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Bioremediation of Azo-dyes by indicator fungi

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ABSTRACT

Indicator fungal isolates of synthetic azo-dyes polluted soils were used for bioremediation process of red and blue azo-dyes. The indicator fungal isolates are *Aspergillus erythrocephalus* NCBT 106, *Cladosporium herbarum* NCBT 147, *Fusarium solani* NCBT 163 and *Humicola fuscorata* NCBT 169. These fungal strains were able to decolorize the azo-red and blue dyes. Decolourization was assayed colorimetrically at 540 nm and percentage of decolourization was calculated. These indicator fungal isolates were able to utilize the dye as nitrogen source. The optimum concentration for both azo-red and azo-blue dye was 200 ppm. The maximum decolourization of azo-red dye by *Aspergillus erythrocephalus* NCBT 106 and azo-blue dye by *Cladosporium herbarum* NCBT 147 fungal strains at the end of 10th day of decolourization experiment were 85% and 80% respectively. These fungal isolates can be exploited as bioremediation agents in bioreactors to remove the pollutants through enzymatic degradation of these indicator fungal metabolism.

Key words: Azo-red, and Azo-blue dyes, Bioremediation, Decolourization, Enzymatic degradation, Fungal isolates, Pollutant.

INTRODUCTION

Azo-dyes constitute the largest group of synthetic dyes with great deal of structural and colour variety (Cripps *et al*., 1990). They are extensively used in textile, leather, food, cosmetics, pharmaceutical and paper industries. Around 10000 dye industries eventually produced more than 7×10^5 metric tons of these dyes annually world wide (Keck *et al*., 1997). Amongst various applications of synthetic dyes about 3,00,000 tons of different dyestuffs are used per year for textile dyeing operations. Thus dye houses are the major consumers of synthetic dyes and consequently are the major cause of soil and water pollution (Bechtold *et al*., 2001). Textile dyes are classified as azo, diazo, cationic, basic, anthraquionone and, metal complex dyes based on the nature of their chemical structure. Further, depending on their application dyes are classified as reactive, disperse, vat, mordant etc. Amongst all, azo-dyes are the most problematic, due to their excessive consumption and high water solubility (Keharia and Madamwar, 2003). Azo-dyes have the same chromophore-N=N-and different auxochromes such as $-NH_2$,-OH and-$N(R)_2$ groups. These dyes are recalcitrant even in conventional aerobic waste water treatment plants (Zimmermann *et al*., 1982). Azo-dyes are potential mutagens and carcinogens which necessitate proper degradation and safer disposal (Vyas and Molitoris, 1995). Azo-dyes can be degraded by microbes both aerobically and anaerobically (Knapp and Newby, 1995; Spadaro and Renganathan, 1994). Anaerobic degradation is easy to achieve but the degradation products are biorecalcitrant under anaerobic condition (Keck *et al*., 1997). Hence, aerobic degradation offers simpler and efficient degradation of this compound with suitable microorganism. Benzidine based azo-dyes 1-(chlorophenyl) azo-2-naphthol (X=H) red and blue azo-dyes are extensively used in textile dyeing and hence these dyes have been chosen in the present study. An attempt was made to isolate the indicator fungal species of azo-dyes affected soil samples and used them as bioremediation microorganism to reduce the impact of azo-dye pollution under laboratory conditions.

MATERIAL AND METHODS

Soil samples were collected aseptically from six different azo-dyes contaminated soil sites in Tirupur Town, Tamil Nadu, India. Soil suspension was prepared by mixing 10 gm of soil in 100 ml of sterile distilled water. Standard serial dilution (Pramer and Schmidt, 1965) was employed for isolation of fungi from these soil samples. Czapek Dox agar medium (Himedia, India) was used for this study. Isolated fungi were identified on the basis of cellular, colonial morphological structures observed by microscopy (Domsch *et al*., 1980). The fungi was immobilised in polyurethane foam and deposited in the culture collection centre at the Department of Botany, National College, Tiruchirappalli for further experimental use.

