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Screening of some Brazilian fungi and bacteria able to decolor a textile effluent and evaluation of effluent toxicity on *Artemia salina*

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ABSTRACT

The screening of organisms capable of decoloring a textile effluent was performed using 4 fungi (F24= *Aspergillus spp*.., F48= *Aspergillus spp*.., F98= *Aspergillus fumigatus*, FTL01=*Trametes lactinea*) and 2 bacteria (T9 = *Bacillus subtilis*, T19 = *Alcaligenes faecalis*). These organisms were submitted to effluent decoloration assays in which the *T. lactinea* fungus demonstrated the greatest decoloration capacity (51.35%); thus, it was selected as a model organism for this study. The effect of pH, temperature and carbon sources on the effluent decoloration rate by *T. lactinea* was studied. The effluent decoloration by *T. lactinea* reached levels higher than 89.77%. Finally, acute toxicity assays on *Artemia salina* revealed that *T. lactinea* treatment reduced the toxicity of raw effluent.

Keywords: biodegradation, textile dyes, water treatment.

Triagem de alguns fungos e bactérias brasileiros capazes de descolorir um efluente têxtil e avaliação da toxicidade do efluente em *Artemia salina*

RESUMO

A triagem dos organismos com capacidade de descoloração de um efluente têxtil foi executada usando 4 fungos (F24 = *Aspergillus spp*.., F48 = *Aspergillus spp*.., F98 = *Aspergillus fumigatus*, FTL01 = *Trametes lactinea*) e 2 bactérias (T9 = *Bacillus subtilis*, T19 = *Alcaligenes faecalis*). Esses organismos foram submetidos ao teste de descoloração do efluente bruto, no qual o fungo *Trametes lactinea* foi o organismo que demonstrou maior capacidade de descoloração (51,35%) e, desse modo, foi selecionado como organismo modelo para este estudo. *T. lactinea* foi submetido ao teste de descoloração na presença de diferentes variáveis (valores de pH, temperaturas e fontes de carbono) e a descoloração por *T. lactinea* chegou a níveis superiores a 89,77% de descoloração. Por fim, os testes de toxicidade aguda em *Artemia*

salina indicaram que houve redução da toxicidade do efluente bruto após o tratamento com *T. lactinea*.

Palavras-chave: biodegradação, corantes têxteis, tratamento de água.

1. INTRODUCTION

Textile chain production is known to use the greatest amount of water, which generates high volumes of effluents associated with large amounts of chemical compounds used during industrial operations: heavy metals, detergents and dyes applied in the dyeing process of the fibers (Kadam *et al.*, 2018). Currently, around 10,000 tonnes of dyes are used in the textile industry, and without proper treatment these effluents will include various compounds used during manufacturing (Chandanshive *et al.*, 2020). Thus, the load of compounds present in effluents can affect aquatic fauna, through bioaccumulation and increased toxicity (Alves, 2013). In addition, the dyes, once discarded in the environment, form a barrier that inhibits the penetration of sunlight, which affects the ecosystem through the alteration of photosynthetic activities (Elgarahy *et al.*, 2021).

Therefore, textile effluents constitute an environmental problem, which requires effective solution systems. With this perspective, the Conselho Nacional do Meio Ambiente (CONAMA) established standards for the disposal of textile effluents (CONAMA, 2011), and required industries to use chemical and physical effluent treatment procedures. However, most of these treatments are costly and are not sustainable alternatives (Akpomie and Conradie, 2020; Deshmukh *et al.*, 2016). Biological models of textile effluent treatments can act on dyes that are difficult to eliminate by conventional methods, and the biological models can be seen as sustainable treatment alternatives in the textile industry (Deshmukh *et al.*, 2016). Biological treatment by organisms may occur by several mechanisms, such as the mechanism of degradation by enzymes (biodegradation) and the mechanism of adsorption of particles or molecules (biosorption) (Roy *et al.*, 2018; Ezike *et al.*, 2020).

Despite the existence in the literature of biological organisms capable of biodegrading textile dyes, such as some species of *Trametes*, few can be applied to the biotreatment of raw effluent effectively, considering that most of the works use synthetic effluents, which generates the need to further studies or prospecting for new strains with favorable biotreatment characteristics (Monsalve *et al.*, 2017; Santana *et al.*, 2021). Therefore, due to the lack of organisms capable of textile effluent's bioremediation, this work aimed to bioprospect new organisms with bioremediation potential, and in this way contribute to the scenario of biotreatment of Brazilian textile effluents.

