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Microbial Degradation and Decolourization of Dyes in Semi-Solid Medium by the Fungus – *Trichoderma harzianum*

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Abstract

Microbial degradation of dyes by the fungus – *Trichoderma harzianum*, along with the effects of these dyes on this fungus during their biodegradation, was investigated using semi-solid medium (PDA, Himedia). A fixed amount of each dye was used in the culture medium to screen out the potential of the fungus for dye degradation. Dye degradation was observed by comparing samples with controlled cultures. Two types of controls, first to compare degradation and second for inhibition of mycelial growth, were used for the study. Degradation of dye was observed as decolourization percentage, while accumulation of dye was observed by the presence of colour in the fungal mycelium. The growth of the fungal mycelium varied for all tested dyes, in term of inhibition. Maximum inhibition (43%) of mycelial growth was recorded in the dye Bromophenol blue, whereas minimum inhibition (9%) took place in the dye Direct green. The dyes, Congo red and Bromophenol blue were clearly degraded by the fungus, while Acid red, Basic blue and Direct green were accumulated by fungal mycelium.

Keywords: Dyes, Degradation, Inhibition, Fungal mycelium.

Introduction

Synthetic dyes are used extensively for textile dyeing, paper printing, colouring cotton, silk, synthetic fibers, jute fibers, wool and colour photography (Maynard, 1983). According to annual report of the Union Ministry of Environment and Forest, some 4.4 million tones of hazardous wastes are being generated by 13,011 units spread over 373 districts of India (Ramaswamy, 2003). Azo dyes are considered as electron-deficient xenobiotic compounds because they possess the azo (N=N) and sulfonic (-SO₃⁻) electron withdrawing groups, generating electron deficiency in

molecule and making the compounds less susceptible to oxidative catabolism by microbes. The colouring processes discharge huge quantity of dye effluents, which pollute local terrestrial habitat, aquatic bodies as well as rivers. Approximately 10,000 different dyes and pigments are used industrially, and these dyes are produced annually worldwide (Zollinger, 1987). About 10 to 15% of the dyes are lost in the effluent during the dye process. Most of these dyes are difficult to be degraded in environment and have varied potential to form carcinogenic breakdown products in the environment (Chung, *et al.*, 1992).

Inefficiency of the dyeing process, poor handling of spent effluent and insufficient treatment of wastes of dyestuff industries lead to dye contamination of the environment such as soil and natural water bodies (Nigam, *et al.*, 1996). A number of physico-chemical methods, such as adsorption, coagulation, precipitation, filtration and oxidation, have been used to treat dyestuff effluents, but these methods have many disadvantages and limitations (Davis *et al.*, 1994). Therefore, there is a need to develop efficient and cost-effective methods for the decolourization and degradation of dyes in industrial effluents and contaminated soil.

Microbial degradation of waste materials (Singh, 2008) and different classes of dyes by fungi (D'Annibale *et al.*, 1998; Martins *et al.*, 1999), especially the representative of white rot fungi (Rodrigues *et al.*, 1999; Swamy and Ramsay 1999) has been reported. Microbial decolourization of reactive azo dyes has been reached by sequential anaerobic-aerobic system (Supaka, *et al.*, 2004). Bumpus and Aust (1986) have also reported that *Phanerocheate chrysosporium*, a ligninolytic white-rot fungus degrade a variety of structurally diverse organo-pollutants. Another white-rot fungus, *Thelephora* sp. was also used for decolourization of azo dyes, such as orange G, congo red and amido black 10B (Selvam *et al.*, 2003). Decolourization of various kinds of azo dyes by various fungi has been reported (Rafii *et al.*, 1990). Wong and Yu (1999) reported the adsorption of acid green 27, acid violet 7 and indigo carmine dyes on the living and dead mycelia of *Tremetes versicolour*. Degradation and decolorization of dye Congo red by fungus *Gliocladium virens* in semi-solid medium was also earlier studied by Singh (2008a). The present study deals with degradation/decolourization of dyes namely, Congo red, Acid red, Basic blue, Bromophenol blue and Direct green by the fungus *Trichoderma harzianum* in semi-solid medium and the effects of these dyes on the growth of the fungal mycelium. Objectives of the present study include i) screening of *T. harzianum* for biodegradation of hazardous dyes and ii) to investigate the adverse effect of hazardous dyes on the growth of this fungus, i.e., to focus on the inhibition of fungal growth by the dyes during their degradation.

