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Biosorption of Textile Dye by *Aspergillus lentulus* Pellets: Process Optimization and Cyclic Removal in Aerated Bioreactor

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Abstract The present study was conducted to maximize the biosorption of dye by utilizing the native (untreated) pellets of *Aspergillus lentulus*. The native (55.0 mg/g) and heat-treated (56.7 mg/g) pellets showed excellent dye biosorption capacity which declined upon alginate immobilization (27.2 mg/g). Fourier transform infrared and EDX spectra revealed that phosphate and $-CH_3$ groups are important in determining the biosorption capacity of the pretreated fungal biomass. The operating conditions of the aerated fed batch reactor were optimized and 90 % removal of Acid Blue 120 in 12 h was achieved after five biosorption–desorption cycles. At the end of the fifth cycle, 508.57 mg/L dye could be removed in 60 h with the removal rate of 8.48 mg/L/h. Further, the potential utilization of fungal biomass for the treatment of complex effluent was validated by studying the dye removal from unprocessed textile effluent wherein 58.0 % dye was removed within 4 h of contact.

Keywords Acid Blue 120 · Biosorption · Pretreatment · Bioreactor · Effluent

1 Introduction

Biosorption has been widely investigated for the removal of dyes, metals and other toxic contaminants present in water (Volesky 2007; Gupta and Suhas 2009). Nevertheless, majority of the reports employing biosorbent essentially employ energy expensive grinding and dehydration steps to obtain powdered biosorbent (Fu and Viraraghavan 2002) which enhance the cost of biosorbent production. Utilization of the biomass in its native state will result in more economical operational cost. Fungal biomass depicts pelletization during growth which offer suitable surface for biosorption without any further preparation step. Several studies reported for the efficient removal of varieties of dyes (Kaushik and Malik 2013; Hadibarata et al. 2012; Kaushik and Malik 2010a) and metals (Mishra and Malik 2013; Sharma et al. 2011) have been reported using fungal pellets in batch cultures.

Several issues are encountered while using the biosorbent in column reactors like clogging (Couto 2009), channelling and complexity in column packing. Utilizing immobilized or entrapped (alginate immobilized) biomass and aggregated fungal pellets in bioreactors can eliminate these difficulties (Couto 2009). However, alginate immobilization is a complicated and time consuming process. Therefore, it shall be worthwhile to compare the performance of alginate immobilized

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versus native fungal pellets improved surface characteristics for dye removal. Some reports indicate that the biosorption capacity can be enhanced by suitable pretreatment of the biomass which results in modification of the surface group (Zeroual et al. 2006; Bayramoglu and Arica 2007; Binupriya et al. 2008; Lata et al. 2008).

For the field applicability of the process, it is important to perform biosorption studies utilizing real textile effluent. The results obtained with dye solutions cannot be extrapolated to complex textile effluents as these contain mixtures of dyes and some other interfering chemicals (Fu and Viraraghavan 2001). Another important prerequisite for industrial application include the regeneration of the biosorbent for multiple cycles with stable performance over wide range of environmental parameters such as temperature, pH, dye concentration, etc.

There are limited data available on the utilization of native biosorbent and its performance in bioreactor as well as in actual wastewater for field application. Thus, in the present work, the performance of native pellets was compared with alginate immobilized and heat-treated pellets in terms of dye removal efficiency and biomass characteristics using Fourier transform infrared (FTIR), SEM and EDX. Process parameter optimization was carried out and same was applied to conduct biosorption of Acid Blue 120 (AB 120) in aerated fed-batch bioreactor.

2 Materials and Methods

2.1 Dyes and Chemicals

The dye used in the present study, AB 120, was provided by Department of Textile Technology, IIT Delhi (India). AB 120 is an anionic azo dye and is extensively used in dyeing of silk, wool and nylon fibres. Absorption maxima of the dye was obtained by scanning the dye solution over the visible range (400–750 nm) for the determination of dye concentration. The stock solution of 10 g/L of AB 120 was prepared in distilled water. Standard curve was plotted by measuring absorbance at absorption maxima of the dye (561 nm).

2.2 The Test Organism

An alkali-, thermo- and halo-tolerant strain of *Aspergillus lentulus* (Kaushik and Malik 2010a) with reported good dye removal efficiency was used in the present study. The fungal isolate was maintained on slants of potato dextrose agar stored at 4 °C. Freshly revived cultures were used in all the experiments. To cultivate the fungal biomass for biosorption studies, sterile composite media (100 mL in each flask) was inoculated with 5 % spore suspension and incubated in a shaker at 180 rpm and 30 °C for 24 h. The resulting biomass was filtered through Whatman filter paper, rinsed with distilled water and termed as native biomass.

2.3 Effect of Biomass Pretreatment

Batch experiments were conducted using three types of fungal biomass (native, heat-treated and calcium (Ca)-alginate immobilized) to remove AB 120 (100 mg/L). Heat treatment modifies the surface properties of the fungal biomass without the addition of strong alkali or acid. Ca-alginate immobilization was adopted to overcome the limitations of channelling, clogging and cracking as encountered with native biomass while working in reactor systems. In the first mode, native biomass was used for removing dye from the solution. In the second mode, heat-treated fungal biomass, produced by autoclaving native biomass for 20 min at 121 °C and 15 psi was utilized for dye removal. In the third mode of application, Ca-alginate immobilized biomass was used for dye removal. For this, 4 % Na-alginate solution and 2 % $CaCl_2$ solution were prepared separately in distilled water. Native fungal biomass was added to Na-alginate solution in the ratio of 1:1 and was stirred thoroughly. The mixture was kept in a burette and was fed drop-wise in the $CaCl_2$ solution kept beneath the burette. The immobilized biomass beads thus formed were kept in a refrigerator at 4 °C overnight and then rinsed with water. Initial dye concentration of 100 mg/L was prepared in conical flasks and loaded with respective biomass: native, heat-treated and immobilized, separately (2 g/L on dry cell weight basis). The flasks were then kept in a shaker at 30 °C and 180 rpm. An aliquot of sample was removed from the flasks at a regular interval and analysed by spectrophotometer for absorbance as described earlier. The dye removal data was fitted into Langmuir and Freundlich adsorption isotherms.

