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Evaluation of the textile dye removal process using the residual substrate from *Pleurotus ostreatus* mushroom production

Avaliação do processo de remoção de corante têxtil utilizando o substrato residual da produção do cogumelo *Pleurotus ostreatus*

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ABSTRACT

The release of textile effluents without treatment, or subjected to ineffective treatment, causes serious problems in aquatic ecosystems. In this context, the objective of this study was to evaluate the capacity and process of removing the textile dye Drimaren Red CL-7B, using the residual compound resulting from the production of the *Pleurotus ostreatus* mushroom. For this purpose, a 3^2 experimental planning was first developed, considering the variables pH and substrate concentration, seeking to evaluate the removal of dye from synthetic textile effluent, during six hours of contact time. The substrate concentrations applied to the effluent were 50, 100 and 150 g L^{-1} and the pH values used were 5, 7 and 9. After determining the best experimental condition among those evaluated, the possible removal mechanisms that occurred and the evaluation of the enzymatic activity of the mycelium present in the substrate were also studied. It was found that the best operational condition was obtained at pH 5 and concentration of 150 g L^{-1}. Under these conditions, the achieved dye removal efficiency was close to 70%. It was observed that the main removal mechanism was adsorption and a contact time of 30 minutes already allows to obtain satisfactory results. Additionally, the results of the laccase activity demonstrated that its efficiency is high under acidic pH conditions and it becomes inactive under alkaline pH conditions. It was concluded that the residual substrate has high potential for the treatment of textile effluents due to the simultaneous action of

RESUMO

O lançamento de efluentes têxteis sem tratamento, ou submetidos a um tratamento ineficaz, ocasiona graves problemas nos ecossistemas aquáticos. Neste contexto, o objetivo deste estudo foi avaliar a capacidade e o processo de remoção do corante têxtil Drimaren Red CL-7B, empregando o substrato residual da produção do cogumelo *Pleurotus ostreatus.* Para tanto, primeiramente elaborado um planejamento experimental 3^2, considerando as variáveis pH e concentração de substrato, buscando-se avaliar a remoção de corante proveniente de efluente têxtil sintético, durante seis horas de tempo de contato. As concentrações do substrato aplicado ao efluente foram 50, 100 e 150 g L^{-1} e os valores de pH empregados foram 5, 7 e 9. Após a determinação da melhor condição experimental dentre as avaliadas, também foram estudados os possíveis mecanismos de remoção ocorridos e a atividade enzimática do micélio presente no substrato. Verificou-se que a melhor condição operacional foi obtida com pH 5 e concentração de 150 g L^{-1}. Sob essas condições, a eficiência de remoção de corante alcançada foi próxima de 70%. Observou-se que o principal mecanismo de remoção foi a adsorção e um tempo de contato de 30 minutos já permite obter resultados satisfatórios. Além disso, os resultados da atividade da lacase demonstraram que sua eficiência é elevada em condições de pH ácido, e que esta fica inativa em condições de pH alcalino. Concluiu-se que o substrato residual apresenta elevado potencial para o tratamento de efluentes têxteis em decorrência da ação simultânea das enzimas

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lignolytic enzymes and adsorption in the removal of dyes, as well as the possibility of valuing an agro-industrial residue.

Keywords: azo dye; textile effluents; bioremediation; mycoremediation; enzymatic degradation; adsorption.

lignolíticas e da adsorção na remoção de corantes, bem como, pela possibilidade de valorização de um resíduo agroindustrial.

Palavras-chave: azo corante; efluentes têxteis; biorremediação; micorremediação; degradação enzimática; adsorção.

Introduction

The textile industry plays a crucial role in societal advancements and the global economy. In Brazil, this sector emerged around 200 years ago, being one of the pioneering activities in the industrialization process (Fujita et al., 2015). According to recent data, the textile sector represents approximately 18.0% of the total industrial production workforce and 6.6% of the total value of the Brazilian manufacturing industry (Abit, 2024). The industrial production of textile articles is a complex process in which natural or artificial fibers are converted into yarns and fabrics and subsequently into finished products. During the fabric finishing stages, water is directly used in washing, dyeing, and softening, and indirectly in heating and cooling processes (Abbas et al., 2020; Azanaw et al., 2022). Among these stages, dyeing and finishing consume most of the water volume and cause the greatest contamination, as dyes and various chemicals are applied to fix colors, with water being fundamental for this application (Samsami et al., 2020).

