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Evaluation of the biosorption potential of *Aspergillus flavus* biomass for removal of chromium (VI) from an aqueous solution

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ABSTRACT

Chromium biosorption potential of live and dead biomass of *Aspergillus flavus* was analyzed by batch experiments under various experimental conditions like pH, adsorbent dose, exposure period, and temperature. Maximum biosorption of hexavalent chromium was observed at pH 3.5 with adsorbent dose 2.5 g at 30°C. Three days were considered as the optimum exposure period for chromium removal for live biomass, whereas 1.3 hours exposure period for dead biomass of *A. flavus.* The equilibrium data were examined by Langmuir and Freundlich isotherms. Freundlich isotherm appeared to be the best fit model. Phytotoxicity test was conducted to check the effect of the treated chromium solution on the seed germination, seedling length, and vigor index of *Vigna radiata.* Only 23% germination was reported in chromium metal-treated *V. radiata* seeds, but germination and growth parameters of mung bean seeds were significantly increased in the chromium solution after treatment with dead and live biomass. The chromium biosorption potential showed the following trend: dead *A. flavus* > live *A. flavus*. Hence, live and dead biomass of *A. flavus* can be applied as a safe and economically feasible biosorbent for hexavalent chromium elimination for the treatment of industrial effluent or wastewater system.

1. INTRODUCTION

Heavy metal pollution in terrestrial and aquatic ecosystems is a major threat. Heavy metals have atomic numbers more than 20 and a density >5 g/cm^3 [1]. They have become a serious threat due to their long-term endurance, toxic and non-degradable nature with a negative impact on human health after entering into the food chain [2]. They interact with organic compounds and transform them into more complicated toxic complexes [3,4]. The heavy metals cannot be removed after they enter in the organs of human beings as they cumulate, which may cause changes in biochemical processes and may lead to chronic and long-term hazardous effects [4]. The presence of a high concentration of chromium in the soil and water due to excessive release from industrial activities has become a serious issue [5]. The hexavalent chromium [Cr (VI)] is released into the environment from various industrial processes such as electroplating, dye, paint, leather, metal finishing, steel, and paper production work [6,7]. It has seven oxidation states in which chromite [Cr (III)] and chromate [Cr (VI)] states are durable natural forms of chromium [8]. Chromium (VI) is an oxidizing agent and its soluble nature and bioavailability converts it into a toxic form of chromium [9]. The International Agency for Research on Cancer has kept chromium under the category of group one carcinogens [10]. The hexavalent chromium in the environment mainly occurs as chromate oxyanions which act as strong mutagen and oxidizing agent [11]. The chromate oxyanions are similar to the sulfate oxyanions and enter in the cells with the help of sulfate transporters available on the cell surfaces [12]. Under normal physiological conditions, hexavalent chromium crosses the cell membrane and reacts with ascorbate and glutathione and generates free radicals, and finally gets converted into trivalent chromium which is stable in nature [13]. The reduction of glutathione produces reactive oxygen species which can damage biomolecules such as sugar, protein, lipid, and nucleic acids [12]. Chromium can show alterations in DNA methylation and histone modification in cells [14].

However, removal of chromium from industrial effluents is an arduous task. Different methods such as ion exchange, evaporation,

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membrane separation, electrochemical treatment, and chemical reduction have been applied for chromium removal from contaminated effluents [15]. All the above mentioned conventional methods are expensive and time-consuming. Hence, there is a need to explore simple, cost-effective, and environmental benign approaches for the elimination of chromium from wastewater. Biosorption is a physicochemical process that utilizes biomass for the removal of heavy metals from aqueous solution [16]. Fungi have an excellent binding ability for heavy metal ions and can be employed as biosorbent. The metal ion binding ability is due to the high electronegativity present on the surface of fungal biomass which can attract and sequester metal ions [17,18]. The dead biomass of microbes can also be used for the recovery of metal ions as it binds metal ions more efficiently [19]. As compared to traditional ways, biosorption has been considered as a cost-effective, eco-friendly, and efficient method to solve the problem of environmental pollution which rose due to heavy metals with regeneration of the biosorbent, recovery of metals, and less sludge formation [20]. Very few reports are available on the application of dead biomass for metal biosorption. To the best of our knowledge, no information is available till date to study the effect of live and dead biomass of *Aspergillus flavus* on the removal of chromium from aqueous solution and effect of treated metal solution on mung bean seeds. Therefore, the present investigation was carried out to analyze live and dead biomass of *A. flavus* ability for chromium removal from aqueous solution under different environmental conditions.

2. MATERIALS AND METHODS

2.2. Maintenance of the Fungal Biomass

Aspergillus flavus was isolated from the soil samples. The isolated fungi were maintained on the potato dextrose agar medium. Cultures were kept at 4°C and sub-cultured in every 15 days. The inoculated slants were incubated for 7 days at 28°C ± 2°C.

