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ORIGINAL PAPER

Decolorization of textile dye by *Candida albicans* isolated from industrial effluents

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Abstract The aim of the present work was to observe microbial decolorization and biodegradation of the Direct Violet 51 azo dye by *Candida albicans* isolated from industrial effluents and study the metabolites formed after degradation. *C. albicans* was used in the removal of the dye in order to further biosorption and biodegradation at different pH values in aqueous solutions. A comparative study of biodegradation analysis was carried out using UV–vis and FTIR spectroscopy, which revealed significant changes in peak positions when compared to the dye spectrum. Theses changes in dye structure appeared after 72 h at pH 2.50; after 240 h at pH 4.50; and after 280 h at pH 6.50, indicating the different by-products formed during the biodegradation process. Hence, the yeast *C. albicans* was able to remove the color substance, demonstrating a potential enzymatic capacity to modify the chemical structure of pigments found in industrial effluents.

Keywords Textile dyes · Biodegradation · Decolorization · *Candida albicans* · Direct Violet 51 · Biosorption

Introduction

Dyes and dyestuff are used in textile, pharmaceutical, leather and cosmetic industries. The main use occurs in textile manufacturing, resulting in wastewater that contains a variety of these pollutants. The release of dyes is a cause of concern due to the effects on the environment and human health [13]. A number of these dyes are very stable in the presence of light, water and chemicals. Degradation is used in the bioremediation of these dyes and is an attractive solution due to its lower cost and environmentally friendlier technology when compared to chemical and physical treatment processes [14].

The annual world production of dyestuff amounts to more than 7×10^5 tons [17]. It is estimated that 10–15% of the total production of colorants is lost during synthesis and dyeing processes [5]. Pigmented industrial effluent is the most obvious indicator of water pollution. The discharge of highly pigmented synthetic dye is not only aesthetically unpleasing, but causes considerable harmful effects when released in bodies of water. Some methods try to solve this problem through the study of decolorization kinetics and the mineralization of reactive azo dyes in aqueous solution through UV/H_2O_2 oxidation as an appropriate procedure in a textile wastewater pretreatment step [15]. However, treatment with biological agents remains the best solution of such a problem. A large number of microorganisms belonging to different taxonomic groups of bacteria, algae, fungi and yeast and have been reported for their ability to decolorize azo dye [10, 11, 20].

Dye removal by yeast occurs through the physical biosorption of the dye in a non-specific manner to the periphery of the cell, followed by specific accumulation in the wall and interior of the cell. Yeast biomass is an inexpensive, readily available source of biomass that has potential for dye accumulation at lower pH values [4]. *Candida tropicalis* was investigated in the removal of the textile dyes Remazol Blue, Reactive Black and Reactive Red. The results reveal that yeast can bioaccumulate the

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selected dyes to different extents. Remazol Blue and Reactive Black were bioaccumulated to a greater extent, whereas Reactive Red was bioaccumulated within a more limited range.

In the present study, *Candida albicans* isolated from industrial effluents was used in the removal of the Direct Violet 51 azo dye in order to further biosorption and biodegradation at different pH values in aqueous solutions.

Materials and methods

Dyes and chemicals

The azo dye Direct Violet 51 CI 27.905 was obtained from Imperial Chemistry Industries, a dye manufacturing unit in Rio Claro, São Paulo, Brazil. The chemicals used were of analytical grade FW 719.71, with a $\lambda_{max} = 549$ nm. Dye content was 36%.

Microorganism and culture conditions

Growth medium

A mineral salt medium (MSM) with the following composition was used for all the studies (g l^{-1}): Na_2HPO_4 (2.0), $(NH_4)_2SO_4$ (3.0), $MgSO_4.7H_2O$ (0.25), $CaCl_2.7\ H_2O$ (0.25) and trace element solution per liter. The trace element solution had the following composition (mg l^{-1}): $ZnSO_4$ (1.0), aspartic acid (100.0), H_3BO_3 (1.0) and Inositol (50.0). The MSM was supplemented with 0.5% (w/v) yeast extract and glucose g.l^{-1} (20). The yeast extract, glucose and dye were added to the sterilized MSM from respective filter-sterilized stock solutions.

