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**Research Article**

# DECOLOURIZATION OF SYNTHETIC DYES BY *ASPERGILLUS NIGER* ISOLATE RECOVERED FROM COAL MINES

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**ABSTRACT**

Biodegradation of synthetic dyes, rose Bengal, nigrosin, congo red, chlorazole black E and safranin have been carried out using a fungal isolate, *Aspergillus niger,* isolated from coal mines. Among the 33 fungal species recovered from coal mines only *A. niger* was found to be a good decolourizer of dyes. This fungus has shown positive results for the decolourization of synthetic dyes. The degradation of dyes was observed by the change in original colour and visual disappearance of colour from the solution. Decolourization of dyes was also observed as accumulation of dyes by the fungal mycelium, and it was confirmed by the presence of colour in the fungal mycelium. Two types of controls: media without dye and with dye were used for studying dye decolourization.



## INTRODUCTION

Due to rapid industrialization and urbanization, a lot of chemicals including synthetic dyes of various types are manufactured and are being used extensively for textile dyeing, paper printing, colouring silk, cotton, wool, jute fibers, synthetic fibers and coloured photography (Maynard, 1983). Worldwide it has been estimated that more than 100, 000 commercial dyes are available (Robinson *et al*., 2001) and nearly 10 to 15% of these dyestuffs are discharged as industrial effluents in the environment. The dyeing industries represent major environmental problems by releasing coloured dyes as industrial effluents and often lead to calamities. It has been reported that the incidence of bladder tumor is higher in dye industry workers than in the general population (Suryavathi *et al*., 2005). These discharged coloured dyes even at very small concentration have a huge impact on the aquatic environment caused by its turbidity and toxicity. The complicated molecular structures of dyes make wastewater difficult to be treated by conventional methods (Sathiya moorthi *et al*., 2007). Although a number of physical and chemical methods are used to remove these dyes including adsorption, precipitation, chemical oxidation, photodegradation or membrane filteration etc (Yeh and Thomas, 1995; Gogate and Pandit, 2004) before discharging into the environment, but they have some limitations like high cost and disposal of large amount of sludge with some toxic byproducts (Robinson *et al.,* 2001). Therefore, economical and ecofriendly techniques are required for the removal of dye waste from the effluents and bioremediation is one such effective tool where indigenous microorganisms are used for the treatment of industrial dye effluents. Many fungal species (especially white rots), actinomycetes and bacteria are used for the treatment of textile effluents (Mielgo *et al*., 2001; Bhatt *et al*., 2005). Researchers have already worked with many microorganisms, the imperative bacteria being *Staphylococcus arlettae* (Elisangela *et al*., 2009); *Pseudomonas pudita* (Leebana *et al*., 2012); *Nocardia atlantica* (Hassan *et al*., 2013) and fungi viz., Basidiomycetes fungi (Machado *et al*., 2006); *Trametes pubescens* and *Pleurotus ostreatus* (Casieri *et al*., 2008); *Aspergillus tamarii* and *Penicillium purpurogenum* (Ramalingam *et al*., 2010); *A. ochraceous* (Tisma *et al*., 2012); *A. niger, F. oxysporium* and *Trichoderma lignorum* (Shahid *et al*., 2013). Another white rot fungus, *Trametes versicolor* has also been used for efficient decolourization of dye industry effluents and azo dyes such as orange G, congo red and amido black 10 B (Selvam *et al.,* 2012). In addition, some species of other genera like *Pleurotus, Bjerkanera, Polyporus, Phelinus, Funalia, Ganoderma* and *Thelephora* are also reported to possess decolourization potential (Fu and Viraraghavan, 2002;

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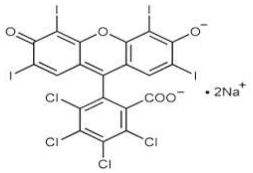
Yesilada *et al*., 2002; Selvam *et al*., 2003; Wesenberg *et al*., 2003). Fungal species are known to involve both enzyme-mediated degradation biosorption in the decolourization of textile dyes (Park *et al*., 2007; Shahid *et al*., 2013). Keeping all this in view, the present investigation was aimed to decolourize five different synthetic dyes by using an isolate of *Aspergillus niger* recovered from coal mines that have the ability to decolourize the dyes using liquid media under shaking as well as stationary conditions.

