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Biosorption of hexavalent chromium using immobilized fungal biomass and optimization of biosorption parameters

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Tannery operations in and around Kolkata extensively use chromium salts for leather tanning, posing significant environmental challenges due to lax handling practices, illegal discharges, and a general lack of awareness or proper infrastructure. Our study not only aims to demonstrate the feasibility of using fungal biomass for chromium removal but also to optimize the conditions under which maximum biosorption can be achieved. By integrating this bioremediation technique into existing tannery operations, it is possible to significantly reduce the environmental impact of chromium pollution, contributing to a healthier ecosystem and improved public health.The immobilized biomass from a Cr (VI) tolerant*Aspergillus nomius*strain was explored or its biosorption capacities and conducting corresponding desorption studies. The immobilized fungal biomass (0.5 gm) yielded maximum biosorption rates at – 98% when an optimum 2% Na-Alginate concentration was used at pH 6. The immobilized beads were recycled for at least 3 rounds and showed decent retention of biosorption efficiency from 98.7% to 60.09% after the 3rd round. The data gives an insight of the potential of immobilized biomass to be used as bisorbent. Desorption studies showed maximum desorption efficiency – of 18% at pH 10, 33% when the biomass was pretreated with sulphuricacid; 8% with hydrochloric acid; 5% with acetic acid and 3% when immobilized beads were used. Comparative analyses were done on the effect of experimenting on pure dichromate solution in lab and tannery effluent with immobilized biomass, taking into account important parameters such as influence of Sodium Alginate strength, pH and pretreatment of biomass – in all cases higher percentages seen with pure dichromate solution.

Keywords : Biosorption, Desorption, Fungal Biomass, Hexavalent Chromium, Immobilization

INTRODUCTION

The removal of toxic heavy metals from aqueous streams is a critical challenge for industries discharging effluents containing these pollutants. Conventional methods like chemical precipitation and reverse osmosis, although widely used, often result in incomplete metal removal, high reagent and energy demands, and the generation of toxic sludges and other waste products that require careful disposal.

Biosorption is defined as a metabolically independent process that entails the removal of inorganic and organic substances from solutions using biological materials (Gadd, 2009; Hansda*et al*. 2016). Despite the broad range of potential pollutants, the majority of biosorption research has concentrated on metal removal. Metals are distinct from other contaminants due to their non-biodegradable nature and propensity to accumulate within the food chain (Abdi and Kazema, 2015; Rao and Prabhakar, 2011; Alluri *et al*. 2007). As an environmentally sustainable and cost-effective alternative, biosorption offers a viable solution for metal removal.

In the pursuit of effective treatment technologies for heavy metal ion removal, the use of microorganisms has garnered significant interest. Notably, the utilization of fungal microorganisms for heavy metal removal has attracted increasing attention. Various fungal species, including *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Saccharomyces*, and *Trichoderma*, have also demonstrated the capability to remove heavy metals from aqueous solutions.Both living and dead fungal cells have been found effective in adsorbing metal ions, with the use of dead cells

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receiving heightened attention due to several advantages..

Dead microbial biomass offers several advantages over living cells: it is low-cost, does not require nutrient media or maintenance of pure cultures, exhibits high sorption and desorption rates, operates over a wide pH range, requires simple equipment, and allows for rapid and easy regeneration of used biomass. The cell wall of microbial cells plays a pivotal role in metal ion removal from aqueous solutions, attributed to the presence of numerous functional groups with diverse charges and geometries, such as carboxyl, hydroxyl, amino, imidazole, sulfate, and sulfhydryl groups (Gadd, 2009).

