Response surface modeling of bioremediation of acid black 52 dye using *Aspergillus flavus*

Arpita Ghosh, Manisha Ghosh Dastidar and T. R. Sreekrishnan

ABSTRACT

Bioremediation is an efficient process to remove metals and dyes from solutions using different micro-organisms. In the present study, the efficiency of growing *Aspergillus flavus* (isolated from the effluent of an electroplating industry) to treat a synthetic solution of acid black 52 dye (a trivalent chromium complex dye) was investigated. Maximum removal of dye and chromium was observed to be 390 and 17.22 mg/L, respectively, at an initial dye concentration of 750 mg/L and at pH 4.5 in 50 hours in a batch bioreactor. The biomass concentration was reduced from 4.1 to 0.4 g/L with increasing dye concentration from 100 to 2,000 mg/L. The response surface modeling for color removal was performed using the range of initial dye concentration 200–400 mg/L, pH 4–6 and time 35–50 hours. The optimum conditions for maximum color removal (76.52%) were observed at initial dye concentration: 200 mg/L, pH: 4.75 and time: 50 hours. The deviation (−0.02%) showed a close agreement between the experimental and predicted values of color removal. The scanning electron microscopic and energy dispersive X-ray analyses indicated bioremediation of the dye.

Key words | acid black 52 dye, *Aspergillus flavus*, bioremediation, removal of color and chromium, response surface modeling

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INTRODUCTION

A worldwide environmental concern has been invited over the past few decades due to the tremendous increase in industrialization and urbanization. Industries such as textile, mining, steel electroplating, etc. generate aqueous effluents containing relatively high levels of synthetic dyes and heavy metals (Madhavan *et al.* 2009; Suditu *et al.* 2013). Chromium complex dyes are generally used in leather and nylon industries (Aksu & Balibek 2010). Hexavalent chromium has a more toxic effect on human beings than trivalent chromium. Exposure to hexavalent chromium may cause hemorrhage, epigastric pain, dermatitis, bronchitis, severe diarrhea, nausea, and also may cause cancer in the digestive tract and lungs (Pillai *et al.* 2013). Trivalent chromium gets absorbed in the digestive tract and develops harmful compounds with proteins, which affect the health of humans and animals (Carmona *et al.* 2012). Due to the toxicity of both chromium and dyes, the effluents from these industries need to be treated prior to their discharge. The acceptable range for trivalent chromium and hexavalent chromium in potable water is 0.1 mg/L as per the rule of the environmental protection agency (Panayotova *et al.* 2007). Different techniques for treatment of wastewater include ion-exchange, oxidation, precipitation, evaporation, electroplating and membrane filtration (Banerjee & Dastidar 2005). However, application of such techniques is limited because of technical or economic constraints (Meunier *et al.* 2003). Extensive studies have been reported on bioremediation of dyes and metals using various micro-organisms such as bacteria, fungi and algae (Mehta & Gaur 2001). Micro-organisms acquire toxicity resistance via different processes such as transport across the cell membrane, biosorption to cell walls and oxidation–reduction reactions, entrapment in extracellular capsules, precipitation, complexation, etc. (Macaskie & Dean 1989; Avery & Tobin 1993). Fungal strains have been reported to remove and degrade various pollutants efficiently due to the high biomass yield and presence of different oxidoreductive enzymes (Anastasi *et al.* 2010). The optimization of process parameters for bioremediation of pollutants by conventional batch process is time consuming and requires many experimental runs. These problems are minimized by optimizing the process parameters together by statistical

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methods such as Taguchi or response surface methods (Preetha & Viruthagiri 2007; Pundir *et al.* 2016).

However, very little information in literature is available on simultaneous removal of chromium and color from a synthetic solution of chromium complex dye. Kalpana *et al.* (2011) reported color removal of a chromium complex dye (Isolan Dark Blue) using growing *Irpex lacteus*. *Aspergillus tamarii* isolated from the sludge of a textile industry in the laboratory of the present authors was reported to remove trivalent chromium complex dye (acid black 52) (Ghosh *et al.* 2016a). Previously, *Aspergillus flavus* was isolated from an electroplating effluent, which was reported to remove different heavy metals and dyes separately from aqueous solutions (Ranjusha *et al.* 2010; Pundir *et al.* 2016). In the present study, an attempt was made to compare the efficiency of *Aspergillus flavus* with that of *Aspergillus tamarii* for simultaneous removal of color and chromium from the solution of acid black 52 dye in a batch bioreactor. Response surface modeling was performed to optimize the parameters for color removal from an aqueous solution of acid black 52 dye using *Aspergillus flavus*.

EXPERIMENTAL

Dye

Acid black 52 was collected from a local textile industry at Delhi National Capital Region (India). This dye was water soluble and was used without any purification in the present study. The dye was mainly complexed with trivalent chromium. Copper and iron were present in the dye molecules as impurities (Ghosh *et al.* 2016a). The concentrations of chromium and copper were observed to be 4.1 and 0.091 mg/L, respectively, using an atomic absorption spectrophotometer (AAS) in a 100 mg/L acid black 52 dye solution (Ghosh *et al.* 2016a, 2016b).

