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Microbial Decolourization of Textile Dyes by the Fungus *Trichoderma harzianum*

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The present investigation was undertaken to study the fungal decolourization of dyes. Fungal strain Trichoderma harzianum was used for decolourization of azo (Acid red) and anthraquinonic (Basic blue) dyes in liquid culture medium (PDB, Himedia). The cultures, containing dyes were filtered and centrifuged at 6000 rpm for 5 minutes and supernatant was used for spectrophotometric observations. Absorbance at 494 nm (for Acid red) and 630 nm (for Basic blue) were recorded for fungal inoculated samples and compared with un-inoculated samples or controls experiments. The applied fungal species was able to decolourize the tested dyes, as revealed by spectrophotometric analyses of the culture filtrates, which showed gradually decreased absorbances of dyes cultures. Continuous increase in the mycelial weight was also recorded during the incubation periods in dyes cultures. The magnitudes of decolourization of dyes were 51 and 70 % for Basic blue and Acid red dyes, respectively.

Key words: Decolourization, Acid red, Basic blue, Textile dyes, *Trichoderma harzianum*.

Various types of synthetic dyes and pigments are extensively used (approximately 100,000 tons/year) worldwide in textile sector (Moreira *et al.,* 2000; Park *et al.,* 2006; Soares *et al.,* 2002). 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). Untreated textile effluents are highly toxic, as they contain a large number of heavy metals like, Cd, Cr, Co, Cu, Hg, Ni, Mg, Fe and Mn. Mutagenic, carcinogenic and toxic potential of the heavy metals present in textile effluents has been extensively studied by Delclos *et al.* (1984). Some of these compounds cause serious threat because of their carcinogenic potential or cytotoxicity (Adedayo *et al.,* 2004). The pollutants aggravated by the presence of free chlorine and toxic heavy metals, cause rapid depletion of dissolved oxygen leading to "Oxygen Sag" in the receiving water. These pollutants are known to destroy microorganisms that lead to a reduction in the self-purification capacity of the stream (Rivera *et al.,* 1999). 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, chloride etc. (Dhaneshvar, *et al.,* 2007).

The role of fungi in the treatment of waste water has been extensively researched. Fungus has proved to be a suitable organism for the treatment of textile effluent and dye removal. Many genera of fungi have been employed for the dye decolourization either in living or dead form

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(Kaushik and Malik, 2009). Dyes are removed by fungi through biosorption, biodegradation, bioaccumulation and enzymatic mineralization using lignin peroxidase, manganese peroxidase, manganese independent peroxidase and laccase (Wesenberg *et al.,* 2002). Fungi have been attracting a growing interest for the bio-treatment of waste water ingredients such as metals, inorganic nutrients and organic compounds (Coulibaly, 2003). Therefore as better alternatives, biological processes are getting more and more attention since it is cost-effective, environment friendly and does not produce large quantity of sludge (Seong *et al.,* 1995). Several combined anaerobic and aerobic microbial treatments have also been suggested to enhance the bio-degradation of textile dyes (Huag *et al.,* 1991). The present study was focused on decolourization and degradation of textile Acid red and Basic blue dyes using the fungus *Trichoderma harzianum* in liquid culture medium.

MATERIALS AND METHODS

Organisms and Dyes Used

The fungus *Trichoderma harzianum* was used for the decolourization of Acid red and Basic blue dyes, which are extensively used in textile industry.

Spore Suspension and Mycelial Dry Weight

Pure cultures (5-7 days old) of fungus *Trichoderma harzianum*, grown on Potato Dextrose Agar (PDA, Himedia), were used for spore suspension. Spores were harvested in sterilized normal saline (0.9 % NaCl in distilled water with a drop of Tween 80) and spore concentration was adjusted to $1x10^8$ spores/ml. Mycelial dry weight was calculated by evaporating the moisture at 80°C for 24 hour.

