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STUDIES ON FUNGAL DECOLORIZATION AND DEGRADATION OF FEW AZO DYES

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ABSTRACT: Utilization of fungi for the removal of dyes and treatment of textile effluents is an eco-friendly, cost effective alternative. In this study, varied fungal forms were isolated from the soils near the Thane creek, Maharashtra. The isolated fungal forms were screened for their azo dye degrading capacities by qualitative methods. The azo dyes used were Congo red, Bromophenol Blue and Methyl orange. A zone of hydrolysis around the fungal colony or change in the original colour of the dye and visual disappearance of colour from the petriplate inoculated with the fungus was observed. Of all the fungal forms *Aspergillus nige* and *Trichoderma* sp. showed high dye decolorization potentials. Percentage decolourization was studied for *Aspergillus niger* and *Trichoderma* sp using uv- visible spectrophotometer analysis. *Aspergillus niger* showed highest percentage decolourization for bromophenol blue (97.39%) whereas Trichoderma sp showed showed highest percentage decolourization for Congo red (87.75%). The decolorization of azo dyes by the fungi can be attributed to the production of extracellular lignin modifying enzymes.

Key words: - Azo-dye, Bromophenol blue, Congo red, Decolorization, fungi, textile effluents

INTRODUCTION :

One of the most important cause for water pollution is the industrial waste water. Among the various industries that release their effluents in the water bodies, the textile industry is the major contributor. The textile industry releases about 10 to 15% of the dye, which finds its way into waste water (Rodríguez *et al.*, 1999). The effluents released contains hazardous chemical compounds such as azo dyes, which are difficult to degrade and pose environmental problems (Fouda *et al.*, 2016).

Dyes are carcinogenic, and can induce allergic and asthmatic reactions to the human body toxic. Since they are stable and non- degradable they cannot be removed by conventional waste water treatment methods. Various physical and chemical procedures such as flocculation, membrane filtration, electrochemical techniques, ozonation, coagulation, adsorption etc. have been employed for elimination of the coloured dyes from the textile effluents (Kapdan and Kargi *et al.*, 2002), however these are highly expensive, time consuming and unsustainable procedures that result in the generation of huge amount of sludge Hence there is a need for alternative effluent treatment method that is low cost, sustainable and eco-friendly. Many researchers have recommended the use of micro-organisms, in particular bacteria and fungi in bioremediation which is a cost effective, eco efficient and widely acceptable approach for the treatment of waste waters and textile effluents (Banat, 1996).

Filamentous fungi have been reported as efficient producers of extracellular lignolytic enzymes including laccase, lignin peroxidase and manganese peroxidase. These enzymes are able to oxidize a variety of substrate molecules such as dyes, PAHs, lignins etc (Raghukumar *et al.*, 1996). Moreover, higher yield of enzyme production compared to bacteria and the ability to produce several auxiliary enzymes that are required for the debranching of the substituted organic compounds make fungi more preferred over bacteria. Several studies demonstrated the

dye degradation activities and partial mineralization capabilities of white rot fungi namely *Phanerochaete chrysosporium*, *Bjerkandera audusta*, *Trametes versicolor* (Revankar and Lele, 2007). The soft rot fungi like *Aspergillus niger*, *Aspergillus foetidus* , *A. flavus*, *Curvularia verruciformis*, *Fusarium* sp. and *Penicillium* sp. also showed the dye degradation activity and hence could be an attractive option for the treatment of effluents (Sumanthi and Manju, 2000; Yang *et al.*, 2003; Sharma *et al.*, 2009). In the present study, diverse fungal forms were isolated from the contaminated soil from Thane creek and screened for the highly potent fungi for their azo dye degrading capacities for possible utilization in the process of bioremediation

MATERIALS AND METHODS:

1. Isolation of fungi

Thane creek located in Thane Maharashtra, India is highly contaminated with industrial effluents. The soil sample was collected in zip lock bags from a location near the creek side of Thane creek, where there is shallow contact with water. One gram of soil sample was used for isolation of fungi by serial dilution technique (Aneja, 2008). The pure culture was then transferred to PDA slants and maintained at 4°C and sub-cultured every month for further use.

2. Morphological identification

The various fungal forms were studied for their macroscopic characters such as colour, shape, appearance, colony growth and diameter of colonies and Microscopic (microstructures) characteristics. The fungal forms were identified using standard books and referring monographs (Gilman, 1957; Raper & Fennell, 1965) and research papers.

