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Biodegradation and detoxification and of an azo dye, Eriochrome black T by genus Penicillium citrinum

Paul Olusegun Bankole
Federal University of Agriculture Abeokuta, Nigeria

Synthetic textile dyes are one among the foremost serious pollutants that contaminate steadily higher amounts of wastewater as industrial ef luents. The dyes are highly recalcitrant due to their chemical structure. Filamentous fungi possess excellent biosorption capacity thanks to the secretion of non-selective extracellular enzymes. The aim of this study is to guage the biodecolorization efficiency of Penicillium citrinum on Ericochrome black T dye. Optimum decolorization (98%) was achieved at a degree (10 mg L -1), temperature (35 °C), and pH 6 during 5 days optimization proportion studies. UV—Vis spectroscopy, HPLC and gas chromatography-mass spectrometry was utilized in analyzing the degraded products of the dye. The GCMS analysis revealed the assembly of three metabolites; naphthalen-1-ol, 2- nitronaphthalene and naphthalene after degradation of Eriochrome black T dye. A possible metabolic pathway for the degradation of Eriochrome black T dye by Penicillium citrinum was proposed. The phytotoxicity study revealed the nontoxic nature of the ultimate metabolites. The detoxified status of the dye metabolites was confirmed with significant growth of plumule and radicle including increase in germination percentage of Vigna uniguiculata and common wheat.

Introduction

Dyes from textile industries may be a problem in large parts of the planet thanks to their chemical compositions. the amount of textile dyes used today exceeds 10 000. Textile dyes are chemicals with complex aromatic structures designed to resist the consequences of laundering and sunshine, for instance. These dyes also are of relatively high relative molecular mass, so it's difficult for several microorganisms to transfer them through their membranes. These dyes are consequently difficult to degrade through microbial processes. Fungi, due their excretion of extracellular enzymes, are known to be ready to degrade – though possibly not completely – structures that are difficult for bacteria to handle. Fungi produce intermediates which will be degraded by bacteria. It is very important to analyse the treated water with reference to the dye content also as intermediates, especially aromatic amines since some are considered carcinogenic. Many earlier studies have focused on UV–vis spectrophotometer analyses of dyes; these analyses show the decolourization and may additionally give a sign of changes of structures of the dyes. Biosorption and biodegradation are two major mechanisms within the biodecolorization of dye wastewater. the previous describes the method mediated by inactivated biomass, and these materials are often mentioned as being biosorbent. In living cells, the 2 mechanisms can act together. Given the big variety of enzymes during a strain and therefore the low selectivity of biosorbents, it's expected that an equivalent strain can decolorize various dyes via different mechanisms, as demonstrated by many examples. The white rot fungus Coriolopsis sp. Was tested to decolorize four pigments of various colors or common backbones, and Phanerochaete chrysosporium showed the decolorization ability for Acid Blue 62, Direct Red 80, and indigo dye. they are doing not, however, separate different intermediates. Some recent studies have used high-performance liquid chromatography (HPLC) to separate the intermediates into different peaks. Other studies have used high-performance liquid chromatography/mass spectrometry (LC/MS) to work out the molecular structures of intermediates. Biodecolorization of a dye, Eriochrome Black T by Penicillium citrinum Science and Technology in Emerging Smart Cities and Sustainable Development. The aim of this study is to guage the potentials of Penicillium citrinum within the biodecolorization of Eriochrome black T dye. Physicochemical parameters were varied to determine the simplest conditions for optimal decolorization. GC-MS and HPLC analyses were carried out to work out the metabolic fates of the dye after the experiment. Toxicity analyses were conducted to verify the non-toxic states of the dye after biodecolorization.