2.4 Effect of Process Parameters on Biosorption of AB 120

Effect of biomass loading on dye biosorption by *A. lentulus* was estimated at different biomass loads

(0.75, 1, 2, and 3 g/L dry weight). Series of flasks containing 100 mL AB 120 solution (initial concentration 200 mg/L, pH 6.5) were prepared. The flasks were then loaded with respective biomass dosage and kept in a shaker at 30 °C and 180 rpm. Effect of initial dye concentration on dye biosorption by *A. lentulus* was studied in the range 100–900 mg/L at biomass loading of 2 g/L (dry weight), pH 6.5 and temperature 30 °C. Effect of pH was estimated for both acidic as well as alkaline ranges (pH 4–10) at an initial dye concentration of 200 mg/L, biomass loading 2 g/L and temperature 30 °C. Effect of temperature was studied between 30 and 45 °C at an initial biomass load of 2 g/L, initial dye concentration 200 mg/L, and pH 6.5.

All the flasks were incubated in a shaker at 180 rpm throughout the experiment and an aliquot of sample from each of the flask was withdrawn at regular intervals and centrifuged at 5,000 rpm for 5 min to separate any suspended biomass. The optical density of the supernatant was measured at 561 nm (absorption maxima of AB 120) using a spectrophotometer (Systronics Visiscan 167). A calibration plot between concentration and absorbance of AB 120 was used for determination of dye concentration. Dye removal (%) was calculated using the equation:

$$\text{Dye removal}\,(\%) = \left(\frac{A_0 - A_t}{A_0}\right) \times 100 \quad (1)$$

where A_0 is the initial absorbance, A_t is the absorbance at incubation time, t.

Biosorption capacity of the fungal biomass was measured for AB 120 using the equation:

$$q = \left(\frac{C_0 - C}{m}\right) \quad (2)$$

where C_0 is the initial concentration of dye in solution, C is the final concentration of dye in the solution and m is the biomass of the fungi present in 1 L of the solution.

2.5 Dye Desorption Studies

Desorption of AB 120 from dye laden pellets was carried out using 0.1 M NaOH while the cell disruption was done with the help of 0.5 M EDTA (Kippert 1995; Liu et al. 1999). The dye laden fungal biomass (8 g wet weight) was suspended in 100 mL 0.1 M NaOH solution for 30 min. After initial surface desorption, the sample containing released dye was collected and its absorbance was measured at 561 nm after centrifuging the sample at 8,000 rpm for 10 min. Later, the fungal biomass was collected by filtering the contents of the flask through Whatman No. 1 filter paper and the biomass was washed with distilled water. The fungal biomass was suspended again in 100 mL 0.5 M EDTA for cell disruption for 30 min. The sample containing released dye was collected and its absorbance was measured at 561 nm. Again, the filtered biomass was subjected to surface desorption by 0.1 M NaOH and the steps for measuring the dye absorbance were repeated.

2.6 Aerated Bioreactor

Due to their proven efficiency over other designs (Binupriya et al. 2008; Zhang et al. 1999), a pneumatic type aerated bioreactor of 4 L capacity (Fig. 1) was employed for conducting biosorption studies in continuous mode. Diffused air was provided in the cylinder with the help of an air pump and diffuser located at the bottom of the cylinder. Dye solution containing AB 120 at an initial concentration of 100 mg/L (total volume 3.5 L) was introduced into the reactor. Fungal biomass pre-grown in composite media at 30 °C for 48 h was thoroughly washed with deionized water and added into the reactor at 20 g/L wet biomass dose (equivalent to 2 g/L dry weight). Samples were withdrawn intermittently during a period of 18 h. After this, all the dye laden biomass from the reactor was collected and subjected to desorption at 30 °C using 100 mL 0.1 M NaOH for 30 min at 150 rpm. The desorbed biomass was further utilized for next biosorption cycle. Approximately 10 % biomass loss was observed after each successive cycle which was made up by introducing fresh biomass along with desorbed biomass. The reactor was run for five consecutive cycles.

2.7 Dye Biosorption from Textile Effluent

The textile effluent (Kaushik and Malik 2010b) obtained from textile unit located in Sanganer (Rajasthan, India) and characterized earlier was utilized for conducting biosorption studies. No adjustments in the initial pH were made prior to the effluent treatment with *A. lentulus*. Dye removal was studied by contacting 100 mL of the neat effluent with 2 g/L (dry weight) fungal biomass and incubating it in a shaker at 180 rpm and 30 °C. Intermittently, samples were withdrawn and analysed spectrophotometrically till 4 h of contact time.

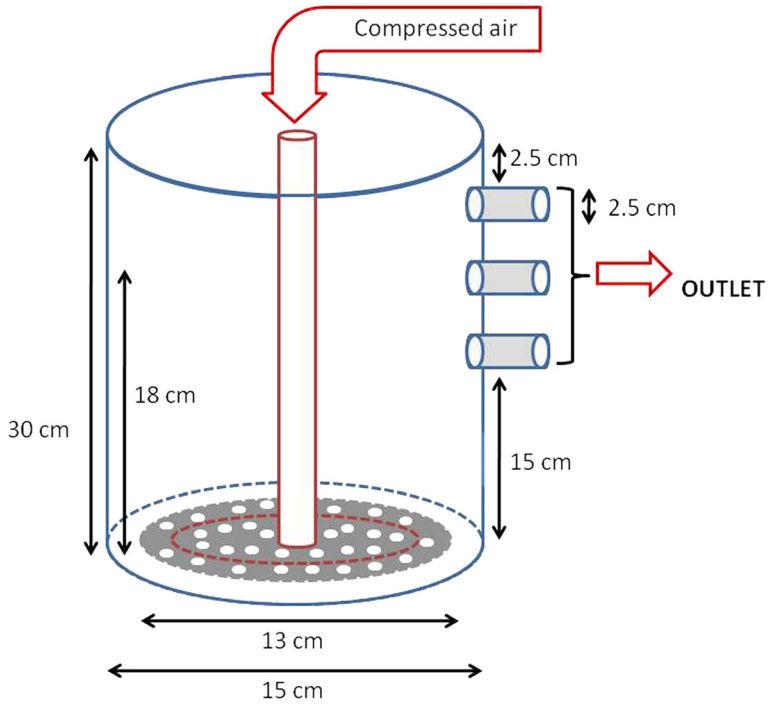


Fig. 1 Schematic representation of aerated bioreactor used for the biosorption of Acid Blue 120 by *A. lentulus*