2.3. Growth Conditions

One disk of fungal biomass (4 mm diameter) was inoculated into modified Czapek's Dox medium (sucrose = 30 g, $NaNO_3$ = 2 g, K_2HPO_4 = 1 g, $MgSO_4.7H_2O$ = 0.5 g, KCl = 0.5 g, and ferrous sulfate traces) at pH 6.9. Flasks were incubated for 7 days at 29°C ± 2°C. By filtration, fungal biomass was separated from culture, washed with autoclaved distilled water, and dried with filter papers to get biosorbent [21].

2.4. Preparation of Dead Fungal Biomass

The live biomass of *A. flavus* was converted into dead biomass by boiling it in 0.5 N NaOH solution for 15 minutes, washed with double distilled water till the pH becomes 7. Biomass was dehydrated at 5°C for 1 day and powdered [22]. Dead biomass was kept in a desiccator for further use. It was observed that approximately 4 g of live biomass (wet weight) was equivalent to 0.35 g of dead biomass of *A. flavus*.

2.5. Removal of Chromium by *A. flavus*

Hexavalent chromium (10 mg/l) was prepared by mixing of potassium chromate in sterilized distilled water which was further used to prepare required concentrations. *Aspergillus flavus* live or dead biomass was mixed in a metal solution (100 ml) in different conical flasks and the metal solution without any live or dead biomass was treated as control. Factors that affect chromium biosorption by fungal biomass were investigated under different experimental conditions. In order to study the effect of exposure time for maximum adsorption of Cr (VI), several equilibrium experiments were conducted at different time period ranging from 0.5 to 96 hours. The effect of pH (1–5) on chromium removal was carried out by maintaining desired pH with NaOH or HCl before mixing biomass. The dependence of chromium uptake on initial chromium concentration was also analyzed by increasing chromium concentration from 20 to 120 mg/l. The effect of different fungal biomass dosages (0.5–3.5 g live and dead biomass respectively) on the Cr (VI) uptake was also studied. The effect of temperature (10°C–50°C) was also investigated on the chromium removal. The flasks were kept in a shaking incubator under different experimental conditions for the optimization of various parameters. At the end of the experiment, the supernatant was used for residual metal analysis [23].

2.6. Chromium Biosorption Capacity

Chromium content was analyzed after the formation of colored compound of chromium with 1,5-diphenylcarbazide and optical density was measured at 540 nm with a spectrophotometer [24]. Biosorption of chromium shows the uptake of metal and it was assessed by the following equation:

$$q_e = (C_i\text{-}C_e)\ V / m$$

where q_e = equilibrium, V = volume of hexavalent chromium, C_i and C_e are initial and final concentrations of hexavalent chromium metal, and m = biosorbent mass.

2.7. Adsorption Isotherm

Two isotherm models were used to interpret sorption equilibrium in the present study. Different concentrations of chromium metal solution (20–120 mg/l) were treated with different adsorbent doses of *A. flavus*. The adsorption capacity and equilibrium solution concentrations were evaluated with suitability of the isotherm.

2.6.1. Langmuir isotherm

The Langmuir isotherm model speculates that adsorption process takes place in monolayer mode [25].

Langmuir equation was expressed as follows:

$$C_e/q_e = 1/q_e K_l + C_e/q_m$$

q_e (mg/g) = chromium metal adsorbed at equilibrium, q_m (mg/g) = chromium metal adsorbed, C_e is equilibrium chromium concentration (mg/l), and K_l is Langmuir constant associated with binding capacity of chromium on fungal biomass.

2.6.2. Freundlich isotherm

Freundlich isotherm explains the distribution of solute molecules between aqueous and solid phases at equilibrium. This isotherm

assumes an exponential disparity in energy of surface-active sorption site and reduction in adsorption heat is logarithmic [26].

The Freundlich equation is as follows:

$$\text{Log } q_e = \log K_f + 1/n \log C_e$$

Freundlich constants, such as K_f and n, are adsorption capacity and adsorption intensity, respectively. Nature of process (n) may be: $n = 1$, a linear process; $n < 1$, a chemical process; and $n > 1$, a physical phenomenon.

2.8. Phytotoxicity Study

The effect of chromium before and after treatment with live and dead biomass of *A. flavus* was studied on the growth parameters of mung bean (*Vigna radiata*) seeds.

2.7.1. Seed germination test

Mung bean (*V. radiata* var. KM2195) seeds were thoroughly washed with tap water to remove dust then sterilized with distilled water / NaOCl (10:1) solution for 5 minutes and washed four to five times again with autoclaved distilled water. Seeds of mung bean were immersed in chromium solution before and after *A. flavus* live and dead biomass treatment for 4 hours. Filter paper was placed on sterilized petri dishes and control and treated mung bean seeds were transferred into petri dishes. The petri dishes were covered with sterilized polythene bags and kept in a seed germinator for a week under 80% relative humidity at 25°C ± 2°C with 12 hours light following the ISTA guidelines [27].

