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A Comparative Study on Decolourization of Industrial Dyes and Real Textile Wastewater by White Rot and Non-white Rot Fungi

Mitali K. Shah, T. Dharajiya Darshan and Bhakti Bajpai*

Department of Biotechnology, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), Affiliated to Sardar Patel University
New V.V. Nagar – 388120, Gujarat, India
✉ bhakti.bajpai@gmail.com

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Abstract: Synthetic dyes are extensively used in several industries including textile, paper, printing, cosmetic and pharmaceutical. Dyes are released in effluent from a wide variety of industries such as textile, tannery, packed food, pulp and paper, paint and electroplating thus threatening various forms of life. Non-white rot fungi *Aspergillus flavus* A6, *Aspergillus fumigatus* A23, *Aspergillus terreus* A2 and white rot fungus *Phanerochaete chrysosporium* were used to decolourize individual dyes, simulated textile effluent (STE) and real textile wastewater (RTW). Fungi could effectively decolourize STE and RTW under optimized conditions of medium (minimal salt medium and potato dextrose agar medium), temperature (40 ºC for *A. flavus* A6 and 30 ºC for *P. chrysosporium*), pH (4.0 for *A. flavus* A6 and 5.0 for *P. chrysosporium*) and agitation (100 rpm for *A. flavus* A6 and *P. chrysosporium*). The decolourization of STE by *A. flavus* A6 and *P. chrysosporium* was 73 and 62% respectively while the decolourization of RTW by *A. flavus* A6 and *P. chrysosporium* was 76 and 68% respectively after 7 d incubation. The mechanism of dye removal by the fungus appeared to be mainly by 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.

Key words: Dyes, biotransformation, decolourization, *A. flavus* A6, *P. chrysosporium*.

Introduction

The total consumption of dyes by textile industry worldwide is more than 107 kg/year and accounts for the largest consumption of dyestuffs, at nearly 80% (Lorimer et al., 2001). There are more than 10,000 commercially available dyes with over 7×10^5 tonnes of dyestuff produced annually across the world (Gong et al., 2005).

Industrial effluents from various industries like textile, dyestuffs, paper and pulp, distillery, olive oil mill and metal industries etc. are the major contributors to water pollution. Colour of textile effluent escalates environmental problem mainly because of its non-biodegradable characteristics. Many dyes are difficult to degrade; they are generally stable to light, oxidizing agents and are resistant to aerobic digestion (McKay and Sweeney, 1980).

A fungal treatment of dyes is an economical and feasible alternative to the physical treatment technologies (Knapp et al., 2001; Singh, 2006). Lignin modifying enzymes (LMEs) secreted by white rot fungi, during the secondary metabolism, mediate decolourization of azo, triphenylmethane and polymeric dyes (Yang et

*Corresponding Author

al., 2009). Besides white rot fungi, few non-white rot fungi such as *Aspergillus niger* (Bhole et al., 2004; Fu and Viraraghavan, 2000), *Rhizopus arrhizus* (Zhou and Banks, 1993) and *Geotrichum candidum* (McMullan et al., 2001) are also reported to have dye decolourization activity. Unlike white-rot fungi, the mechanism of dye removal in non-white rot fungi is mainly by adsorption and/or absorption, in some cases followed by biodegradation through microbial metabolism (Fu and Viraraghavan, 2001).

The paper compares the potential of indigenous non-white rot fungus *Aspergillus flavus* A6 with widely acclaimed white rot fungus *Phanerochaete chrysosporium* MTCC 787 to decolourize textile dyes, simulated textile effluent (STE) and real textile wastewater (RTW).

Materials and Methods

Chemicals

All the chemicals and readymade media used for the present study were of analytical grade and purchased from Merck (India), Hi media (India) and Sigma (USA). Textile dyes used in 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. The dyes were of commercial grade purchased from Sunit Chemicals, Gujarat.

Microrganisms

The mother culture of white rot fungus *Phanerochaete chrysosporium* MTCC No. 787 was procured from Microbial Type Culture Collection (MTCC), Chandigarh, India. Cultures of non-white rot fungi *Aspergillus flavus* A6 (ITCC designation no. 6949.08), *A. fumigatus* A23 (ITCC designation no. 6950.08), and *A. terreus* A2 (ITCC designation no. 6948.08) were isolated from dye infested soil and identified in a separate study. The cultures were maintained on PDA + ampicillin slants and stored at 4 ºC.

Sample Collection

The real textile wastewater (RTW) was collected from Surat (Gujarat, India), a textile hub, located at 21°10′N latitude and 72°50′E longitude. RTW was collected from G.I.D.C area of Katargam, Surat, where most of the textile industries are located.