2.8 SEM, EDX, and FTIR Analysis

The samples for SEM were washed with 0.15 M saline phosphate buffer (pH 6.8) and subsequently fixed in 2.5 % glutaraldehyde. Fixation was done for 12–18 h at 4 °C. After this, cells were washed in fresh phosphate buffer. After several washes in buffer, the specimens were dehydrated in ethanol series following 30 %, 50 %, 70 %, 90 %, and absolute alcohol. These fixed and dehydrated cells were then dried using critical point dryer for 20 min. Dried samples were mounted on aluminum stubs. The samples were gold-coated by cathodic spraying (Polaron gold) and observed under a scanning electron microscope (ZEISS EVO 50). The SEM observation was done under the following analytical condition: EHT=20.0 kV, Signal A=SE1. For EDX, samples were carbon-coated and analysis was performed at 20 kV.

FTIR spectroscopy analysis was also done for native, heat-treated and Ca-alginate immobilized biomass. For this, native and pretreated fungal biomass materials were first air-dried and then mixed with KBr to form pellets. The FTIR spectra were recorded using Spectrum One (Perkin Elmer) spectrometer equipped with DTGS detector. IR absorbance data were obtained for the wave number range of 650–4,000 cm^{-1}. Data point resolution of the spectra was 2 cm^{-1} and four scans were accumulated for each spectrum.

2.9 Statistical Analysis

All the studies were conducted in triplicates and the results are presented as means of the replicates along with standard deviation. One-way ANOVA was used to statistically analyse the results with the limit of significance set at $P<0.05$.

3 Results and Discussion

3.1 Biosorption by Native and Pretreated Biomass

The present study deals with optimization of biosorption on *A. lentulus* biomass. In our previous study (Kaushik and Malik 2013), this fungal isolate was utilized for the removal of various classes of textile dyes in bioaccumulation as well biosorption mode. Acid Blue 120 was found to be non-toxic and its removal followed second-order rate kinetic model. Hence, in the present study, attempts were made to maximize AB120 biosorption through process optimization and biomass pretreatment.

Three types of fungal biomass (native or untreated, heat-treated and Ca-immobilized biomass) were utilized at an initial biomass loading of 2 g/L (dry weight). Visual observation showed distinct nature of the native and heat-treated *A. lentulus* biomass. The native fungal biomass was in form of small pellets while the heat-treated biomass turned into flakes.

In the first application mode where native biomass was used, 91 % dye removal was achieved in 1 h (Fig. 2a). A large fraction (81 %) of dye was removed within the first 10 min of contact time with the dye solution. Biosorption capacity of the native fungal biomass for AB 120 (100 mg/L) at equilibrium was found to be 55.0 mg/g. Kinetic data suggest that the biosorption of AB 120 on *A. lentulus* biomass follows a first-order kinetic model (Kaushik and Malik 2013). Hence, the *A. lentulus* biomass seems to be an efficient biosorbent for AB120 dye. However, the biosorption capacity varies depending upon the nature of the strain and operating conditions such as dye concentration and biomass loading. For instance, Fu and Viraraghavan (2002) reported biosorption capacity of 12.1 mg/g with native biomass of *Aspergillus niger* (2.66 g/L) for the dye Congo Red (50 mg/L). Similarly, Maurya et al. (2006) reported that biosorption capacity of *Phellinus igniarius* was 36.8 mg/g and that of *Fomes fomentarius* was 25.1 mg/g for the dye Rhodamine B (100 mg/L).

In the second mode of application, heat-treated biomass was used for dye removal. After 1 h of contact time, 94.4 % dye removal was achieved (Fig. 2a). This is only marginally higher than the native one. The dye removal within initial 10 min of contact time was 91 % which is considerably higher when compared with the native biomass. The increased efficiency of heat-treated biomass could be attributed to the increase in the number of binding sites due to denaturation of proteins present on the cell wall structure. It is reported that after heat treatment, hydrophobic entities on the fungal cell surface are removed (Bayramoglu and Arica 2007). Similar results were obtained by Arica and Bayramoglu (2007) where heat-treated biomass had better dye removing efficiency (182.9 mg/g, biosorption capacity) as compared to the native biomass (117.8 mg/g) of *Lentinus sajor-caju* in removing Reactive Red-120 (800 mg/L, pH 3).

In the third mode of application, where Ca-alginate immobilized biomass was utilized for dye removal (Fig. 2a), only 49.6 % dye removal was observed after 5 h of contact time (36.5 % in 2 h). Ca-alginate beads (without fungal biomass) showed negligible amount of dye removal. It signifies that the fungal biomass provides the major sites for binding of the dye which gets masked when immobilized in Ca-alginate beads. Overall, the dye removal efficiency after 5 h of contact time showed the following trend: heat-treated (98.5 %) > native (98.3 %) > immobilized biomass (49.9 %). Similar trend was observed for the biosorption capacity of the differently treated fungal biomass at equilibrium: heat-treated (56.7 mg/g) > native (55.0 mg/g) > immobilized (27.2 mg/g). Further to explain the differences obtained in the dye removal efficiency of variously pretreated fungal biomass, FTIR and EDX spectra were obtained.

