

Optimization of Culture Conditions for Enhanced Decolorization of Azo Dyes by *Aspergillus flavus* Isolated from Dye Contaminated Soil



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Abstract Dyes released by the textile industries pose a threat to the environmental safety. Recently, dye decolorization through biological means has gained impetus as these are cheap and can be applied to wide range of dyes. The main focus of the present investigation is to evaluate the bioremediation potential of *Aspergillus flavus* isolated from textile dye contaminated soil for the decolorization of synthetic acid dyes. Among the isolated four fungal strains namely *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus sp.* and *Aspergillus terreus*, *Aspergillus flavus* was capable of decolorising 96 and 97% for Acid Blue (AB) 193 and Acid Violet (AV) 90, respectively under optimum conditions of pH 6.0 and temperature 25 °C. Hence from the results, it can be concluded that this fungus can be used as eco-friendly and economically effective tool to decolorize textile dye effluents.

Keywords Acid blue 193 · Acid violet 90 · *Aspergillus flavus* · Decolorization · Effluent

1 Introduction

Synthetic dyes are widely used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetic and leather industries (Rafii et al. 1990; Kuhad et al. 2004; Rodriguez Couto 2009). Since 1856, over 105 different dyes have been produced worldwide with an annual production of over 7×10^5 metric tons (Chen et al. 2003). Of all the familiar dyestuffs in the world, azo dyes make up about a half, making them the largest group of synthetic colorants that are released into the environment (Zhao and Hardin 2007; Sen et al. 2016). Effluents from the textile industries containing dye are highly coloured and are therefore visually identifiable (Kılıç et al. 2007) and are visible even at 1 mg/ml concentration (Pandey et al. 2007). The discharge of colored effluents into the environment is detrimental, not

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only because of their colour but due to the lethal and carcinogenic properties of dye compounds and their end products (Weisburger 2002).

Different approaches are available for the remediation of dye effluents which include a number of physical, chemical and biological methods namely, oxidation and reduction, adsorption, photocatalysis, coagulation, microbial degradation methods, etc., (Fu and Viraraghavan 2001). Nevertheless, these technologies are usually ineffectual in the removal of color, costly and less adaptable to an extensive range of dye waste waters (Banat et al. 1996). Biological methods are generally considered environmentally approachable as they can lead to complete mineralization of organic pollutants at low cost (Pandey et al. 2007).

Among the biological methods, bioremediation is promising tool for the elimination of toxic pollutants and it is the application of microorganisms (fungi, bacteria, actinomycetes, yeasts and algae). Microorganisms which are naturally having the tolerant ability to utilise dye compounds as their sole energy source are of extraordinary interest for scientific community to eliminate the environmental contaminants that results from the industrial sources (Ali et al. 2010). Dye degradation by bacteria predominantly depends on the enzymatic transformation where the azo dyes are cleaved with the enzyme azo-reductase along with its coenzyme (Zimmermann et al. 1982; Moutaouakkil et al. 2003; Saroj et al. 2015), whereas fungal degradation of azo dyes are catalyzed by extracellular enzymes i.e., ligninolytic peroxidases (Young and Yu 1997; Selvam et al. 2003; Máximo et al. 2003). Although bacterial dye degradation have been widely studied, in recent days fungal have gained importance in dye degradation due to their unique extra cellular enzyme systems. Fungi are capable of degrading the complex dye structures when compared with bacteria (Forss and Welander 2009). Fungal mycelia have an additive advantage over single cell organism by solubilizing the insoluble substrates by producing extracellular enzymes (Gajera et al. 2015). Higher concentration of dye toxicants can be easily degraded by fungi due to their advantageous enzyme arrangements and improved cell to surface ratio. Various studies have reported that fungi like white rot decolorize the synthetic dyes and the processes are mediated by lignin peroxidase (Ollikka et al. 1993), manganese peroxidase (Heinfling et al. 1998) and laccase (Abadulla et al. 2000; Soares et al. 2001; Murugesan and Kalaichelvan 2003). Based on the fact that the fungi have the capacity of degrading wide variety of organic and inorganic dye components, the present study was carried out to isolate and screen fungal strains for decolorization of azo dyes (AB 193 and AV 90) and optimize the pH and temperature for better understanding the process.

2 Materials and Methods

2.1 Dye Stuffs and Chemicals

Acid Blue 193 (AB 193) and Acid violet 90 (AV 90) commonly used in local textile processing industries were obtained from Rajasthan Dye Chemical Ltd., Chennai, India. Dyes were dissolved in double distilled water to prepare a stock concentration (1000 mg L^{-1}) and solutions of the desired concentrations were obtained by successive dilutions. Media components and chemicals were purchased from Himedia Labs, Bombay, India.