For the dyeing stage, synthetic dyes are the most used and can be classified according to their origin, method of application to the fiber, and chemical structure (Vázquez-Ortega et al., 2020). Azo class synthetic dyes represent approximately 70% of commercially available dyes and are widely employed in the textile industry due to their versatile application to different materials, high fixation, and wide spectrum of available colors (Benkhaya et al., 2020; Selvaraj et al., 2021). However, this class of dyes is also recognized for its high toxicity and recalcitrant characteristics, making them resistant to various treatment alternatives (Almeida and Corso, 2014).

In this context, highly colored effluents from the dyeing phase can be considered the most problematic, as they not only exhibit coloration but also high chemical and biochemical oxygen demand, toxic compounds, salts, metals, and suspended solids (Yaseen and Scholz, 2019). Thus, it is highlighted that the discharge of textile effluents containing residual dyes can cause numerous environmental impacts on the receiving water bodies, such as depletion of dissolved oxygen concentration, reduction of sunlight penetration, and consequent harm to photosynthetic activity, as well as direct toxic effects on aquatic fauna and flora (Selvaraj et al., 2021).

The treatment of textile effluents generally involves a combination of conventional physicochemical and biological processes (Otto et al., 2021). Biological treatment systems, such as activated sludge, are quite common and allow for high removal of organic matter and solids but are inefficient at removing dyes (Albahnasawi et al., 2020; Azanaw et al., 2022). To overcome these limitations, the physicochemical processes of coagulation followed by flocculation and advanced oxidation processes have been considered as solutions. However, despite their proven efficiency, they often require the use of large amounts of chemicals and specific equipment, resulting in high operational costs, which limits their application (Samsami et al., 2020; Al-Tohamy et al., 2022).

In this scenario, adsorption processes appear as a very promising alternative for the removal of dyes from textile effluents, mainly due to their high efficiency combined with lower operational costs (Rashid et al., 2021; Sintakindi and Ankamwar, 2021). Different materials have been studied for this application, including zeolites, alumina, silica gel, and activated carbon, and more recently, the focus has also been on the search for low-cost alternative adsorbents, such as bioadsorbents (Sintakindi and Ankamwar, 2021; Al-Tohamy et al., 2022).

Among the studied bioadsorbents, fungi present high potential for application in the treatment of textile effluents, as they have high porosity and a wide diversity of functional groups in their structure, allowing good interaction with dyes (Sintakindi and Ankamwar, 2021). Additionally, studies indicate that extracellular ligninolytic enzymes produced by some fungi, such as basidiomycetes, are also capable of degrading, reducing, and mineralizing complex and recalcitrant molecules, such as those usually present in textile effluents (Stamets, 2005; Sintakindi and Ankamwar, 2021; Al-Tohamy et al., 2022).

Basidiomycete fungi are produced on a large scale for edible purposes, but the substrate used for their cultivation, once the nutrient source is exhausted, is usually discarded as it has no profitable destination for producers (Battistelli et al., 2019). However, considering that after the fruiting cycle is completed, the substrate in question is fully colonized by fungal mycelia; it is believed that this material can be used for dye removal through adsorption and for enzymatic degradation, characterizing it as an economical and environmentally viable alternative, as it allows the valorizing of a residue that would originally be discarded (Sintakindi and Ankamwar, 2021; Negi and Das, 2023).

Nevertheless, it is noteworthy that the available information in the literature on this topic is scarce, especially regarding the operational conditions to be employed and the removal mechanisms involved in the process. In this context, the present study aimed to evaluate the capacity and process of removing the textile dye Drimaren Red CL-7B using the residual substrate resulting from the production of the mushroom Pleurotus ostreatus, seeking to identify the possible removal routes and evaluate the enzymatic activity.

Materials and Methods

Residual substrate from mushroom production

The material added to the textile effluent in the tests was a residual substrate from the production of P. ostreatus fungi, provided by the company Fungitake, located in the municipality of Umuarama (PR), Brazil. The substrate was composed of sawdust, soybean hulls, agricultural gypsum, calcium carbonate, water, and the spores of P. ostreatus fungus. It is worth noting that at the end of cultivation, the blocks are discarded but their structure is completely colonized by hyphae and mycelia that contain ligninolytic enzymes capable of breaking down complex structures (Stamets, 2005). For use in the tests, the substrate in question was manually disintegrated, resulting in a material with heterogeneous granulometry.

Textile industry effluent

The procedure adopted by Spagni et al. (2010) was followed to prepare the synthetic textile effluent used in the present study. The dye used was the reactive azo dye Drimaren Red CL-7B, along with auxiliary chemical substances, as employed in the textile industry. Table 1 presents the composition of the synthetic effluent and the respective concentrations of the substances used.

