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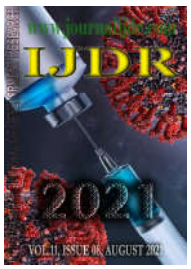

RESEARCH ARTICLE OPEN ACCESS

FUNGAL POTENTIAL FOR THE DECOLORIZATION OF TEXTILE DYES

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ABSTRACT

The textile dyes used in industry are formed by complex structures with high coloration and are often difficult to treat for decolorization. These dyes, present in high concentration, have a toxic effect on the ecosystem and bioremediation methods using microorganisms cause less toxicity impact on the environment. In this work, four distinct *Aspergillusspp*. strains, capable of decolorizing dyes used in the textile industry, congored and indigo carmine, diluted in Normal Decolorization Medium, were used. The screening of the best fungus was performed to evaluate the decolorization capacity in liquid medium. The best decolorization results were chosen to carry out the ecotoxicity assays and characterization of the biotreated dye parameters. The four strains evaluated showed a percentage of up to 96.10% of decolorization of the tested dyes. Phytotoxicity tests were carried out, which, in comparison with the negative control group, showed significant and promising results, with cucumberseed germination and root growth. The characterization of the dye demonstrated biological oxygen demand (BOD), chemical oxygen demand (COD), turbidity and pH according to established parameters. Therefore, the removal of dyes was achieved through decolorization carried out by fungal microorganisms, and this corresponded to a decrease in the phytotoxicity of congored.

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INTRODUCTION

Over the years, in parallel to population development,it was detected the generation of large amounts of effluents with different compounds that cause various environmental problems (Thuy *et al.*, 2020). Studies suggest the importance of evaluating different strategies aimed at sustainable development by applying methods capable of treating and mitigating damages caused by textile effluents, including the use of microorganisms and/or active biomolecules of biotechnological interest (Kasiri, 2019; Lellis *et al.*, 2019). In this context, the water bodies that compose the ecosystem form a final disposal site for various activities from industry, commerce and domestic activities that end up contaminating the environment. The industrial synthetic products are not only toxic to human health, but also to aquatic life, land animals and plants, being harmful in a chain effect throughout the environment (Athira and Jaya, 2018).

The textile industry uses a wide range of chemical products, including dyes, which are important constituents, during the different stages of manufacturing, producing a colored wastewater, called textile effluent (Khan and Malik, 2018). Effluents from textile industries are considered oneof the largest generators of polluting liquid effluents, discarding tons of textile dyes into the environment. They affect the aquatic ecosystem, soil fertility, plant growth and productivity as well as plant susceptibility to pathogens, that is, the entire development of fauna and flora around where it is being discarded (Saratale *et al.*, 2020). According to the study by Khan and Malik (2018), the textile industry consumes a huge amount of drinking water, that is, for the production of 1 kg of dyed fabric, about 200 L of water are used. As a result, a large amount of wastewater is discharged into various bodies of water, generally, without adequate prior treatment, which results in serious pollution of the water containing dyes and other hazardous chemicals generated by the textile industry, such as heavy metals and sulphides(Khan and Malik, 2018).

