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Original Research Paper

Decolorization of Simulated Textile Effluent by *Phanerochaete chrysosporium* and *Aspergillus fumigatus* A23

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ABSTRACT

Synthetic dyes are released in the effluent from a wide variety of industries such as textile, tannery, packed food, pulp and paper and paint, thus threatening various forms of life. Bioremediation is always considered as cost effective and eco-friendly way for the treatment of recalcitrant dyes and effluents. Non-white rot fungus *Aspergillus fumigatus* A23 and white rot fungus *Phanerochaete chrysosporium* were used for comparative study of decolorization of individual dyes and simulated textile effluent (STE). Both the fungi could effectively decolorize STE under optimized conditions of medium (potato dextrose agar medium), temperature (40°C for *A. fumigatus* A23 and 30°C *P. chrysosporium*), pH (4.0 for *A. fumigatus* A23 and 5.0 for *P. chrysosporium*) and agitation (100 rpm for *A. fumigatus* A23 and *P. chrysosporium*). The decolorization of STE by *A. fumigatus* A23 and *P. chrysosporium* was 86% and 62% respectively after 7d incubation. The key mechanisms involved in dye removal by the fungus appeared to be adsorption and absorption and the biotransformation occurred only after absorption of the dye. Analysis of samples before and after treatment with fungus using TLC indicated the biotransformation of dye.

INTRODUCTION

Effluents are wastewater, unwanted fluids and chemicals in liquid forms having complex composition that is extremely variable and are discharged as industrial wastewater responsible for major environmental problems. Mostly the effluents from textile, dyeing, leather, food processing, cosmetics, paper and dye manufacturing industries are important sources of water pollution. Coloured wastewater containing dyes has large inauspicious effects on the aquatic environments due to its turbidity and strength of pollution, which is very difficult to treat, since the dyes are recalcitrant organic molecules, stable to light (Sathiya et al. 2007, Tisma et al. 2012). In various parts of the world, wastewater from textile industry constitutes a serious threat to the environment due to the presence of the toxic degradation products of textile dyes (Sathiya et al. 2007, Thakur et al. 2014). It has been reported that among the total dyestuff consumption, textile industry accounts for 67% of the total dyestuff market (Rajamohan & Karthikeyan 2004).

Azo dyes are a group of compounds characterized by the presence of one or more azo bonds (-N=N-) along with one or more aromatic systems. Azo dyes comprise the largest and most diverse group of dyes used in industrial applications. In spite of their toxic effect on receiving water bodies, these dyes create an aesthetic problem and their colour discourages the downstream use of wastewater (Adebayo et al. 2010). Textile effluent can seep into the aquifer and pollute the underground water or the area where it is discharged without proper treatment (Ezeronye & Ubalua 2005). The presence of dyes in the water stream adversely affects the aesthetic merit, gas solubility, turbidity and water transparencies, even if present in small amount.

The removal of colour from wastewater has been rated to be relatively more important than the removal of soluble colourless organic substances, which usually contribute the major fraction of biochemical oxygen demand (Rajamohan & Karthikeyan 2004). For the removal of dyes from wastewater, several physicochemical methods have been employed; unfortunately they comprise some limitations like excessive use of chemicals and energy, high cost of operation and disposal as well as regeneration of used material (Crini 2008). Therefore, use of biological agents is the most desirable approach for cleaning up many environmental pollutants including removal of dyes from wastewater. Bioremediation is a pollution control technology that uses biological systems to catalyse the biodegradation or biotransformation of various toxic chemicals to no or less harmful

forms (Ashoka et al. 2002).