To obtain the concentrations indicated in Table 1, the reagents were weighed on an analytical balance, followed by the dilution of each reagent in distilled water. Samples of synthetic textile effluent with pH values of 5, 7, and 9 were prepared using a bench pH meter (Gehaka PG1800). A textile company from Brusque (SC) provided the azo dye used in the research; however, no citations were found in the scientific literature regarding it.

Experimental procedure

The experiment was conducted on a laboratory bench scale, using glass beakers containing 400 mL of effluent, to which the previously disintegrated substrate was added in contact with the synthetic textile effluent with a Drimaren Red CL-7B dye concentration of 40 mg L^{-1}.

Table 1 – Composition of the synthetic textile effluent.

Chemical products	Concentration (mg L^{1})
Dye Drimaren Red CL-7B	40.0
Ammonium Chloride	23,0
Dipotassium Phosphate	9,0
Monopotassium Phosphate	17.0
Ferric Chloride (III)	1.25
Sodium Acetate	1400.0
Sodium Chloride	126.0
Sodium Bicarbonate	668.0

Source: adapted from Spagni et al. (2010).

Then, the mixture of effluent and substrate was placed under constant agitation using a magnetic stirrer (1500 rpm) for six hours (Battistelli et al., 2019). At the end of the experiments, 10 mL samples were taken, centrifuged at 3900 rpm for 30 minutes, and filtered through qualitative filter paper with a weight of 80 g m^{-2} for subsequent determination of the residual dye concentration.

For the quantification of the dye concentration, a spectral scan of the effluent was first performed using an ultraviolet/visible (UV/Vis) absorption spectrophotometer HACH 6000, between wavelengths of 380 to 740 nm (visible light), to identify the absorption peak corresponding to the dye (517 nm). Then, an analytical curve was inserted into the equipment using different dye concentrations (between 10 and 50 mg L^{-1}), which enabled the readings to be carried out.

Next, the percentage of decolorization was calculated to determine the dye removal efficiency, according to Equation 1:

$$R = \frac{(Ci - Cf)}{C_i} * 100 \quad (1)$$

Where:
R=dye removal (%);
Ci=initial concentration of the effluent (g L^{-1});
Cf=final concentration of the effluent (g L^{-1}).

Factorial design developed to determine the best experimental conditions for dye removal

Throughout the tests, different operational conditions were tested regarding variables pH and substrate concentration which are presented in Table 2. Table 3 presents the distribution of the tests that were carried out according to the 3^2 factorial design, aiming to evaluate the effect of pH and substrate concentration on dye removal.

The softwares Statistica and RStudio were used for statistical analyses, based on data corresponding to a contact time of six hours. A multiple linear regression model was employed to evaluate the effects and interactions between the variables. The model residuals were examined for normality, homogeneity of variances, and independence of errors using the Shapiro-Wilk and Bartlett tests. The experimental error was estimated from the residuals of the linear regression model. Additionally, an analysis of covariance (ANCOVA) was conducted to identify significant differences in dye removal between the different levels of the variables at a significance level of 5% (α=0.05).

Table 2 – Levels of factors used in the factorial design of the textile effluent decolorization tests.

	Level (-)	Level (0)	Level (+)
pH	5	7	9
Substrate concentration (g L^{-1})	50	100	150

Table 3 – Factorial design developed to evaluate the effect of pH and substrate concentration on dye removal.

Test	X1	pH	X2	Substrate concentration (g L^{-1})
1	-1	5	-1	50
2	-1	5	0	100
3	-1	5	+1	150
4	0	7	-1	50
5	0	7	0	100
6	0	7	+1	150
7	+1	9	-1	50
8	+1	9	0	100
9	+1	9	+1	150

X1 and X2: factor levels.

Complementary tests conducted to evaluate dye removal mechanisms

Based on the results achieved through the tests conducted in item 2.4, the operational condition among those evaluated that allowed for the highest dye removal efficiency was determined by considering the variables related to substrate concentration and pH. Subsequently, complementary experiments were conducted in triplicate, based on this operational condition, aiming to evaluate the possible mechanisms of dye removal in the textile effluent, involving adsorption on the substrate, adsorption on the fungal structure, and enzymatic degradation. In this context, it became possible to estimate the contribution of each of these processes to the dye removal in question. To this end, the operational configuration that enabled the highest dye removal efficiency was reproduced considering different variations, as follows:

- Test I: The system was operated under agitation of 1,500 rpm for six hours, maintaining the operational conditions corresponding to the configuration that enabled the highest dye removal efficiency. However, samples were collected after 15 minutes and then at 30-minute intervals to assess the variation in dye removal over time and determine the appropriate contact time for the other tests;
- Test II: Sodium sulfite (Na_2SO_3) at a concentration of 160.0 mg L^{-1} and cobalt chloride ($CoCl_2$) at a concentration of 2.0 mg L^{-1} were applied as catalysts to reduce the dissolved oxygen concentration in the system to a value close to 0.00 mg L^{-1} (Puskeiler and Weuster-Botz, 2005). Additionally, a continuous flow of nitrogen gas (N_2) was introduced into the sample throughout the experiment to maintain the system under anaerobic conditions. This methodological approach was selected considering the oxygen dependency for the occurrence of the enzymatic degradation process (Kaushik and Malik, 2009). Thus, it was possible to evaluate dye removal without the interference of the enzymatic degradation process;
- Test III: Conducted with operational parameters similar to the configuration that enabled the highest dye removal efficiency, but with constant aeration of the sample throughout the operation period using a porous stone associated with a compressor. This configuration was tested to evaluate the enzymatic activity of the fungi under oxygen saturation conditions, with a concentration of approximately 7.5 mg L^{-1};
- Test IV: Performed with operational parameters similar to the optimized configuration that enabled the highest dye removal efficiency, but the effluent was replaced with distilled water. This allowed for the investigation of the potential release of substances from the residual substrate that could be mistakenly identified at the same wavelength used for reading the dye concentration;
- Test V: A sample of substrate not colonized by fungi was used, thus eliminating the possibility of dye removal processes through enzymatic degradation and adsorption on the fungal structure.

Characterization of laccase

The enzymes contained in the substrate were extracted using a 0.1 M sodium acetate buffer solution, with the pH adjusted to 5 and a substrate concentration of 150 g L^{-1}, maintained under constant agitation at 100 rpm on a magnetic stirrer. This procedure was carried out over an interaction period of one hour, kept at room temperature to ensure extraction efficiency (Schallemberger et al., 2023a).

Subsequently, the resulting solution was subjected to centrifugation at a speed of 4,000 rpm for a period of five minutes to separate the phases. The obtained supernatant was then used in further investigations. It is noteworthy that all subsequent analyses related to the characterization of the laccase enzyme were conducted in duplicate.

Enzymatic activity of laccase

The enzymatic activity was quantified in International Units (U), with one unit of enzymatic activity defined as the minimum amount of enzyme required to catalyze the oxidation of 1 µmol of substrate per minute, as established by Buswell et al. (1996). For the evaluation of laccase enzymatic activity, Equation 2 was employed.

$$\frac{U}{L} = \frac{\Delta abs * Vt * 10^6}{\varepsilon * d * Va * t} \quad (2)$$

Where:
Δabs=difference between final and initial absorbance;
Vt=total reaction volume (mL);
ε=molar extinction coefficient (36000 L M-1 cm-1 for ABTS);
D=path length (cm);
Va=enzyme sample volume (mL);
T=reaction time (min).

The activity of the enzyme laccase was quantified according to a colorimetric method, based on the oxidation of the substrate called 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and

subsequent reading at a wavelength of 420 nm, as described by Bourbonnais and Paice (1990).

Effect of pH on laccase activity

To determine the optimal pH of the enzyme, the same protocol used in the laccase activity assay with the ABTS substrate was followed, as described in item 4.6.1, but employing different buffer solutions. The pH values evaluated were 2, 4, 6, 8, and 10, using McIlvaine buffer (0.1 M citric acid and 0.2 M disodium phosphate) for pH values 2 to 8 and borate buffer (PanReac AppliChem: 3.092 g of boric acid 3.728 g of potassium chloride and 2.34 mL of 50% sodium hydroxide) for pH 10.

Results and Discussion

Study of the effects of substrate concentration and pH

In Figure 1, the effect of the predictor variables and their interactions is presented, making it possible to determine the best experimental conditions, among those evaluated, for the treatment of synthetic textile effluent using the residual substrate from the production of the mushroom *P. ostreatus*.

From the response surface plot, it is possible to observe that, regarding the substrate concentration factor, the highest dye removal efficiencies were obtained in the experimental condition with the highest concentration tested (150 g L^{-1}). These results indicate that increasing the substrate concentration can improve the dye removal capacity. For the pH factor, it is noted that the highest removal efficiencies were obtained at pH 5, indicating that dye removal is favored under acidic conditions.