Powdered dead fungal biomass has been the preferred choice in numerous studies for the adsorption of toxic metal ions from aqueous solutions. This form has been conveniently used in batch adsorption studies. However, in an industrial context, operating in batch mode may pose challenges in separating the biomass from aqueous streams post-adsorption. Upon contact with water, the biomass becomes soft, and its small particle size, low density, and strength can complicate column applications and the separation of biomass from treated effluent. Immobilization of fungal biomass powder in a solid matrix can address these issues. Immobilized biomass facilitates easier separation from aqueous solutions, enhances biomass strength, and improves handling.Live fungal cells have been immobilized in sand and biomass support particles, though specific details on the support materials used are often lacking. Various materials have been employed for immobilizing dead fungal biomass, including textile fibers, polyacrylamide, alginate, polysulfone, and inorganic compounds. Polysulfone has also been used to immobilize peat for metal ion removal from aqueous streams.Despite the promising applications, there is limited information available on the immobilization of dead fungal cells specifically for metal ion removal from aqueous solutions.

Microbial biosorbents often exhibit characteristics such as small size and low density, which can lead to challenges including insufficient mechanical stability and low elasticity. In dynamic flow mode biosorption, additional difficulties may arise, such as issues with liquid solid phase separation, sorbent swelling, clogging, and low regeneration rates. These challenges underscore the need for strategies to enhance the performance and efficiency of microbial biosorbents in dynamic flow systems.One effective approach to addressing these challenges is the immobilization of microbial biomass onto a suitable carrier. This process can produce particles ranging from 0.5 to 1.5 mm in size, characterized by good porosity and physical and chemical stability. Immobilized biomass offers the advantage of easy regeneration and reusability, making it suitable for incorporation into both fixed and fluidized bed columns

However, living cells are susceptible to heavy metal toxicity and adverse operating conditions. Therefore, using metal-resistant cells can be preferable for metal removal (Malik, 2004).Immobilized biomass offers several advantages such as easy regeneration and reusability, which are beneficial for incorporation into fixed and fluidized bed columns (Ramrakhiani, *et al* 2016;Andrès, *et al*. 2003). The immobilization technique is crucial for the practical application of biosorption processes (Volesky, 2003).Among these, sodium alginate with a crosslinking agent like $CaCl_2$ is one of the most frequently employed natural carriers for immobilization, owing to its high biocompatibility and the simplicity of the gelation process (Ivánová*et al*. 2010). The biosorption capacity of primary biomass can be enhanced by chemically modifying it before immobilization, which involves introducing additional functional groups to facilitate metal ion binding, thereby increasing biosorption efficiency (Mao *et al*. 2015; Li*et al.* 2013).

The efficiency of metal ion biosorption by immobilized microbial biomass is influenced by various factors, including the properties of the metal ions (such as ionic radius, degree of oxidation, and covalent index), process conditions (such as medium strength, initial metal ion concentration, biosorbent dosage and size), density of sorption centers (which depends on the biomass used and its pretreatment), as well as the carrier and immobilization technique

employed. Experimental data obtained from biosorption studies are often modeled and simulated to elucidate the process mechanism, evaluate changes in operating parameters, and optimize the biosorption process. Numerous models applicable in batch or continuous column modes have been documented in the literature to provide insights into the biosorption mechanism and optimize the efficiency of metal ion removal (Michalak *et al.* 2013).

Despite the advantages, biosorption using immobilized biosorbents presents certain drawbacks, including additional costs associated with immobilization, higher mechanical diffusion resistance, reduced biosorbent capacity compared to free biomass, and potential interactions between the carrier and the active sites of the biosorbent. These limitations have hindered the widespread commercialization of biosorption with immobilized microbial biomass for metal removal from natural and wastewater sources. This lack of commercialization is due to the aforementioned drawbacks and the incomplete understanding of the exact mechanism of the process. Further research is needed to address these challenges and unlock the full potential of biosorption with immobilized biosorbents for practical applications (Dhankar and Hooda, 2011; Fosso Kankeuand Mulaba-Bafubiandi, 2014).

The current research article explores the use of immobilized biomass of *Aspergillus nomius* for biosorption and desorption studies of hexavalent chromium and optimization of various process parameters for maximum biosorption efficiency. A comparative study was conducted with pure chromium solution and tannery effluent to test the effect of biosorption and desorption under actual field condition.

MATERIALS AND METHODS

Microorganism

Isolation and thereafter, identification of the most chromium tolerant strain was done by fungal ITS sequencing analysis and phylogeny. The strain was identified as *Aspergillus nomius* (Guha *et al*. 2020).This strain was maintained on Czapekdox agar medium with regular subculturing after every 15 days and preserved at 4^0C.

