

Removal of Synthetic Textile Dyes using *Aspergillus* Species Isolated from Soil

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Studies on decolourization of textile dyes using brown rot fungi are very scarce. In the present investigation, nine such brown rot fungi were isolated and screened for their ability to decolourize brilliant green, bromophenol blue and methyl red. Three promising isolates, *A. flavus* LCJ 51, *A. nidulans* LCJ 52 and *A. niger* LCJ 55 were selected on the basis of this qualitative screening and used for decolourization study in liquid medium by monitoring the decrease in absorbance of each dye. All three *Aspergillus* sp. (*A. flavus* LCJ 51, *A. nidulans* LCJ 52 and *A. niger* LCJ 55) effectively decolourized brilliant green, bromophenol blue and methyl red, but they differed in decolourization ability depending on various culture conditions which have been reported. Our result proposes that *Aspergillus* sp. could effectively be used as an alternative to the conventional physico-chemical method of dye decolourization.

Key words: Dye decolourization, Biological treatment, Brown rot fungi, *Aspergillus* species.

The increasing demand for textile products has proportionately increased the number of textile industry and the amount of wastewater discharged making it one of the main sources of rigorous pollution problems worldwide. Dye serves as a major compound in textile industries and nearly 10,000 different dyes and pigments are used in dyeing and printing industries worldwide. Reports by Palmieri *et al.* (2005) and Levin *et al.* (2004) emphasize that the total world colourant production is estimated to be 8, 00,000 tons per year and about 10% of these dyestuff enters the environment through wastes. This wastewater has substances that are toxic, carcinogenic and genotoxic to living

being (Weisburger, 2002). Due to the complex aromatic structure of the dyes, the conventional dye containing wastewater treatment remains ineffective.

The methods for the treatment of wastewater containing dyes fall into three categories: physical, chemical and biological. The physico-chemical methods have been reviewed extensively earlier (Robinson *et al.*, 2001; Forgacs *et al.*, 2004; Joshi *et al.*, 2004) but the major drawback of physico-chemical methods are expensive, low efficiency, limited versatility, interference by other wastewater constituents and the handling of the waste generated. However, biological method are cost-effective and receiving much attention for treatment of textile dye wastewater (Zee and Villaverde, 2005). Biological dye degradation technique is either based on partial or complete biodegradation of dyes by pure and mixed culture of bacteria, fungi and algae (Arora and Chander, 2004). Degradation of dyes has been reported through biosorption (Fu and Viraraghavan, 2000) or enzymatic degradation by lignin peroxidase, manganese peroxidase,

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manganese independent peroxidase and laccase (Ferreira *et al.*, 2000; Wesenberg *et al.*, 2003; Jebapriya and Gnanadoss, 2013). Considering the metabolizing properties, fungi have been either used directly or the culture filtrate containing enzymes were used for the degradation of dyes. The biodegradation of dyes was first reported in white rot fungi (Glen and Gold, 1983). Besides white rot fungi, brown rot fungi also play an important role in the degradation of dyes. Fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus foetidus*, *Penicillium* sp. and *Trichoderma viride* have been found to be efficient in decolorization of textile dyes (Fu and Virarghavan, 2001; Sumathi and Manju, 2000).

In the present study, the ability of three *Aspergillus* species in decolorizing the dyes namely brilliant green, bromophenol blue and methyl red was evaluated.

MATERIAL AND METHODS

Dyes and chemicals

Brilliant green, Bromophenol blue and Methyl red were obtained from Hi-media and Merck (India). All the other chemicals were procured from Hi-media (India).

Microorganism and culture condition

The fungi used for this study were isolated from soil and purified. The pure cultures were maintained on Potato Dextrose Agar (PDA) slants at 5 °C.

Dye decolorization on agar plates

PDA was supplemented with brilliant green, bromophenol blue and methyl red at 0.05% (w/v) in separate flasks. The medium was poured in Petri plates and allowed to solidify. The dye containing PDA plates were then inoculated by mycelial disc, obtained from an actively growing culture. The formation of decolorization zones under and around developing mycelia was noted.

