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Effect of Spent Mushroom Substrate on Azo Dye Removal and Effluent Treatment

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HIGHLIGHTS

- SMS was applied to remove the mixture of two dyes in textile effluent.
- The best conditions of pH, temperature and SMS concentration were determined.
- The process resulted in color removal, but increased other parameters
- The treatment did not increase the toxicity.

Abstract: This study evaluated the removal mechanisms of azo dyes from synthetic textile wastewater using spent mushroom substrate (SMS). The simultaneous adsorption and enzymatic degradation were analyzed. The effect of pH, SMS concentration and temperature were studied. The maximum enzymatic degradation (16.36%) was obtained at pH 4 and 65 g SMS L^{-1}, whereas the maximum adsorption (62.86%) at pH 8 and 155 g SMS L^{-1}, both at 25°C. The simultaneous enzymatic degradation and adsorption led to the removal of 75.67%. with increased color change. The kinetic study showed that dye adsorption was obtained in the first 30 minutes while the enzymatic degradation increased slowly over the time. The treatment with SMS resulted in the removal of 28.75% of the true color and no increase of effluent toxicity.

Keywords: Spent mushroom substrate; Enzymatic degradation; Adsorption.

INTRODUCTION

The textile industry holds worldwide importance and stands out in the economic market. However, the production of textiles results in effluents discharges with important environmental impacts [1]. Azo dyes are one of the factors that can contribute to the toxicity of textile effluents, which requires greater attention in

wastewater treatment plants and efforts must be directed towards the removal of these compounds using viable and effective methods.

Textile dyes are synthetic and complex molecules, stable to light, and resistant to microbial attack, which makes their elimination difficult. In water, these compounds are visible even at low concentrations and reduce the solar radiation penetration, altering biological cycles and impairing the survival of aquatic organisms. In addition, textile dyes can cause acute and/or chronic effects on living beings such as lethality, genotoxicity, mutagenicity, and carcinogenicity, depending on the concentration and duration of exposure [2]. In the study by Arl and coauthors [3] the toxicity of azo pigments to the microcrustacean *Daphnia magna* and the cell line HaCaT was investigated. The authors associated the toxicity due to the presence of azo compounds in their compositions, which resulted in an overproduction of reactive oxygen species (ROS). These results show the importance of treating effluents containing these compounds.

Among the various physical and chemical methods for removal of textile dyes, such as coagulation, membrane filtration, and advanced oxidative processes, adsorption has advantages due to its ease of operation, non-use of chemicals, and low cost. In addition to being an environmentally appropriate destination, the use of agro-industrial waste as adsorbent materials contributes to the reduced costs [4]. In this sense, the residual substrate from the production of edible mushrooms (SMS) stands out.

The SMS consists of remaining biomass after the mushroom harvest, usually consisting of wood and agricultural residues such as bark and seeds, mixed with the fungal mycelium. An average farm generates about 24 tons of SMS per month, representing a problem for farmers [5]. Inadequate disposal of SMS in the environment can result in contamination of soil, and surface and groundwater due to the high concentration of organic matter and inorganic salts released by SMS [6]. Therefore, SMS applications with less environmental impact and greater appreciation of this waste must be developed.

The presence of functional groups on the surface and a porous structure constitutes SMS as a potential adsorbent for pollutants [7]. Some edible mushrooms are white rot basidiomycetes, for example those belonging to the *Pleurotus* genus, which release ligninolytic enzymes such as laccase, manganese peroxidase, and lignin peroxidase which are capable of degrading chemical structures such as those of textile dyes. As the industrial enzymes production is a time-consuming and expensive process, and the commercial enzymes are expensive for environmental applications, the use of readily available enzymes in residues is an attractive alternative [8].

The use of SMS for the removal of textile dyes by the combined adsorption and enzymatic degradation eliminates costs involved in the preparation of the adsorbent and costs with enzymatic formulation. In addition, natural sources of enzymes may contain natural mediators for laccase, contributing to the catalytic process of dye degradation by enzymes [9]. Several studies have already evaluated the capacity of SMS as an adsorbent [7,10,11] and the degradation efficiency of enzymes extracted from SMS [5,8,12]. However, detailed studies of the simultaneous performance of both processes in the removal of dyes in synthetic textile effluent when using SMS without any treatment are scarce in the literature.

This study proposes the removal of azo dyes from textile effluents using SMS, offering an economical and sustainable treatment for a recalcitrant pollutant. The adsorption and enzymatic degradation mechanisms were evaluated, and the treated effluent analyzed in terms of toxicological and water quality parameters.

MATERIAL AND METHODS

SMS source and characterization

The SMS of *Pleurotus ostreatus* production consisted of sawdust and wheat bran, supplemented with calcium carbonate. SMS collected after 114 days of inoculation and 75 days after the second induction was used, when the SMS was no longer useful for the producing company and became a waste. The SMS was kept at 5ºC until its use. Prior to the experiments, the SMS blocks were unstructured and homogenized manually.

The morphology of the SMS was evaluated by scanning electron microscopy (SEM) with a JEOLJSM-6390LV microscope equipped with energy dispersive spectroscopy (EDS).

Tests for the removal of textile dyes

The synthetic textile wastewater composition was based on Mo and coauthors [13], consisting of two anionic azo dyes (Levafix Brilliant Red E4BA and Remazol Black B 133%), and auxiliary chemicals (Polyvinyl alcohol, NaCl, and Na_2SO_4) in order to simulate real textile wastewater. Their composition is presented in Table 1.

Table 1. Composition of synthetic textile wastewater.

