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ORIGINAL PAPER

Decolorization and removal of toxicity of textile azo dyes using fungal biomass pelletized

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Abstract

Industrialization and other human impacts have placed increasing pressure on aquatic environments, with the generation of large quantities of toxic aqueous effluents containing different substances, such as synthetic dyes and other organic pollutants. It is estimated that between 10 and 15% of all dyes used in textile processes and other industries are discharged into wastewater, causing extensive aquatic pollution. Biological methods have been employed for the removal of color and toxicity from effluents containing azo dyes. Therefore, biosorption tests were performed with the dyes Acid Blue 161 e Procion Red MX-5B in simple and binary solutions, whereas biodegradation treatment was performed with the dyes only in binary solution. For biosorption, the dyes were removed by the fungi *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus oligosporus*. The fungal biomass demonstrated good adsorption capacity to these compounds. The elimination of the toxicity of the solution after biosorption demonstrated the effectiveness of the treatment. Intense molecular changes after biodegradation treatment with the *A. terreus* fungus were demonstrated by the FTIR analysis. However, toxicity tests with *Lactuca sativa* seeds and *Artemia salina* nauplii indicated the presence of highly toxic metabolites in the reaction medium at the end of the treatment. Based on the findings, biosorption is more suitable for this type of treatment, since it was also capable of removing the molecules from the medium, with the advantage of impeding the formation of highly toxic by-products.

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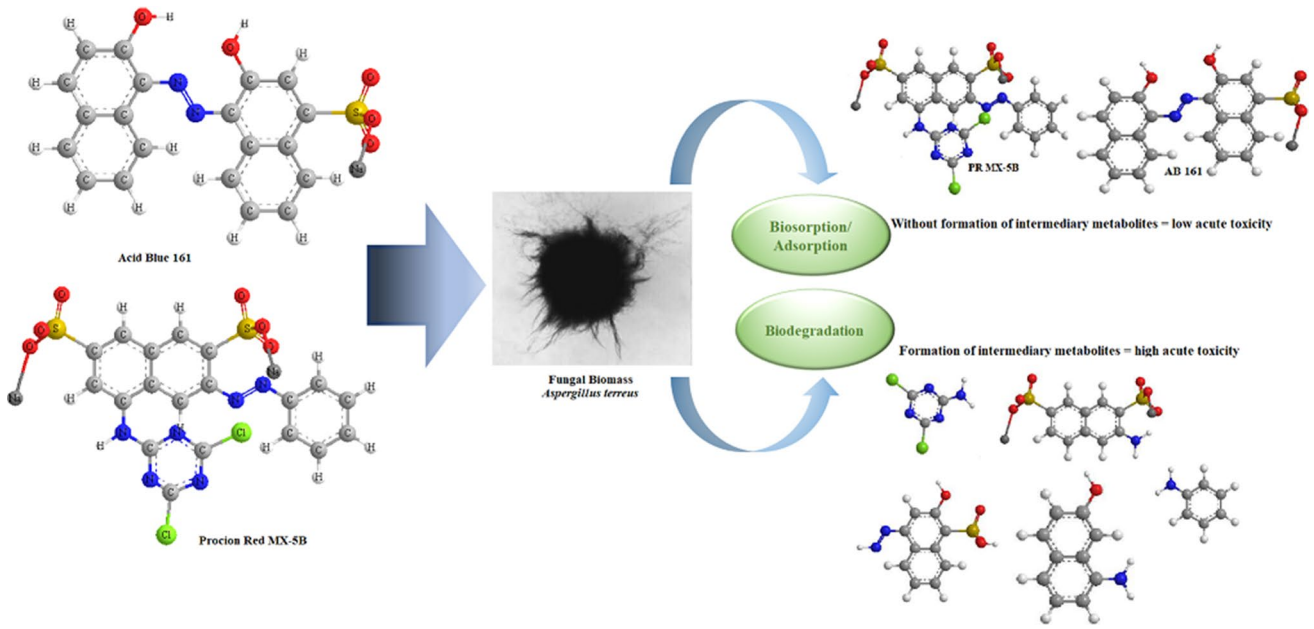
Graphical abstract



Keywords *Artemia salina* · Biological treatments · Filamentous fungi · *Lactuca sativa* · Synthetic dyes · Toxicity tests

Introduction

Industrialization and other human impacts have placed increasing pressure on aquatic environments, with the generation of large quantities of toxic aqueous effluents containing different substances, such as synthetic dyes and other organic pollutants (Fomina and Gadd 2014). The textile industry is the major consumer of synthetic dyes, which are an essential element in the dyeing process of fabrics. The main challenge facing this industry is the indiscriminate use of such compounds and the inefficiency of the dyeing process (Mahmoud et al. 2017). Indeed, textile manufacturers are among the largest consumers of water in industrial facilities, producing 50–100 L of wastewater per kg of finished product (Arslan-Alaton et al. 2008). Moreover, it is estimated that between 10 and 15% of all dyes used in textile processes and other industries are discharged into wastewater, causing extensive aquatic pollution (Edison et al. 2016).

