



## RESEARCH ARTICLE

### DEGRADATION OF TEXTILE DYES BY *ASPERGILLUS FUMIGATUS* STRAIN AND THEIR CULTURE OPTIMIZATION

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#### ABSTRACT

Synthetic dyes used in textile industries, if not treated prior to its disposal, can enter our water systems and cause pollution. The present investigation focused on the decolorization of the textile dyes like Congo red, Malchite green. The present *Aspergillus fumigatus* were isolated from marine soil samples collected from Bay of Bengal, Kakinada, East goghavari district, Andhrapradesh, India. Different parameters such as various carbon source, nitrogen source, temperature, pH and salinity concentrations were optimized for decolorization of textile dyes Congo red Malchite green by *Aspergillus fumigatus* showed maximum dye decolorization of 90% at the end of 5th day under optimum condition and found to be more efficient in dye decolorization. All parameters studied in this paper were found to be effective for all isolates. The results reported here warrant further investigation to establish the usefulness of these isolates for bioremediation and biodegradation application such as waste water treatment.

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#### INTRODUCTION

Environmental pollution from human activities is a major challenge for the present world (Srivastava, 2012). Textile, cosmetics, pharmaceuticals and dying industry effluents constitute a major source of water pollution. Dyes or their breakdown products are known to be highly toxic and carcinogenic for living organisms (Khehra et al., 2006). The high concentration of dyes leads to ulceration of the skin and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and on ingestion may cause vomiting, hemorrhage and diarrhea (Mittal, et al., 2005). Azo dyes account for most textile dyestuff produced and are most commonly used synthetic dyes in textile industry (Zollinger, 1992). About 10-15 % of dyes go unused in textile effluents (Selvam et al., 2003; Wesenberg et al., 2003). Most synthetic dyes are not degraded by conventional physical and chemical processes (Robinson et al., 2001; Ahn et al., 1999). Fungi or their oxidative enzymes can decolorize textile wastewater either by adsorption of dye on fungal mycelium or by oxidative degradation of dye molecule (Fu and Viraraghavan, 2001). Biological methods of removal involve the use of

microorganisms such as fungi, bacteria, algae and actinomycetes (Perumal et al., 2012) to convert the pollutants into non-toxic harmless substances. Biological processes convert organic compounds to water and carbon dioxide (Ponraj et al., 2011), have a low cost, sustainable and are easy to use. Decolorization of azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes by white rot fungus *Phanerochaete chrysosporium* have been reported (Jeffery and Gold, 1983; Paszczynski et al., 1992). *P. chrysosporium* produces extracellular manganese peroxidase (MnP), which may be responsible for degradation of xenobiotic compounds from wastewater (Mester et al., 1995) effluent decolorization and bioleaching of kraft pulp (Field et al., 1993). MnP oxidizes Mn(II) to Mn(III), which is responsible for oxidation of compound like phenolic compounds (Moreira et al., 1997). In contrast, the present investigation we reported here that the strains of *Aspergillus fumigatus* also able to degrade textile dyes.

#### MATERIALS AND METHODS

##### Isolation, screening and identification of dye degrading fungi

Marine soil samples were collected from Bay of Bengal, Kakinada. The sample were mixed in sterile water and serially

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diluted from  $10^{-1}$  to  $10^{-6}$  and 0.1 ml of diluted samples spread on Potato dextrose agar (PDA) plates separately. Plates were incubated at  $25^{\circ}\text{C}$  for 5 to 7 days till the appearance of fungal colonies. The colonies were further streaked on the respective agar medium to get pure culture and observed under the light microscope for the identification of fungal isolate. All isolates were preserved on PDA slant in refrigerator.

### Spore suspension preparation

Total 20-25 mycelium disc of 5 mm diameter obtained from a 5 to 7 days old culture plates of fungus were transferred to 50 ml PDA in a 250 ml conical flask and incubated at  $25^{\circ}\text{C}$  temperature for 5 to 7 days. At the end of the incubation period 30 ml sterile water was added to each culture and the flasks were shaken with shaker. Then the content of each conical flasks were filtered through glass wool. The filtrate contained spores and were used for spore count on PDA. The same spore suspension was used in the experiments described below.