Chromium (VI) solution preparation

Aqueous (stock) solution of chromium (VI) concentration 1 g/L was prepared by dissolving 2.83 g of potassium dichromate salt in 1000 ml distilled water. Concentration of chromium was varied as required.

Colorimetric assay of Cr (VI) by Diphenylcarbazide (DPC) method and standard curve graph preparation

Hexavalent chromium forms a purple colored complex with diphenyl carbazide and methanol at low pH and at very low concentrations. The test is very sensitive but at lower concentrations. Known concentrations of hexavalent chromium was assayed at 540nm and recorded in order to prepare standard curve for future experiments and unknown Cr (VI) concentration determination (USEPA).

Biosorption efficiency calculation

Biosorption efficiency (%) was calculated using the following equation (USEPA):

$E= (C_i-C_f /C_i) \times 100$

Where,

E=Percentage removal of hexavalent chromium

C_i= initial metal ion concentration, g/L

C_f= final metal ion concentration, g/L

Each experiment was repeated three times in order to get accurate results and the average was taken for the calculation of biosorption efficiency.

Immobilization methods

For the immobilization of biomass by entrapment method the powdered biomass was immobilized by entrapment in the polymeric matrix of sodium alginate beads. A slurry of 100ml sodium alginate was prepared in hot (60^0C) distilled water. After cooling, 0.5g of biomass was added and stirred. The alginate–biomass slurry was then extruded into 3% (w/v) $CaCl_2.2H_2O$ for polymerization and bead formation. The resultant beads were of 2-3

mm diameter and were cured in the polymerizing medium for 3 hrs. The beads after biosorption experiments were also studied for their re-usability.

Effect of varying Sodium Alginate concentration on biosorption by Immobilized Biomass

0.5 g of powdered biomass was mixed with different concentrations of Sodium alginate (1%, 2% and 3%) to form the beads and biosorption was studied in 100 of 0.001 g/L concentration of chromium solution, at pH as 6.0 and temperature 37ÚC. A control sample solution (2% and without biomass) was also made. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540 nm.

Immobilization with varied amount of biomass loading and its effect on biosorption

Beads were preparedby mixing 2% Sodium-Alginate and varied amount of biomass load (0.5g, 1g and 2g)and biosorption was studied in 100 ml and 0.001 g/L concentration of chromium solution, at pH as 6.0 and temperature 37ÚC. Then the flasks were agitated in a shaker at 150 rpm and after 24 hours, the samples were taken out, filtered and analyzed using DPC assay at 540 nm.

Effect of pH on biosorption with Immobilized biomass

Powdered fungal biomass (0.5g) was mixed with 2%sodium alginate to form the beads. The biosorption efficiency was studied using 100 ml of 0.001 g/L concentration of chromium solution at pH 4, 6 and 8 respectively at temperature of 37ÚC. Then the flasks were agitated in a shaker at 150 rpm and after 24 hrs, the samples were taken out, filtered and analyzed using DPC assay at 540 nm.

Reusability of the biomass Immobilized beads
After one round of biosorption with the biomass immobilized beads, the beads were washed twice with double distilled water and used for the next round of biosorption.

Desorption studies with treated and immobilized biomass

It is essential to remove the metal ions from the biosorbents after the completion of biosorption to test for the desorption potentials. Hence, desorption experiments were carried out with NaOH as the desorping agent. The dead fungal biomass and biomass immobilized beads after biosorption was collected and weighed. The desorbing reagent was prepared at the concentration of 0.5 N (100ml) and the 0.5g adsorbed fungal biomass and the used immobilized beads were added separately and kept in shaker for 24 hours. After incubation, the reaction mixture was centrifuged and the supernatant was subjected to the estimation of chromium (desorbed) by DPC method.