The textile dyes used in the industry are formed by complex structures with high coloration and are often difficult to treat. Even going through a treatment process to be discarded in water courses, they cause damage to the environment. The dyes present in high concentration have a toxic effect on the ecosystem in addition to not allowing the penetration of sunlight through the water body, inhibiting and harming biological processes(Samchetshabam *et al.*, 2017). Congo red and indigo carmine are among the most widely used emerging industrial dyes around the world. They are very toxic to the environment and have carcinogenic effects. They are formed by complex aromatic molecular structures and even in small amounts are undesirable because they are difficult to degrade (Ismail *et al.*, 2019).
The CONAMA Resolution nº. 430/11 (Brasil, 2005) provides on the conditions, parameters, standards and guidelines for the discharge of effluents into bodies of water. The effluents resulting from industrial processes, such as those from textile dyeing plants, must be passed through treatment processes before being discarded. The companies that generate these effluents must ensure that the standards required for treatment are within the specifications requested in the resolution. In addition, one of these parameters is that the wastewater from the textile process is decolorized. Biodegradation or bioremediation is a biological mechanism for recycling waste that can otherwise be used and reused by other organisms. It is one of the possible techniques of easy application that can cause fewer environmental impacts of toxicity (Yadu *et al.*, 2020). The use of bioremediators is a viable option in specific actions for the recovery of the environment, and for that, in-depth studies are needed on how the manipulation should be done for a better control of its application (Brasil, 2005). Microorganisms are involved through their enzymatic pathways acting as natural biocatalysts, facilitating the progress of biochemical reactions that degrade the target pollutant. These microorganisms act against pollutants depending on access to a variety of nutritional compoundsand generating energy for their growth, replication and activity. The efficiency of bioremediation depends on many factors, including, the chemical nature and concentration of pollutants, the physicochemical characteristics of the environment and its availability for microorganisms (Abatenh *et al*., 2017). Fungi or fungal enzymes proved to be potent bioremediators, due to associations resulting from biodegradation or biotransformation of waste (Przystas *et al.*, 2015). They are found in all environments and release extracellular enzymes according to their environmental condition, being able to act easily on any amount of pollutant, even in high concentration, which makes their easy survival (Baghel *et al.*, 2020). Fungi can be excellent candidates for dye removal. Different strains of fungi have been reported to decolorize or biosorb dyes found in textile effluents. Most of them use an extracellular enzyme system that transforms complex substances into more assimilable substances. Fungi are highly studied as bioremediators as they produce non-specific enzymes that efficiently degrade different dyes found in the textile industry (Przystas *et al.*, 2015). In this context, the present study aims to evaluate the fungal potential in the decolorization of dyes used in the textileindustry and analyze the toxicity of the biologically treated dye.

MATERIALS AND METHODS

Microorganisms-growing conditions: *Aspergillusspp*. strains 1, 2, 3 and 4, obtained from the culture collection of the research group of the *Laboratório de Microbiologia Ambiental e Industrial* (LAMAI-UFPE), were multiplied under culture conditions in a liquid Sabouraud medium (glucose 0.4 g; agar 1.5 g; distilled water, 100 ml) incubated for 120 h at 30 °C in stationary condition and, subsequently, inoculated in solid Sabouraud medium and incubated for 168 h, at 30 °C.

Dyes: Commercial indigo carmine dyes (5.5′-indigodisulfonic acid sodium salt, indigoid sulfonate class, CI: 73015)Sigma-Aldrich Corporation, St. Louis, Missouri, USA and congored (sodium salt of benzidine diazo-bis-1-naphthyl amine-4-sulphonic acid, diazo-sulfonate class)Cromato Chemical Products Corporation, Diadema, São Paulo, Brazil,were used. Aqueous solutions in ultrapure water were prepared, for the dissolution of the dyes (synthetic effluents), at a concentration of 25 ppm.

Screening of decolorization in liquid medium: Screening to assess the decolorization capacity in liquid medium was performed in 250 ml Erlenmeyer flasks, containing 60 mlof modified Normal Decolorization Medium (NDM), consisting of: 0.25% yeast extract, 2% glucose and 25 ppm of textile dye, inoculated with 6 discs of fungal mycelium with a diameter of 9 mm,cultivated in Sabouraud agar, after 7 days of cultivation. Flasks were kept for 192 h at 25 °C in biological oxygen demand (BOD). The assays were performed in triplicate, the control assay was performed using the NDM culture medium, added with dye without the inoculum of the fungal strains, and, every 24 h, 1 mlaliquots were removed and centrifuged for 20 min at 5,000 rpm (Ramalho*et al*., 2004). The percentage of decolorization was calculated according to the equation:

$$D = \left(\frac{AbsCTRL - AbsTEST}{AbsCTRL}\right) X100$$

D% = decolorization percentage.
AbsCTRL= absorbance of the control at 610 nm for indigo carmine and 497 nm for congored.
AbsTEST = absorbance with fungal treatment at 610 nm for indigo carmine and 497 nm for congored.
The maximum absorbance length for the readings was determined by mass spectrometer in the visible range from 195 to 1100 nm.

Phytotoxicity assay: For the phytotoxicity tests, 10 cucumber seeds (*Cucumis sativus L.*) were employed per plate. Deionized water was used as a positive control, and dyes at 25 ppm were applied as a negative control. The seeds were placed in Petri dishes (9.5 cm in diameter) with qualitative filter paper substrate (porosity 1 µm) and moistened with 5 ml of the biotreated dye. To maintain moisture, the Petri dishes were wrapped up in paper film and incubated in BOD for 7 days at 25°C (Table 1). At the end of the experimental period, the number of germinated seeds was evaluated, considering only those that presented root protrusion. Test solutions were prepared with deionized water. The methodology and statistics were performed from Tiquia (1996), modified (Tiquia *et al*., 1996).