Nevertheless there have been numerous attempts to develop biological processes for the treatment of textile effluents either based on partial or complete biodegradation of dyes by pure and mixed cultures of bacteria, fungi and algae (Arora & Chander 2004, Banat et al. 1996, Bhatt et al. 2000, Chen et al. 2003, Ferreira et al. 2000, Govindwar et al. 2014, Jin et al. 2007, Ma et al. 2014, Machado et al. 2006, McMullan et al. 2001, Mohan et al. 2013, Muthukumaran & Mathumitha 2013, Sandhya et al. 2005, Saratale et al. 2013, Semple et al. 1999, Wesenberg et al. 2003, Zheng et al. 1999). White rot fungi that produce ligninolytic enzymes, such as lignin peroxidase, manganese peroxides and laccase have been studied extensively because of their ability to degrade various complex organic compounds. Adsorption is considered to be a promising technology for its simplicity, good removal performance, and tolerance to toxic material. To reduce the cost of adsorption process, studies have been conducted to efficiently use low-cost biological and agricultural materials (Fu & Viraraghavan 2001, Crini 2008).

However, great deal of expertise is a prerequisite in screening, selection and application of right organism(s) against specific dyes under different physicochemical conditions to achieve optimum results. The present work was carried out to compare the potential of indigenous isolated non-white rot fungus *Aspergillus fumigatus* A23 with widely acclaimed white rot fungus *Phanerochaete chrysosporium* (MTCC No. 787) to decolorize textile dyes and simulated textile effluents (STE).

MATERIALS AND METHODS

Dyes: Textile dyes used in the present study were Direct Orange 39, Acid Black 52, Acid Red 18, Direct Red 31, Direct Yellow 12, Golden Yellow HER, Reactive T Blue and Reactive Violet 5R procured from Sunit Chemicals, Ankleshvar, Gujarat, India. The dyes were of commercial grade and used without any further purification. The λ_{max} of each dye was determined by scanning on UV-VIS double beam spectrophotometer (Optizen 3220 from Mecasys, Korea) from 300-700 nm.

Organisms: The mother culture of white rot fungus *Phanerochaete chrysosporium* MTCC 787 procured from microbial type culture collection (MTCC), Chandigarh, India. Cultures of non-white rot fungi *Aspergillus flavus* A6 (ITCC 6949.08), A. *fumigatus* A23 (ITCC 6950.08) and A. *terreus* A2 (ITCC 6948.08) were used in the present study.

The fungal cultures were purified and maintained on PDA slants (containing ampicillin) at 4°C till further use.

Screening for Dye Decolorization

Decolorization of individual dye in liquid medium: Each of the eight dyes, Acid Red 18, Acid Black 52, Direct Orange, Direct Red, Direct yellow, Reactive T Blue, Reactive Violet 5R and Golden Yellow HER were added separately at a concentration of 100 mg L^{-1} to flasks as the source of dye and inoculated with 5 mm agar plug of each fungus. Autoclaved un-inoculated medium served as a control. The flasks were incubated at 30±2°C on an orbital incubator shaker for 7 d at 120 rpm, culture samples were harvested aseptically after every 24 hr and centrifuged at 10,000 rpm for 20 min. The absorbance of supernatant was determined spectrophotometrically (Systronic spectrophotometer 166, Naroda Ahmedabad, India) at λ_{max} of individual dye upto 4d. The percentage decolorization was calculated according to Kalyani et al. (2008) as given below:

$$\% \text{ Decolourization} = \frac{\text{Initial absorbance-Final absorbance}}{\text{Initial absorbance}} \times 100$$

***Selection of fungal strains*:** White rot fungus (*P. chrysosporium*) and non-white rot fungus (*A. fumigatus* A23) were selected for further decolorization of simulated textile effluent (STE).

Decolorization of Simulated Textile Effluent (STE)

Preparation of STE: STE was prepared by adding acetic acid (99.9%) 0.150 mL, KH_2PO_4 67 mg, $NaHCO_3$ 840 mg, $MgSO_4.7H_2O$ 38 mg, $CaCl_2$ 21 mg, $FeCl_3.6H_2O$ 7 mg, Acid Red 18 25 mg, Acid Black 52 25 mg, Direct Yellow 12 25 mg and Direct Orange 39 25 mg per litre of distilled water (Ali et al., 2009). STE was centrifuged at 10,000 rpm for 15 min to remove insoluble material and then it was scanned on spectrophotometer (UV VIS double beam spectrophotometer Optizen 3220 from Mecasys, Korea) from 300-700 nm to record the λ_{max}. Characteristics of different textile dyes used to prepare STE are given in Table 1.

