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# DESCOLORIZATION OF TEXTILE EFFLUENTS BY A CONSORTIUM OF MIXED MICROORGANISMS OF FUNGI AND BACTERIA IN A BIOREACTOR SIMPLE AND SEQUENTIAL BATCHES

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**ABSTRACT**

This work aims to optimize the treatment of industrial effluents from the Jeans laundry treatment station using microorganism consortia. For the formation of the consortium, 1 strain of fungus and one of bacteria was used. The consortium formed by *Phanerochaetechrysosporium* (F101) and *Bacillussubtillis* (T9) discolored the effluent in percentage above 70, after 6 days in both single and sequential batch, however, the toxicity was reduced by 73% for single batch and 84,39% in the sequential batch and the enzymes of the lignolytic group were detected.The determination of total proteins produced in batch effluent treatments. In both treatments the best specific activities can be observed with 2 days of treatment. After successive increments of untreated effluents, the sequential batch presented higher dosages than the simple one, reaching 0.4246mg/mL with 8 days of treatment and at the end of the assay with 0.3168mg/mL of total proteins.



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## INTRODUCTION

The textile industry uses in its processing several types of chemical compounds including dyes, in the dyeing and washing of textile fibers and fabrics a large of effluents is generated. And when released into water bodies cause great disturbance to organisms present at these sites due to the different chemical constituents present in these effluents, besides having carcinogenic and mutagenic properties. It is also known that a small amount of dye causes significant color changes in the waters of rivers and lakes, preventing the transmission of sunlight to living beings causing them to die and aesthetically repudiating society.Miranda 'et al'(2013); Afreen'et al' (2016); Raman 'et al' (2016). In view of this, various technologies such as chemical oxidation, coagulation-flocculation, membrane filtration, photo-catalysis and biodegradation are developed to treat textile effluents and minimize the high volumes of water that are discarded.

There are physical, chemical and biological treatments, however some of them are economically unfeasible and besides not having the expected efficiency, they can generate undesirable by-products.Zucca'et al' (2015). The bioremediation by microorganisms has proved efficient and in many cases the costs are used in pure cultures or consortia in the treatment of textiles and textile dye waste. Fungi have some mechanisms of degradation and discoloration of dyes and effluents such as biosorption (adsorption is retention of pollutant molecules on the cell wall surface), biodegradation (there is a breakdown of dye molecules), mineralization (is the complete metabolization of molecules dye or textile effluent into CO2 and water). Choosing the bioremediation method for wastewater recovery from textile industries is an essential step because of its composition. Dellamatrice 'et al' (2016). The most used enzymatic biodegradation occurs through extracellular oxidase pathways, including lignin peroxidase

(LiP), manganese peroxidase (MnP) and lacase (Lac), which participate in the degradation of lignin, that is, they could oxidize phenolic compounds. They are classified into phenoloxidities, widely applied in the treatment of dyes and textile effluents, divided into two groups, those dependent on peroxide or peroxidase, manganese peroxidase (MnP) and lignin peroxidase (LiP), and the laccase group (Lac) that do not depend on peroxide. Several studies have reported the use of these enzymes in the degradation of synthetic dyes and textile effluents. Lu 'et al' (2016). MnP (E.C 1.11.1.13) are glycoproteins, have the ability to oxidize various substrates through reactions that oxidize Mn + 2 to Mn + 3. These glycoproteins act as a redox mediator of phenolic substrates and amines. LiP EC (1.11.1.14) is a glycoprotein containing protoporphyrin IX capable of oxidizing phenolic and non-phenolic substrates in the presence of hydrogen peroxide. Through the transfer of electrons and forming intermediate radicals. Lacase is an oxidoreductase enzyme (EC 1.10.3.2.) Has been found in plants and fungi, bacteria, and algae, contains in its active site copper, capable of oxidizing aromatic and phenolic compounds and is therefore part of the group of polyphenoloxidities. Basically, the action is the removal of an electron from hydroxy phenolic or amino aromatic groups generating free radicals or phenoxyls. Esposito & Azevedo (2010). The objective of this work was to analyze the discoloration of carmine indigo dye and textile effluent from industrial laundry of jeans by microbial consortium in batch test.