Table 1. Specifications for the phytotoxicity assay of textile dyes with *Cucumis sativus* seeds to obtain %G, %CR and IG.

Parameters	Condition
Seed species	*C. sativus*
Seed desinfection	By immersion in a solution of distilled water and 2% commercially available sodium hypochlorite for 3 to 5 min. Then with 6 passes of autoclaved deionized water.
Incubation	Static, 25°C for 7 days in Petri dishes containing paper filters as the wet substrate for growth.
Wetting	Every 48h, 5 ml
Light quality	Dark
Chosen biotreatments	4 treatments indigocarmine, 4 treatments congored, filtered in a syringe filter.
Replicates	3
Controls	Positive: only water Negative:25ppm dye diluted in water.
Endpoint	Count the number of sprouts and measurement of roots.
Not acceptable	Ungerminated and less than 65% growth.
Formulas for calculations	Germination percentage (% G) = Average of germinated test seeds X 100/ Average of germinated control seeds. Root growth percentage (% CR) = Average of growth of test seeds X 100/ Average of root growth in the control. Germination index (GI) = (Seed germination) X (% Root growth)/100 Root growth percentage (%CR) = Average of root growth of test seeds X 100/ Average of rootgrowth in the control. Germination index (GI) = (Seed germination) X (%Root growth)/100

Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD): To carry out the tests of this methodology, we based our efforts on the Standard Methods for the Examination of Water and Wastewater(Rice *et al.*, 2017), forBOD and chemical oxygen demand (COD). The tests were carried out by the *Instituto de Tecnologia de Pernambuco* – ITEP. To obtain the results of COD, it was necessary to dilute the sample 100 times. The samples chosen to perform BOD and COD were the ones that obtained the best results in congored decolorization and phytotoxicity. The pH and turbidity of all tested samples were also verified.

RESULTS AND DISCUSSIONS

Every production process is followed by the generation of waste, and among the various technologies investigated in waste cleaning, bioremediation emerged as the most desirable approach to decontaminate many environmental pollutants. The ability of microorganisms to transform a variety of chemicals has led to their use in bioremediation processes (Bhattacharya et al., 2011). Several microorganisms, therefore, are being studied to develop their degradative abilities in the remediation of pollutants. In this context, the present work shows the decolorization analysis of the two textile dyes, congored and indigo carmine, by the 4 different *Aspergillus spp*. strains, as shown in Figure 1. The best result for congored dye decolorization was 96.1%, using strain 4 in 72 h of incubation at 25°C. The other three strains used in this work also showed high decolorization rates (94.75% to 96.10%) in the same cultivation time (72 h), Figures1 and 2. Amen et al. (2021), evaluating the degradation of azo dyes, found that *Aspergillus* strains decolorized 85% of the dyes in synthetic wastewater containing congored. Assis et al (2018) obtained 98% decolorization of congored at 25 ppm after 144 h of treatment using *A.niger*. Mohammed *et al*., (2015), in studies with *A. terreus* and *A. flavus* for the biodegradation of congored dye, obtained a percentage of 46.89% after 72 h of incubation.