### Screening of decolorizing fungi

Screening of fungal strain from the marine soil samples were carried out to their ability to degrade the textile dyes by absorbance method (Moorthi et al., 2007). Fungal disc of 5 mm diameter cut from the 5 to 7 days old culture was placed in flask containing 50 ml mixed textile Congo red and Malchite green. After 5 to 7 days, effective decolorization was seen visually.

### Decolorization assay

Decolorization activity in terms of percentage of decolorization was determined by following method described by Moorthi et al. 10 ml of sample was centrifuged at 2000 rpm for 4 minutes. The decrease in absorbance was monitored at 486nm for Orange 3R. Decolorization activity was calculated according to the following formula (Moorthi et al., 2007).

$$D = [A_0 - A_f / A_0] \times 100$$

Where, D- decolorization;  $A_0$ - initial absorbance;  $A_f$ - final absorbance.

### Optimization of dye decolorization

Decolorization of Congo red and Machite green textile dyes (0.05g/ 100ml) in PDB broth by selected fungal isolate were optimized with respect to the effect of 1%, carbon sources (glucose, maltose, fructose, sucrose), 1%, organic nitrogen sources (peptone and yeast extract) and inorganic nitrogen sources (Yeast extract, Beef extract, Sodium nitrate, Ammonium sulphate), pH (4, 7 and 9) and temperature (25, 35 and  $45^{\circ}\text{C}$ ). All experiments were arrived out with 1% (w/v) inoculums of  $10^{-5}$  spores/ml and PDB broth without culture was served as control. All the flasks were incubated at  $25^{\circ}\text{C}$  under shaking conditions for 5 days.

## RESULTS

### Screening of Dye Decolorization

In PDB broth the fungal isolate showed maximum decolorization. Congo red showed 90% decolorization and Malchite green showed 60% decolorization. When compared

to these two dyes Congo red showed maximum decolorization by fungal isolate after 5 days of incubation. Only the rate of decolorization of dye and final percent of color removal varied for each isolates. This was confirmed with the earlier findings of (Nehra et al., 2008; Moorthi et al., 2007; Spadaro et al., 1992).

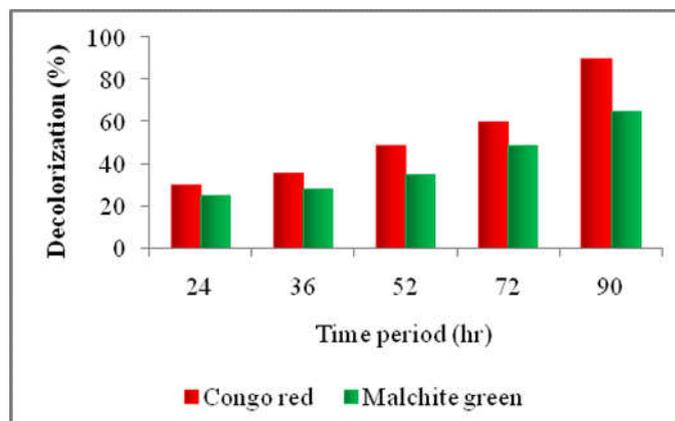


Fig. 1. Dye decolorization of Congo red and Malchite green by *Aspergillus fumigatus*



Fig. 2. Dye decolorization of Congo red and Malchite green by *Aspergillus fumigatus*

### Optimization of Dye decolorization

For the optimization of decolourization of the textile dyes Congo red and Malchite green by the selected fungal isolate, experiments were conducted for optimization of carbon source, nitrogen source, salinity, pH and temperature.