The total number of seeds germinated was calculated using the following formula:

Germination percentage = Total number of seeds germinated / Total number of seeds taken for germination × 100

2.7.2. Seedling length

The radicle and plumule length of mung bean seedlings were measured with a measuring scale.

2.7.3. Vigor index

The vigor index was calculated as follows:

Vigour index = Total seedling length (mm) × percentage of germination [28].

2.8. Statistical Analysis

Treatments were exhibited as randomized block design with three replications. Data were statistically analyzed using analysis of variance by using SPSS software (version 16 SPSS, US).

3. RESULTS AND DISCUSSION

To identify suitable conditions for chromium elimination, comparative analysis of live and dead biomass of *A. flavus* was carried out by changing various parameters like exposure time, pH, chromium amount, temperature, and adsorbent dose through the batch experiments.

3.1. Effect of Exposure Time

Batch experiments were conducted to investigate *A. flavus* live and dead biomass effect on chromium removal under different exposure periods. The results showed that the biosorption capacity was increased rapidly with increasing contact time (Figs. 1 and 2). The 72 hours was considered as the optimum exposure period for chromium removal for live biomass, whereas 1.3 hours exposure time for dead biomass of *A. flavus* (Figs. 1 and 2). It was observed that after the optimum exposure period, chromium removal efficiency was decreased gradually with increasing contact time. The high rate of biosorption at the initial stage was due to the

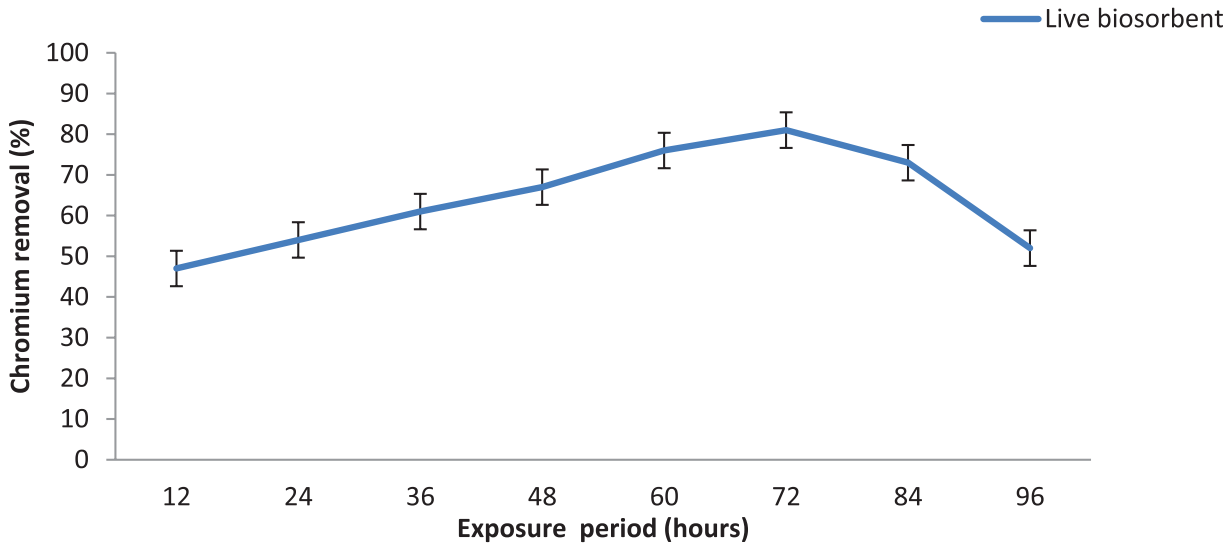

Figure 1: Exposure time effect on chromium elimination by live *A. flavus*. Data are mean ± standard error of three replicates. Data were found to be significant at $p < 0.05$.

