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ORIGINAL ARTICLE

Textile Effluent Containing Azo Dyes Eco-Friendly Bioremediation using *Lentinus* sp. in a Packed-Bed Bioreactor with Pine Sawdust as an Organic Inducer

Camila Gómez Garcés[1*], **Luisa Múnera Porras**[2], **Lida Arias Marín**[1]

Edited by
Angela Johana Espejo Mojica
editorus@javeriana.edu.co

1. Research group Biomicro, Escuela de microbiología, Universidad de Antioquia, Colombia.
2. Research group salud y sostenibilidad ambiental, Escuela de microbiología, Universidad de Antioquia, Colombia.

*camila.gomezg@udea.edu.co

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Abstract

Industrial textile effluents containing azo dyes pose a major environmental challenge due to their persistence and toxicity. This study evaluated the capacity of a native white-rot fungus (*Lentinus* sp.) to treat a real textile effluent polluted with an azo dye (Red 40) in a packed-bed bioreactor. Fungal biomass was immobilized on low-cost lignocellulosic supports (*Luffa cylindrica*), and pine sawdust was added as a biological inducer to stimulate ligninolytic enzyme production. Treatment conditions were first optimized in Erlenmeyer flasks (0.15 L), achieving 94.0 ± 0.1 % decolorization. Under scaled-up conditions (6 L, 30 °C, 12-day hydraulic retention time, batch mode, no agitation), the system removed 61.5 ± 0.2 % of dye color and reduced chemical oxygen demand (COD) by 99.0 ± 0.3 %. Enzymatic assays revealed manganese peroxidase activity, while laccase was not detected. Nuclear magnetic resonance (NMR) analysis confirmed structural modifications of the dye through azo bond cleavage. These findings demonstrate the potential of native ligninolytic fungi as sustainable and cost-effective biotechnological tools to treat azo dye-polluted industrial effluents, supporting their applicability at larger scales.

Keywords: Azo; *Luffa cilyndrica*; dye removal efficiency; *Lentinus* sp; wastewater; laccase; peroxidase

1. Introduction

Industrial wastewater is one of the main sources of environmental pollution, as it contains several contaminants originating from production processes that are released into natural water bodies through effluents [1]. The textile industry is one of the leading contributors to the global economy and is also the second-largest industry that causes water pollution. Textile effluents usually contain high levels of organic matter, salts, and dyes, most notably azo dyes [2, 3]. Each year, the textile industry uses approximately 10 000 tons of dyes, releasing between 10 % and 15 % of them into wastewater during the production process [3, 4].

In most cases, these colored effluents are discharged without prior treatment, causing adverse environmental effects such as changes in color, increased turbidity, reduced photosynthetic activity, and elevated chemical (COD) and biochemical oxygen demand (BOD) (ranges between 1062 mg/L – 2200 mg/L for COD and 100 mg/L – 300 mg/L for BOD) [5], among others, which negatively affect biodiversity. In humans, some chemical structures of dyes can cause adverse effects, including DNA mutations, neurological issues, and carcinogenic effects [6–9].

In the search for alternatives to mitigate the impact of azo dye-contaminated effluents from the textile industry, various strategies have been proposed, including physical treatments (*e.g.*, activated carbon adsorption and membrane filtration) and chemical treatments (*e.g.*, coagulation, flocculation, and advanced oxidation processes). However, these methods are often expensive and may generate intermediate compounds more toxic than the parental dye [6, 10, 11].

This has driven the pursuit of treatment alternatives leveraging the metabolic versatility of microorganisms as a cost-effective and sustainable option for degrading these substances [12]. Among these, the metabolic potential of fungi, particularly white-rot fungi (WRF), stands out for its ability to treat industrial wastewater. Fungi can colonise large surfaces and tolerate fluctuations in environmental physicochemical conditions. Notably, WRF have demonstrated efficiency in degrading or transforming high-molecular-weight organic and inorganic compounds through a non-specific extracellular multienzyme complex composed of laccase (Lac), manganese peroxidase (MnP), and lignin peroxidase (LiP) enzymes which are capable to degrade complex molecules like lignin and cellulose in wood, making them widely used in the transformation of similar compounds, such as aromatic compounds and synthetic dyes [13].