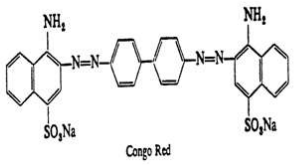
## MATERIALS AND METHODS

### *Dyes used*

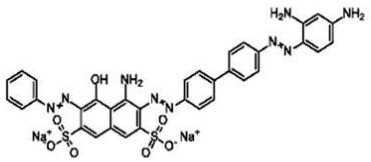
Five different dyes used in the present investigation included rose Bengal ($C_{20}H_2Cl_4I_4Na_2O_5$), congo red ($C_{32}H_{22}N_6Na_2O_6S_2$), safranin ($C_{20}H_{19}ClN_4$), nigrosin ($C_{22}H_{14}N_6Na_2O_9S_2$) and chlorazole black E ($C_{34}H_{25}N_9Na_2O_7S_2$), whose chemical structures are depicted in figure 1.



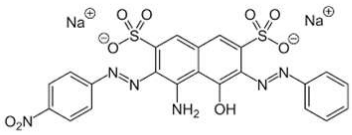
a Rose Bengal



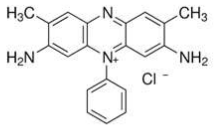

b Congo red



c Chlorazole black E



d Nigrosin



e Safranin

**Figure 1** Chemical structure of dyes

### *Isolation of fungi*

Coal samples were collected from two mines viz., Moghla and Kotla coal mines situated in Rajouri and Reasi districts respectively of Jammu Province. Two methods viz., dilution pour plate method (Waksman, 1927) and Warcup method (Warcup, 1950) were used for the isolation of fungal flora. Five replicates were prepared and incubated at 28º ± 2ºC for 7 days or till proper growth of the fungal colonies was obtained.

### *Identification and maintenance of fungi*

The recovered fungal sp. was identified by studying its cultural and morphological characters. For the purpose of identification, the recovered fungi was grown and made to sporulate on different culture media, such as potato dextrose agar medium (PDA), malt extract agar medium (MEA) and Czapek yeast agar medium (CYA).

Relevant literature and key given by Raper and Fennel (1965) used for the identification of fungal species. The purified and identified fungal culture was maintained on sterilized PDA slants at 10ºC and subsequent sub-culturing was done after every 6 months. Isolate was cultured and maintained in duplicate.

### *Preparation of media*

To analyze the decolourization efficacy of recovered fungal flora, the method of Purewal (2014) was followed, since different methods can show different efficiencies in the treatment. In this method, the test fungi were grown in a medium prepared by using the following four stock solutions:

| | | | |
|---|---|---|---|
| **Stock-A**: | $NaNO_3$ | - | 40g/L |
| | KCl | - | 10g/L |
| | $MgSO_4 .7H_2O$ | - | 10g/L |
| | $FeSO_4.7H_2O$ | - | 0.2g/L |
| **Stock-B:** | $K_2HPO_4$ | - | 20g/L |
| **Stock-C:** | $ZnSO_4.7H_2O$ | - | 1g/100ml |
| **Stock-D:** | $CuSO_4.7H_2O$ | - | 0.5g/100ml |

Added equal volume (50 ml) each of Stock-A and Stock-B, 1ml each of Stock-C and Stock-D, 30g sucrose as a carbon source in a flask and prepared final volume 1L by adding distilled water. The dyes were dissolved in the medium in the concentration of 100mg/L. After dissolving the dyes, culture medium was sterilized in an autoclave at 15 lbs p.s.i for 20 minutes. Then the medium was inoculated with 5 days old culture disc (8mm in diameter) and incubated at 28±2ºC for a period of 10 days (3 days shaking and 7 days static condition).

