Euro Analytica 2020:Detoxification and biodegradation of an azo dye, Eriochrome black T by Penicillium citrinum- Paul Olusegun Bankole - Federal University of Agriculture.

Abstract:

Paul Olusegun Bankole, Adedotun Adeyinka Adekunle Department of Pure and Applied Botany, College of Biosciences, Federal University of Agriculture Abeokuta, Ogun tate, Nigeria

Filamentous fungi are known for their effective and efficient biosorbent properties. In this study, Penicillium citrinum strain LAG decolorized Eriochrome black T dye within 5 days. Maximum decolorization (98%) was achieved at a concentration (10 mg L^{-1}), temperature (35 °C), pH 6 and 2.0 g cell biomass during optimization scale up studies. The enzymes activities showed 63% and 55% induction of laccase and lignin peroxidase respectively. UV—Vis spectroscopy, HPLC and gas chromatography-mass spectrometry was used in analyzing the degraded products of the dye. The GCMS analysis revealed the production 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 Aspergillus niger was proposed. The detoxified status of the dye metabolites were confirmed with significant growth of plumule and radicle coupled with increase in germination percentage of Phaseolus mungo and Triticum aestivum.

The present study deals with the decolorisation, biodegradation and detoxification of Direct Black-38, a benzidine based azo dye, by a mixed microbial culture isolated from an aerobic bioreactor treating textile wastewater. The studies revealed a biotransformation of Direct Black-38 into benzidine and 4-aminobiphenyl followed by complete decolorisation and biodegradation of these toxic intermediates. From cytotoxicity studies, it was concluded that detoxification of the dye took place after degradation of the toxic intermediates by the culture. Synthetic textile dyes are one of the most serious pollutants that contaminate steadily higher amounts of wastewater as industrial ef luents. The dyes are highly recalcitrant owing to their chemical structure. Filamentous fungi possesses excellent biosorption capacity due to the secretion of non-selective extracellular enzymes. The aim of this study is to evaluate the biodecolorization ef iciency of Penicillium citrinum on Ericochrome black T dye. Optimum decolorization (98%) was achieved at a concentration (10 mg L -1), temperature (35 °C), and pH 6 during 5 days optimization scale up studies. UV—Vis spectroscopy, HPLC and gas chromatography-mass spectrometry was used in analyzing the degraded products of the dye. The GCMS analysis revealed the production 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 final metabolites. The detoxified status of the dye metabolites were confirmed with significant growth of plumule and radicle coupled with increase in germination percentage of Vigna uniguiculata and Triticum aestivum. INTRODUCTION Dyes from textile industries is a problem in large parts of the world due to their chemical compositions. The number of textile dyes used today exceeds 10 000 (Nilsson et al. 2006). Textile dyes are chemicals with complex aromatic structures designed to resist the effects of laundering and sunshine, for example. These dyes are also of relatively high molecular Biodecolorization of an Azo Dye, Eriochrome Black T by Penicillium citrinum Science and Technology in Emerging Smart Cities and Sustainable Development weight, so it is difficult for many microorganisms to transfer them through

Paul Olusegun Bankole, Federal University of Agriculture.
Contact number: +2348058110550

their membranes. These dyes are consequently difficult to degrade through microbial processes. Fungi, due their excretion of extracellular enzymes, are known to be able to degrade – though possibly not completely – structures that are difficult for bacteria to handle. Fungi produce intermediates that can be degraded by bacteria (Andersson, 2001; Rosen et al., 1998). It is very important to analyse the treated water with regard to the dye content as well as intermediates, especially aromatic amines since some are considered carcinogenic. Many earlier studies have focused on UV–vis spectrophotometer analyses of dyes (Axelsson et al., 2006; Deng et al., 2008; Nilsson et al., 2006; Silveira et al., 2009); these analyses show the decolourization and might also give an indication of changes of structures of the dyes. Biosorption and biodegradation are two major mechanisms in the biodecolorization of dye wastewater. The former describes the process mediated by inactivated biomass, and these materials are often referred to as being biosorbent. In living cells, the two mechanisms can act together. Given the wide variety of enzymes in a strain and the low selectivity of biosorbents, it is expected that the same 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 different colors or common backbones, and Phanerochaete chrysosporium showed the decolorization ability for Acid Blue 62, Direct Red 80, and indigo dye. They do not, however, separate different intermediates. Some recent studies have used high-performance liquid chromatography (HPLC) to separate the intermediates into different peaks (Asad et al., 2007; Shedbalkar et al., 2008; Supaka et al., 2004). Other studies have used high-performance liquid chromatography/mass spectrometry (LC/MS) to determine the molecular structures of intermediates (Libra et al., 2004; Plum and Rehorek, 2005). Biodecolorization of an Azo Dye, Eriochrome Black T by Penicillium citrinum Science and Technology in Emerging Smart Cities and Sustainable Development The aim of the present study is to evaluate the potentials of Penicillium citrinum in the biodecolorization of Eriochrome black T dye. Physicochemical parameters were varied to determine the best conditions for optimal decolorization. GC-MS and HPLC analyses were carried out to determine the metabolic fates of the dye after the experiment. Toxicity analyses were conducted to confirm the non-toxic states of the dye after biodecolorization. MATERIALS AND METHODS Isolation and Culture Medium Penicillium citrinum was isolated from a dumpsite at Orita, Ilaro, Ogun State (7°50’35’’ N, 5°23’47’’E). The medium consisted of the following: 2 gL -1 D-glucose, 2.5 gL -1 NaNO3, 2 gL -1 KH2PO4, 1 gL -1 MgSO4·7H2O in 250 mL Erlenmeyer flasks containing 60 mL of sterile medium were incubated in a controlled incubator at 150 rpm for 4 days at 30 °C. The pH of the medium was adjusted to 5.0 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 in a 250 mL Erlenmeyer flask containing a 60 mL autoclaved solution of culture medium with dye of the desired 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 Biodecolorization of an Azo Dye, Eriochrome Black T by Penicillium citrinum Science and Technology in Emerging Smart Cities and Sustainable Development buffer. The intracellular enzyme was harvested by centrifugation, and the extrace

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Paul Olusegun Bankole, Federal University of Agriculture.
Contact number: +2348058110550