

Performance evaluation of two *Aspergillus* spp. for the decolourization of reactive dyes by bioaccumulation and biosorption

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Abstract A biological method was adopted to decolourize textile dyes, which is an economic and eco-friendly technology for textile wastewater remediation. Two fungal strains, i.e. *Aspergillus lentulus* and *Aspergillus fumigatus*, were used to study the removal of low to high concentrations (25 to 2000 mg L⁻¹) of reactive remazol red, reactive blue and reactive yellow dyes by biosorption and bioaccumulation. The biosorption was successful only at the lower concentrations. *A. lentulus* was capable of removing 67–85% of reactive dyes during bioaccumulation mode of treatment at 500 mg L⁻¹ dye concentration with an increased biomass uptake capacity. To cope up with the high dye concentration of 2000 mg L⁻¹, a novel combined approach was successful in case of *A. lentulus*, where almost 76% removal of reactive remazol red dye was observed during bioaccumulation followed by biosorption. The scanning electron microscopy also showed the accumulation of dye on the surface of fungal mycelium. The results signify the application of such robust fungal strains for the removal of high concentration of dyes in the textile wastewaters.

Keywords Reactive dyes · Fungus · Bioaccumulation · Biosorption · Biomass · Dye uptake capacity

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Introduction

The increasing industrialization has led to introduction of toxic chemicals and polluted effluents in the air, soil and water bodies on a massive scale (Kaushik et al. 2012; Bhattacharya et al. 2015; Gola et al. 2016b). The scientific communities are, therefore, concentrating to cope up with this pollution for the removal or remediation of these chemicals which are hazardous to the human health (Gola et al. 2017). The textile industry is also one of the biggest contributors to this toxic effluent generation which release high concentrations of dyes, mordants and other auxiliaries in the environment (Gola et al. 2015). According to the published reports, out of total synthetic textile dyes used by the textile industries, almost 15–20% unused dye is drained away as a textile discharge from the wastewater treatment plant into the water streams and rivers (Weber et al. 1990). The hazardous health effects of textile dyes to humans and environment are very well tested and documented by various researchers (Schneider et al. 2004; Tsuboy et al. 2007; Carneiro et al. 2010).

Dyes are classified into various categories like anionic, cationic, acid, basic, azo, reactive and Vat dyes; the cocktail of these highly concentrated dye effluents becomes even more difficult to handle and remediate by existing chemical methods. Reactive dyes exhibit a unique property in the classes of dye because it provides a wide range of bright coloration to the fabric and fastening tendency. It forms a covalent bond with the fabric due to the presence of 2-sulfatoethyl-sulfone moiety in most of dyes of this group (Weber et al. 1990). Due to these hindrances, there is a need for implementation of the biological methods, which are able to remove more than one dye from the effluent. Researchers have indicated a successful removal of dyes from textile wastewater using extremophilic microorganisms (Amoozegar et al. 2015) and microaerophilic/aerobic bacterial consortium

(Abraham et al. 2003; Shah et al. 2014). Qi et al. (2017) have studied azoreductase enzyme from *Rhodococcus opacus* ICP strain as a biocatalyst to decolourize azo dye at certain acidic conditions. Apart from these, the dead biomass of fungus and yeast along with other chemical adsorbents were also compared for the removal of several reactive dyes at a concentration not more than 200 mg L⁻¹ (Kumari and Abraham 2007). Few more studies have also shown successful decolourization of dyes and textile effluents using fungus (Novotný et al. 2004; Taskin and Erdal 2010; Przystaś et al. 2013; Singh et al. 2015). Our previous reports have established the versatility of *Aspergillus lentulus* on various anionic, cationic and azo dyes like acid navy blue, orange HF, methylene blue fast red and acid magenta (Kaushik and Malik 2010, 2013). However, majority of the reports select lower dye concentrations (25 µg L⁻¹ to 200 mg L⁻¹) for removal studies while the industrial utilization of dyes occurs at much higher doses (10–12 g L⁻¹). As a result, the experimental results may not be reliable for actual application. Overall, biological removal of reactive dyes at higher concentrations is not well studied. Therefore, in the present study, two *Aspergillus* strains, i.e. *A. lentulus* and *A. fumigatus*, were used to study the removal efficiency for reactive remazol red, reactive yellow and reactive blue dyes at concentrations ranging from 25 to 2000 mg L⁻¹ by biosorption, bioaccumulation and an integrated process mode. The purpose of using both these modes of removal and its combination was to study its potential to remove high concentrations of dyes in the solution, which may not be possible on using any one type of mode. A novel approach of sequential integration of the two modes of dye removal, i.e. bioaccumulation followed by biosorption, shows its potential to be used at the commercial scale for the removal of highly contaminated dye effluents from the industries.

