

ORIGINAL RESEARCH ARTICLE

Efficient Removal of Chromium (VI) ions from Aqueous Solutions using *Aspergillus flavus*: Biosorption and Adsorption Mechanisms

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ABSTRACT

This study examines the efficiency of fungal biosorption and adsorption in removing chromium (VI) ions from aqueous solutions. *Aspergillus flavus*, a common effluent fungus, was used as the test fungus in this study. The fungus, known for its adaptability to harsh environments, was subjected to batch experiments to assess its chromium removal capabilities. Several variables, including temperature, pH, contact time, and initial chromium concentration, significantly influenced the biosorption and adsorption processes. The results of this study demonstrate that *A. flavus* effectively removes chromium (VI) ions across a range of concentrations. The optimal pH for removal was identified as 4.0 and 6.0, with maximum % removal (99.96 %) and adsorption (50.42 mg/g) achieved at pH 4.0. Kinetic studies revealed that biosorption and adsorption occur rapidly, reaching equilibrium after 15 minutes ($Q_t = 159.201$ mg/g). The maximum % biosorption and adsorption capacity were determined to be 99.96 % and 723.223 mg/g, respectively. These findings suggest that *A. flavus* is an efficient biosorbent for chromium (VI) ions, offering a promising solution for reducing chromium concentrations in aqueous solutions. The study sheds light on the mechanisms underlying chromium binding to fungal biomass and emphasizes the significance of optimizing operational parameters to enhance biosorption efficiency. These results advance our knowledge of fungal-based approaches for heavy metal removal from aqueous environments, with potential implications for sustainable water treatment practices. Further research is warranted to investigate the scalability and practical applications of *Aspergillus flavus* in real-world chromium-contaminated effluent scenarios.

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KEYWORDS

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INTRODUCTION

One of the major global environmental challenges of considerable concern is the continuous release of heavy metals into the environment due to technological, industrial, and agricultural activities (Mehwish, 2015). The advancement of human civilization, industrial growth, urbanization, and population expansion contribute to the increasing environmental contamination (Marandi, 2011). The indiscriminate discharge of industrial effluents into agricultural lands and water bodies has increased, particularly in developing countries (Akhtar et al., 2013). The untreated wastewater containing metals is often discharged into water sources, accumulating various hazardous heavy metals in water bodies, creating severe environmental problems. These metals harm aquatic

organisms and pose a significant threat to human health (Subbaiah et al., 2008; Ali et al., 2019; Sanone et al., 2020).

Many heavy metals, including arsenic, lead, copper, cobalt, chromium, nickel, and cadmium, have been found in industrial effluents. These heavy metals accumulate in the soil, posing a threat to freshwater sources, groundwater quality, and soil health, all of which are major concerns. Improper waste and wastewater management from households, industries, and farms are already negatively impacting the environment (Akhtar and Mannan, 2020). Heavy metals in crops irrigated with industrial effluents and shallow groundwater can lead to various health issues in humans and animals. Prolonged exposure to high metal concentrations is associated with an increased risk of

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neurological disorders, lung cancer, cardiovascular disease, gastrointestinal disorders, and carcinogenesis (Akhtar *et al.*, 2013).

The primary sources of toxic chemicals released into the environment and ecosystems are residential, commercial, and industrial activities. The influx of contaminated substances into water bodies greatly impacts organisms' survival, behavior, reproduction, growth, and development. Heavy metals tend to accumulate in organisms' bodies and bioaccumulate up the food chain due to their resistance to decomposition, unlike organic pollutants. The inert nature of heavy metals, such as chromium, cobalt, arsenic, lead, cadmium, and others, often leads to DNA disruption and poses various health hazards (Hanif and Bhatti, 2015).

According to Akhtar *et al.* (2013), the chemical and physical techniques used for removing heavy metal pollution come with high capital and operating expenses. As biological methods are cost-effective, eco-friendly, and without adverse effects, they are increasingly being researched as alternatives for metal cleanup. Numerous studies have focused on microorganisms like algae, fungi, bacteria, and yeast (Talukdar *et al.*, 2020). Certain microorganisms can adsorb heavy metals onto their surfaces and sequester them within their structures through bioaccumulation and/or passive adsorption processes (Hyde *et al.*, 2019). Fungi are of particular interest to scientists due to their distinct outcomes in both liquid and solid phases and the diverse mechanisms they employ (Oladipo *et al.*, 2016). Mycoremediation, a globally recognized technology, is currently gaining attention from researchers as one of the most environmentally friendly and effective biological approaches (Lakhani *et al.*, 2022).

