

Fungal Decolourization of Direct Azo Dyes and Biodegradation of Textile Dye Effluent

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Keywords	Abstract
Decolourization Degradation Direct azo dyes Textile dye effluent Fungi	Decolourization of Direct azo dyes by fungi isolated from textile dye effluent was investigated. Seven different fungal species were isolated and identified. The fungal isolates were identified as <i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Aspergillus fumigatus</i> , <i>Fusarium oxysporum</i> , <i>Penicillium chrysogenum</i> , <i>Mucor</i> sp. and <i>Trichoderma viride</i> . The fungal inoculums were inoculated into flasks containing Direct azo dyes (500 mg/l) with trace amounts of yeast extract, glucose and sucrose and then sterilized and incubated for 12 days. <i>Aspergillus niger</i> completely decolourized the Congo Red within 6 days. The best decolourizer of Viscose Orange-A was <i>Aspergillus fumigatus</i> (88.70%). <i>Mucor</i> sp. (69.73%) was identified as the best decolourizer of Direct Green – PLS. The dye Direct Violet-BL was completely decolourized by <i>Aspergillus niger</i> within 9 days and <i>Trichoderma viride</i> within 12 days. The dye Direct Sky Blue-FF was completely decolourized by <i>Aspergillus flavus</i> within 9 days and <i>Mucor</i> sp. within 12 days. <i>Penicillium chrysogenum</i> have the capacity to completely decolourized the dye Direct Black-E within 12 days. Fungal biodegradation was assessed by physicochemical analysis.

1. Introduction

The textile industry is one of the industries that generate a high volume of waste water. Strong colour of the textile waste water is the most serious problem of the textile waste effluent. The disposal of these wastes into receiving water causes damage to the environment. Dyes may significantly affect photosynthetic activity in aquatic habit because of reduced light penetration and may also toxic to some aquatic life due to the presence of aromatics, metals, chlorides and other toxic compounds (Husseiny, 2008).

Dyes are major source of metals like; Cd, Cr, Co, Cu, Hg, Ni, Mg, Fe and Mn. Sediments and suspended solids particles are important repositories for trace metals in waste water. 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. The metals and some other contaminants tend to persist indefinitely; circulating and eventually accumulating throughout the food chain (Rivera *et al.*, 1999).

Azo dyes are largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries (Maximo *et al.*, 2003). Azo dyes are characterized by the presence of one or more azo groups – $N = N -$, which are

responsible for their colouration and when such a bond is broken the compound loses its colour. They are the largest and most versatile class of dye, but have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water system. Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and during washing. Many are carcinogenic and may trigger allergic reactions in man. It is estimated that over 10% of the dye used in textile processing does not bind to the fibers and is therefore released to the environment. Some of these compounds cause serious threat because of their carcinogenic potential or cytotoxicity (Adedayo *et al.*, 2004).

Virtually all dyes from all chemically distinct groups are prone to fungal oxidation but there are large differences between fungal species with respect to their catalyzing power and dye selectivity. A clear relationship between dye structure and fungal dye biodegradability has not been established (Fu and Virarahavan, 2001). Degradation by fungi is known as mycoremediation. Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints. Beyond the production of such relevant metabolites, fungi have been attracting a growing interest for the biotreatment of waste water ingredients such as

metals, inorganic nutrients and organic compounds (Coulbaly, 2003).

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. The fungal mycelia have an additive advantage over single cell organisms by solubilizing the insoluble substrates by producing extracellular enzymes. Due to an increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with the environment. The extra-cellular nature of the fungal enzyme is also advantageous in tolerating high concentration of the toxicants. Many genera of fungi have been employed for the dye decolourization either in living or dead form (Prachi Kaushik and Anushree Malik, 2009).

Dyes are removed by fungi through biosorption, biodegradation, bioaccumulation and enzymatic mineralization (Lignin peroxidase, Manganese peroxidase, Manganese independent peroxidase and Laccase) (Wesenberg *et al.*, 2002). Biosorption is defined as binding of solutes to the biomass by processes which do not involve metabolic energy or transport. Biodegradation is an energy dependent process and involves the breakdown of dye into various products through the action of various enzymes. Bioaccumulation is the accumulation of pollutants by actively growing cells by metabolism. The present study was focused on decolourization and degradation of textile direct azo dyes and biodegradation of textile dye effluent by using bacteria and fungi isolated from textile dye effluent.

2. Materials and Methods

Sample collection and preservation

The dye house effluent was collected from a dyeing unit in Tirupur region (Tamil Nadu, India). It was refrigerated at 4°C and used without any preliminary treatment.