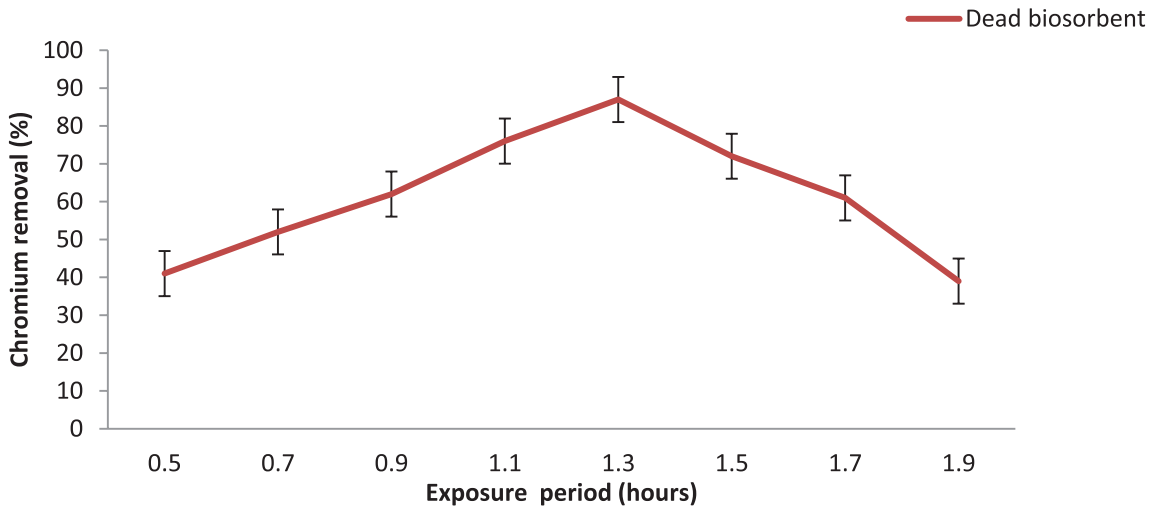

Figure 2: Exposure time effect on chromium removal by dead biomass of *A. flavus*. Data are mean ± standard error of three replicates and data were found to be significant at $p < 0.05$.

presence of more active centers on the surface of *A. flavus*. Later biosorption was merely an attachment-controlled process due to less available sorption sites.

3.2. Effect of pH

Chromium removal by live and dead *A. flavus* biomass at different pH is shown in Figure 3. The pH is an important parameter in the chromium metal biosorption process. Cr (VI) biosorption was enhanced with escalation in pH from 1 to 3 for both live and dead biosorbents and reflected maximum adsorption capacity at pH 3.5. At pH 3.5, maximum chromium removal of 75% and 83% was observed with live and dead biomass, respectively. The reports revealed that in aqueous solution chromium is present in the form of acid chromate, dichromate, and other oxyanions. At a low pH, acid chromate is the main form, but it gets converted into CrO_4^{2-} and $Cr_2O_7^{2-}$ with the increase in pH.

The reduction in chromium ion removal efficiency at a high pH is due to competition between chromate and hydroxyl ions [29]. At low pH, chromate ions are in $Cr_2O_7^{2-}$ and $HCr_2O_4^-$ forms and amino groups are protonated. Park *et al*. [30] observed that at low pH hexavalent chromium converts into the trivalent form.

3.3. Effect of Initial Chromium Concentration

Initial chromium metal concentration effect on Cr (VI) adsorption on the live and dead *A. flavus* biomass is shown in Figure 4. Cr (VI) elimination was enhanced by escalation in initial concentration of Cr (VI) ions. It may be due to the saturation

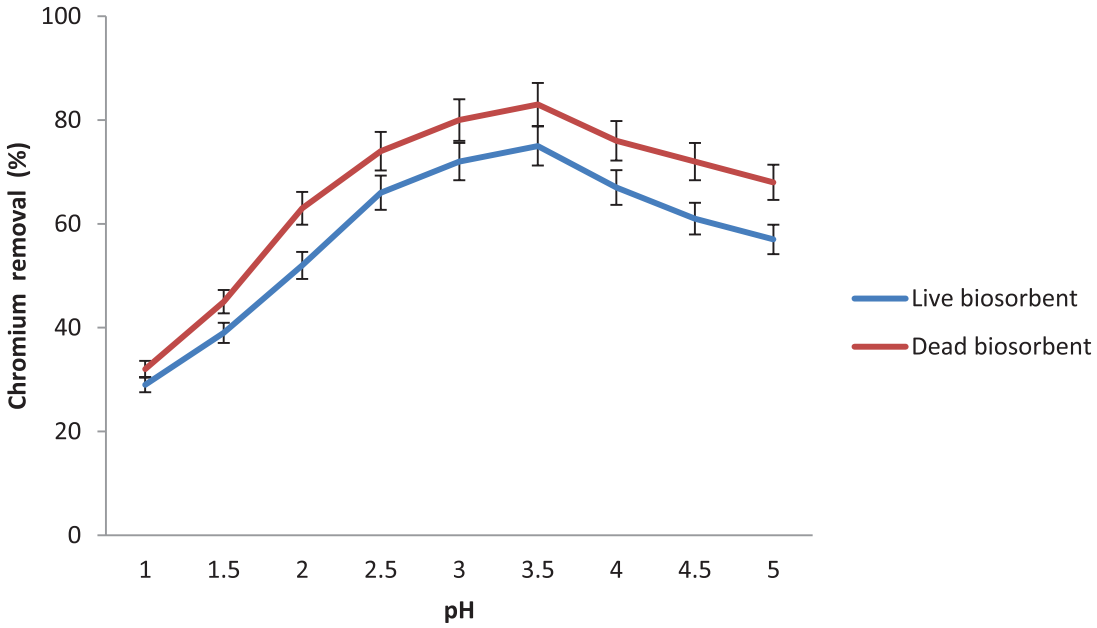

Figure 3: Biosorption of chromium by live and dead *A. flavus* biomass at different pH. Data are mean ± standard error of three replicates. Data were found to be significant at $p < 0.05$.