Water contamination is a primary cause of 70–80% of all illnesses in underdeveloped countries, especially those that affect women and children, according to reports from the WHO and UNICEF (2000); Bagotia *et al.*, 2021). Rapid industrialization, population growth, and insufficient control over environmental pollution and waste management pose serious threats to food safety and the environment in numerous developing nations, Nigeria among them (Abdullahi and Mohammed, 2020). Untreated wastewater from the textile and tannery industries is among the primary sources of heavy metals (HMs) in the environment, according to Danjuma and Abdulkadir (2018). Additionally, it was noted that the effluents from Kano's chemical processing firms contained levels of Cr, Zn, Cu, and Pb that were higher than the top limits advised by the World Health Organization and the Federal Environmental Protection Agency of Nigeria (FEPA).

Heavy metal accumulation above the recommended levels poses a significant threat to all organisms, including humans, highlighting the need for prompt, effective, reliable, and sustainable solutions. Bioremediation is one of the most environmentally friendly methods for reducing heavy metal concentrations and offers numerous

advantages over traditional approaches. It is feasible, cost-effective, user-friendly, time-efficient, and generates less harmful sludge (Mungasavalli *et al.*, 2007). It can easily complement existing physical and chemical processes while ensuring permanent pollutant removal (Ahmed *et al.*, 2021). The study aimed to assess the diversity of mycoflora and their bioremediation potential in specific industrial effluents within certain regions.

MATERIAL AND METHODS

Preparation of Chromium (VI) Stock Solution

A solution of chromium (VI) was prepared using the standard procedure from potassium chromate salt (K_2CrO_4) as adopted by Garg *et al.* (2004). The molecular mass of the overall salt is 194, with potassium (K_2) having $39 \times 2 = 79g$, chromium (Cr) with 52g, and oxygen (O_4) having $16 \times 4 = 64g$, totaling 194g MM. To obtain 1g of Cr from the 194g mixture (K_2CrO_4), $194g \div 52g = 3.73076$, approximating 3.731g. 1000 cc of distilled water dissolved 3.731g of K_2CrO_4 to create a 1000 ppm Cr solution. Experimental solutions with the required concentrations were prepared through a series of dilutions. The initial pH values ranged from 2.0 to 10.0 and were adjusted by adding diluted HCl or NaOH solutions.

Isolation of Heavy Metal-Resistant Mycoflora

The effluent samples, collected in 2 L sterilized sample bottles following the procedures of Daizee and Raman (2015) and Abdullahi and Machido (2017), were allowed to stand, settle, and concentrate through sedimentation at room temperature on a thoroughly sterilized and disinfected laboratory bench for 30 minutes. The supernatant was decanted to about 50% of the total volume and then vigorously shaken. Subsequently, 10 ml of each sample was transferred separately into sterile centrifugation tubes in replicates and spun at 250 rpm for 10 minutes using a centrifugation machine (Model: 800D) to further concentrate the spore propagules before aseptically transferring a 0.1 ml aliquot of the suspension onto freshly prepared sterile potato dextrose agar and yeast extract agar following the method described by Ezeonuegbo *et al.* (2014). All plates were incubated aerobically inside an incubation room at $28 \pm 2^\circ C$ on a disinfected cupboard for 7 days. Subcultures were performed continuously until pure cultures were obtained. Part of the colonies was selected for further analysis, while the remaining ones were utilized for molecular identification as outlined by Ramachandran *et al.*, 2022).

Preparation of Fungal Adsorbent

The adsorbent was prepared using the method adopted by Kalaimurugan *et al.* (2020). To generate high biomass of conidiophores, the isolated fungal strains were cultivated on potato dextrose broth (PDB) medium and incubated at $28^\circ C$ with agitation at 100 rpm on an orbital shaker for 5 days. The biomass was centrifuged at 12,000 rpm for 15 minutes in a speed centrifugation machine with rotor no.