Determination of RTW Characteristics

The temperature of RTW sample was directly measured with thermometer and pH was measured using digital pH meter (Digital pH meter 335 from Systronics, Ahmedabad, India). The biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS), total dissolved solids (TDS) and total suspended solids (TSS) of RTW were determined using standard methods described by Ademoroti (1996).

Determination of λ_{max}

RTW was thoroughly mixed and then centrifuged at 10,000 rpm for 15 minutes to remove insoluble suspended material. The supernatant was scanned from 300 nm to 700 nm on spectrophotometer (UV VIS double beam spectrophotometer Optizen 3220 from Mecasys, Korea). λ_{max} of individual dye and STE was also recorded by same method.

Screening for Dye Decolourization

The qualitative and quantitative decolourization of eight structurally different textile dyes viz. Acid Red 18, Acid Black 52, Direct Orange, Direct Red, Direct Yellow, Reactive T Blue, Reactive Violet 5R, and Golden Yellow HER was performed. In all the experiments dye stocks were autoclaved separately.

Agar Plate Method

The cultures of *P. chrysosporium*, *A. flavus* A6*, A. fumigatus* A23 and *A. terreus* A2 were spot inoculated on PDA plates containing different dyes at a concentration of 100 mg l^{-1} and incubated at 30°C for six days. The development of halo/clear zone at the inoculation site was noted as an index of dye decolourization. The diameter of the halo was measured and used for calculation of decolourization efficiency as below:

$$\text{Decolourization efficiency} = \frac{\text{Decolourization diameter (mm)}}{\text{Growth diameter (mm)}} \times 100$$

Broth Method

Quantitative determination of dye decolourization in broth was carried out in 100 ml Potato Dextrose Broth (PDB) at dye concentration of 100 mg/l. The flasks were inoculated with 5 mm agar plug of each fungus, then incubated at 30 ± 2°C on an orbital incubator shaker for seven days at 120 rpm. The samples were drawn aseptically after every 24 h and centrifuged at 10,000 rpm for 20 min; the absorbance of supernatant was determined spectrophotometrically at respective λ_{max}. The percentage decolourization was calculated according to Kalyani et al. (2007) as given below:

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

Decolourization of Simulated Textile Effluent (STE)

The STE was prepared according to Anastasi et al. (2010) and contained Acetic acid (99.9%) (0.150 ml), KH_2PO_4 (67.0 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) to prepare one litre, pH was adjusted to 8.0. The final concentration of dye was 100 mg/l. Autoclaved uninoculated STE served as control. The flasks were incubated at 100 rpm. The samples were taken aseptically after every 24 hrs and were centrifuged at 10,000 rpm for 20 min. The absorbance of supernatant was determined spectrophotometrically (Systronic spectrophotometer 166, Ahmedabad, India) at 520 nm (λ_{max} of STE). The percent decolourization was calculated as described earlier.

Decolorization of Real Textile Wastewater (RTW)

The RTW was centrifuged at 10,000 rpm for 20 min to remove insoluble material and then used in place of water to prepare PDB media. Autoclaved uninoculated medium served as control. Absorbance was read at 305 nm (λ_{max} of RTW). Percent dye decolourization was calculated as described earlier.

Optimization of Culture Conditions for Decolourization

Five different media viz. Czapek Dox Broth (CDB), Sabouraud Dextrose Broth (SDB), Potato Dextrose Broth (PDB), Minimal Salt Medium (MSM) (Patel and Suresh, 2008), and Tien and Kirk's medium (Tien and Kirk, 1988) were used in order to establish the most suitable medium for decolourization of STE and RTW; temperature employed were 20, 30, 40 and 50 °C; pH varied from 3.0 to 8.0; agitation speed was 0, 50, 100 and 150 rpm.

Analysis of Degradation Products by Thin Layer Chromatography (TLC)

Preparation of Adsorbed Dye and Absorbed Dye Fraction for TLC

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 hr. The dye, that leached out from the biomass, was considered as adsorbed dye fraction. Next, the fungal biomass was thoroughly washed with distilled water and resuspended in 100 ml of 0.1M NaOH. After 6 h the biomass was macerated and the supernatant obtained was used as absorbed dye fraction.

TLC Analysis

A preliminary investigation of the presence of dyes in the RTW and STE before and after treatment was done using thin layer chromatography (TLC). The TLC plates (Merck SIL G 60 F254, Damstadt, Germany, 20 × 20, 250 cm layer) were activated at 100 °C for one hour. At the end of the incubation (after seven days) culture were centrifuged and supernatant (their metabolites), adsorbed sample, absorbed sample and effluent (control) were concentrated to 2.0 ml in a rotary evaporator corresponding to 100 ml of sample and spotted onto the TLC plates. The TLC plates were developed in a solvent system of *n*-Butanol:GAA:H_2O (60:10:30). After development of the plate, the dyes were detected by visual inspection and reference factor (R_f) values were recorded.