***Decolorization of STE*:** STE with 100 mg L^{-1} of dye was prepared and inoculated with 5 mm agar plug of *A. fumigatus* A23 and *P. chrysosporium* separately. Autoclaved un-inoculated medium served as control. The flasks were incubated at 30°C on an orbital incubator shaker at 100 rpm. The samples were taken aseptically after every 24 h and were centrifuged at 10,000 rpm for 20 min. The absorbance of supernatant was determined spectrophotometrically at λ_{max} of STE. The percent decolorization was calculated.

Optimization of culture conditions for decolorization: Five different media, viz. Czapek Dox Broth (CDB), Sabouraud Dextrose Broth (SDB), Potato Dextrose Broth (PDB), Minimal Salt Medium (MSM) (Patel and Suresh, 2008), Tien and Kirk's medium (Tien & Kirk 1988) were

used in order to establish the most suitable medium for decolorization of STE by selected fungal strains. For the determination of optimum temperature, flasks were incubated at varying temperature (i.e. 20±2°C, 30±2°C, 40±2°C and 50±2°C) on an orbital shaker incubator for 7 d at 120 rpm. pH was varied from 3.0 to 8.0 and agitation speed was 0, 50, 100 and 150 rpm for decolorization of STE by both the fungal strains. STE samples were harvested aseptically after every 24 hr and centrifuged at 10,000 rpm for 20 min. Decolorization of dyes in the supernatant was determined upto 7 d as described previously.

Preparation of adsorbed dye and absorbed dye fraction for TLC: After completion of incubation period, the broth was centrifuged at 10,000 rpm for 15 min and pellet was washed with distilled water. Then dye loaded fungal biomass was treated with 10 mL acetone for 1 h. The dye, that leached out from the biomass was considered as an adsorbed dye fraction. Next, the fungal biomass was thoroughly washed with distilled water and re-suspended in 100 mL of 0.1M NaOH. After 6h the biomass was macerated and the supernatant obtained was used as absorbed dye fraction (Shah et al. 2013).

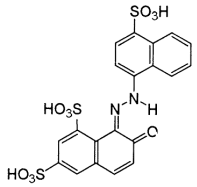
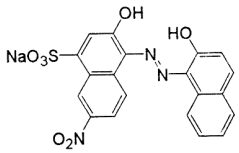
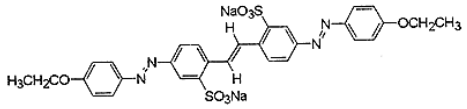
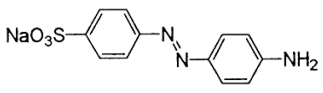
TLC (Thin Layer Chromatography): A preliminary investigation of the presence of dyes in STE was done using thin layer chromatography. The TLC plates (Merck SIL G 60 F254, Damstadt, Germany, 20×20, 250cm layer) were activated at 100°C for 1 hr. Attempts were made to separate components of effluent sample and their metabolites by TLC. After 7 d incubation, culture supernatant was obtained by centrifugation; 100 mL each of culture supernatant (dye degradation products) and effluent (control) were concentrated to 2 mL volume in rotary evaporator. These were then spotted onto the TLC plates. The TLC plates were developed in a solvent chamber containing 20 mL of a mixture of *n*-butanol: glacial acetic acid: water (60: 10: 30). After development of the plate, the dyes were detected by visual inspection and retention factor (R_f) values were recorded.

$$R_f = \frac{\text{Distance moved by analyte from origin}}{\text{Distance moved by analyte from origin}}$$

RESULTS

Decolorization of individual dye in liquid medium: All the three non-white rot fungi used in the study, *A. flavus* A6, *A. fumigatus* A23, *A. terreus* A2, showed better decolorization of dyes within 4 d than the widely used white rot fungus *P. chrysosporium* (Fig. 1). Dye decolorization by *P.*

Table 1: Characteristics of different textile dyes used to prepare simulated textile effluent (STE)