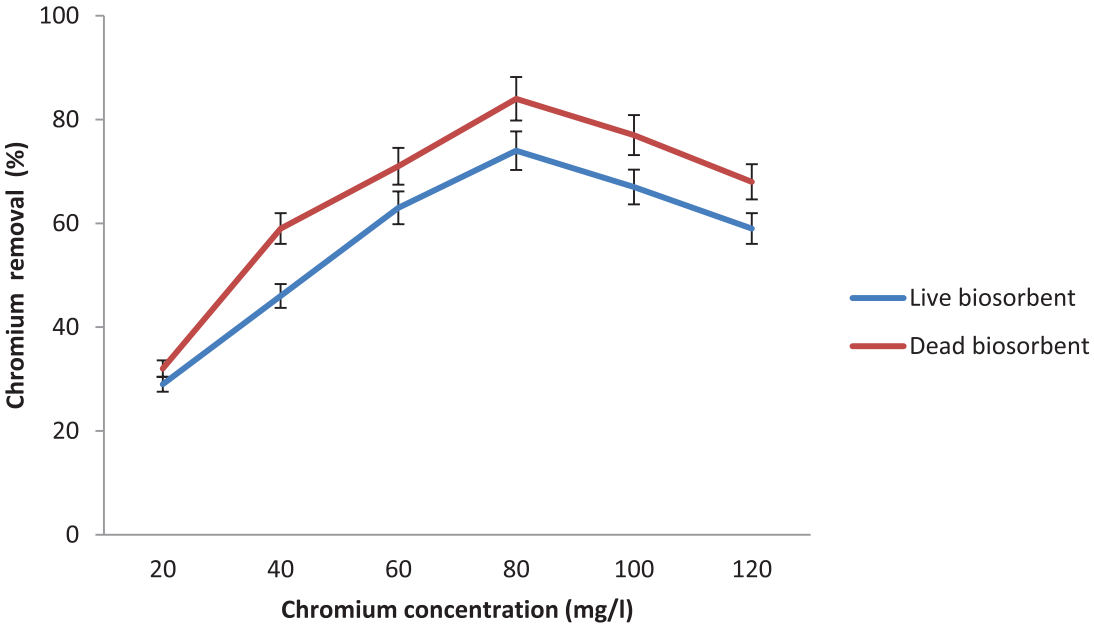

Figure 4: Impact of initial chromium concentration on chromium removal. Data are mean ± standard error of three replicates. Data were found to be significant at $p < 0.05$.

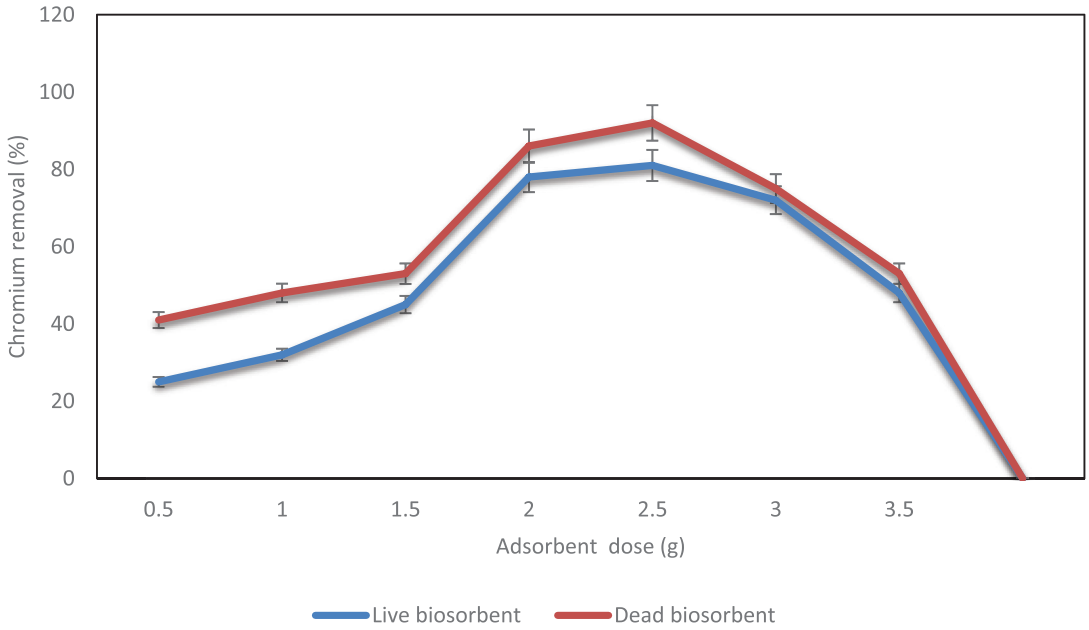

Figure 5: Effect of the adsorbent dose on chromium elimination. Data are mean ± standard error of three replicates. Data were found to be significant at $p < 0.05$.

of available active centers of fixed amount of adsorbent. The results reflected that the adsorption capacity increased with increasing initial concentration of hexavalent chromium. The metal ions provide the required driving force to exceed the mass transfer resistance of hexavalent chromium between liquid and solid adsorbent. The enhancement in initial chromium ion concentration enhanced the interaction between chromium ions in aqueous phase and biomass surface and enhanced chromium uptake by *A. flavus*. It might be due to the more number of active groups available for biosorption and reduction because of the increased amount of *A. flavus* biomass.