About 100,000 different types of synthetic dyes and pigments are used on an industrial scale and approximately 700,000 tons are consumed per year. The most important group is azo dyes, which are characterized by the presence of one, two or three azo bonds (R_1–N=N–R_2). These dyes account for 60–70% of all synthetic dyes produced worldwide (Konicki et al. 2017; Wang et al. 2017a, b) and constitute a potential hazard to aquatic environments and human health. When improperly discarded into aquatic ecosystems, effluents containing these substances can cause serious disturbances, such as a reduction in the water solubility of oxygen and adverse effects due to the toxicity, mutagenicity and carcinogenicity of their molecules and/or their intermediates (Mittal et al. 2013; Chequer et al. 2015; Song et al. 2017). Considering an important class of environmental pollutants, azo dyes are resistant to many types of treatment, difficult to mineralize and persistent in aquatic environments due to their bio-recalcitrance.

Physical, chemical and biological methods have been employed for the removal of color and toxicity from effluents containing azo dyes, such as coagulation–flocculation (Lau et al. 2014), advanced oxidation processes (Abdi et al. 2017), biodegradation (Song et al. 2017) and adsorption/biosorption (Guerrero-Coronilla et al. 2015). With biological treatments, the mechanism of decolorization occurs through biosorption, enzymatic degradation (biodegradation) or a combination of both.

The advantage of biosorption/adsorption is that the process occurs without the formation of metabolites. Biological materials, such as chitin, chitosan, peat, yeast and fungi biomass, are used as chelating and complexing sorbents to concentrate and remove dyes from a solution. These biosorbents and their derivatives contain a variety of functional groups that can form complexes with dyes through physicochemical interactions. However, the efficiency and

selectivity of adsorption by biomass is mainly due to ion exchange mechanisms (Crini et al. 2006).

The biodegradation process occurs with the formation of intermediates (metabolites), which are often more toxic than the parent dye. Therefore, it is important for any bioremediation technology to assess the toxicity of the pollutants and metabolites formed after dye degradation to determine the efficiency of the method (Sen et al. 2016).

The aim of the present study, performed in the Bioscience Institute, Biochemistry and Microbiology Department, São Paulo State University, Rio Claro, São Paulo (2012–2013) was to analyze the use of adsorption and biodegradation methods for the treatment of the azo dyes Procion Red MX-5B and Acid Blue 161 in aqueous solutions employing pelletized biomasses of the filamentous fungi *Aspergillus niger*, *Aspergillus terreus* and *Rhizopus oligosporus*. Acute toxicity of the solutions before and after treatments was analyzed using *Lactuca sativa* seeds and *Artemia salina* nauplii.

Materials and methods

Azo dyes

The azo dyes Acid Blue 161 (AB 161) and Procion Red MX-5B (PR MX-5B) were analyzed in simple and binary solutions (Fig. 1). The main characteristics of these dyes are:

AB 161 (EC Number: 235-628-6) was acquired from the Sigma-Aldrich Inc. (São Paulo, Brazil) and has the following characteristics: molecular weight: 394.40; water soluble; $\lambda_{max} = 602$ nm; and dye content: ~45%.

And the PR MX-5B (EC Number: 241-776-2) was acquired from I.C.I. Brazil Inc. (Rio Claro, São Paulo, Brazil) and has the following characteristics: molecular weight: 615.33; water soluble; $\lambda_{max} = 538$ nm; and dye content: ~40%.

Microorganisms

Decolorization tests were performed with the filamentous fungi *A. niger* (CCT 1435), *A. terreus* (CCT 2679) and *R. oligosporus* (CCT 3762) obtained from the culture collection of the André Tosello Foundation for Research and Technology. The fungi were used in their paramorphogenic physical form (Marcanti-Contato et al. 1997).

Toxicity tests

Toxicity tests were performed with the brine shrimp *A. salina* and *L. sativa* seeds. The test with *A. salina* was based on the mortality of nauplii after 48 h of exposure to the PR MX-5B and AB 161 dyes in simple and binary solutions. The test was conducted in test tubes containing dye solution at different concentrations and 10 *A. salina* nauplii before and after decolorization treatments with the filamentous fungi.

The lethal concentration (50% mortality rate (LC_{50})) of the dye solutions was determined using the trimmed Spearman–Karber method (Hamilton et al. 1977). The test