Effect of biomass pretreatment and immobilized biomass on desorption

The fungal biomass was pretreated with 1(N) Sulfuric Acid, Hydrochloric Acid and Acetic Acidseparately asthat worked best as we learned from our previous studies(Guha *et al*. 2021). The pretreated biomass were then subjected to Cr (VI) recovery experiments using 0.5 N solutions of NaOH to neutralize the acid pretreated Cr (VI) biosorbed biomass. For this desorption experiment, 0.5g dry biomass pretreated with 1 (N) Sulfuric Acid, Hydrochloric Acid and Acetic Acidrespectively were mixed with 100 ml Cr (VI) solution (0.001 g/l). After 24 h of biosorption, the bound Cr ions were allowed to desorb in 100 ml of the NaOH solution. The same procedure was also followed with the beads immobilized with the fungal biomass. Percentage desorption was calculated as the percentage release of Cr ions initially bound to the biomass.

Comparison of biosorption and desorption efficiency between pure Cr (VI) solution and tannery effluent

A comparative analysis was done to determine the biosorption and desorption efficiency under different conditions such as effect of pre-treatment, contact time, dosage, pH, temperature, Na-alginate concentration, etc. with 100 ml of pure Cr (VI) solution and tannery effluent to test the ability of the biosorbent under field condition.

RESULTS AND DISCUSSION

The results of biosorption experiments obtained by using immobilized beads with varying Sodium Alginate concentrations were studied. During comparison it was observed that increase in Sodium Alginate concentration from 1% to 2% increased metal biosorption from 58.3% to 98.2%. However, further increase in concentrations beyond 2% resulted in a decrease in Cr adsorption

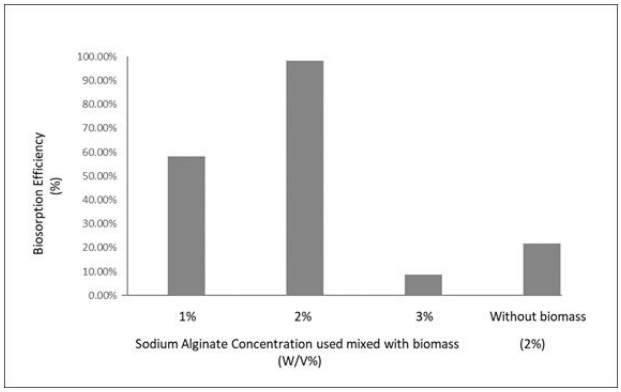

Fig.1: Effect of Na-Alginate Strength on biosorption efficiency

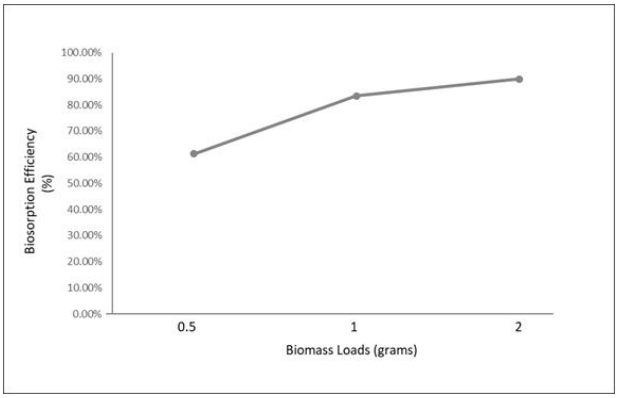

Fig.2*:* Effect of varying biomass loads within Na-Alginate beads on biosorption efficiency

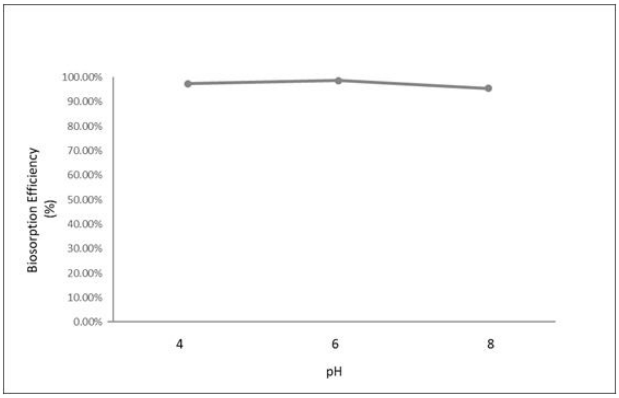

Fig.3: Effect of varying pH for immobilized beads with biomass on biosorption efficiency