2.2 Sample Collection

Soil samples were collected from the textile industry at Tirupur, Tamil Nadu, India and were stored at 4°C until further process.

2.3 Screening and Identification of AB 193 and AV 90 Tolerant Fungi

The soil samples were serially diluted using sterile distilled water and from the 10^{-2} to 10^{-7} dilutions, 0.1 mL of sample was transferred onto Czapek-Dox agar plate containing AB 193 and AV 90 (50 mg L^{-1}). Spread plate method was performed and the plates were incubated at $30 \pm 1^\circ\text{C}$ for 4 days. Fungal colonies were identified using steriobinocular microscope. Fungi which showed decolorization in Czapek-Dox agar medium were screened further by transferring in a fresh Czapek-Dox agar plates containing different dye concentrations (50 and 100 mg L^{-1}). Colonies were selected on the basis of their ability to decolorize the dye on the plates. Isolated colonies were then subsequently transferred to Czapek-Dox agar for further study. Fungal strains with greatest dye tolerance capacity were identified by its different biochemical characteristics and structural arrangements. Wet mount was prepared by suspending culture in a few drops of lacto phenol cotton blue solution. The slides were examined under high and low power magnification with the aid of a dissecting microscope (Cappuccino and Sherman 2004).

3 Decolorization Experiments

3.1 *Optimization of Incubation Time on Decolorization of Azo Dyes*

The isolated fungus was tested for its ability to decolorize AB 193 and AV 90 (100 mg L⁻¹). About 50 mL of C-limited Czapek-Dox medium was amended separately with the dyes and subsequently inoculated with 5 ml spore suspension (10⁸ spore/ml) from a slant culture. The flasks were incubated at 30 ± 1 °C for 12 days on a rotary shaker (150 rpm) and the samples were withdrawn at 2 days intervals and centrifuged at 10,000 rpm for 10 min. Supernatant collected was assessed for the decolorization of AB 193 and AV90 in UV–Vis spectrophotometer (UV–Vis 1700, Shimadzu, Japan) at its maximum absorbance wave length of 586 and 526 nm, respectively. Two control flasks (i) dye + medium without inoculum and (ii) medium with inoculum without dye were maintained. The percentage decolorization was calculated according to the following formula Bergsten-Torralba et al. (2009)

$$\text{Decolorization (\%)} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100 \quad 0.11.1$$

3.2 *Optimization of pH and Temperature on Decolorization of Azo Dyes*

For optimization of physical parameters, the fungal inoculated shake flasks were incubated for 10 days at different initial pH values from 2 to 10 (2, 4, 6, 8, 10) to determine the optimal pH for dye decolorization. For investigating the effect of temperature, the shake flasks inoculated with fungal inoculum were incubated at varying temperatures from 20 to 35 °C (20, 25, 30 35 °C) at optimum pH for 10 days. The experimental conditions were maintained as mentioned earlier. Samples were withdrawn at regular intervals and analyzed for color removal.

3.3 *Optimization of Initial Dye Concentration on Decolorization of Dyes*

Fungal isolates were added into a 250 mL medium containing varying concentrations (25, 50, 75, 100 and 125 mg L⁻¹) of AB 193 and AV 90 individually. At optimum pH and temperature, the flasks were incubated in a rotary shaker (Orbitek) at 150 rpm.

Table 1 Dye decolorization pattern of fungal isolates

Organisms	Acid blue 193 (AB 193)		Acid violet 90 (AV 90)	
	50 mg L ⁻¹	100 mg L ⁻¹	50 mg L ⁻¹	100 mg L ⁻¹
<i>Fusarium oxysporum</i>	–	–	+	+
<i>Rhizopus</i> sp.	+	+	–	–
<i>Aspergillus terreus</i>	+	–	–	–
<i>Aspergillus flavus</i>	+	+	+	+

+ decolorization zone observed

–decolorization zone not observed

4 Results and Discussion

4.1 Screening and Selection of Dye Tolerant Fungi

In the present study one fungal strain was selected among the four isolated strains, *Aspergillus flavus*, *Fusarium oxysporum*, *Rhizopus* sp. and *Aspergillus terreus*, *Aspergillus flavus* for the decolorization of AB 193 and AV 90 based on its high dye decolorization ability (Table 1). This selected strain was identified as *Aspergillus flavus* based on various cultural characteristics, morphology and by the presence of conidiospores (Table 2). It was observed that the *Aspergillus flavus* and *Rhizopus* species tolerated upto 100 mg L⁻¹ of AB 193 and in case of AV 90, *Aspergillus flavus* and *Fusarium oxysporum* tolerated upto 100 mg L⁻¹, respectively. Above results reveals that decolorization by *Aspergillus flavus* was found to be high (100 mg/L) for both AB 193 and AV 90. Hence for further studies, *Aspergillus flavus* was selected as an efficient strain for decolorization.