## MATERIAL AND METHODS

**Textile effluent:** The textile effluent was obtained from the storage tank located in a denim textile industry located in the city of Caruaru (PE / Brazil). The following physicochemical characteristics of the textile effluent were determined: temperature, color, pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), organic load, total hardness, total phenols, total nitrogen, total dissolved solids (TDS), total suspended solids (TSS), total carbon (TC), total chromium, total phosphorus, oils, and greases. The absorbance scan on the UV-Vis absorption spectrum of the textile effluent tested in the experiments was obtained by spectrophotometer (Thermo Fisher Scientific, model EVO 60) in the range between 400-900 nm.

**Microorganisms:** For this study, 10 strains of filamentous fungi and bacteria, provided by the Micoteca (URM) of the Department of Mycology and of the Culture Collection ofmicroorganisms (UFPEDA) of the Department of Antibiotics of the Federal University of Pernambuco, Brazil. The maintenance of the isolates was done successive transfers every two months, in Petri dish and test tubes, containing Potato dextrose agar (BDA) medium for fungi and Nutritious agar (AN) for bacteria.

**Selection of strains for discoloration of dye and textile effluent:** The strains were tested for degradation potential of the indigo carmine textile dye using a synthetic medium composed of the dye and the textile eluent (1ppm) plus agar. The strains were incubated in growth-specific media and transferred in blocks of agar to plates with dye medium. The strains that presented a degradation halo greater than 0.5 cm after 72 hours of incubation were selected.

**Consortium selection for textile effluent discoloration:** A petri dish antagonism test was performed with the tested strains (Fungi and Bacteria), to prove that there was no inhibition of growth.The strains were inoculated in Petri dishes containing SAB and AN culture medium. After 5 days of incubation at 30ºC, three blocks of mycelial growth agar were removed from each microorganism, forming mixed consortia between a fungus and a bacterium, and inoculated in Erlenmeyer flasks (250 mL) containing 50 mL of the textile effluent. Mtui et al'(2008). And incubated for 5 days at 30ºC, all experiments were performed in triplicate and in static condition. For dye discoloration analysis aliquots were taken at the end of incubation and subjected to spectrophotometer absorbance readings (Thermo Fisher Scientific, model EVO 60) at the wavelength obtained by scanning. This analysis was performed by reducing the absorbance obtained in the control, the discoloration percentage (% D) was calculated using the formula: %D = initial time absorbance - final time absorbance/ initial time absorbance *100.

**Enzyme Quantification:** The consortia, which obtained effluent discoloration equal to or greater than 70%, were inoculated in Petri dishes containing specific medium plus carmine indigo (5 mg/L) and incubated at 28ºC for 5 days. From these three blocks of agar were removed and inoculated into Erlenmeyer flasks (250mL) containing liquid culture medium according to Miranda 'et al' (2012), (0.05 g yeast extract, 0.2 g $KH_2PO_4$, 0.05 g $MgSO_4$, 0.02 g $CuSO_4$, 0.016 g $MnSO_4$, pH 7.5, 1 Liter of textile waste) at 30 °C for 10 days under static conditions. Analyzes were performed from samples taken from the flasks at the end of incubation for protein and enzyme analysis Lacase (Lac), lignin peroxidase (LiP), manganese peroxidase (MnP) through spectrophotometer absorbance readings. Lac activity was performed according to the following methodology of Buswell'et al' (1995), in which 0.1 ml of 0.1 M sodium acetate buffer (pH 5.0); 0.8 ml ABTS solution in 0.03% (w/v); 0.1 ml enzyme extract. The absorbance was read at 420 nm at 5 and 10 min, and the coefficient used to calculate U (international enzyme units per liter) was E = 36000 M-1 cm -1. An enzyme unit (1U) was defined as the amount of enzyme capable of oxidizing 1.0 mol of substance per minute per mg of protein. For MnP according to de Kuwahara'et al' (1984), 250 µL of the enzyme extract; 100 µl phenol red (0.01% w / v); Manganese sulfate $MnSO_4$ (2 mM); Hydrogen peroxide (20mM, pH 4.5). The mixture was incubated at 30 °C for 10 min and the reaction was stopped by 100 µL NaOH (2N). The absorbance was read at 610 nm. One unit of enzyme (1U) equals the amount of enzyme capable of oxidizing 1.0 mol phenol red / L / min per mg protein. The coefficient used to calculate U was E = 4466 M-1 cm -1. For LiP was followed the methodology of Buswell'et al' (1995), 1mL sodium tartrate buffer 125mm pH 3.0; 500µL of 10mM veratrylic alcohol; 500µL of 2mM hydrogen peroxide; 500 µL of the enzyme extract. Absorbance was read at 310 nm at 0 and 5 min. One unit of enzyme (1U) equals 1µmol of product formed per minute per mg of protein. The coefficient used to calculate U was E = 9300 $M^{-1}$ $cm^{-1}$. Specific enzyme activity was determined in µmol $minute^{-1}$mg protein-1 (U/mg $protein^{-1}$).