3.2 Characterization of Native and Pretreated Biomass by FTIR, SEM and EDX

Figure 2b represents the FTIR spectra obtained for three different types of fungal biomass: native, heat-treated and immobilized. The peaks observed on native biomass includes 3,279 cm^{-1} (bonded hydroxyl groups, OH^-; –COOH; primary and secondary amines and amides stretching, N–H stretching), 2,926.66 cm^{-1} (CH stretching), 1,634.75 cm^{-1} (primary and secondary amines and amides stretching, N–H bending), 1,405.41 cm^{-1} (C–H bending, CH_3), 1,020–1,076.20 cm^{-1} (amines, C–N stretching; C–O stretching of COOH), 1,076 cm^{-1} (orthophosphate, PO_4^{3-}), and 1,020 cm^{-1} (P–OH), 933.33 cm^{-1} (C–H bending). Band region at 870 cm^{-1} suggests the presence of sulphonate group in the native fungal biomass.

The previous study on dye removal by *A. lentulus* (Kaushik and Malik 2010a) suggests the probable role of certain groups present on fungal biomass such as bonded –COOH and –OH groups; primary and secondary amine and amides, N–H stretching; N–H bending; phosphate bonds, C–N stretching; and C–H bending in the binding of dye molecules to the fungal biomass. However, in the present study, it was observed that heat treatment (autoclaving) resulted in the reduction of peaks at 1,400 cm^{-1} (CH_3) and 1,640–1,550 cm^{-1} (amines). Heat treatment has been reported to increase the hydrophilicity of the fungal biomass by removing hydrophobic entities on the fungal cell surfaces (Bayramoglu and Arica 2007). Reduction in hydrophobicity can be linked to reduction in CH_3 groups which are said to be responsible for hydrophobic nature of the biomass. It seems that similar changes might have occurred during the heat treatment of *A. lentulus* biomass.

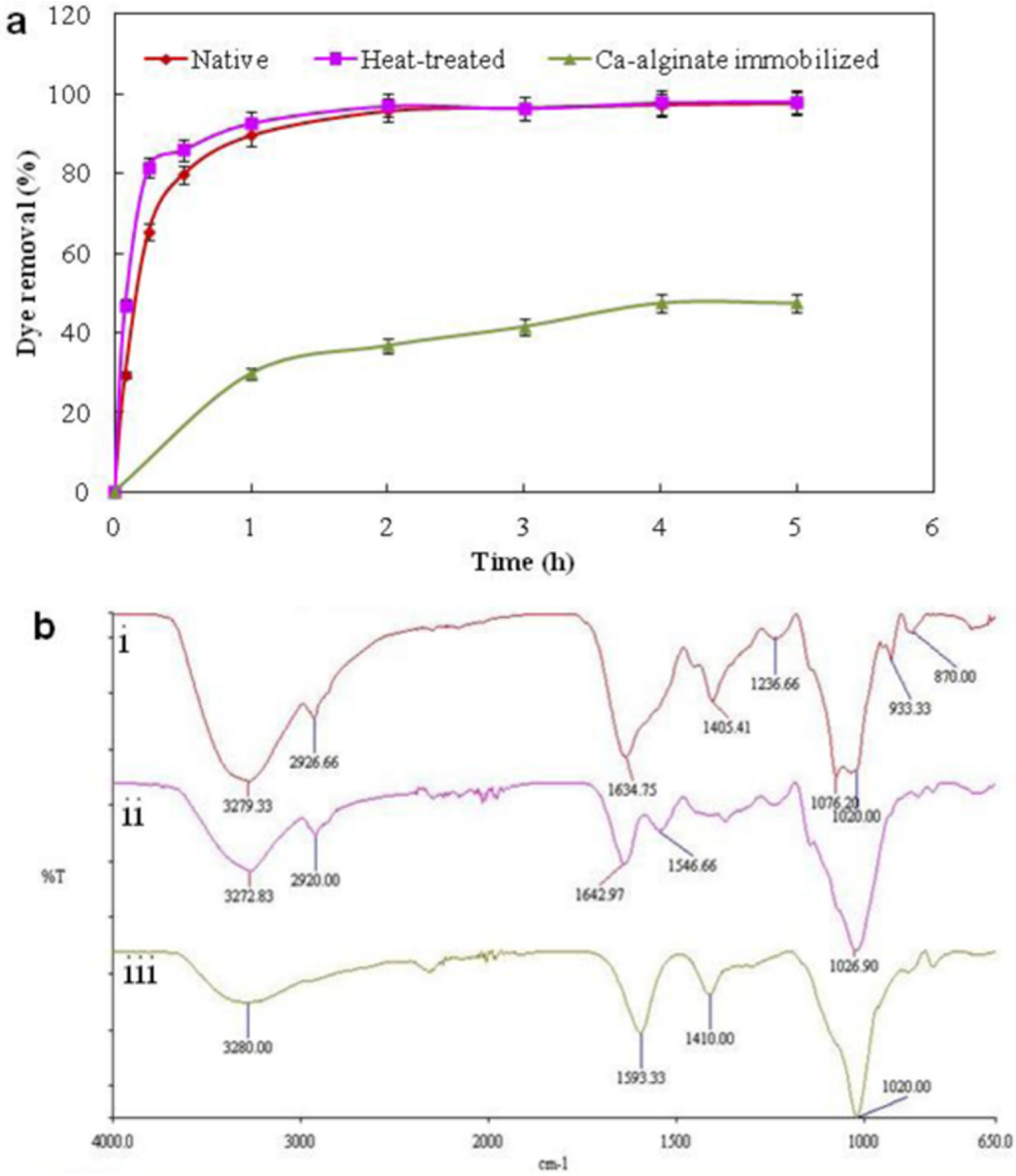


Fig. 2 **a** Effect of different treatments on the removal of Acid Blue 120 by *A. lentulus* with time (initial dye concentration, 100 mg/L; temperature, 30 °C and pH, 6.5); **b** FTIR spectra showing different peaks on (*i*) native biomass (*ii*) heat-treated biomass and (*iii*) Ca-alginate immobilized biomass

Autoclaving also causes the disruption of the fungal structure which leads to increased porosity (Aksu 2005). Therefore, more dye molecules can enter the expanded pores in autoclaved biomass. Hence, better biosorption in case of heat-treated biomass may be related to both physical changes and changes in the functional groups. As compared to the native biomass, Ca-alginate immobilized biomass showed reduction in the intensity of the peaks responsible for dye binding: (1,596 cm^{-1}, primary and secondary amines; 3,280 cm^{-1}, –COOH, OH, amines; 2,913 cm^{-1}, CH stretching; 1,540 cm^{-1}, primary and secondary amines; and 1,076 cm^{-1}, PO_4^{3-}.