Materials and methods

Test organism and growth conditions

Dye decolourization was tested using two fungal strains, i.e. *Aspergillus lentulus* (accession no. FJ172995) and *Aspergillus fumigatus* (accession no. KY241789), previously isolated in lab from textile effluent and industrial wastewater, respectively. The cultures were maintained in potato dextrose agar (PDA) slant. The potato dextrose broth was used for the growth of fungal pellets by inoculating spore suspension (~ 10⁷ spores per mL). The growth conditions were maintained at 30 °C at 150 rpm.

Dyes and chemicals

The dyes used in the experiments, i.e. reactive remazol red (RRR) RGB (C.I. Reactive Red 198), reactive blue (RB) CP

(C.I. Reactive Blue 2) and reactive yellow (RY) 3RS (C.I. Reactive Yellow 176), were obtained from Vardhaman Textiles Ltd., Budhni (MP), India. A stock solution of 10 g L⁻¹ (10,000 mg L⁻¹) was prepared in distilled water and was autoclaved for bioaccumulation experiments. Absorption maxima of each dye were estimated by scanning the dye solution over the visible range (400–700 nm). Other chemicals were of analytical grade obtained from Merck, Himedia and Qualigens.

Biosorption

The biosorption, which is a metabolically passive process for the removal of toxicants by live/dead/inactive biomass, was carried out using pre-grown fungal biomass of *A. lentulus* and *A. fumigatus*. At lower dye concentrations (25, 50 and 100 mg L⁻¹), *A. lentulus* biomass was analysed for the dye removal. At 500 mg L⁻¹ concentration, both the fungal strains were compared for the removal efficiencies. The pre-grown fungal biomass (4–5 g L⁻¹ dry weight) was mixed with the dye solutions of respective concentrations in a 250-mL Erlenmeyer flask and was incubated at 30 °C at 150 rpm. The experiment was done for 2–4 h (depending upon the removal efficiencies), and removal efficiencies after centrifugation at 10,000 rpm for 10 min were observed every 30 min in terms of optical density and residual dye concentration in the supernatant. The control of each concentration was run simultaneously under similar conditions and monitored every 30 min for any abiotic removal of dye.

Bioaccumulation

Bioaccumulation is an active process which accumulates the dyes within the fungal biomass during growth. Shake flask experiment for bioaccumulation was performed by inoculating the spore suspension (10⁷ spores per mL) of both the fungus in the potato dextrose broth (pH 6) mixed with dye solution at the desired concentration (50, 100 and 500 mg L⁻¹). The experiment was done in the sterilized conditions. The fungal biomass was allowed to grow at 30 °C and 250 rpm for 72 h (3 days). The dye removal was estimated after every 24 h of inoculation. The removal efficiency in abiotic control for each interval was also monitored. At the end of experiment, the biomass was filtered in a pre-weighed Whatman no. 1 filter paper to estimate the dry weight, and dye uptake capacity was calculated.

Two-stage process: bioaccumulation followed by biosorption

An integrated two-stage approach was applied to monitor the dye removal at a high concentration of 2000 mg L⁻¹.

For this, the spores of fungal strains (*A. lentulus* and *A. fumigatus*) were inoculated in dye containing media for bioaccumulation experiment. After 5 days, the residual dye in the media was subjected to biosorption mode of removal by a freshly grown fungal biomass of 4–5 g L⁻¹ dry cell weight (DCW) at 30 °C and 150 rpm. The removal efficiency was observed in terms of optical density at regular interval of 30 min.