JLA. 16. 250, at 20 °C. The pellets were pretreated by washing them in distilled water for 15 minutes (deactivation), followed by another wash in distilled water, filtration through a 2 mm pore size muslin cloth, and drying at 80 °C in a hot air oven for 72 hours. Finally, they were ground into a powder for adsorbent use. The supernatant was discarded after centrifugation.

Batch Adsorption Experiment

This is one of the most widely used experiments to determine adsorption equilibrium and kinetics from solutions. It is also referred to as an immersion experiment and was conducted following the methods described by Brandani (2021) with slight modifications. A known mass of adsorbent (0.1 g) was transferred into a 250 ml conical flask containing a known initial concentration (100 to 500 ppm) of chromium solution. The experiment was carried out at a neutral pH of 7, with an agitation speed of 100 rpm, a temperature of 25 +/- 2 °C, and varying agitation periods. The mixtures were prepared in duplicate, and after a specific time interval, they were micro-centrifuged for five minutes at 12,000 rpm. The supernatants were mixed with 200 µl of 2,5-diphenyl carbazide, and the absorbance was measured using a spectrophotometer. Subsequently, the concentration of heavy metals in the post-incubated filtrate of the fungal biomass was calculated from the absorbance using the following formulae:

$$\text{Metal Uptake (Q)} = \frac{V(C_i - C_f)}{M(\text{mg})} \quad \text{while}$$

$$\text{Removal Efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where:

- Q = Metal uptake (mg metal per g of biosorbent),
- V = Liquid sample volume (ml),
- C_i = Metal in the solution the Initial concentration (mg/L),
- C_f = Final concentration of the metal in the solution (mg/L) and
- M = Amount of the added biosorbent on a dry basis (mg).

RESULTS AND DISCUSSIONS

Nine metal-tolerant isolates were analyzed for their susceptibility; MIC results showed that all were resistant to Cr (VI), with one isolate exhibiting the best response, which was further characterized and utilized for metal absorption experiments. Following morphological identification, the isolate's partial sequence of ITS1 and ITS4 matched perfectly with *Aspergillus flavus*. The nuclear region was sequenced using universal primers: ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') followed by ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The resulting sequence was blasted in the NCBI database, revealing a 100% identity, 0.0 E-value, 89% queried cover, and a total score of 1350 against the *Aspergillus flavus* GenBank accession (MN095167.1). The phylogenetic position of

the studied and sequenced *Aspergillus flavus* is illustrated in Figure 1.

Figure 2 displayed the metal uptake profile for the biomass of *A. flavus*. After attaining equilibrium at 15 min Q_t = 159.201 mg/g, the metal absorption capability of Cr (VI) increased steadily concerning the increase in contact time, although it eventually stabilized. Similarly, up to 10 minutes (99.65%), the percentage removal rose along with the contact period, and after that point, there was no discernible rise in the Cr (VI) removal. From chromium-contaminated locations, high concentrations of *Aspergillus* sp. chromium-resistant fungi were identified, and these fungi can all convert chromium's toxic effects into non-toxic forms (Viegas *et al.*, 2017). *Aspergillus niger* and *Aspergillus fumigatus* biomass combined with nanoparticles showed a Q_{max} of 249.9 mg/g, adsorption of Cr (4) ions via a nanolayer type (Saravanan *et al.*, 2022). Research indicates that when coupled with *Azadirachta indica* oil cake, bacteria that are highly tolerant of Cr (VI) could adsorb 64.4% of the substance (Govarthanan *et al.*, 2019).