Czapek Dox liquid medium was prepared, in which red and blue azo-dyes were individually incorporated at different concentrations *viz*. 100, 200, 300, 400 and 500 ppm. On this fungal isolates were inoculated and incubated for 10 days at 28° ± 1 C to identify the colour reduction phenomenon due to bioremediation process by the fungal isolates. Colorimetric analysis was employed for monitoring dye decolourization by the fungal isolates in Czapek Dox liquid (broth) cultures. Prior to this, the absorbance maxima of the dye incorporated broth was determined. The absorption maxima was 410 nm for red dye and 440 nm for blue dye incorporated broth and hence decolourization was assayed at these absorption maxima. During the colorimetric analysis of fungal culture broth, in order to avoid the interference of fungal cell or mycelia in the absorbance value, the culture broth was centrifuged at 4000 rpm for 5 minutes and they were removed as pellets. The supernatant was analysed colorimetrically (410 and 440 nm). Aliquots were withdrawn from the culture broth at 24 h interval and the extent of decolourization was monitored colorimetrically for 10 days.

Decolourization assay

Decolourization was calculated in terms of percentage, using the following formula as described by Sani and Banerjee (1999).

$$\text{Percentage of decolourization} = \frac{\text{Initial absorbance value - Final absorbance value}}{\text{Initial absorbance value}} \times 100$$

The fungal isolates were tested for their capacity to utilize the dye as C/N source. Two sets of mineral salt medium (Walker *et al*., 1993) of 100 ml capacity were prepared. To one set, 1% glucose was added as carbon source and the dye was added at three different concentrations of 100, 200 and 300 ppm as nitrogen source. To another set ammonium nitrate (0.03 gm / 100 ml) was added as nitrogen source and the azo-dye was incorporated at three different concentrations (100, 200 and 300 ppm) as carbon source. Both sets were inoculated with fungal cultures and incubated at 28° ± 1° C for 10 days. At the completion of the incubation periods, the culture broth was assayed colorimetrically for dye decolourization.

RESULTS AND DISCUSSION

From the azo-dyes contaminated soil samples, azo-dye resistant fungal species were isolated. The fungal species were identified and designated as *Aspergillus erythrocephalus* NCBT 106, *Cladosporium herbarum* NCBT 147, *Fusarium solani* NCBT 163 and *Humicola fuscorata* NCBT 169. These fungal isolates were identified as indicator organisms of azo-dye polluted soils. The azo-dye decolorization percentage of these isolates are presented in Tables 1 and 2. The percentage of decolourization by azo-red dye (Table 1) was observed as 85% in the case of *Aspergillus erythrocephalus,* 75% for *Cladosporium herbarum*, 70% for *Humicola fuscorata* and 60% for *Fusarium solani* at 200 ppm dye concentration. Decolourization percentage was low in the rest of the concentrations and hence 200 ppm dye concentration was found to be optimum for these fungal isolates. For azo-blue dye decolourization (Table 2), it was maximum in the case of *Cladosporium herbarum* (80%), followed by *Humicola fuscorata* (75%), *Aspergillus erythrocephalus* (70%) and *Fusarium solani* (60%) at 200 ppm dye concentration and in this case also 200 ppm was found to be optimum concentration. There are many reports regarding the decolourization of dye by fungi such as *Geotrichum candidum, Trametes versicolor, T. modesta, T. pocas, T. cigulata, Clitocybulla dusenii, Pleurotus ostreatus, Bjerkandera adusta, Pycnoporus cinnabarinus* and *Datronia concentria* was found to exhibit the

biodegradability of azo-dyes as reported by Nyanhongo *et al*. (2002), Tekere *et al*. (2001), Schliephake *et al*. (2000), Heinfling *et al*. (1997), Kim *et al*. (1995), Shin and Kim (1998), Wesenberg *et al*. (2002) and Verma and Madamwar (2002). The present investigation is yet another source of report, that *Aspergillus erythrocephalus* can be utilized to decolourize the azo-red dye whereas *Cladosporium herbarum* can be utilized for blue dye biodegradation. Application of these fungi for biodegradation treatment of azo-red and azo-blue dyes for industrial use is possible under two important conditions as stated by Keharia and Madamwar (2003).