Dye decolorization in liquid medium

The basal medium used for decolorization studies composed of Acetic acid - 0.075 mL, Urea - 54 mg, Potassium phosphate - 33.5 mg, Sodium bicarbonate - 42.0 mg, Magnesium sulphate - 19 mg, Calcium chloride - 10.5 mg, Ferric chloride - 3.5 mg, Sucrose - 3000 mg and Distilled water - 1000 mL. The dyes were added at a concentration of 0.05% (w/v). The basal liquid

medium containing the respective dyes were autoclaved at 121°C for 15 minutes. The mycelial discs of the fungi were separately inoculated into the conical flasks under sterile condition. They were then incubated on the rotary shaker at 120 rpm and maintained at room temperature. The OD of each of the sample was taken at the respective absorption maxima of each of the dyes (630, 590 and 523 nm) at different time interval. Each experiment was done in triplicates. The percentage of decolorization was calculated as per the following equation:

$$\text{Decolorization (\%)} = \frac{(I-F)}{F} \times 100$$

where

I = Initial absorbance

F = Final absorbance

Initial Absorbance

The effect of dye concentration, inoculum size, pH, agitation, dried mycelia, culture filtrate and immobilized cell on the decolorization of the dyes was examined in basal medium containing respective dyes with actively grown fungal biomass. Experiments were also conducted to study the effect of dried mycelia, where dried biomass was used instead of actively grown fungal biomass. The dye decolorization was monitored on different time interval and results were calculated as per the formula mentioned above.

RESULTS AND DISCUSSION

Studies on the decolorization of dyes using brown rot fungi are very few (Ali *et al.*, 2008; Ambrosio *et al.*, 2004; Husseiny, 2008). However, the current study has significantly vitalized the role of such fungal isolates. The present study was carried out to examine the decolorization of dyes using three different isolates of *Aspergillus* sp. (LCJ51, LCJ52 and LCJ55). Nine different *Aspergillus* species were tested for their dye (brilliant green, bromophenol blue, methyl red) decolorization activity on agar plates (Table 1). Among the nine isolates tested, three promising fungal isolates were selected for further studies. Based on the morphological and microscopical identification, the promising three fungal isolates were identified as *A. flavus* LCJ 51, *A. nidulans* LCJ 52 and *A. niger* LCJ 55.

All the three fungal isolates selected for the present study were able to remove textile dyes (brilliant green, bromophenol blue and methyl red). Dye removal in the present study was mainly due to biosorption or bioadsorption by the fungal hyphae. Likewise, few other studies have also clearly mentioned biosorption and bioadsorption

of certain brown rot fungi such as *A. niger* and *A. foetidus* (Ali *et al.*, 2008; Fu and Viraraghavan, 2000; Sumathi and Manju, 2000). Biosorption is reported to be the primary dye removal process in wood rotting basidiomycetes (Balan *et al.*, 2001; Fu and Viraraghavan, 2000). Similar mechanism was evident in the present study. However, the fungal

Table 1. Dye decolourization by the fungal strains on agar plate

Fungal strain	Decolourization zone		
	Brilliant green	Bromophenol blue	Methyl red
LCJ51	+++	++	+++
LCJ52	+++	++	++
LCJ53	+	+	+
LCJ54	-	-	-
LCJ55	+++	+++	+++
LCJ56	++	+	++
LCJ57	-	++	+
LCJ58	+	-	-
LCJ59	++	+	++

(+++ very high, ++ moderate, + less and - negative)

Table 2. Influence of different conditions on dye decolourization using *Aspergillus* sp