2. MATERIAL AND METHODS

2.1. Effluent samples and organism selection

The effluent samples were collected from the Denim Laundry Treatment Station, located in the Clothing Pole in the Agreste Region of Pernambuco, specifically in the Textile Industrial Pole in Caruaru City-State of Pernambuco. The effluent pH and temperature were determined. The organisms used in this work were provided by the Microorganism Collection of the Department of Antibiotics at the Federal University of Pernambuco, from which 6 organisms previously identified were used in this work (F24 = *Aspergillus spp*..; F48 = *Aspergillus spp*..; F98 = *Aspergillus fumigatus;* FTL01 = *T. lactinea,* T9 = *Bacillus subtilis,* T19 = *Alcaligenes faecalis*). The biological organism selected as a model was identified through analyses of the ribosomal DNA regions ITS and LSU in the work of Santana *et al.* (2021).

2.2. Screening of biological organism assays

Initially, the fungi were cultivated in Potato Dextrose Agar (BDA) medium and the bacteria were cultivated in Brain Heart Infusion (BHI) for 5 days at 25°C. At the end of the pre-inoculum stage, 5 blocks of 9 mm agar were removed and added into an Erlenmeyer flask containing 25 mL of crude effluent and 5% glucose (v'm); the flasks were incubated for 5 days at room temperature (± 28ºC), in triplicate. The supernatants were subjected to spectrophotometry readings at a wavelength between 600-700 nm. To select the organism with greater decoloration capacity, the data obtained in absorbance were submitted to the calculation of the decoloration index (%) following the methodology of Chen (2002), using Equation 1:

$$\% D = \frac{Abs\ o - Abs\ fi}{Abs\ o}\ x\ 100 \quad (1)$$

Which:

% D – Percent decoloration

Abs O - Initial absorbance

Abs FI - Final Absorbance

2.3. Influences of factors on discoloration assay

To verify the influence of factors on decoloration, tests were carried out for pH values (4, 5, 6 and 7), distinct temperatures (25°C, 30°C, 40°C and 45°C) and carbon sources 0.05 g L^{-1} (galactose, glucose, maltose, starch and sucrose). These assays were carried out in triplicate for 5 days in an Erlenmeyer flask with 25 mL of the raw effluent associated with the organism obtained from the screening. Aliquots were withdrawn every 24-h for 120-h and were analyzed by spectrophotometry and using Equation 1.

2.4. Toxicity effluent assay

Acute toxicity tests were performed using the *Artemia salina* model following the methodology of Meyer *et al.* (1982) and ISO/TS 20787:2017 standards (ISO, 2017). The Artemia cysts were obtained from a local aquarium shop. Initially, with adaptations from the research group, a solution was prepared using 30 g L^{-1} of NaCl in autoclaved distilled water and the pH value was adjusted (8-9) with sodium hydroxide (NaOH). Artemia cysts were added to 200 mL of saline solution in an Erlenmeyer flask and incubated in positive photoperiod for 48-h with constant aeration using an oxygen pump for aquariums. After the period of eclosion of the cysts, aliquots of the solution of eclosion were used to make dilutions with the test effluents (20, 40, 60, 80, 100). The dilution solution was associated with NaCl and the pH was regulated (8-9). Each dilution was added to 6 well plates in a volume of 10 ml per well. Each exposure was carried out in triplicate in positive photoperiod and the density was ten nauplii of Artemia (Instar II) per well. The immobility rate of Artemia nauplii was evaluated within 24-h and 48-h of exposure following the ISO/TS 20787:2017 standards.

2.5. Statistic assays

For data interpretation, the results were submitted for INOVA using the statistical program Graphpad.

3. RESULTS AND DISCUSSION

3.1. Screening of organisms with best decoloration

Based on the absorbance and using Equation 1 it was possible to establish the percentage of decoloration of each test organism to choose the best strain to proceed with the following

experiments. Table 1 shows the percentages of discoloration of each organism tested, in which the highest rates of discoloration were F48, FTL01 and BT9 with percentages of discoloration of 42.56, 51.35 and 44.93%, respectively.