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

Results

Screening for Dye Decolourization Activity

Agar Plate Method

In general, all the dyes under study were decolourized more efficiently by non-white rot fungi as compared to white rot fungus (Figure 1) except for Direct Red 31 which was decolourized only by *P. chrysosporium*. Among non-white rot fungi, *A. flavus* A6 has emerged as the potential fungus showing more than 100% decolourization (154.5%-0%) of all the seven dyes. Further, *A. fumigatus* A23 (125%-0%) decolourized more variety of dyes as compared to *A.terreus* A2 which could not decolourize Acid Black, Direct Red 31, Reactive T Blue and Reactive Violet 5R.

Decolourization of Individual Dye in Broth

Like PDA, in PDB too *A. flavus* A6, *A. fumigatus* A23 and *A. terreus* A2 showed better performance than the widely used *P. chrysosporium*. Non-white rot fungi were able to decolourize most of the dyes within three days whereas decolourization was slower in *P. chrysosporium* requiring at least seven days for maximum decolourization. Except for Golden Yellow HER, *A. flavus* A6 could effectively decolourize rest of the dyes (65-97%). It is evident from Figure 2 that the *A. flavus* A6 showed maximum decolourization of dyes followed by *A. fumigatus* A23, *A. terreus* A2 and *P. chrysosporium*.

Therefore, for further comparative dye decolourization studies *A. flavus* A6 (a non-white rot fungus) and *P. chrysosporium* (a white rot fungus) were selected. Direct Yellow 12, Direct Orange 39, Acid Red 18, and Acid Black 52 were decolourized maximally by all the strains, hence used as model dyes for further studies.

Decolourization of Simulated Textile Effluent (STE)

The rate of decolourization of Simulated Textile Effluent (100 ppm) by *A. flavus* A6 and *P. chrysosporium* was high in the initial 24 h (42%), later it slowed down showing 70 and 60% decolourisation respectively after 168 h (Figure 3).

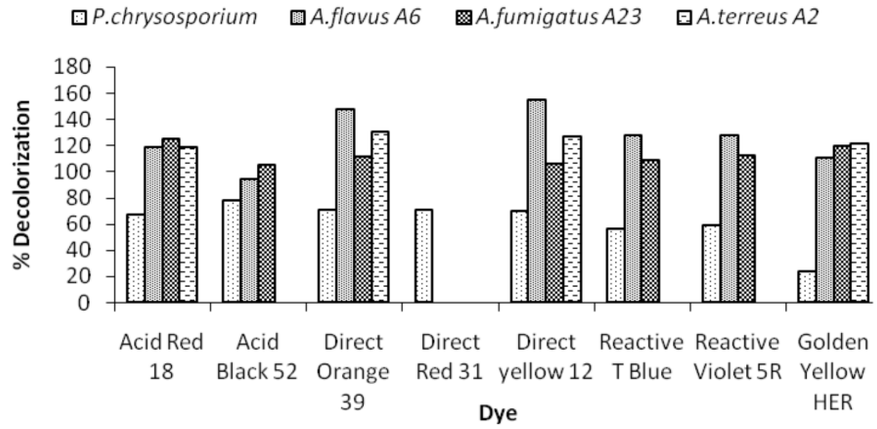


Figure 1: Screening of dyes for decolourization by different fungal strains using agar plate method.

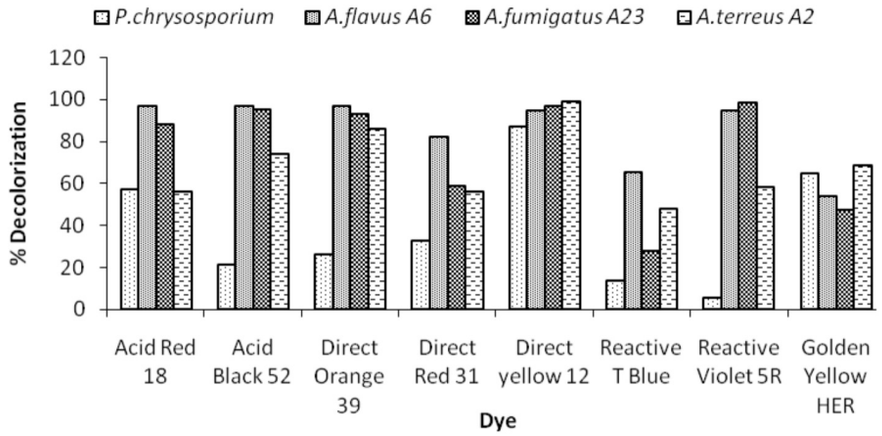


Figure 2: Screening of dyes for maximum decolourization by different fungal strains in liquid medium.