**Textile effluent discoloration in batches:** The selected consortium was used in the tests to discolor the textile effluent. A pre-inoculum was prepared with three mycelial-growing agar blocks of each selected strain, inoculated into 6 Erlenmeyer flasks (250 mL) containing 50 mL of non-autoclaved textile effluent totaling 300 mL of pre-inoculum

and incubated for 48 hours at 30 °C. Control consisted of the same conditions, but without inoculum. After the incubation period the pre-inoculum was transferred to a biological reactor containing textile effluent with a final volume of 6 liters. The experiment was performed in two batches, one reactor received the effluent only once. While the second received 3 liters of untreated effluent in sequential batches every 96 hours. 10mL aliquots were taken every 48 hours of incubation for discoloration percentage analysis and enzymatic quantitation. To analyze the discoloration percentage of the textile effluent, the samples were centrifuged and analyzed by spectrophotometer (Thermo Fisher Scientific, model EVO 60) to obtain the UV-Vis absorption spectrum and then the percentage of discoloration (% D) was calculated.

**Protein quantification in textile wastewater treatment:** To quantify Total Protein Activity (mg/mL), aliquots were taken every 48 hours from each biological reactor and subjected to the total protein dosing methodology according to the Bradford (1976) method.

**Toxicity:** To analyze the toxicity of biotreatment products *Cucumissativus* (cucumber) seeds were used according to Tiquia'et. al' (1996). For this experiment, 5 mL of the product, filtered on a 0.22µm syringe filter from each batch assay, was inoculated into qualitative double filter paper Petri dishes and sterilized with 10 bean seeds placed equidistant from each other. The seeds were disinfected in 1% hypochlorite and sterile distilled water. A positive control with sterile distilled water, a negative control with cell free textile effluent was also added. Experiment performed in triplicate, incubated at 25 ° C for 7 days. After the incubation period, the germination index was analyzed using the formula IG = (% Seed Germination) x (% Root Growth) ÷ 100. To obtain the seed germination percentage (% G = (average germinated seed / average germinated control seeds) * 100) and the average root growth percentage (% CMR = (root mean growth / control root mean growth) * 100).

## RESULTS AND DISCUSSION

**Characterization of textile efluente:** The physicochemical characterization of textile laundry effluent was performed by the Pernambuco Institute of Technology (ITEP), using the Standard Methods for the Examination of Water and Wastewater, 22st edition, 2012. The following parameters were observed. Temperatura: 28 $^{o}$C; pH: 6,5; Color: >500; COD: 1000 mg.L$^{-1}$; BOD: 208 mg.L$^{-1}$; COD/BOD: 0,83 KgBODQm$^{3}$; Total hardness: 135,7 mg $CaCO_3$/L; Total Fenol: 0,09 mg/L; Total Nitrogen 8,2 mg/L; TSD 2121 mg/L; TSS 2028 mg/L; Fat oils: 113 mg/L; Chrome <0.03 mg/L Cr; Phosphorus: 4.79 mg/L P.The data confirm that the textile effluent under study is a potentially harmful waste if released into untreated water bodies, according to Lau and Ismail, (2009);Brink 'et al' (2017). The chemical composition of textile effluents varies widely, and dyes present chemical substances as surfactants, acids, bases, among others. The pH 6.5 and temperature 28 °C observed in the textile effluent under study are within the standards determined by Brazil legislation (Art. 21 Res. CONAMA No. 20) and of the São Paulo States (Art. 18 Dec. No. 8468), Rio de Janeiro (Art. 19 Dec. No. 14,250) and Pernambuco (Art. 29 Dec. No. 7,269), which are pH 5.0-9.0 and Temperature <40 °C. Brazil legislation also requires that color levels be up to 75 pt while the analyzed textile effluent was >500 above what is allowed for disposal into the environment. As for BOD and COD rates, the states of São Paulo and Rio de Janeiro require industries to reduce BOD by 60% CETESB (2009). In the textile effluent scan the maximum absorbance was found at 670nm (Figure 1), confirming that in this effluent there are other dyes besides the carmine indigo, so all the following experiments were analyzed in the absorbance observed in the textile effluent at 670nm.