Name of dye	Characteristics		
	λ_{max}(nm)	Solubility	Chemical structure
Acid Red 18	510	In water	
Acid Black 52	589	In water	
Direct Yellow 12	400	In water	
Direct Orange 39	470	In water	

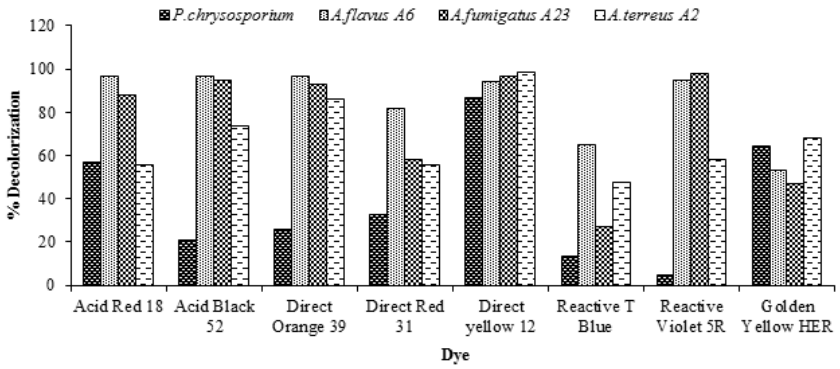


Fig. 1: Screening of different fungal strains for dye decolorization efficiency in liquid medium.

chrysosporium was slow and took 7 d. Among the non-white rot fungi, *A. fumigatus* A23 could effectively decolorize Reactive Violet 5R (98.15%) followed by Direct Yellow 12 (96.47%) and Acid Black 52 (94.87%); least decolorization was observed with Reactive T Blue (27.30%). *P. chrysosporium* showed maximum decolorization of Direct Yellow 12 (86.70%) followed by Golden Yellow HER (66.41%) and Acid Red 18 (58.50%) and minimum decolorization of Reactive Violet 5R (5.04%).

On the basis of better dye decolorization potential *A. fumigatus* A23 (a non-white rot fungus) was selected for further study and *P. chrysosporium* (a white rot fungus) was included for comparison purpose. Direct Yellow12, Direct Orange 39, Acid Red 18 and Acid Black 52 were decolorized maximally by all the strains, hence used as model dyes for further studies.

The value of λ_{max} of different azo dyes used in the study is given in Table 2.

Decolorization of simulated textile effluent (STE): Percent decolorization was estimated spectrophotometrically at λ_{max} of simulated textile effluent i.e. 520 nm. Both the fungi showed the maximum rate of decolorization during the first 24 h which, however, continued up to 120 h at a slower rate. The percent decolorization of STE by *P. chrysosporium* and *A. fumigatus* A23 after 144 h were 58.0 and 82.0%. After 168 h *A. fumigatus* A23 could decolorize up to 86.0% of STE (Fig. 2).

Table 2: λ_{max} of different azo dyes and simulated textile effluent (STE) used in the present study.

Name of dye	λ_{max}(nm)
Acid Red 18	510
Acid Black 52	589
Direct Orange 39	470
Direct Yellow 12	400
Direct Red 31	515
Golden Yellow HER	405
Reactive T Blue	625
Reactive Violet 5R	555
Simulated Textile Effluent (STE)	520

Optimization of Culture Conditions

***Medium composition**:* As shown in the Fig. 3, both the fungi, *A. fumigatus* A23 and *P. chrysosporium,* decolorized simulated textile effluent maximally in PDB medium being 85.0 and 71.0 % respectively. While in other four media, percent decolorization by *A. fumigatus* A23 ranged from 49.0 to 69.0% and 52.0 to 67.0% for *P. chrysosporium*. Both the fungi showed lowest decolorization of STE in T & K medium.