Analytical techniques

The dye concentrations were measured in terms of optical density on their corresponding absorption maxima for dyes. The absorption maxima for the dyes RRR, RY and RB were 520, 420 and 610 nm, respectively. The calibration curve correlating absorbance and concentrations of dyes was prepared to calculate respective dye concentrations. The dye removal efficiencies were calculated using the following:

$$\text{Dye removal (\%)} = \frac{(A^0 - A^t)}{A^0} \times 100$$

where *A*⁰ is the initial absorbance and *A*^{*t*} is the absorbance at incubation time, *t*.

Phase contrast and scanning electron microscopy

The control and experimental fungal biomass was viewed under phase contrast (Nikon Eclipse Ti-U) and scanning electron microscope (Zeiss Evo 40) to observe the change in morphological characteristics after dye exposure. For phase contrast microscopy, pellets were centrifuged and a single pellet was observed under microscope. For scanning electron microscopy (SEM) analysis, the samples were prepared by washing it twice with 0.15 M phosphate-buffered saline (PBS) to remove the media followed by fixing it 2.5% glutaraldehyde at 4 °C for 12–18 h. The samples were again washed with 0.15 M PBS followed by its freeze-drying in a lyophilizer (Allied Frost FD3). The lyophilized sample was then subjected to the gold coating by cathodic spraying and viewing under scanning electron microscope (Gola et al. 2016a).

Statistical analysis

All the experiments were done in duplicates and the results are expressed in terms of mean of the replicates with its standard deviation (error bars in the figure represent the standard deviation).

Results and discussion

Biosorption

The fungal strain *Aspergillus lentulus* isolated in the lab has been one of the most versatile fungus for removal of cationic, anionic and azo dyes as reported in our earlier studies (Kaushik and Malik 2010, 2013). In the present study, the efficacy of these strains was also established on reactive dyes. The dyes used in the present study were obtained from one of the leading textile industries, Vardhaman Textiles Ltd., Baddi, Himachal Pradesh, where it is commonly being used to colour cotton and wool fabric. The biosorption of these dyes at lower concentrations (25, 50 and 100 mg L⁻¹) by *A. lentulus* was highly efficient, the results of which showed that reactive yellow was removed up to 98.4% at the concentration of 100 mg L⁻¹. In case of reactive blue dye, more than 96 and 97% removal was obtained in 25 and 50 mg L⁻¹ dye concentration. But a limitation in removal capacity was observed on increasing the concentration up to 100 mg L⁻¹, where only 49% decolourization was seen by the end of the experiment after 4 h (Fig. 1).

A comparative evaluation of biosorption ability among two *Aspergillus* strains, i.e. *A. lentulus* and *A. fumigatus*, was performed for the dye removal at a higher concentration, i.e. 500 mg L⁻¹ (Fig. 2). The results indicated that both the fungal strains were not actively removing the dyes in biosorption mode. In case of *A. lentulus*, the residual dye concentration was estimated to be ranging from 360 to 400 mg L⁻¹ out of 500 mg L⁻¹ (20–28% removal). The other fungal strain, i.e. *A. fumigatus*, was not able to remove more than 174–182 mg L⁻¹ dye (35–36%) at the end of the experiment. Earlier studies on biosorption of reactive dyes from textile wastewater have shown passive mode of adsorption of dye on dead/inactive fungal biomass, which showed a better

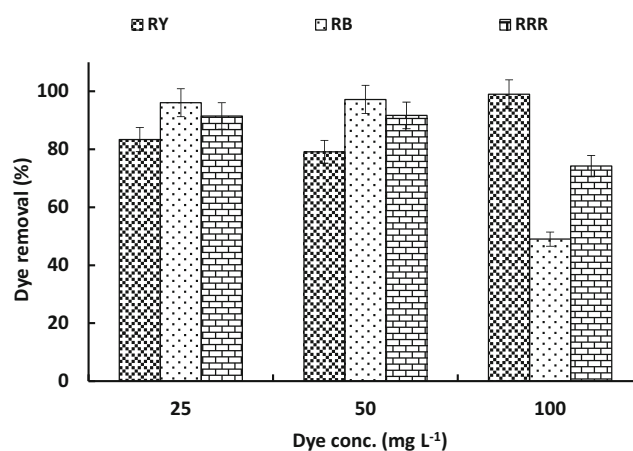


Fig. 1 Dye removal (%) of all three reactive dyes at lower concentration (25, 50 and 100 mg L⁻¹) obtained by *A. lentulus* during the biosorption mode by 72 h pre-grown fungal biomass at 30 °C and 150 rpm