Bioreactors are frequently employed in bioremediation procedures involving azo dyes mediated by WRF, as these systems provide a suitable environment for microbial growth and metabolic activity. Establishing such bioreactors requires considering factors such as i) device geometry and design (*e.g.,* packed-bed, airlift, stirred tank), ii) operation mode (batch, fed-batch, continuous), iii) hydraulic retention time, and iv) culture conditions (*e.g.,* inducers, temperature, agitation) to ensure efficient dye removal from wastewater [14, 15]. Bioreactors inoculated with fungal biomass offer a viable and efficient alternative for promoting contaminant transformation and removal in industrial wastewater treatment. They enhance fungal biomass activity over extended periods, and batch operation eliminates the need for additional nutrients, thereby reducing system operating costs [16]. Some studies employing WRF have reported decolorization efficiencies of 100 % for Brilliant Green in 24 hours [17], 30 % for Reactive Blue in 30 days [18], and 8 % for Remazol Black in 24 hours [19]. However, significant laboratory-scale research has been conducted on this type of process; few studies have evaluated the treatment of wastewater using immobilized native WRF strains at larger scales and with real textile industry effluents [20, 21]. In recent years, agro-industrial residues such as sawdust have been increasingly investigated not only as low-cost support matrices but also as organic inducers of ligninolytic enzyme production. For instance, sawdust substrates have been successfully employed in solid-state fermentation to stimulate the secretion of laccase and manganese peroxidase by white-rot fungi, outperforming other lignocellulosic residues due to their porosity and nutrient content [22]. Moreover, the inclusion of sawdust in fungal cultures has been shown to promote oxidative enzyme activities more effectively than inert supports, likely by supplying phenolic fragments or trace nutrients that act as inducers [23]. Therefore, the use of pine sawdust as a biological inducer in the present bioreactor system not only represents an environmentally friendly valorization of an agro-industrial byproduct but also aligns with previous findings highlighting its potential to enhance ligninolytic enzyme production and dye degradation efficiency.

The main purpose of this study was to evaluate the bioremediation potential of a native white-rot fungus (*Lentinus* sp.) for the treatment of a real textile effluent containing azo dyes in a 6-L packed-bed bioreactor, using pine sawdust as a natural inducer of ligninolytic enzyme activity.

2. Materials and Methods

2.1. Microorganisms and Culture Conditions

The fungal strain used in this study belongs to the genus *Lentinus sp.*, isolated as part of a previous bioprospecting study conducted by the research group. The strain is registered in the Microorganisms Collection of the School of Microbiology (CM-EM-UdeA). The working strain was activated and grown on potato dextrose agar (PDA) at 30 °C for 5 days. Working cultures were stored at 4 °C until use.

2.2. Industrial Wastewater Effluent Characterization

The azo dye-containing wastewater was sourced from the effluent of a textile dye manufacturing company. Physicochemical characterization indicated that the industrial effluent contained high levels of dissolved and suspended matter, including solids (17–22% w/v), salts (25% to 40% w/v), organic compounds (15% to 25% w/v), and color (30% to 45% w/v). According to standard wastewater examination protocols [24], these parameters are typically expressed as concentrations (*e.g.,* mg/L); however, the textile manufacturer reports them as percentage composition of the bulk concentrate. Color concentration was quantified in this study using UV–Vis spectrophotometry at 504 nm with an initial value of 122 g/L.

Color and COD removal efficiency was calculated using Equation 1:

$$\text{Removal efficiency } (\%) = \frac{C_i - C_t}{C_i} \times 100 \quad (1)$$

where C_i (initial concentration mg/L) and C_t (final concentration mg/L), were measured after the hydraulic retention time ($t = 12$ days). All treatments were performed in triplicate.

To validate the significance of the results, exploratory statistical analyses (boxplots) and variance analyses (ANOVA) were conducted. Normality and homoscedasticity of the data were verified using Tukey and Kolmogorov tests, with a significance level of $p < 0.05$. Analyses were performed using R Studio (R Core Team, 2021.09.2).

2.3. Evaluation of Dye and Organic Matter (COD) Removal.

2.3.1. Dye Removal and Culture Conditions.

Preliminary decolorization assays were conducted at laboratory scale using Erlenmeyer flasks (150 mL, 70% working volume) to determine culture condition effects on azo dye removal. Industrial effluent containing Red 40 was added to a liquid culture medium (malt extract, glucose, and peptone) to obtain a final dye concentration of 100 mg/L. Each flask was inoculated with three agar plugs (4 × 4 mm) of actively growing *Lentinus* sp. mycelium. To evaluate the effect of biological induction, pine sawdust (30 g/L) was included in chosen treatments as a lignocellulosic substrate to stimulate ligninolytic enzyme production. Cultures were incubated at 30 °C for 15 days under two agitation conditions: static (no agitation) and stirred (80 rpm). The experimental design, therefore, consisted of four treatments: (i) pine sawdust + agitation, (ii) pine sawdust + static culture, (iii) without inducer + agitation, and (iv) without inducer + static culture. Samples were taken every 2 days to monitor residual dye concentration by UV–Vis spectrophotometry

at 504 nm. Decolorization efficiency was calculated with Equation (1). All treatments were performed in triplicate, and results were presented as mean ± standard deviation (SD). Statistical comparisons between treatments were conducted by one-way ANOVA ($p < 0.05$).

To assess the decolorization process, two abiotic controls were included in all experiments: (i) colored effluent at 100 mg/L supplemented with pine sawdust, in order to evaluate potential dye adsorption onto the lignocellulosic substrate, and (ii) colored effluent (100 mg/L) mixed with culture medium without fungal inoculation, to rule out decolorization caused by abiotic factors.