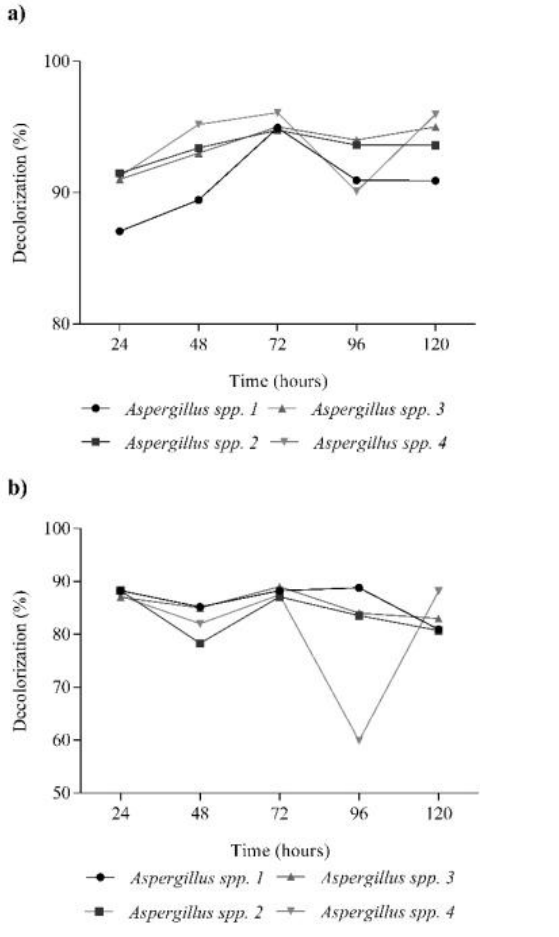
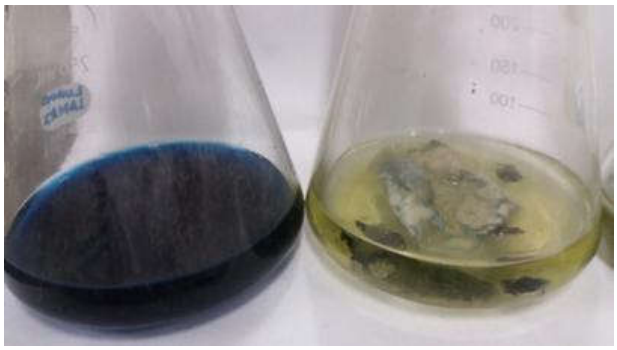


Figure 1. Kinetics of textile dye decolorization in MND culture medium inoculated with *Aspergillus spp*. strains 1, 2, 3 and 4; (a) congored at 25 ppm and (b) indigo carmine at 25 ppm. T = 25°C.

Thus, it can be referred that fungal species have been presented as potential candidates for removal of congored from textile wastewater and its safe disposal. Azo dyes are toxic and recalcitrant environmental pollutants in wastewater and soil, and are widely used in the textile industry;among these dyes is congored. Due to its high toxicity, several decolorization studies were able to find fungal species useful in wastewater treatment and soil remediation efforts (de Almeida *et al.*, 2021; Sumera AfzalKhan *et al.*, 2020). Throughout the indigo carmine screenings, a decolorization of more than 80% was noted from the second day (48 h) of incubation. A slight drop in the effluent decolorization activity throughout the experiment, with the activity in the 4 selected microorganism strainsallowed above 80% of bioremediation (Figure 2). Throughout the indigo carmine screenings, a decolorization of more than 80% was noted from the second day (48 h) of incubation. A slight drop in the effluent decolorization activity throughout the experiment, with the activity in the 4 selected microorganism strainsallowed above 80% of bioremediation (Figure 2).

(A)



(B)

Figure 2. *Aspergillus spp. 4* -Decolorization of (A) congored and (B) indigo carmine dyes

In plants, water plays an important role in regulating biotic and abiotic stress responses caused by contaminants (Wang *et al.*, 2019). Toxicity assays are of great importance and should be carried out after microbial decolorization tests. These assays provide insight into the physiological impact of contaminants on crops, to design more appropriate treatments for contaminants (Werrie *et al.*, 2020). The removal of the color from the dyes by the fungal strains led to a decrease in toxicity in the growth of cucumber seeds from the culture solution after the decolorization process. The control results, in both textile dyes used, were characterized as toxic, since they showed the lowest growth compared to the others. According to Tiquia (1994), GI valuesabove 80% indicate the reduction or elimination of toxic effects in the decolorization process. The best congo red decolorization conditions were displayed in the phytotoxicity test;it was demonstrated that fungal strains had better results with the culture solutions after the congo red decolorization process (Figure 3). These results showed that even with satisfactory decolorizations of the culture solutions, the indigo carmine treatments were not adequate for the phytotoxicity assays.