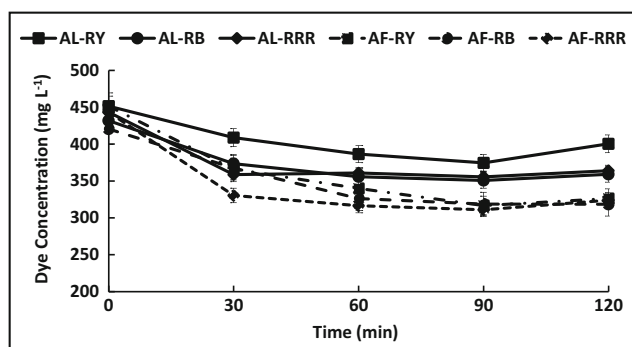


Fig. 2 Comparative profile of removal of reactive dyes by *A. lentulus* and *A. fumigatus* at 500 mg L⁻¹ dye concentration during the biosorption mode by 72 h pre-grown fungal biomass at 30 °C and 150 rpm. AL-RY *A. lentulus* reactive yellow, AL-RB *A. lentulus* reactive blue CP, AL-RRR *A. lentulus* reactive remazol red, AF-RY *A. fumigatus* reactive yellow, AF-RB *A. fumigatus* reactive blue CP, AF-RRR *A. fumigatus* reactive remazol red

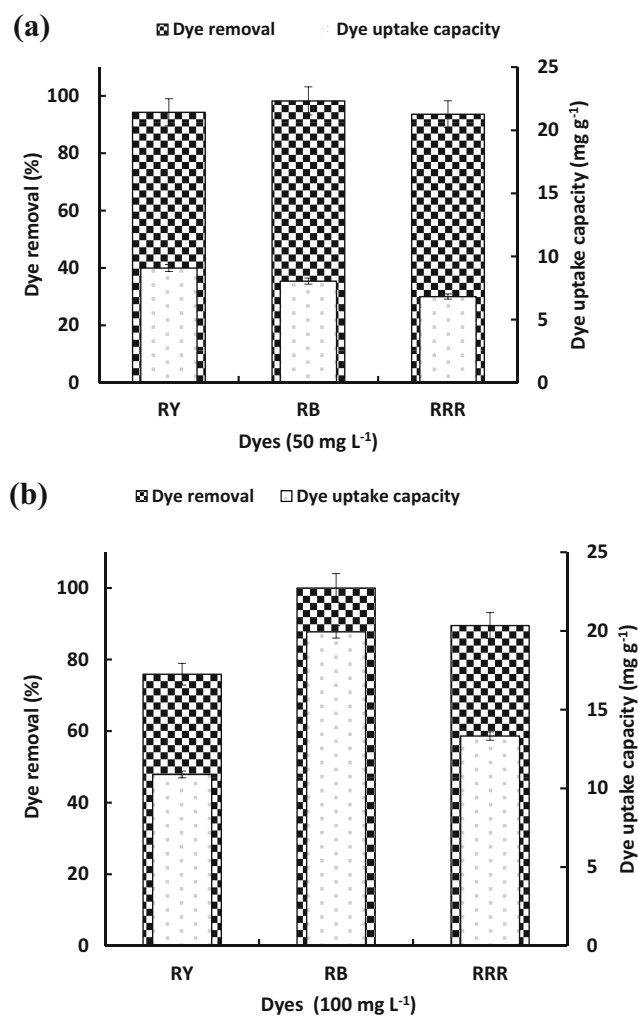


Fig. 3 Dye removal (%) and dye uptake capacity (mg g⁻¹) of reactive dyes at concentrations **a** 50 and **b** 100 mg L⁻¹ during the bioaccumulation experiment carried out at 30 °C and 150 rpm

