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## Mycoremediation of Coomassie Brilliant Blue by *Aspergillus* spp.

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### ABSTRACT

Aqua pollution is one of the major global threats. Untreated industrial effluent discharged into ecosystems pose a serious problem to the aqua living organism, plants and human beings. Among pollution causing Industries, textile industry accomplish a major attention by environmentalists due to consumption of large volume of water, dyes and chemicals for various processing of textiles. Textile effluents contain carcinogenic aromatic amines, dyes, organic and inorganic materials. Azo, anthro quinone and triphenyl methane dyes are the major classes of synthetic colorants, which are difficult to degrade and have received considerable attention. Nevertheless, during the last few years it has been demonstrated that several fungi, under certain environmental conditions, are able to transfer azo dyes to non toxic products. Removal of colored compounds from textile industry effluents by physic co - chemical and biological methods is currently available.

### KEYWORDS

Mycoremediation; Color removal; Dyes; Textile effluent; Azo dyes; Wastewater treatment.



## INTRODUCTION

Release of toxic and recalcitrant chemicals including synthetic dyes from industries profoundly affects soil fertility and aquatic life. Use of physical and chemical methods for removal of dyes creates disposal problem of remaining dye sludge, whereas biotechnological approach provides viable, less sludge and eco-friendly method.

Dyes are in regular usage in the textile printing industries. This is the major cause of environmental pollution, because large amount of dye wastewater is discharged from the printing units. It is estimated that about 10 - 15% dyes are released into processing water during this procedure<sup>[16]</sup>. There are different class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as  $-C=C-$ ,  $-N=N-$  and  $-C\equiv N-$ , which are responsible for the dye colours, and of functional groups responsible for their fixation to fibres, for example,  $-NH_2$ ,  $-OH$ ,  $-COOH$  and  $-SO_3H$ <sup>[11]</sup>. Dyes may also significantly affect photosynthetic activity in aquatic life by reducing light penetration intensity and may also be toxic to some aquatic fauna and flora due to the presence of aromatics, metals, chlorides, etc.<sup>[5]</sup>. White-rot fungi though effectively decolorizes dyes in simulated textile effluents but decolorization of dyes in real textile effluent is not encouraged due to less competitive ability with other fungi, grows well on wood/lignin, and also relatively low decolorization rates.

However, bacterial degradation have some limitations and in recent years, there has been an alternative research on fungal decolorization of dyes present in wastewaters, and it is turning into a promising alternative to replace or supplement for present treatment processes<sup>[13]</sup>.

## MATERIALS AND METHODS

### Organism and dyes used

The fungus *Aspergillus* spp was used for the degradation of Coomassie Brilliant Blue, which is extensively used in textile industry.

### Preparation of media and sample

Potato Dextrose Agar (PDA) medium was prepared by dissolving the medium ingredients in 1.0% (w/v) dye solution. The medium was autoclaved at 15 lb/inch<sup>2</sup> for 16 minutes and about 20 ml ( $\pm$  1) of medium was poured into each previously sterilized Petri plates, which were allowed to cool inside the UV chamber. The Petri plates were inoculated with the help of inoculation rod, and apical part of 5-day old fungal mycelium was used as inoculant. All inoculated Petri plates were kept in an incubator at 28°C. A little amount (20  $\mu$ g/1000 ml) of streptomycin was also added in the medium to minimize the bacterial interference.

### Preparation of controls

For control experiments, two types of controls, first without dyes and second with dyes were used. The first control was used to compare the fungal growth in the medium with and without dyes. The second control was used to compare the visual disappearance of colour from the inoculated Petri plates.

### Monitoring for decolorization

Decolorization of dyes from the *Aspergillus* sp. treated Petri plates were assessed by the change in original color (as compared to control) and by the visual disappearance of color from the petri plates. The agar plate screening was performed, using glass petri plates (90 mm in diameter), containing 20 ( $\pm$ 1) ml of the dye medium. The radial growth of fungal mycelium and change in the color was measured after a fix interval (48 hours). The culture plates containing dyes were examined for the visual disappearance of color from the media of the petri plates, when compared to their respective controls.

### Percentage of inhibition

Percentage of inhibition of fungal growth during degradation/ decolorization was measured and calculated by using the following formula:

$$I = C - T / C \times 100$$

where, I = Percentage of inhibition in fungal growth, C = Growth in terms of colony diameter in control and T = Growth in terms of colony diameter in the sample.

### Statistical analysis

The statistical analysis was conducted for all the experiments and standard deviation (SD) was calculated, and given as mean  $\pm$  SD values in representation<sup>[10]</sup>.

## RESULTS

In the present study, results for dye degradation/decolorization by *Aspergillus spp* were positive, and the accumulation of dyes by the fungus also took place. The disappearance of color and change in original color in the fungus-treated medium were observed. The evaluation of degradation /decolorization was assessed as the disappearance of color from the petri plate, during the growth of the fungal mycelium. For the dye Coomassie Brilliant Blue, the applied fungus has shown positive result for biodegradation and the blue color of this dye was turned into yellow, a zone of yellow color was present around the mycelium and finally it became clear (figure). A small fraction of dye was also accumulated by the applied fungus, and its mycelium turned into blue colour.