2.3.2. Bed-packed bioreactor

A cylindrical packed-bed polycarbonate bioreactor was designed and built, featuring a total volume of 6 L, a height of 30 cm, and an internal diameter of 18 cm (**Fig. 1**). The bioreactor was cleaned with an active oxygen solution and exposed to ultraviolet light in a laminar flow cabinet for 30 minutes. The bioreactor's inoculum was prepared by growing *Lentinus* sp. in 1 L of liquid culture medium containing pine sawdust (30 g/L) at 30 °C without agitation for 4 days. Fungal biomass was immobilized on discs (8 cm diameter) made of low-cost lignocellulosic material (*Luffa cylindrica*), chosen for its low dye absorption rate [6, 24]. The discs were sterilized at 121 °C for 15 minutes and placed inside the bioreactor, with one disc at the top of the reactor's working volume (6 L) and another submerged at 2/4 parts of the total reactor height.

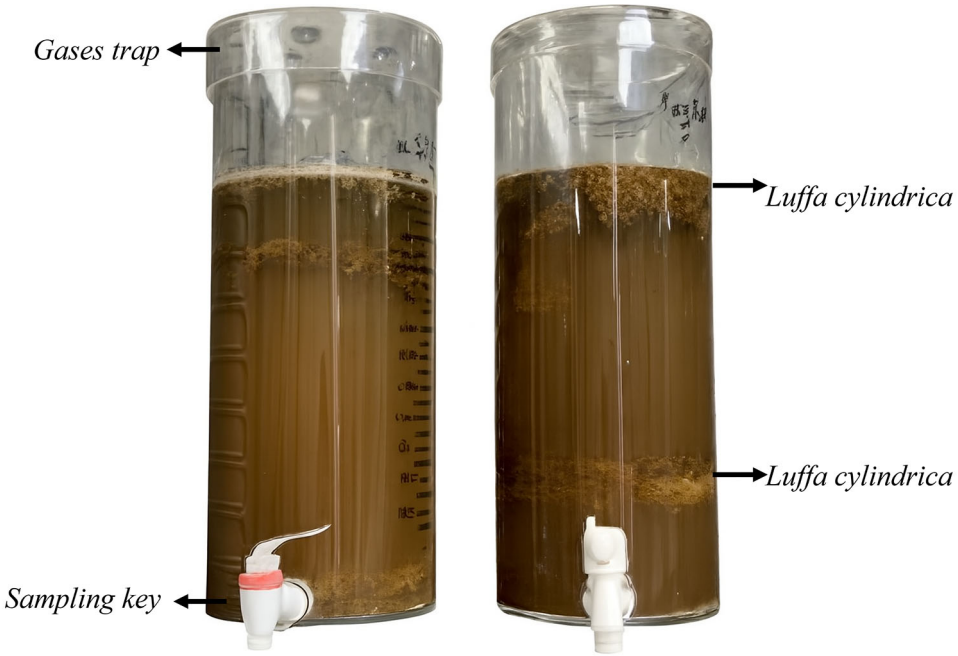

Figure 1. Bioreactor system design.

The bioreactor mixture consisted of liquid culture medium, pine sawdust, fungal biomass inoculum, and colored wastewater, adjusted to a dye concentration of 100 mg/L, for a total effective volume of 6 L. The bioreactor operated in batch mode at 30 °C for 12 days without agitation. Samples were taken from the bioreactor every 2 days to analyze color removal, enzyme activity, and possible biotransformation. For COD analysis, samples were taken on day 1 (initial) and day 12 (final). Bioreactor assays were performed in duplicate, and an abiotic control was included under the same operating conditions. The control treatment contained the lignocellulosic support (*Luffa cylindrica* and pine sawdust) but no fungal inoculum, to verify that dye removal was not attributable to physical adsorption onto the matrix.

2.4. Enzyme Activity and Nuclear Magnetic Resonance Analysis

Laccase (Lac) activity was determined using 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid, ABTS) as a substrate, following Niku-Paavola *et al.* method (1988). Manganese peroxidase (MnP) was determined using the DMAB/MBTH colorimetric assay. Reaction mixtures (1.00 mL total) contained 50 mM sodium malonate buffer (pH 4.5), 1.0 mM $MnSO_4$, 0.5 mM 3-dimethylaminobenzoic acid (DMAB), and 0.5 mM 3-methyl-2-benzothiazolinone hydrazone (MBTH). The reaction was initiated by adding H_2O_2 to a final concentration of 0.10 mM and incubated at 30 °C. After exactly 3 minutes, the reaction was stopped by quenching residual peroxide with sodium metabisulfite (final 10 mM). Enzyme activities were calculated based on the change in absorbance per unit of time using the following general equation:

$$\text{Enzymeactivity (UI/L)} = \frac{(\Delta A/\Delta t)(V total)}{(\varepsilon)(l)(V enzyme)} \tag{2}$$

where $\Delta A/\Delta t$ is the slope of absorbance versus time (Abs min^{-1}), V_{total} is the total reaction volume (mL), V_{enzyme} is the sample volume added (mL), l is the path length of the cuvette (1 cm), and ε is the molar extinction coefficient of the oxidized product. Both enzyme activity assays were performed in triplicate and expressed in International Units (IU), where one unit represents the amount of enzyme that oxidizes 1 μmol of substrate per minute.

