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Biodegradation of Textile Dyes, Bromophenol Blue and Congored by Fungus *Aspergillus Flavus*

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Abstract

In the present study, *Aspergillus flavus* was employed for biodegradation of two commercially used textile dyes, Bromophenol blue and Congo red. The biodegradation of these dyes by this fungus was studied in Potato Dextrose Agar (PDA) medium, while a fixed amount of dye solution (1.0 % w/v) in each case was used in culture medium. This fungus has shown positive results for the degradation/ decolourization for textile dyes. The degradation of dyes was observed by the change in original colour and visual disappearance of colour from the fungus-treated Petri plates. Degradation/decolourization of dyes was also observed as accumulation of dyes by the fungal mycelium, and it was confirmed by the presence of colour in the fungal mycelium or coloured fungal mycelium in fungus-treated Petri plates. Two types of controls: media without dye and with dye were used for studying dye degradation and inhibition of fungal growth. The addition of dyes, Bromophenol blue and Congo red, in the culture medium separately, inhibited the growth of *A. flavus* to varying degrees, when compared to their respective controls.

Keywords: *Aspergillus flavus*, Biodegradation, Bromophenol blue, Congo red Decolourization, Textile dyes

Introduction

Large number chemicals, including dyes are used in textile processing. Recently, environmental contamination has been pointed as one of the greatest problems of modern society, mainly due the population explosion and the increased industrial activities. The textile industry stands out, as it produces a large amount of effluents which can cause serious environmental problems. It is estimated that about 10-15% dyes are released into processing water during this procedure (Selvam, *et al*., 2003). Dyes make up an abundant

class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as ─C═C─, ─N═N─ and ─C≡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 (Molinari, *et al*., 2004). 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. (Dhaneshvar *et al*., 2007).

At present, there is no satisfactory method to economically decolourize textile wastewater. Recent studies point out the potential of fungal wastewater treatment for textile industries. Several combined anaerobic and aerobic microbial treatments had been suggested to enhance the biodegradation of textile dyes (Huag, *et al*., 1991). In recent years, there has been an alternative research on fungal decolourization of dye present in wastewaters, and it is turning into a promising alternative to replace or supplement for present treatment processes (Ramya *et al*., 2007). The ability of white rot fungi to degrade synthetic chemicals, such as dyes, is well known, and a white rot fungus, *Phanerocheate chrysosporium* has been reported to decolourize dyes with enzymes involved in lignin degradation, such as lignin peroxidase (Lip), manganese peroxidase (MnP) and laccase (Swamy *et al*., 1999; Robinson *et al*., 2001). The fungus *Trichoderma harzianum* has also been reported earlier for the degradation of textile dyes (Singh and Singh, 2010). In the present work, we have investigated microbial degradation/decolourization of textile dyes, Bromophenol blue and Congo red, using a fungus, *Aspergillus flavus*.

Materials and Methods

Organism and Dyes Used

The fungus *A. flavus* was used for the degradation of Bromophenol blue and Congo red, which are 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^2 for 16 minutes and about 20 ml (± 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^{o}C. A little amount (20 µg/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 (Figure 1) 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 (Figures 2 and 3).

Monitoring for Decolourization

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

Percentage of Inhibition

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

$$I = C\text{-}T/C \times 100$$

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 ± SD values in representation (Mead and Currow, 1983).

Results

In the present study, results for dyes degradation/decolourization by *A. flavus* were positive, and the accumulation of dyes by the fungus also took place. The disappearance of colour and change in original colour in the fungus-treated medium were observed. The evaluation of degradation/decolourization was assessed as the disappearance of colour from the Petri plate, during the growth of the fungal mycelium.

For the dye Bromophenol blue, the applied fungus has shown positive result for biodegradation and the blue colour of this dye was turned into yellow and finally, a zone of yellow colour was present around the mycelium. A small fraction of dye was also accumulated by the applied fungus, and its mycelium turned into blue colour (Figure 4).

In case of fungal degradation of Congo red also, the results were positive, but it was degraded more efficiently than Bromophenol blue and the disappearance of colour from the Petri plate was observed in the fungus-treated dye (Figure 5).

The inhibition of fungal growth in dye-containing medium was also observed during dye degradation (Table 1).

Table 1 Biodegradation of textile dyes, Bromophenol blue and Congo red by *A. flavus* and percentage inhibition of growth of this fungus in response to these dyes in Potato Dextrose Agar (PDA) medium.