The results were further validated by the SEM-EDX data obtained for variously pretreated fungal biomass (Fig. 3). SEM micrograph of native fungal biomass (Fig. 3a) shows the presence of compact structure of the mycelial mass while thread-like scattered mycelia

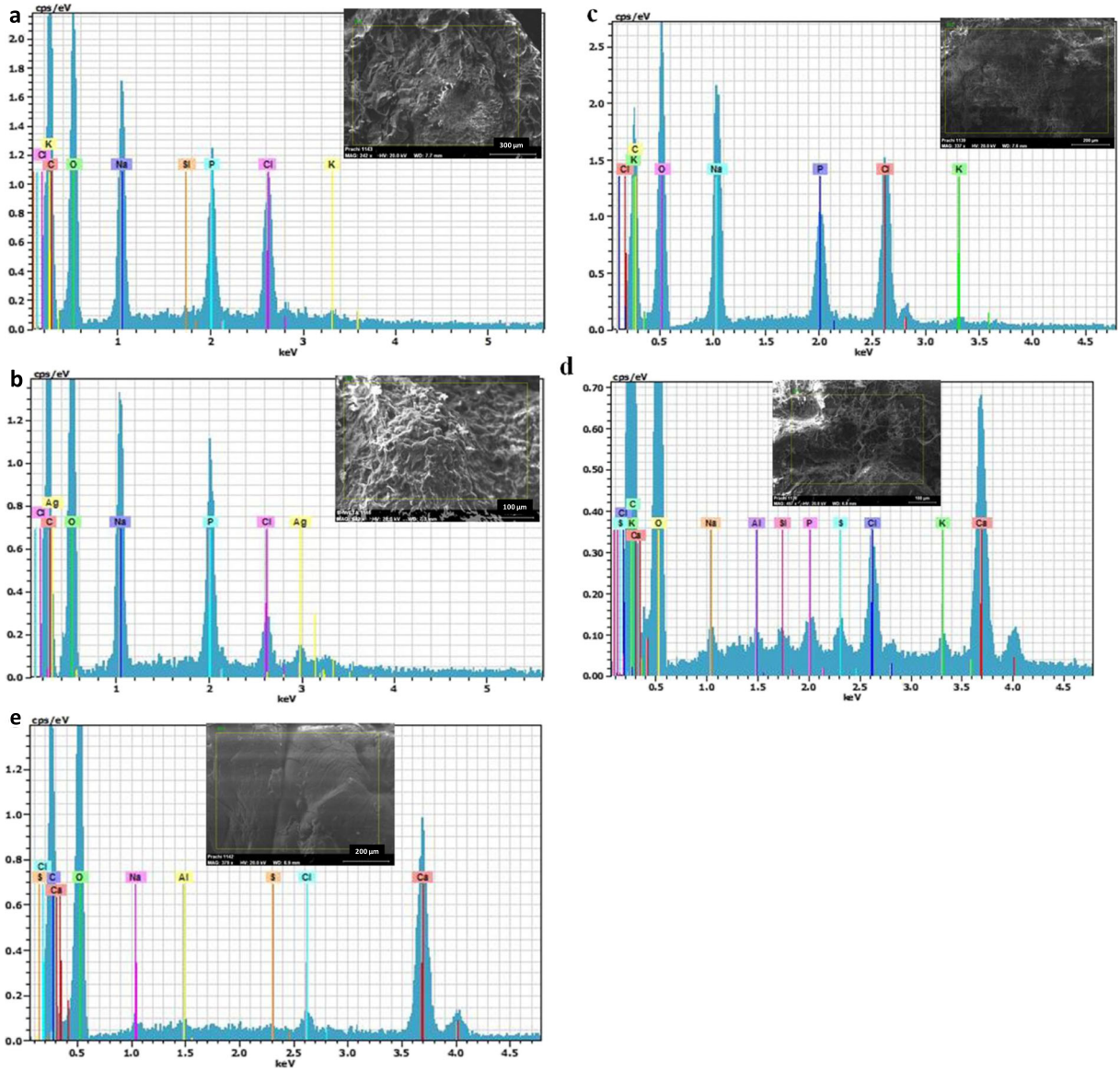


Fig. 3 SEM micrographs and EDX profiles showing the effect of different pretreatments on the biomass of *A. lentulus* **a** native fungal biomass; **b** dye-laden native fungal biomass; **c** heat-treated fungal biomass; **d** Ca-alginate immobilized fungal biomass beads and **e** dye-laden Ca-alginate immobilized fungal biomass beads

were seen in heat-treated fungal biomass (Fig. 3c). In contrast to the above, the biomass beads (Fig. 3d) displayed fungal mycelia entangled in the alginate matrix. This explains the similar performance and better dye removal by native and heat-treated biomass as compared to immobilized biomass.

As compared to native biomass (Fig. 3a), the peaks in dye-laden native biomass (Fig. 3b) corresponding to phosphorus (2 keV), sodium (1 keV), chlorine (2.6 keV), and potassium (0.25 keV) were reduced. This further confirms the role of PO_4^{3-} groups in the binding of dye molecules. The EDX data for heat-treated biomass (Fig. 3c) shows the reduction in carbon (0.3 keV) peak validating the finding of FTIR data regarding the reduction in $-CH_3$ peak. The EDX data for heat-treated biomass also shows the increase in sodium (1 keV), and chlorine (0.1 and 2.6 keV), whereas no marked difference was observed for oxygen (0.5 keV) and phosphorus (2 keV) peaks, when compared to native biomass (Fig. 3a).