Chemical product	Concentration (mg L^{-1})
Levafix Brilliant Red E4BA	25
Remazol Black B 133%	25
Polyvinyl alcohol	125
Sodium chloride (NaCl)	250
Sodium sulphate (Na_2SO_4)	200

The tests for the removal were carried out in a thermostatic bath (Dubnoff brand, model 252) with 150 mL of textile effluent in Erlenmeyers (250 mL) under agitation at 200 rpm. Different values of pH (4, 6, 8 and 10), temperature (25, 35, 45, and 55°C) and SMS concentration (65, 95, 125, and 155 g L^{-1}) were used to determine the best conditions for the removal of textile dyes. pH values were adjusted with 0.5 M HCl and 0.5 M NaOH. Kinetic analysis was performed with sample collection after 0, 15, 30, 60, 120, 180, and 240 minutes. Samples containing only the effluent and only the SMS in distilled water were considered controls.

To evaluate the individual contribution of adsorption and enzymatic degradation in the removal of dyes, SMS with and without enzymes (after enzymatic inactivation) was used. The SMS was pre-treated at 90ºC for 15 minutes for denaturation of the enzyme and loss of activity. Enzymatic degradation was estimated by the difference between total removal percentage and that removal obtained by SMS after enzymatic inactivation.

Dyes concentration and laccase activity were measured using centrifuged (11,000 rpm, 5 min) samples. The concentration was determined using a spectrophotometer (Hach, DR 3600), at 549 nm.

The experiments were conducted in replicates, and the results presented as mean values. Statistical analysis was performed the analysis of variance ANOVA and the means compared to the Tukey test with a 5% probability. The normality of the data was confirmed by the Anderson-Darling test. These analyses were carried out using Origin® 2017 software.

The soluble and total chemical oxygen demand (COD_s, COD_t), ammonia nitrogen (NH_4-N), total phosphorus (P_t), true color, apparent color, turbidity, and pH were evaluated as wastewater quality parameters [14]. These parameters were analyzed under the most favorable conditions obtained in previous experiments.

Laccase activity assay

The enzymatic activity of the enzyme laccase was determined by increasing the absorbance with the oxidation of the 2,2-azinobis (3-ethylbenzathiazoline-6-sulfonic acid) (ABTS) substrate. The reaction mixture contained 4 ml of ABTS (1.8 mM), 0.5 ml sodium acetate buffer (0.1 M; pH 5), and 0.5 ml of the sample. The reaction mixture was kept at 40°C for 5 minutes in a water bath, and the resulting absorbance was spectrophotometrically measured at 420 nm (Hach, DR 3600) [15]. Two control samples were prepared, one replacing the sample with the buffer and the other replacing the ABTS with the buffer. Enzymatic activity was expressed in International Units (U), with one unit of enzyme activity defined as the amount of enzyme needed to oxidize 1 µmol of the substrate per minute.

Toxicological analysis

The toxicological tests were carried out at the Environmental Toxicology Laboratory (LABTOX) at UFSC. For toxicological analyses, the freshwater microcrustacean *Daphnia magna* was selected. This organism was chosen due to its ease of culture and testing, its availability, vast literature and because there is local legislation that indicates its use as a model for toxicological evaluation of effluents. The culture of organisms occurred in accordance with ISO 6341 [16] and NBR 12713 [17] standards. The daphnids were maintained in 2 L beakers with culture medium M4 and a density of one adult organism per 50 mL of medium, temperature at 20 ± 2ºC and photoperiod of 16 h of light/8 h of dark. The medium was changed three times a week and the fed was performed with the green alga *Scenedesmus subspicatus* (approximately 10^6 cells mL^{-1} per organism) [18]. For the acute toxicity assays, the raw effluent, and that treated with SMS under the best conditions for two hours was evaluated. The toxicity of the distilled water kept in contact with the SMS during the test also was assessed to verify the possible release of toxic compounds by SMS. A total of 20 *D. magna* neonates with 2 to 26 hours of life were exposed to different dilution factors (1, 2, 4, and 8) from the same sample, at a temperature of 20 ± 2°C for 48 hours. The negative control of the test was performed by exposing the daphnids to medium at the same conditions [16,17]. The tests were performed in duplicate and after 48 hours the number of immobile organisms was observed. The results were expressed as $EC_{50,48h}$ using

Trimmed Spearman−Karber method for statistical analysis and as Dilution Factor (DF), that is, the highest dilution in which no toxic effect was observed after 48 hours.

RESULTS AND DISCUSSION

SMS characterization

The SMS images with and without enzymes (after enzyme inactivation) obtained by SEM are shown in Figure 1. from which it is possible to verify the morphological structure of the SMS and the presence of fungal mycelium. The SMS has an irregular and porous surface, formed by the growth of the fungus that uses the substrate as a source of carbon and energy. In comparisons made between the SMS with, and without enzymes, no morphological changes were observed due to heating to deactivate the enzymes.