Figure 1

The figure indicates total degradation of Coomassie Brilliant Blue by *Apergillus spp* from blue color to colorless.

**TABLE 1: Biodegradation of Coomassie Brillaint Blue by *Aspergillus spp* and percentage inhibition of growth of this fungus in response to dye in Potato Dextrose Agar (PDA) medium**

Dye tested	Fungal growth in Control (in cm) as C	Fungal growth in sample (in cm) as T	Inhibition (in %)
Coomassie Brilliant Blue	3.2 ( $\pm 0.05$ )	1.8 ( $\pm 0.05$ )	43.75

## DISCUSSION

The present study was carried out to examine the fungal degradation of hazardous dye in semi-solid medium, taking a fungus, *Aspergillus* spp, as the experimental organism and textile dye, Coomassie Brilliant Blue as the testing dye. The applied fungus has shown positive results for dyes degradation/ decolorization, as was indicated by the change and disappearance of color of the dyes from the dye-containing media of the petri plates. A zone of different color around the fungal colony was also observed which might be due to the production of extracellular enzymes by the applied fungus, during the biodegradation of dye. Microbial degradation of Coomassie Brilliant Blue by *Gliocladium virens*<sup>[18]</sup>, various hazardous dyes likes, Congo red, Acid red, Basic blue and Bromophenol blue, Direct green by the fungus *Trichoderma harzianum*<sup>[17]</sup> and biodegradation of plant wastes materials<sup>[19]</sup> by using different fungal strains has been investigated earlier. Our results were similar to biodegradation of Congo red and Bromophenol blue by the fungus *Trichoderma harzianam* in semi-solid medium<sup>[17]</sup> and biodegradation of Methylene blue, Gentian violet, Crystal violet, Cotton blue, Sudan black, Malachite green and Methyl red by few species of *Aspergillus*<sup>[12]</sup> in liquid medium. Cripps *et al.* (1990) also reported the biodegradation of three azo dyes (Congo red, Orange II and Tropaeolin O) by the fungus *Phaenerocheate chrysosporium*. In the present study dye might be degraded by the production of extracellular enzymes as well as adsorption of dyes by the mycelium of *Aspergillus* spp during its growth in the dye-containing medium.

Adsorption of dyes to the microbial cell surface is the primary mechanism of decolorization<sup>[9]</sup>. In our study, the adsorption of dyes by the fungal mycelium was also observed, as it was confirmed by the change in the color of fungal mycelium in tested dyes. Wong and Yu (1999) also reported the adsorption of Acid green 27, Acid violet 7 and Indigo carmine dyes on living and dead mycelia of *Trametes versicolor*. Most of the color removal during first stage might be due to dye adsorption by the mycelium of the fungus, during its growth.

Extracellular enzymes, such as laccase, are produced by fungal strain, like *Aspergillus*<sup>[2,24,14]</sup>. Breakdown of most of organo-pollutants by fungi is closely linked with ligninolytic metabolism. Decolorization of dye is related to the process of extracellular oxidases, particularly manganese peroxidases<sup>[6]</sup>. Liginin peroxidase (Lip), manganese dependant peroxidase (MnP) and laccase, all of which are involved in lignin degradation, have been reported to decolourize dyes<sup>[22]</sup>. In the present study, the degradation and decolourization of tested dye by *Aspergillus* spp appeared to be due to the production of extracellular enzymes by this fungus in the dye-containing medium. It is quite clear that the change in color might be due to the biochemical (metabolic) reactions of fungal strain. Bavendamm (1928) suggested that the presence the phenoloxidases was correlated with fungi, causing white rot decay and that, only these fungi were able to completely decompose lignin. The fungal strain used in the present study was responsible for biodegradation/decolorization of textile dyes, and it was also responsible for change in dye color from reddish to a light color ring around the mycelium

## REFERENCES

- [1] W.Bavendamm; Originalabhandlungen, Uber das Vorkommen und den Nachweis von Z.Pflanzenkrank Pflanzenschutz, 38, 257–276 (1928).
- [2] R.Berka, M.P.Schneider, E.J.Golightly, S.T.Brown, M.Madden, K.Brown, M.T.Halkier, K.Mondorf, F.Xu; Characterization of the gene encoding an extracellular laccase of Myceliophthora thermophila and analysis of the recombinant enzyme expressed in Aspergillus oryzae, Applied and Environmental Microbiology, **63**, 3151-3157 (1997).
- [3] C.Cripps, J.ABumpus; Biodegradation of azo and heterocyclic dyes by Phanerochaete chrysosporium, Applied and Environmental Microbiology, **56**, 1114-1118 (1990).
- [4] R.J.Davis, J.L.Gainer, G.O' Neal, I.W.Wu; Photocatalytic decolourization of waste water dyes, Water Environmental Research, **66**, 50-53 (1994).
- [5] N.Dhaneshvar, M.Ayazloo, A.R.Khatae, M.Pourhassan; Biological decolourization of dye solution containing malachite green by microalgae Cosmarium sp, Bioresource Technology, **29**, 1-7 (2007).