Conditions	Percentage of Decolourization								
	<i>A. flavus</i> LCJ 51			<i>A. nidulans</i> LCJ 52			<i>A. niger</i> LCJ 55		
	BG	BB	MR	BG	BB	MR	BG	BB	MR
Dye Conc. (mg/L)									
20	70	09	28	35	09	03	71	79	66
40	37	06	18	25	03	01	33	05	09
60	37	03	31	14	01	01	34	01	10
Inoculum size (g/L)									
0.20	12	10	16	05	02	14	16	05	17
0.40	15	14	19	10	02	21	24	09	24
0.60	32	16	24	12	06	22	32	12	29
Static	24	04	02	16	05	09	06	08	02
Shaking	41	31	42	03	05	06	38	37	40
pH									
6	13	01	8	25	02	04	17	17	03
7	09	01	02	06	02	01	11	11	03
8	08	01	01	00	01	01	05	05	00
Dried biomass	09	05	01	07	05	12	19	11	10
Culture filtrate	11	01	38	27	00	32	74	03	05
Immobilized cells									
Scotch brite	23	04	02	09	03	14	07	05	10
Foam	54	08	25	05	22	34	03	07	03

(BB-Brilliant green; BB-Bromophenol blue; MR-Methyl red)

biomass gradually turned colourless after the bioadsorption of dyes. These observations are in line with the findings of Balan and Monterio (2001) and Radhika *et al.* (2013), where they reported the removal of dyes by fungal adsorption and extracellular degradation. Bioadsorption has been linked to electrostatic pull between the negatively charged dyes and the positively charged cell wall components (Aksu and Tezer, 2000).

The dye solutions inoculated with the fungi showed a steady decrease in the absorption with increase in the days of incubation indicating that the dye had undergone decolourization. Moreover, there was also a marked change in the absorption spectrum with increase in time. The decolourization percentage ranges from 80 to 99% after the 5th day (Fig. 1-3). The fungi have shown positive results for dyes decolourization, as indicated by the gradual decrease in the intensity of colour and eventually the disappearance of colour from the dye-containing basal medium. This might be due to the production of extracellular enzymes by the fungi, during the biodegradation of tested dyes. Similar results were also observed in biodegradation of methylene blue, gentian violet, crystal violet, cotton blue, sudan black, malachite green and methylred using few species of *Aspergillus* (Muthezhilan *et al.*, 2008). Earlier, decolourization of dyes by using different fungal strains were studied extensively example *Glocladium virens* for congo red (Singh, 2008),

Trichoderma harzianum for congo red, acid red, basic blue and bromophenol blue, direct green (Singh and Singh, 2010). Cripps *et al.*, (1990) also reported the biodegradation of three azo dyes (congo red, orange II and tropaeolin O) by the fungus *Phaenerocheate chryso sporium*.

The growth of the fungi was affected by the presence of dyes at toxic concentrations. In the present study, the decolourization efficiency of bromophenol blue, brilliant green and methylred were studied at different concentrations of the dyes (20 to 60 mg/L). It found that more than 75% of dyes were decolourized when the dyes were used in low concentration. The percentage of removal of dyes decreased with an increase in dye concentration (Table 2). In addition, the growth of fungi was also strongly inhibited at higher concentration of the dyes. This might be due to the reason that higher concentration of dye may be toxic to cellular activities. Similar kind of observation is also reported by Ramya *et al.*, (2007). The effect of inoculum size of *Aspergillus* sp. (0.20 to 0.60 g/L of actively grown biomass) and time on decolourization of brilliant green, bromophenol blue and methyl red at 20 µg/mL initial concentration were studied (Table 2). Results showed that, there was a significant increase in decolourization with increasing inoculum size. The maximum decolourization (29 to 32%) was observed with 0.60 g/L for all three *Aspergillus* sp. (*A. flavus* LCJ 51, *A. nidulans* LCJ 52 and *A. niger* LCJ 55).

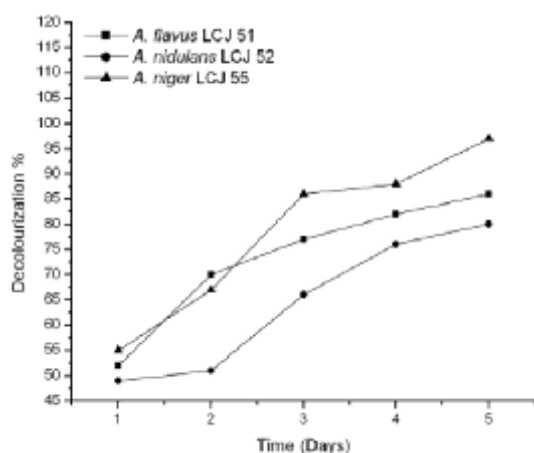


Fig. 1. Decolourization of Brilliant green using *Aspergillus* sp.