adsorption when the biomass of *A. niger* was autoclaved as compared to gamma irradiation. The strain showed maximum removal of 85% with 8 g L⁻¹ autoclaved biomass at pH 3, temperature 30 °C after 18 h (Khalaf 2008). The reason for the better performance of dead biomass was explained by the authors as autoclaving breaks the fungal pellets and all the binding sites were exposed for dye binding. Another study has depicted biosorption potential of live but immobilized fungal biomass of *Phanerochaete chrysosporium* for the removal of remazol brilliant blue R dye (100–500 mg L⁻¹). The biosorption capacity in this study was estimated to be 101.06 mg g⁻¹ of biomass when the initial dye concentration was 500 mg L⁻¹ (Iqbal and Saeed 2007). The earlier reports on biosorption of higher concentrations of dye show a better performance when the biomass was either inactive or immobilized onto a substrate. In our study, any pre-treatment of fungal biomass was not done for dye removal; therefore, this method was only successful in the removal of dye at low concentration, i.e. up to 100 mg L⁻¹. As a general observation, any pretreatment using energy and cost-intensive technique imbalances the overall techno-economic feasibility of the process at large scale. Therefore, to avoid any such processes, another mode for dye removal, i.e. bioaccumulation, was preferred and performed in the subsequent experiments.

Bioaccumulation

Bioaccumulation is a better approach than biosorption as reported in our previous studies (Kaushik and Malik 2013). The lower concentrations of dye (50 and 100 mg L⁻¹) showed almost complete removal (more 95%) by *A. lentulus* (Fig. 3a, b). But as soon as the dye concentration was increased up to 500 mg L⁻¹, the removal efficiency of this strain declined with only 13 to 35% removal after 3 days (325.9–430.8 mg L⁻¹ residual dye in supernatant). Therefore, another fungus *A. fumigatus* was tested for the dye removal potential

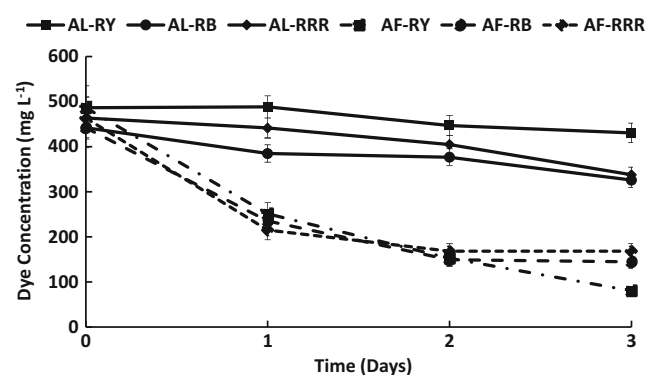


Fig. 4 Comparative profile of removal of reactive dyes by *A. lentulus* and *A. fumigatus* at 500 mg L⁻¹ dye concentration during the bioaccumulation experiment carried out at 30 °C and 150 rpm. (The nomenclature, i.e. AL-RY, AL-RB, AL-RRR, AF-RY, AF-RB, AF-RRR, is the same as given in Fig. 2)

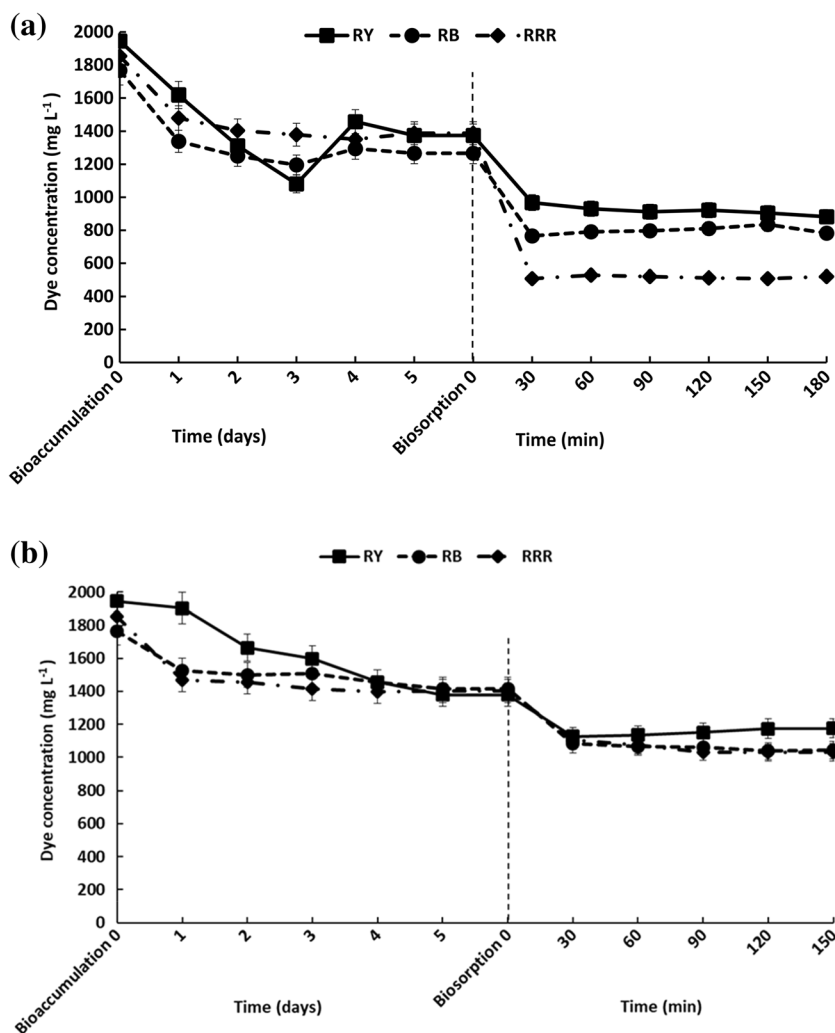
Table 1 Dye uptake capacity of *A. lentulus* and *A. fumigatus* for all three reactive dyes in bioaccumulation mode at concentrations 500 and 2000 mg L⁻¹