Effect of Biomass Concentration and Contact Time

The impact of biomass concentration (0.025 g to 0.150 g) and contact period (5 min to 120 min) was investigated on the metal absorption. *Aspergillus flavus* biomass was utilized to examine the biosorption of Cr ions while maintaining consistent levels of other parameters. The percentage of removal increases upon increasing the adsorbent dosage from 0.025 to 0.125 mg/L, with a maximum removal rate of 99.56% at 0.125 mg/L. Conversely, the pattern for uptake capacity was inversely correlated with the dosage of the adsorbent. As the dosage of the adsorbent is increased, the uptake capacity decreases. The highest uptake (723.223 mg/g) was measured at a dosage of 0.025 g/L, while the lowest uptake (132.701 mg/g) was recorded with a dosage of 0.15 g/L (Figure 3). A value of 398.490 mg/g was achieved at a dosage of 0.05 g/L. The increased binding sites the biomass provides, which enhances the biosorption behavior of *A. flavus* biomass, might cause the maximum absorption seen at 0.025 g/L. The results contradict other studies that found a clear correlation between the rise in absorption capacity and the concentration of the biosorbent (Ayele *et al.*, 2021; Zhou *et al.*, 2021). However, the decline in removal beyond the optimal dose may be related to the adsorbent and adsorbate reaching equilibrium under the given ideal conditions. This could be the case since, initially, there is a greater surface accessible for the adsorption of Cr (VI) ions (Kumar *et al.*, 2008).

The graph illustrates the observed Cr (VI) adsorption as a function of contact time. The results indicated that the percentage of Cr (VI) removal increased as the contact duration extended up to 10 minutes (99.65%). Beyond ten minutes, there was no significant increase in Chromium (VI) elimination. Similarly, the uptake capacity increased over time before reaching a plateau at 15 minutes, Q_t = 159.201 mg/g (Figure 3). This stabilization can be

attributed to the reduced accessibility of the surface area over prolonged periods, hindering the binding of additional metal to the biosorbent's surface. These findings are consistent with those of Ramachandran *et al.* (2022).

Effects of pH on Biosorption of Chromium (VI) ions

The investigation examined the ability of the *Aspergillus flavus* strain to adsorb chromium (VI) across a pH range of 2 to 12, considering the initial solution (Figure 4). The results indicated that the solution's pH significantly influences Cr (VI) absorption. The removal percentage and absorption capacity of Cr (VI) were highest at pH 4 and 6. Specifically, a pH of 4 yielded the maximum removal of 99.96% and absorption of 50.42 mg/g, while a pH of 6 resulted in the second-highest removal percentage of 99.89% and absorption of 50.23 mg/g. Conversely, the lowest absorption (49.05 mg/g) and removal percentage (98.11%) were observed at pH 10. Consequently, for the optimization process, a pH of 4 was selected. The experiment conclusively demonstrated that a pH of 4 is optimal when utilizing *Aspergillus flavus* biomass for biosorption. The sluggish adsorption behavior at low pH levels can be attributed to the high concentration of hydrogen ions, which compete with the positively charged Cr (VI) cations, diminishing their ability to bind to surfaces, in line with the findings of Rose and Devi, (2015); and Vilar *et al.* (2005). As the pH increases, the surface functional groups of the adsorbent ionize, enhancing the overall negative charge and facilitating adsorption (Rose and Devi, 2015).

Effects of Initial Concentration on Biosorption

Figure 5 illustrates the behavior of Cr (VI) removal at pH 4 and 0.1 g/L adsorbent dose, with initial Cr (VI) concentrations ranging from 50 mg/L to 500 mg/L. The findings suggest a positive correlation between the initial Cr (VI) concentration and absorption capacity. However, the removal percentage decreased, peaking at 99.91% at

300 ppm and declining to 500 ppm. 500 ppm was the concentration at which the best absorption uptake was achieved ($Q_t = 249.13$ mg/L). Reusing material after the procedure is finished is crucial since biosorbent is inexpensive. The negatively charged functional set of biosorbent surfaces reacted with the cation of Cr (VI).

In most cases, there was far less interaction between the metal ions and the biosorbent than with other types. Synthetic water containing metal ions interacts electrostatically with the biosorbent's surface (Han *et al.*, 2020). When interacting with metal ions like copper, lead, and cadmium, absorption demonstrated a similar result (Ouyang *et al.*, 2019).