3.4. Effect of Adsorbent Dose

Different amounts of live and dead biomass of *A. flavus* were utilized to compare their biosorption ability for chromium. The increase in the biosorption rate of chromium was observed with an increase in the fungal biomass (Fig. 5). The biosorption capacity

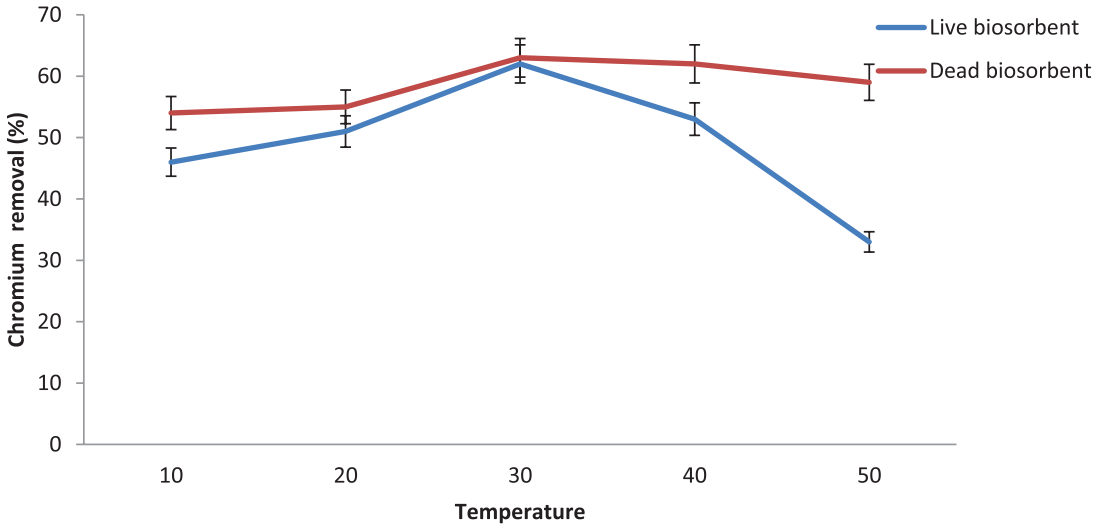

Figure 6: Effect of temperature on the chromium removal. Data are mean ± standard error of three replicates. Data were found to be significant at $p < 0.05$.

Table 1: Isotherm constants for chromium adsorption by the dead biomass of *A. flavus*.

Isotherm	Equation	Plot	Parameters	Value
Langmuir	$C_e/q_e = 1/q_e K_1$ `+ C_e/q_m	C_e/q_e versus C_e plot showed straight line of slope $1/q_m$ and an intercept of $1/(K_a q_m)$	q_m (mg/g)	0.5337
			K (l/mg)	147
			R^2	0.9485
Freundlich	Log q_e = log K_f + $1/n$ log C_e	K_f and $1/n$ were evaluated from intercept and slope of linear plot of lnq_e versus lnC_e, respectively	n	0.8407
			K_f (mg/g)	7.795136
			R^2	0.9486

of live and dead fungi showed the following trend: 2.5 > 2 > 3 > 3.5 > 1.5 > 1 > 0.5 g. Therefore, 2.5 g/100 ml was considered as the optimal biosorbent dose due to its high chromium adsorption capacity. Sarikaya [31] reported that enhancement in biosorbent dosage increased Cr (VI) *Agaricus campestris* biosorption. The results reflected that dead biomass was effective in removal of chromium as compared to live fungal biomass (Fig. 5). It is because of more absorptive surface area and binding sites availability on dead biomass as compared to live biomass. Maximum chromium removal 92% was observed with 2.5 g *A. flavus* dead biomass, but the addition in adsorbent dose could not alter adsorption. This might be due to the absence of active sites on biomass and establishment of equilibrium between chromium on biosorbent and solution. However, after some time equilibrium biosorption capacity was reduced. It was due to the reduction in the number of effective active centers for binding of hexavalent chromium by biosorbate amount available in the medium.

3.5. Effect of Temperature

Temperature plays a significant role in the biosorption process. The biosorption process was increased with the increase in temperature from 10°C to 50°C with live *A. flavus* (Fig. 6). It shows that the interaction between chromium and *A. flavus* was endothermic in nature. With the rise in temperature above the optimum value, biosorption ability of live biomass declined. The dead biomass of *A. flavus* did not show any significant alterations in biosorption with alteration in temperature as it could not modify functional groups with the increase in temperature.