The presence of transformation or degradation products formed in the bioreactor was analyzed using nuclear magnetic resonance (NMR) spectroscopy at 600 MHz (Bruker Avance HD III, BioSpin) with a 5 mm TCI cryoprobe at 300 K. Liquid samples (600 μL of treated effluent or abiotic control) were prepared with 60 μL of 1.5 M phosphate buffer (pH 6.2) in deuterium oxide. The pure dye (Red 40) was prepared to a concentration of 80 μg/mL in Type II water. Spectra were acquired using a 90° pulse length and low-power radiofrequency irradiation for water signal presaturation.

3. Results and discussion

3.1. Azo dye (red 40) removal and culture conditions

White-rot fungus *Lentinus* sp. culture conditions and decolorization rates were optimized at a small scale (150 ml) using real colored wastewater, with performance values shown in **Table 1**, depending on incubation conditions, (i) pine sawdust addition and (ii) presence or absence of agitation.

Table 1. Evaluation of the decolorization potential of an azo dye (100 mg/L) on liquid culture media inoculated with WRF *Lentinus* sp., after 12 days of incubation time.

WRF	Inducer	Incubation condition	Dye removal (%)
Lentinus sp.	Pine sawdust	Stirred 80 rpm	$66 \pm 0,002$
	Pine sawdust	Without agitation	$94 \pm 0,001$
	Without inducer	Stirred 80 rpm	$11 \pm 0,5$
	Without inducer	Without agitation	$57 \pm 0,4$

The use of fungal biomass as a biotechnological tool implies the establishment of adequate culture conditions given the diverse composition of the wastewater and the chemical structure of the dye molecule to be treated, which constitute unique growth environments for the microorganisms, favoring the production of one enzyme over another within the extracellular enzymatic complex and leading to variation in color removal efficiencies of [27, 28]. In this initial preliminary assessment phase, different culture conditions were evaluated (*e.g.*, agitation and the addition of lignin-rich compounds) to identify the optimal combination that enhances dye removal efficiency. The interaction of these factors, among others, directly influences the rates of color removal in the treatment of colored wastewater using fungi [25].

In this study, a liquid culture with sawdust addition and no agitation (Table 1) led to the highest removal efficiency (94 ± 0.001 %). Regarding the addition of a lignocellulosic substrate, previous studies demonstrated that wood composition significantly affects extracellular enzyme production [25]. Based on this observation, three types of wood were tested: walnut, eucalyptus, and pine. No growth was observed in cultures supplemented with walnut or eucalyptus sawdust (data not shown), a phenomenon attributed to the presence of tannins with antifungal properties [29, 30]. In contrast, cultures supplemented with pine sawdust exhibited abundant biomass growth and, consequently, the production of lignolytic enzymes, such as laccase (Lac) and manganese peroxidase (MnP). Similar studies emphasizing the stimulation of extracellular enzyme production have reported decolorization percentages of 76 % for remazol blue and 100 % for reactive blue 220 in submerged cultures inoculated with ligninolytic basidiomycetes (LBM) of the genus *Lentinus* and supplemented with lignin- and cellulose-rich agroindustrial residues such as citrus pulp and sugarcane bagasse [31, 32]. Similarly, in azo dye degradation (amaranth and cibacron blue) using submerged cultures inoculated with *Lentinus arcularius*, decolorization percentages ranged between 87 % and 100 % [33].

3.2. Packed-Bed Bioreactor Treatment of Industrial Colored Effluent

Once preliminary fungal culture conditions for wastewater decolorization were established, these were then scaled up to a 6-L bioreactor system. The upsacled system setup included culture medium, pine sawdust, a natural cellulose matrix, a pre-inoculum of *Lentinus* sp., and industrial wastewater containing an azo dye, for a total effective volume of 6 L. The defined hydraulic retention time (HRT) for the system operation was 12 days, after which a decolorization percentage of 61.45 ± 0.0014 % (38 mg/L) was achieved, starting from an initial dye concentration of 100 mg/L (**Fig. 2**). Increasing the system volume is a critical aspect of biotechnological processes upscaling, as it impacts factors such as homogenization and mass transfer within the system [34]. Similar systems have reported azo dye removal rates ranging from 77 % to 95 % for mordant blue 9 and direct red 80 using *Phanerochaete chrysosporium* in a 5 L rotary drum reactor [35] and 69 % removal for indigo blue using immobilized *Bjerkandera* sp. biomass in a 5-L bioreactor [6]. In addition to dye removal, a 99 % chemical oxygen demand (COD)