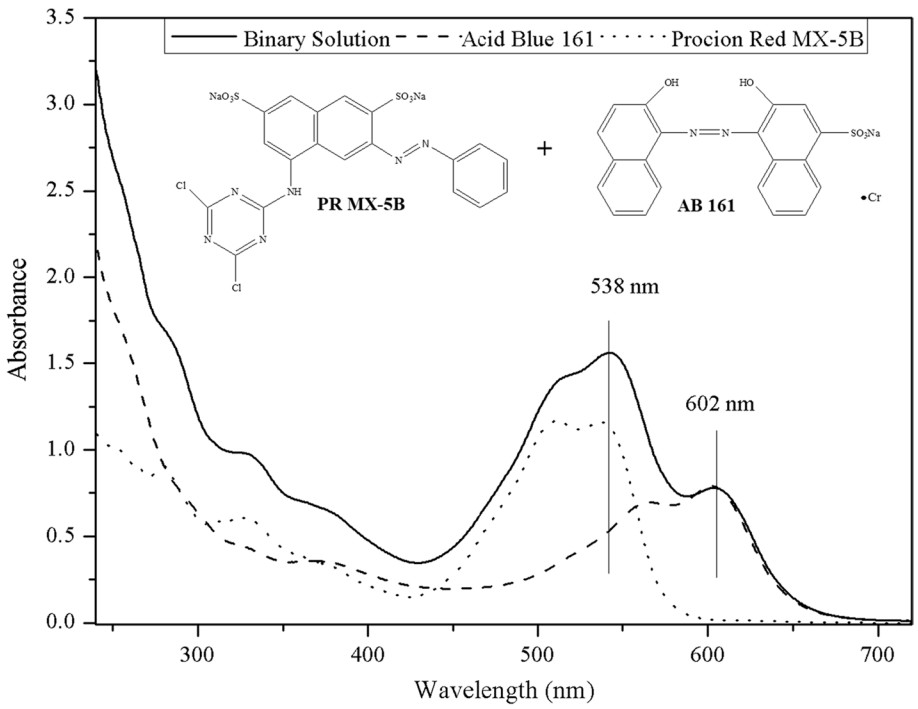


Fig. 1 UV–Vis spectra of dyes AB 161 and PR MX-5B in simple and binary solutions

is considered valid when the survival rate in artificial seawater (pH 8, salinity: 3.2%) is equal to or greater than 90% and the LC_{50} for sodium dodecyl sulfate (SDS) is within the sensitivity range of 13.95–20.23 mg L^{-1}. The sensitivity determined in the present study was 19.30 mg L^{-1} (Platte et al. 2002), demonstrating that the test was considered valid.

For the toxicity test with *L. sativa* (TopSeed® Garden), the first aim was to determine the inhibition of root growth of the seeds before the adsorption and biodegradation treatments to determine the LC_{50} of each dye studied in the simple and binary solutions. The test was conducted in Petri dishes with filter paper, 20 seeds and 3 mL of the test solution in a climatic chamber at 21 ± 1 °C in the absence light for 72 h. The positive control was $ZnSO_4$ 0.05 N and the negative control was distilled water. After this period, measurements of the roots were taken and growth inhibition (GI) is calculated using Eq. (1).

$$GI(\%) = \frac{(\text{root growth negative control} - \text{root growth dye solution})}{(\text{root growth negative control})} \times 100 \quad (1)$$

Adsorption treatment

Adsorption was performed with 10 mL of each dye solution at a concentration of 100 µg mL^{-1} and pH 4.0 for the PR MX-5B and AB 161 dyes in simple solutions. For the binary solution, the concentration was of 200 µg mL^{-1} (100 µg mL^{-1} AB 161 + 100 µg mL^{-1} PR MX-5B). The solutions were placed in contact with 1, 2, 3, 4 and 5 mg mL^{-1} of pelletized biomass of each fungus tested for 3 h at a temperature of 30 ± 1°C. The values specified for time, pH and initial concentration were selected based on data obtained from previous studies.

Decolorization of the solutions was monitored using UV–Vis spectrophotometry (Shimadzu UV–Vis 2401 PC). After the period of contact between the adsorbent biomass and dye, the solutions were centrifuged at 5000 rpm for 10 min. The rate of decolorization was calculated from the results of absorbance at λ_{max} = 602 (AB 161) and 538 (PR MX-5B) nm. The UV–Vis scans were performed using a quartz cuvette with an optical path of 5 mm.

Adsorption isotherms and adsorption capacity of mycelial pellets

Adsorption isotherms are important to describing interactions between adsorbate molecules and the biosorbent surface as well as distribution between the liquid and solid phases in a state of equilibrium (Rosales et al. 2016). After the adsorption test with the filamentous fungi (*A. niger*, *A. terreus* and *R. oligosporus*), the Freundlich and Langmuir isotherms were applied. The isotherms proposed by Wang et al. (2017a, b) are employed using Eqs. (2) and (3).

Freundlich isotherm equation

$$\log qe = \log K + \frac{1}{n} \cdot \log Dr \quad (2)$$

in which qe = concentration of adsorbed per unit of biomass (mg g^{-1}); Dr = dye remaining (mg mL^{-1}); log K is the y intercept; and n is the slope.

Langmuir isotherm equation

$$\frac{Dr}{qe} = \frac{1}{K1 \cdot K2} + \frac{Dr}{K2} \quad (3)$$

in which $K1$ = is the y intercept and $K2$ = amount of solute that saturates a unit of biomass with a monolayer adsorbent (mg mL^{-1}).

The sorption capacity of mycelial pellets of fungi tested is calculated using Eq. (4) proposed by Asgher and Bhatti (2010).

$$q = \frac{(Di - De) \cdot V}{W} \quad (4)$$

in which q = amount of dye adsorbed per unit of adsorbent biomass (mg g^{-1}), Di and De = initial and final concentrations of dyes (mg mL^{-1}), v = volume of solution tested (mL) and W = amount of adsorbent biomass (g).

Biodegradation treatments

Biodegradation treatments were performed with the PR MX-5B and AB 161 dyes in binary solutions and using the fungus *A. terreus,* based on findings in other biodegradation studies. Biodegradation was performed with the 3-mg mL^{-1} of *A. terreus* biomass in 100-mL Erlenmeyer flasks containing 25 mL of binary solution at a concentration of 200 µg mL^{-1} and sterilized by autoclaving at 120º C and 1 atm for 15 min. The flasks were then incubated at 30 ± 1 °C. The solutions were analyzed at intervals of 24, 240 and 336 h of treatment. For the UV–Vis, FTIR (Shimadzu-8300) and toxicity analysis, the solutions were centrifuged at 5000 rpm for 10 min. Changes in the UV–Vis spectra of each dye before and during treatment enabled evaluating the degradation of the molecules based on ratio absorbance (Abs^{537nm}/Abs^{327nm}) (Glenn and Gold, 1983). After treatment, FTIR analysis was performed using the method proposed by Vitor and Corso

(2008) for the determination of the molecular structure of the dyes in the binary solution.