Isolated strain and growth-media

The fungal strain *Aspergillus flavus*, isolated previously in the laboratory from an effluent of an electroplating industry, was used to remove heavy metals such as copper, zinc, nickel and remazol black b, methylene blue, acid orange 80 in separate studies (Ranjusha *et al.* 2010; Kumar *et al.* 2014; Ghosh *et al.* 2016c; Pundir *et al.* 2016). A growth medium containing glucose: 10.00 g/L; K_2HPO_4: 0.5 g/L; NaCl: 1 g/L; $MgSO_4$: 0.1 g/L; NH_4NO_3: 0.5 g/L and yeast extract: 5.0 g/L was prepared and the required quantity of acid black 52 dye was added to the media, which was autoclaved and inoculated with *Aspergillus flavus* (Ranjusha *et al.* 2010). The pH for maximum growth of *Aspergillus flavus* in the absence of dye was reported to be 4.5, which was maintained in the batch experiments.

Bioremediation studies

The sterile growth medium (100 mL) containing acid black 52 dye of different initial concentrations (100–2,000 mg/L) was inoculated with the actively growing cells of *Aspergillus flavus* in 250 mL conical flasks. This was incubated aerobically at 27 °C under shaking condition (110 rpm) in an Orbitek shaker for up to 50 hours. The samples were collected from the conical flasks after set time intervals and centrifuged at 4,000 rpm (Eltek centrifuge, Model no. TC 4100F). The concentrations of metals (copper and chromium) and dye were determined using AAS and a UV-visible spectrophotometer, respectively. The biomass collected after centrifugation was dried at 60 °C in a hot air oven (Ambassador, India) and estimated gravimetrically to determine the biomass concentration. The dried fungal biomass after bioremediation of dye (100 mg/L) was also preserved in an air-tight glass container for scanning electron microscopic (SEM) and energy dispersive X-ray (EDX) analyses.

Optimization of process parameters

In the present study, optimization of process parameters was performed to treat a known synthetic dye solution for academic purposes, whereas optimization of the process parameters to treat the actual effluent for practical applications is quite difficult. Statistically based response surface modeling (RSM) was performed to find out the optimum conditions of process parameters for maximum color removal from the acid black 52 dye solution. Different RSM models such as the Box-Behnken method and central composite design (CCD) are available. The advantage of CCD is that a high range prediction can be possible within and outside the design range as compared to the Box-Behnken method, which can be applied within the design range only (Ghosh *et al.* 2015).

Hence, a 2^3 full factorial CCD was used for the modeling. Design Expert Version 7.0.0 (Stat Ease, USA) was used to optimize the parameters and to evaluate of the combined effects of the parameters. For response surface modeling, the ranges of parameters were dye concentration: 200–400 mg/L, pH: 4–6 and time: 35–50 hours. The ranges of independent process parameters examined in the study are shown in Table 1. The

Table 1 | Experimental range and levels of independent process variables

	Range and levels (coded)				
Independent parameters	$-\alpha$	**−1**	**0**	**+1**	$+\alpha$
Initial dye concentration, mg/L (A)	97.7311	200	350	500	602.269
pH (B)	3.31821	4	5	6	6.68
Time, hours (C)	29.8866	35	42.50	50	55.1134

quadratic equation for determination of the optimal conditions is shown according to Equation (1):

$$Y = \beta o + \sum_{i=1}^{K} \beta_i X_i + \sum_{i=1}^{K} \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j + \in \quad (1)$$

where Y is the predicted response, X_i and X_j refer to the independent variables, βo, β_i, β_{ii}, and β_{ij} are the regression coefficients and $\in$ is the statistical error. Twenty experiments were conducted at factorial points (coded to the usual ± 1 notation), axial points ($\pm\alpha$) and center points (0). Each experiment was conducted in duplicate. The percentage removal of color was the response of the system, which was the dependent variable. A confirmatory experiment in batch mode was performed at optimum conditions as suggested by the RSM model.

Assay techniques

The total chromium was determined using AAS (Perkin-Elmer AAnalyst 200), whereas the hexavalent chromium was determined using a UV-Vis spectrophotometer 117 (Systronics). The amount of trivalent chromium was determined by subtracting the amount of hexavalent chromium from the amount of total chromium. The UV-Vis spectral analysis of the acid black 52 dye in the wavelength range of 200–900 nm before bioremediation indicated maximum absorbance at 569.6 nm. SEM analysis was conducted using a ZEISS EVO Series Scanning Electron Microscope Model EVO 50 to investigate the changes in surface morphology of the fungal biomass after bioremediation of acid black 52 dye. EDX analysis was performed to determine the presence of multi-metal in the biomass after bioremediation of acid black 52 dye, using the Bruker-AXS EDX System. Initially, the biomass samples were kept on a stainless steel stub and then under a vacuum condition gold and carbon were plated respectively for SEM and EDX analyses.