In present study *A. flavus* decolorized dye by forming clear zone on agar plates, which may due to their extracellular, nonspecific and nonstereoselective enzyme system (Hofrichter 2002). Fungal decolorization capacity can be confirmed by their ability to form a clear halo zone on agar plates containing dye AB 193 or AV 90 (Yamini et al. 2012). Previous studies disclose that fungal strains were able to decolorize dyes on agar plates (Jarosz-Wilkolazka et al. 2002; Eichlerova et al. 2006).

4.2 Effect of Incubation Time on AB 193 and AV 90 Decolorization by *Aspergillus Flavus*

Initially, the decolorization was very low on the second day that is 42 and 43% for AB 193 and AV 90, respectively. Then it gradually increased with increase in incubation time. The decolorization efficiencies of *Aspergillus flavus* were 48 and 45% for AB

Table 2 Identification of fungal isolates by macroscopic and microscopic appearance

Organisms	Macroscopic appearance	Microscopic appearance
<i>Aspergillus flavus</i>	White colonies become greenish—blue as culture matures	Single-celled spores (Conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophore, the vesicle; long conidiophores arise from a septate mycelium
<i>Fusarium oxysporum</i>	Wolly, white, fuzzy colonies changing colour to pink	Multicelled spores (conidia) are oval or crescent-shaped and attached to conidiophores arising from a septate mycelium
<i>Aspergillus terreus</i>	White colonies become greenish—blue as cultures matures	Single-celled spores (Conidia) in chains developing at the end of the sterigma arising from the terminal bulb of the conidiophore, the vesicle; long conidiophores arise from a septate mycelium
<i>Rhizopus</i> sp.	White colonies become gray—yellowish brown as cultures matures	Single-celled spores (Sporangia) are globose to ovoid shaped and nonseptate or sparsely septate broad hyphae. They are rhizoids and unbranched usually

193 and AV90, respectively and it slowly increased and reached maximum of 96 and 97% within 10 days of incubation time as represented in Figs. 1 and 2.

Fig. 1 Effect of incubation time on decolorization of AB 193 by *Aspergillus flavus*

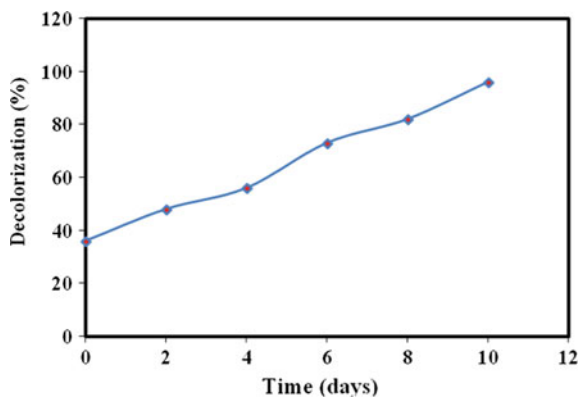
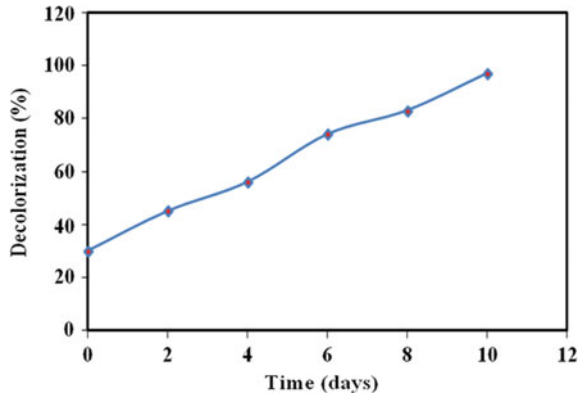


Fig. 2 Effect of incubation time on decolorization of AV 90 by *Aspergillus flavus*



4.3 Effect of pH and Temperature for AB 193 and AV 90 Decolorization by *Aspergillus Flavus*

At initial pH 2, the decolorization of both dyes was low. Maximum decolorization of AB 193 (96%) and AV 90 (97%) were observed around pH 6 by *Aspergillus flavus* (Fig. 3 and 4). When the pH was increased to 10 the decolorization of dyes was decreased in both cases. It is observed that the fungus showed higher decolorization efficiency in acidic condition for both the dyes.