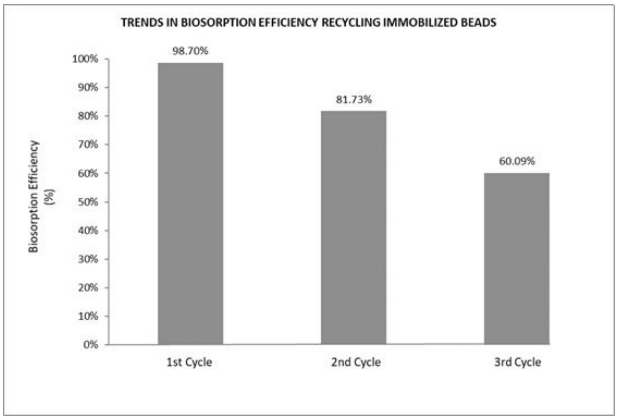

Fig. 4: Effect of recycling immobilized beads with biomass on biosorption efficiency

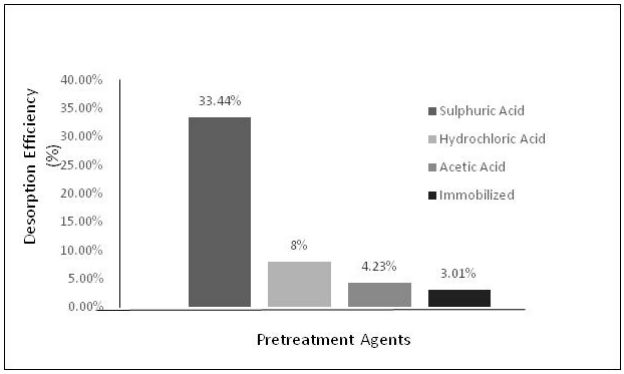

Fig.5: Effect of varying fungal biomass pretreatments and using immobilized biomass on desorption efficiency

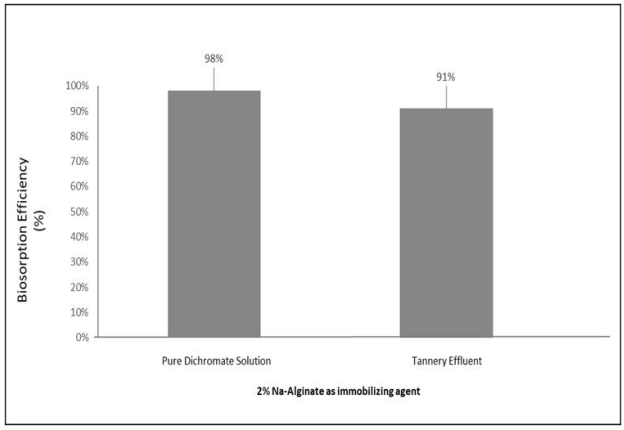

Fig.6: Comparative Analysis for Estimating the Effect of Sodium Alginate concentration for Immobilization of Biomass on Biosorption with Pure Dichromate Solution Vs. Tannery Effluent

i.e. when 3% Sodium Alginate concentration was used for bead formation, the biosorption plummeted to 8.8% (Fig.1). This can be attributed to the difference in porosity of the beads.Similar results were also reported by previous workerswho also found 2% Sodium Alginate concentration as most efficient in their immobilization studies(Riaz *et al.* 2009;

Geethanjali and Subhas, 2013). While 2% has the optimal porous nature for entry and trapping of the Cr (VI) ions, lower concentrations are too porous so there is an easy movement and less entrapment of ions and higher concentrations altogether reduces the porous nature and also surface area for trapping of ions. The control bead without any biomass made using 2% Sodium Alginate concentration independently showed biosorption of 21.8%.