3. Qualitative screening of fungi

For the present study, three different azo dyes used namely Congo red, Bromophenol blue and methyl orange were commercially obtained. The isolated fungal forms were screened on PDA medium supplemented with 0.01% of each dye by dye agar plate assay techniques (Arora *et al.*, 2005). The uninoculated plates were kept as control. The diameter of the growing colony and the zone of hydrolysis was measured daily. Based on the diameter of zone of hydrolysis the highly potent fungal frorm was selected for quantitative studies.

4. Inoculum preparation

Three mycelial plugs (8 mm diameter) from a 7 day old culture PDA plate were cut with a potato borer and added aseptically to the sterilized 250ml Erlenmeyer flasks containing 10 ml of Sabouraud's broth. The inoculated flasks were incubated at 28° ±2°C on an orbital shaker at 150 rpm for 48 hrs. to obtain large quantity of active mycelia.

5. Dye Decolorization

Submerged fermentation was carried out for the azo dye degrading fungi and their activities were studied spectrophotometrically (Saranraj *et al.*, 2010). 30 ml of Sabouraud's dextrose broth containing 0.01% of the selected azo dyes was taken in100ml flask. pH was adjusted to 7. Then the flasks were autoclaved at 121° C for 15 minutes. The autoclaved flasks were inoculated with 5ml of fungal inoculums of highly potent fungal isolates. The uninoculated flasks were kept as control. All the flasks were kept on orbital shaker with 150 rpm for 8 days. 10 ml of the dye solution was filtered and centrifuged at 3000rpm for 10 mins.

Dye Decolourization assay

Decolourization was assessed by measuring the absorbance of the supernatant with the help of uv- spectrophotomether at maximum wavelength (λm) of respective dye.

Decolourization activity (%) was calculated as described below:

$$\text{Percentage Decolorization} = \frac{\text{Initial OD - Final OD}}{\text{Initial OD}} \times 100$$

RESULT & DISCUSSION:

Nine fungal isolates were obtained from the contaminated soil sample. These were identified as : *Aspergillus niger* (four isolates), *A. flavus* (two isolates), *Trichoderma* species (two isolates) , *Fusarium* sp (one isolate) (Fig1) . Similar results were reported by Raju *et al.*, (2007). They isolated *Aspergillus niger, A. flavus, Fusarium oxysporium* and *Penicillium notatum* from heavily contaminated textile dye effluent water bodies. However Ali *et al.*, (2008a) isolated *Aspergillus flavus, A. terreus, Alternaria sp* and *Penicillium sp* from storage pond of textile effluent.

The primary screening of the isolated fungi for azo dye degradation capabilities were carried out by qualitative methods. Methyl orange is deep orange red in colour. The gradual decrease in the intensity of the deep orange colour of Methyl orange to yellow colour shows the dye decolorizing ability of the fungus. The disappearance of the blue colour of Bromophenol blue during the fungal growth and the formation of yellow colour around the fungal colony indicates the production of enzymes for dye decolorization. Maximum yellow colour was observed around the *Aspergillus niger* colony. Congo red is also a pH indicator dye . The red colour dye is decolorized and a light colour ring is observed around the fungal colony.

In present study it was observed that *Aspergillus niger* and *Trichoderma sp* showed maximum zone of hydrolysis with all the three dyes used viz., Methyl orange, Bromophenol blue and Congo red (Fig2, Fig3 and Fig 4). Minimum dye degradation was observed with *Aspergillus flavus*. Therefore *Aspergillus niger* and *Trichoderma sp* were selected for percentage decolorization assay studies. Karthikayan *et al.*, (2012) also reported that among 31 fungal isolates from soil sample polluted with textile dyes from textile hub of Tamil nadu, India, *Aspergillus niger* HM11 was most efficient to decolorize ongo red and Reactive blue 140 dye by qualitative method. Other studies emphasized that *Aspergillus flavus* decolorize Bromophenol blue and congo red at 1% w/v on PDA medium (Singh and Singh, 2010)

Percentage dye decolorization studies with the highly potent fungi were carried out for the highly potent fungi *Aspergillus niger* and *Trichoderma sp. It was observed that Aspergillus niger* was highly potent fungus as compared to *Trichoderma* sp in *decolorizing the azo dyes* (Graph 1). Salem *et al.*, (2019) also reported *Aspergillus niger* as highly efficient fungus with dye decolorizing abilities. According to Sadhasivam *et al.*, (2005) no studies were recorded in literature using *Trichoderma viride*, although *T. harzianum* was used for decolorization of Trypan Blue. Isil and Tugba (2008) reported that some species of *Trichoderma* showed decolorization of methyl orange in the range of 6- 52%. These results are in accordance with the present study.