RESULTS AND DISCUSSION

Batch-bioremediation

Bioremediation experiments were carried out to determine the color and chromium removal during the growth period of the fungal strain at initial concentrations of dye ranging from 100 to 2,000 mg/L. No growth of the fungi was observed at 3,000 mg/L initial dye concentration. *Aspergillus flavus* was able to grow and remove color and chromium up to 2,000 mg/L dye concentration. It was expected that the simultaneous removal of chromium along with dye would take place under growing conditions of *Aspergillus* sp.

Figure 1 shows the removal (%, mg/L) of color and chromium and the concentrations of dye and chromium removed

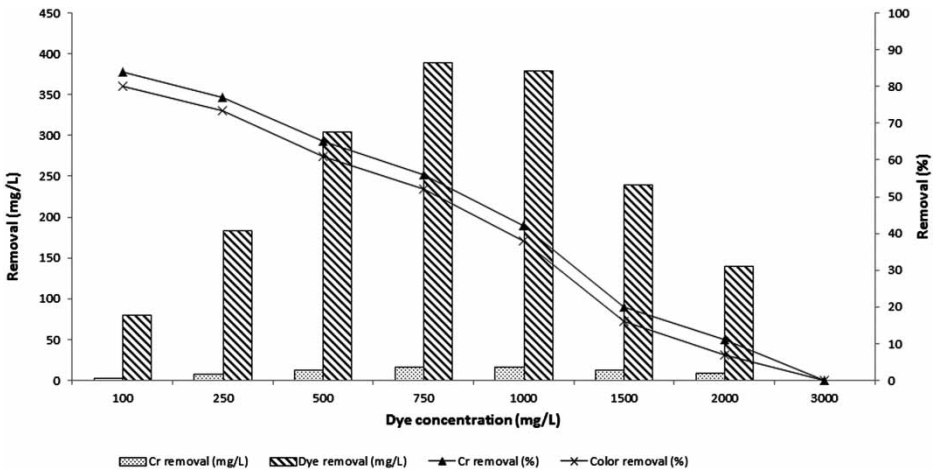

Figure 1 | Removal of color and chromium at different initial dye concentrations.

at different concentrations of dye at pH 4.5 up to 50 hours. Color and chromium removal were decreased from 80 to 7% and from 84 to 11%, respectively, with increasing dye concentration from 100 to 2,000 mg/L. An increase in removal of concentrations of dye and chromium was observed in the range of 100 to 750 mg/L due to the increased availability of dye and chromium. Maximum removal of dye and chromium was observed to be 390 and 17.22 mg/L, respectively, at initial dye concentration 750 mg/L. The removal of dye and chromium was reduced with increasing initial dye concentration above 750 mg/L. The lower removal at higher dye concentration may be due to the crowding effect of dye molecules on the binding sites of the cells.

Figure 2 shows the biomass concentration and specific removal of dye and chromium at an initial dye concentration ranging from 100 to 2,000 mg/L. The biomass concentration was reduced from 4.1 to 0.4 g/L with increasing dye concentration from 100 to 2,000 mg/L. This is due to the toxicity of the dye as well as trivalent chromium to the fungal cells at higher concentrations. The specific removal of dye and chromium was increased from 19.51 to 350 mg/g and from 0.84 to 22.55 mg/g, respectively, with increasing dye concentration from 100–2,000 mg/L. The higher values of specific removal may be due to more availability of dye and chromium to the fungal cells at higher concentrations of dye. Further, at a higher concentration of dye, the growth of the biomass decreased significantly, which led to higher values of specific removal of dye and chromium.

During bioremediation, the chromium complex dyes might be distributed in the extracellular and intracellular space using growing cells. Due to the filamentous structure of the fungus, the chromium complex dye is also expected to adhere to the surface of the cell. The living cells can remove pollutants in two ways, active and passive uptake (Velásquez & Dussan 2009). The availability of active functional groups such as carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups on the cell wall for binding dye and chromium (Kapoor *et al.* 1999) and the initial concentration of dye are important factors for the removal of dye and chromium. The cell surface is negatively charged due to the presence of these functional groups, which favor binding of positively charged molecules (Congeevaram *et al.* 2007). Also, the presence of different oxidoreductive enzymes might be responsible for color removal of dye (Anastasi *et al.* 2010). Biodegradation and biosorption of different chromium complex dyes such as Isolan Dark Blue 2SGL-01 and Acid Orange 80 have been reported using different fungi such as *Irpex lacteus* and *Aspergillus tamarii*, respectively (Kalpana *et al.* 2011; Ghosh *et al.* 2016b). In the present study, colored biomass obtained after bioremediation of the acid black 52 dye also suggested that biosorption had taken place. SEM analysis of the fungal biomass in the absence of dye (Figure 3(a)) and after bioremediation (Figure 3(b)) of acid black 52 dye solution (100 mg/L) strongly indicated distorted cell shape after bioremediation.