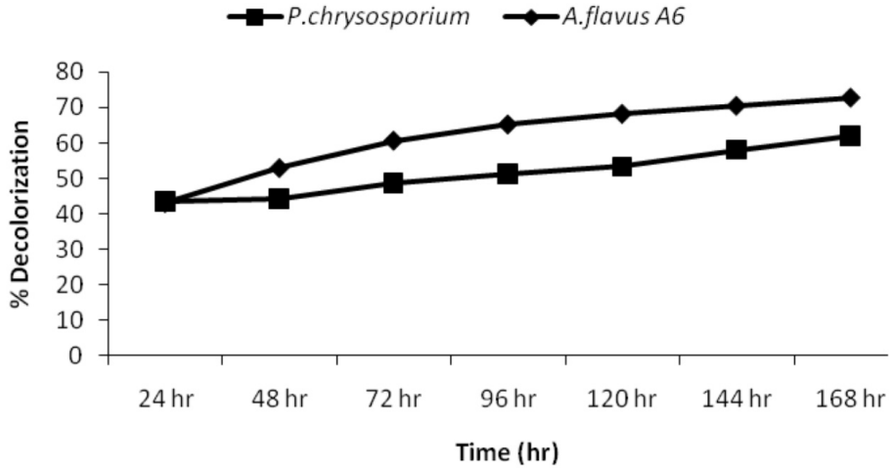


Figure 3: Decolourization of simulated textile effluent by *A. flavus* A6 and *P. chrysosporium*.

Decolourization of Real Textile Wastewater (RTW)

A. flavus A6 showed maximum decolourization (65%) of 50% RTW in just 72 h as against 35% decolourization by *P. chrysosporium* in the same time period (Figure 4), 65% decolourization of RTW by *P. chrysosporium* was achieved after 7 d (168 h). When 100% RTW was used for medium preparation the percent decolourization was 76 and 67% by *A. flavus* and *P. chrysosporium* respectively after 7 d.

Determination of Effluent Parameters

P. chrysosporium showed 50 and 60% reduction of BOD and COD respectively, whereas *A. flavus* A6 showed 79 and 75% reduction in BOD and COD respectively (Table 1). From the results obtained it can be concluded that *A. flavus* A6 shows better BOD and COD reduction rate than *P. chrysosporium* in RTW.

Optimization of Culture Conditions for STE and RTW Decolourization

Medium

As shown in Figure 5, among the four media tested maximum decolourization of STE and RTW by both the fungi was observed in PDB. *A. flavus* showed 83 and 68% decolourization of STE and RTW respectively and *P. chrysosporium* showed 64 and 66% decolourization of STE and RTW respectively in PDB. While in other four media percent decolourization by *A. flavus* A6 and *P. chrysosporium* was in the range of 68 to 73% after

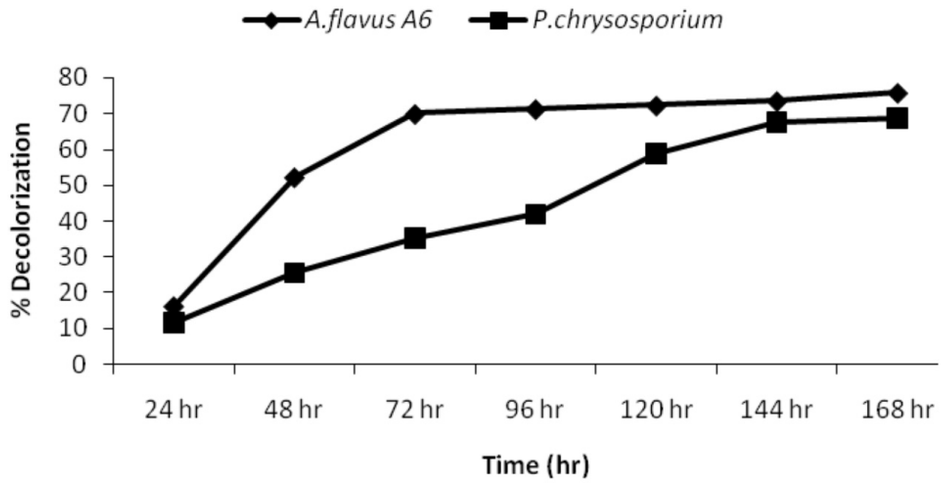


Figure 4: Decolourization of RTW by *A. flavus* A6 and *P. chrysosporium*.