Isolation and screening of dye decolorizing microorganisms

The MSM broth was inoculated with 10% (w/v) sludge/soil samples collected from waste disposal sites of an aromatic and peroxide manufacturing unit near the city of Rio Claro, SP (Brazil). The flasks were incubated at 35 °C under agitation (150 rpm). After 48 h of incubation, 1.0 ml of culture broth was appropriately diluted and plated on MSM-agar containing 10 mg l^{-1} of dye. Morphologically distinct bacterium, yeast and fungus isolates exhibiting clear zones around their colonies due to the decolorization of dye were selected for further studies. The pure culture stocks of these isolates were stored at 4 °C. Identification of the selected yeast was performed using ChromAgar methods and the IDC32C biochemistry test [3].

Dye decolorization experiments

The sterilized MSM was inoculated with 1 ml of the selected yeast, *C. albicans*, cultured in 250-ml Erlenmeyer flasks with 100 ml of medium and incubated at 150 rpm for 24 h at 37 °C. The biomass was removed from the flask (10 ml) and inoculated in 500-ml Erlenmeyer flasks containing 80 ml of sterilized water with pH adjusted at 2.50, 4.50 and 6.50, and 10 ml of dye at a concentration of 1 mg ml^{-1} The initial concentration of dye for the experiment was 100 μg ml^{-1} Flasks were stopped with cotton gauze, cannula and syringe. The same method was used for the biomass autoclaved at 121 °C in 1 atm for 15 min. Flasks in both tests were maintained at 30 °C. Aliquots from 0 to 96 h were withdrawn and centrifuged at $3{,}779 \times g$ for 15 min, for UV–vis spectrophotometer analysis. For the biodegradation test, comparisons were performed between the control dye and samples extracted at different time intervals (0–280 h).

Decolorization assay

The cell free supernatant color was read at the maximum absorbance spectrum (λ_{max}) of dye used—549 nm for Direct Violet 51—using a Shimadzu UV 2401 PC spectrophotometer. Thus, clear supernatant was used to measure decolorization at the absorbance maxima of the dye. An abiotic control (with no microorganism) was always included.

The percentage of decolorization was calculated as follows [16]:

$$\%\ \text{Decolorization} = \frac{\text{Initialabsorbance} - \text{Observedabsorbance}}{\text{Initial absorbance}} \times 100$$

where initial absorbance ($A_{\lambda 549\ nm}$) is the absorbance of the sample with dye but without the microorganism and observed absorbance ($A_{\lambda 549\ nm}$) after any time of contact between the living or dead microorganism and the dye (0–96 h).

Biodegradation analysis

Decolorization was monitored through UV–vis spectroscopic analysis (Shimadzu UV 2401PC) at different time intervals. For the biodegradation analysis, 100 ml of culture broth were taken at different time intervals: 0–72 h for samples at pH 2.50; 0–240 h for pH 4.50; and 0–280 h for pH 6.50. UV–vis spectral analysis was carried out and changes in the absorption spectra (200–700 nm) were recorded.

For the FTIR analysis, samples were dried at 105 °C and mixed with spectroscopically pure KBr at a ratio 1:149. Pellets were fixed in a sample holder and analyses were carried out using a Shimadzu FTIR 8300. Changes in absorbance at different frequencies (4,000–400 cm^{-1}) were observed.

Results

Decolorization assay

The yeast was tested for its ability to decolorize the dye under three different pH conditions as well as with living and dead cells. The overall decolorization of up to 73.2% in non-autoclaved conditions and 87.26% in autoclaved conditions demonstrate the efficiency of dye removal in both cases.