Dyes

Direct azo dyes were used in this study. The dye samples were commercially graded and kindly supplied by the dealers of "ATUL Dyes". Direct azo dyes used in this research are, Congo Red ($\lambda_m = 580$ nm), Viscose Orange – A ($\lambda_m = 480$ nm), Direct Green PLS ($\lambda_m = 580$ nm), Direct Violet BL ($\lambda_m = 550$ nm), Direct Sky Blue FF ($\lambda_m = 580$ nm) and Direct Black – E ($\lambda_m = 600$ nm).

Isolation and identification of dye decolourizing fungi from textile dye effluent

Isolation of dye decolourizing fungi

Pour plate technique was used for the isolation of dye decolourizing fungi.

Maintenance of fungal isolates

Well grown fungal colonies were maintained on Sabouraud dextrose agar slants and stored at 4°C.

Identification of the fungal isolates

Identification of the fungal isolates was carried out by the routine mycological methods i.e., Lactophenol cotton blue staining and plating on Sabouraud dextrose agar medium.

Screening of fungal isolates for textile direct azo dye degradation

Inoculum preparation

The suspension of 4 days old cultures of fungi were used to investigate their abilities to decolourize dyes. They were prepared in saline solution (0.85% Sodium chloride). The fungal cultures were inoculated into 50 ml of saline and incubated at room temperature for 5 hours (Benson, 1994).

Dye decolourization experiments

Dye decolourization experiments were carried out in 100 ml flask containing 50 ml of Direct azo dyes (500 mg/l), traces of yeast extract, sucrose and glucose. The pH was adjusted to 7 ± 0.2 using Sodium hydroxide and Hydrochloric acid solution. Then, the flasks were autoclaved at 121°C for 15 minutes. The autoclaved flasks were inoculated with 5 ml of fungal inoculums of each isolates. The flasks were kept in mechanical shaker at room temperature for 12 days. Samples were drawn at 3 days intervals for observation. 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. Decolourization was assessed by measuring absorbance of the supernatant with the help of UV-Spectrophotometer at wavelength maxima (λ_m) of respective dye.

Decolourization assay

Decolourization assay was measured in the terms of percentage decolourization using UV-Spectrophotometer. The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{InitialOD} - \text{FinalOD}}{\text{InitialOD}} \times 100$$

Biodegradation of textile dye effluent by fungal consortium

Fungal biodegradation of textile dye effluent was carried out in 1000 ml flask containing 800 ml of dye effluent. To the dye effluent 8 g of peptone and 32 g of dextrose was added and then sterilized by autoclaving at 121°C for 15 minutes. The pH was adjusted to 5.6 ± 0.2 . The autoclaved flask was inoculated with 5 ml of fungal inoculums of each microorganism. The flask was kept in mechanical shaker and incubated at room temperature for 12 days. Biodegradation was assessed by physico-chemical analysis.

3. Results and Discussion

Biodegradation of textile effluent and commercially available textile dyes namely, Congo Red, Viscose Orange-A, Direct Green- PLS, Direct Violet-BL, Direct Sky Blue-FF and Direct Black-E were studied against seven fungal isolates which have been isolated from the dye effluent sample by Pour plate method and percentage decolourization was shown in the graphs accompanying the results. Finally physico-chemical analyses were done with untreated and microbially treated textile effluent.

Seven different fungi were isolated from the dye effluent. Based on Lactophenol cotton blue staining and colony morphology on Sabourauds dextrose agar, they were identified as, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichoderma viride*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Mucor* sp.