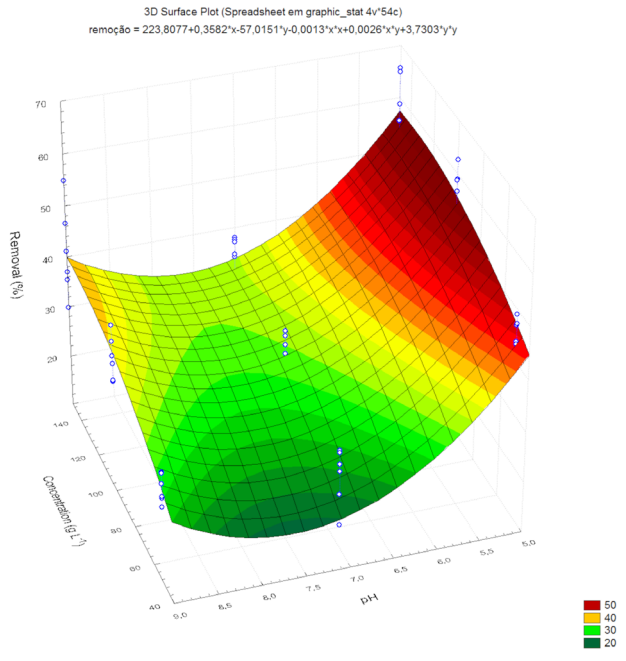

Figure 1 – Response surface plot developed based on dye removal.

It is also notable that the removal values achieved at three concentration levels at pH 5 were more satisfactory than those obtained under neutral and alkaline conditions. However, with the increase in the amount of substrate in contact with the effluent, higher decolorization values are observed, indicating an interaction between the studied variables. Therefore, the best operational condition among those evaluated in the present study was obtained at pH 5 and a substrate concentration of 150 g L^{-1}, in which the observed dye removal was close to 70%. To gain a better understanding of the results obtained from the experimental design, Figure 2 illustrates the interaction and effect of the predictor variables. Additionally, the Pareto diagram ranks the effect of the predictor variables on the final result, emphasizing the impact of pH and its interaction with concentration.

According to the Pareto diagram, it is possible to verify that the pH variable, in isolation and the interaction between pH and substrate concentration, exert significant influence on the dye removal process. From the analysis of these results, together with the behavior evidenced in the response surface plot, it is found that the dye removal efficiency was favored under acidic pH conditions and with higher substrate concentration.

Regarding the influence of substrate concentration, it is believed that the observed increase in efficiency can be mainly attributed to the expansion of the surface area and the number of available active sites, which are directly proportional to the adsorption capacity of the material (Rashid et al., 2021; Sintakindi and Ankamwar, 2021). Additionally, the greater amount of fungi may have also contributed to increased enzymatic activity, maximizing the dye degradation process (Negi and Das, 2023).

Similarly to the present study, Sintakindi and Ankamwar (2020) evaluated the removal of methylene blue dye using different types of mushrooms in various dosages and found that increasing the adsorbent concentration resulted in improved dye removal capacity, which was essentially attributed to the adsorption process. Schallemberger et al. (2023b), in turn, conducted a study aiming to evaluate the removal of azo dyes from synthetic textile effluent using residual mushroom production substrate.

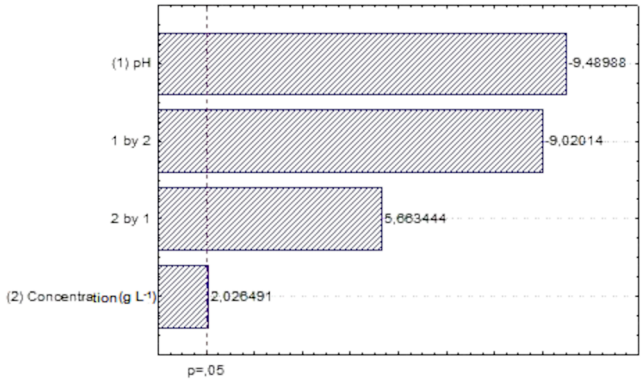

Figure 2 – Pareto diagram of the effects of pH and substrate concentration variables, as well as their interactions.

According to these authors, increasing the substrate concentration from 65 to 155 g L^{-1} allowed for an enhancement in adsorption capacity from 43.60 to 62.86%. Additionally, laccase activity also increased from 16.20 to 34.29 U L-1 under these same conditions.

Sintakindi and Ankamwar (2021) identified pH as another factor that can also influence the adsorption process once the pH change of the solution caused ionic alterations in the functional groups present in the adsorbent's cell walls. Alhujaily et al. (2020) evaluated the potential of using residual mushroom production substrate for the

removal of different anionic textile dyes and noted that the process efficiency was inversely proportional to pH, with the best condition obtained at pH 2, as the electrostatic interactions between the positively charged adsorbent surface and some negatively charged adsorbate groups were benefited.