Biodecolorization analysis

The UV–vis scan (200–700 nm) of supernatants at different time intervals and pH values (2.50, 4.50 and 6.50) revealed decolorization and decreased dye concentration in the culture batch. Peak observed absorbance at 549 nm (λ_{max}) decreased with time. The peak at 347 nm after 72 h was absent in pH 2.50. The same occurred after 240 h in pH 4.50 and 280 h in pH 6.50, which indicates the start of the biodegradation process [5].

Biodegradation analysis

The FTIR spectral comparison between the control dye and samples extracted at different time intervals revealed degradation in different metabolites of Direct Violet 51 by *C. albicans*. FTIR spectra of DV 51 revealed the presence of different peaks at 3430.87, 3238.33, and 1104.92 cm^{-1}, which indicates that the N–H stretch represents the formation of primary and secondary amines. The new peak expressed the breakdown of DV51 in various compounds, exhibiting C=O and N–H stretching as well as N–H vibration and C–N deformation at 1636.09 cm^{-1} [9]. The peak at 609.41 cm^{-1} indicates a gain of aromaticity. These peaks appear faster in the reduced medium with a more acid pH (pH 2.50 in 72 h, 4.50 in 240 h and pH 6.50 in 280 h). It seems that azo-reductase from the microorganism catalyzes the reductive cleavage of the azo bond and produces the metabolites identified by the FTIR method [8]. These products are further degraded into aliphatic amines, which may have been facilitated by oxidative enzymes.

Discussion

A number of studies with *C. albicans* indicate that yeast has the potential to develop enzymatic activity involving the production of reductase to degrade several substrates, including the dyes [1, 7, 12, 19]. The best biosorption in the decolorization of direct azo dye occurred in pH 2.50 and the autoclaved biomass exhibited a higher biosorption capacity than the non-autoclaved biomass (Figs. 1, 2). The chemical structure and visible spectrum of the dye are displayed in Figs. 3, 4 and 5. As yeast adsorption and transformation reduced the intensity of the dye in solution, it was necessary to measure soluble dye absorbance at two wavelengths. Absorbance at both wavelengths decreased significantly, whereas the absorbance ratio (A_{549}/A_{347}) remained nearly constant. This was predictable, as adsorption leads to a proportional decrease in absorbance at all wavelengths, resulting in a minimal change in absorbance ratios [6]. Similar results were obtained with the dye in pH 2.50 at 48 h, but at 72 h the 347 nm peak practically disappeared. The same applies to pH values 4.50 and 6.50 after 240 and 280 h, respectively. At these time intervals, the two wavelengths indicated were chosen to produce the greatest change in the absorbance ratio as the dye was degraded. Possible genotoxic effects of textile dyes are discussed most often with respect to certain azo dyes, which contain an azo group (–N=N–) able to split off genotoxic and carcinogenic amines (e.g. Acid Red 85, which releases benzidine). The use of these dyes has been drastically reduced in Europe due to national regulations (e.g. the German Food and Commodities Act; similar regulations exist in the Netherlands and France), but may still be a

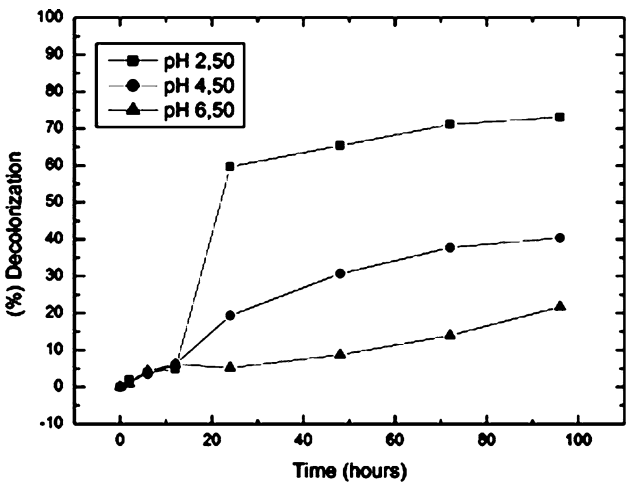

Fig. 1 Temporal evolution of non-autoclaved wild *Candida albicans* and dye decolorization under different pH conditions at an initial dye concentration of 100 μg ml^{-1} in minimum nutrient broth