In the EDX data of immobilized biomass (Fig. 3d), markedly visible Ca peaks appeared at 0.4 and 3.7 keV along with some minor peaks corresponding to aluminium (1.5 keV) and sulphur (2.3 keV), whereas no such peaks were present in the native biomass (Fig. 3a). In addition to this, the intensity of the phosphorus peak observed at 2 keV reduced in immobilized biomass thus resulting in lower dye biosorption. Dye-laden Ca-immobilized beads (Fig. 3e) show the disappearance of potassium (0.25 keV and 3.3 keV) and phosphorus (2 keV) peaks as compared to control Ca-alginate immobilized biomass (Fig. 3d). Also, an increase in the Ca peak is seen at 3.7 keV. Thus, FTIR and EDX observations point out the unfavourable changes in the biomass subsequent to immobilization.

3.3 Adsorption Isotherms

The Langmuir and Freundlich isotherm models (Freundlich 1906; Langmuir 1918) which are widely used to analyse data for water wastewater treatment applications were applied to describe the biosorption equilibrium obtained with the different biomass treatments. The Langmuir model can be expressed by the equation:

$$q = \frac{q_m b C_{eq}}{1 + b C_{eq}} \tag{3}$$

where q (mg/g) is the amount of adsorbed dye on the biosorbent surface, C (mg/L) is the concentration of the dye in solution, constant b (l/mg) is related to the energy of adsorption, and constant q_m (mg/g) represents the adsorption capacity at the complete saturation of biosorbent binding sites.

The Freundlich equation is described as:

$$q_{eq} = K_F \left(C_{eq}\right)^{1/n} \tag{4}$$

where K_F and n are the Freundlich adsorption isotherm constants characteristic of the system. K_F and n are indicative of the extent of the adsorption capacity and adsorption intensity, respectively. The value of n and K_F is given by slope and intercept, respectively, of the plot between log q and log C.

The dye biosorption data of AB 120 dye at various initial dye concentrations (50–300 mg/L) on differently pretreated biomass was fitted with Freundlich and Langmuir plots (Sup. Fig. 1). The data shows good correlation coefficients (r^2) with Freundlich isotherm (native, 0.98; heat-treated, 0.97; ca-alginate immobilized, 0.98). Thus suggesting that biosorption of AB 120 on different biomass of *A. lentulus* follow Freundlich model showing heterogenous nature of adsorption. Also, value of n (adsorption intensity) for heat-treated (2.83), native (2.78) and Ca-alginate immobilized (2.27) is >1, this indicates positive cooperativity in binding of dye molecules to the biomass. Highest value of K_F (measure of adsorption capacity) was obtained for heat-treated biomass (50.97) followed by native (45.98) and Ca-alginate immobilized (3.98) biomass (Table 1). Lower r^2 values were obtained when the dye biosorption data was fitted with Langmuir isotherm (native, 0.95; heat-treated, 0.95; Ca-alginate immobilized, 0.97) suggesting that the data cannot be well represnted by this model (Table 1). Langmuir constants were obtained by plotting the curve between $1/q_e$ versus $1/C_e$. The slope and intercept give the value of $1/bQ_{max}$ and $1/Q_{max}$, respectively.

3.4 Biosorption of AB 120 and Effect of Process Parameters

Table 2 shows the biosorption of dye on fungal biomass at different biomass loadings. As the biomass dose was increased from 0.75 to 3 g/L (dry weight basis), the removal (%) of the dye increased. The kinetics of fungal biosorption (data not shown) showed almost complete dye removal from the solution at 3 g/L biomass load within 4 h at an initial dye concentration of 100 mg/L, pH 6.5 and 30 °C. Maximum biosorption took place in

Table 1 Freundlich and Langmuir isotherm constants obtained for biosorption of Acid Blue 120 on *A. lentulus*

Pretreatment	Freundlich constants			Langmuir constants		
	K_F	Q_{max}	r^2	b (l/mg)	Q_{max} (mg/g)	r^2
Native	45.98	57.90	0.98	1.59	101.01	0.95
Heat-treated	50.97	60.83	0.97	1.81	104.17	0.95
Ca-alginate immobilized	3.98	22.73	0.98	0.02	45.66	0.97

Table 2 Effect of various process parameters on biosorption of AB 120 by *A. lentulus* after 4 h of contact time

Experimental conditions	Parameter	Dye removal (%)	Biosorption capacity (mg/g)
Initial dye concentration, 100 mg/L; pH, 6.5; temperature, 30 °C; agitation, 180 rpm	Biomass loading (g/L)		
	0.75	38.66a	53.33a
	1	57.65b	58.22b
	2	96.50c	54.60a
	3	98.28c	33.89c
Biomass loading, 2 g/L; pH, 6.5; temperature, 30 °C; agitation, 180 rpm	Initial dye concentration (mg/L)		
	100	96.33a	54.55a
	200	90.15b	95.59b
	400	84.53c	165.49c
	600	62.54d	177.28d
	700	56.44e	205.28e
	900	46.85f	210.74f
Initial dye concentration, 200 mg/L; biomass loading, 2 g/L; temperature, 30 °C; agitation, 180 rpm	Initial pH (pH at equilibrium)		
	4 (6.5)	94.45a	99.41a
	5 (6.8)	91.68b	96.58b
	6 (7.1)	90.09b,c	95.89b
	7 (7.4)	88.84c	95.35b
	8 (7.0)	83.46d	88.42c
	9 (8.5)	83.10d	89.37c
	10 (8.1)	82.00d	87.97c
Initial dye concentration, 200 mg/L; biomass loading, 2 g/L; pH, 6.5; agitation, 180 rpm	Process temperature (°C)		
	30	89.98a	94.70a
	35	95.17b	98.93b
	40	95.64b	99.43b
	45	95.45b	99.23b

Numbers followed by same letter (a–f) within a column for different parameters are not significantly different ($P<0.5$)

the initial 30 min. However, attainment of equilibrium was delayed at lower biomass dosage. Since efficient dye removal (96.5 %) as well as high biosorption capacity (54.6 mg/g) was found at 2 g/L biomass load, the same was considered to be the optimal.