The results of biosorption experiments obtained by using immobilized beads with varying fungal biomass loads were studied. For all matrices compared, increase in biomass loads from 0.5g to 2g increased metal adsorption from 61.23% to 89.9%(Fig.2). This could be due to the fact of using 2% Sodium Alginate forming beads that provided ample porosity for the biomass to get trapped and enhance the biosorption potential of the bead itself by providing optimal surface area for the Cr (VI) ions to get biosorbed and trapped.

The results of biosorption experiments obtained by using immobilized beads with 0.5g fungal biomass loads were studied for varying pH. For all matrices compared, increase in pH showed increased metal adsorption optimally at pH 6 (98.7%). Beyond pH 6 biosorption slowly started to drop (Fig.3). This could be due to the fact that the lower pH causes the protonation of binding sites in the microbial surface, and thereby imparts negative charge to microbial surface, which in turn contributing metal binding of Cr (VI) ions.DoðaçandTeke 2(013) reported pH 6 as optimum for their immobilization studies. The point of zero charge for the biomass would be around pH 6 and as mentioned for that reason. Further increase in pH caused reduction in biosorption. Amino groups become positively charged at low pH levels. Metal ions are attracted to these groups electrostatically, and complex formation involving N or O donor atoms, such as those present in chitin and chitosan, is also possible (Volesky, 2003; Velkova*et al.* 2018).

In this study, the Sodium Alginate beads with fungal biomass, which were used for biosorption once, was collected and washed carefully with double distilled water. This biosorbent was subsequently used again in fresh biosorption studies, repeating the process for a total of 3 cycles to assess its reusability potentials.The results suggest gradual loss of biosorption potential with each cycle, which may be due to the gradual deterioration of biomass or increasing occupied sorption sites or the adverse effect of elution on sorption sites(Fig.4).Ma *et al.* (2018) also reported similar trend of decreasing efficiency of immobilized beads with each cycle in their study.

The desorption results using 0.5 N NaOH on variedly pretreated biomass and immobilized biomass was obtained. It was observed that Sulphuric Acid treated biomass was the most efficient bisorbent assisting desorption with efficiency at around 33%, while HCl treated biomass showed desorption efficiency of 8%, Acetic Acid treated biomass with desorption efficiency of 4.23% and finally immobilized biomass with desorption efficiency of 3.01%. With the immobilized beads the rate of desorption was very less may be due to the fact as the biomass along with the metal ions got entrapped within the beads it was very difficult to again elute it back into the solution(Fig.5).

Biosorption experiments were carried out with fungal biomass mixed with varying concentration of Na-Alginate for preparing the immobilized beads and then they were used on pure dichromate solution and tannery effluent respectively. When 2% Na-Alginate was used for making the immobilized biomass beads, maximum biosorption efficiencies of 98% and 91% was recorded for pure dichromate solution and tannery effluent respectively. The lower biosorption efficiency with the tannery effluent might be due to the fact that many impurities present in the effluent may have interfered with the biosorption process.

The fungal biomass was pretreated with 1 N H_2SO_4 and then were used to prepare the immobilized beads for biosorption and then desorption experiments were carried out with the immobilized biomass on pure dichromate solution and tannery effluent respectively. Maximum desorption efficiencies of only 33% with pure dichromate solution and 21% from tannery effluent were recorded in this case. Due to pure and clear nature of the dichromate solution maybe

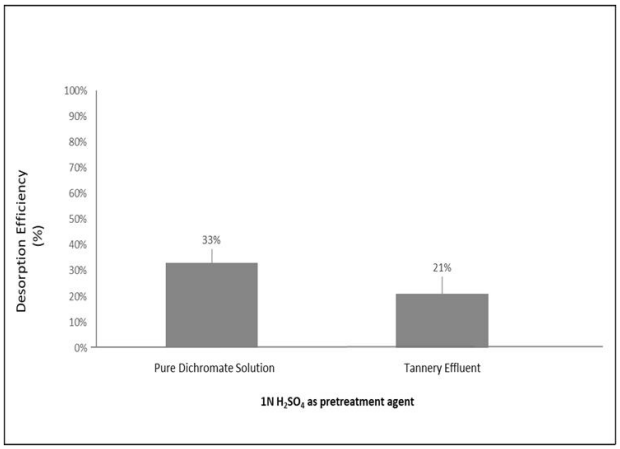

Fig. 7*:* Comparative Analysis for Estimating the Effect of Pretreatment of the Immobilized Biomass on Desorption with Pure Dichromate Solution Vs. Tannery Effluent