Figure 1: Decolourization of Congo Red by fungi

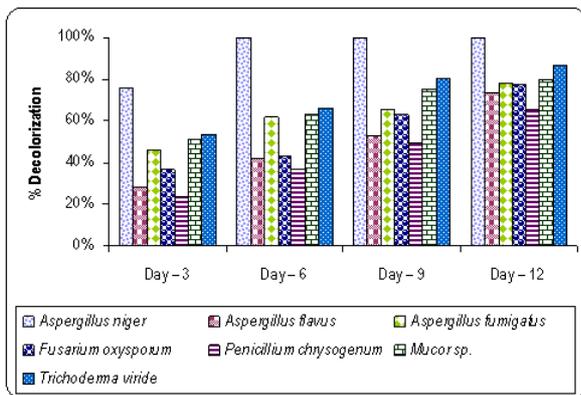


Figure 2: Decolourization of Viscose Orange-A by fungi

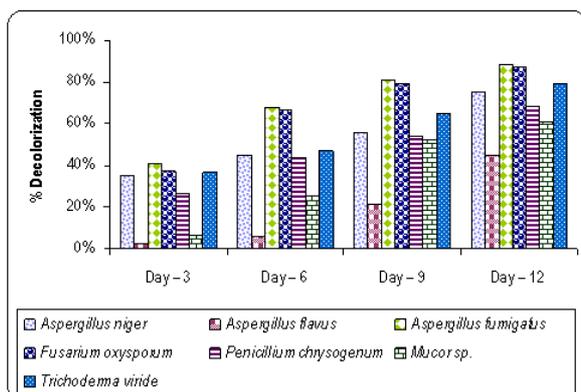


Figure 3: Decolourization of Direct Green-PLS by fungi

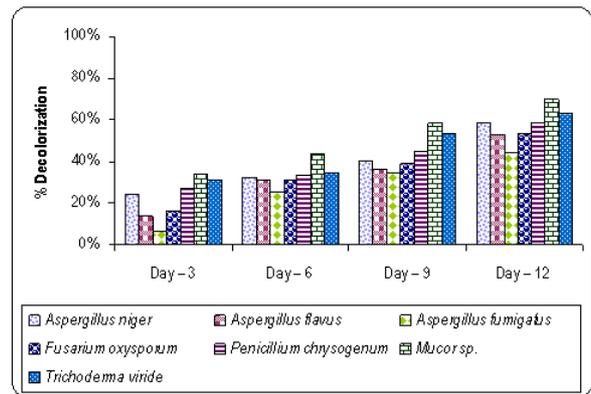


Figure 4: Decolourization of Direct Violet-BL by fungi

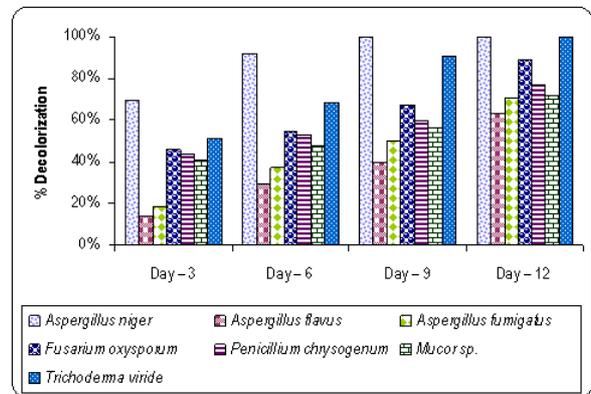


Figure 5: Decolourization of Direct Sky Blue-FF by fungi

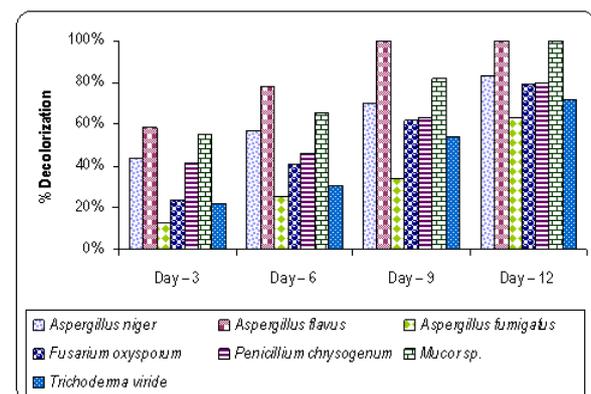
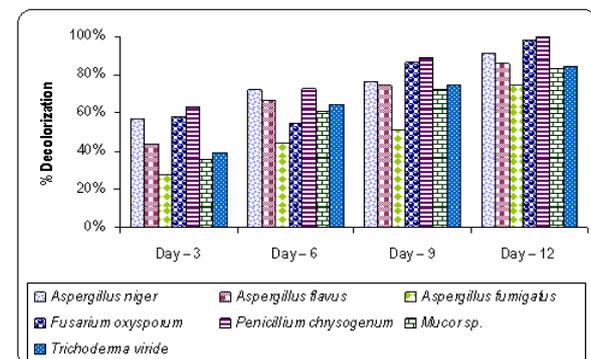


Figure 6: Decolourization of Direct Black-E by fungi



In this study, fungal dye degradation was studied using UV-Spectroscopic analysis. The fungal inoculums was inoculated into the flasks containing direct azo dyes with trace amount of carbon sources and incubated for 12 days. The decolourization was expressed in terms of percentage decolourization. *Aspergillus niger* completely decolourized the Congo Red within 6 days. The best decolourizer of Viscose Orange-A was *Aspergillus fumigatus* (88.70%). *Mucor* sp. (69.73%) was identified as the best decolourizer of Direct Green-PLS. The dye Direct Violet – BL was completed decolourized by *Aspergillus niger* within 9 days and *Trichoderma viride* within 12 days. The dye Direct Sky Blue – FF was completed decolourized by *Aspergillus flavus* within 9 days and *Mucor* sp. within 12 days. *Penicillium chrysogenum* have the capacity to completely decolourized the dye Direct Black-E within 12 days.