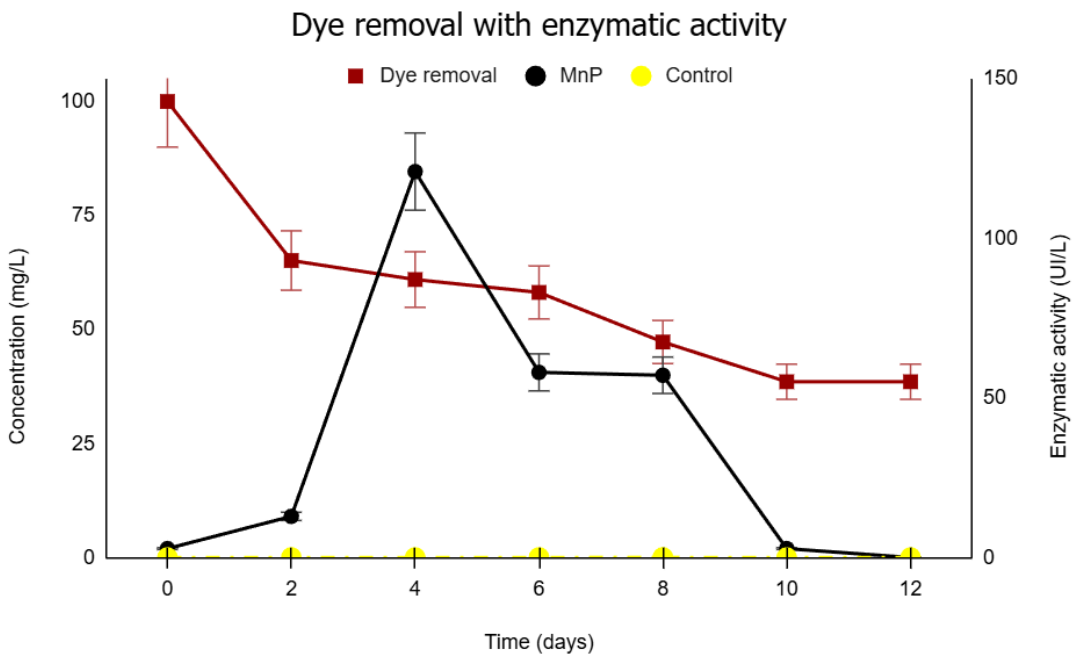

Figure 2. Decolorization kinetics of an azo dye (Red 40) using the fungus Lentinus sp., from an initial dye concentration of 100 mg/L. Tracking activities of the enzymes manganese peroxidase (MnP) and laccase (Lac).

reduction was achieved, with a final value of 253.75 mg/L. Similar COD removal rates (70 %) have been reported during the decolorization of textile wastewater using fungal genera such as *Aspergillus* sp. and *Penicillium* sp. in fluidized bed reactors operated in batch mode, with biomass immobilized on synthetic sponges [23]. In both cases, the results suggest the potential of fungi as a biotechnological alternative for implementing colored wastewater treatment systems.

Regarding the production of extracellular enzymes during the decolorization process, MnP reached a maximum concentration of 120 U/L after 4 days of system operation, whereas Lac remained undetectable in all samples throughout the entire assay span (Fig. 2).

This enzymatic dynamic was previously observed during flask trials (data not shown), suggesting that, under the established conditions, the decolorization process is influenced by the presence of the MnP enzyme. A recent study conducted under similar conditions (decolorization mediated by ligninolytic basidiomycetes in a static reactor) also failed to detect laccase production [36]. These authors concluded that laccase production is arguably influenced by the presence of decolorization and carbon-source degradation by-products, which inhibit the enzyme's active site. For cultures inoculated with *Lentinus* sp., it has been observed that solid-state fermentation processes favor Lac production compared to submerged fermentation, which results in greater MnP production [37]. These findings align with the results of the present study. Notably, MnP is a peroxidase with a strong active site for binding complex molecular products [38], highlighting its ability to oxidize the azo bond in dyes. This feature promotes the removal of various azo dyes such as amaranth, reactive black 5, and cibacron yellow, with removal rates of 95 %, 76 %, and 46 %, respectively [39, 40]. Nevertheless, the role of extracellular enzymes in decolorization processes remains under study, as it is not always possible to attribute dye removal exclusively to the extracellular enzymatic activity of ligninolytic basidiomycetes [33]. This observation is consistent

with a previous study reporting low extracellular enzyme activity alongside a decolorization rate of 69 %, suggesting that the amount of secreted enzyme is not directly related to the efficiency of the decolorization process [6].

3.3. Nuclear magnetic resonance analysis

Fig. 3 shows the structure of the azo dye present in the industrial-colored effluent, the 1H-NMR spectrum of the dye, and the signal assignment for the observed molecule. The spectrum reveals the presence of all expected protons, consistent with the dye's chemical structure.