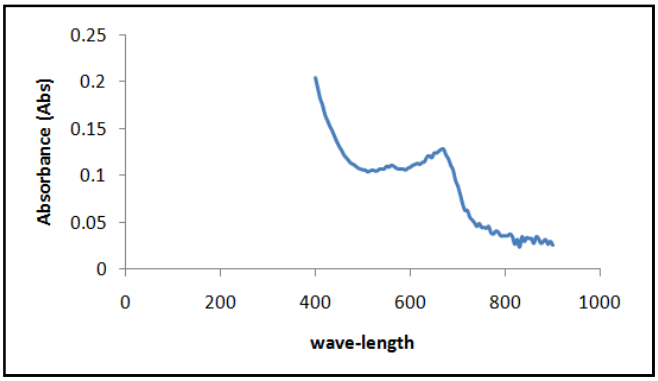


**Figure 1. Floor length scan of the collected textile effluent**

**Consortium selection for textile effluent discoloration:** For the composition of the consortia, the strains were previously submitted to an antagonism test. That consists of submitting the microorganisms to the conditions of growth together and checking the growth inhibition.Of the tested strains, only one composition presented a growth inhibition halo between the fungus F22 and the bacterium T16. Thus, these microorganisms are unable to form a consortium. In the other consortia, the following results were obtained regarding the discoloration of the dye (Figure 2).

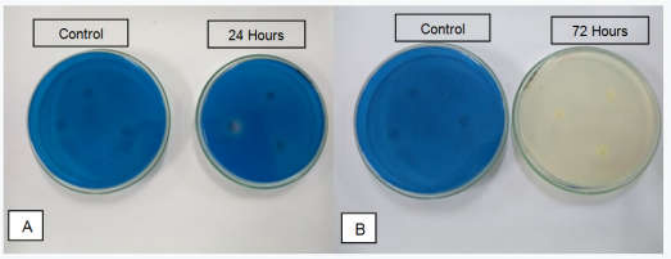


**Figure 2. Selection of strains for discoloration of dye and textile effluent using Petri dishes and indigo carmine dye. (A) Shows the assay with 24 hours of incubation. The strain shown in the image is from the Phanerochaetechrysosporium (F101).and (B) Shows the assay with 72 hours of degradation.The strain shown in the image is from the Phanerochaetechrysosporium (F101)**

Fifteen consortia presented a discoloration percentage equal to or higher than 70%. These were selected for being good degraders. Highlighting the consortium between the fungus *Phanerochaetechrysosporium* (F101) and the bacterium *Bacillus subtilis* (T09) (Figure 3). Yang 'et al' (2016) also observed a good performance of 13 fungal strains out of 92 strains tested isolated from freshwater for discoloration of synthetic dyes reactive red 11, acid red 73 and reactive blue 74, acid blue 40, 62.113, 193. The results corroborate those of several studies that used microorganisms for dye discoloration and degradation, such as Rani 'et al' (2014) observed that the fungi Phanerochaetechrysosporium and Aspergillus niger presented better percentages of basic fuchsin dyes discoloration (81.85% ), nigrosine (77.47%), malachite green

(72.77%) and a mixture of dyes (33.08%) under stirring conditions, which according to the authors favored the discolouration of the dyes, however in our study the fungi were able to discolor the dye in static condition. Many other works describe the potential of fungi in the discoloration of textile dyes. Batista-García'et al' (2017).Lu 'et al' (2017) also showed that the Aspergillus niger ZJUBE-1 strain, in pellet form, discoloured the Congo red azo dye through the biosorption mechanism. The fungus *Trametesversicolor* was also effective in 100% discoloration of reactive black dye 5 under alkaline condition Ottoni'et al' (2016).