CONCLUSION:

Dye decolorization during fungal growth can be attributed to the production o extracellular enzymes as well as to the adsorption of dyes by the mycelium. Lignin modifying enzymes such as laccase, lignin peroxidase and manganese peroxidase are involve in lignin degradation and breakdown of mot of the organo-pollutants.. In our study *Aspergillus niger* and *Trichoderma* sp are highly potent fungi for their azo dye decolorization abilities.

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Table 1: Mycelial growth of fungi and dye decolorization zone

Fungal isolate	**0.01% methyl orange**		**0.01% Bromophenol blue**		**0.01% congo red dye**	
	diameter of mycelial growth (cm)	**diameter of dye decolorization zone (cm)**	**diameter of mycelial growth (cm)**	**diameter of dye decolorization zone (cm)**	**diameter of mycelial growth (cm)**	**diameter of dye decolorization zone (cm)**
Aspergillus flavus	**7.5**	**8**	7.2	7.8	6.2	6.6
Aspergillus niger	**7.5**	**8.3**	7	8	6.2	7.8
Fusarium sp	**6**	**6.7**	4	4.6	4.8	5.3
Trichodema sp	**6.8**	**7.6**	7.0	7.8	6.1	7.2

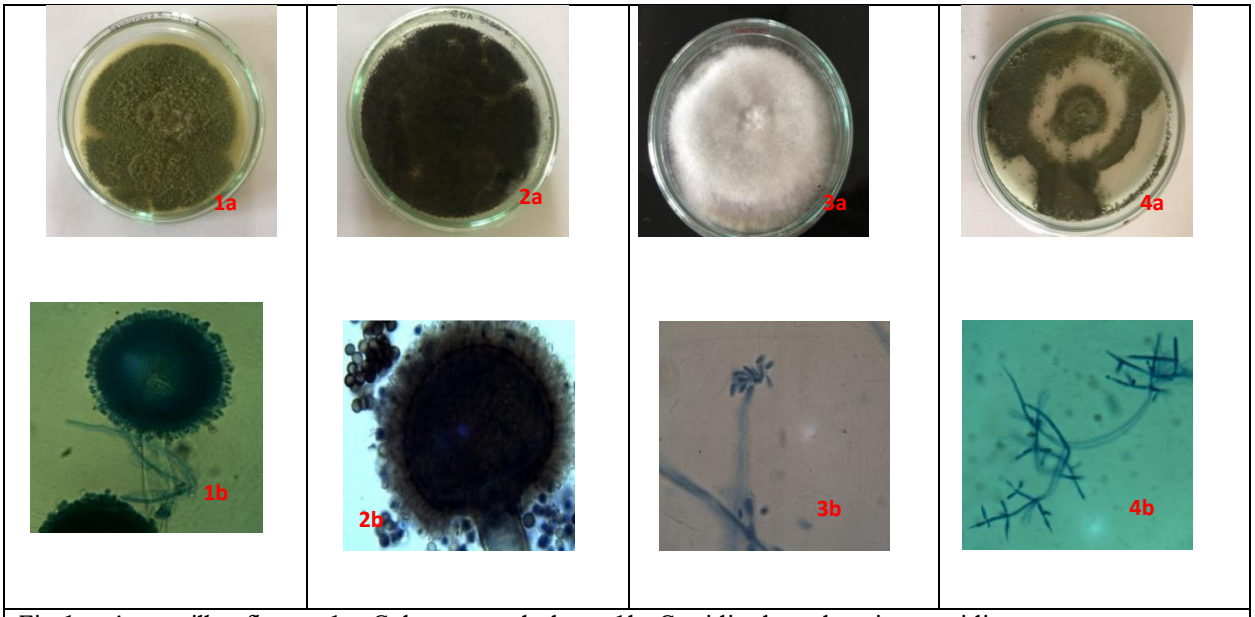


Fig 1. *Aspergillus flavus:* 1a. Colony morphology 1b. Conidiophore bearing conidia., *Aspergillus niger* : 2a. Colony morphology 2b. Conidiophore bearing conidia., *Fusarium sp.* : 3a. Colony morphology 3b. Conidiospores bearing conidia., *Trichoderma sp.* 4a. Colony morphology 4b. Conidiophore bearing conidia