Materials and Methods

Organism and Dyes Used

The organism used in the present study was *Trichoderma harzianum* against the dyes such as Acid red, Basic blue, Bromophenol blue, Congo red and Direct green. These dyes have been reported as carcinogenic and were less biodegradable in environment.

Preparation of Media and Sample

A fix amount (0.5%, w/v) of each dye was added to Potato-Dextrose Agar (PDA) medium, and the medium was finally autoclaved at 15 lb/inch² for 16 minutes, this medium was poured into previously sterilized Petri plates. The Petri plates were inoculated with the apical part of 5 days old fungal mycelium. All Petri plates were kept in an incubator at 28°C. A little amount (20 µg/1000 ml) of streptomycin was added in the medium to minimize the bacterial interference.

Preparation of Controls

Two types of controls, first the medium without dye and inoculated with same fungus, second with similar percentage of dye and medium but without inoculation. The first control was used to compare the fungal growth with and without the dye. The second control was used to compare the visual disappearance of colour from the inoculated plate.

Dyes and Monitoring of Their Decolourization

Decolourization of dyes in the medium was assessed by the visual disappearance of colour from the inoculated Petri plate. The agar plate screening was performed using glass Petri plates (90 mm in diameter), containing 20 (±1) ml of the medium with dye. Each plate was inoculated with the fungus with the help of inoculation needle. Five to seven days old mycelium was used for inoculation and incubated for ten days at 28°C. The radial growth and change in the colour intensity was measured after every 24 hours intervals. The dye-containing plates were examined for the visual disappearance of colour from the background of the semi-solid medium of the plates.

Percentage of Inhibition

Percentage of inhibition of fungal mycelial growth in semi-solid medium was calculated by using the formula

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

where, I = Percentage 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 was calculated, and given as mean ± values in representation (Mead and Curnow, 1983).

Results

In the present study, the results with dyes were positive for degradation/ decolourization, and there was also an interested phenomenon, i.e., accumulation of dye by the fungus, *Trichoderma harzianum* was observed. Fungal growth was also

inhibited by all dyes at different levels. The maximum inhibition of the mycelial growth was observed in Bromophenol blue, while minimum inhibition took place in Direct green dye (Table 1).

Acid Red

The result with this dye was positive, as there was accumulation of the dye by the applied fungus. The mycelium of the fungus became coloured due to the accumulation of dye. The dye also showed the adverse effect on the fungal growth, and about 18% inhibition in the growth of the fungal mycelium was observed with this dye.

Basic Blue

The results with this dye were also positive, as there was also accumulation of this dye by *T. harzianum*. Inhibition in the fungal growth with the dye was around 13%.

Bromophenol Blue

The evaluation of degradation/decolourization was assessed as the disappearance of colour during the growth of fungus. The blue colour of the dye turned into yellow colour after the inoculation of fungus. A ring of yellow colour was observed around the growing colony or mycelium of the fungus. This dye is a pH-indicator dye and shows yellow colour at pH 2.8 and violet at pH 4.6. About 43% inhibition in the growth of the fungal mycelium was observed with this dye.

Congo Red

Congo red is also a pH indicator dye and shows violet colour at pH 3.0 and orange red at pH 5.0. The fungal strain used for the study was responsible for dye degradation/decolourization, and there was also a change in the dye colour from reddish to a light coloured ring around the mycelium. Inhibition of fungal mycelium or growth was shown to be about 29% in this dye.

Direct Green

This dye did not show degradation or decolourization clearly, but there was accumulation like the others dyes. *T. harzianum* was capable only to accumulate the dye from the medium, and the whole mycelium became coloured. The fungal growth was also inhibited by this dye, and about 9% inhibition in fungal growth was recorded.