Results and discussion

Determination of LC_{50}

Among environmental factors, water availability and quality influence the germination and development of plants. The absorption of water by seeds causes the hydration of tissues, leading to the intensification of respiration and all other metabolic activities, which result in the supply of energy and nutrients for the development of the embryonic axis (Palácio et al. 2009). Therefore, the LC_{50} (Fig. 2, graph A) of the PR MX-5B and AB 161 dyes in simple and binary solutions was determined in the first stage of the experiment.

The AB 161 dye was more toxic, as demonstrated by the 1.5-fold lower LC_{50} in comparison with the PR MX-5B. In the binary solution, the LC_{50} was 1400 µg mL^{-1} of each of the dyes, indicating that the combination did not make the solution more toxic to *L. sativa* seeds.

The nauplii of the microcrustacean *A. salina* are considered a good indicator of the presence of toxic substances in the aquatic environment and are easy to handle in the laboratory, providing fast, reliable results (Liwarska-Bizukojc et al. 2005). The results enabled the calculation of LC_{50} (Fig. 2, graph B) values for each solution, which were 2175 µg mL^{-1} for AB 161, 2570 µg mL^{-1} for PR MX-5B and 2600 µg mL^{-1} for the binary solution.

Adsorption treatments: decolorization, adsorption capacity of mycelial pellets, isotherms and toxicity tests

The decolorization rate was proportional to the amount of fungal biomass (Fig. 3, graphs 1a, 2a and 3a) used in each treatment, with an increase in biomass leading to an increase in decolorization. The fungus *A. niger* achieved the highest removal rates of the PR MX-5B and AB 161 dyes in the simple and binary solutions. AB 161 was more easily removed from the reaction medium by the three filamentous fungi studied, indicating that this dye has greater affinity with the cell walls of these microorganisms.

The fungal cell wall consists mainly of polysaccharides, proteins, lipids and several functional groups. Dyes may interact with these active groups on the cell wall surface through physical (Van der Waals forces) or chemical (ion exchange or covalent bond) mechanisms (Crini 2006).

Kabbout and Taha (2014) studied the decolorization of the cationic dye methylene blue by a dead fungal biomass of *Aspergillus fumigatus*. Using 5 g of biomass, the results revealed rapid adsorption, with a rate decolorization of 58% in 30 min and 68% in 120 min. Peak adsorption (71%) occurred at 210 min. In the present investigation using the same amount of live biomass, maximum decolorization in the treatment of the binary solution by *A. niger* occurred after 180 min. The greater efficiency of dead fungal biomass regarding the decolorization of the solutions is likely due to the fact that dead biomass does not present any resistance to the adsorption process of the dyes.

Regarding the adsorption capacity (Fig. 3, graphs 1b, 2b and 3b), the biosorbent pellets present as a tangled mass of hyphae surrounded by many loose hyphae on the surface

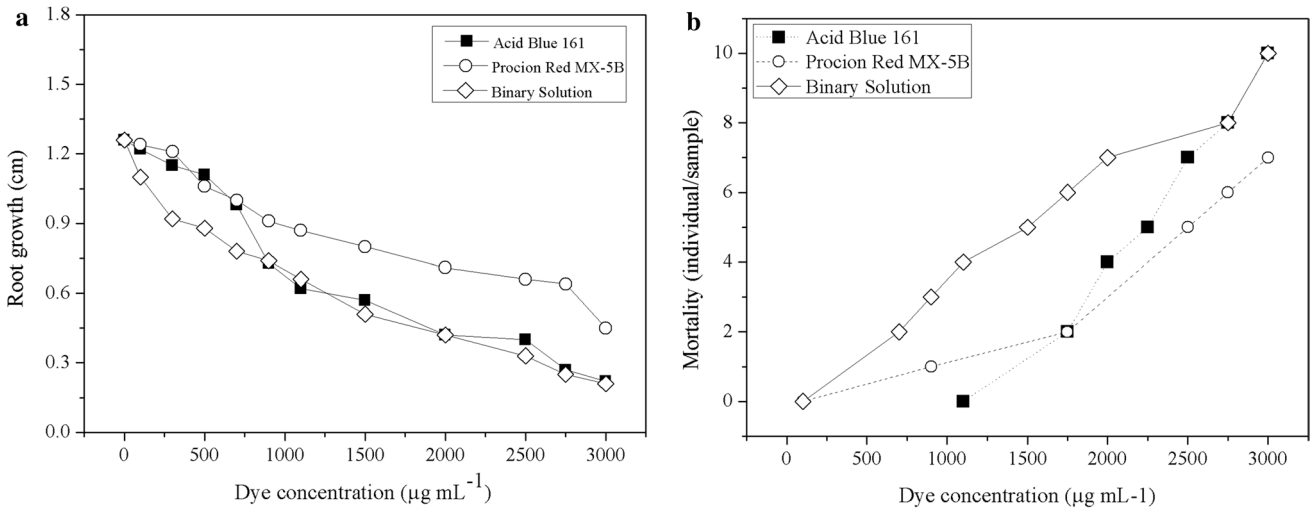


Fig. 2 **a** Inhibition of root growth of *L. sativa* seeds and **b** mortality of *A. salina* nauplii exposed to different concentrations of AB 161, PR MX-5B and binary solution