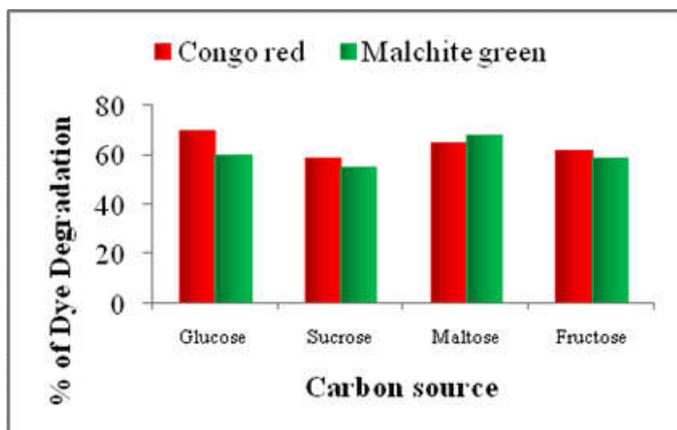


Fig. 3. Effect of Carbon source on % degradation of Congo red and Malchite green

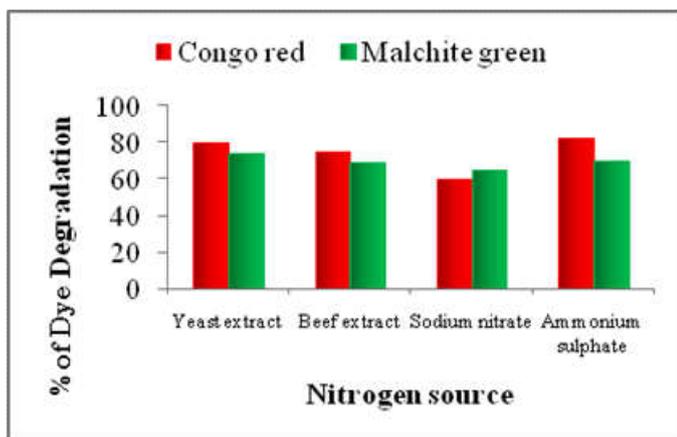


Fig. 4. Effect of Nitrogen source on % degradation of Congo red and Malchite green

To explore carbon effect, experiments were performed with different carbon sources (maltose, glucose, sucrose and fructose) keeping other conditions constant (pH 6.5, dye conc. 0.05g / 100ml). Initially there was an increase in Congo red dye degradation rate up to 70% with glucose and Malchite green dye degradation up to 60% afterward dye degradation decreased (Fig.2). Maximum degradation was observed with 1% glucose in both dyes. When compared these two dyes Congo red showed Maximum dye degradation up to 70% and experiments performed with different nitrogen sources (Yeast extract, Beef extract, Sodium nitrate, Ammonium sulphate). Initially increase in Congo red and Malchite green dye degradation up to 80% and 74% (Fig-3) after 5 days of incubation. Congo red and Malchite green exhibit maximum degradation with 1% Yeast extract. Effect of pH (4.0-9.0) was investigated, keeping other parameters constant. As pH increased from highly acidic conditions (pH 7 to pH 9), congo red decolorization increased from 85 to 95% (Fig-4). Optimum decolorization of dye (90%) was found at pH 9.0, with further decrease 90% and 85% for pH 4.0 and 7.0 respectively. Malchite green decolorization increased from 82% to 90% (Fig-4). Optimum decolorization was found at  $P^H$ -9(90%) and  $P^H$ -4(89%),  $P^H$ -7(82%) respectively.

