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Isolation of laccase producing fungi: *Aspergillus niger* from Sri Lankan textile wastewater effluents and its potential applicability on decolorization of an azo dye: CI Direct Blue 201

E. M. M. S. Ekanayake[1], I. Wijesekara[2] and Pathmalal M. Manage[1]*

[1]Centre for Water Quality and Algae Research, Department of Zoology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
[2]Department of Food Science and Technology, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
*pathmalal@sjp.ac.lk

The complex aromatic structural nature of synthetic dyes show resistance to natural oxidation processes and persist in the surface water and sediments for a long time. The existing physical and chemical treatment methods are costly and create secondary pollution. Therefore, the present study was focused on the degradation of an azo dye: CI Direct Blue 201 (DB 201), by myco-remediation. *Aspergillus niger*, a filamentous fungus, was isolated from textile wastewater effluent site in Sri Lanka and pure cultures were maintained on Potato Dextrose Agar (PDA) plates. Four cylinders (10 mm diameter in each) of actively growing *A. niger* cultures were cut and inoculated into mineral salt medium consisted of 50 mgL^{-1} DB 201 dye. All the experiments were carried out in triplicates, while controls were maintained without addition of the fungus. Flasks were incubated at 28 °C for seven days with shaking at 100 rpm. Three milliliters of sample aliquots were removed at 6 hrs intervals, centrifuged and the changes of the absorbance in the supernatant was analyzed through UV-Vis spectrophotometer at 570 nm. The laccase activity was determined by measuring the increase in the optical density at 420 nm. The reaction mixture for laccase assay contained 5 mM of 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) in 50 mM sodium acetate buffer (pH 4.5) and 50 µL of decolorized dye solution (ε_{420} = 36000 M^{-1} cm^{-1}). Decolorized dye sample was analyzed through Fourier Transform Infrared (FTIR) Spectroscopy. A Bio-sorption test was carried out by providing the same incubation conditions using 3-day-old live and autoclaved fungi. The control without adding fungus, remained the same without showing any decolorization. The enzyme activity of laccase has increased during the decolorization processes from 18 Uml^{-1} to 254 Uml^{-1}. The changes of the FTIR spectra relevant to the N=N Vibration (1723.3 cm^{-1}), S=O Stretching (1227.3 cm^{-1}) and N-O Stretching (742.88 cm^{-1}) indicated the changes of the initial DB 201 dye structure after the treatment by *A. niger*. Furthermore, the bio-sorption assay by live (100%) and autoclaved fungi (12 ± 2%) confirmed the decolorization and the degradation of DB 201 dye would be based on the metabolic activity of the fungus rather than surface adsorption. Therefore, the present study emphasizes the potency of *A. niger* as an eco-friendly candidate for degradation of azo dyes. Further studies regarding the application of enzymes for real textile dye treatments are currently in progress.

Keywords: Degradation, Laccase, *Aspergillus niger*, Mycoremediation

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