Table 1: Analysis of RTW parameters before and after fungal treatment

	P. chrysosporium			*A. flavus A6*		
	Before treatment (mg/l)	*After treatment (mg/l)*	*% reduction*	*Before treatment (mg/l)*	*After treatment (mg/l)*	*% reduction*
COD	3600	1440	60	3600	900	75
BOD	145	72	50	145	30	79
TSS	233.3 mg/l					
TDS	1666.6 mg/l					
TS	2066.6 mg/l					
Temperature	30°C (at collection site)					
pH	6.64					
Colour	Brown					

4 days, lowest decolourization of both STE and RTW was observed in SDB medium even after prolonged incubation.

Temperature

The optimum temperature for decolourization of STE and RTW by *A. flavus* A6 was found to be 40°C, showing 79 and 69% decolourization respectively (Figure 6). The rate of decolourization of RTW and STE increased with increase in temperature from 20° to 40°C beyond which the decolourization rate decreased sharply. The optimum temperature for decolourization of STE and RTW by *P. chrysosporium* was 30 °C with 65% decolourization.

pH

pH played great influence in decolourization of RTW and STE. An initial pH of 4.0 was found to be most suitable for the decolourization of STE (81%) and RTW (78%) by *A. flavus* A6, which declined steeply in the basic pH range. On the other hand, *P. chrysosporium* showed maximum decolourization of STE (65%) and RTW (67%) at initial pH of 5.0 (Figure 7).

Agitation

Irrespective of the fungi, decolourization of both STE and RTW improved under shaking condition as against static condition (0 rpm) being maximum at 100 rpm (Figure 8).

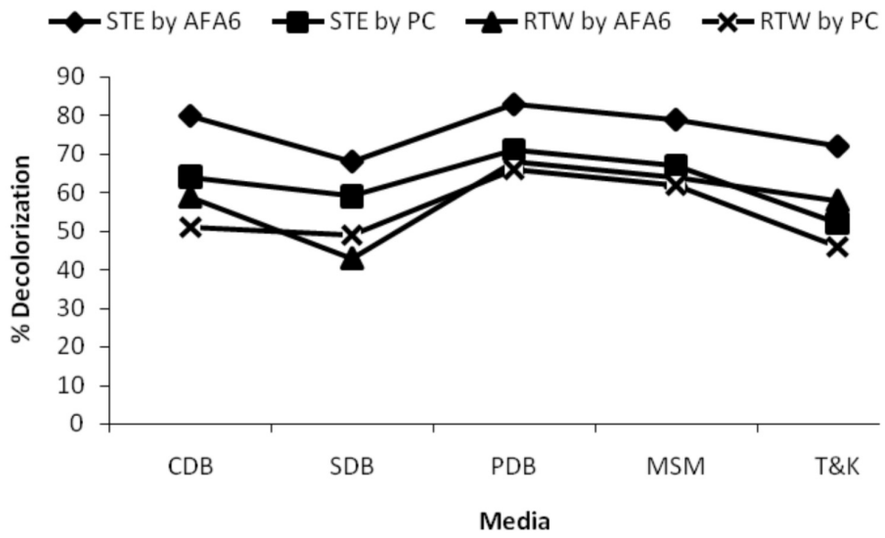


Figure 5: Effect of medium composition on decolourization of STE and RTW by *A. flavus* A6 (AFA6) and *P. chrysosporium* (PC) respectively.

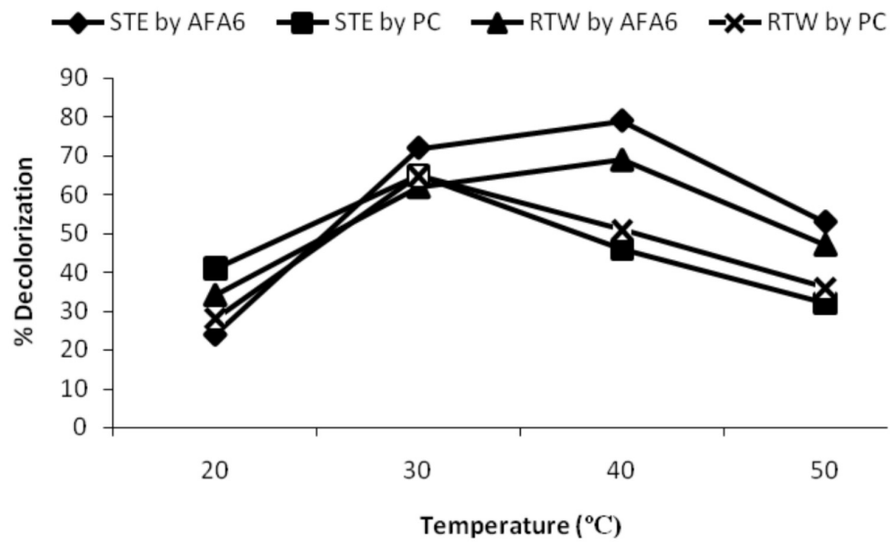


Figure 6: Effect of temperature on decolourization of STE and RTW by *A. flavus* A6 (AFA6) and *P. chrysosporium* (PC) respectively.