Preparation of Media and Samples and Controls

Media were prepared by dissolving the ingredients in dye stock solution (1 %) replacing the distilled water and autoclaved at 15 lb/inch^2 for 16 minutes. Medium (40 ml) was poured into pre-sterilized 250 ml Erlenmeyer flasks. Next day these flasks were inoculated with 1 ml of spore suspension (1×10^8 spores/ml) and placed for incubate at 28°C for ten days statically. Spectrophotometric observations were made after an interval of 24 hours of incubation. A little amount (5 µg/1000 ml) of streptomycin was also added in the medium to minimize the bacterial interference. For control experiments, sample-flasks were kept un-inoculated.

Monitoring of Dyes Decolourization

Decolourization of dye was observed visually by disappearance of colour as well as spectrophotometrically by using Varian Cary 8 Spectrophotometer with the wavelength range 250-700 nm. Measurements were recorded at 494 nm (for Acid red) and 630 nm (for Basic blue), of liquid culture's supernatants. The cultures were filtered with Whatman filter paper No. 1 and the filtrates were centrifuged at 6000 rpm for 5 minutes.

Percentage of Dyes Decolourization/Degradation

Percentage of dye decolourization/ degradation was calculated by the formula given by Awaluddin *et al.,* (2001) which is given as follows:

$$\text{Percentage of degraded dye } \frac{\text{Initial absorbance - Final absorbance}}{\text{Initial absorbance}} \times 100$$

Statistical Analysis

The statistical analysis was conducted for all experiments and standard deviation (SD) was calculated, and given as mean ± SD values in representation (Mead and Currow, 1983). All experiments were done in triplicate manner under identical conditions.

RESULTS

The results clearly indicated the decolourization/degradation of tested dyes, Acid red and Basic blue, by the applied fungus *Trichoderma harzianum*. The magnitudes of decolourization of Basic blue and Acid red dye were recorded as 51 and 70 %, respectively (Tables 1 & 2). The initial degrees of decolourization (after 24 hours) were 2 and 17 % for Basic blue and Acid red dyes, respectively. Visual disappearances in colour as well as gradual decreased absorbances took place in fungal inoculated culture media; whereas, no colour removal was observed in un-inoculated sample-flasks or controls of static cultures. Increased mycelial weight in respect of incubation period were also recorded (Tables 1 & 2).

Table 1. Dry weight of mycelium and spectrophotometric assessment of decolourization of Acid red dye by the culture of *T. harzianum* at different hours of its growth. Absorbance of control (dye + medium, i.e initial absorbance) at 494 nm was 2.066 ± 0.653

Hours of growth	Dry wt. of mycelium (gm/40ml) (± S.D.)	Absorbance of culture filtrate at 630 nm (± S.D.)	Percentage of decolourization
24	0.11 ± 0.0050	1.721 ± 0.0200	16.69 %
48	0.17 ± 0.0050	1.545 ± 0.1350	25.21 %
72	0.29 ± 0.0050	1.351 ± 0.0300	34.60 %
96	0.38 ± 0.0173	1.216 ± 0.0950	41.33 %
120	0.45 ± 0.0081	1.109 ± 0.0055	46.32 %
144	0.54 ± 0.0095	1.065 ± 0.0090	48.45 %
168	0.69 ± 0.0129	1.032 ± 0.0110	50.04 %
192	0.82 ± 0.0189	0.944 ± 0.0850	54.30 %
216	1.06 ± 0.0170	0.782 ± 0.0150	62.14 %
240	1.16 ± 0.0191	0.618 ± 0.0050	70.08 %

Table 2. Dry weight of mycelium and spectrophotometric assessment of decolourization of Basic blue dye by the culture of T. harzianum at different hours of its growth. Absorbance of control (dye + medium, i.e initial absorbance) at 630 nm was 3.830 ± 0.0515