The EDX micrograph (Figure 3(c)) shows that chromium was absent in the fungal biomass before bioremediation. The EDX micrograph (Figure 3(d)) shows the presence of chromium, copper and iron in the fungal biomass after bioremediation of acid black 52.

Table 2 shows the comparison of color and chromium removal from solutions of dye and chromium using different

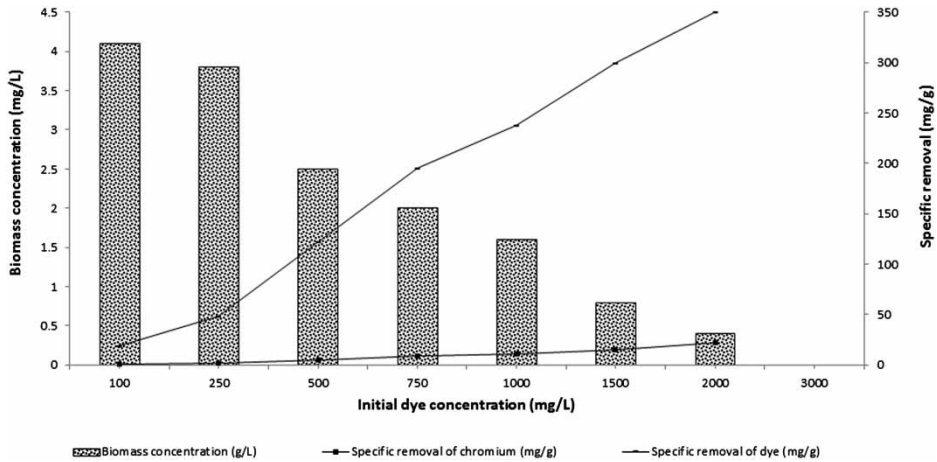

Figure 2 | Biomass concentration and specific removal of dye and chromium at different initial dye concentrations.