The pH of the medium plays a vital role in decolorization of dyes. The effects of pH may be related to the transport of dye molecule across the cell membrane (Kodam et al. 2005). Study of Asgher et al. (2008) shows *Schizophyllum commune* can work in acidic media, and decolorize maximum (77%). Murugesan and Kalaichelvan (2003) also reported that *G. lucidum* laccase prefers acidic orange for higher decolorization extent. The maximal decolorization was observed at pH 4.0 for both RB-5 and Remazol Brilliant Blue R (RBBR). However Yesilada et al. (2003) studied that *Funalia troglia* had effective decolorization capacity in broad pH range of 6–11 and

Fig. 3 Effect of pH on decolorization of AB 193 by *Aspergillus flavus*

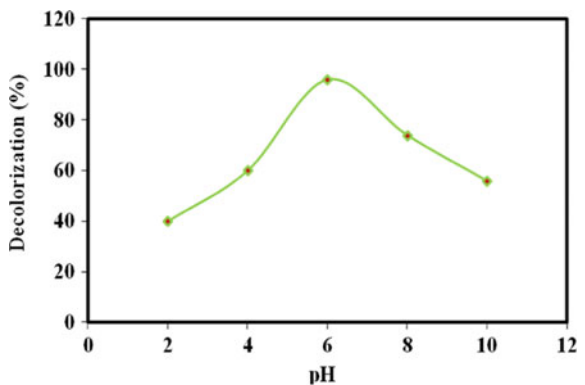
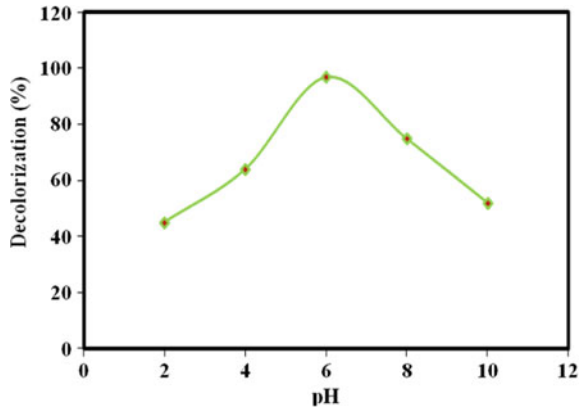


Fig. 4 Effect of pH on decolorization of AV 90 by *Aspergillus flavus*



this results reveals that the optimum pH of fungus may be affected by its chemical nature.

Dye decolorization was evaluated at varying temperatures (20–35 °C) for AB 193 and AV 90 and the results are shown in Figs. 5 and 6. The fungus exhibited better dye decolorization at 25 °C for both the dyes in 10 days of incubation time. At 20 °C, decolorization of dyes was 50 and 60% for AB193 and Av 90, respectively. The decreasing trend of dye decolorization of 42 and 74% were observed at higher temperatures 30 °C for AB 193 and 35 °C AV 90, respectively.

The decolorization of dye varies at different temperatures, since the temperature changes lead to a sudden alteration in the activation energy which is concerned with the cell viability (Yu et al. 2001). Decolorization of *A. flavus* reached maximum within 10 days at lower concentrations of dye which indicates that increasing the dye concentration gradually decreases the decolorization rate, probably due to the toxic effect of dyes with regard to the fungal biomass concentration (Tony et al. 2009).

The dye decolorization is inversely related to the concentration of the dye due to which it shows inhibitory effects of high dye stuffs in the solution. Similar results