Table 1. Decoloration (%) of raw effluent by organism tested -F24 = *Aspergillus spp*.., F48 = *Aspergillus spp*.., F98 = *Aspergillus fumigatus*, FTL01 = *Trametes lactinea*, T9 = *Bacillus subtilis*, BT19 = *Alcaligenes faecalis.*

Organism	% Decoloration
F24	41.89
F48	42.56
F98	22.29
FTL01	51.35
BT9	44.93
BT19	20.27

In terms of biodegradation of textile effluent, especially in terms of decoloration, bacteria are used in the biodegradation of dyes. Although in the present study *A. faecalis* (BT19) did not demonstrate satisfactory decoloration (20.27%), this bacterium has already been reported in the literature with the ability to efficiently remove textile dyes, as demonstrated in the study by Hossen *et al.* (2019) who used *A. feacalis* and obtained 90% decoloration of the azo dye Novacron Super Black (200 mg L^{-1}) after 96-h of cultivation.

B. subtilis (BT9) was the bacterium with the greatest decoloration capability (44.93%). *B. subtilis* is already described in the literature as a bacterium capable of removing dyes used in the textile industry, as shown in the work by Upendar *et al.* (2016), who used *B. subtilis* immobilized in a calcium alginate matrix to remove methylene blue organic dye and reached a removal rate of 90%. Furthermore, Sarim *et al.*, (2019) studied the ability of *B. subtilis* to remove the Congo red dye; at the end of the experiment they obtained a removal of 92.8% at a temperature of 35°C and pH 7.0 and the mechanism of action associated with the adsorption process.

The genus *Aspergillus* have already been associated with the ability to remove dyes, including *A. tubingensis*, *A. niger* and *A. fumigatus* (Ghany *et al.*, 2019; Hamad and Saied, 2021; Karatay *et al.*, 2021). However, the fungi of the genus *Aspergillus* (F24, F48, F98) did not show satisfactory decoloration in relation to the other organisms tested in this work. With regard to biodegradation, *Trametes* is a fungus related to wood degradation; its enzymatic apparatus and its adsorption capacity favor the degradation and removal of recalcitrant molecules such as those of dyes present in textile effluents (Ezike *et al.*, 2020).

Thus, several applications of different species of *Trametes* are reported in the literature in relation to the biodegradation of dyes used in the textile industry. For example, in the work of Noreen *et al.* (2021), who used laccase enzymes from *T. versicolor* immobilized in a matrix of polyvinyl alcohol (PVA) and alginate for the decoloration of various textile dyes and achieved a decoloration of 92% of the Blue reactive dye and 77.4% of the Acid Black 172. Thampraphaphon *et al.* (2022) tested the ability of the fungus *T. hirsuta* to decolorize a mixed textile dyes (Navy EC-R, Ruby S3B and Super Black G) in a real sample of textile effluent, thus achieving an optimal decolorization of 95.39% in the presence of ammonium nitrate. The species *T. lactinea* was shown as a source for biotreatment of textile effluent with its ability to remove indigo-carmine dyes with a percentage of 85.06% (Santana *et al.*, 2021). However, there are few articles in the literature that report this organism and its ability to decolor and biodegrade textile effluents. The fungus FTL01 showed a decoloration of 51.35%; it was

selected as an organism (model) for the other assays in this work.

3.2. Variables that influence discoloration by *T. lactinea*

Figure 1 shows the influence of pH value on decoloration by *T. lactinea* and demonstrates that in the first 24 h the tests with pH values of 5, 6 and 7 revealed higher percentages of decoloration whereas the pH values 4 and 8 showed lower efficiency in the initial decoloration. The pH value of 5 induced the highest response in the initial and final time of the assay, and in this condition the fungi reached a decoloration of 87.55%; thus, the pH 5 proved to be the best for the discoloration test.