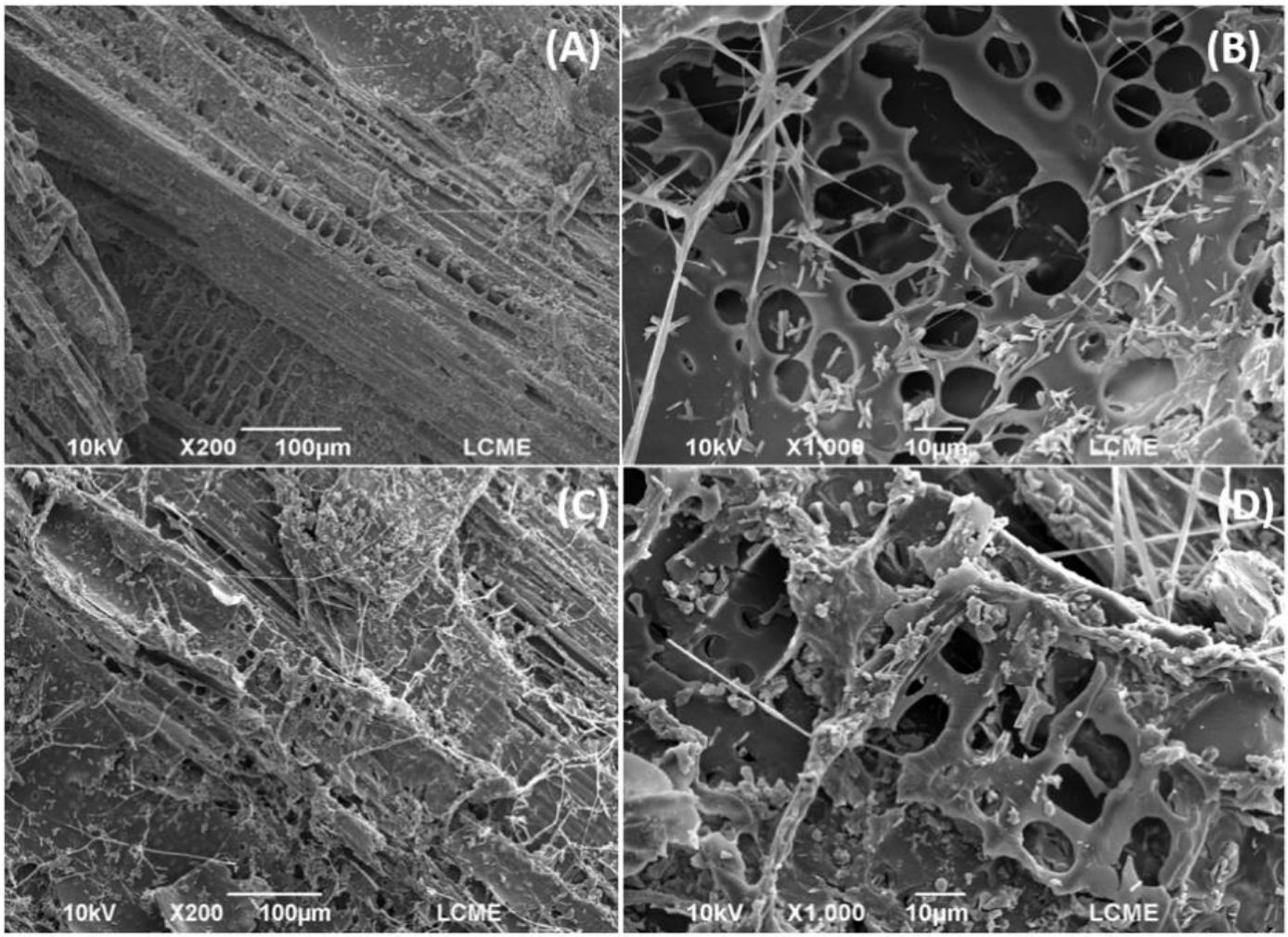

Figure 1. SEM of the SMS without enzymes after heating treatment, 200x (A) and 1000x (B) magnification. SMS with enzymes and no heating treatment, 200x (C), 1000x (D).

The results obtained from EDS of SMS is shown in Table 2, where carbon (C), oxygen (O), and calcium (Ca) where the predominant elements. The presence of Ca in the SMS can be attributed to the calcium carbonate added to the substrate to neutralize the acidity. Alhujaily and coauthors [10] also found that C, O, and Ca are the main elements in SMS.

Table 2. EDS analysis of the SMS with and without enzymes.

Element	Point	Mass percentage (%)	
		SMS without enzymes	SMS with enzymes
C	1	78.92	83.96
	2	82.47	87.44
O	1	8.36	6.14
	2	6.52	5.99
Ca	1	12.71	9.26
	2	11.00	6.12
Mg	1	-	0.64
	2	-	0.45

SMS is rich in lignocellulose content that could contain (%) up to 48.7 cellulose, 34 hemicellulose, and 39.8 lignin, depending on the source of the mushroom cultivation medium [19]. The SMS has its structure affected by the lignocellulolytic enzymes produced during the fungi (*Pleurotus ostreatus*) growth. Cellulases, hemicellulases and laccases degrade the lignocellulosic structure of the wheat bran and sawdust contained in the SMS to access the monomeric substrates.

The effect of pH on the adsorption and enzymatic degradation of textile dyes using the SMS was evaluated at 65 g SMS L^{-1}, 25°C, during 120 mintures, and the results are shown in Figure 2. The total dyes removal ranged from 43.79 to 52.24% at pH 4 and 8, respectively. The highest dye removal due to enzymatic degradation was 16.36% at pH 4 and decreased with an increasing pH, although there was no significant difference; while the laccase activity did not show much variation, except in pH 6.0 (increasing to 20 U L^{-1}). Unlike degradation, adsorption was favored at alkaline pH and reached a maximum removal of 43.60% at pH 8, with no significant difference from pH 6 to 10.