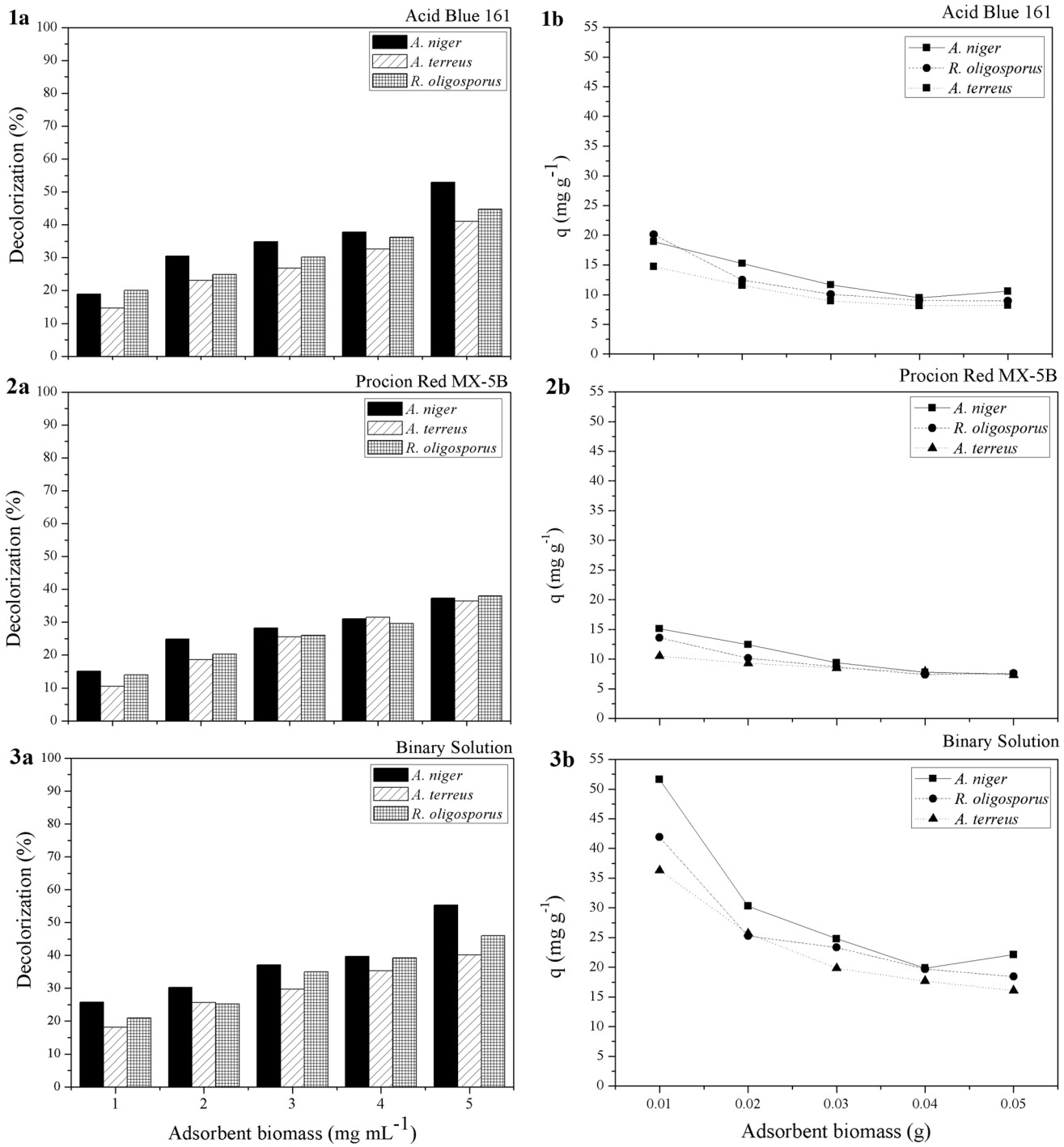


Fig. 3 Decolorization and adsorption capacity of mycelial pellets after 3 h of adsorption treatments. (**1a**, **1b**) AB 161, (**2a**, **2b**) PR MX-5B and (**3a**, **3b**) binary solution

(Fig. 4, image 1). This conformation enables the adherence of dye molecules, which explains why an increase in quantity of pellets in the solutions tested led to a reduction in the sorption capacity of the biomass. Compaction of mycelium occurs in the presence of a greater amount of biomass, reducing the contact surface of the biomass with the dye molecules. Therefore, although decolorization was greater in solutions with a larger amount of biomass, each pellet used in the adsorption process was less efficient under these conditions.

The Freundlich and Langmuir isotherms were applied to evaluate how the interaction between the adsorbent substrates and dyes occurred. In the Langmuir isotherm, all