Dyes tested	Fungal growth in Control (in cm) as C	Fungal growth in sample (in cm) as T	Inhibition (in %)
Bromophenol blue	2.1 (±0.05)	1.6 (±0.05)	23.80
Congo red	2.1 (±0.05)	1.4 (±0.05)	33.33

Discussion

The present study was carried out to examine the microbial degradation of two hazardous dyes in semi-solid medium, taking a fungus, *Aspergillus flavus*, as the experimental organism and two textile dyes, Bromophenol blue and Congo red as the testing dyes. The applied fungus has shown positive results for dyes degradation/decolourization, as was indicated by the change and disappearance of colour of the dyes from the dye-containing media of the Petri plates. A zone of different colour around the fungal colony was also observed (Figures 4 and 5), which might be due to the production of extracellular enzymes by the applied fungus, during the biodegradation of tested dyes. Microbial degradation of Congo red by *Gliocladium virens* (Singh, 2008a), various hazardous dyes likes, Congo red, Acid red, Basic blue and Bromophenol blue, Direct green by the fungus *Trichoderma harzianum* (Singh and Singh, 2010) and biodegradation of plant wastes materials (Singh, 2008b) 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 (Singh and Singh, 2010) and biodegradation of Methylene blue, Gentian violet, Crystal violet, Cotton blue, Sudan black, Malachite green and Methyl red by few species of *Aspergillus* (Muthezhilan *et al.*, 2008) 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 dyes might be degraded by the production of extracellular enzymes as well as adsorption of dyes by the mycelium of *A. flavus* during its growth in the dye-containing medium.

Adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization (Knapp *et al*., 1995). In our study, the adsorption of dyes by the fungal mycelium was also observed, as it was confirmed by the change in the colour 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 colour removal during first stage might be due to dye adsorption by the mycelium of the fungus, during its growth.

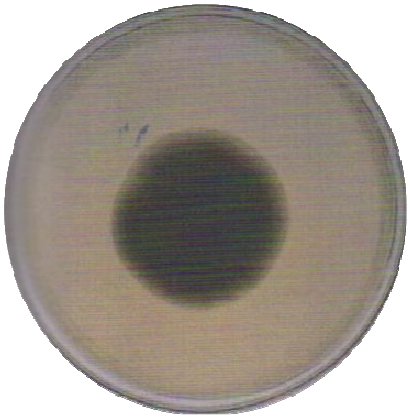

Figure 1

Figure 2

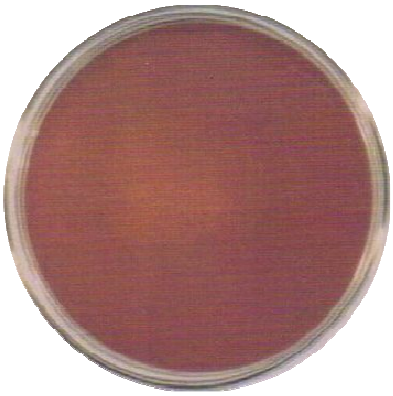

Figure 3

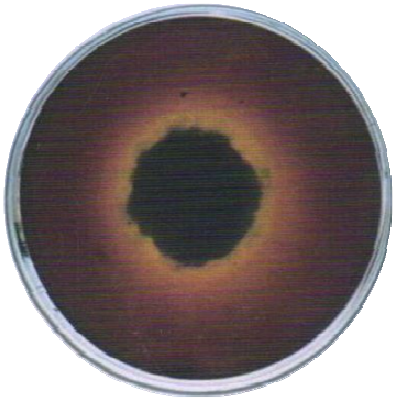

Figure 4

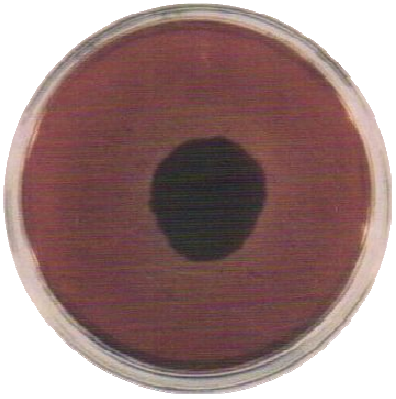

Figure 5

Figure 1 Control (to compare the growth of fungal mycelium in dye-containing medium); Figures 2 and 3 Controls (for visual comparison of disappearance of dye colours, Figure 2 for Bromophenol blue and Figure 3 for Congo red from the fungus-treated media); Figures 4 and 5 Growth of fungal mycelium during biodegradation of Bromophenol blue (Figure 4) and Congo red (Figure 5).

Extracellular enzymes, such as laccase, are produced by fungal strain, like *Aspergillus* (Berka *et al*., 1997; Yaver *et al*., 1999; Record *et al*., 2003). Breakdown of most of organo-pollutants by fungi is closely linked with ligninolytic metabolism. Decolourization of dye is related to the process of extracellular oxidases, particularly manganese peroxideases (Gold *et al.*, 1988). Liginin peroxidise (Lip), manganese-dependant peroxidase (MnP) and laccase, all of which are involved in lignin degradation, have been reported to decolourize dyes (Vyas and Molitores, 1995). In the present study, the degradation and decolourization of tested dyes by *A. flavus* appeared to be due to the production of extracellular enzymes by this fungus in the dye-containing medium. It is quit clear that the change in colour 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/decolourization of textile dyes, and it was also responsible for change in dye colour from reddish to a light colour ring around the mycelium (Figure 5). Further investigations on isolation and purification of enzyme(s), involved in the biodegradation of hazardous dyes, are in progress in the research laboratory of the authors at Delhi University.

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