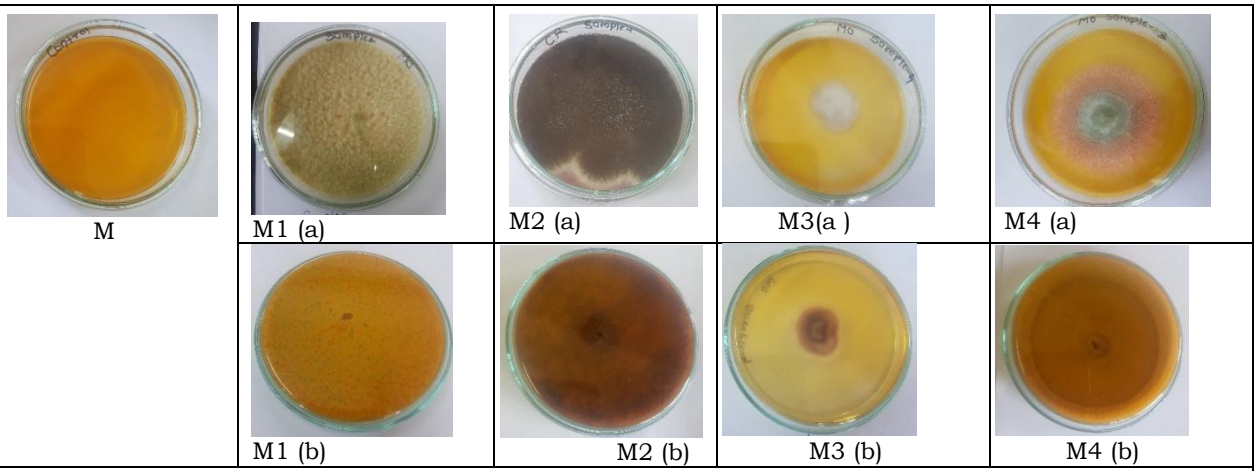
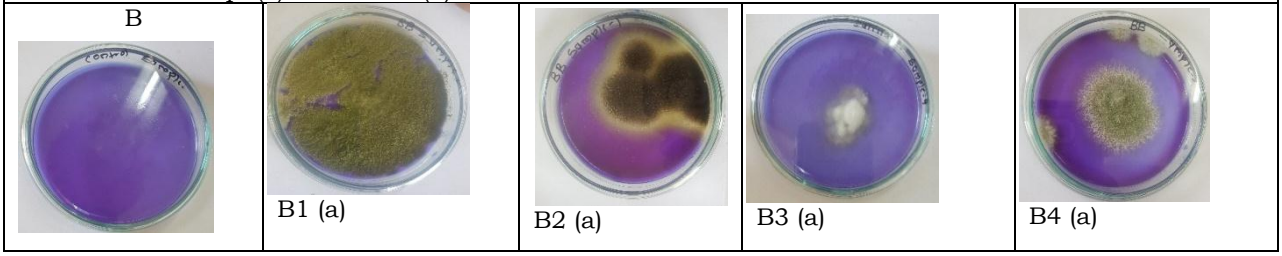


Fig2 : M- control plate with 0.01% methyl orange , M1.*Aspergillus flavus* (a) front view, (b) back view , M2.*Aspergillus niger* (a) front view, (b) back view, M3.*Fusariumsp.* (a)front view (b) back view , M4.*Trichoderma sp.* (a) front view (b) back view



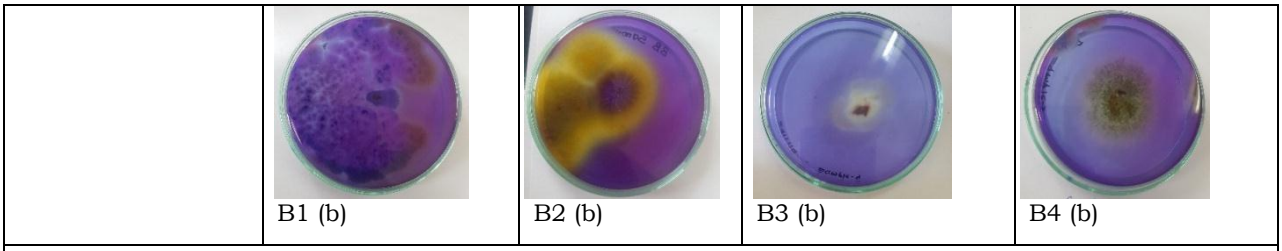


Fig 3: B.control plate with 0.01% bromophenol blue ,B1*Aspergillus flavus* (a) front view, (b) back view , B2.*Aspergillus niger* (a) front view, (b. back view, B3. *Fusariumsp.* (a)front view (b) back view, B4.*Trichoderma sp.* (a) front view (b)back view