Materials and methods

Isolation and medium

Penicillium citrinum was isolated from a dumpsite at Orita, Ilaro, Ogun State. The medium consisted of the following: 2 gL-1 D-glucose, 2.5 gL-1 NaNO3, 2gL-1 KH2PO4, 1 gL-1 MgSO4·7H2O in 250 mL Erlenmeyer flasks containing 60 mL of sterile medium were incubated during a controlled incubator at 150 rpm for 4 days at 30 °C. The pH of the medium was adjusted to five .0 with NaOH. Chemicals Eriochrome black T dye was procured from Sigma Aldrich, UK. All the reagents were of high purity and analytical grade (>98%). Mycelia Preparation for Decolorization. The pellets were harvested after cultivation of the fungus and washed several times with distilled water before being inactivated at 121 °C for 20 min. The dried mycelia were stored in the refrigerator and used for biodecolorization experiments.

Enzyme assay preparation

To grow the fungal biomass, 4.3 × 10 4 spores' mL −1 of fungal suspension on a Potato Dextrose Agar medium (PDA) was inoculated during a 250 mL Erlenmeyer flask containing a 60mL autoclaved solution of medium with dye of the specified concentration. The flasks were agitated at 150 rpm and at 30 °C for 4 days. The pellets were separated from the fermentation broth by centrifugation and homogenized in 0.05 mol/L, pH 7.0, phosphate buffer. The intracellular enzyme was harvested by centrifugation, and the extracellular enzyme was harvested by ultrafiltration of the cell culture filtrate. The crude enzyme liquid was obtained using a mixture of extracellular enzyme. The assay

was thereafter used for the evaluation of laccase, lignin peroxidase and manganese peroxidase activities during decolorization.

Decolorization Experiments

To grow fungal biomass, 4.3 × 10 4 spores' mL −1 of the fungal suspension on a PDA plate was inoculated in an Erlenmeyer flask containing a 60 mL autoclaved solution of culture medium with dye of the desired concentration. The flasks were agitated at 150 rpm at 30 °C. Sample solution (1 mL) was collected from the flasks after 8 days and separated by centrifugation (Allegra 25R, Beckman, USA) at 8000 rpm for 10 min; the supernatant fraction was analyzed for the remaining dye ions. The results, presented as averages, were obtained from experiments performed at least three times. To evaluate the influences of pH, temperature and dye concentration, one experiment was performed at pH 3 to 11, temperature 15, 20, 25, 30, 35, 40 °C and dye concentration 100, 200, 300, 400 and 500 mg L-1 in triplicates. Absorbance readings were taken at maximum wavelength (ʎ=452 nm). Percent decolorization was evaluated with the formula:

(i)where A0 is the initial absorbance and A is the final absorbance. Gas Chromatography – Mass Spectrometry Analysis Restek column (nonpolar; XTI-5, 0.25mm id, 60m long) was set at temperature programming mode with ionization voltage 70 eV for Gas chromatography analysis. The column temperature was linearly raised from 80 °C for 2 min to 280 °C at 10 °C per minute and maintained further for 7 min. Temperature 290 °C was obtained at the GCMS interface, that of injection port was kept at 280 °C. Helium gas with a flow rate of 1.0 ml min−1 was used as carrier gas. Shimadzu QP 2010 GCMS Engine (Shimadzu Corporation, Japan) was used following the earlier procedure reported by Patil and Jadhav, 2013. Comparison of retention time, mass spectra obtainable in the GCMS solution software and fragmentation pattern were used in identifying the metabolites.

High Performance Liquid Chromatography Analysis

Filtration of the metabolites extracted ab initio was done using through 0.22 µl membrane filter and then analyzed HPLC engine fully equipped with Waters 2690, UV–visible detector and C18 hypersil column (4.6 mm×250 mm) with a mobile phase of methanol (80%) mand deionized water (20%) at a flow rate of 1 ml min−1 for 10 min. An aliquot of 20 µl was injected and analyzed by UV–visible detector at a wavelength of 505 nm.