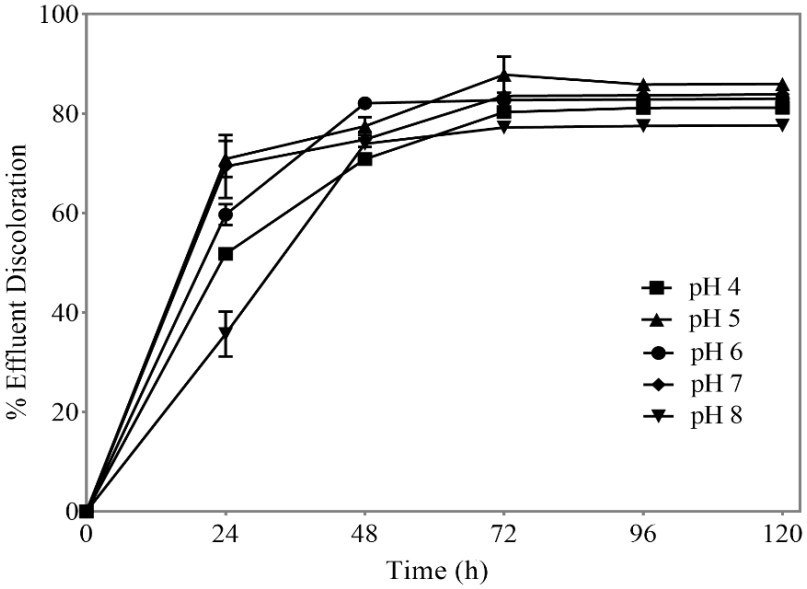

Figure 1. Influence of pH value in decoloration of textile effluent by *T. lactinea*.

The pH 5 value gave the best decoloration response in this experiment; which corroborates with data reported in the literature, especially when the mechanism of action of the organism was by the biodegradation method using enzymes such as the enzyme laccase. Thus, in the work of Ezike *et al.* (2020) they have done purification and characterization of a new laccase from *T. polyzona* and the enzyme showed stability at a pH value of 5.5-6.5. Furthermore, laccase was reported with better activity and stability at pH 5 value in the work of Rangelov and Nicell (2015). Thus, one can hypothesize that the production and activity of enzymes may be one of the mechanisms of action of decoloration by *T. lactinea* in this work. For this reason, the best pH value for decoloration may coincide with the pH value of greater stability and activity of these enzymes (Baccar *et al.*, 2011).

In addition, based on Figure 2, which shows the influence of temperature on decoloration, it can be inferred that in the first 24-h the temperature of 30°C was the temperature that generated greater efficiency in decoloration followed by temperatures of 25°C and 40°C. The best temperatures for decoloration at the end of the decoloration process were 25°C, 30°C and 40°C reaching proximally 78.96% of decoloration.

The temperatures attributed to the biodegradation of recalcitrant molecules by *Trametes* were between the 20°C, 30°C and 40°C spectra and when the temperatures were increased there was a reduction of biodegradation (Zdarta *et al.*, 2018). Thus, the results shown in this present work corroborate the literature data, mainly with regard to the temperatures of the enzymes involved in the degradation of dyes, such as laccase which in previous works showed better activity of the enzymes in the temperature range between 20-60°C (Ezike *et al.*, 2020).

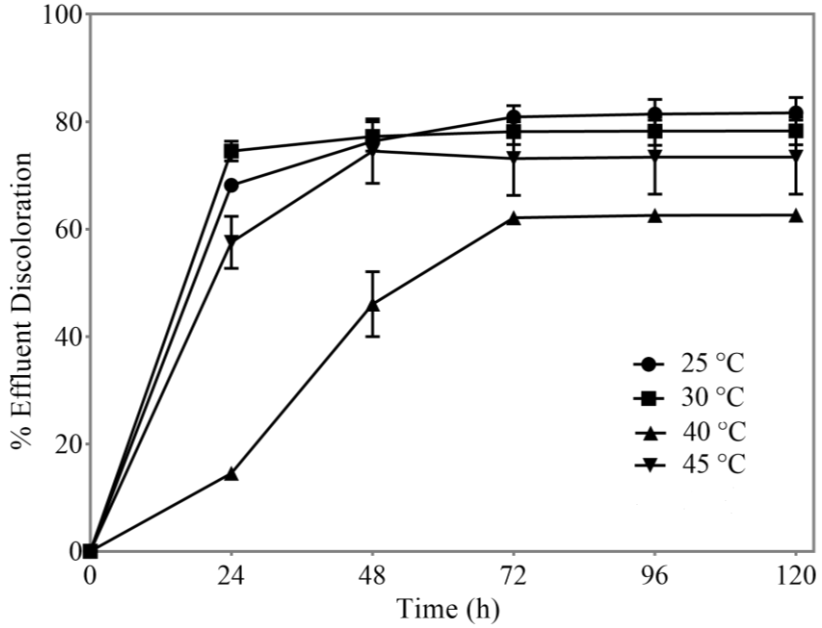

Figure 2. Influences of temperatures in decoloration of textile effluent by *T. lactinea*.

