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Biosorption of Trivalent Chromium Using Ca-alginate Immobilized and Alkali-treated Biomass

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***Abstract*-Immobilized and alkali treated biomass showed a higher affinity towards trivalent chromium than live free biomass. Alginate, as a natural biopolymer, plays a key role in the removal of trivalent chromium. Alginate is a polysaccharide containing hydroxyl groups as function groups, which replace H_2O molecules in $[Cr(H_2O)_6]^{3+}$. In addition to that, chromium ions replace calcium ions in Ca-alginate matrices. Release of calcium ions in samples after treating with alginate is evidence of ion exchange. The removal percentages of trivalent chromium were 98.6, 98.5, 27 and 91.2% after treating 2 mM of chromium chloride hexahydrate solution with alginate free beads, alginate biomass beads, biomass live free and biomass live treated with Na_2CO_3, respectively. We reached the safe limit of discharge of trivalent chromium, which is lower than 5 ppm.**

Keywords- Biosorption; Trivalent Chromium; Alginate Free Beads; Alginate Biomass Beads; Alkali Treated Biomass; Inductively Coupled Plasma Spectroscopy

I. INTRODUCTION

Pollution of the surrounding environment accelerated after the industrial revolution, leaving a legacy faced by modern society. The growth of industry caused a considerable increase in the discharge of industrial waste to the environment, chiefly soil and water, which has led to the accumulation of heavy metals, especially in urban areas. Several of these heavy metals are toxic even at very low concentrations, e.g. arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc etc. They are considered carcinogenic and cytotoxic. Tannery effluent is a source of trivalent chromium (basic chromic sulfate), which is converted under certain environmental conditions to hexavalent chromium. Hexavalent chromium is more toxic than trivalent chromium and is also carcinogenic. Wastewater treatment methods include conventional methods like physical and chemical methods and unconventional methods like bioremediation. Bioremediation methods are currently receiving favorable publicity as promising, environmentally-friendly treatment technologies for the remediation of heavy metal. Moreover, biological methods can have an edge over the physicochemical treatment regimes, including chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies and adsorption on activated carbon in removing heavy metals, as they offer cost effective in situ biosorption of heavy metals by the microorganisms. Chemical precipitation and electrochemical treatment are ineffective, especially when the aqueous metal concentration is in the range of 1-100 ppm, and these treatments also produce sludge as byproduct, which is difficult to treat. Ion exchange, membrane technologies and activated carbon adsorption process are expensive, especially when used at large scale. Volesky (2001) [1] briefed the pros and cons of these conventional treatment methods.

Fungi as a biosorbent has high metal resistance to toxic heavy metals like chromium in both valence states, trivalent and hexavalent. The fungal cell wall structure of chitin also gives it an advantage as a good biosorbent. Certain fungal species have high reduction potential towards hexavalent chromium, where fungi species reduce hexavalent chromium to less toxic trivalent forms [2]. It is well known that fungi grow in tropic and humid areas. Temperature is one of the important factors that affects the bioremediation potential of fungi species.

Some methods are found to enhance metal biosorption, such as Alkali treatment of fungal biomass, which significantly increased the biosorption capacity [3, 4]. Grinding the biomass cells to a very small size offers a large surface area and thus more available binding sites [5]. An alkali pretreated biomass of *Mucor rouxii* was investigated for biosorption of divalent metal cations [6].

For industrial use of biosorption, it should utilize an immobilization technique to prepare eco-friendly, cost-effective biosorbents retaining the capability of the biomass to biosorb metal(s) during the continuous treatment process. Alginate was considered one of the most important immobilization materials [7]. Alginate has been widely used due to its biocompatibility and ease of modification. Because of its biodegradability, immunogenicity and ability to form gels with a variety of cross-

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linking agents and divalent cations, such as calcium, it produces thermally irreversible and water insoluble gel. It is widely used for metal ion remediation from aqueous solutions via adsorption [8]. Alginate is a bionatural polymer and is named after the algae species that are its sources. Alginate is a polysaccharide containing hydroxyl groups as function groups which can replace H_2O molecules in $[Cr(H_2O)_6]^{3+}$. The aim of this study is to compare the biosorption potential of Ca-Alginate-biomass beads and alkali treated biomass in the removal of trivalent chromium.