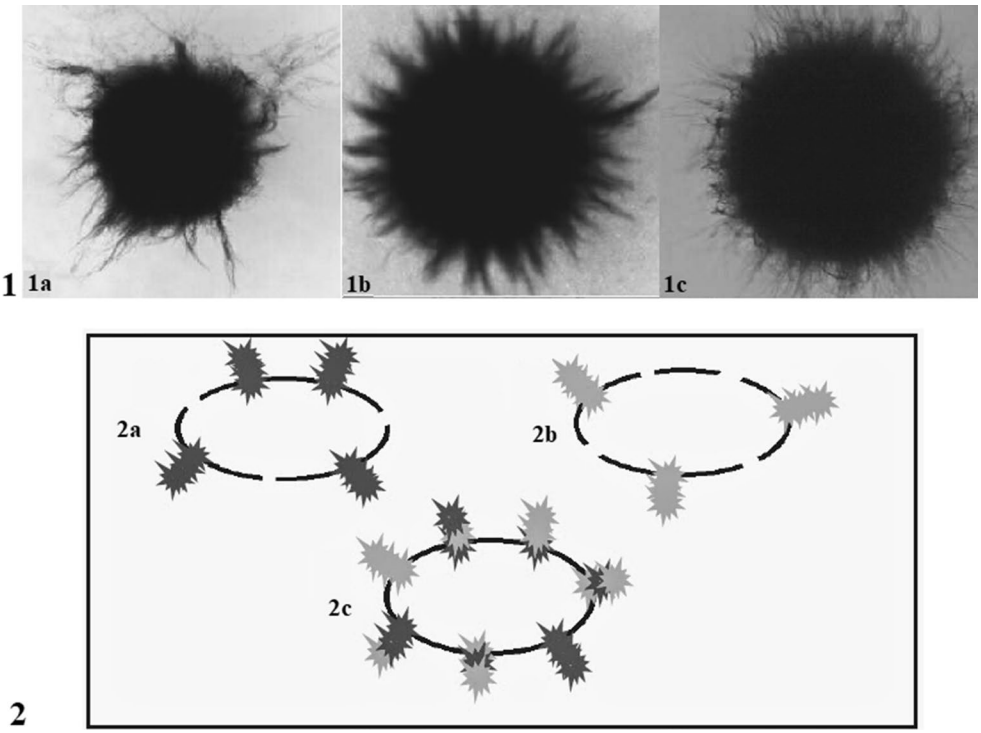


Fig. 4 **1** Fungal pellets (*a*) *A. niger,* (*b*) *R.oligosporus* and (*c*) *A. terreus* after adsorption process in binary solution; **2** Adsorption scheme of dyes through cell wall of pelletized fungal biomass. (*a*) AB 161, (*b*) PR MX-5B and (*c*) binary solution

Table 1 Correlation coefficients of Freundlich and Langmuir equations

Acid blue 161		Procion red MX-5B		Binary solution	
Freundlich	Langmuir	Freundlich	Langmuir	Freundlich	Langmuir
A. niger					
0.5073	0.2947	0.8650	0.6642	0.3015	0.2516
A. terreus					
0.7399	0.1489	0.9937	0.0322	0.9078	0.8503
R. oligosporus					
0.5869	0.1769	0.7309	0.2788	0.7053	0.2741

forces acting on adsorption are similar in nature to those involving a chemical reaction and adsorption occurs in a single layer of dye molecules on the surface of the adsorbent. In the Freundlich isotherm, adsorption occurs in multiple layers; this isotherm is useful for describing adsorption on highly heterogeneous surfaces. The isotherms were analyzed from their correlation coefficients (R^2) (Table 1) obtained from the analysis of the results of the adsorption test.

Analyzing the correlation coefficients, the adsorption with the pelletized fungal biomass occurred in accordance with the Freundlich isotherm, that is, the removal of the dyes occurred through multiple layers. This adsorption model explains the decolorization results, which indicate that the dye removal rate was smaller in the simple solution than the binary solution. This suggests the presence of specific active sites (Fig. 4, image 2) for each of the dyes. In the binary solution, all available fixation sites on the surface of the pellets were filled, thereby increasing the adsorption capacity of the pellets and consequently increasing the decolorization rate in the binary solution. Moreover, no toxicity to *A. salina* nauplii or *L. sativa* seeds was found after the adsorption process with the filamentous fungi *A. terreus*, *A. niger* and *R. oligosporus*.

Binary solution: biodegradation treatments with *A. terreus*

Biodegradation of the binary solution was performed with the fungus *A. terreus* (Fig. 5, graph A). After 24 h of treatment, the fungal biomass presented greater affinity for AB 161, removing 84% of the dye. Moreover, decolorization remained constant until the end of treatment. For PR MX-5B, 40% of decolorization occurred in 24 h of treatment, totaling 60% decolorization of the binary solution. At 240 and 336 h of treatment, this dye was completely removed. At the end of the treatments, 92% decolorization of the initial solutions was obtained.

The absorbance ratios were calculated from the UV–Vis analyzes of the solutions. According to Glen and Gold (1983), adsorption is the predominant process if these values remain constant in relation to the control solution and biodegradation of the molecules is indicated when these values exhibit significant changes. Analyzing the results, it's possible to observe (Fig. 5, graph B) a significant variation in the absorbance ratios of the solution occurred in the first