Corroborating these results, the enzymes extracted from the SMS of *Pleurotus sajor caju* (LiP as the main enzyme) showed optimum pH to be in the range of 4 and 4.5 for degradation of other dyes [5], and for the laccase of *Trametes trogiies,* pH 4 was ideal for the degradation of reactive black dye 5 [20]. For Levafix Brilliant Red E4BA, a crude enzymatic preparation containing laccase of *Pleurotus sajor caju* (30 U L^{-1}) was not able to degrade it at any of the evaluated pH values (2.4 to 5.0) [21].

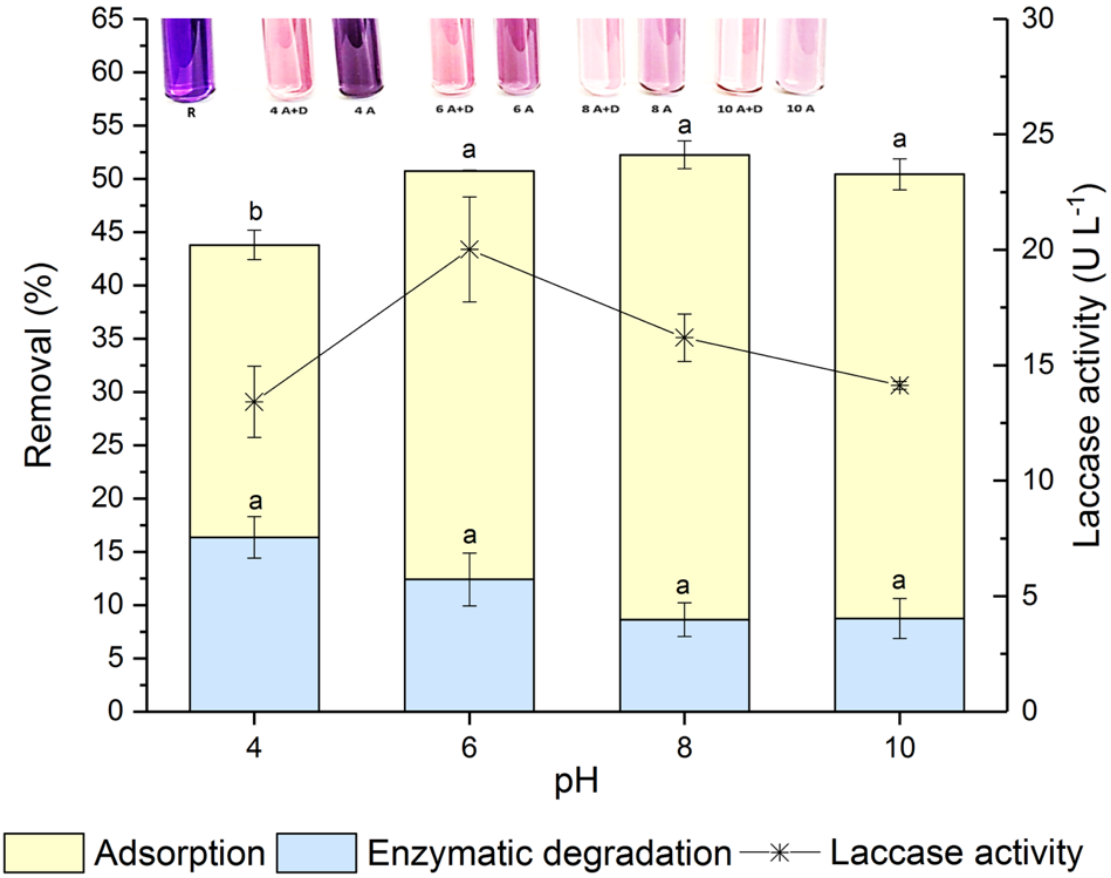

Figure 2. Effect of pH on adsorption and enzymatic degradation of textile dyes using SMS. Insert: image of the raw effluent (R), treated after adsorption (A), and enzymatic degradation (D) at different pH values.

According to Schallemberger and coauthors [22], the zero charge point of SMS is 5.5, indicating that below 5.5 the SMS has positive charge and above 5.5 has negative charge, resulting in better adsorption for cationic species. The main difference in dye removal was observed changing pH 4 to 6, as presented in Figure 2. This behaviour is related to the presence of protons (H^+) and hydroxyl (OH^-) depending on the pH. The unexpected increase in adsorption with pH above 5.5 indicates that other adsorption mechanisms are involved besides the electrostatic interaction.

However, Alhujaily and coauthors [23] and Toptas and coauthors [24] found that the adsorption of anionic dyes by SMS was favored at acidic pH. The adsorption of anionic dyes by SMS modified by cationic surfactant increased from pH 3 to 5 and decreased slightly at pH 7 to 10 [10]. In the present study, the adsorption of dyes by the SMS increased from 27.43 to 43.60% with an increase in pH from 4 to 8 and reduced to 41.69% at pH 10.

Figure 3 shows the effect of temperature on the adsorption and enzymatic degradation of the dyes at pH 8, 65 g SMS L^{-1}, during 120 minutes. For both adsorption and enzymatic degradation, the increase in temperature promoted a reduction in the removal percentage. The adsorption increased significantly from 29.90% to 43.60% at temperatures of 55°C and 25°C, respectively. Enzymatic degradation decreased significantly from 8.63% at 25°C to zero at 45°C, while the laccase activity showed little variation and remained between 13.32 and 19.25 U L^{-1}.

It is noteworthy that enzymatic degradation and adsorption also took place in higher temperatures. This is of special interest when working with industrial textile wastewater with higher temperatures due to the industrial process.