- Use of the dye degrading enzymes extracted from the culture medium and
- Use of whole cell active cultures directly for transformation of dyes.

Mechanism of dye decolourization

The mechanism of dye decolourization involve a two-step process, *viz*., the physical absorption and the enzymatic degradation. According to Knapp and Newby (1995) the fungal mediated dye decolourization is due to absorption mechanism by the fungal cell surface. Young and

Table 1: Efficiency of azo-red dye decolorization by indicator fungal strains

S. No.	Fungal Strain	Dye Concentration (ppm)	Percentage of decolorization (10 days of incubation)
1.	*Aspergillus erythrocephalus* NCBT 106	100	70
		200	85
		300	70
2.	*Cladosporium herbarum* NCBT 147	100	65
		200	75
		300	60
3.	*Fusarium solani* NCBT 163	100	55
		200	60
		300	50
4.	*Humicola fuscorata* NCBT 169	100	60
		200	70
		300	70

Table 2: Efficiency of azo-blue dye decolorization by indicator fungal strains

S. No.	Fungal Strain	Dye Concentration (ppm)	Percentage of decolorization (10 days of incubation)
1.	*Aspergillus erythrocephalus* NCBT 106	100	60
		200	70
		300	60
2.	*Cladosporium herbarum* NCBT 147	100	70
		200	80
		300	60
3.	*Fusarium solani* NCBT 163	100	50
		200	60
		300	50
4.	*Humicola fuscorata* NCBT 169	100	60
		200	75
		300	60

Yu (1997) suggested that the binding of dyes to the fungal hyphae and physical adsorption and enzymatic degradation by extracellular and intracellular enzymes as reasons for the colour removal.

In the present investigation the maximum colour decolourization of azo-red dye by *Aspergillus erythrocephalus* and azo-blue dye by *Cladosporium herbarum* might have been mediated by the cellular and intracellular enzymes of the fungi as reported by Young and Yu (1997). Dyes with different structures are decolourized at different intrinsic enzymatic rates and high dye concentration results in slower decolourization rate (Abadulla *et al*., 2000). Such mechanisms would have occurred in the present experimental condition and the 200 ppm concentration is found to be optimum for both azo-red and azo-blue dyes.

CONCLUSION

The fungal isolates *Aspergillus erythrocephalus* NCBT 106 can be utilized to decolourize the azo-red dye by extracting the enzymes from the culture medium whereas *Cladosporium herbarum* NCBT 147 can be utilized to decolourize the azo-blue dye as whole cell active cultures directly to decolourize the dye.