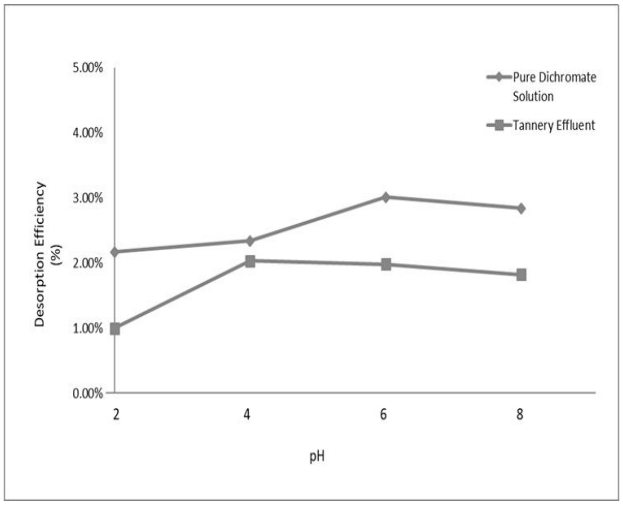

Fig.8*:* Comparative Analysis for Estimating the Effect of pH for Immobilized Biomass on Desorption with Pure Dichromate Solution Vs. Tannery Effluent

it was easier for biosorption due to less blockage of the micropores and also during desorption it may have been easier to release the Cr (VI).

Desorption experiments were carried out with immobilized fungal biomass at varying pH with pure dichromate solution and tannery effluent respectively. Maximum desorption efficiencies of only 3% for pure dichromate solution and 2% for tannery effluent respectively were recorded.

CONCLUSION

Our study on the use of sodium alginate-immobilized fungal biomass for the biosorption of Cr (VI) from tannery effluent has yielded several important conclusions, underscoring its potential in sustainable bioremediation applications.

The investigation revealed the optimum parameters for efficient biosorption by *Aspergillus nomius* biomass, immobilized in sodium alginate beads. We achieved maximum Cr (VI) removal under optimal conditions: 2 grams of fungal biomass in a 2% sodium alginate solution, reacting for 24 hours at room temperature, pH 6.0, and a reaction volume of 100 ml with an initial Cr (VI) concentration of 0.001 g/L. Notably, the immobilized biomass maintained a commendable biosorption efficiency of 60% even after three cycles of biosorption, indicating its potential for repeated use.

Comparative studies demonstrated that using 2% sodium alginate for immobilizing the fungal biomass resulted in maximum biosorption efficiencies of 98% for pure dichromate solution and 91% for tannery effluent. Biosorption experiments conducted at varying pH levels highlighted that maximum desorption efficiencies of only 3% for pure dichromate solution and 2% for tannery effluent were achieved. The fungal biomass, pretreated with 1 N H2SO4, exhibited desorption efficiencies of 33% with pure dichromate solution and 21% from tannery effluent. These results suggest that the clearer nature of the dichromate solution facilitated both biosorption and desorption processes due to reduced blockage of the micropores.

The desorption experiments using 0.5 N NaOH on pretreated and immobilized biomass showed that sulfuric acid-treated biomass was the most efficient biosorbent, achieving a desorption efficiency of approximately 33%. In contrast, HCl-treated biomass showed an efficiency of 8%, acetic acid-treated biomass 4.23%, and immobilized biomass 3.01%.

Through these studies, we have successfully determined and optimized various parameters crucial for Cr (VI) biosorption using immobilized fungal biomass. These findings not only demonstrate the feasibility and efficiency of this bioremediation approach but also provide a foundation for further research and development to enhance its practical application in treating tannery effluent.

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DECLARATION

Conflicts of interest. Authors declare no conflict of interest.

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