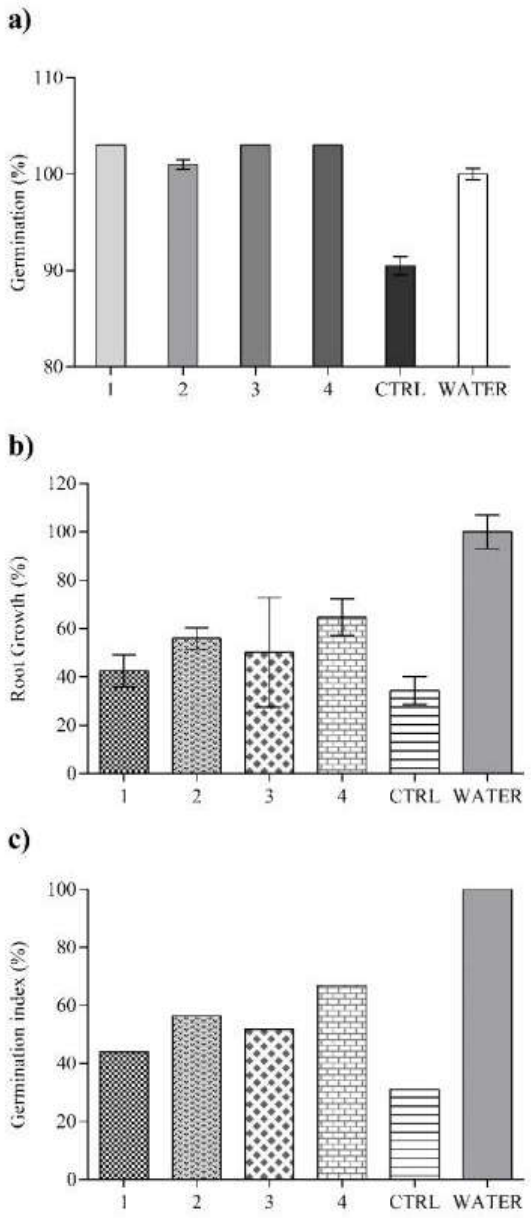


Figure 3. Congo red phytotoxicity assay.(a) Germination percentage, (b) root growth percentage, (c) germination index.*Aspergillus spp*. strains 1, 2, 3 and 4; CTRL = control

Considering ecotoxicity tests, the absence of color does not necessarily translate into the absence of toxicity (de Almeida *et al.*, 2021). There was germination of more than 80% of the seeds of *C. sativus* (Figure 3a and 4a). If compared to the positive and negative control group, there is a promising improvement in germination after bioremediation with the four strains. In the root growth, nuances were verified in the different fungi compared to the control groups, since they were biotreated dyes from four different *Aspergillus spp*. strains.A significant growth was detected in germinated seeds when compared to the negative control group (Figure 3b). Finally, the germination indices were also satisfactory, since the indices of the negative control group are lower when compared to the groups after bioremediation.

The germination, root growth and germination index percentage represented in Figure 4 showed a satisfactory and higher growth percentage when compared to the 100% germination negative control group. However, throughout the growth test there was no root development and growth. The germination index was satisfactory under all conditions of the culture solution after the congored decolorization process by fungal strains. A decrease in toxicity due to root growth was confirmed when compared to the control group.