***Preparation of controls***

For this experiment, two controls were prepared. One type of control consisted of medium without dye and inoculated with test fungus, whereas the second control consisted of medium with dye but without the test fungus. The first control was used for comparing the fungal growth obtained in the medium with and without the dye. The second control was used for visual comparison of the decolourization efficacy of the test fungus.

## RESULTS AND DISCUSSION

With few exceptions all synthetic dyes are aromatic organic compounds and the fungi degrade these dyes during secondary metabolism. *Aspergillus niger* decolourized all the five synthetic dyes that were used in the present investigation (Figure 2 &3). Further, it was observed that *A. niger* resulted in maximum decolourization of safranin, nigrosin, chlorazole black E and rose Bengal on the 10$^{th}$ day of incubation, whereas it could completely decolourize congo red on the 7$^{th}$ day of incubation itself (3 days shaking and 4 days static condition). Increased biomass and maximum surface area provided by fungal mat helps in the adsorption of dye on its surface and results in clearance of dye containing solution. Infact, adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization (Knapp *et al*., 1995).

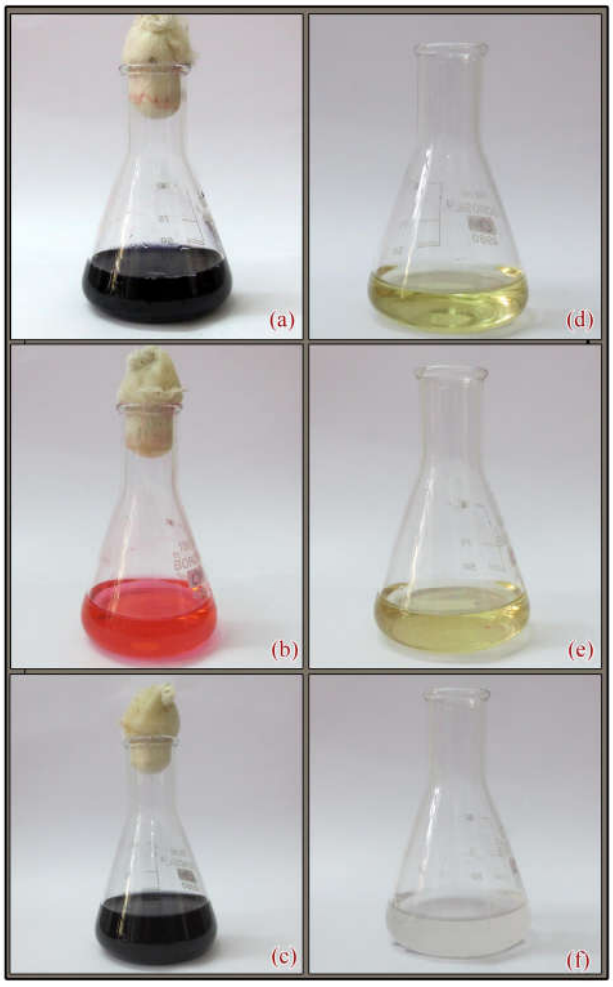



**Figure 2** Initial colour of the medium containing (a) nigrosin (b) safranine (c) chlorazol black E(d,e,f) clear solution after incubation with *Aspergillus niger* on 10$^{th}$ day.