In relation to the influence of the carbon source on the decoloration shown in Figure 3, the tested carbon sources revealed statistically differences in the first 24-h. At the end of the experiment the galactose, starch and sucrose were statistically similar with decoloration in the range of 70%. On the other hand, the maltose, although it reached one of the best decolorations in the first 24-h, was the one that presented the lowest decoloration at the end of the process remaining in the range, and it was demonstrated in other works mainly for the production of lignocellulosic enzymes as in a research that used the species *T. versicolor* (Iqbal *et al.* 2011). Furthermore, in the work by Ado *et al.* (2019) glucose proved to be one of the best sources for the production and activity of the laccase enzyme by *Trametes sp*.

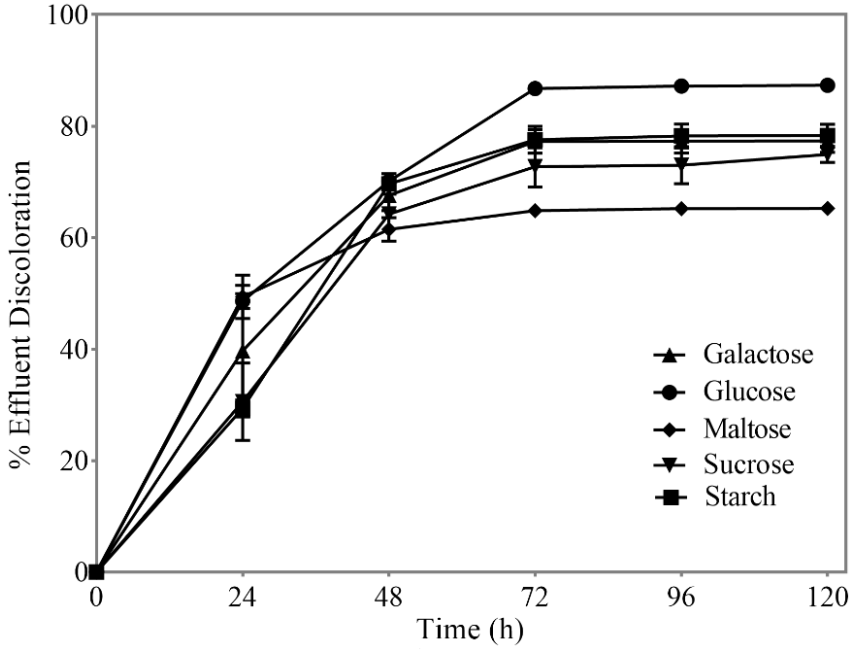

Figure 3. Influences of carbon sources in decoloration of textile effluent by *T. lactinea*.

3.3. Toxicity on *Artemia salina*

The *A. salina* toxicity assay is widespread as a model for acute toxicity tests in treatments of textile effluents of various types, such as in physical treatments of electrocoagulation, electro-oxidation and adsorption on charcoal, in the work of Gilpavas *et al.* (2020). Furthermore, Kalivel *et al.* (2020) also used this test to infer the influence of intermediate products from the electrocoagulation treatment of textile effluents. With the methodology used in this work, it was possible to obtain data that related immobilization and the different concentrations of effluents (20%, 40%, 60%, 80% and 100%) after biological treatment with *T. lactinea* in comparison with raw effluent. According to Figure 4, the treated effluent by *T. lactinea* showed reduction of immobilization in all results when compared with the same dilution in the raw effluent. In addition, in the raw effluent the highest immobilization was at 80% dilution in 48-h test, whereas in the treated effluent the maximum mortality only occurred at the 100% dilution, indicating that there was a reduction in the toxicity of the effluent when compared to the raw effluent.