Dye concentration (mg L ⁻¹)	Dye uptake capacity of <i>A. lentulus</i> (mg g ⁻¹)		Dye uptake capacity of <i>A. fumigatus</i> (mg g ⁻¹)	
	500	2000	500	2000
RRR	32 ± 1.6	151 ± 7.55	72 ± 3.6	89.6 ± 4.48
RB	36 ± 1.8	189 ± 9.45	63 ± 3.15	69.5 ± 3.5
RY	12 ± 0.6	165 ± 8.25	95 ± 4.75	108.7 ± 5.4

at higher concentration, the results of which depicted a better removal (Fig. 4). The reactive yellow dye was removed up to 80 mg L⁻¹ from the initial concentration of 500 mg L⁻¹, showing almost 85% removal of this dye. The concentrations of other dyes like reactive blue and reactive remazol red were reduced up to 145 and 168 mg L⁻¹ in the supernatant after 3 days of incubation showing 75 and 67% removal, respectively, by *A. fumigatus*. The resultant biomass concentration

after the termination of experiment showed that there was no hindrance in the biomass production due to the presence of reactive dyes in the medium at lower concentrations as it showed 5–6 g L⁻¹ DCW. On increasing the concentration of reactive dyes up to 500 mg L⁻¹, the biomass production reduced to 3–4 g L⁻¹. This affects the final uptake capacity of the dye per unit biomass (Table 1). The better performance of fungal strains in bioaccumulation mode as compared to biosorption mode can be explained by the presence of surface groups present on the fungal biomass reported in our earlier studies (Kaushik and Malik 2010; Bhattacharya et al. 2017). In both the fungal strains, there was presence of peaks corresponding to the carboxyl (–COOH) and –OH groups along with amines, amides and N–H stretching between 3250 and 3500 cm⁻¹ and primary, secondary amines and amides with N–H bending in the surface of control fungal biomass (without dye). The peaks were shown to have been reduced and shifted on accumulating the dye with the biomass with the introduction of a distinct peak corresponding to the dye spectrum (acid navy blue) (Kaushik and Malik 2010). Since, the

Fig. 5 The two-stage process for dye removal at a concentration 2000 mg L⁻¹ by **a** *A. lentulus* and **b** *A. fumigatus*, where bioaccumulation was carried out up to 5 days and the residual dye was subjected to biosorption 150 min using 72 h pre-grown fungal biomass



pre-grown biomass was used in the biosorption experiments, the presence of these peaks must have interfered in the uptake of the dye within the biomass. Bioaccumulation mode of the fungus has been studied by several researchers for the removal of synthetic dyes. Wang and Hu (2008) have used immobilized *A. fumigatus* biomass for the removal of reactive brilliant blue KN-R dye. The immobilized spores were inoculated in dye containing media of different dye concentrations ranging from 70 to 734 mg L⁻¹. The bioaccumulation efficiency was 100 and 98% at concentration of 70.3 and 213.1 mg L⁻¹ but upon increasing it to 374.3, 570.3 and 734.1 mg L⁻¹, it dramatically reduced to 57.6, 31.7 and 20.6%, respectively. The biomass also decreased on increasing the dye concentration depicting inhibition of fungal growth due to the presence of dyes.