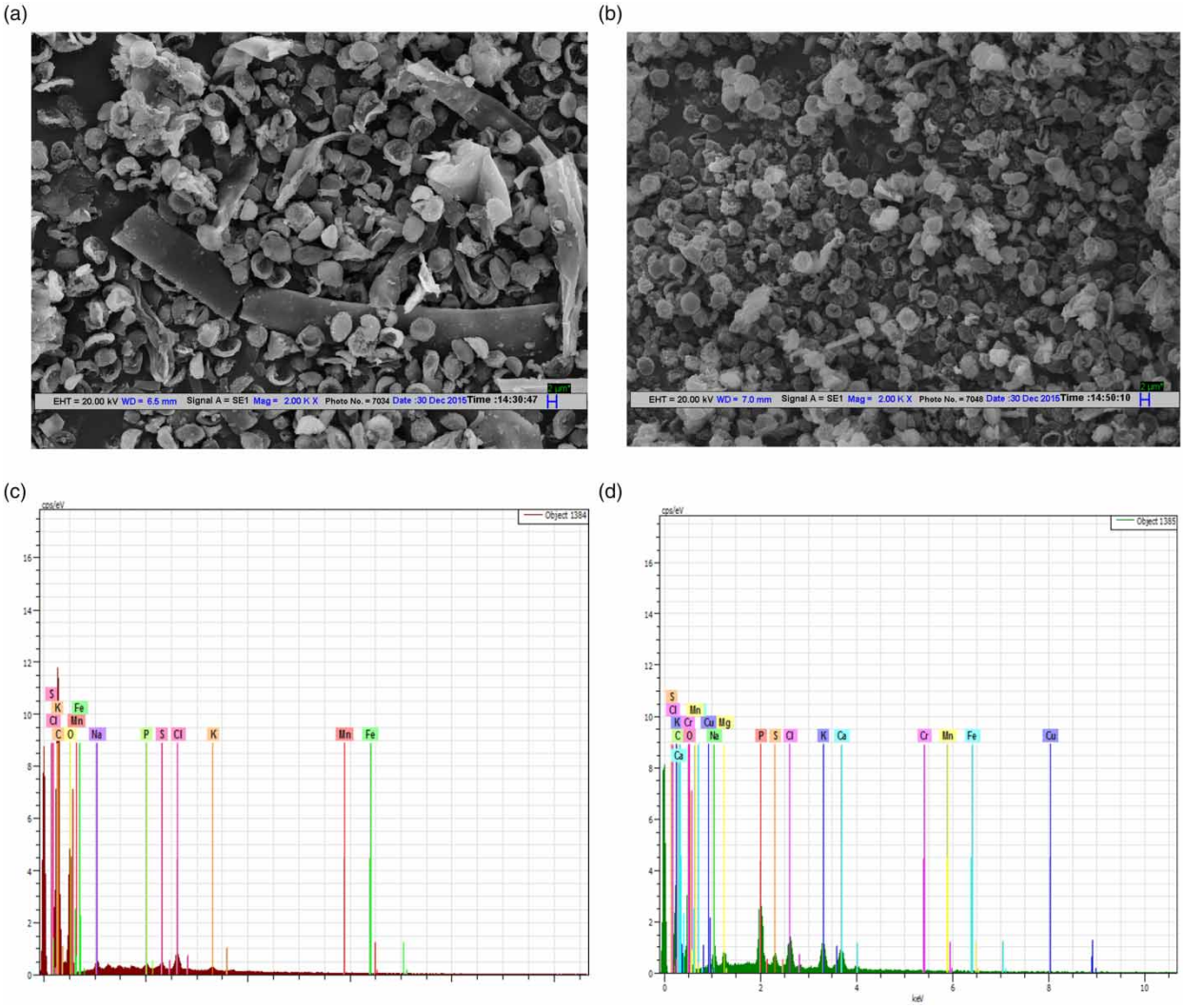

Figure 3 | SEM and EDX analyses of fungal biomass (a) and (c) in absence of dye and SEM and EDX analyses of fungal biomass (b) and (d) after bioremediation of acid black 52 dye.

growing *Aspergillus* sp. In general, higher removal of color was observed from the dye solution which was not complexed with chromium. Similarly, higher removal of chromium was observed when it was not complexed with dye. The lower values of color and chromium removal were obtained in the present study due to the complexity of the structure of acid black 52 dye.

RSM

Twenty experiments were performed under different combinations of dye concentration, pH and time as designed by RSM for optimization study. Removal of color obtained in bioremediation experiments using the CCD matrix are presented in Table 3.

The quadratic model equation relating the color removal and process parameters such as dye concentration (A), pH (B) and time (C) has been expressed by the following Equation (2):

$$\begin{aligned}\%\ \text{Color removal} = {} & -349.12549 - 0.15620A \\ & + 132.55634B + 4.70404C \\ & + 0.016667AB - 0.0004444AC \\ & - 0.36667BC + 0.000108897A^2 \\ & - 12.36371B^2 - 0.023066C^2 \end{aligned} \quad (2)$$

This quadratic equation was used to calculate predicted color removal as shown in Table 3. Percentage deviation between experimental and predicted color removal was calculated. It is observed that there is a close interaction (−0.02) between experimental and predicted color removal.

Table 2 | Removal of color and chromium by different *Aspergillus* sp. during growth

Micro-organisms	Dye/chromium	Conditions	Removal (%)/specific removal (mg/g)	References
Aspergillus niger	Chromium (VI) (Synthetic solution)	Batch; Concentration: 50 mg/L; pH: 3.5; Temperature: 30 °C; Shaking speed: 150 rpm; Time: 170 hours	Chromium: 6.6 mg/g	Dursun *et al.* (2003)
Aspergillus niger	Chromium (VI) (Synthetic solution)	Batch; Concentration: 250 mg/L; pH: 6; Temperature: 30 °C; Shaking speed: 100 rpm; Time: 15 days	Chromium: 19.8 mg/g	Srivastava & Thakur (2006)
Aspergillus versicolor	Chromium (VI) (Synthetic solution)	Batch; Initial chromium concentration: 50 mg/L; pH: 6; 30 °C; 100 rpm; 7 days	Chromium: 99.89%	Taştan *et al.* (2010)
Aspergillus oryzae	Chromium (III) (Synthetic solution)	Batch; Initial chromium concentration: 240 mg/L; pH: 5.5; 30 °C; 150 rpm; 30 hours	Chromium: 98%	Sepehr *et al.* (2012)
Aspergillus niger	Chromium (III) (Synthetic solution)	Batch; Initial chromium concentration: 240 mg/L; pH: 5.3; 30 °C; 150 rpm; 30 hours	Chromium: 95%	Sepehr *et al.* (2012)
Aspergillus niger	Direct violet dye (Synthetic solution)	Batch; Initial dye concentration: 50 mg/L; pH: 5; 30 °C; 150 rpm; 72 hours	Color: 77%	El-Rahim *et al.* (2009)
Aspergillus ochraceus	Reactive blue 25 (Synthetic solution)	Batch; Initial dye concentration: 100 mg/L; pH: 5; 30 °C; 150 rpm; 7 days	Color: 100%	Parshetti *et al.* (2007)
Aspergillus versicolor	Remazol blue dye (Synthetic solution)	Batch; Initial dye concentration: 100 mg/L; pH: 6; 30 °C; 100 rpm; 7 days	Color: 95%	Taştan *et al.* (2010)
Aspergillus flavus	Remazol black b dye (Synthetic solution)	Batch; Initial dye concentration: 100 mg/L; pH: 4.5; 30 °C; 150 rpm; 60 hours	Color: 89%	Ranjusha *et al.* (2010)
Aspergillus tamarii	Acid orange 86 dye (Synthetic solution)	Batch; Initial dye concentration: 100 mg/L; pH: 5; 27 °C; 110 rpm; 50 hours	Color: 98.2% Chromium:100%	Ghosh *et al.* (2014)
Aspergillus tamarii	Acid black 52 dye (Synthetic solution)	Batch; Initial dye concentration: 100 mg/L; pH: 5.0; 27 °C; 110 rpm; 50 hours	Color: 87% Chromium: 92%	Ghosh *et al.* (2016a)
Aspergillus flavus	Acid black 52 dye (Synthetic solution)	Batch; Initial dye concentration: 100 mg/L; pH: 4.5; 27 °C; 110 rpm; 50 hours	Color: 80% Chromium: 84%	Present study