In addition to interfering with the adsorption process, pH can also significantly influence the enzymatic activity of fungi. Fithri et al. (2020), in studies related to laccase characterization, observed more significant enzymatic activity in environments with pH below 7. Similar results were reported by Schallemberger et al. (2023a), who evaluated the enzymatic degradation of azo dyes using mushroom production residues at different pH levels (2, 4, 6, and 8) and found that laccase activity was favored under pH 2 conditions. Therefore, it is believed that the higher dye removal efficiency observed in the present study under acidic pH conditions can be explained by both the improvement of the adsorption process and the enhancement of the degradation process resulting from laccase enzymatic activity.

Additional results regarding dye removal mechanisms

As evidenced in the previous section, the experimental condition among those evaluated that allowed achieving the highest dye removal efficiency at pH 5 and a substrate concentration of 150 g L^{-1}. Therefore, to understand the processes involved in dye removal, additional tests were conducted based on the aforementioned condition. Figure 3 presents the dye removal results over time, obtained from the execution of Test I.

Kinetic analysis is of great relevance as it shows how contact time affects the amount of contaminant removed (Müller et al., 2019). In the present study, it was found that the highest dye removal capacity occurred in the first 30 minutes, reaching approximately 0.135 mg.g^{-1}. After this period, stabilization was observed up to 120 minutes, followed by a gradual decrease over time, reaching about 0.10 mg.g^{-1} after 360 minutes.

Considering the possibility of the adsorption process, it is believed that the reduction in efficiency after the first 120 minutes may have occurred due to the saturation of the active sites of the adsorbent. Thus, once the maximum removal point is reached, a decrease in active sites is observed, resulting in the stabilization of adsorption and the onset of the repulsion process between the adsorbed dye molecules and the free molecules, leading to their desorption (Schallemberger, 2023a). Studies conducted by Yan and Wang (2013) AND Alhujaily et al. (2018) also found higher dye removal rates at the beginning of the process, corroborating the results obtained in the present study.