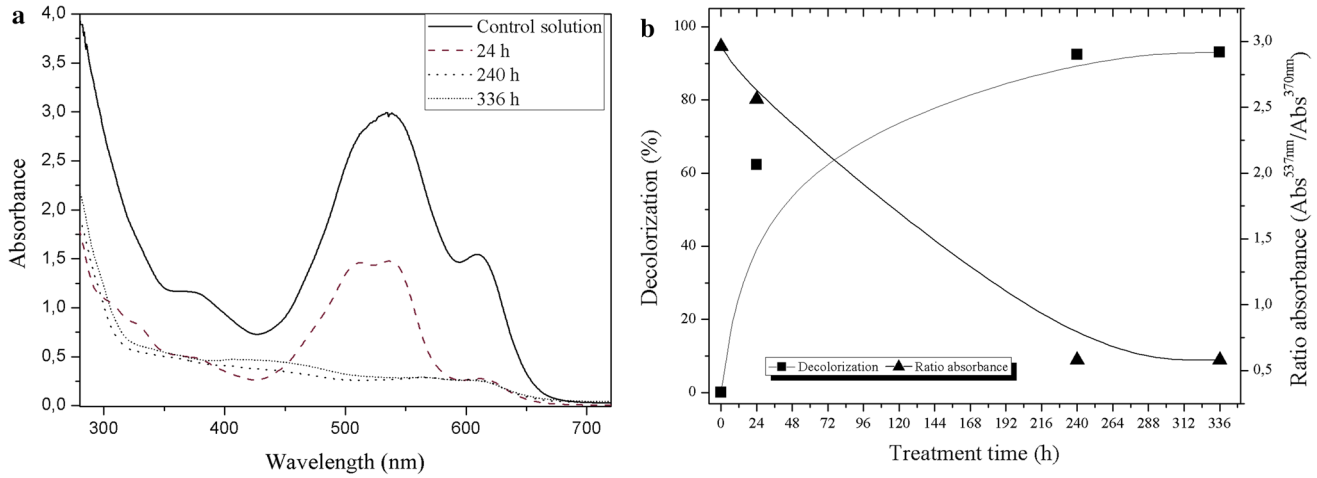


Fig. 5 **a** Decolorization and **b** ratio absorbance of binary solution after treatment with *A. terreus*

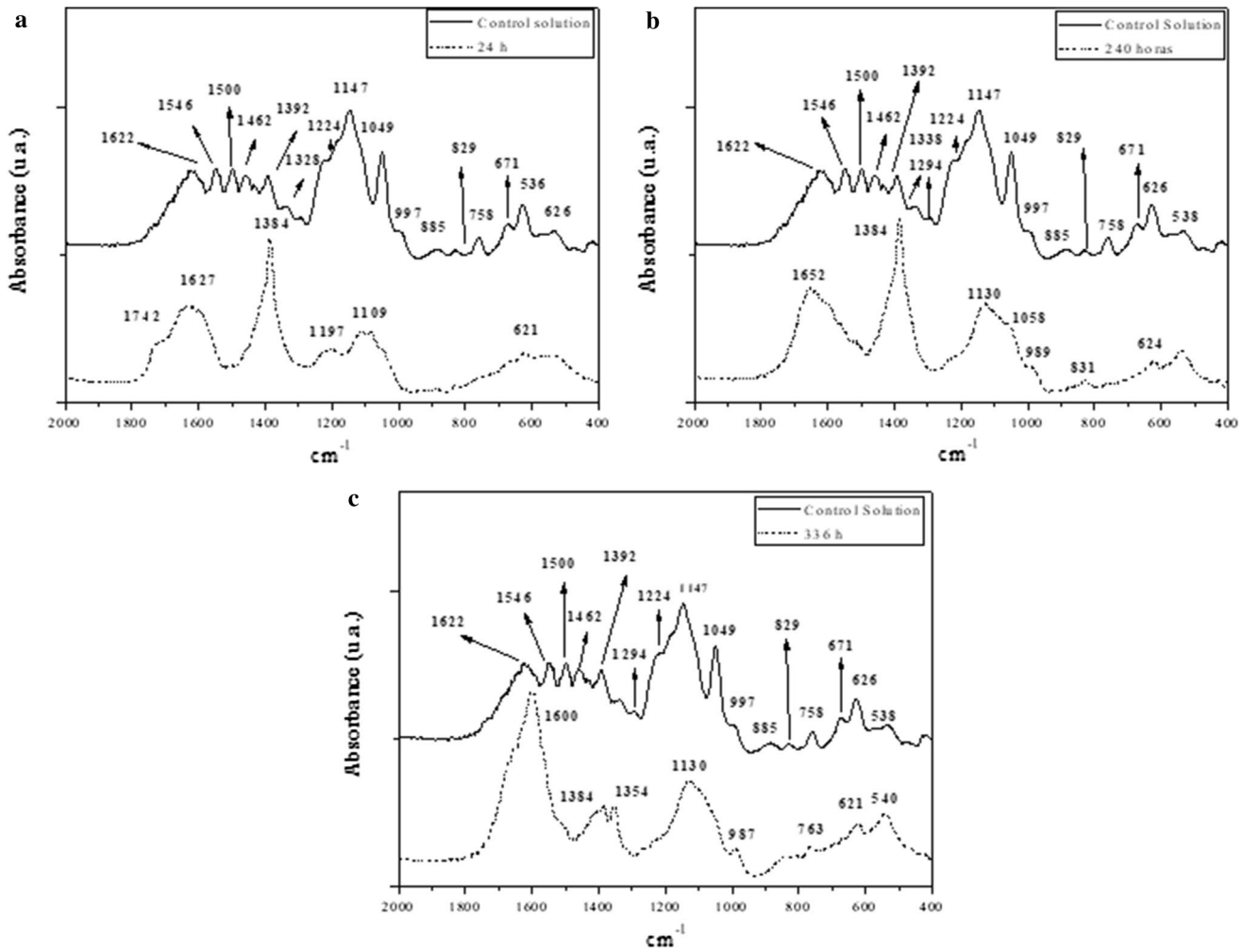


Fig. 6 FTIR spectra of binary solution before and after biodegradation treatments