Two-stage process for dye removal

Since the biosorption was effective only at lower concentrations and bioaccumulation showed good removal of dye at 500 mg L⁻¹, attempts were made to integrate both the approaches in sequential manner for effective dye removal at a concentration as high as 2000 mg L⁻¹ (Fig. 5a, b). The results showed that both the fungal strains were able to accumulate 600–750 mg L⁻¹ of all three dyes after 5 days of incubation showing uptake capacity of 165, 189 and 151 mg g⁻¹ biomass

for *A. lentulus* and 108.7, 69.5 and 89.6 mg g⁻¹ biomass for *A. fumigatus*, respectively (Table 1). There was marked increase in the removal efficiency of residual dyes after bioaccumulation by *A. lentulus* as compared to *A. fumigatus*. The former strain showed best removal within the first 30 min of the biosorption experiment, where the dye concentration was reduced up to 508, 765 and 967 mg L⁻¹ for reactive remazol red, reactive blue and reactive yellow, respectively. After 30 min, the fungal pellets showed saturation for the sorption of reactive yellow and reactive remazol red, but a decrease in concentration was observed in reactive blue dye even after 30 min up to 881 mg L⁻¹. In case of *A. fumigatus*, the sequential biosorption of residual dye could not remove more than 206, 372 and 371 mg L⁻¹ after 150 min of incubation. Therefore, the overall reduction of dye was 74.6, 62.2 and 51.65% for RRR, RB and RY, respectively, by *A. lentulus* and 48.5, 47.8 and 41.2% for RRR, RB and RY, respectively, by *A. fumigatus*. Interestingly, the results of dye removal at 500 mg L⁻¹ showed a better bioaccumulation efficiency of *A. fumigatus*, as compared to *A. lentulus*. This contradiction can be very well explained by the phase contrast microscopy. The dye accumulation at the centre of *A. fumigatus* pellet and no colouration towards the periphery indicated the saturation of the pellet up to a particular concentration and thereafter even if the pellet was growing, no accumulation was taking place (Fig. 6a). Moreover, in our earlier studies, the 3-[4,5-