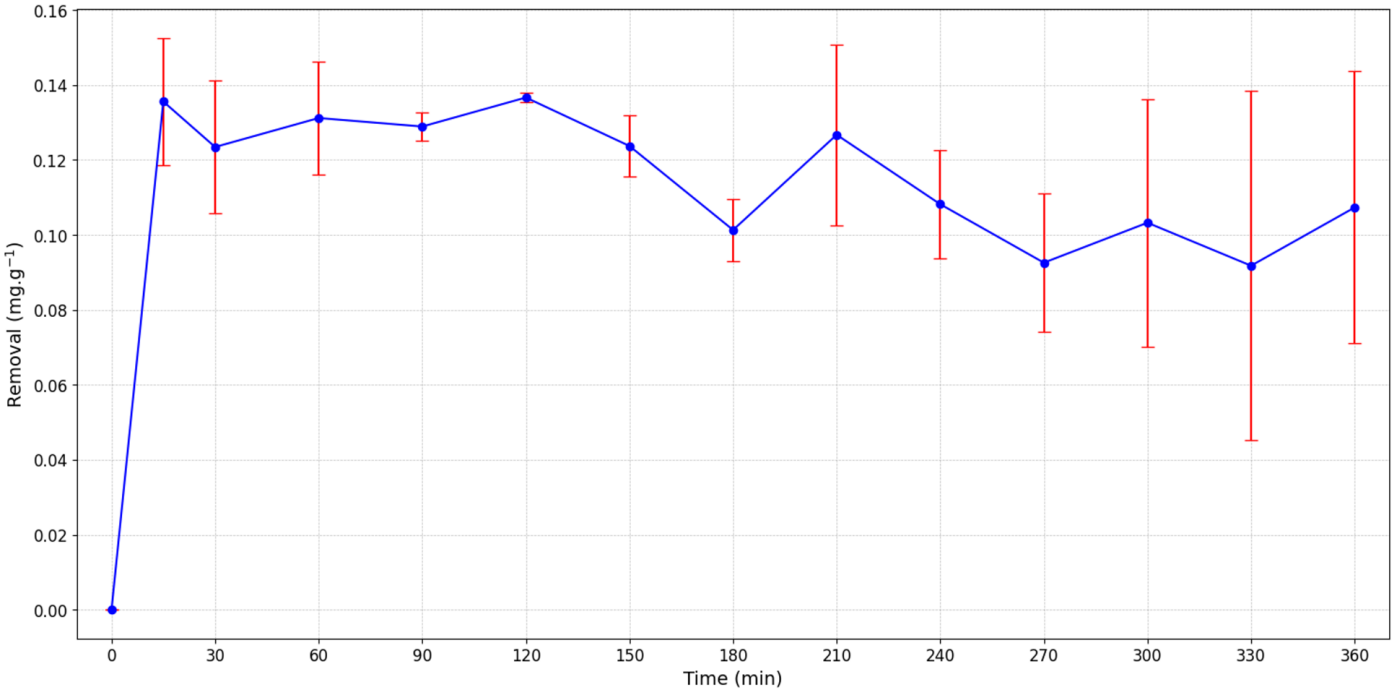

Figure 3 – Dye removal capacity over time.

These results indicate that, despite a decrease in the removal rate over time, dye removal continued to occur until the end of the experiment, suggesting that the substrate can be effective for dye removal over a prolonged period. However, it is believed that the contact time used could be reduced for the application of this process on a real scale. Therefore, it was decided to perform out the remaining additional tests with a contact time of two hours. The results corresponding to each of these tests are detailed in Table 4.

It is possible to observe that in test I, conducted under the same conditions that allowed for achieving the best results in the experimental planning tests, the removal efficiency obtained was equally satisfactory, reaching 67.0%. As previously discussed, it is believed that such removal is the result of the simultaneous occurrence of adsorption processes on the surface of the substrate and fungi, as well as enzymatic degradation (Sintakindi and Ankamwar, 2021).

In test V, operational conditions identical to those of test I were employed; however, the substrate used had not been colonized by fungi, which made the occurrence of enzymatic degradation and adsorption processes on the mushroom surface unfeasible. In this context, it is believed that the 40.1% efficiency achieved can be attributed to the adsorption process on the substrate surface, highlighting the relevance of this mechanism in dye removal. According to Chikri et al. (2020), sawdust has a high potential for treating textile effluents since this material contains numerous functional groups in its structure, which can be favorable for adsorbing a wide variety of dyes. Müller et al. (2019) evaluated the adsorption of methylene blue dye using two species of wood, and both demonstrated high efficiency. In the tests conducted by Shrestha (2021), in turn, adsorption with sawdust allowed achieving 98.4% dye removal. These results help explain the removal obtained in the present study, as sawdust is the main component of the substrate used.

It is also important to emphasize that in test II, conducted with the substrate colonized with fungi, but under anaerobic conditions, the removal efficiency achieved 54.8%, about 15% higher than in test V. Considering that the presence of oxygen is a prerequisite for the occurrence of enzymatic activity (Kaushik and Malik, 2009), it is believed that adsorption was also the predominant removal mechanism in this test. However, in addition to the substrate, the presence of fungal mycelia may have contributed to the expansion of the surface area and, consequently, to the increase in active sites, resulting in the improvement of the overall process efficiency.

The results obtained in test III, in turn, indicate that forced aeration did not result in improved dye removal, as the efficiency obtained under this condition (47.5%) was even lower than that observed in test II (54.8%), conducted in the absence of oxygen. A possible explanation for these results is that, although enzymatic activity may have been maximized due to the high availability of oxygen (Beltrán-Flores et al., 2023), the turbulence generated by the aerator bubbles may have impaired dye adsorption. This behavior occurs because the adsorption process is directly influenced by the turbulence of the medium, with very high speeds potentially favoring desorption, causing a reduction in efficiency (Kołodyńska et al., 2017; Tay et al., 2020).

It is noteworthy that the results obtained in test IV indicate that the substrate released substances that impart color to the effluent, which may have been mistakenly identified as dye according to the method used. Thus, the actual dye removal efficiency may have been even higher. Further studies are recommended to clarify this interference and identify the released substances.

From the analysis of the complementary test results, it is possible to ascertain that the predominant dye removal mechanism was adsorption, both on the substrate and in the structure of the fungus itself. In any case, the contribution of the enzymatic degradation process is not ruled out, as in test I, which allowed the occurrence of both processes, the highest efficiency among the tested conditions was obtained.

Table 4 – Residual dye concentrations and removal efficiencies obtained from the additional tests after two hours of contact.

Test	Description	Residual dye concentration (mg L^{-1})	Removal efficiency (%)
I	Best condition	13.8±1.2	67.0±1.0
II	Absence of O_2	18.9±2.1	54.8±5.1
III	With aeration	22.1±5.5	47.5±13.9
IV	Distilled water	11.0±2.1	-
V	Substrate without fungus	24.0±3.7	40.1±10.4

±: standard deviation.