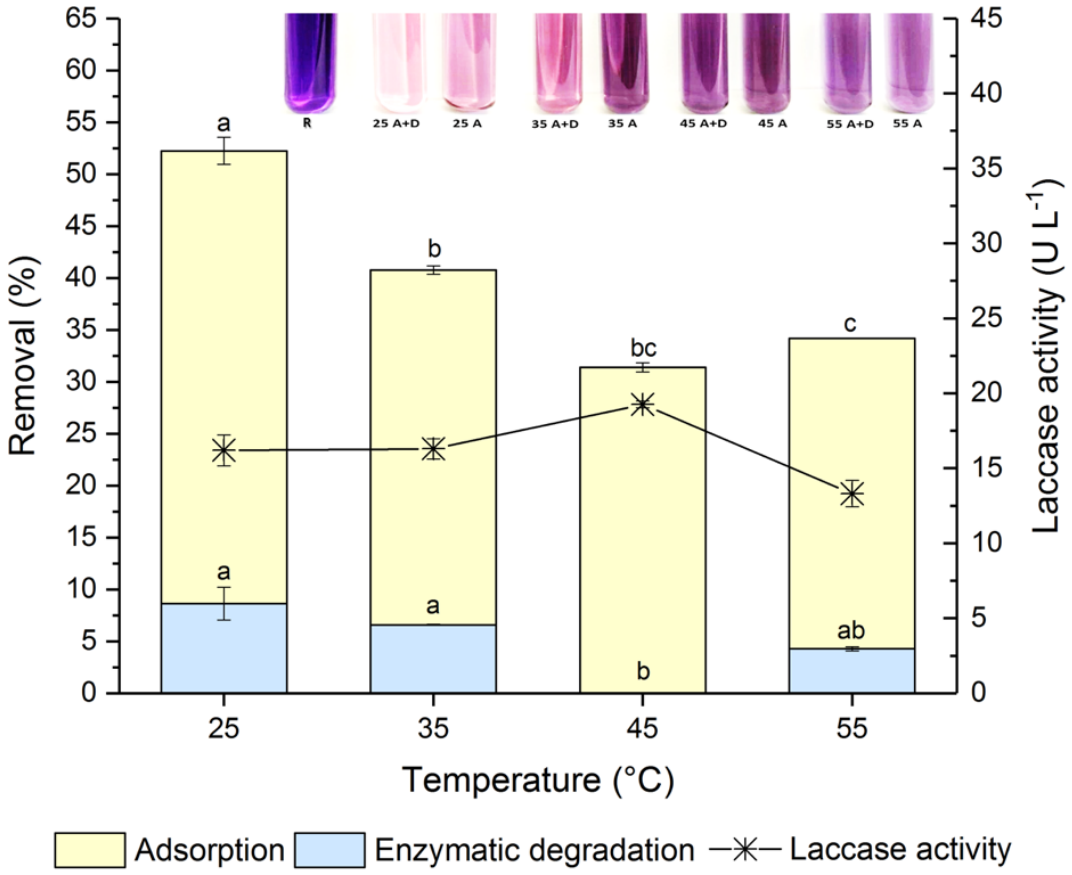

Figure 3. Effect of temperature on adsorption and enzymatic degradation of textile dyes using SMS. Insert: image of the raw effluent (R), that treated after adsorption (A), and enzymatic degradation (D) at different temperatures.

The ideal temperature for the degradation of different dyes by enzymes extracted from the SMS [5] and for the laccase enzyme broth of *Pleurotus sajor caju* was in the range of 30 to 35°C [21]. These authors attributed the low removal percentage at temperatures above 35°C to the thermal denaturation of the enzymes. In contrast, higher percentages of enzymatic degradation of dyes were obtained at high temperatures by the crude laccase of *Trametes trogii* at 60°C [25] and the purified laccase of *Armillaria* sp. F022 (0.5 U mL^{-1}) at 40°C [26].

Regarding adsorption, it is known that the increase in temperature promotes an increase in the diffusion rate of the dye molecules that go beyond the outer boundary layer and reach the internal pores of the adsorbent, allowing a greater number of molecules to be adsorbed. However, the adsorption of dyes by the SMS was not favored by the increase in temperature, which may indicate an exothermic nature of the adsorption process [24]. The decrease in the adsorption percentage with increasing temperature is attributed to the weakening of the adsorptive forces between the dye molecules and the adsorbent. This is in addition to the desorption process that can result in the clogging of the SMS pores by the formation of a dye layer on the adsorbent surface [25,26].

The temperatures used in the adsorption tests are not enough to change the SMS structure. High temperatures like 120ºC are frequently used for pretreatment purposes aiming the release or reducing sugars. However, the maximum temperature used in the adsorption experiments was 45ºC. Wu and coauthors [29] showed that the reducing sugars concentrations was not significantly affected (12-18 g/kgSMS) when using SMS pretreatment with hot water and temperatures of 50, 100 and 121ºC during 1h. It is noteworthy that no significant difference was observed between 35ºC and 45ºC, as well as 45ºC and 55ºC, according to the statistical results expressed in the Figure 3. The heat treatment at 90ºC for 15 minutes was used just to those tests to inactivate the enzymes, to observe the isolates adsorption.

When evaluating the potential of SMS as a dye adsorbent, Wu and coauthors [7,27] verified that the increase in temperature caused a reduction in the removal percentage, obtaining maximum efficiency at 15°C. In contrast, a modified SMS promoted greater adsorption at higher temperatures, however this increase was only 5% from 20 to 50°C for reactive black dye 5 [10].

The effect of SMS concentration was evaluated under the optimal conditions for total removal (pH 8 and 25°C) during 120 minutes, and the results are shown in Figure 4.