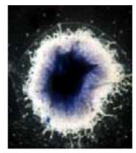
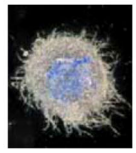
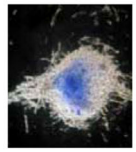
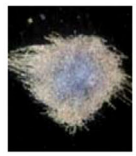
Effect of initial dye concentration on dye removing efficiency of *A. lentulus* was studied in the range 100–900 mg/L for an initial biomass loading of 2 g/L, pH 6.5 and 30 °C. Data obtained at 4 h of contact time has been tabulated in Table 2. It was observed that as the initial dye concentration was increased from 100 to 900 mg/L, biosorption capacity increased from 54.6 to 210.7 mg/g which could be attributed to the saturation of binding sites in fungal biomass at higher dye concentration. However, in spite of the considerable increase in biosorption capacity, decrease in percentage dye removal was observed, which could be attributed to the saturation of the available sites for dye biosorption on fungal biomass. As a result, beyond 200 mg/L initial dye concentration, the process of biosorption becomes ineffective.

The initial pH of a dye solution influences the chemistry of both dye molecules and fungal biomass in an aqueous solution. Hence, effect of initial pH was tested for various pH values ranging from pH 4 to 10 (initial dye concentration 200 mg/L, temperature 30 °C). With the increase in pH, decrease in dye removal was observed (Table 2). At 4 h of contact time, maximum dye removal was observed at pH 4 (94.4 %) which declined gradually to 82.0 % at pH 10. The higher dye removal at acidic pH may be explained by the electrostatic interactions between the fungal biomass and the dye molecules. In the aqueous solution, the AB 120 dye is first dissolved and the sulfonate groups of the acid dye ($D\text{-}SO_3Na$) are dissociated and converted to anionic dye ions ($D\text{-}SO_3^-$). The weak base groups such as amines (pK_a 5–6) on the fungal biomass get protonated at acidic pH ranges and attain a net positive charge (O'Mahony et al. 2002). Hence, the attraction between the negatively charged dye anions and positively charged biomass increases. On the other hand, deprotonation of the fungal biomass at high pH results in net negative charge on the biomass. This increases the repulsion between the anions of AB 120 dye and fungal biomass thus lowering the dye removal. During biosorption, only the initial pH was maintained at the respective value. At the end of biosorption, equilibrium pH was also measured. The equilibrium pH was higher (Table 2) than the initial pH (in acidic ranges) indicating the possibility of either release of OH^- ions or uptake of H^+ ions from the solution.

Various dye bearing effluents are produced at a relatively high temperature which can be an important factor for the application of fungal biomass in treating coloured effluents at industrial scale. In the present study, dye removal after 4 h increased from 90 % at 30 °C to 95 % at 35 °C but further increase in temperature to 45 °C did not lead to any significant change in dye removal (Table 2). Increased biosorption could be attributed to the increased surface energy and kinetic energy of the dye molecules. Similar observation was

made by Iqbal and Saeed (2007) where they observed an increase in biosorption of Remazol Brilliant Blue R by *Phanerochaete chrysosporium* with increase in temperature up to 30 °C.

Table 3 Dye desorption obtained from dye laden fungal biomass

Parameter	Value	Remaining dye load on biomass
Initial dye concentration (mg/L)	112 mg/L	Fungal biomass prior to dye biosorption 
Dye removed (mg/L)	110.1 mg/L	Fungal pellet after dye biosorption  13.76 mg/g (on wet weight basis)
Dye removal (%)	98.3	
Surface desorption (mg/L)	50.08 mg/L	Fungal pellet after surface desorption  7.50 mg/g (on wet weight basis)
Surface desorption (%)	45.49	
Desorption after cell disruption (mg/L)	45.28 mg/L	Fungal pellet after cell disruption 
Desorption after cell disruption (%)	41.1	
Total desorption (mg/L)	95.36 mg/L	Fungal pellet after cell disruption and further desorption  1.84 mg/g (on wet weight basis)
Total desorption (%)	86.6	

3.5 Desorption Studies

In order to understand the detailed mechanism of dye binding, desorption studies were carried out. When 0.1 M NaOH was used as an eluant to desorb AB 120 dye from dye-laden fungal biomass, 45.5 % desorption was obtained. Desorption with NaOH may be explained on the basis of electrostatic repulsion between the negatively charged sites on the fungal biomass and the anionic dye molecules. The electrostatic repulsion between fungal biomass and anionic dye increases at alkaline pH resulting in desorption. Sumathi and Manju (2000) obtained 85 % desorption of Drimarene Blue dye from the biomass of *A. foetidus* when 0.1 M NaOH was used as the eluant.

However, as compared to the above report (Sumathi and Manju 2000), desorption obtained for AB 120 from the biomass of *A. lentulus* is poor, indicating that the dye is firmly bound to the fungal biomass. Initially, when dye-laden fungal biomass (obtained after biosorption) was subjected to surface desorption with 0.1 N NaOH, only 45.5 % (6.3 mg/g) dye could be desorbed. This figure is only a fraction of the total dye load present on the fungal biomass (13.8 mg/g). Therefore, cell disruption using 0.5 M EDTA was attempted and further desorption by 0.1 N NaOH was carried out to estimate the internalized dye. This resulted in substantial dye desorption (45.3 %). The results indicate that apart from surface binding, intracellular compartmentalization of dye also occurs during biosorption (Table 3). Phase contrast micrographs of the fungal pellet (Table 3) during each desorption stage depict how the dye got desorbed with the eluant used in the study. Increased cell permeability due to EDTA and release of internalized dye is also prominently visible. Dye biosorption along with internalization has also been described for the cells of *Myrothecium verrucaria* for Orange II dye (Brahimi-Horn et al. 1992).