Characterization of laccase

To evaluate the enzymatic activity of the residual substrate of *P. ostreatus*, the laccase activity was analyzed over a pH range of 2 to 10, using ABTS as the reaction substrate, as shown in Figure 4.

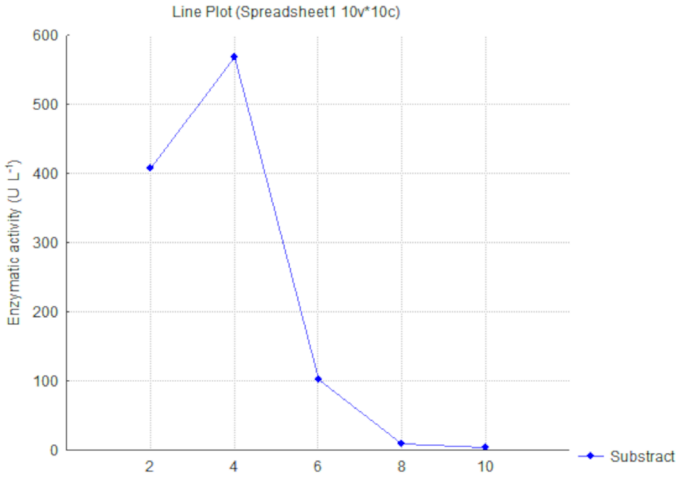

Figure 4 – Effect of pH on laccase activity.

The results of laccase activity demonstrated that its efficiency is higher under acidic pH conditions (2–6), and it becomes inactive under alkaline pH conditions (8–10). According to Alhujaily et al. (2020), pH intrinsically affects mycelial growth and enzymatic activity, which are favored under acidic conditions. Additionally, laccase activity can be altered by the conditions of the medium or by the characteristics of the enzyme itself (El-Batal et al., 2015). Given the potential of fungi to adapt to extreme conditions, an excess of hydroxide (OH-) or (hydrogen ion) H+ can interfere with the enzyme's characteristics, reducing its action (Semchenko et al., 2018).

In the present study, it was identified that the pH resulting in the maximum laccase activity was 4, which corroborates the results obtained in the tests to determine the best experimental conditions, as shown in Figure 1. As stated by Baldrian (2006), the ideal pH for laccase activity is directly linked to the substrate used. In the case of ABTS, the optimal pH ranges between 2 and 5. Studies conducted by Fernandes (2023) revealed that the point of maximum laccase enzymatic activity was recorded at pH 5.

Thus, it is believed that the high enzymatic activity observed may be related to the characteristics of the residual substrate, as this is the development environment of the fungus, characterized by an abundant presence of hyphae. These fungal hyphae act as a matrix that retains extracellular enzymes, resulting in a higher concentration of enzymes (Stamets, 2005). Additionally, the cultivation of basidiomycete fungi in solid environments also provides higher rates of enzymatic activity (Alexandrino et al., 2007). These results indicate the possibility of extracting a commercially high-cost enzyme with various applications from an agro-industrial residue, which can be better evaluated in future studies.

Conclusion

From the results of the experimental design, it was found that the variable pH, as well as its interaction with the substrate concentration, have a significant influence on dye removal. Among the levels evaluated, the reduction in pH and the increase in concentration resulted in improved process efficiency. Thus, the best result was obtained in the experimental condition at pH 5 and a substrate concentration of 150 g L^{-1}.

From the complementary tests, it was found that dye removal can be attributed essentially to the adsorption process, both on the substrate and in the fungi structure itself. However, the contribution of enzymatic degradation was not ruled out, as the condition that allowed this process to occur achieved higher removal efficiency than the others. Additionally, from the tests to determine enzymatic activity, high laccase activity was identified under acidic pH conditions, particularly at pH 4.

It was concluded that, due to the high efficiency of adsorption in dye removal, combined with the action of ligninolytic enzymes, the use of the residual substrate from the production of the mushroom P. ostreatus shows great potential for the treatment of textile effluents. Furthermore, it is noteworthy that this alternative can be considered a sustainable solution, as it allows for the utilization and valorization of an agro-industrial residue.

Authors' Contributions

Silva, A.T.R.: conceptualization, data curation, formal analysis, funding, acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing. **Ferreira**, A.T.: writing – review & editing. **Battistelli**, A.A.: conceptualization, data curation, formal analysis, funding, acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing. **Martins**, K.G.: data curation, formal analysis, investigation, methodology, resources, software, supervision, validation, visualization. **Souza**, C.M.V.: funding, acquisition, writing – review & editing. **Souza**, J.B.: conceptualization, data curation, formal analysis, funding, acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing.

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