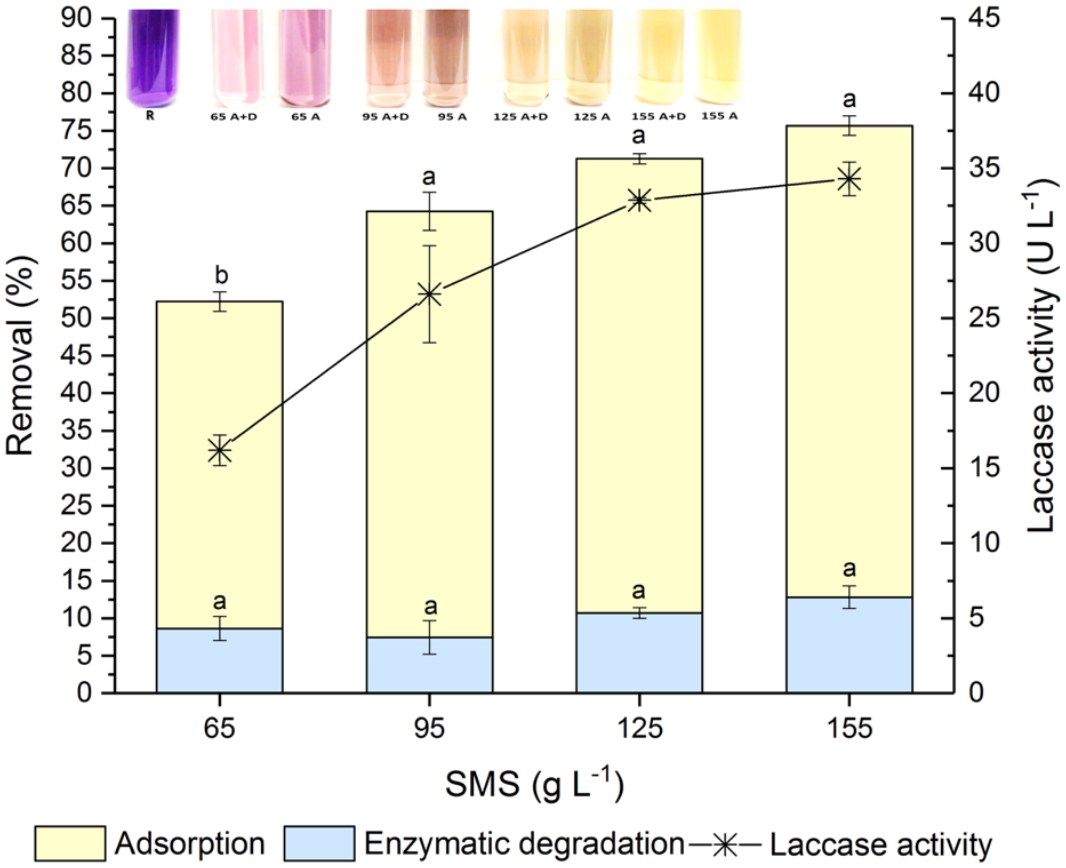

Figure 4. Effect of SMS concentrations on adsorption and enzymatic degradation of textile dyes. Visual comparison of the removal of textile dyes with different concentrations of SMS. Insert: image of the raw effluent (R), that treated after adsorption (A), and enzymatic degradation (D) at different SMS concentrations.

Higher concentrations of SMS resulted increased adsorption from 43.60% in 65 g L^{-1} to 62.86% in 155 g L^{-1}, however there was no significant difference in concentrations from 95 to 155 g L^{-1}, indicating a saturation condition. Although the enzymatic degradation increased from 8.63% to 12.81% in 65 to 155 g L^{-1}, this difference was not significant. Corroborating the degradation results, the laccase activity varied from 16.20 to 34.29 U L^{-1} in 65 to 155 g L^{-1}, respectively.

For different direct and reactive dyes, adsorption increased at higher concentrations of the SMS adsorbent. For reactive black 5, the removal rates were 44% and 100% with the dose increase from 8 to 30 g dry SMS L^{-1}, respectively [24]. A maximum adsorption rate of approximately 60% of the reactive dye Levafix Braun E-RN was obtained with 2 g dry SMS L^{-1} [25]. For comparison with the present study, the SMS concentrations in dry weight were 27.3 (65 g L^{-1}) to 65 g SMS L^{-1} (155 g L^{-1}).

These cited studies obtained higher removal percentages even with lower concentrations of SMS, which can be attributed to the fact that SMS has been washed and dried, which facilitates the contact of the dye with the active sites of the adsorbent. In addition, the SMS was crushed and used in smaller sizes, which results in a larger surface area, requiring a smaller amount of adsorbent for high removal percentages. However, the objective of this study is to use SMS without any type of treatment to preserve and take advantage of the enzymatic activity of the residue and facilitate its application on a full scale, making it possible to obtain a total removal percentage of 75.67% with 65 g of dry SMS L^{-1}.

Figure 5 shows the kinetics at pH 4 (A) and 8 (B) with 125 g SMS L^{-1} and 25°C for 240 minutes, respectively. At pH 8, the initial dye concentration was 51.66 to 18.64 mg L^{-1} in 240 minutes, however at this pH no enzymatic degradation was found. At pH 4, both processes contributed to the removal of dyes, obtaining a final dye concentration of 12.47 mg L^{-1}. Considering the separate processes, the final concentration for adsorption was 17.91 mg L^{-1}, and for enzymatic degradation, 46.22 mg L^{-1}. The maximum activity of the laccase was 37.15 U L^{-1} at pH 8 and 90.25 U L^{-1} at pH 4.0, both after 240 minutes. While the highest dye removal by adsorption occurred in the first 30 minutes, enzymatic degradation increased slowly over the time of contact.