Analysis of Degradation Products by TLC

STE

As evident from Table 2 and Figure 9, the separation of STE by TLC showed six different spots; however no spot was observed in culture supernatant inoculated with either *A. flavus* A6 or *P. chrysosporium* indicating complete removal of dye from the medium. In case of *A. flavus* A6, adsorbed fraction showed six spots and absorbed fraction showed three spots with R_f values different from that of control. The spots with similar R_f value in control and adsorbed fraction (where new spots in absorbed fraction), suggested that dye modification did not occur at all in the culture supernatant; however the biotransformation occurred after absorption.

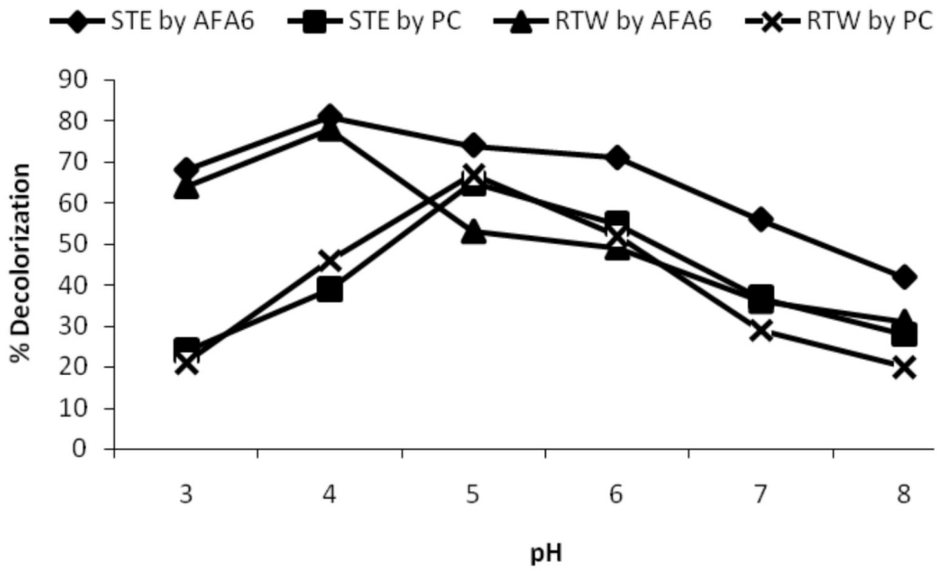


Figure 7: Effect of pH on decolourization of STE and RTW by *A. flavus* A6 (AFA6) and *P. chrysosporium* (PC) respectively.