3.6. Adsorption Isotherm

The adsorption isotherm model provides information on adsorption mechanism, surface property, and adsorbent capacity. This assessment helps to construct better biosorbent for future research. Langmuir isotherm deals with the homogeneous distribution of active sites on adsorbent surface and shows single molecular layer of adsorbate molecules. Freundlich isotherm model applies to heterogeneous system, and is not restricted to monolayer formation and display interaction between adsorbed molecules. It indicates that with the increase in adsorbate concentration, adsorbate amount on the adsorbent surface also enhances and sorption energy reduces with non-availability of sorption centers on adsorbent. Hence, isotherm data of chromium adsorption on *A. flavus* biomass was assessed by using Langmuir and Freundlich models (Table 1).

In this investigation, Freundlich isotherm showed better fitting model as compared to Langmuir due to high correlation coefficient (R^2 = 0.9486). After calculation by the equation, Langmuir constants reflected the following values: q_m = 0.5337 mg/g, k = 147 mg^{-1}, and R^2 = 0.9485, and Freundlich constants were K_f = 7.795136 and n = 0.8407 (Fig. 7a and b).

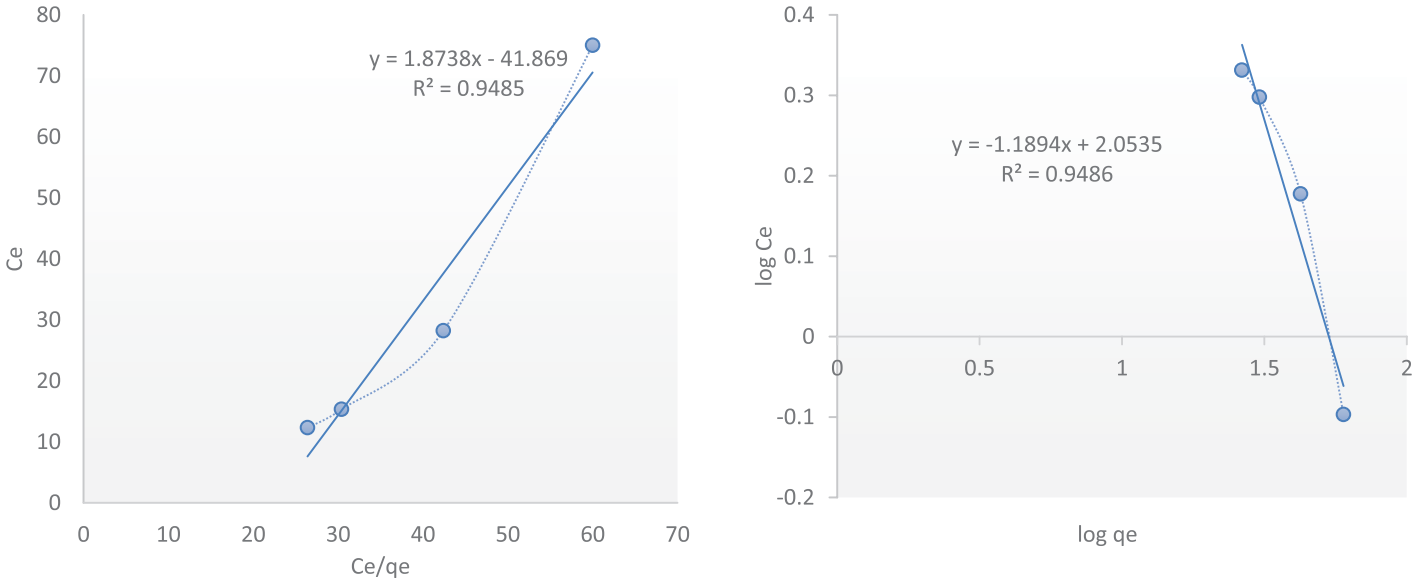

Figure 7: (A). Langmuir isotherm for chromium adsorption by the dead biomass of *A. flavus*. (B). Freundlich isotherm for chromium adsorption by the dead biomass of *A. flavus.*

Table 2: Effect of chromium before and after treatment with live and dead biomass of *A. flavus* on germination and growth parameters of *V. radiata.*

Treatment	Seed germination (%)	Radicle length (cms)	Plumule length (cms)	Vigour index
Control	96.59 ± 0.74[a]	3.1 ± 0.22[a]	8.2 ± 0.72[a]	10,901
Chromium (VI)	23.43 ± 0.66[c]	0.34 ± 0.01[c]	2.8 ± 0.25[c]	738.21
Chromium solution treated with live fungal biomass	72.85 ± 0.67[b]	1.8 ± 0.09 [b]	5.4 ± 0.42[b]	5,245
Chromium solution treated with dead fungal biomass	92.63 ± 0.73[a]	2.7 ± 0.18[a]	7.6 ± 0.51[a]	9,539

Data are mean ± standard error of three independent experiments. Values followed by different letters show a significant difference at $p < 0.05$ between treatment according to analysis of variance and Duncan's Multiple Range Test (DMRT).