Table 3 | The experimental conditions and percentage color removal

Run	Dye concentration, mg/L (A)	pH (B)	Time, hours (C)	Experimental color removal (%)	Predicted color removal (%) (approximate)	Deviation (%) (approximate)
1	200.00	6.00	35.00	50.00	50.51	−1.02
2	350.00	5.00	42.50	65.70	66.13	−0.66
3	350.00	6.68	42.50	31.00	29.84	3.74
4	200.00	4.00	35.00	51.00	51.67	−1.32
5	350.00	5.00	42.50	65.00	66.13	−1.74
6	350.00	3.32	42.50	31.80	32.63	−2.61
7	500.00	6.00	50.00	57.00	56.67	0.58
8	350.00	5.00	42.50	67.00	66.13	1.30
9	97.73	5.00	42.50	78.00	76.98	1.31
10	200.00	6.00	50.00	56.00	57.33	−2.37
11	500.00	4.00	35.00	44.00	43.01	2.24
12	350.00	5.00	42.50	67.00	66.13	1.30
13	500.00	4.00	50.00	59.00	58.83	0.29
14	500.00	6.00	35.00	51.00	51.85	−1.67
15	350.00	5.00	42.50	66.00	66.13	−0.20
16	602.27	5.00	42.50	68.6	69.15	−0.80
17	350.00	5.00	55.11	72.00	71.98	0.03
18	350.00	5.00	42.50	66.00	66.13	−0.20
19	200.00	4.00	50.00	70.00	69.49	0.73
20	350.00	5.00	29.89	53.40	52.95	0.85

Figure 4 shows the graph between actual and predicted color removal.

The 3-D contour plots of response surfaces were drawn to find out the combined effect of different parameters on percentage color removal of acid black 52 dye. The analysis of variance (ANOVA) is believed to be practicable to test statistical significance of response surface model.

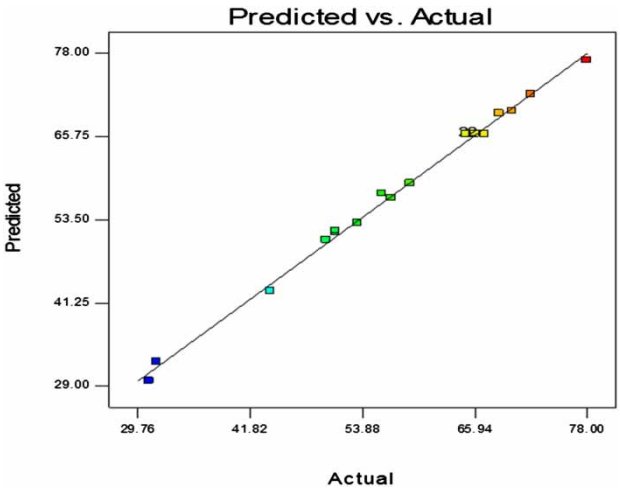

Figure 4 | Actual color removal versus predicted color removal.

The ANOVA results (Table 4) of the quadratic model indicate that the model was highly significant, as indicated from the Fisher's F value (higher F value, i.e. 300.31) with a low probability value ($P < 0.0001$). The 'Lack of fit F value' of 2.73 was insignificant, which proves that the model is fit for the study (Ghosh & Saha, 2012). The value (0.9963) of the determination coefficient (R^2) expresses

Table 4 | ANOVA for the response surface quadratic model for percentage color removal

Source	Sum of squares	Degree of freedom (df)	Mean square	F value	Probablity value (*P* value)
Model	3,035.51	9	337.28	300.31	<0.0001
Residual	11.23	10	1.12		
Lack of fit	8.22	5	1.64	2.73	0.1470
Pure error	3.01	5	0.60		
Cor total	3,046.74	19			

$R^2 = 0.9963$; adjusted $R^2 = 0.9930$; predicted $R^2 = 0.9771$.

that more than 99% of the data deviation can be explained by the model. The predicted correlation coefficient (predicted $R^2 = 0.9771$) also shows good agreement with the adjusted correlation coefficient (adjusted $R^2 = 0.9930$).

Table 5 shows the regression analysis of color removal by using CCD. In this model, the *P* values of A, B, C, AB, BC, A^2, B^2 and C^2 express that the terms are significant.

Combined effect of process parameters on color removal

The 3-D contour plot shows the combined effect of dye concentration and pH on color removal at 50 hours time (Figure 5).