3.6 Aerated Bioreactor

The results obtained during cyclic removal of AB 120 dye by *A. lentulus* biomass in the aerated fed-batch bioreactor are shown in Fig. 4. The results obtained have been presented in Fig. 4. In the first cycle, 82.4 % dye removal was attained in the initial 4 h of contact time which increased to 96.7 % after 12 h. Although a gradual decline in dye biosorption was observed with each consecutive cycle of biosorption–desorption, still 79.9 % dye was removed during the fifth cycle by the fungal biomass in 4 h. Further, comparing the 12 h data, only slight decrease was observed during the fifth cycle (94.05 %). The results indicate that efficient dye removal could have been obtained for successive cycles of biosorption if it was tested beyond the fifth cycle.

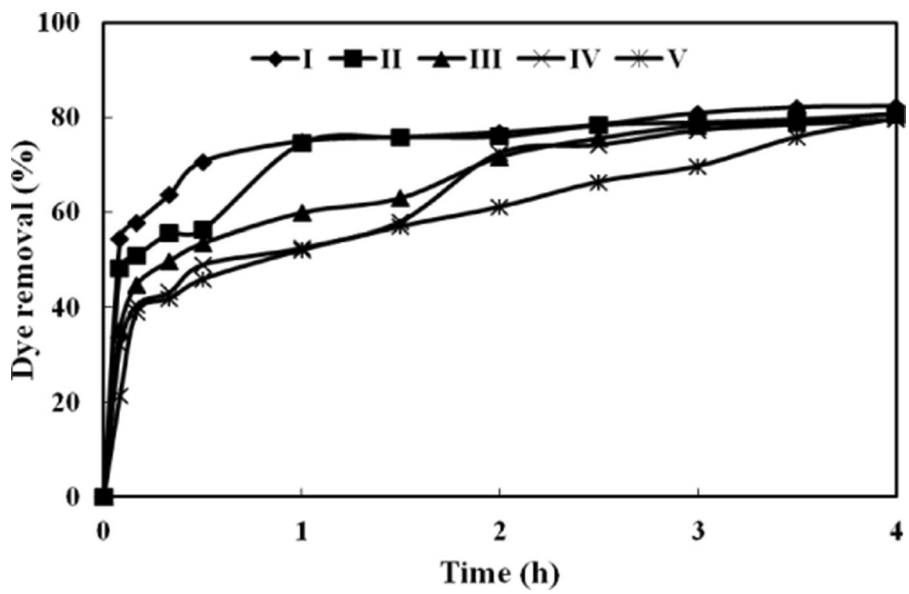


Fig. 4 Cyclic biosorption (I-V cycles) of AB120 dye by *A. lentulus* pellets in aerated fed batch reactor

Amount of dye desorbed at each consecutive cycle also increased from the first cycle to the fifth cycle. Exhaustion of the dye binding sites during initial biosorption cycle may have reduced the affinity of dye molecule towards available sites on fungal biomass resulting in enhanced desorption in successive desorption cycles. At the end of each cycle, the total biosorption capacity of fungal biomass was found to be 52.5 (first cycle), 104.0 (second cycle), 153.6 (third cycle), 203.4 (fourth cycle) and 254.3 mg/g (fifth cycle). At the end of the fifth cycle, 508.6 mg/L dye was removed by the biomass of *A. lentulus* in 60 h with the removal rate of 8.48 mg/L/h. The results are at par with that obtained by El-Naggar et al. (2004) where they tested the removal of crystal violet dye by agar-alginate immobilized beads of *Pseudomonas aeruginosa* in air bubble column reactor for 19 cycles and reported removal of 705 mg/L dye in 171 h with an average rate of 4.15 mg/L/h. Similar study made by Akar et al. (2013) reported the utilization of *Thamnidium elegans* for seven continuous cycles of biosorption–desorption for the removal of Methyl Violet dye in continuous flow mode reactor. The results thus obtained for *A. lentulus* in reactor mode show a high dye removing ability of fungal biomass and prove its utility in continuous cycles of biosorption.

3.7 Dye Biosorption from Industrial Effluents

In order to prove the industrial application of *A. lentulus* in small or medium size enterprises, it is essential to demonstrate the dye removal from actual industrial effluents. Native biomass of the fungal isolate was utilized for the removal of dye from previously characterized textile effluent (Kaushik and Malik 2010b). After 4 h of incubation, 58 % dye was removed, thereby suggesting the utility of *A. lentulus* in treating industrial effluents. It is important to note here that lower dye removal obtained with actual textile effluent as compared to single dye solution could be attributed to the high concentration of dissolved matter present in the effluent (1,021 mg/L). Also, no pH adjustment was done prior to the experiment and biosorption was conducted at pH 8.11 which was the original pH of the effluent (Kaushik and Malik 2010b). Pretreatments such as filtration and acidification of the effluent could further increase the dye removal from the effluent.

4 Conclusions

Native biomass of *A. lentulus* exhibited equally good biosorption capacity for the removal of AB120 dye as shown by heat-treated biomass; however, Ca-alginate immobilization reduced it significantly. Biosorbent characterization conducted through FTIR studies suggest the predominant role of bonded –COOH and –OH groups, primary and secondary amines, and amides and phosphate bonds in the binding of dye molecules to fungal biomass. Further, desorption studies revealed that other than surface binding, compartmentalization of dye molecules within the fungal cell wall is a predominant mechanism for dye removal in the native biomass.

Under optimized conditions (2 g/L biomass loading, 200 mg/L initial dye concentration, pH 4–6 and 35–45 °C temperature), *A. lentulus* was able to remove more than 90 % dye within 4 h of contact time. Biosorption conducted with AB 120 and *A. lentulus* pellets in an aerated fed-batch reactor run under the optimized conditions resulted in more than 90 % dye removal even after five biosorption–desorption cycles. Also, 58.0 % dye removal was obtained with unprocessed real textile effluent within 4 h of contact time thus establishing the potential utilization of *A. lentulus* biomass in treating dye containing effluents.

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