Fig. 5 Effect of temperature on decolorization of AB 193 by *Aspergillus flavus*

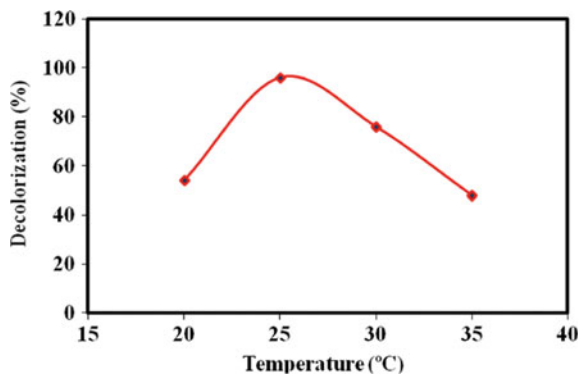
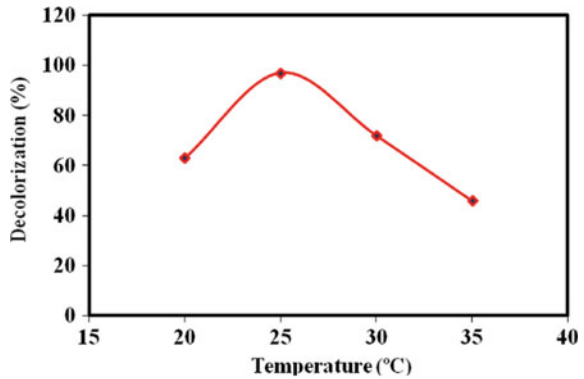


Fig. 6 Effect of temperature on decolorization of AV 90 by *Aspergillus flavus*



has been observed in decolorization of acid fuchsin, acid violet and evans blue up to 83–91% within 2 h whereas it slowly decreased to 45% for bromophenol blue within 8 h. The difference in the decolorization efficiencies for the dyes is from the different redox potentials of the colored substrates (Chivukula and Renganathan 1995).

4.4 Effect of Initial Dye Concentration on AB 193 and AV 90 Decolorization

The decolorization efficiencies of *Aspergillus flavus* against AB 193 and AV 90 reached 100% till 100 mg/L whereas when the concentration of dye was increased slowly, decreased decolorization was observed which is shown in Figs. 7 and 8. It was observed that at concentrations 100 mg/L the decolorization was quite efficient, and it reached up to 96% (AB 193) and 97% (AV 90) respectively. When the concentration was gradually increased to 125 mg/L, the decolorization of AB 193 and AV 90 decreased up to 65 and 56%, respectively.

Fig. 7 Effect of initial dye concentration on decolorization of AB 193 by *Aspergillus flavus*

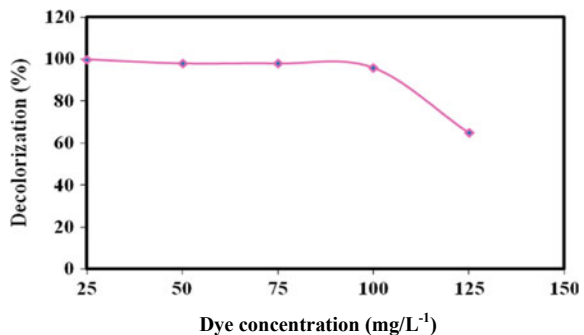
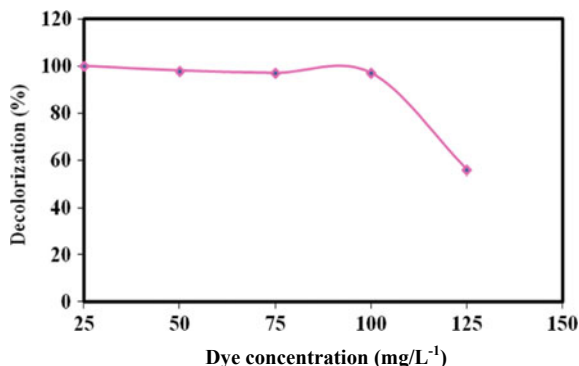


Fig. 8 Effect of initial dye concentration on decolorization of AV 90 by *Aspergillus flavus*



The growth of the fungi may be affected by the presence of dyes at toxic concentrations and in turn shows effect on decolorization efficiency. Also, the structure of the dye is also influential in deciding the extent to which dye is decolorized. As reported earlier (Kapdan et al. 2000) that at lower dyestuff concentration 0.05% complete decolorization of everzol turquoise blue G by *C. versicolor* was observed whereas only partial decolorization could be achieved for dyestuff concentrations above 0.05%. *Phanerochaete chrysosporium* showed a color removal efficiency of 95–100% on direct blue 15 in repeated batches (Pazarlioglu et al. 2005) following first order kinetics with respected to initial concentrations of dye.

5 Conclusion

In summary, the present study indicates that the fungal isolate *Aspergillus flavus* is an excellent strain for the decolorization of azo dyes. The optimum pH and temperature for enhanced decolorization is at 6 and 25 °C, respectively. This implies the fungus *Aspergillus flavus* serve as a potential candidate for the decolorization of textile effluents containing the dyes. In future, further approach has to be focused on enzyme responsible for decolorization of azo dyes.

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