With increasing pH up to 4.75, color removal was observed to increase. At pH above 4.75, color removal was observed to decrease. Maximum removal of color was observed at pH 4.75. The solution pH value affects the charge of the functional groups on the cell wall. Initially, at higher acidic pH, competition occurs between positively charged hydrogen ions and acid black 52 dye molecules for free binding sites on the cell wall. The concentration of hydrogen ions decreases in the solution with increasing pH, which favors binding of dye molecules to the free cell wall groups. Color removal decreased with increasing dye concentrations above 200 mg/L. Maximum removal of color (76.52%) was observed at dye concentration: 200 mg/L, pH: 4.75 and time: 50 hours.

The 3-D contour plot shows the combined effect of initial solution pH and time on color removal at the initial dye concentration of 200 mg/L (Figure 6). The removal of color was increased with increasing time up to 50 hours. Maximum color removal (76.52%) was observed at

Table 5 | Regression analysis of color removal by using CCD

Model term	Coefficient estimate	Standard error	F value	*P* value	Remarks
A	−2.33	0.29	65.97	<0.0001	Significant
B	−0.83	0.29	8.39	0.0159	Significant
C	5.66	0.29	399.39	<0.0001	Significant
AB	2.50	0.37	44.52	<0.0001	Significant
AC	−0.50	0.37	1.78	0.2116	
BC	−2.75	0.37	53.87	<0.0001	Significant
A^2	2.45	0.28	77.03	<0.0001	Significant
B^2	−12.36	0.28	1,961.49	<0.0001	Significant
C^2	−1.30	0.28	21.60	0.0009	Significant

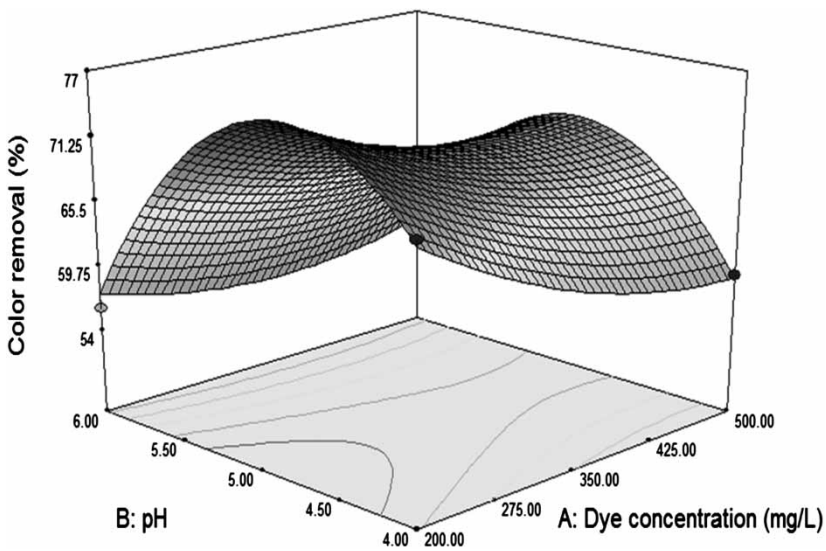

Figure 5 | The combined effect of initial dye concentration and pH on percentage color removal.

50 hours of time at pH 4.75. An increase in time to over 50 hours does not show any improvement in color removal.

The 3-D contour plot shows the combined effect of initial dye concentration and time on color removal of acid black 52 dye at pH 4.75 (Figure 7). At 50 hours, maximum color removal is observed at different concentrations of acid black 52. It is evident from the 3-D contour plots that all the independent parameters such as dye concentration, pH and time have strong effects on the responses, i.e. color removal. Growth of the fungal strain and corresponding color removal were strongly influenced by dye concentration, pH and time. Figure 8 shows the ramp of desirability (0.984) for the RSM model.

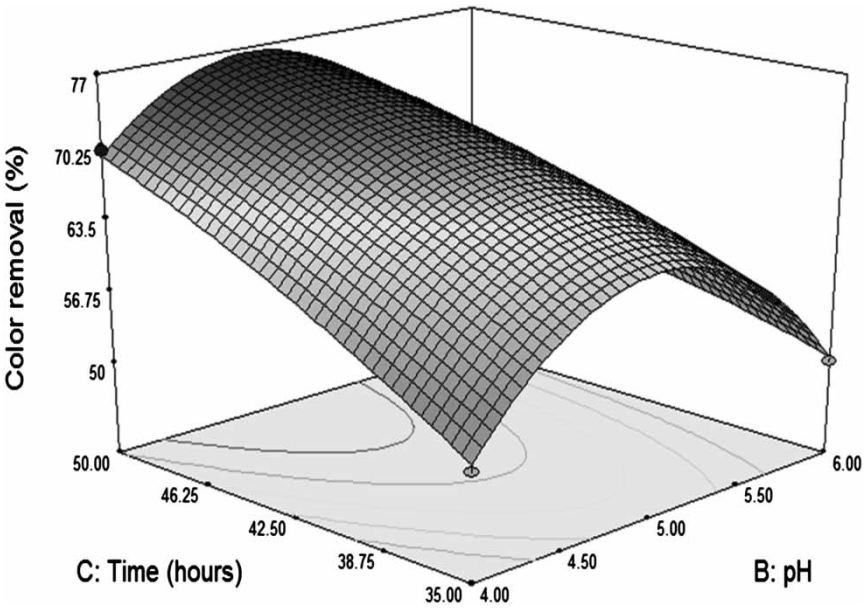

Figure 6 | The combined effect of time and pH on percentage color removal.