Hours of growth	Dry wt. of mycelium (gm/40ml) (± S.D.)	Absorbance of culture filtrate at 630 nm (± S.D.)	Percentage of decolourization
24	0.05 ± 0.0050	3.765 ± 0.0180	01.69 %
48	0.11 ± 0.0050	3.619 ± 0.1581	05.50 %
72	0.24 ± 0.0050	2.892 ± 0.0120	24.49 %
96	0.28 ± 0.0057	2.758 ± 0.0540	27.98 %
120	0.35 ± 0.0057	2.433 ± 0.0190	36.47 %
144	0.42 ± 0.0057	2.170 ± 0.0570	43.34 %
168	0.51 ± 0.0050	2.104 ± 0.0468	45.06 %
192	0.63 ± 0.0129	2.075 ± 0.0320	45.82 %
216	0.72 ± 0.0095	1.982 ± 0.0960	48.25 %
240	0.94 ± 0.0095	1.885 ± 0.0970	50.78 %

DISCUSSION

In the present investigation, results clearly indicated that the decolourization of tested dyes by the fungus *Trichoderma harzianum* was positive. In the present scenario dyes were removed by the fungus through biosorption, bioaccumulation, biodegradation and enzymetic mineralization. It was evident from the change in colour of used fungal mycelium and decreased absorbances during the decolourization. The phenomena of biosorption, accumulation and adsorption of dyes by fungal mycelium were reported by earlier studies. Adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization (Knapp *et al.,* 1995) and the fungal biomass displayed good sorption capabilities (Valeria *et al.,* 2008). The different strains of the fungi *Aspergillus* and *Trichoderma* were also used earlier for azo dyes and textile effluents decolourization (Saranraj et al. 2010). 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*. The decolourization/degradation of some hazardous textile dyes by *Trichoderma* has been reported and studied earlier by Singh and Singh (2010). Our findings were similar and support these earlier studies, as the dye colour was adsorbed by fungal mycelium and finally turned

into dye colour during the incubation period. Dyes decolourization was due to the physical processes such as biosorption, accumulation and adsorption, but role of enzyme(s) could not be ignored in the study. Although, enzyme(s) were not isolated but their role has been extensively studied by many workers. Abadulla *et al.* (2000) have reported that the anthraquinonic dyes were decolourized faster than the azo dyes by *Trametes hirsuta*, which was due to the action of enzyme laccase in that organism. Howksworth *et al.* (1995) decolourized azo and anthraquinonic dyes by fungal extracellular redical-generating enzymes or non-oxidative factors possessed by them. Heinfling and Bergbauer (1997) reported 95 % colour removal of HRB 8 dye, by *Bjerkandera adusta* and *T. versicolor* within four days. Extracellular enzymes, such as laccase, are produced by the fungal strains (Berka *et al*., 1997; Yaver *et al*., 1999; Record *et al*., 2003) and the decolourization of dye is related to the process of extracelluar oxidases, particularly manganese peroxidases (Gold *et al*., 1988). Lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase, all of which are involved in lignin degradation, have also been reported to decolourize dyes (Vyas and Molitores, 1995). Kim and Shoda (1999) have purified and characterized the novel peroxidase (Dyp) that is responsible for the dye decolourizing activity of *Geotrichum candidum* Dec 1. In the present study, the decolourization of the dyes was also due to physical and chemical processes, i. e. both adsorption of dyes and enzymatic decolourization took place. Increased mycelial weight (Tables 1 & 2) indicated that increased surface area was provided by the fungus for accumulations of dyes, as well as enzyme(s) were produced in sufficient amount for dyes decolourization. The use of ligninolytic enzymes were also investigated for industrial applications; and commercial enzymes, such as xylanases, were produced in large quantities by *Trichoderma reesei* to be used for pulp bleaching (Bajpai, 1999).

CONCLUSION

Microbes have a good potential for degradation of dyes and other hazardous wastes. Although, other methods for waste degradation are also in use, but bio-degradation is a good alternative among all of them. Bio-degradation is much safe and cost-effective too. Bio-treatment offers easy, cheaper and effective alternative for colour removal from textile dyes. Thus, by the present study, we concluded that the fungus *Trichoderma harzianum* could be effectively used as a good microbial source for wastewater treatment system.

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