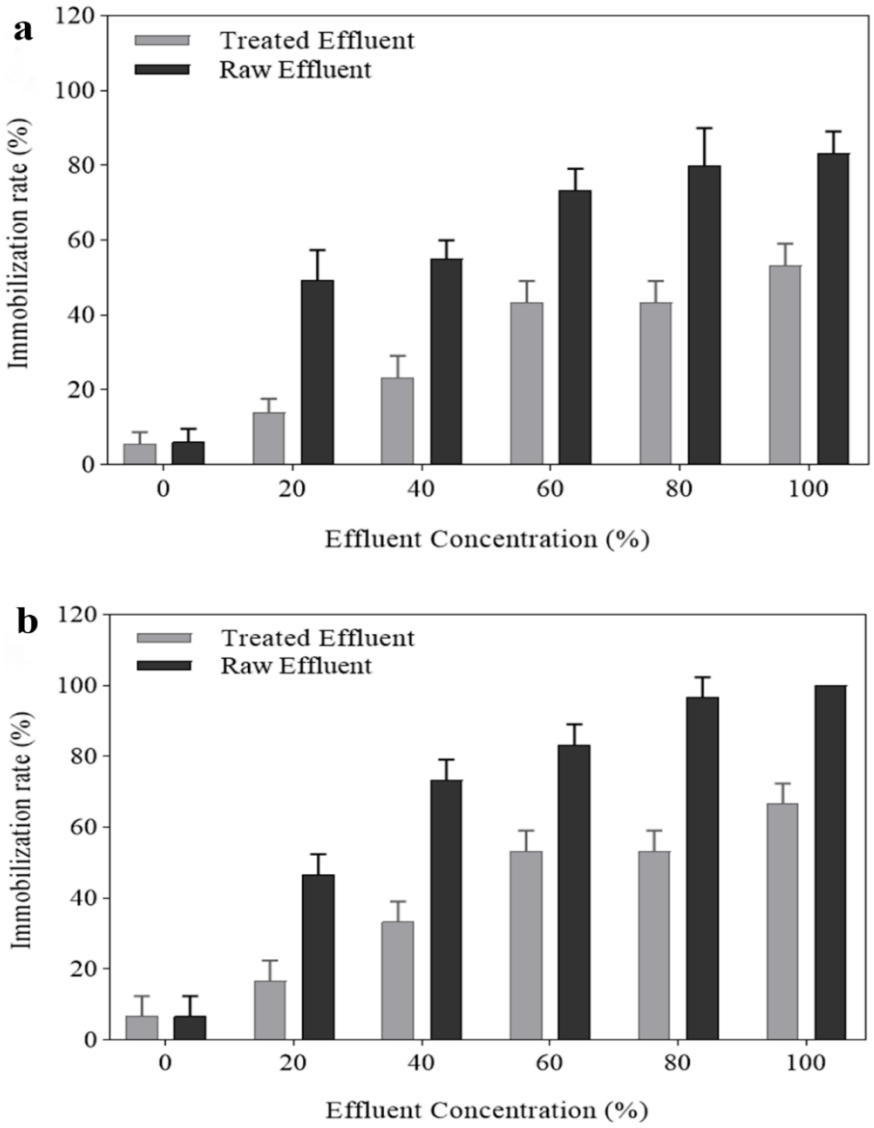

Figure 4. Immobilization rate (%) of *A. salina* in the presence of different concentrations of treated effluent and raw effluent. **a**. After 24-h. **b**. After 48-h.

4. CONCLUSIONS

Based on the results achieved in this work, it was possible to screen the organisms capable of decoloring textile effluent, and consequently, to obtain the fungus *T. lactinea* as a model. There was also an improvement in the decoloration by this fungus when changing the carbon source factors, regulating the pH values and changing the temperature, thus reaching a decoloration of 89.77%. *T. lactinea* was able to reduce the toxicity of the raw effluent through the acute toxicity assays on *A. salina* and thus the fungus was not only able to decolorize efficiently but also reduced the toxicity of the raw effluent. In future projects, tests will be necessary to indicate which mechanism *T. lactinea* is using to cause effluent decoloration. This will enhance the use of *T. lactinea* as a promising source of biotreatment model of textile effluents.

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