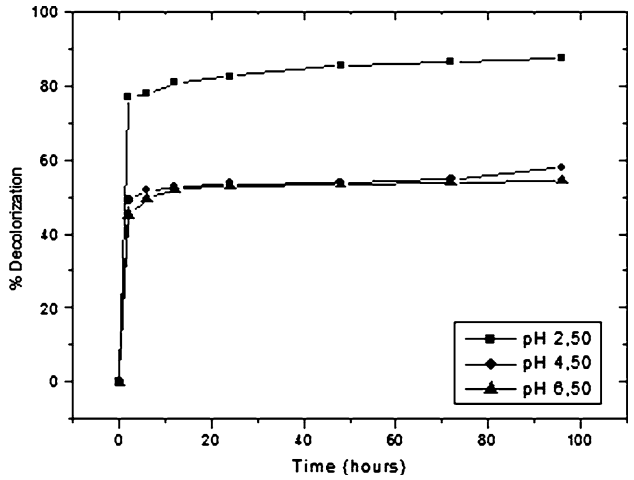

Fig. 2 Temporal evolution of autoclaved wild *Candida albicans* and dye decolorization under different pH conditions at an initial dye concentration of 100 μg ml^{-1} in minimum nutrient broth

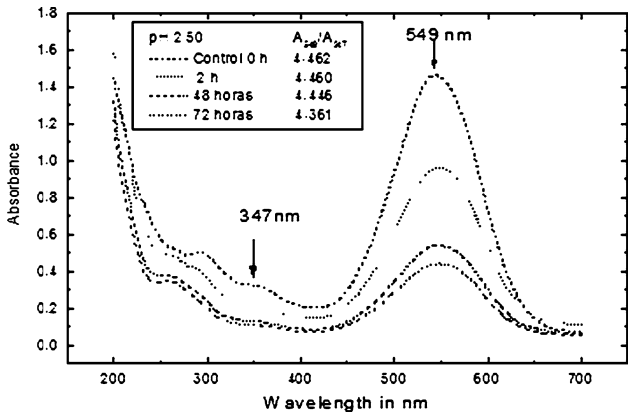

Fig. 3 UV–vis spectral analysis of culture supernatant extracted during decolorization of Direct Violet 51 at different time intervals in pH 2.50

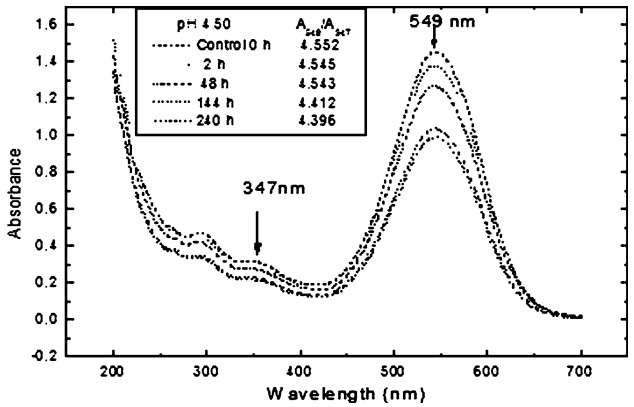

Fig. 4 UV–vis spectral analysis of culture supernatant extracted during decolorization of Direct Violet 51 at different time intervals in pH 4.50

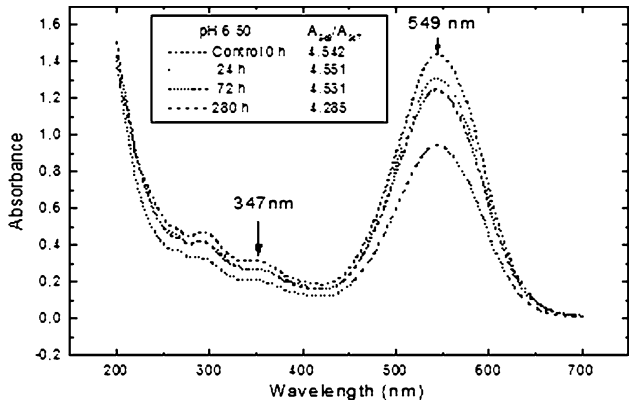

Fig. 5 UV–vis spectral analysis of culture supernatant extracted during decolorization of Direct Violet 51 at different time intervals in pH 6.50

problem in non-European countries [18], where the majority of industries that manufacture these dyes are located and dump their sewage into the environment.