- [6] M.H.Gold, J.K.Glenn, M.Alic; Use of polymeric dyes in lignin biodegradation assays, *Methods in Enzymology*, **161**, 74-78 (1988).
- [7] W.A.Huag, B.Schmidt, D.C.Nortemann, A.Hempel, Stolz, H.J.Knackmuss; Mineralization of sulfonated azo dyes mordant yellow 3 by a 6-aminoaphthalene- 2-sulfonate degrading bacterial consortium, *Applied and Environmental Microbiology*, **57**, 3144-3149 (1991).
- [8] A.J.Jadeja; Studies on Decolorization of Textile Dye Effluent by Lignin Degrading Fungi, Ph.D Thesis, Maharaja Krishnakumarsinhji Bhavnagar University (2011).
- [9] J.S.Knapp, P.S.Newby, L.P.Recee; Decolourization of dyes by wood-rotting basidiomycete fungi, *Enzyme and Microbial Technology*, **17**, 664-668 (1995).
- [10] R.Mead, R.N.Currow; Statistical methods in agricultural and experimental biology, Chapman and Hall, London UK., 335 (1983).
- [11] R.Molinari, F.Pirillo, M.Falco, V.Loddo, L.Palmisano; Photocatalytic degradation of dyes by using a membrane reactor, *Chemical Engineering Process*, **43**, 1103-1114 (2004).
- [12] R.Muthezhilan, N.Yoganath, S.Vidhya, S.Jayalakshmi; Dye degrading mycolflora from industrial effluents, *Research Journal of Microbiology*, **3(3)**, 204- 208 (2008).
- [13] M.Ramya, B.Anusha, S.Kalavathy, S.Devilaksmi; Biodecolourization and biodegradation of reactive blue by *Aspergillus* sp., *African Journal of Biotechnology*, **6**, 1441-1445 (2007).
- [14] E.Record, M.Asther, C.Sigoillot, S.Pages; Overproduction of the *Aspergillus niger* feruloyl esterase for pulp bleaching application, *Applied Microbiology and Biotechnology*, **62**, 349-355 (2003).
- [15] T.Robinson, B.Chandran, P.Nigam; Studies on decolourization of an artificial textile-effluent by white rot fungi in N-rich and Microbial Technology, **29**, 575-579 (2001).
- [16] K.Selvam, K.Swaminathan, K.S.Chae; Decolourization of azo dyes and a dye industry effluent by white rot fungus *Thelephora* sp., *Bioresource Technology*, **88**, 115-119 (2003).
- [17] L.Singh, V.P.Singh; Microbial degradation and decolourization of dyes in semisolid medium by the fungus – *Trichoderma harzianum*, *Environment & We: International Journal of Science & Technology*, **5(3)**, 147-153 (2010).
- [18] L.Singh; Exploration of fungus *Gliocladium virens* for textile dye (congo red) accumulation/degradation in semi-solid medium: A microbial approach for hazardous degradation” paper presented in conference “ISME 12” held on August, 2008, in Cairns, Australia, 17-22 (2008).
- [19] L.Singh; Isolation and characterization of fungal strains for biodecomposition of plant wastes” poster presented in “108th General Meeting of American Society for Microbiology (ASM)”, held on June 1-5, 2008, in Boston, Massachusetts, U.S.A (2008).
- [20] N.Supaka, K.Juntongjin, S.Damronglerd, M.L.Delia, P.Strehaiano; Microbial decolourization of reactive azo dyes in a sequential anaerobic-aerobic system, *Chemical Engineering*, **99(2)**, 169-176 (2004).
- [21] J.Swamy, J.A.Ramsay; The evaluation of white-rot fungi in the decolourization of textile dyes, *Enzyme and Microbial Technology*, **24**, 130-137 (1999).
- [22] B.R.M.Vyas, H.P.Molitores; Involvement of an extracellular H<sub>2</sub>O<sub>2</sub>-dependent lignolytic activity of the white rot fungus *Pleurotus ostreatus* in the decolorization of Remazol Brilliant Blue R, *Applied and Environmental Microbiology*, **61**, 3919- 3927 (1995).
- [23] Y.Wong, J.Yu; Laccase-catalyzed decolorization of synthetic dyes, *Water Research*, **3**, 3512-3520 (1999).
- [24] D.S.Yaver, M.D.C.Overjero, F.Xu, B.A.Nelson, K.M.Brown, T.Halkier, S.Bernauer, S.H.Brown, S.Kauppinen; Molecular Characterization of Laccase Genes from the Basidiomycete *Coprinus cinereus* and Heterologous Expression of the Laccase Lcc1, *Applied and Environmental Microbiology*, **65**, 4943-4948 (1999).