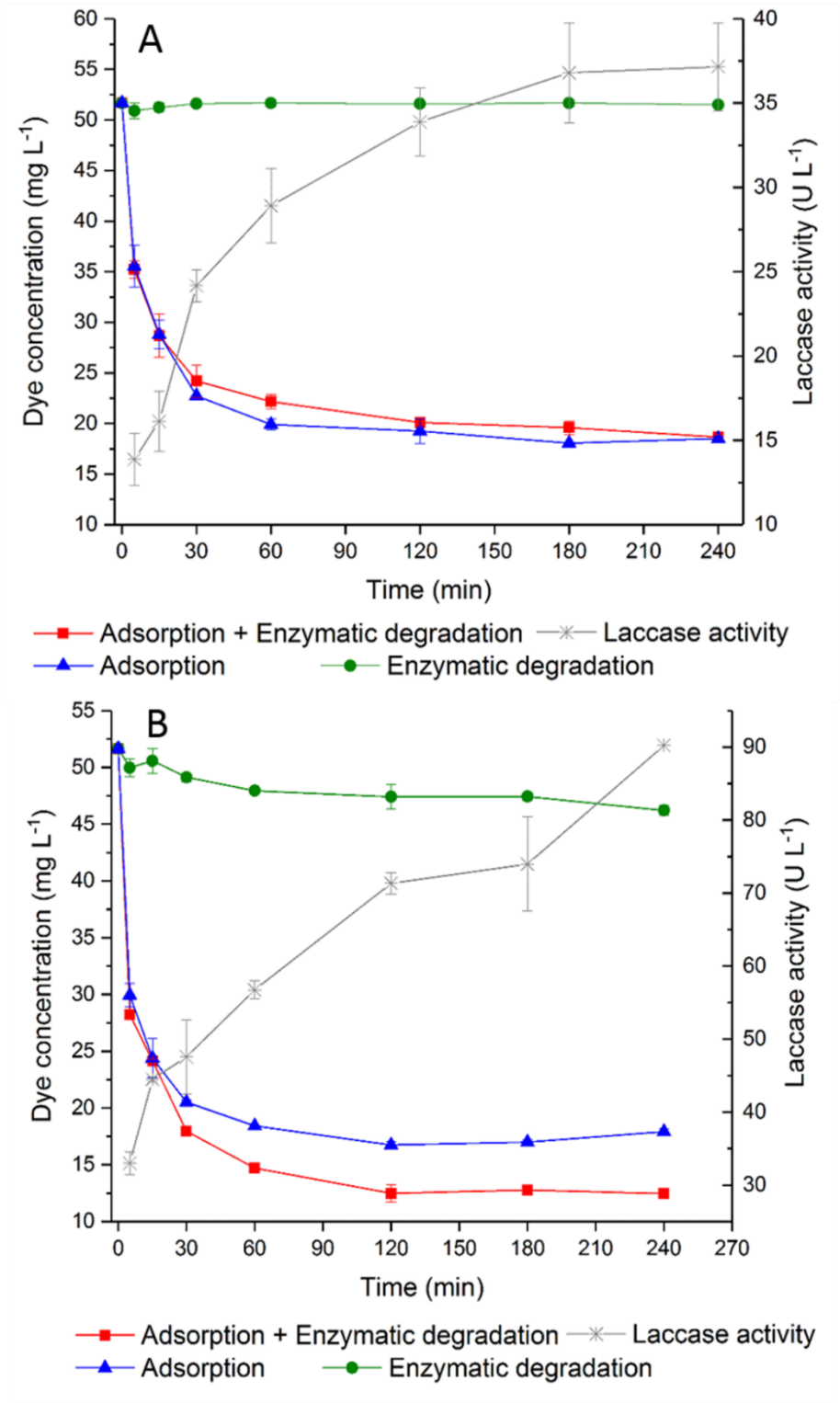

Figure 5. Kinetics for dye removal through adsorption and enzymatic degradation at pH 8 (A) and pH 4 (B).

Table 4 presents the results of the acute toxicity tests with *D. magna* for the raw wastewater, treated wastewater, and distilled water (control) after two hours of contact with 125 g SMS L^{-1}. The decolorization tests were carried out with the pH of the raw wastewater adjusted to 4.0, in order to consider the performance of the enzymatic activity in the degradation of the dyes. Thus, the raw and treated wastewater samples showed pH values below the interval of 5 to 9 established by the standards for toxicity tests with *D. magna*. Also, it is worth to mention that the Brazilian legislation for the release of effluents indicates that they can only be released into the receiving body if their pH values are within the range of 5 to 9 [34]. To ensure the analysis in the required release condition and to avoid interferences in the toxicity, parallel tests were performed with the pH adjusted to 7 ± 0.2 with 1 M NaOH.

Effluent Analysis

The effect of using SMS on textile wastewater treatment parameters was measured, considering the possible release of compounds to the liquid medium. Table 3 presents the results for the quality parameters of the raw and treated wastewater, at pH 4.0, 125 g L^{-1} of SMS, 25°C, and contact time of 120 minutes.

In this test, the total removal of textile dyes was 65.78% and the laccase activity was 39.79 U L^{-1}. The treatment with the SMS promoted an increase in COD, apparent color, P_t, turbidity, and pH of the wastewater. The increase in COD in the treated wastewater is attributed to the high content of organic matter in the SMS.