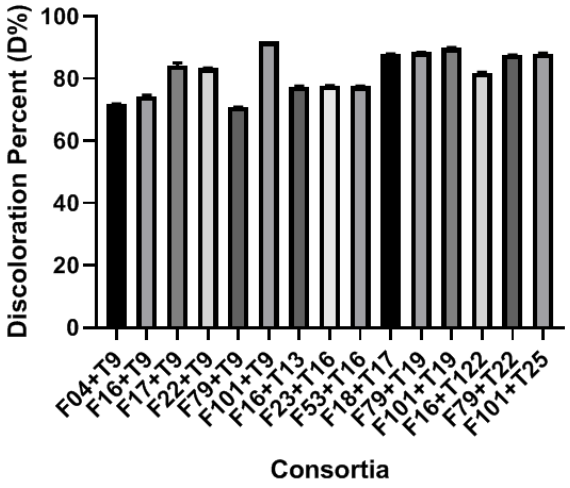


**Figure 3. Percentage of effluent discoloration by microbial consortia**

**Enzymatic quantification of selection assays:** The production of Lac, MnP and LiP enzymes by the selected consortia can be observed in figure 4. All consortiums presented production of the tested enzymes, with better production of Manganese Peroxidases. The consortium x among the tested ones presented better production of the enzymatic group tested, producing 0.495 U/mg of MnP, 0.236 U/mg of LiP and 0.034 U/mg of Lac. According to Kumari'et al' (2016) extracellular enzymes from fungi and fungal coculturescan remove dyes present in recalcitrant textile effluents from the environment. According to Zerva'et al' (2016) lignolytic enzymes have the potential for discoloration and detoxification of dyes and textile effluents, as shown by the work of textile effluents. Neves 'et al' (2013) reported that when working with industrial effluent degradation using fungi, which are better producers of phenoloxidase group enzymes, they obtained yields between 13 to 550 U/mg of Lacase, 0 to 1295 U/mg of lignin Peroxidase and 0 to 155 U/mg Manganese Peroxidases. The specific activity of bacterial lipases isolated from sewage from various genera is in the range of 0.044 to 1.952 U/mg protein.

**Textile effluent discoloration in batches:** The consortium that presented the best discoloration percentage (% D) of the textile effluent in the flask assay was selected for the biological reactor assay. Figure 5 shows that the single batch biological reactor presented a percentage of textile effluent discolouration after 2 days of incubation with 57.69%. On the sixth day, the discoloration rate reached 72.09%, reaching the end of the trial with 79.28%. As can be seen from Figure 6 the sequential batch assay showed a 2 and 4 day treatment discoloration of 57.63% and 57.46% respectively, like that of the single batch. However, after the first effluent increment the percentage of discoloration increased to 74.48% and went from 80% on the eighth day of treatment (81.27%). After the second batch the% D decreased to 79.11% and increased again at the end of the assay reaching 81.80% discoloration.

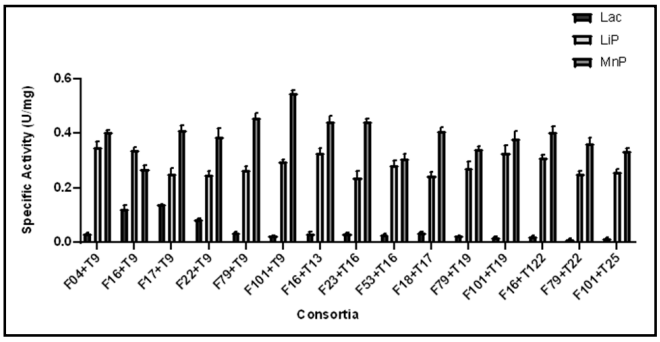


**Figure 4. Activity of Lac, LiP and MnP enzymes from consortium assays in international units per milligram of protein (U/mg) per liter**