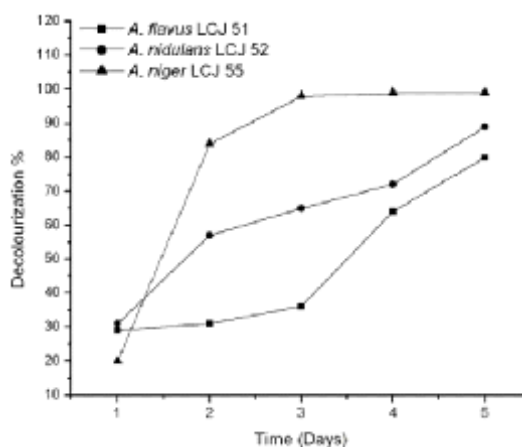


Fig. 2. Decolourization of Bromophenol blue using *Aspergillus* sp.

Similarly, the decolourization of dyes was found to increase from 45 to 80% when the inoculum size increased from 0.5 to 5.0 g/L (Mehna *et al.*, 1995). Thus, the present result clearly emphasizes that when the quantity of biomass increased the dye decolourization percentage also increased. This was consistent with the study by Assadi *et al.*, 2001.

pH of the medium had a significant effect on the decolourization of brilliant green, bromophenol blue and methyl red by *Aspergillus* sp. The result shows that the highest colour removal (16 to 25%) was detected at pH 6 when compared to neutral and basic pH. At the pH of 8, the decolourization was highly inhibited. The result provides an information that acidic pH is required for growth and decolourization. Similar results have been reported by Ramya *et al.*, 2007. The effect of pH on decolourization of solar golden yellow R by *Schizophyllum commune* has shown that maximum decolourization efficiency (73%) was observed at pH 4.5 after 6 days. The efficiency decreased from 59 to 8% as pH was increased from 5 to 6 (Asgher *et al.*, 2008). Hence, it seems that for majority of the fungi the optimum pH for dye decolourization lies in the acidic range. However, such a low pH is not suitable for the wastewater treatment and hence, fungal isolates capable of decolourizing dye efficiently at wide pH ranges are desirable for industrial applications.

Cultural stability proved to have an important role in fungal growth and associated dye removal and it is notable in the present study. In the present study, removal of dyes under static and shaking condition have exhibited a different result. Under shaking condition *Aspergillus* sp. (*A. flavus* LCJ 51, *A. nidulans* LCJ 52 and *A. niger* LCJ 55) exhibited higher removal of brilliant green, bromophenol blue and methyl red (30 to 40%). On the contrary, in static condition the same fungal isolates showed lower percentage of removal when compare to shaking condition (Table 2). Knapp *et al.* (1997) reported that there was only 45% decolourization of orange II after 23 hours of incubation in static conditions whereas in shaking conditions 97.5% decolourization occurred. Fungi being aerobic organisms normally show better dyes degradation activities under facilitated agitated condition (Ge *et al.*, 2004). Shaking increases the mass (substrate) and oxygen transfer from the

culture medium to the cells, thereby, it facilitates optimum fungal growth and enzyme (oxidase) production (Swamy and Ramsay, 1999).