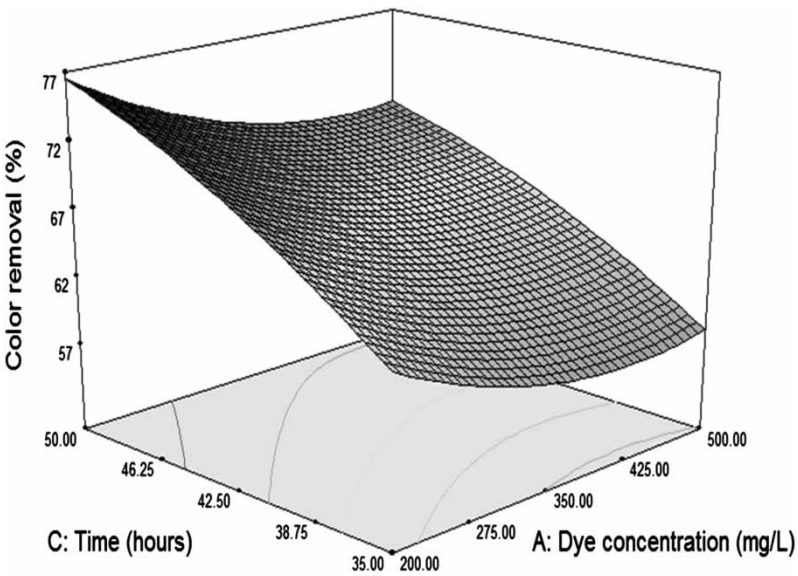

Figure 7 | The combined effect of initial dye concentration and time on percentage color removal.

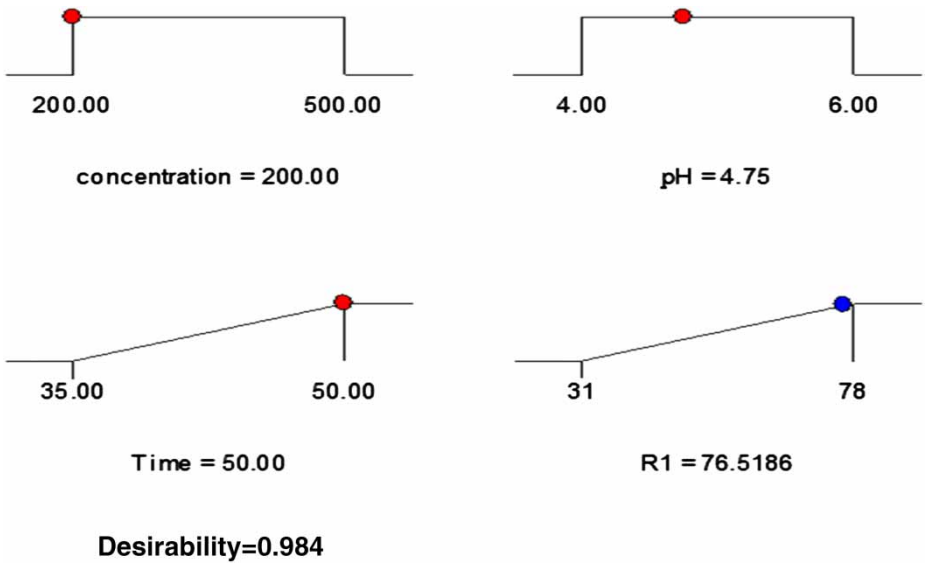

Figure 8 | The desirability ramp of response surface modeling.

Confirmatory experiment

The confirmatory experiment was performed under the optimum conditions (dye concentration: 200 mg/L, pH: 4.75 and time: 50 hours) as suggested by the RSM model. Under the optimum conditions, removal of color was up to 75.80%, and the RSM predictive value was 76.52%, with a marginal deviation.

CONCLUSION

Aspergillus flavus was found to be efficient in removing color and chromium from a synthetic solution of acid black 52 dye under growing conditions. The specific removal of dye and chromium was increased from 19.51 to 350 mg/g and from 0.84 to 22.55 mg/g, respectively with increasing dye concentrations from 100–2,000 mg/L at pH 4.5 in 50 hours in a batch bioreactor. Based on response surface modeling, the optimum conditions for 76.52% color removal were observed as: pH 4.75, initial dye concentration 200 mg/L and time 50 hours. The SEM analysis indicated distortion of the cell surface after bioremediation of acid black 52, and the EDX analysis showed chromium uptake by the fungal cell. *Aspergillus flavus*, therefore, seems to have the potential to biologically treat effluent contaminated with dye as well as chromium.

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