Fig. 4 shows the 1H-NMR results for two samples collected during the treatment process (incubation days 4 and 12), compared to the untreated dye spectrum. A reduction in dye signal (identified between 7.50 and 7.35 ppm) is accompanied by the appearance of two new signals between 7.35 and 7.30 ppm. These signals may be associated with the addition of protons to the molecular structure, resulting from the breakdown of the parental molecule upon reduction of the azo group during cultivation with the HPB *Lentinus* sp.

The protons (H) highlighted in red in Fig. 4B correspond to the new proton signals observed in the spectra of the samples throughout the bioreactor treatment. Regarding the degradation mechanisms of azo dyes by ligninolytic fungi, the extracellular enzymes produced by these organisms are noteworthy for their role in breaking the azo bond [30]. This cleavage represents the initial step in the degradation pathway of these dyes, leading to the loss of the chromophore group and the transformation of the parent molecule into simpler compounds, such as aromatic amines [39, 40]. The NMR data provide molecular-level evidence that the treatment resulted in true dye transformation rather than mere adsorption on the lignocellulosic matrix.

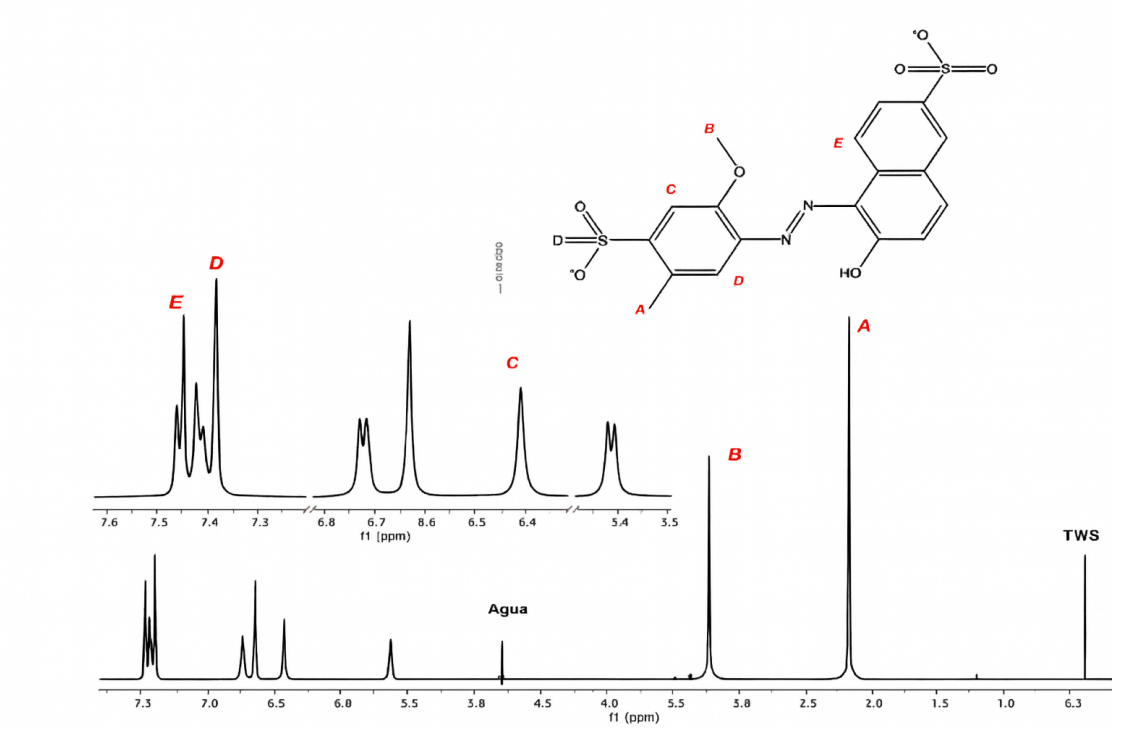

Figure 3. Decolorization kinetics of an azo dye (Red 40) using the fungus Lentinus sp., from an initial dye concentration of 100 mg/L. Tracking activities of the enzymes manganese peroxidase (MnP) and laccase (Lac).