Temperature: The decolorization of STE was observed in the temperature range from 20 to 50°C with the optimum temperature being at 30°C; at this temperature *A. fumigatus* A23 and *P. chrysosporium* showed 83.0% and 65.0% decolorization respectively (Fig. 4). The rate of decolorization of STE increased with increase in temperature from 20°C to 30°C beyond which the decolorization efficiency of fungi decreased.

pH: pH plays a great influence in decolorization of STE. An initial pH of 3.0 was found to be most suitable for *A. fumigatus* A23 for the decolorization of STE (83.0%), which declined steeply with increase in pH (Fig. 5). While *P. chrysosporium* showed maximum decolorization (65.0%) of STE at an initial pH of 5.0.

Agitation: Decolorization of STE improved under stirred condition as compared to static (Fig. 6). Maximum STE decolorization, 78.0% by *A. fumigatus* A23 and 65.0% by *P. chrysosporium,* was observed at 100 rpm and decreased upon increasing or decreasing the agitation rate.

TLC: Fig. 7 depicts the TLC profile of treated and untreated STE samples. Separation of STE (control) by TLC showed six different coloured spots, the R_f values were 0.05 (red),

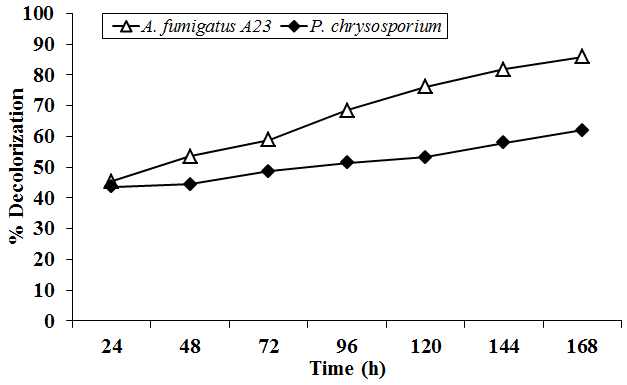


Fig. 2: Decolorization of simulated textile effluent (STE) by *A. fumigatus* A23 and *P. chrysosporium.*

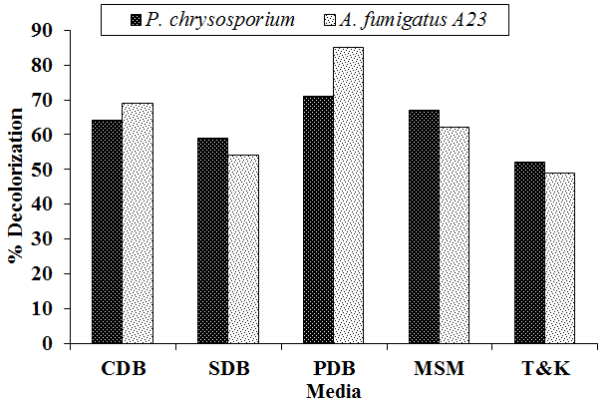


Fig. 3: Effect of medium composition on STE decolorization efficiency of *A. fumigatus* A23 and *P. chrysosporium*.

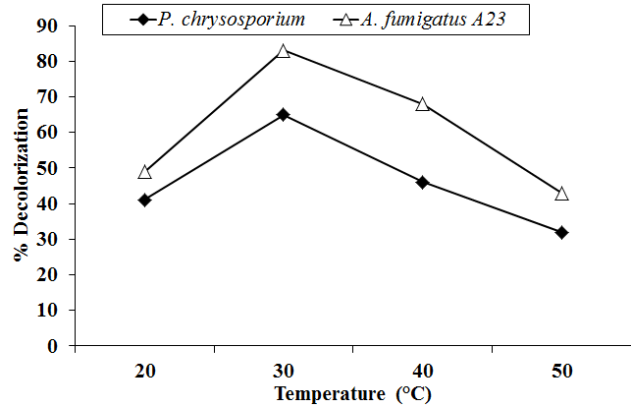


Fig. 4: Effect of temperature on STE decolorization efficiency of *A. fumigatus* A23 and *P. chrysosporium*.

0.15 (orange), 0.35 (reddish), 0.37 (black), 0.44 (red), 0.6 (yellow) and 0.7 (pink); no spot was observed in culture supernatant indicating complete removal of dye.