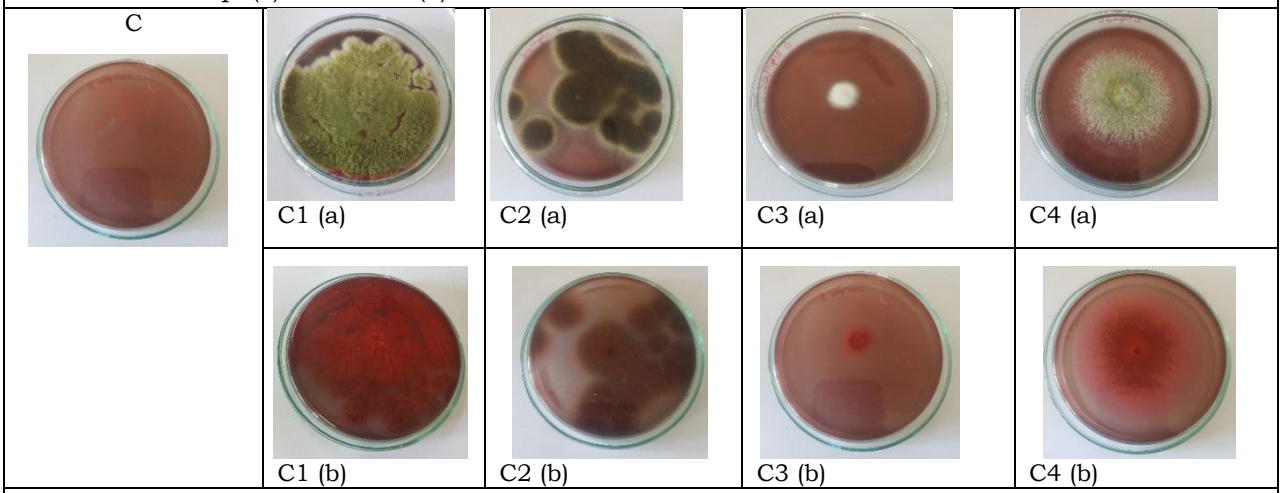
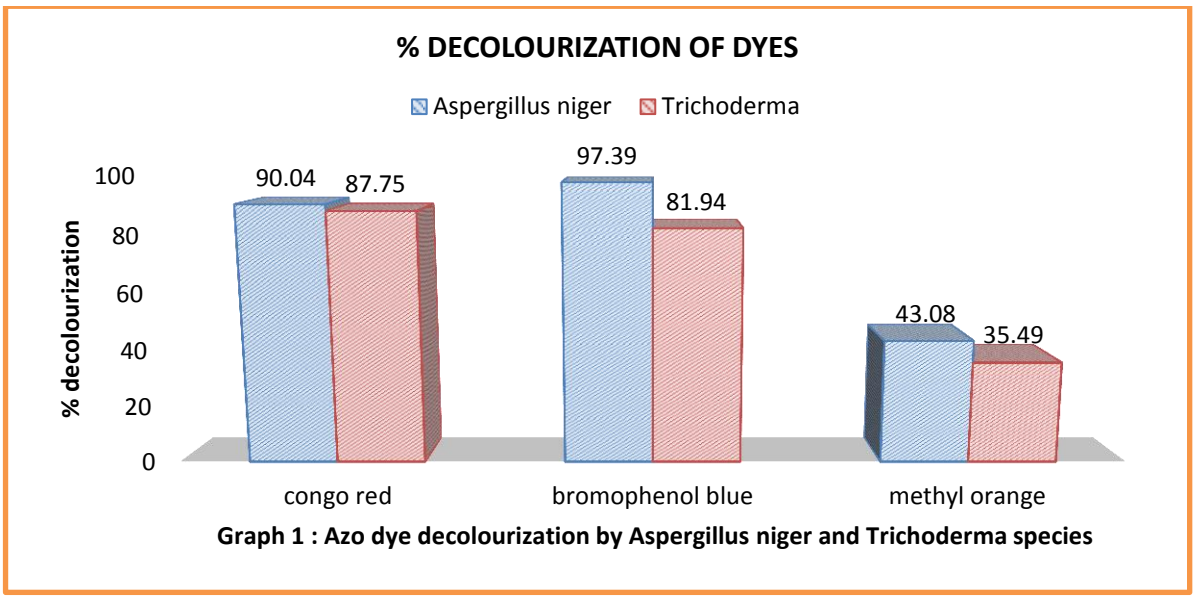


Fig 4 : C - control plate with 0.01% Congo red , C1.*Aspergillus flavus* (a) front view, (b) back view, C2.*Aspergillus niger* (a) front view, (b) back view, C3.*Fusarium sp.* (a)front view (b) back view , C4.*Trichoderma sp.* (a) front view (b) back view



Graph 1 : Azo dye decolourization by Aspergillus niger and Trichoderma species