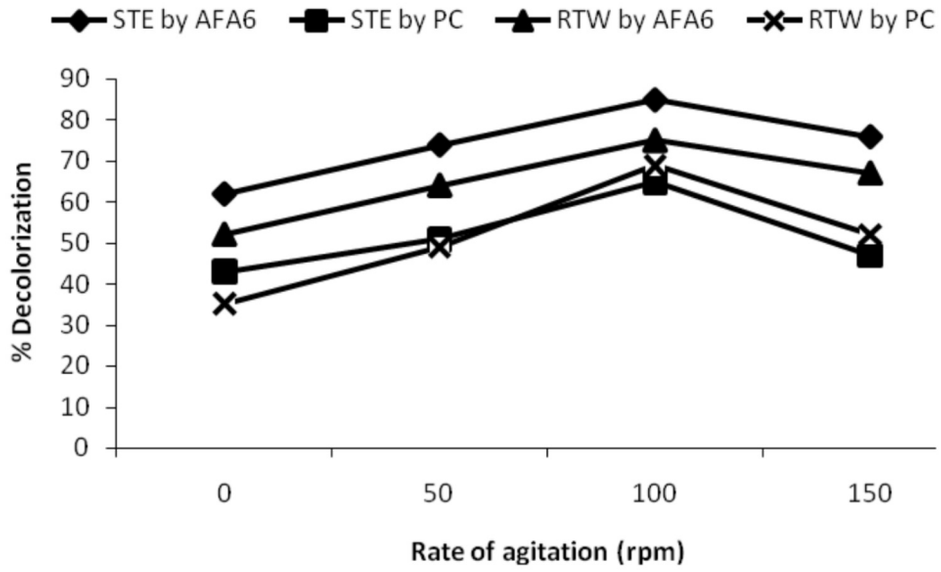
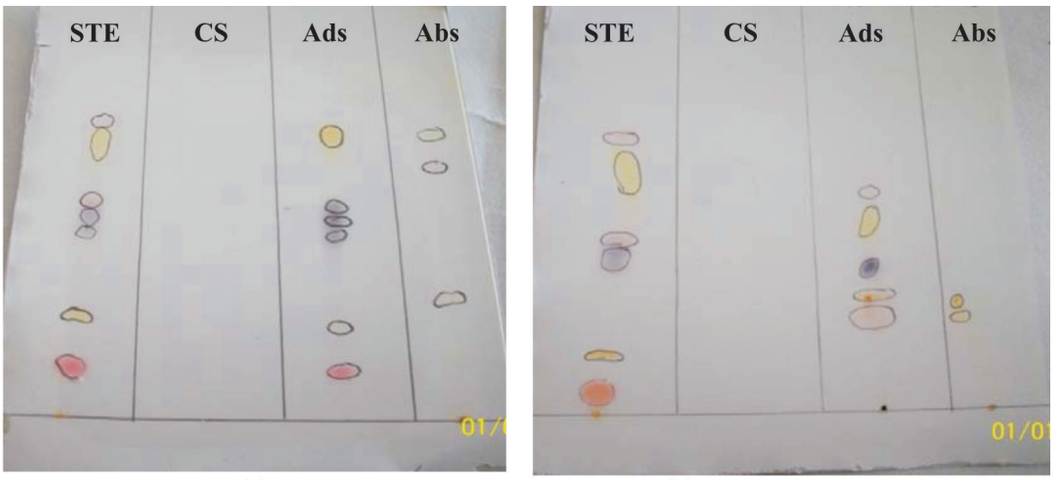


Figure 8: Effect of rate of agitation on decolourization of STE and RTW by *A. flavus* A6 (AFA6) and *P. chrysosporium* (PC) respectively.

Table 2: TLC analysis of STE samples

	STE control		*Adsorbed fraction*		*Absorbed fraction*	
Spot No.	*Colour*	*R_f value*	*Colour*	*R_f value*	*Colour*	*R_f value*
			A. flavus A6			
1	Red	0.05	Red	0.095	Orange	0.25
2	Orange	0.15	Orange	0.238	Pink	0.66
3	Red-orange	0.35	Red-orange	0.430	Yellow	0.69
4	Black	0.37	Black	0.472		
5	Purple	0.44	Purple	0.533		
6	Yellow	0.60	Yellow	0.695		
			P. chrysosporium			
1	Same as above		Pink	0.23	Yellow	0.22
2			Orange	0.30	Orange	0.25
3			Black	0.36		
4			Yellow	0.56		
5			Pink	0.60		



(a) (b)

Figure 9: TLC of STE samples: (a) *Aspergillus flavus* A6 and (b) *P. chrysosporium*, where STE = Simulated Textile Effluent control, CS = Culture supernatant, Abs = Absorbed dye sample and Ads = Adsorbed dye sample.

The difference in the R_f value of spots observed in control, adsorbed and absorbed fractions of *P. chrysosporium* indicated the possible modification of dye by the extracellular lignin modifying enzymes of *P. chrysosporium*.

RTW

The separation of RTW by TLC showed four different spots with different R_f values 0.85 (red), 0.88 (violet), 0.92 (pink) and 0.96 (yellow) as shown in Table 3 and Figure 10; no spot was observed in culture supernatant of either *A. flavus* or *P. chrysosporium*. In adsorbed fraction of *A. flavus* A6, three spots had R_f values similar to the control though the absorbed fraction showed three new spots. Likewise, adsorbed fraction of *P. chrysosporium* showed three spots with R_f values similar to control and absorbed fraction showed two spots with entirely new R_f values as compared to control.

Discussion

Bioremediation can be achieved by two mechanisms: biodegradation and biosorption. Although many researchers have used white rot fungi (WRF) for dye degradation process (Levin et al., 2004) however,

Table 3: TLC analysis of RTW samples

	RTW control		*Adsorbed fraction*		*Absorbed fraction*	
Spot No.	*Colour*	*R_f value*	*Colour*	*R_f value*	*Colour*	*R_f value*
			P. chrysosporium			
1	Red	0.85	Violet	0.91	Red	0.18
2	Violet	0.88	Pink	0.93	Yellow	0.36
3	Pink	0.92	Yellow	0.96		
4	Yellow	0.96				
			A. flavus A6			
1	Same as above		Violet	0.90	Yellow	0.193
2			Pink	0.93	Orange	0.30
3			Yellow	0.97	Red	0.68