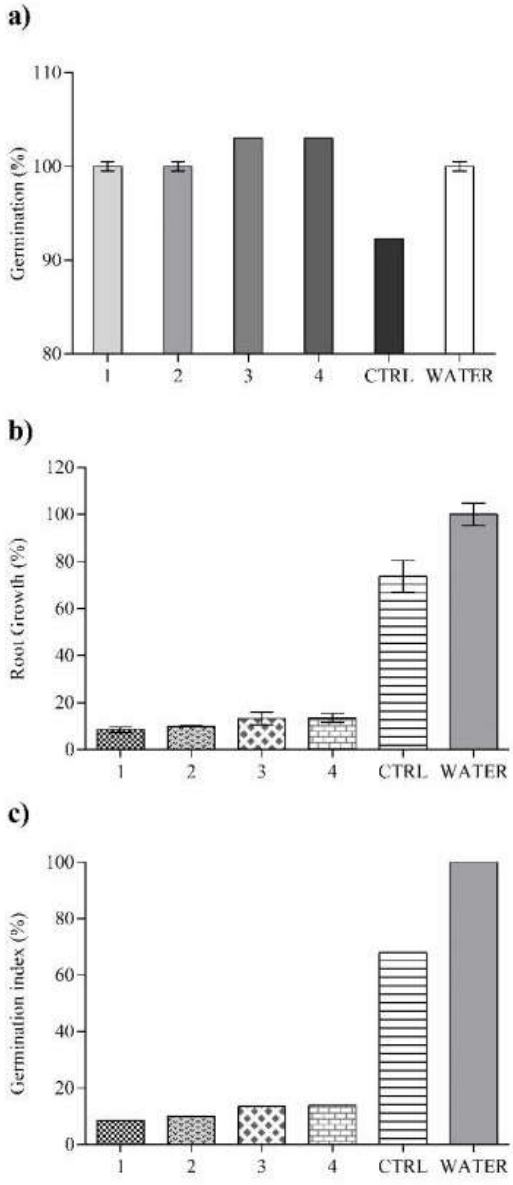


Figure 4. Indigo carmine Phytotoxicity Assay. (a) Germination percentage, (b) root growth percentage, (c) germination index. *Aspergillus spp*. strains 1, 2, 3 and 4; CTRL = control

All four strains tested for congored decolorization showed promise bothin color removal and in the detoxicity in the growth of cucumber seeds (Figure 5 and 6).

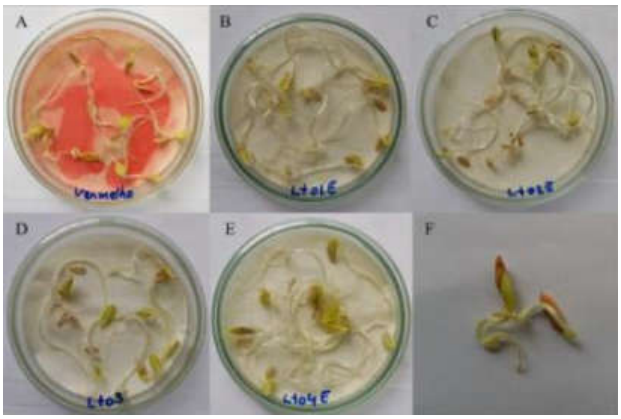


Figure 5. Cucumber seed phytotoxicity assays. Using the culture solutions after the congored decolorization process. A-negative control, B strain 1, C-strain 2, D- strain 3, E-strain 4 and F-atrophy of the roots of the negative control

However, evaluating the results of the toxicity tests of cucumber seeds with the culture solution after the process of decolorization of indigo carmine, it was not possible to detect promising results in seed growth (figure 6). In other studies, such as Przrystas (2018), it was shown that there is a decrease in the toxicity of dyeing solutions after the fungal decolorization processes. Then,decolorization significantly reduces the negative influence of textile dyes on plant germination, root and stem growth. Similar results were reported using the yeast *Pichia kudriavzevii SD5* for decolorization, in the tests by Delane and collaborators (2020); the results effectively demonstrated that there was a reduction in dye decolorization and also a reduction in toxicity rates.

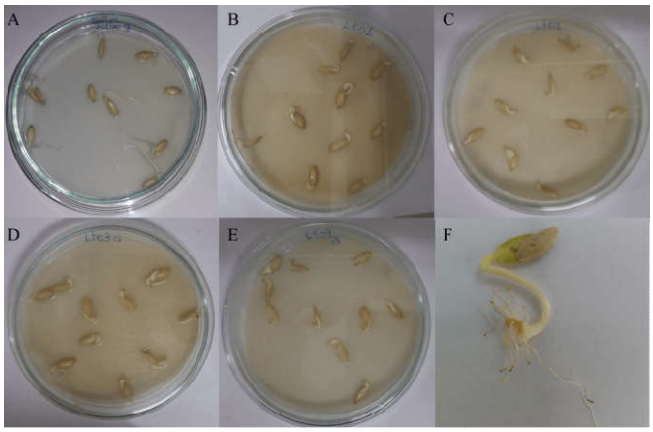


Figure 6. Cucumber seed phytotoxicity assays using the culture solutions after the indigo carmine. decolorization process. A-negative control, B-strain 1, C-strain 2, D-strain 3, E-strain 4 and F-atrophy of the roots of the negative control

Textile effluents can only be released directly or indirectly if they are properly treated and comply with standard norms.The pH of most samples throughout the decolorization was kept in accordance with the parameters established by CONAMA in art. 16 and 17, of class 3 and 4 waters, indicated by pH values obtained between 6 and 9. Only strain 2 showed a low pH (Table 2) (Brasil, 2005). The pH values did not show any sharp differences during the incubation period. On the other hand, turbidity values increased progressively. Even though the absorbances showed low values of decolorization throughout the experiment, the values obtained were high. This is due to the fact that the fungal strains developed in the decolorization medium and even performing theirdecolorizing activity, the turbidity becomes progressively high due to the fungal growth, making unachievable the necessary transparency for the collection of lower values (Table 2).