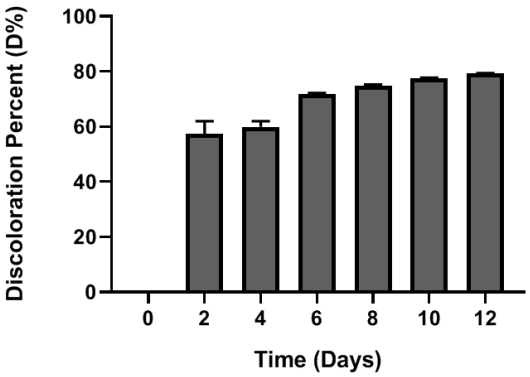


**Figure 5. Textile effluent discoloration in a single batch biological reactor**

The consortium used in this process were able to discolor the textile effluent as reported by Miranda 'et al' (2013) who obtained 98% and 93% discoloration of textile effluent using the fungi *P. crysosporium* URM 6181 and *Curvularialunata* URM 6179 respectively in bioreactor after 10 days of treatment. Fungi of the genus Penicillium have been reported in various works on textile dye discoloration processes, which makes it an efficient fungus for discoloration and degradation of textile effluents. This fungus was reported by Xin 'et al' (2010) in bioaccumulation processes of the Cu-reactive dye in aqueous pellet solutions. In this study it was demonstrated that there was thickening of the hyphae confirming the accumulation of dye in the cytoplasm. *Penicillium* SAR-3 has also been reported to have the potential to degrade various direct and reactive azo dyes, red acid 183, direct red 75, blue acid 161, red acid 88, blue acid 45, black reactive 5 at concentrations of 100-300mg / L .Saroj'et al' (2014). *Fusarium oxysporum* were also able to discolor a mixture of dyes with bright green (triphenylmethane) and evan blue (azo), with *Pleurotusostreatus* presenting the best result around 90%, confirming its potential in the treatment of textile effluents. Przystas'et al' (2015).

**Total protein quantification in batch assays:** Figure 7 shows the determination of total proteins produced in batch effluent treatments. In both treatments the best specific activities can be observed with 2 days of treatment with 0.6265 mg / mL in the single batch and 0.7446 mg / mL in the sequential.

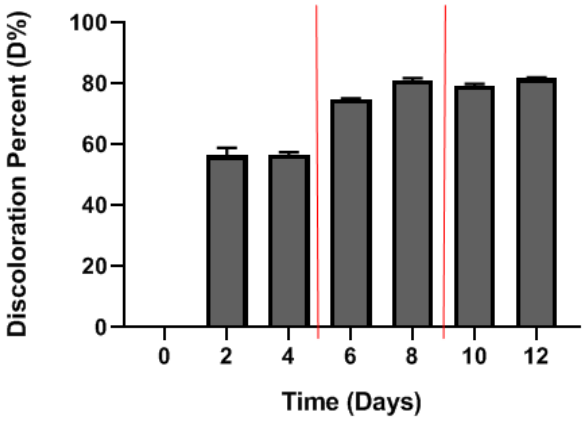


**Figure 6. Textile effluent discoloration in sequential batch biological reactor. The red lines mark the increment of untreated effluent**

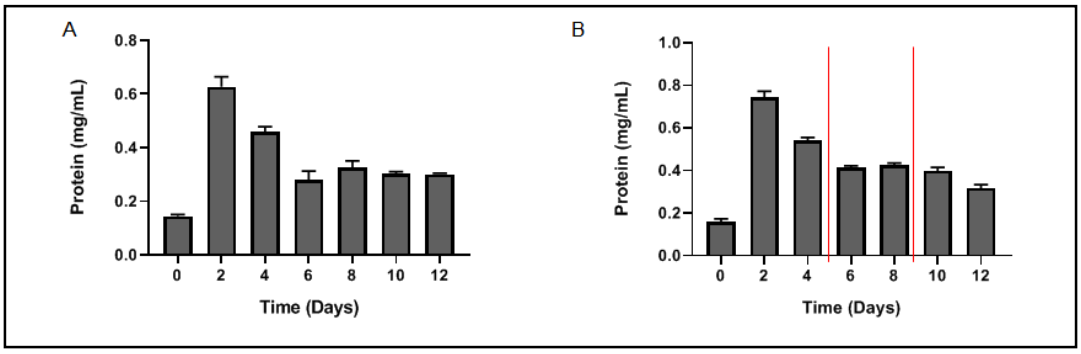


**Figure 7. Total protein dosage by Bradford's method (1976) in the treatment of textile effluent: A) Simple batch; B) Sequential batch. The redlines mark the increase of untreated effluent**

