R was degraded in 15 hours using a higher laccase concentration of 4.2 $mUmL^{-1}$. Toxicity analysis of CBB and CR treated with unpurified laccase revealed a significant decrease in toxicity. This research demonstrates the potential of utilizing spent *P. ostreatus* substrate as a sustainable source for laccase production, offering a promising approach for bioremediation of textile wastewater.

Keywords: Spent substrate; Mushrooms; Agro-industrial co-product.

1 INTRODUCTION

The Brazilian textile industry represents a significant economic driver, with a revenue of R$ 193.2 billion and the creation of 1.3 million jobs in 2022. This highlights its crucial role in the national economy. Approximately half of the industry is concentrated in the southeastern region, with Minas Gerais ranking third nationally, accounting for 12% of textile industries, behind Santa Catarina (19.7%) and São Paulo (25.5%).[1] The textile production process utilizes substantial quantities of dyes, surfactants, salts, heavy metals, reducing agents, and water. Consequently, the resulting effluent contains a diverse array of contaminants, with dyes posing a significant challenge for wastewater treatment.[2]

Textile dyes are classified based on their chemical structure into distinct categories including azo, anthraquinone, metallized, indigoid, phthalocyanines, methine and polymethines, di- and triarylmethanes, nitro and nitroso, and sulfurous dyes. The azo class of synthetic dyes represents the most prevalent and widely utilized class of dyes in the commercial market, with applications in the coloring of food, cosmetics, and textiles. They constitute over 65% of commercially available dyes and are characterized by the presence of the (-N=N-) group as a chromophore. Triphenylmethane dyes also find application in the textile industry, with some finding additional use as fungicides and ectoparasitic ides in aquaculture.[3,4] Synthetic dyes are composed of recalcitrant, mutagenic, and carcinogenic substances, which pose a significant environmental threat due to improper disposal. [5]

Laccases are copper-containing polyphenol oxidases with a remarkable ability to degrade a wide range of phenolic and non-phenolic substrates. This includes recalcitrant pollutants such as textile dyes, bisphenol A, endocrine-disrupting chemicals, pesticides, pharmaceuticals, and even contributes to the degradation of complex organic matter in composts.[6]

2 MATERIAL & METHODS

A crude enzymatic extract was prepared from spent Pleurotus ostreatus substrate donated by a small producer in Barbacena, Minas Gerais, Brazil. Sodium acetate buffer (50 mM, pH 5.5) was added to the spent substrate in a 5:1 ratio and stirred for 40 minutes at room temperature. The mixture was then centrifuged at 4500 rpm for 10 minutes to obtain the crude enzymatic extract, which was stored at -18 °C.

Laccase activity was determined using a modified literature method.[7] In a cuvette, 975 µL of 50 mM sodium acetate phosphate buffer (pH 5.5) and 25 µL of 1 mM ABTS solution were mixed. The absorbance change at 420 nm was monitored for 5 minutes at 25 ± 2 °C. One unit of laccase activity was defined as the amount of enzyme that oxidizes 1 µmol of ABTS per minute.[7]

The decolorization assay was performed in 96-well microplates. Each well contained 125 µL of a dye mixture composed of Chromotrope 2R (234 mg L^{-1}), Congo red (50 mg L^{-1}), and Coomassie Brilliant Blue (16 mg L^{-1}), along with laccase at four different activities (0.312, 0.525, 0.785, and 1.050 mU per well). The final volume of each well was adjusted to 250 µL with 50 mM sodium acetate phosphate buffer (pH 5.0). Decolorization was monitored spectrophotometrically at 505 nm for Chromotrope 2R, 500 nm for Congo red, and 585 nm for Coomassie Brilliant Blue over an 18-hour period. The percentage decolorization was calculated using Equation 1.

$$Decolorization\ (\%) = \frac{A_0 - A_t}{A_0} \cdot 100 \quad (1)$$

A_0 = initial absorbance, A_t = Absorbance at different evaluated times (from 0 to 18 hours).

Data analysis was performed using Origin 2024 software. Analysis of variance (ANOVA) was employed to assess the significance of differences between treatments, followed by the Tukey test for pairwise comparisons.

The phytotoxicity assay was performed using the best decolorization condition for each dye. The phytotoxicity of the decolorized dye solutions was evaluated using lettuce (Lactuca sativa) seeds. Fifteen seeds were transferred to filter paper placed in Petri dishes and moistened with 5.0 mL of the decolorization assay solution. The plates were incubated in the dark at 25 ± 2°C for 48 hours. Subsequently, the plates were transferred to a growth chamber with a 12-hour light/12-hour dark cycle for 7 days.[8] The radicle lengths of germinated seeds were measured using a caliper. All assays were performed in triplicate, and the percentage of seed germination was calculated using Equation 2.

$$Germination\ \ (\%) = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} \cdot 100 \quad (2)$$