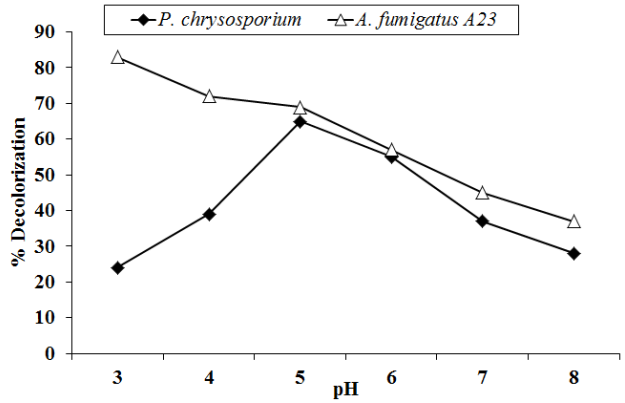


Fig. 5: Effect of pH on STE decolorization efficiency of *A. fumigatus* A23 and *P. chrysosporium.*

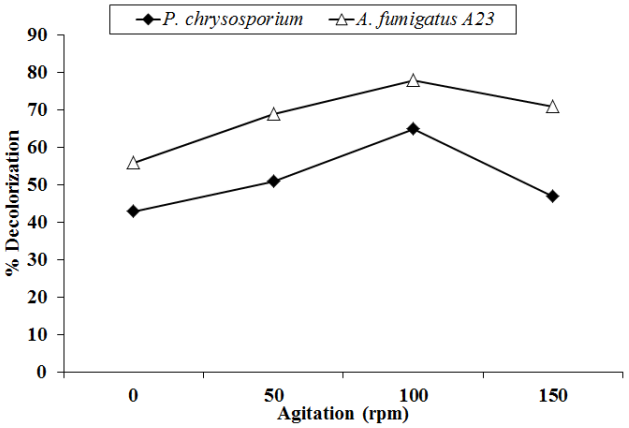


Fig. 6: Effect of rate of agitation on STE decolorization efficiency of *A. fumigatus* A23 and *P. chrysosporium*.

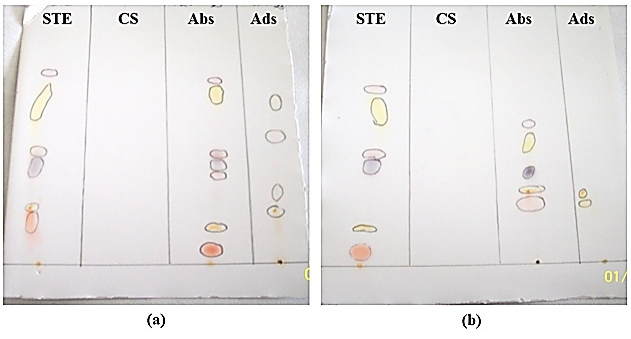


Fig. 7: TLC analysis of simulated textile effluent (STE), culture supernatant (CS), absorbed fraction (Abs), adsorbed fraction (Ads) samples after decolorization of STE by (a) *A. fumigatus* A23 and (b) *P. chrysosporium.*

In case of *P. chrysosporium,* adsorbed fraction of STE showed 5 spots with R_f values 0.23 (pink), 0.3 (orange), 0.36 (black), 0.56 (yellow) and 0.6 (pink); absorbed fraction

showed two spots with R_f value of 0.22 (yellow) and 0.25 (orange). There is difference in the R_f value of spots observed in control, adsorbed and absorbed fractions which is due to the modification of the dye by the possible lignin modifying enzymes of the fungi.

Similarly, *A. fumigatus* A23 treated culture supernatant did not show any spot indicating complete removal of the dye from the medium, adsorbed fraction showed seven spots with R_f values 0.066 (red), 0.142 (orange), 0.33 (red), 0.352 (black) 0.419 (reddish), 0.619 (yellow) and 0.685 (pink); absorbed fraction showed four spots with R_f value of 0.19 (orange), 0.219 (grey), 0.476 (pink) and 0.609 (yellow).

The new spots were observed in adsorbed and absorbed fractions which were not there in the control sample; this suggests that modification of dye occurs only after adsorption and absorption.