Phytotoxicity Study

The phytotoxicity of acid red 88 dye was performed in order to assess the toxicity of dye before and after degradation, in the concentration range 500 ppm. The studies were carried out using Vigna unguiculata and Triticum aestivum seeds. Twenty (20) seeds of each were grown in the petri plates with the daily supply of 5 ml sample and incubated at room temperature at the same environmental conditions. Control set was done simultaneously by watering the seeds daily with 5 ml of sterile distilled water. After five days growth, length of plumule and radicule was measured.

RESULTS AND DISCUSSION

There was relative disappearance (at day 5) of the peak observed at 503 nm (control-day 0) in the UV/Vis absorbance spectra of EBT dye. The relative reduction and disappearance of the peaks at day 5 suggested the reduction of the dye components thus decolorization. The drastic decrease in absorption peak at day 5 of the spectrum showed decolorization in concordance with the visual observation of the Erlenmeyer flasks.

Decolorization at different pH

Relative decrease in decolorization efficiency was observed from acidic pH 4 to alkaline pH 9. Although in this present study, decolorization was observed at pH 4, 5, 6, 8 and 9 but optimum decolorization (97.85 and 99.97 %) was however observed at pH 5 and 6 respectively. Biosorption is highly favored at lower pH owing to strong electrostatic forces existing between charged dye molecules and the fungal cell. This further suggested that in acidic medium, protonation of functional groups on fungal biomass surface is greatly and optimally enhanced. Decolorization of dyes is largely due to the electrostatic interactions between the negatively charged EBT dye anions and positively charged Penicillium citrinum cells reported of adsorption depletion owing to electrostatic repulsion brought about by increases in the negatively charged sites on the biomass surface as a result of attendant increase in pH respectively.

Decolorization at varying concentrations

Decreasing decolorization efficiency (99.1, 96.54, 91.44, 89.67, 85.76 and 82.44%) was directly proportional with increasing concentration of indigo dye (10, 20, 30, 40, 50 and 60 mg L -1).

Decolorization at varying concentrations

This suggests that, at higher dye concentrations adsorption is negatively affected. Lower dye concentration of EBT dye propels and favors mass transfer resistance between solid and aqueous surfaces. Dye decolorization capacity of the fungus is greatly depleted with increasing dye concentration due to surface saturation action on the fungal surface Decolorization at different temperatures While significant decolorization were recorded at lower temperatures (20 and 25 °C), optimum decolorization (99.95%) of EBT dye was observed at almost ambient temperature (40 °C).

Decolorization by biosorption is actively aided through increasing temperature of the reaction medium of the biomass and EBT dye Our study characteristically showed that myco-removal of colour usually decreases with attendant increase in temperature.

Degradation studies (HPLC Analysis)

HPLC spectra of EBT dye showed the peaks at retention time 1.668, 2.440, 3.007, and 3.287 min and the metabolites obtained after its degradation by Penicillium citrinum showed the peaks at retention time 1.487, 2.505, 2.859, 3.036, and 3.314 min.

Phytotoxicity study

The phytotoxicity experiment results showed significant effect on the % germination and length of the plumule and radicle of the EBT dye solution (1,000 ppm) wetted seeds. The germination percentage of Triticum aestivum and Vigna unguiculata seeds was higher (100%) when treated with water than the dye 5 days decolorized metabolites on treatment with P. citrinum. Significant growth was observed in the length of plumule and radicle when wetted with the dye decolorized products. The results showed that Penicillium citrinum exhibited detoxifying efficiency after dye treatment. This may be due to the removal of aromatic amines by the fungus used in this study.

CONCLUSION

The degradation process of EBT dye by Penicillium citrinum proved to be dependent largely on the pH of the solution, temperature and concentration of the dye. The study reported three major intermediate metabolites after degradation of EBT dye. EBT degraded dye products exhibited fewer toxic potentials on V. unguiculata and T. aestivum than the control dye (EBT). Conclusively, Penicillium citrinum has proven to be a cheap, economic, effective, efficient and eco-friendly alternative in the bio-removal of azo dyes from polluted environment