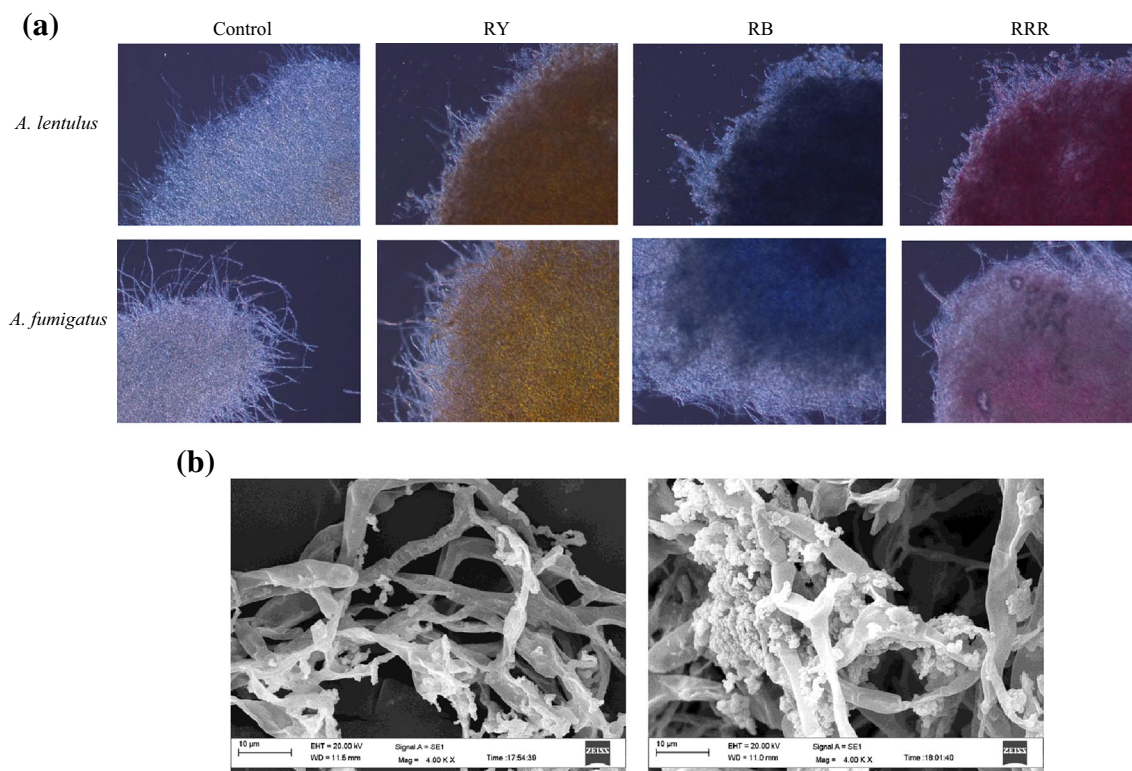


Fig. 6 a Phase contrast micrographs of control as well as dye laden biomass after bioaccumulation studies using *A. fumigatus* and *A. lentulus*. b Scanning electron micrographs of control (left) and dye-treated biomass of *A. lentulus* (right) (2000 mg L⁻¹)

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay results of the *A. fumigatus* have revealed the non-viability/less viability of the fungus beyond 72 h (Bhattacharya et al. 2017). This could be one of the reasons that most of the dye was removed in the first 2 or 3 days of pellet formation and there was no further accumulation of the dye on the fourth and fifth day of the incubation. On the other hand, in spite of a bad bioaccumulation capacity at 500 mg L⁻¹, *A. lentulus* showed increased bioaccumulation capacity on increasing the dye concentration. This can be explained due to the nutrient uptake for growth which indirectly relates to the dye uptake capacity of the fungus. Since, *A. fumigatus* has a higher specific growth rate as compared to *A. lentulus* (data not shown), attaining an early exponential and lag phase during growth. This results into a faster consumption of the available nutrients by *A. fumigatus* than *A. lentulus*, reducing its potential to uptake dye in later stages of growth. On the contrary, our earlier dye removal studies have depicted functionality of *A. lentulus* even after the 13 days of incubation (Kaushik and Malik 2013). The maximum removal was observed in reactive remazol red, where almost 1492 mg L⁻¹ was removed by this hybrid process of bioaccumulation and biosorption. The SEM analysis was also done for *A. lentulus* after the fifth day of growth during bioaccumulation experiment, which depicted slight modifications in the mycelial surface with depositions and precipitations of dye on the surface (Fig. 6b). This clearly indicates the affinity of dyes for the fungus which helps in its removal from the dye solution. The removal of such high dye concentrations have not been reported so far. Moreover, there have been no earlier reports on the use of this hybrid process for the increase in the removal capacity, which has been validated by the results. The introduction of dye peak in the dye laden biomass as indicated from the FTIR spectra of *A. lentulus* in our previous reports also supports the results of the present study showing that the dye is actually being captivated into the layers of the fungal pellets (Kaushik and Malik 2010). This study has also reported modifications and shifting of peaks in the dye laden biomass as compared to the control biomass, depicting this process to be a pure biological phenomenon rather than a physical adsorption of dye to the fungal biomass.

Conclusion

The comparative evaluation of *Aspergillus lentulus* and *Aspergillus fumigatus* depicted a better performance of the former strain in removing reactive dyes from low (25 mg L⁻¹) to higher concentrations (2000 mg L⁻¹). *A. fumigatus* was effective in dye concentration of 500 mg L⁻¹, but showed a limitation beyond it due to its non-viability after 3 days of incubation. The microscopic analysis and the dye uptake capacity for each fungal strain also

support the results. The present study also signifies the importance of *Aspergillus lentulus* to be used for the removal of reactive dyes at a high concentration of 2000 mg L⁻¹. The integrated approach is a newer technique which helps in mediating this removal, which otherwise is not possible with the individual processes. The results indicate its potential for further scale up and commercial usage, which require further investigations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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