The elucidation of degradation pathways is of special interest regarding health and environmental priorities. This was confirmed by [2], who demonstrated cleavage of the azo bond. The FTIR spectral comparison between the control dye and samples extracted at different time intervals and pH values detected the presence of different peaks, which expressed the breakdown of the dye into various compounds, such as primary and secondary amines and an absence of benzene rings (Figs. 6, 7) [9]. These metabolites still require even more vigorous enzymatic activity to reduce the maximum levels of toxicity of such compounds.

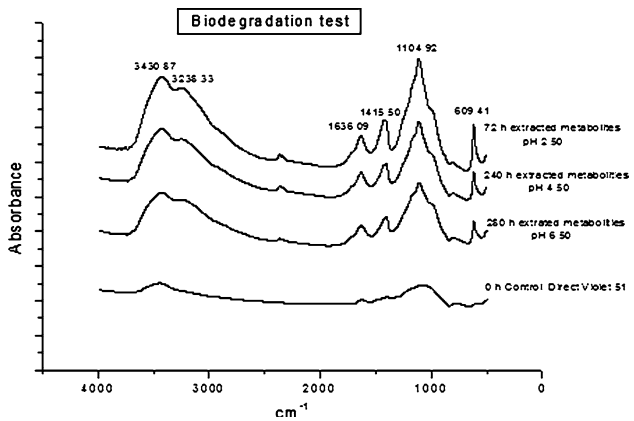

Fig. 6 FTIR comparison of control dye Direct Violet 51 and metabolites formed at different time intervals and pH values during degradation with *Candida albicans*

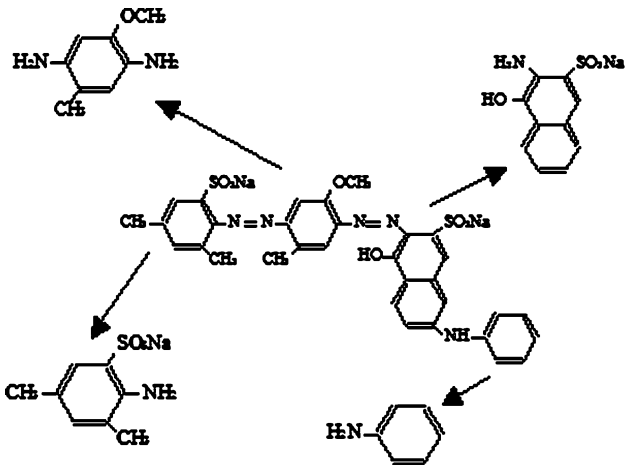

Fig. 7 Proposed pathways for degradation of azo dye Direct Violet 51 by *Candida albicans.* The compounds represent the first possibility of metabolites formed under any pH values at different time intervals during degradation

Conclusion

In all the experiments on biosorption interactions carried out between the biomass and the dye, the autoclaved cells achieved better results than non-autoclaved cells. Comparative UV–vis and FTIR analyses of the biodegradation revealed significant changes in peak positions when compared with the dye spectrum. The changes in the dye structure appeared after 72 h at pH 2.50, after 240 h at pH 4.50 and after 280 h at pH 6.50. This alteration reveals pH-dependent results and indicates the emergence of amine-free primary and secondary metabolic products of dye biodegradation.

The yeast *C. albicans* was able to remove the pigmented substance, demonstrating a potential enzymatic capacity to modify the chemical structure of this dye, which is widely found in industrial effluents.

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