**Table 2. Profile of germination index (GI%) in the treatment of textile effluent with simple and sequential batches**

| Batches | |
|---|---|
| Simple | Sequential |
| 10.00 ±0.0 | 10.00±0.0 |
| 22.36 ±0,12 | 25.75 ±0.11 |
| 31.87 ±0.31 | 33.33 ±0.25 |
| 55.33 ±0.21 | 60.25 ±0.18 |
| 67.24 ±0.45 | 61.46 ±0.35 |
| 69.57 ±1.0 | 72.60 ±0.8 |
| 73.33 ±0.38 | 84.39 ±0,36 |

After successive increments of untreated effluents, the sequential batch presented higher dosages than the simple one, reaching 0.4246mg / mL with 8 days of treatment and at the end of the assay with 0.3168mg / mL of total proteins. According to Kerem'et al' (1992) Laccase can both detoxify substrate compounds and oxidize phenolic groups, acting as the initial enzyme in cleavage of side chains and aromatic rings of phenolic moieties of lignin. Other enzymes of oxidative nature, such as alcohol veratril oxidases, may play an important role in lignin degradation, catalyzing the reaction of veratril alcohol to hydrogen peroxide required for peroxidase activity. Kamida'et al' (2005). According to Kumaran 'et al' (1997) Laccase and manganese peroxidase should act synergistically. Studies on the discoloration of industrial dyes, including indigo, emphasize the role of these enzymes produced by *Pleurotus* species in discoloration. Enzymatic treatment with peroxidase results in the removal of toxic phenolic clusters from industrial effluents. Many of the dyes employed in the textile industry have phenolic clusters in their chemical structure, Durán (2003), and the application of oxidative enzymes represents a promising alternative for the removal of color from textile effluents.

**Phytotoxicity:** To analyze the toxicity of metabolites formed from batch treatment in the study, phytotoxicity was observed, as textile effluents may present toxicity and mutagenicity, as explained by Hemachandra&Pathiratne (2016) who analyzed the cytotoxicity and genotoxicity of textile effluents. two industries using Allium cepa system and erythrocyte comet assay Oreochromisniloticus, which demonstrated dangerous potential of these effluents. Table 1 shows the Germination Index (GI%) profile for single and sequential batches, both trials showed a decrease in toxicity reaching the end of treatment with germination indexes of 73.33% for single batch and 84.39. % in sequence. According to Zucconi'et al' (1985), a GI between 50 and 80% is considered moderately toxic, above 80% is considered non-toxic. It was also observed that the control (without inoculum) presented the IG only around 10%, considered toxic. According to Neoh'et al' (2015) it is necessary to perform toxicity tests of the discoloration treatments because it is possible to produce metabolites that increase the degree of toxicity. As shown by the work of Pizato'et al' (2016) who observed the color removal of textile effluent in 91.2% by the fungus *Lasiodiplodiatheobromae*

MMPI but there was no biological detoxification, reduction of phenolic compounds and no chemical oxygen demand.

**Conclusion**

The consortium discoloration study in this paper showed that fifteen compositions had a discoloration percentage above 70. And a low (<0.6 IU) production of lignolytic group enzymes, which may be an indication of using another metabolic pathway to discolor this textile effluent. The sequential batch study showed higher discoloration percentages than the single batch. The sequential batch assay showed a higher protein profile than the simple one, especially after successive increments. The batches tested efficiently reduced their textile effluent toxicity. The strains used in the consortium were *Phanerochaetechrysosporium* (F101) and *Bacillus subtillis* (T9). And the consortium tested in sequential batch is capable of discoloring and detoxifying the textile effluent analyzed from industrial laundry denim.

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