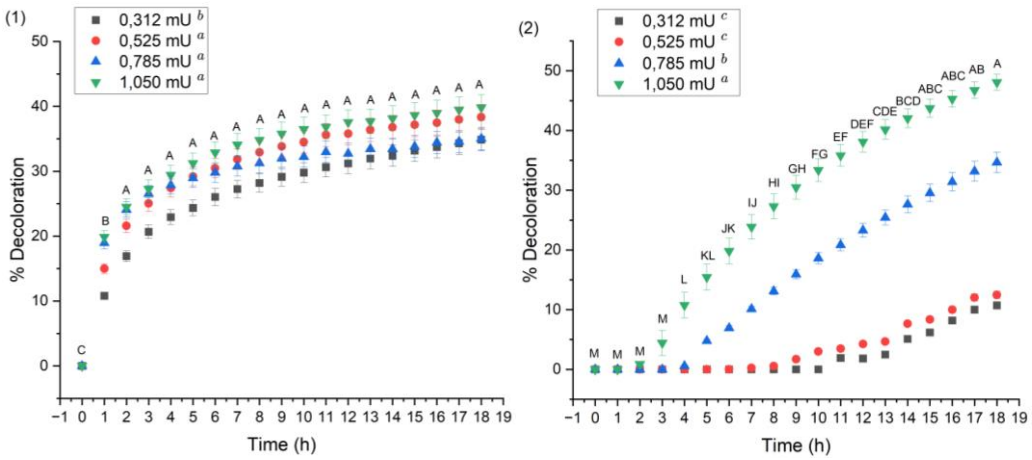
3 RESULTS & DISCUSSION

The activity of the unpurified laccase recovered from the spent substrate of *P. ostreatus* was determined to be 21 UL^{-1}. A previous study reported a laccase activity of 50 UL^{-1} for laccase extracted from spent *P. ostreatus* cultivation at pH 5.0 and 35°C, representing a 2.5-fold increase compared to the activity observed in this work.[9] Several parameters, including the time of spent substrate utilization, cultivation time, and temperature, are likely to influence the activity of laccase recovered from spent substrates.

Among the three dyes tested, Chromotrope 2R, a monoazo dye, exhibited the highest percentage of decolorization (48.08% ± 1.30%), followed by Congo red, a diazo dye (40.88% ± 5.60%), and Coomassie brilliant blue, a triphenylmethane dye (39.83% ± 2.60%), in the presence of laccase (Figure 1). No statistically significant differences in the percentage of degradation were observed for CBB, CR, and C2R dyes after 3 and 15 hours of incubation, respectively. Laccase concentrations of 0.525, 0.787, and 1.05 mU per well did not significantly affect the decolorization of CBB and CR dyes. However, for C2R dye, the highest percentage of decolorization was achieved with 1.05 mU of laccase per well (Figure 1). Therefore, the optimal decolorization parameters used in the phytotoxicity assay were 3 hours and laccase 0.525 mU/well for CBB and CR dyes, and 15 hours and laccase 1.05 mU/well for C2R dye.

Moreover, a maximum decolorization of 28.9% for Congo red (CR) and 35.8% for Coomassie brilliant blue (CBB) was observed when 30 µL of laccase was used under optimized conditions for 168 hours at 30°C.[10]

Table 1 presents the results of the phytotoxicity assay. The decolorized CBB solution exhibited the highest germination rate (40%), followed by the decolorized CR solution (26.67%) and the decolorized C2R solution (13.33%). Notably, a comparable germination rate of 16.15 ± 2.15 using L. sativa was reported for CR treated with laccase from Stenotrophomonas maltophilia.[11] Despite achieving the highest decolorization percentage, C2R displayed the lowest germination rate among the decolorized dye solutions. Furthermore, no germination was observed in the assay containing only unpurified laccase. These observations suggest the presence of inhibitory substances within the crude enzyme extract that potentially hinder seed germination.



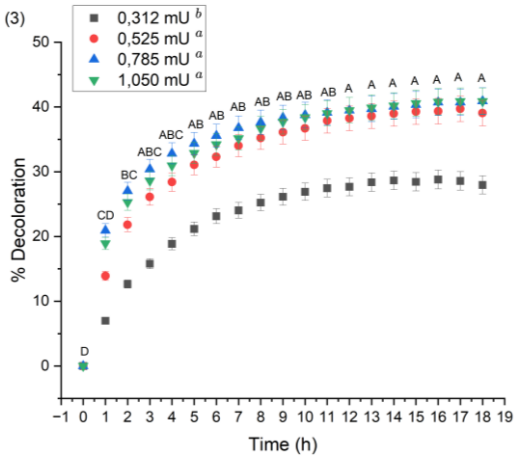


Figure 1 Decolorization of: (1) Coomassie Brilliant Blue G-250, (2) Chromotrope 2R, and (3) Congo Red, unpurified laccase. The decolorization data were subjected to statistical analysis using the Tukey test. Different capital letters (A, B, etc.) indicate statistically significant differences ($P < 0.05$) between treatment times. Similarly, different lowercase letters (a, b, etc.) indicate statistically significant differences ($P < 0.05$) between enzyme activities. Letters that are the same do not differ significantly from each other.

Table 1 Germination rates of Lactuca sativa seeds treated with decolorized dye solutions

Treatments	Seed germination (%)	Treatments	Seed germination (%)
CBB^{CT}	0,00	E	0,00
CBB	40,00	$C2R^{CT}$	4,55
CR^{CT}	6,67	C2R	13,33
CR	26,67	DW	100

DW= Distilled water, CT= control dye, E= unpurified laccase

4 CONCLUSION

This study demonstrates the potential of laccase as an effective tool for decolorizing and mitigating the phytotoxicity of textile dyes. However, optimizing decolorization conditions for each dye is crucial to minimize the generation of toxic byproducts. Further research is necessary to identify and eliminate any inhibitory substances present in the crude enzyme extract.

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