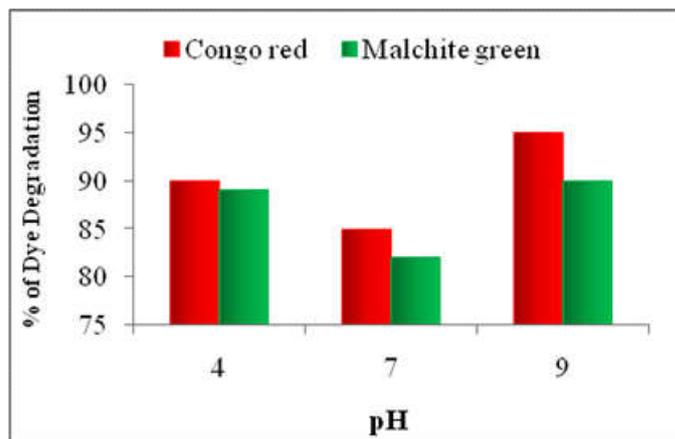


Fig. 5. Effect of  $P^H$  on % degradation of Congo red and Malchite green

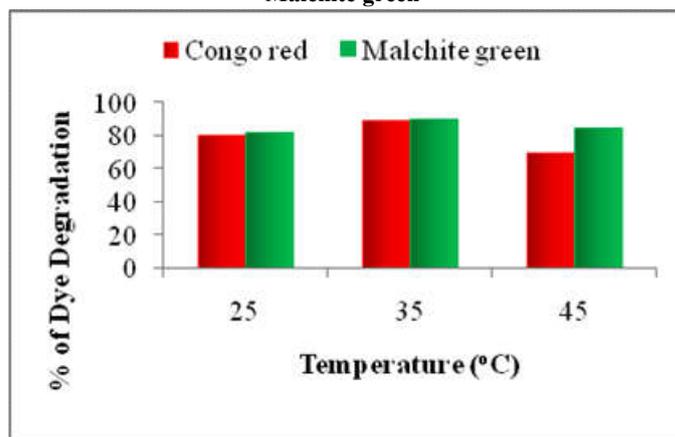


Fig. 6. Effect of Temperature on % degradation of Congo red and Malchite green

Maximum removal of color was observed at 5th day for all studied pH. Since no significant change in removal of dyes by studied fungus was observed after 5th day, thus 5th day of incubation. To explore temperature effect, experiments were performed at different temperatures (25-45°C) keeping other conditions constant (pH 6.5, dye conc. 0.05g/100 ml). Initially there was an increase in congo red dye degradation rate up to 89% at 35°C and afterward dye degradation decreased 80% and 70% at 25°C and 45°C temperatures respectively (Fig-5). Maximum degradation was observed between 30- 35°C. Malchite green dye degradation rate up to 90% at 35°C and afterward dye degradation decreased 82% and 85% at 25°C and 45°C temperatures respectively. Maximum degradation was observed in 35°C

### DISCUSSION

The present study was performed to examine the microbial degradation of hazardous dye in semi-solid medium, taking a fungus, *Aspergillus fumigatus* strain as the experimental organism and a textile dyes, Congo red and Malchite green as the testing dyes. The fungal isolate has shown positive results for dyes degradation, as was indicated by the change and disappearance of colour of the dyes from the dye-containing media of the flasks. Microbial degradation of various hazardous dyes likes, Congo red, Acid red, Basic blue and Bromophenol blue, Direct green by the fungus *Trichoderma harzianum* (Singh and Singh, 2010) and biodegradation of plant wastes materials (Singh, 2008) by using different fungal strains has been investigated earlier. Our result was 90% of

Congo red dye and 60% of Malchite green dye was to best biodegradation by *Aspergillus fumigatus* Cripps and Bumpus (Cripps and Bumpus, 1990) also reported the biodegradation of three azo dyes (Congo red, Orange II and Tropaeolin O) by the fungus *Phanerochaete chrysosporium*. In the present study dyes might be degraded by the adsorption of dyes by the mycelium of *Aspergillus* during its growth. Adsorption of dyes to the microbial cell surface is the primary mechanism of decolorization (Knapp et al., 1995). In our study biodegradation of dye was also observed based on the change of colour. Muthezhilan et al 1995 also reported the biodegradation of Methylene blue, Gentian violet, Crystal violet, Cotton blue, Sudan black, Malachite green and Methyl red by few species of *Aspergillus* in liquid medium. Most of the colour removal during first stage might be due to dye degradation by the mycelium of the fungus, during its growth. The fungal strain used in the present study was responsible for biodegradation/decolorization of textile dyes, and it was also responsible for change in dye colour in the medium with dye containing flasks (Bavendamm, 1995).

## Conclusion

The isolated fungal strains exhibit dye decolorization activities of textile dyes like Congo red and Malchite green in liquid culture medium. Carbon source, nitrogen source, temperature, pH and NaCl concentration had a major influence on dye removal by *Aspergillus fumigatus*

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