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REFERENCES

1. Abadulla, E., Tzanov, T., Costa, S., Robra, K. H., Cavaco-Paulo, A. and Gubitz, G. M., Decolourization and detoxification of textile dyes with a laccase from *Trametes hirsute*. *Appl. Environ. Microbiol.* **66**: 3357 (2000).
2. Bechtold, T., Burtscher, E. and Aurora, T., Cathodic decolourization of textile waste water containing reactive dyes using a multi-cathode electrolyser. *J. Chem. Technol. Biotechnol.* **76**: 303 (2001).
3. Cripps, C., Bumpus, J. A. and Aust, S. D., Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **56**: 1114-1118 (1990)..
4. Domsch, K. H., Gams, W. and Trante-Heidi Anderson., *Compendium of Soil Fungi*. Vol. 1. Academic Press, London (1980).
5. Heinfling, A., Bergbauer, M. and Szewzyk, U., Biodegradation of azo and phthalocyanine dyes by *Trametes versicolor* and *Bjerkandera adusta. Appl. Microbiol. Biotechnol.* **48**: 261 (1997).
6. Keck, A., Klein, J., Kudlich, M., Stolz, A., Knackmuss, H. J. and Mattes, R., Reduction of azo-dyes by redox mediators originating in the Napthalene sulfonic acid degradation pathway of *Sphingomonas* sp. strain BN-6. *Appl. Environ. Microbiol.* **63**: 3684-3690 (1997).
7. Keharia, H. and Madamwar, D., Bioremediation concepts for treatment of dye containing waste water: A Review. *Indian J. Expt. Biol.* **41**: 1068-1075 (2003).
8. Kim, S. J., Ishikawa, K., Hirai, M. and Shoda, M., Characteristics of a newly isolated fungus, *Geotrichum candidum* Dec 1, which decolorizes various dyes. *J. Ferment. Bioeng.* **79**: 601 (1995).
9. Knapp, J. S. and Newby, P. S., The microbial decolourization of an industrial effluent containing a diazo-linked chromospheres. *Water Res.* **29**: 1807 (1995).
10. Nyanhongo, G. S., Gomes, J., Gübitz, G. M., Zvanya, R., Read, J. and Steiner, W., Decolourization of textile dyes by laccases from a newly isolated strains of *Trametes modesta*. *Wat. Res.* **36**: 1449 (2002).
11. Pramer, D. and Schmidt, E. L., *Experimental Soil Microbiology*. Burgess Publishing Co., Minneapolis (1965).

12. Sani, R. K. and Banerjee, U. C., Screening for organisms applicable to the decolourization of triphenylaniline dyes and optimization of biotransformation conditions in stirred tank reactor. *Indian. J. Environ. Ecoplan.* **2**: 1-9 (1999).
13. Schliephake, K., Mainwaring, D. E., Lonergan, G. T., Jones, I. K. and Baker, W. L., Transformation and degradation of the disazo dye Chicago sky blue by a purified laccase from *Pycnoporus cinnabarinu*. *Enzyme Microb. Technol.* **27**: 100 (2000).
14. Shin, K-S. and Kim, C-J., Decolourization of artificial dyes by peroxidase from the white rot fungus *Pleurotus ostreatus*. *Biotechnol. Lett*. **20**: 569 (1998).
15. Spadaro, J. T. and Renganathan, V., Peroxidase catalysed oxidation of azo-dyes: Mechanism of disperse yellow 3 degradation. *Arch. Biochem. Biophys.* **312**: 301 (1994).
16. Tekere, M., Mswaka, A. Y., Zvauya, R. and Read, J. S., Growth, dye degradation and ligninolytic activity studies on Zimbabwean white rot fungi. *Enzyme Microb. Technol.* **28**: 420 (2001).
17. Verma, P. and Madamwar, D., Comparative study of transformation of azo-dyes by different white rot fungi. *Indian J. Biotechnol.* **1**: 321 (2002).
18. Vyas, B. R. M. and Molitoris, H. P., Involvement of an extracellular H_2O_2-dependent ligninolytic activity of the white rot fungus *Pleurotus ostreatus* in the decolourization of Remazol Brilliant Blue R. *Appl. Environ. Microbiol.* **61**: 3919 (1995).
19. Walker, A., Parekh, W. R., Roberts, S. J. and Welch, R., Evidence for the enhanced biodegradation of napropamide in soil. *Pestic. Sci.* **39**: 55-60 (1993).
20. Wesenberg, D., Buchon, F. and Agathos, S. N., Degradation of dye-containing textile effluent by the agaric white-rot fungus *Clitocybula dusenii*. *Biotechnol. Lett.* **24**: 989 (2002).
21. Young, L. and Yu, J., Ligninase-catalysed decolourization of synthetic dyes. *Water Res.* **31**: 1187 (1997).
22. Zimmerman, T., Kulla, H. G. and Leisinger, T., Properties of purified orange-II azo reductase, the enzyme initiating azo dye degradation by Pseudomonas KF 46. *Eur. J. Biochem.* **129**: 197 (1982).