Muhammad Asgher *et al.*, 2008) screened the decolourization capacity of five indigenous white rot fungi *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Coriolous versicolor*, *Ganodermalucidum* and *Schizophyllum commune* against four vat dyes, Cibanon Red, Cibanon Golden-Yellow, Cibanon Blue and Indanthrene Direct Black. It was observed that the *Coriolous versicolor* could effectively decolourized all the four vat dyes at varying incubation times but best results were shown on Cibanon Blue (90.7%) after 7 days, followed on Cibanon Golden-Yellow (88%), Indanthrene Direct Black (97.7%) and Cibanon Red (74%). *Phanerochaete chrysosporium* also showed good decolorization potential on Cibanon blue (87%), followed by Cibanon Golden-Yellow (74.8%), Cibanon Red (71%) and Indanthrene Direct Black (54.6%).

The fungal isolates like *Aspergillus niger*, *Penicillium* sp. and *Bacillus* sp. were used for decolourization activity of reactive and direct dyes. *Aspergillus niger* and *Penicillium* sp. were found to be the most efficient isolates. The maximum degradation activities of *Aspergillus niger* for direct dye was under static condition at pH 4, 28°C and after 4 days of incubation period but for *Penicillium* sp. it was static condition, at pH 4.5, 35°C and after 4 days of incubation period (Husseiny, 2008).

In this work, after inoculation of isolated fungal consortium in textile dye effluent, the colour was changed from black to dark brown. The pH was brought from 9.3 to 7.2. The biological oxygen demand was reduced from 1646 mg/l to 813 mg/l and the chemical oxygen demand was reduced from 3279 mg/l to 1347 mg/l. The fungal biomass displayed good sorption capabilities giving rise to decrease in chemical oxygen demand upto 58% (Valeria Prigione *et al.*, 2008).

4. Conclusion

Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and eco-friendly techniques using fungi can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. Thus, by this present study I concluded that the fungal isolates like *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Mucor* sp. and *Trichoderma viride* were used as a good microbial source for waste water treatment.

Bibliography

- Adedayo, O., Javadpour, S., Taylor, C., Anderson, W.A., and Moo-Young. 2004. Decolourization and detoxification of Methyl Red by aerobic bacteria from a waste water treatment plant. *World Journal of Microbiology*. 20: 545-550.
- Benson, W.J. 1994. Microbiology applications: laboratory manual in general microbiology. Wm. C. Bron Commication., U.S.A.
- Coulibaly, L. 2003. Utilization of fungi for biotreatment of raw waste waters. *African Journal of Biotechnology*. 2(12): 620-630.
- Fu, Y.Z., and Virarahavan, T. 2001. Fungal decolourization of dye waste waters. *Bioresource Technology*. 79: 251-262.
- Husseiny, M. 2008. Biodegradation of the Reactive and Direct dyes using Egyptian isolates. *Journal of Applied Science Research*. 4(6): 599-606.
- Maximo, C., Amorim, M.T.P., and Costa Ferreira, M. 2003. Biotransformation of industrial reactive azo dye by *Geotrichum* sp. *Enzyme and Microbial Technology*. 32: 145-151.
- Muhammad Asgher, Sahaheerah Batool, Haq Nawaz Bhatti, Razia Noreen, Rahman and Javid Asad. 2008. Laccase mediated decolourization of vat dyes by *Coriolous versicolor* IBL-04. *International Biodeterioration and Biodegradation*. 62: 465-470.
- Prachi Kaushik, and Anushree Malik. 2009. Effect of the nutrient composition on dye decolourization and extra cellular enzyme production by *Lentinus edodes* on solid medium. *Enzyme Microbial Technology*. 30 : 381-386.
- Rivera, C.J., Holdsworth, G. M., Dempsey, C.R., and Dostal, K.A. 1999. Biodegradation of textile azo dyes by microbes. *Chemosphere*. 22 : 107-119.
- Valeria Prigione, Cizinia Pezzella and Antonella Anastasi. 2008. Decolourization and detoxification of textile effluents by fungal biosorption. *Water Research*. 42: 2911-2920.

Wesenberg, D., Buchon, F., and Agathos, S.N.
2002. Degradation of dye containing textile
effluents by the agaric white-rot fungus

Clitocybula dusenii. *Journal of Biotechnology*. 24 :
989-993.