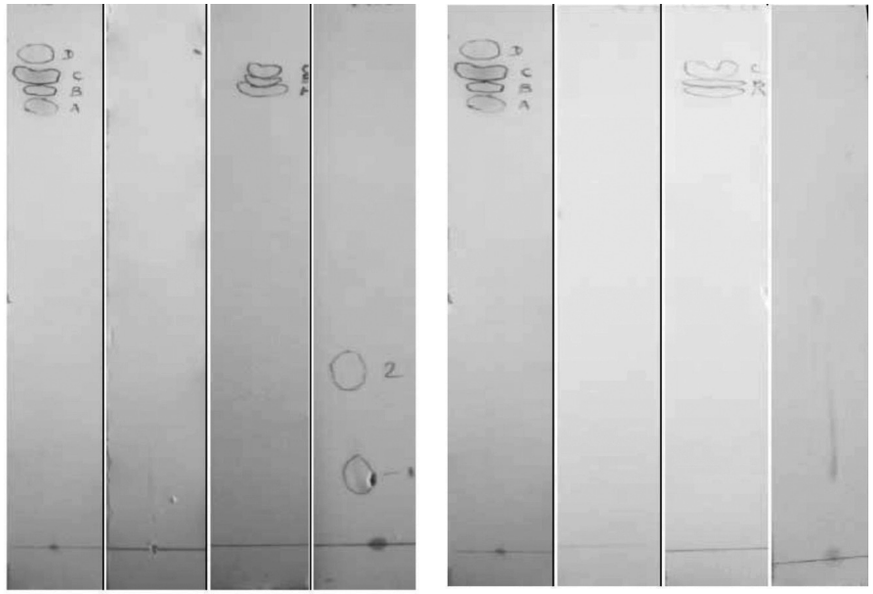


Figure 10: (a) TLC of RTW samples of *P. chrysosporium*, (b) TLC of RTW samples of *Aspergillus flavus* A6, where RTW = Real Textile Wastewater control, CS = Culture supernatant, Abs = Absorbed dye sample and Ads = Adsorbed dye sample.

in the present study it was found that the rate of decolourization as well as percent decolourization was more by non-white rot fungus *A. flavus* A6 as compared to *P. chrysosporium,* a white rot fungus.

STE was more efficiently decolourized by both the fungi as compared to RTW, which may be due to the presence of toxic dye mixture and high concentration of heavy metals salts in textile effluents (Mathur et al., 2005). STE as well as RTW both were decolourized better in PDB medium as compared to other media; this can be explained on the basis of medium composition. PDB is a nitrogen limiting medium and other reports also suggest higher decolourization percentage in nitrogen limiting medium. Harazono and Nakamura (2005) stated that a mixture of four reactive dye were decolourized 90% by *Phaerochaete sorida* in N-limited medium.

Many workers have found that maximum decolourization is in the temperature range of 20-40°C and a subsequent reduction in decolourization potential when temperature was further increased (Park et al., 2007), this is in accordance with our finding that increasing temperature beyond the optimum decreased the percentage decolourization.

Fu and Viraraghavan (2000) reported that the initial pH of the dye solution significantly influences the chemistry of both dye molecules and the fungal biomass. Fungi, both WRF and non-WRF, prefer acidic pH range for effective decolourization . Our study correlates with the above findings where maximum decolourization was found at acidic pH 4. From the present study it can be said that BOD and COD removal by *A. flavus* A6 was better than that by *P. chrysosporium*. Esposito et al. (1991) also reported that *Lentinula edodus* removed COD more efficiently as compared to *P. chrysosporium*, thus from economical as well as environmental point of view *A. flavus* A6 could be a good alternative for BOD and COD reduction.

We found that 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 acetone; further the appearance of colour in NaOH after maceration of biomass indicated the internalization of dye by mycelia. Similar observation was also made by Sumathi and Manju (2000). This observation was also supported by TLC of control, culture supernatant, adsorbed and absorbed dye fractions. Thus, *A. flavus* A6 removed the dye from broth mainly by biosorption in two steps (i) adsorption of dye onto the fungal biomass and (ii) subsequent uptake of the adsorbed dye by absorption process followed by its biotransformation inside the mycelia. The efficient decolourization of STE and RTW by *A. flavus* A6 is indicative of robust nature of the fungus and its possible application in bioremediation of textile effluent.

Conclusion

Aspergillus flavus A6, an indigenous non-WRF, is a potent degrader of various industrially important textile dyes, STE and RTW, the decolourization activity was even better than the widely used WRF *P. chrysosporium*. The efficient decolourization of toxic STE and RTW by *A. flavus* A6 is indicative of robust nature of the fungus and its possible application in bioremediation of textile effluent.

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