3.7. Phytotoxicity Study

The phytotoxicity test was conducted to check the suitability of chromium-treated water for irrigation purposes. The effect of chromium metal ions and treated chromium solution with live and dead biosorbent of *A. flavus* was compared on the growth parameters of *V. radiata*. Several changes were observed under different treatment for seed germination and morphological properties of mung bean seeds (Table 2). Maximum 97% seed germination was reported in control and chromium metal-treated seeds showed only 23% seed germination. The radicle and plumule length were 3.1 and 8.2 cm in control which were significantly reduced to 0.34 and 2.8 cm in chromium metal solution, respectively. The significant increase 211.19% and 295.68% in mung bean seed germination was reported with chromium solution treated with live and dead fungal biomass, respectively, over chromium metal solution. The dead fungal biomass-treated chromium solution showed significant increase in radicle and plumule length as compared to live fungal biomass treated chromium solution. Vigor index of mung bean seeds showed trend: control > chromium solution treated with dead biosorbent > chromium solution treated with live biosorbent solution > chromium.

Chromium accumulation in crop fields shows reduction in plant growth and development process at cellular or genetic level as chromium incited toxicity enhances production of free radicals [32]. Tomko *et al*. [33] reported 90%–95% removal efficiencies of copper, aluminum, and antimony from their respective aqueous solution by fungal biomass. Javaid *et al*. [34] used pretreated *Aspergillus niger* as adsorbent for copper and nickel metals removal. Rao and Bhargavi [35] observed that pretreated fungal biomass can be used as biosorbent for the removal of nickel and lead. Mali *et al*. [36] found that *A. flavus* can be applied as an adsorbent for removal of zinc metal. The biosorption of lead was reported by *Aspergillus terreus* [37] and *Aspergillus versicolor* [38]. Application of *Bacillus* species for lead adsorption was reported by Garcia *et al*. [39]. Mahish *et al*. [40] found that *Penicillium oxalicum* showed 90% lead adsorption capacity.

Table 3: Comparison of chromium removal efficiency by various biosorbents.

Metal	Biosorbent	Adsorption capacity (mg/g)	References
Hexavalent chromium	*Agaricus campestris*	56.21	Sarikaya [31]
	Sugarcane bagasse	4.4	Garg *et al*. [41]
	Jatropha oil cake	4.8	Garg *et al*. [41]
	Maize corncob	3	Garg *et al*. [41]
	Aspergillus niger	11.79	Mondal *et al*. [22]
	Aspergillus flavus	0.53	This study

3.8. Performance of *A. flavus* as Compared to Other Adsorbents

The efficiency of *A. flavus* for chromium removal was compared with other adsorbents and is given in Table 3. Chromium removal efficiency is in consonance with previous findings, showing that chromium can be easily adsorbed on *A. flavus* biomass. It indicates that fungal biomass can be used as cost-effective and environmentally benign biosorbent for chromium removal.

To the best of our knowledge, only few reports have compared live and dead biosorbent capacity for metal removal from aqueous solution or wastewater system [38,42]. The biosorption of chromium metal with live and dead biomass of *A. flavus* has not been reported earlier. Khadivinia *et al*. [43] found that dead biosorbents can be used as a better alternative as compared to live biomass. Paul *et al*. [44] found that heat treatment to bacterial biosorbent enhanced their capacity to adsorb metal ions, and it may be because of cell wall degradation and exposure of binding sites for metal ions. Cheng *et al*. [45] reported that dead biosorbents have many benefits as compared to live biomass like more efficiency, no need of nutrients, less sludge production, and not expensive. The live biomass can transport adsorbed heavy metals inside the cells and converts metal ions into less toxic forms [46]. The live biosorbents can easily eliminate heavy metals even at very low concentrations [47]. The live and dead fungi have been recognized as a promising low-cost biosorbents for the removal of heavy metal ions due to their advantages [48]; hence, it needs to be explored further to take maximum advantage for treatment of industrial wastewater.

4. CONCLUSION

The present investigation has revealed that both live and dead biomasses of *A. flavus* were effective in removing hexavalent chromium from aqueous solution. The non-viable biomass reflected higher metal biosorption capacity as compared to the live biomass. Equilibrium data were fitted well with the Freundlich adsorption isotherm. Hence, it can be concluded from the findings of the present paper that hexavalent chromium can be efficiently removed from aqueous solution by using both live and dead biomasses of *A. flavus*. Further investigations are required for optimization of environmental parameters for its application in effluent treatment at contaminated sites under natural conditions.

5. ACKNOWLEDGMENTS

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6. CONFLICT OF INTEREST

There is no conflict of interest.

7. ETHICAL APPROVAL

Not applicable.

8. AUTHORS' CONTRIBUTION

R.T. Kapoor carried out the experiments and prepared the manuscript.

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