The higher apparent color of the treated wastewater is in accordance with turbidity values that also increased after the treatment. However, the true color of the treated wastewater reduced by 28.75%, which is associated with the adsorption and enzymatic degradation of the dyes. The dissolved and suspended particles released by the SMS resulted in a yellowish and cloudy color of the wastewater, which explains the high turbidity and true color. Also, lower COD_S for the treated effluent reveals that particulate matter from SMS had strong influence in the experiment. The increased pH is probably related to the calcium carbonate in the SMS.

The NH_4-N concentration in the raw wastewater was below the measurement range (< 0.4 mg L^{-1} NH_4-N). In relation to Pt, the SMS contributed 82 mg L^{-1} to the wastewater. This result may be related to the presence of these nutrients in the SMS, as found by other authors [33–35].

In view of these results, the removal of azo dyes should consider other phases of the treatment of textile wastewaters. The organic load released by SMS is easily removed by conventional biological processes and the removal of dyes prior to this phase mitigates the impact on the biological community. Removal of nutrients is also a necessary step after treatment with SMS and is a consolidated process in most treatment plants. The study and development of new ways of using SMS that preserve the enzymatic activity and reduce the release of compounds are also necessary, such as column or immobilization techniques.

Table 3. Quality analysis of the raw wastewater and treated with the SMS.

Parameter	Raw effluent	Treated effluent	Removal (%)
COD_S (mg L^{-1})	-	4308± 140	-
CODt (mg L^{-1})	251 ± 0	4605 ± 125	-
NH_4-N (mg L^{-1} NH_4-N)	-	ND	-
Apparent Color (mg L^{-1} PtCo)	1215 ± 25	1900 ± 30	-
True Color (mg L^{-1} PtCo)	1165 ± 5	830 ± 10	28.75 ± 0.55
P_t (mg L^{-1} PO_4^{3-})	0	82	-
Turbidity (NTU)	0.56	378 ± 21	-
pH	4.04	5.18 ± 0.035	-
Dye (mg L^{-1})	50.01	20.66 ± 0.94	65.78 ± 0.95
Laccase activity (U L^{-1})	0	39.79 ± 6.84	-

ND: Not detectable under the detection limits; CODS: soluble COD; CODT: total COD.

Table 4 presents the results of the acute toxicity tests with *D. magna* for the raw wastewater, treated wastewater, and distilled water (control) after two hours of contact with 125 g SMS L^{-1}. The decolorization tests were carried out with the pH of the raw wastewater adjusted to 4.0, in order to consider the performance of the enzymatic activity in the degradation of the dyes. Thus, the raw and treated wastewater samples showed pH values below the interval of 5 to 9 established by the standards for toxicity tests with *D. magna*. Also, it is worth to mention that the Brazilian legislation for the release of effluents indicates that they can only be released into the receiving body if their pH values are within the range of 5 to 9 [34]. To ensure the analysis in the required release condition and to avoid interferences in the toxicity, parallel tests were performed with the pH adjusted to 7 ± 0.2 with 1 M NaOH.

Table 4. Results of toxicological tests with *D. magna*.

Sample	pH	$EC_{50,48h}$ (%)	DF	pH	$EC_{50,48h}$ (%)	DF	Average immobilization in 100% of sample (% ± SD)
Raw effluent	7.0 ± 0.2	NT	1	4.05	NC	2	23 ± 11
Treated effluent	7.0 ± 0.2	NT	1	4.71	NC	2	20 ± 07
Control	7.0 ± 0.2	NT	1	4.95	NT	1	0

NT: Non-toxic; DF: Dilution factor; EC: Effective concentration; NC: Not calculable SD: Standard deviation.

In the tests with pH correction there was no immobilization of the organisms even at the concentration equivalent to 100% of the samples, both for the raw and treated wastewater and for the control sample. However, without pH correction, an average immobilization of 23±11% in 100% of the sample after 48 hours with the raw effluent and 20±07% with the treated wastewater. As in the sample with pH corrected to 7.0±0.2, the control sample without pH correction did not cause the immobilization of the organism, inferring that SMS does not release toxic compounds for *D. magna*.

Considering that the immobilization of the organisms in the tests without pH correction was detected only in the textile wastewaters, it is inferred that at pH values of 4.05 and 4.71, the toxic effect were observed due to the low pH values and, at pH 4.95, no toxicity was detected since the pH value is close to the minimum limit for *D. magna*. Within the required pH range for the release of effluents, the raw sample did not show toxicity toward *D. magna* and the treatment with SMS did not increase the toxicity of the textile effluent. For the samples without pH correction, the treatment with SMS showed a downward trend when comparing the average immobilization in 100% of sample. This can be attributed to the higher pH value found after treatment. In addition, the results presented in Table 4 are in accordance with the regional standards for ecotoxicological tests [35] where textile effluent disposal is limited by the dilution factor value of 2.

CONCLUSION

Removal of textile dyes by simultaneous adsorption and enzymatic degradation processes was satisfactory. Adsorption is the dominant removal mechanism, but enzymatic degradation supports the azo dye removal and degradation. The treated textile effluent did not demonstrate acute toxicity toward *D. magna*, although new methods of SMS application must be developed to reduce the release of compounds that modify effluent quality parameters. This treatment is an attractive alternative due to the low cost, and the reuse of this residue. Although the disposal of SMS after adsorption limits its use, the enzymatic activity may reduce the harmfulness of the residual SMS. An integrated approach is necessary for the practical application of residues such as SMS in environmental applications, to prevent the production and release of new pollutants to the environment.

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Conflicts of Interest: The authors declare no conflict of interest.

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