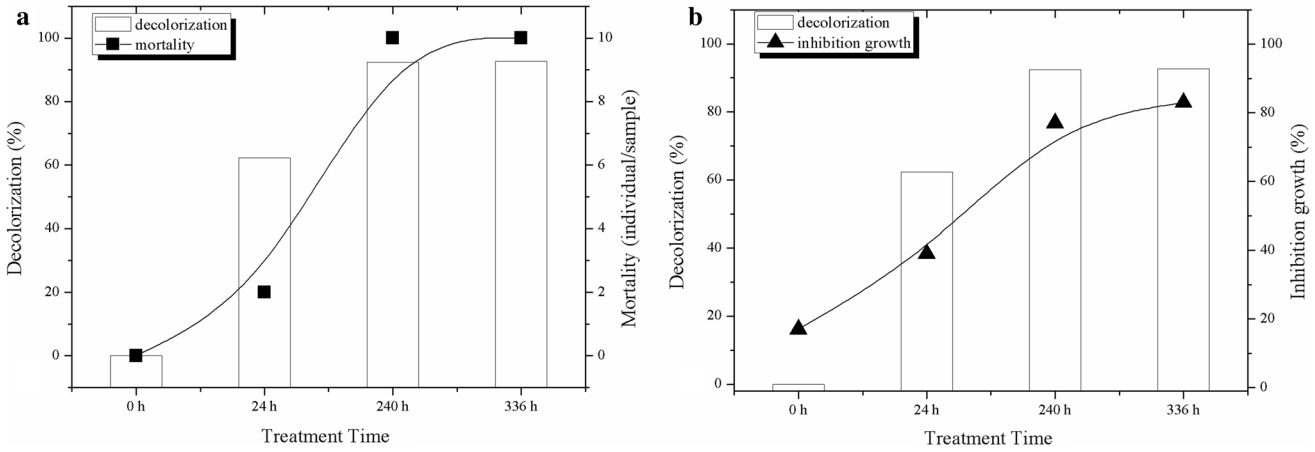


Fig. 7 **a** Mortality of *A. salina* nauplii after biodegradation treatment with *A. terreus*; **b** Growth inhibition of *L. sativa* seeds after biodegradation treatment with *A. terreus*

24 h of treatment, indicating high microbial activity and the consequent degradation of dye molecules.

The FTIR spectra of the binary solution exhibited significant changes after the biological treatment (Fig. 6). In all treatments, spectral changes occurred in the regions of 1622, 1500–1550 and 1462 cm^{-1}, which are characteristics of azo bonds and stretching vibration of the C–N bond (Gao et al. 2010). These changes indicate the breakage of these bonds, leading to the formation of aromatic amino compounds as intermediates. The formation of these metabolites suggests that some peaks corresponding to vibrations of the NH_2 bond of aromatic amines appear more intensely after contact with the fungal biomass. These peaks can be observed in the region of 1109 cm^{-1} at 24 h, 1678 cm^{-1} at 240 h and 1600 cm^{-1} at 336 h of treatment (Pacchade et al. 2009; Olukanni et al. 2010).

The peak that appears in the region of 1742 cm^{-1} at 24 h of treatment is characteristic of the change in the C=O stretching band of the carboxyl group (Zhang et al. 2017). This peak disappears at 240 and 336 h, which indicates complete breakage of these chemical bonds during biodegradation treatment. The 987 and 540 cm^{-1} regions correspond to aromatic compounds and the changes having occurred after treatment indicate cleavage of the chemical bonds of these functional groups.

At 24, 240 and 336 h of treatment, bands appeared in the regions of 1384, 1354, 624 and 621 cm^{-1}, indicating the presence of sulfonic groups (Rawat et al. 2018; Harisha et al. 2017). Sulfonated radicals are found in both dyes, and the enzymatic action on the molecules may have led to the cleavage of the bonds of these groups, releasing them into the reaction medium, which generated an increase in the intensity of the signals of these bands.

The results of the toxicity tests for *A. salina* nauplii and *L. sativa* seeds (Fig. 7) showed a 100% mortality rate of the larvae occurred at the end of the 336 h of degradation treatment. The final solution after the treatment contained residual AB 161 dye and metabolites formed during the biodegradation steps. These metabolites were responsible for the increase in larval mortality.

The microbiological treatment of the binary solution also led to increased inhibition of root growth of *L. sativa* seedlings. The percentage of root growth inhibition increased from 17% (toxicity control binary solution) to 83% at the end of 336 h of treatment.

Conclusion

Analyzing the findings, the more efficient treatment was biosorption with the pelletized biomasses of the fungi *A. terreus*, *A. niger* and *R. oligosporus*. The fungal biomass was able to remove the dye from the medium without degrading the molecules, which was very favorable due to the non-formation of highly toxic by-products. In the biosorption treatment, each dye bounds to specific active sites on the fungal cell walls, as demonstrated by the greater removal of these molecules in the binary solution, indicating more efficient treatment due to the filling of all active sites. The high rate of decolorization obtained during treatment confirmed dye removal by biosorption in multiple layers. While the biodegradation treatment was quite efficient in achieving decolorization, the formation of by-products with high toxicological potential reduces the usefulness of the method. Biodegradation treatments with longer exposure times may be more efficient, since greater exposure to microorganisms enables the complete degradation and consequent mineralization of these metabolites, which are highly persistent in the environment and cause considerable harm to aquatic fauna and flora.

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