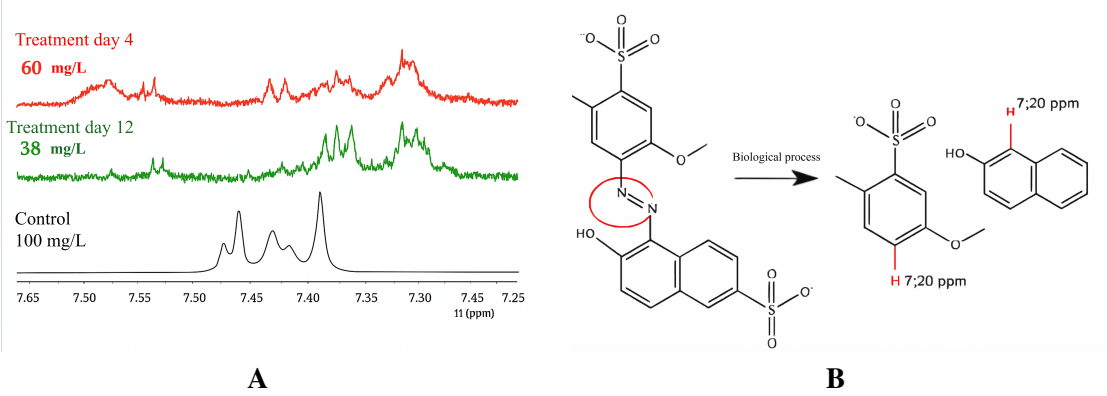

Figure 4. A) 1H-NMR spectrum of the dyed industrial effluent. Black line: effluent without treatment. Red line: spectrum of sample collected after 4 days of incubation; green line: spectrum of sample collected after 12 days of incubation. **B)** Chemical structure of azo dye and generated by-products.

The downshift and redistribution of aromatic proton signals alongside the attenuation of resonances associated with the conjugated chromophore are consistent with the disruption of the azo bond (N=N). Classical NMR analyses of azo compounds show that changes in aromatic regions (approximately 7–8 ppm) and diagnosed variations linked to azo/quinone reflect alterations in the electron distribution of the dye structure, supporting the information suggested in this study of chromophore deconjugation after enzymatic attack [41].

The structural modifications experienced by the dye molecule (red 40) are aligned with what is expected to be obtained from the WRF metabolism. MnP can promote oxidative pathways capable of attacking the dye structure. Furthermore, early seminal work with ligninolytic peroxidases demonstrated direct transformation of industrial azo dyes, reinforcing the plausibility that peroxidase-mediated pathways drive the structural changes captured by NMR in this study. From the perspective of this study, hydrogen peroxide doses could be added to the reactor to increase MnP activity and avoid saturation of its active sites [42]. Comparable literature, using high-resolution analytics, corroborates this interpretation. For immobilized *Trametes hirsuta* D7, UPLC–FTICR–MS tracking of biodegradation shows structure-dependent pathways (such as efficient transformation of Reactive Black 5, Acid Orange 7, and Acid Blue 113), with product patterns indicative of chromophore breakdown [43] These reports converge with our NMR observation of newly formed aromatic signatures.

From an environmental perspective, azo-bond cleavage is typically regarded as a primary detoxification step, even though aromatic amine intermediates may form transiently and require attention as residual hazards. Studies with *Phanerochaete chrysosporium* have demonstrated decolorization and detoxification of several azo dyes [35]; however, toxicity assays are necessary to confirm the safety of the treated effluents [30]. Our NMR results, while confirming chemical transformation, should therefore be interpreted as primary evidence of detoxification and must be aided by toxicological assays to support this conclusion.

Finally, the NMR-inferred structural changes are coherent with other parameters evaluated in this study, namely substantial color removal and COD reduction. Together, these outcomes support a biodegradation-dominant mechanism (rather than adsorption to lignocellulosic supports), in line with abiotic control inclusions (at flask and bioreactor scales) and with the established behavior of ligninolytic fungi treating azo dye effluents. The results obtained with the native strain of the ligninolytic basidiomycete *Lentinus* sp. align with the mechanisms recently described in the

literature, emphasizing the successful establishment of the culture in a bioreactor system with a native strain and its demonstrated capacity to remove dye from real industrial wastewater effluents. These findings corroborate the biotechnological potential of the evaluated strain and underscore the opportunities arising from bioprospecting strategies.

4. Conclusion

This study highlights the potential of the native white-rot fungus *Lentinus* sp. as a sustainable biotechnological alternative for treating real textile effluents containing azo dyes. The packed-bed bioreactor achieved 61.5 % color removal and a 99 % COD reduction after 12 days of operation, confirming the system's efficiency under low-energy, nutrient-limited conditions. NMR analysis indicated azo bond cleavage and molecular transformation of the dye, evidencing true biodegradation rather than mere adsorption, with manganese peroxidase identified as the key active enzyme.

The integration of pine sawdust as a natural and low-cost inducer enhanced enzymatic activity, promoting an environmentally friendly process that valorizes agroforestry residues. These results suggest that the proposed fungal system can be feasibly scaled up for pilot and industrial applications, offering a promising and sustainable approach for the remediation of dye-polluted industrial effluents.