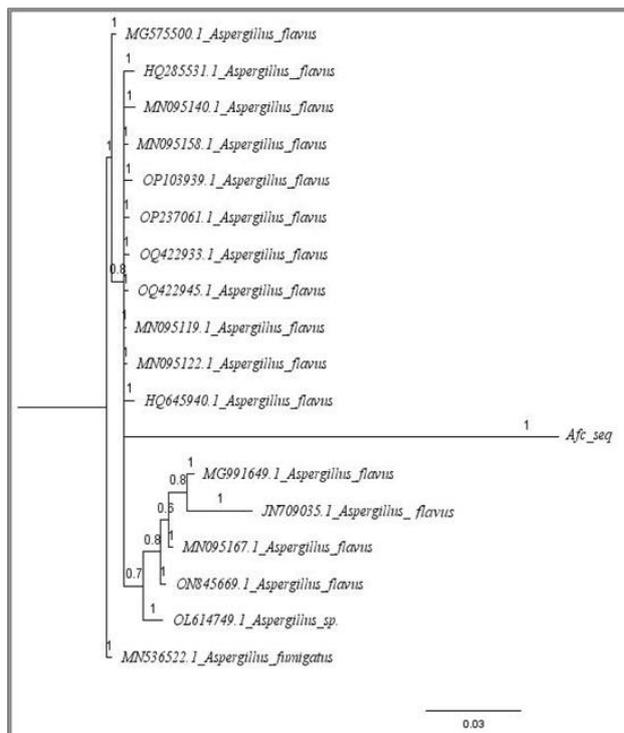


Figure 1: Phylogenetic Tree of the Isolated strain of *Aspergillus flavus* (Afc seq)

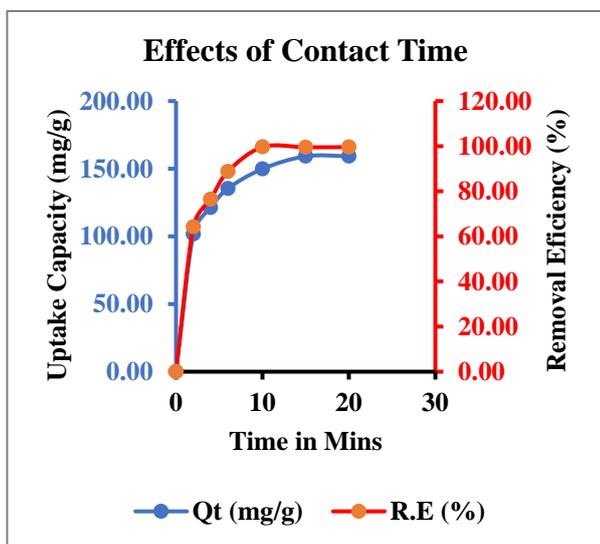


Figure 2: Effects of Contact Time on Biosorption.

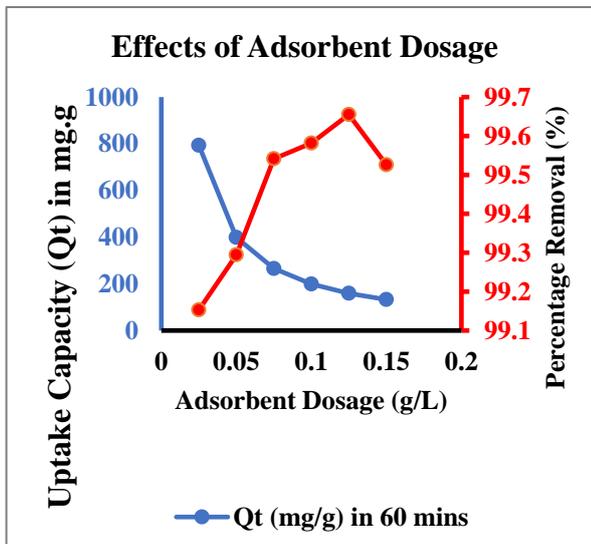


Figure 3: Effects of Adsorbent Dosage on Biosorption.