Table 2. Results of the analysis of Biological and Chemical Oxygen Demand (BOD and COD) with pH and turbidity of congored dye treated with the strains described and positive and negative controls for comparison

Samples	BOD ($x10^3$)	COD ($x10^4$)	BI ((BOD/COD) $x10^{-2}$)	pH	Turbidity
1	4.92	4.0	12.3	6.13	18.40
2	0.2	3.6	0.6	5.50	16.70
3	0.1	5.4	0.2	6.26	39.70
4	4.42	5.8	7.6	6.06	28.10
CTRLl +	1.61	5.6	2.9	6.46	18.80
CTRLl -	2.01	2.0	10.1	6.45	6.19

Aspergillus spp. strains 1, 2, 3 and 4; CTRL = control.

Since the main objective of the treatment of textile, effluent waters significantly reduced the amount of organic matter that can be measured, this organic matter must be converted into inert mineralized products under controlled conditions and high variations. Given this information, the two most common parameters used to recognize the composition of effluent water were tested: BOD and COD. BOD is a measure of how dissolved oxygen is consumed by aerobic bacteria in 5 days at 20 °C, which indirectly assesses the concentration of biodegradable organic matter in water through the oxygen demand produced by microorganisms in respiration. COD is the measured chemical oxygen demand, being an indicator of organic matter that is based on the concentration of oxygen consumed to oxidize the organic matter present in the studied medium, in an acidic medium and under energetic conditions of a strong oxidizing chemical agent(Abdalla and Hammam, 2014; Mohsenpour *et al.*, 2021; Valente *et al.*, 1997). When considered the strains that stood out the most in the BOD and COD parameters, we can highlight strains 2 and 3 for having lower values (Table 2). High COD values indicate the presence of substances capable of consuming oxygen, for example, Fe^{2+}, Mg^{2+} e NH_4(Fenzl, 1988; Pizato*et al.*, 2017). In addition, the results indicate that the dyes used have low biodegradability, as the BOD and COD values are very far from each other (Pizato*et al.*, 2017; Von Sperling, 2005).The presence of organic matter in high concentrations can cause deleterious effects on the concentration of dissolved oxygen, the trophic state and the well-being of the fauna and flora of the water(Mohsenpour *et al.*, 2021). Thus, making a correlation between BOD and COD becomes a useful tool to assess the conditions of the effluent and as an indicator of biodegradation capacity. The ratio between the two parameters (BOD/COD) is called the biodegradability index (BI) and is used as a cut-off point between biodegradable and non-biodegradable waste.Analyzing the results of the physicochemical determinations presented in Table 2, it was observed that the BI obtained for each of the strains was well below the recommended values for different types of waste. Strains 2 and 3 stood out in relation to the other two strains and to the control conditions (Table 2). Generally, the BOD/COD ratio is typically 0.5:1 (0.5) for raw domestic effluents, and can reach 0.1:1 (0.1) for a well-stabilized secondary effluent, as is the case of the tested samples.Thus corroborating to show that the strains were efficient in decolorization when kept under well-established test conditions, in order to assess their capacity to improve effluent water quality. However, biodegradability index values can range from 0.4 to 0.8 for raw municipal effluents. Ratio may exceed 10 for industrial effluents (Abdalla and Hammam, 2014). Thus, with the results presented, it is necessary to elucidate the substances present in the experiment and which, directly and indirectly, influenced the BOD and COD values.

CONCLUSION

The decolorization by the *Aspergillus spp*. strains used showed values according to the quality parameters. More specific studies are still needed for the introduction of this treated effluent in waters below class 4, with the objective that their disposal in the environment will not be harmful neither to consumption nor to human contact, or to faunaand flora.It was demonstrated that the strains decolorized the textile industrial dyes achieving the purpose of the article.Research in this area of textile effluents should be encouraged and, consequently, expanding the number of parameters analyzed, covering more specific characteristics, in order not to harm the environment.

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