Discussion

The present study has been carried out to examine the microbial degradation/decolourization of hazardous dyes in semi-solid medium, using the fungus *Trichoderma harzianum* as an experimental organism. From the present investigation it was observed that *T. harzianum* exhibited its growth inhibition in the media containing Acid red, Basic blue, Bromophenol blue, Congo red and Direct green, in

comparison to the control (growth in the medium without dye). Out of these dyes, Bromophenol blue was found to show maximum growth inhibitory effect (43%). The observations of the present investigations also suggested that the fungus was sensitive towards the tested dyes.

Table 1 Growth of fungal mycelium in semi-solid medium without (C) and with dye (T) and growth inhibition in terms of percentage.

Dyes tested	Fungal growth in Control (in cm) as C	Fungal growth in sample (in cm) as T	Inhibition in percentage
Acid red	1.8 (± 0.05)	2.2 (± 0.07)	18.18
Basic blue	1.9 (± 0.05)	2.2 (± 0.07)	13.63
Bromophenol blue	0.8 (± 0.07)	1.4 (± 0.05)	42.85
Congo red	1.0 (± 0.05)	1.4 (± 0.05)	28.57
Direct green	2.0 (± 0.08)	2.2 (± 0.05)	9.09

Interestingly enough, the data indicated that the growth inhibitory effect of Direct green dye was minimum (9%), it was also noticed that Basic blue dye exhibited little (13%) inhibitory effect on the growth of the fungus in comparison to Bromophenol blue, which exhibited maximum inhibition (43 %) on the fungal growth during its bio-degradation (Table 1). Although, many studies have been carried out to look for the ability of microorganisms to degrade an array of dyes (Singh, *et al.*, 2006) of industrial interests, which serve as pollutants, posing potential threat to both human and animal health as well as the environment (Upadhyay, 2002), but the adverse effect of the dyes on the growth of fungi have not yet been investigated in detail. The observations on the extent of inhibitory effects of these dyes on the growth of *T. harzianum* in semi-solid medium as well as the ability to degrade or decolourize or even to accumulate these dyes, clearly showed that the fungus degraded/decolourized the pH indicator dyes such as Bromophenol blue and Congo red, but accumulated the textile dyes such as Acid red, Basic blue and Direct green. The decolourization of dyes, as indicated by the change in colour of the dyes due to change in pH of the medium around the fungal colonies might be due the degradation of the dyes tested.

Many other workers have described non-specific fungal oxidative enzymes (lignin peroxidases, manganese peroxidases, laccase) which attack a wide range of azo and anthraquinonic dyes outside the cell (Cripps *et al.*, 1990; Ollika *et al.*, 1993; Rodrigues *et al.*, 1999), but the participation of these enzymes in dye decolourization was not obvious. However, in the present study, the decolourization of the dyes under investigation might be purely due to the accumulation and production of extracellular enzymes by *T. harzianum*. This has already been reported by Abadulla *et al.* (2000) and Gold *et al.* (1988), who found that some dyes serve as good substrates for assaying lignin peroxidases, manganese peroxidases and laccase enzymes of fungal origin, which could be responsible for the degradation/decolourization of the dyes. However, the findings of present investigation do not rule out the possibility of involvement of some factor(s)/enzymes in the degradation/decolourization of dyes by fungal species tested. Further studies are required to investigate the enzymatic mechanism for dye degradation/decolourization and the phenomenon by which fungal growth is suppressed in dye medium, which can provide one of the important viable

options for bioremediation of hazardous dyes. Use of microbes (fungi) is a good approach for bioremediation *in vitro* and *in vivo*, but their growth is also inhibited by some hazardous chemicals during the degradation of such hazardous wastes at a large scale. Study also indicates that dye wastes have an adverse effect on the growth of the fungus, which otherwise is involved in their degradation, when tested. This might be due to the specific structure of the dye compounds or heavy metals (in heavy metal based dyes), which inhibit the fungal growth during their degradation. Inhibition in fungal mycelial growth by these compounds shows that they act as a barrier for natural breakdown of such compounds by soil microbes. There is a need for further investigation to develop and to characterize the fungal strain(s) for biodegradation of such hazardous wastes.

Authors's contributions: **Lokendra Singh**, performed these experiments for his M.Phil. thesis, wrote the manuscript and also corresponding author of the manuscript; **Ved Pal Singh** (Professor), Supervisor for M. Phil. thesis of Lokendra Singh, contributed in experiment design and editing of the finally accepted manuscript.

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