The uptake or accumulation of chemicals by microbial mass has been termed biosorption (Kumar *et al.*, 1998). Dead bacteria, yeast and fungi have all been used for the purpose of decolourizing dye containing effluents. Textile dyes vary greatly in their chemical composition, and therefore their interactions with microorganisms depend on the chemistry of a particular dye and the specific chemistry of the microbial biomass (Polman and Brekenridge, 1996). Depending on the dye and the species of microorganism used different binding rates and capacities will be observed. It can be said that certain dyes have a particular affinity for binding with microbial species. *Aspergillus niger* has proved to be a promising biosorbent for dye removal compared to activated carbon. Waranusantigul *et al.* (2003) used dried *Spirodela polyrrhiza* biomass as an adsorbent for the removal of the basic dye methylene blue from aqueous solutions. The dried biomass used in the present study also showed the removal of textile dyes brilliant green, bromophenol blue and methyl red with great extent of decolourization percentage (12 to 18%) at 480 minutes of incubation.

Decolourization of dye is related to the process of extracellular oxidases, particularly manganese peroxidases (Gold *et al.*, 1988). Lignin peroxidase (Lip), manganese dependant peroxidase (MnP) and laccase, all of which are involved in lignin degradation, have been reported to

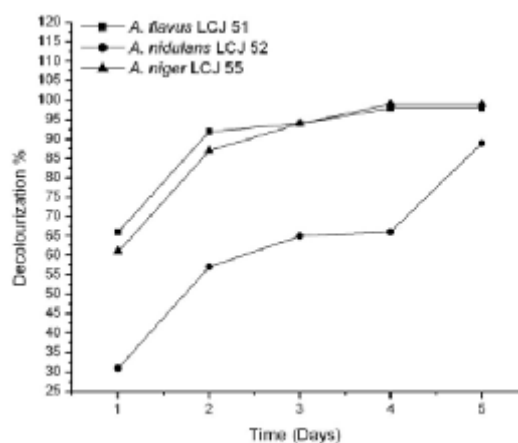


Fig. 3. Decolourization of Methyl red using *Aspergillus* sp.

decolourize dyes (Vyas and Molitores, 1995). Bavendamm (1928) suggested that of presence of phenoloxidases was correlated with fungi, causing white rot decay and that, only these fungi were able to completely decompose lignin. The fungal isolates used in the present study were responsible for biodegradation/decolourization of textile dyes mainly due to the presence of enzymes this statement is proved by removal of dyes using culture filtrate which showed 30 to 74% of decolourization of brilliant green, bromophenol blue and methyl red (Table 2).

Effect of immobilized cell on decolourization in various time intervals using the dyes (bromophenol blue, brilliant green and methylred) at 20 µg/L initial concentration of dye is presented in Table 2. Generally, immobilized fungal cells have several advantages over dispersed cells such as simple reuse of the biomass, easier liquid-solid separation and minimal clogging in continuous flow systems (Tieng and Sun, 2000). In addition, immobilized cultures tend to have a higher level of activity and are more resilient to environmental perturbations such as pH, or exposure to toxic chemical concentrations than suspension cultures (Shin *et al.*, 2002) and immobilization protects the cells from shear damage (Vassilev and Vassileva, 1992). Moreover, cell immobilization lowers the apparent broth viscosity and makes the rheological features more favourable for oxygen supply and mass transfer (Thongchul and Yang, 2003). Iqbal and Saeed (2006) developed a novel immobilization technique by using a structural fibrous network of papaya wood as an immobilizing matrix for the entrapment of *Aspergillus terreus* cells. Wang and Hu (2008) investigated the removal of reactive brilliant blue KN-R using growing *Aspergillus fumigatus* immobilized on carboxymethylcellulose beads. Similarly, the present results have also shown the decolourization of brilliant green, bromophenol blue and methyl red by immobilized cell of *Aspergillus* sp. (*A. flavus* LCJ 51, *A. nidulans* LCJ 52 and *A. niger* LCJ 55) using scotch brite and foam as substrates. Cells immobilized on foam effectively decolourized brilliant green, bromophenol blue and methyl red (3 to 54%) when compared to those immobilized using scotch brite.

CONCLUSION

Results of present study clearly indicate that removal of dyes cannot simply be linked to the white rot fungi alone. Other groups of fungi like, brown rots can also play a significant role in the biodegradation of textile dyes. However, further research on understanding the mechanism of dyes reduction (decolourization) after taken up onto the fungal biomass would help in the advancement of bioremediation technologies.

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