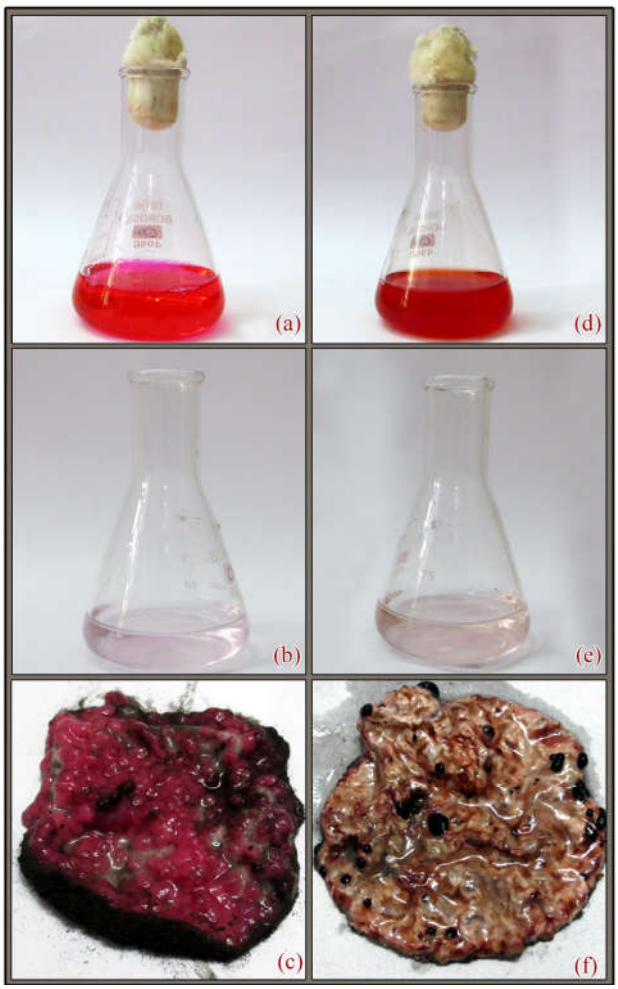



**Figure 3** Initial colour of medium containing (a) rose Bengal (d) congo red (b,e) Clear solution after incubation with *Aspergillus niger* on 10th day and (c,f) fungal mat showing adsorption of (c)rose Bengal (f) congo red.

Adsorption of the tested dyes on the fungal mycelium was also observed by a change in the colour of mycelium. Similar observations have been recorded earlier by Wong and Yu (1999) and Singh and Singh (2010) while studying adsorption of acid green, acid violet, indigo carmine, congo red and bromophenol dyes. As found in the present investigation, Jaidev *et al*. (2009) also reported decolourization of rose bengal by *A. niger*, whereas Singh and Singh (2010) reported decolourization of congo red by *A. flavus*. Later, Namdhari *et al.* (2012) found *A. niger* along with two other species (*A. allhabadi* and *A. sulphureus)* to be efficient for decolourization of reactive blue MR, a textile dye. Recently, Gnanadoss and Jebapriya (2013) studied decolourization of congo red and erichrome black T by using two species of *Aspergillus* (*A. niger* and *A. nidulans*) and found that both of them could be used as an alternative to the conventional physico-chemical methods. Similarly, biodegradation efficiency of *Aspergillus awamori* against rose Bengal dye (100mg/l) has been reported by Purewal (2014). In addition to *Aspergillus* species, few *Penicillium* and *Rhizopus* species are also reported elsewhere to be efficient decolourizers (Iscen *et al.,* 2007; Kumari and Abraham, 2007). Likewise, the basidiomycete, *Phanerochaete chrysosporium*, a white rot fungus has also been used extensively for decolourization of dyes in wastewaters (Murugesan *et al.,* 2007).

## CONCLUSION

The decolourization of dyes was studied under stationary as well as shaking conditions, but maximum decolourization of all the dyes was obtained after 10 days except that of congo red which was completely decolourized after 7th day of incubation. In this study we have observed decolourization under shaking as well as static conditions, which could be due to better oxygenation of the fungus under shaking conditions. Moreover agitation also helps the fungus to grow better. Disappearance of dye color is due to adsorption of dye by the fungal mycelium. Due to its cost effectiveness and little disturbance in the environment, this environmental friendly technique can be used as an effective tool for the bioremediation of synthetic dyes from the industrial effluents. Hence, it can be concluded that the *Aspergillus niger* isolate from coal mines can be utilized as an effective treatment for decolourization of dyes from wastewaters.

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