5. Conflict of interest.

Authors declare no conflict of interests

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Efluentes Textiles Con Colorantes Azo: Biorremediación Ecoamigable Usando *Lentinus* Sp. En Un Biorreactor De Lecho Empacado Con Aserrín De Pino Como Inductor Orgánico

Resumen: Los efluentes textiles industriales que contienen colorantes azo representan un desafío ambiental importante debido a su persistencia y toxicidad. En este estudio se evaluó la capacidad de un hongo nativo de pudrición blanca (*Lentinus* sp.) para tratar un efluente textil real contaminado con un colorante azo (Rojo 40) en un biorreactor de lecho empacado. La biomasa fúngica se inmovilizó en soportes lignocelulósicos de bajo costo (*Luffa cylindrica*), y se añadió aserrín de pino como inductor biológico para estimular la producción de enzimas ligninolíticas. Inicialmente, las condiciones de tratamiento se optimizaron en matraces Erlenmeyer (0,15 L), logrando una decoloración del 94,0 $\pm$ 0,1 %. Bajo condiciones a escala ampliada (6 L, 30 °C, 12 días de tiempo de retención hidráulica, modo batch, sin agitación), el sistema removió el 61,5 $\pm$ 0,2 % del colorante y redujo la demanda química de oxígeno (COD) en un 99,0 $\pm$ 0,3 %. Los ensayos enzimáticos evidenciaron actividad de manganeso peroxidasa, mientras que no detectaron lacasa. El análisis por resonancia magnética nuclear (NMR) confirmó modificaciones estructurales del colorante mediante la ruptura del enlace azo. Estos hallazgos demuestran el potencial de los hongos ligninolíticos nativos como herramientas biotecnológicas sostenibles y de bajo costo para tratar efluentes industriales contaminados con colorantes azo, respaldando su aplicabilidad a mayor escala.

Palabras Clave: Aguas residuales Azo; Eficiencia de remoción de colorante; Lacasa; *Lentinus* sp.; *Luffa cylindrica*; Peroxidasa

Efluentes Têxteis Com Corantes Azo: Biorremediação Ecológica Usando *Lentinus* Sp. Em Um Biorreator De Leito Empacotado Com Serragem De Pinus Como Indutor Orgânico

Resumo: Os efluentes têxteis industriais que contêm corantes azo representam um importante desafio ambiental devido à sua persistência e toxicidade. Este estudo avaliou a capacidade de um fungo nativo de podridão branca (*Lentinus* sp.) para tratar um efluente têxtil real contaminado com um corante azo (Vermelho 40) em um biorreator de leito empacotado. A biomassa fúngica foi imobilizada em suportes lignocelulósicos de baixo custo (*Luffa cylindrica*), e serragem de pinus foi adicionada como indutor biológico para estimular a produção de enzimas ligninolíticas. Inicialmente, as condições de tratamento foram otimizadas em frascos Erlenmeyer (0,15 L), alcançando 94,0 ± 0,1% de descoloração. Em condições ampliadas (6 L, 30 °C, 12 dias de tempo de retenção hidráulica, modo batelada, sem agitação), o sistema removeu 61,5 ± 0,2% do corante e reduziu a demanda química de oxigênio (COD) em 99,0 ± 0,3%. Ensaios enzimáticos evidenciaram atividade de manganês peroxidase, enquanto lacase não foi detectada. A análise por ressonância magnética nuclear (NMR) confirmou modificações estruturais do corante por meio da quebra da ligação azo. Esses achados demonstram o potencial de fungos ligninolíticos nativos como ferramentas biotecnológicas sustentáveis e de baixo custo para o tratamento de efluentes industriais contaminados com corantes azo, apoiando sua aplicação em maior escala.

Palavras-chave: Águas residuais; Azo; Eficiência de remoção de corante; *Lentinus* sp.; *Luffa cylindrica*; Lacase; Peroxidase

Camila Gómez Garcés

Industrial and environmental microbiologist with an M.Sc. in Environmental Engineering and currently Ph.D. candidate in Environmental Engineering at the Universidade Federal de Ouro Preto, professor at Universidad de Antioquia and researcher at Grupo de investigación de bioprocesos microbianos (BIOmicro). Her research focuses on environmental microbiology, with an emphasis on sustainable solutions for contaminated water treatment, bioremediation of microcontaminants, and the development of strategies for advanced wastewater treatment.

ORCID: 0009-0005-6573-363X

Luisa María Múnera Porras:

Luisa María Múnera Porras is a microbiologist with a MSc in Biology and a PhD candidate in Biology at the University of Antioquia, Colombia. Her research focuses on microbial ecology, environmental microbiology, and bioremediation, with particular interest in plant–microbe interactions and microbiomes in polluted environments. She has experience in metagenomics, bioinformatics, and academic teaching.

ORCID: 0000-0002-0215-9993

Lida Arias Marín

Ph.D. in Microbiology and professor at the University of Antioquia. She is the founder and coordinator of Grupo de investigación de bioprocesos microbianos (BIOmicro). Her research focuses on developing sustainable solutions for the treatment of emerging contaminants in soil and water, with a strong emphasis on environmental biotechnology and microorganism-based technologies.

ORCID: 0000-0003-2835-070X