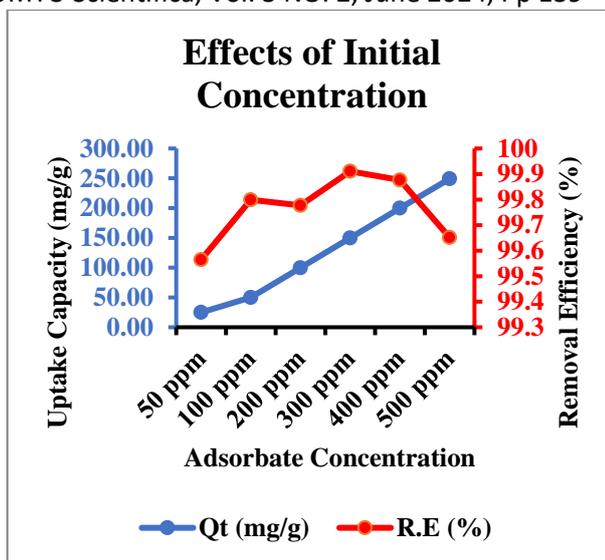
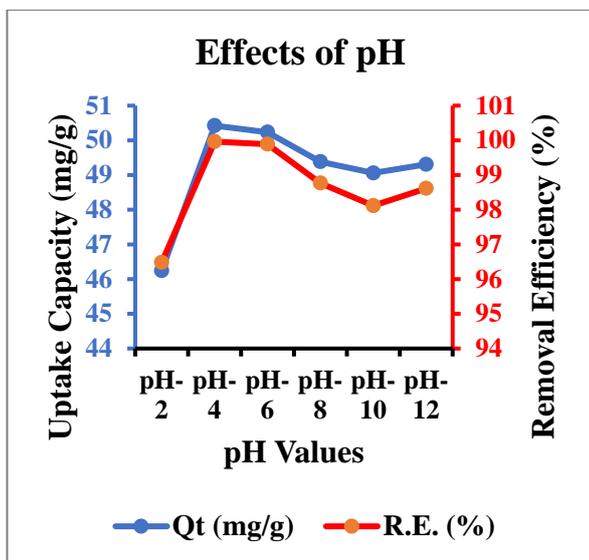


Figure 4: Effects of pH on Biosorption.

Figure 5: Effects of Initial Concentration on Biosorption.

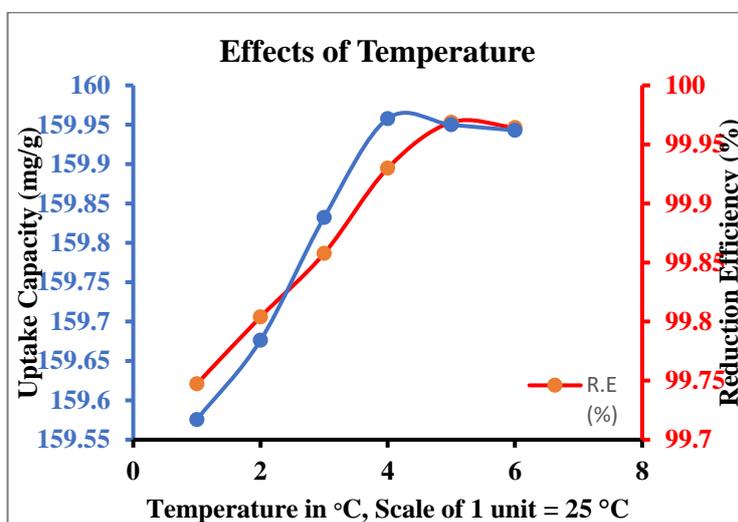


Figure 6: Effects of Varying Temperature on Biosorption.

Effects of Varying Temperature on Biosorption

Effects of temperature were similarly observed to increase both the removal efficiency and the uptake capacity with a rise in temperature from the initial temperature of 20 °C to 35 °C in uptake capacity (159.96 mg/g) and 40 °C in removal efficiency (99.97%). Both the percentage removal and the uptake capacity slightly decreased at 45 °C, with 99.97% and 159.94 mg/g, respectively (Figure 6). Time also affects adsorption as the availability of binding sites keeps reducing over time. Initially, many empty surface sites were available for adsorption, but adsorption slowed significantly beyond the ideal contact period due to the exhaustion of these sites and the repulsive force between the solute molecule and the bulk phase (Bishnoi *et al.*, 2004; Kumar *et al.*, 2008).

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