DISCUSSION

Industrial dyes can be released into the environment from two major sources: as effluents from dye synthesizing plants and from dye-using industries, such as textile factories. Effluents are wastewater, with unwanted fluids and chemicals in liquid form that are discharged as industrial waste. Large amounts of chemically different dyes are employed for various industrial applications including textile dyeing. Among the total dyestuff consumption, it has been reported that textile industry accounts for 67% of the total dyestuff market (Rajamohan & Karthikeyan 2004). Azo dyes constitute the largest and most diverse group of dyes used in commercial applications. In the present study, decolorization of eight industrially important dyes by three non-white rot fungi viz., *Aspergillus flavus* A6, *A. fumigatus* A23, *A. terreus* A2 and one white-rot fungus, *Phanerochaete chrysosporium* was investigated. There are plenty of reports on dye decolorization by white rot fungi owing to their non-specific lignin modifying enzymes (LMEs) (Chander & Arora 2014, Sumandono et al. 2015, Moreira et al. 2015, Si et al. 2014). However, we observed significantly more dye decolorization with non-white rot fungi as compared to white rot fungus. Sharma et al. (2009) observed 85% of decolorization of textile azo dye Orange II by *P. chrysosporium* whereas, Zope et al. (2007) stated that in wastewater non-white rot fungus perform better as compared to white rot fungus owing to their slow growth rate and degradation rate.

In the present study, *A. fumigatus* A23 significantly decolorized (up to 82%) STE containing 100 mg L^{-1} dye within 144 h. Decolorization of Acid Red 151 (20 mg L^{-1}) was well over 95% by *Aspergillus niger* SA1 under different conditions (Ali et al. 2009).

STE was decolorized better in PDB medium by both the fungi as compared to other four media tested. PDB is a nitrogen limiting medium and other reports also suggest higher decolorization percentage in nitrogen limiting medium. Harazono & Nakamura (2005) stated that a mixture of four reactive dye was decolorized 90% by *P. sorida* in N limited medium.

Many workers have found that maximum decolorization is in the temperature range of 20-40°C and a subsequent reduction in decolorization potential when temperature was further increased (Park et al. 2007, Fang et al. 2004), this is in accordance with our finding that increasing temperature beyond 30°C decreased the percentage decolorization. Fu & Viraraghavan (2001) reported that the initial pH of the dye solution significantly influences the chemistry of both the dye molecules and the fungal biomass. Fungi, both white rot fungus and non-white rot fungus, prefer acidic pH range for effective decolorization. Our study correlates with the above findings where maximum decolorization was observed at pH 3 for *A. fumigatus* A23 and pH 5 for *P. chrysosporium.*

It is well documented that the white rot fungus removes dye by the enzymatic degradation (Moredo et al. 2003, Radha et al. 2005, Ghasemi et al. 2010). The dye removal by non-white rot fungus was mainly by adsorption and absorption. The adsorption of dye on the mycelia was stable as the dye was not released into the medium and could only be extracted from biomass with methanol; further the appearance of colour in NaOH after maceration of biomass indicated the internalization of dye by mycelia. Similar observations were also made by Sumathi & Manju (2000). TLC of control (untreated), culture supernatant, adsorbed and absorbed dye fractions provided further evidence in support. Bioremediation can be achieved by two mechanisms: biodegradation and biosorption. *A. fumigatus* A23, a non white rot fungus removed dye from the broth in two steps: (1) adsorption of dye onto the fungal biomass and (2) subsequent uptake of the adsorbed dye by absorption process followed by its biotransformation inside the mycelia.

CONCLUSION

In the present study, it was found that the rate of decolorization as well as percent decolorization was more with non-white rot fungus *A. fumigatus* A23 as compared to *P. chrysosporium*, a commonly used white rot fungus for decolorization purpose. *A. fumigatus* A23 could efficiently decolorize different azo dyes and simulated textile effluent, therefore a process may be designed for treatment of dye containing industrial effluents using *A. fumigatus* A23, a new fungus, which has not been reported so far. The fungus removed the dye mainly by adsorption and absorption process.

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