II. MATERIALS AND METHODS

A. Biomass Production and Treatment

The chromate tolerant fungi strain, fungal endophyte culture-collection STRI: ICBG-Panama: TK1285, was cultured in a liquid medium using the shake flask method. Spore and mycelium from the SPD agar plate culture were transferred to 500 ml Erlenmeyer flasks containing 200 ml growth medium. This growth medium had the following composition (g/l): dextrose 20g, peptone 10 g, NaCl 0.2 g, $CaCl_2.2H_2O$ 0.1 g, KCl 0.1 g, K_2HPO_4 0.5 g, $NaHCO_3$ 0.05 g, $MgSO_4$ 0.25 g, $FeSO_4.7H_2O$ 0.005 g. The pH of the medium was adjusted to 4 using 0.1 N HCl before autoclaving. Once inoculated, flasks were shaken on a rotary shaker at 150 rpm for 5 days at 30 ^{0}C. The dry weight of the biomass was determined after washing the harvested mycelia twice with de-ionized water and drying it to a constant weight at 105 ^{0}C. To maximize removal of Cr^{3+}, part of the biomass was treated with 0.5 M Na_2CO_3. After treatment, the powdered biomass was filtered and washed with deionized water until the pH of the wash solution was in the neutral range (pH = 7) [2].

B. Immobilization of Biomass

The powdered biomass of fungal endophyte culture-collection STRI: ICBG-Panama: TK1285 was immobilized by entrapment in a polymer matrix of Ca-Alginate. A 2% (w/v) suspension of sodium alginate was prepared in distilled water. After cooling, 5% (w/v) of the biomass was added and stirred with a magnetic stirrer. The alginate biomass suspension was introduced into a solution of 0.1 M $CaCl_2.2H_2O$ for bead formation using a 5ml syringe. The formed beads were of 4 mm diameter. The alginate biomass beads were left in this solution for 1 h and washed twice with 200 ml of sterile distilled water. Alginate free beads were also prepared and stored at 4 ^{0}C in 5 mM of $CaCl_2.H_2O$ solution until they were used [9].

C. Biosorption Studies

Biosorption studies were done in 100 ml Erlenmeyer flasks where the volume of the Cr (III) solution (2mM) was 50 ml, the temperature was 37 ^{0}C, the wet weight of the alginate beads was 10 g, the wet weight of the alginate biomass beads was 10 g (i.e., biomass dose= 0.5 g) and the pH was 3. Three replicates of all assays were performed.

D. Surface Characterization Using FTIR

The biomasses of fungal endophyte culture-collection STRI: ICBG-Panama: TK1285 before and after treating the chromium solution were analyzed using an infrared spectrophotometer (IR) (Model 470, Shimadzu corporation) adopting the KBr disk technique [10, 11].

E. Determination of Cr (III)

The trivalent chromium was measured in the samples after filtration with micro-filter paper of pore diameter 0.2 um using inductively coupled plasma spectroscopy (ICP).

III. RESULTS AND DISCUSSION

The 99% confidence interval is displayed as error bars in Fig. 1. The final chromium (III) concentrations were 1.52, 1.62, 75 and 9.02 ppm after treating 2mM of chromium Chloride hexahydrate solution with alginate free beads (i.e., free from biomass), alginate biomass beads, biomass live free (i.e., non-immobilized) and biomass live treated with Na_2CO_3, respectively. The removal percentages of trivalent chromium were 98.6, 98.5, 27 and 91.2% after treating 2 mM of chromium Chloride hexahydrate solution with alginate free beads, alginate biomass beads, biomass live free and biomass live treated with Na_2CO_3, respectively. The pretreated biomass of fungal endophyte culture-collection STRI: ICBG-Panama: TK1285 had a higher affinity towards trivalent chromium compared to native biomass (without pretreatment). The biomass of fungal endophyte culture-collection STRI: ICBG-Panama: TK1285 pretreated either by entrapment of the biomass in a Ca-alginate matrix or alkali treatment of biomass increased the negative charge density on the biomass surface and thus increased the attraction forces between the positively charged chromium ions and the negatively charged biomass surface. Ca-alginate played the key role in the biosorption of trivalent chromium in the case of Ca-alginate immobilized biomass beads, and that can be concluded easily from the results of the removal percentages of both Ca-alginate beads and Ca-alginate biomass beads. Both of them gave approximately the same removal percentages under the same conditions. The negatively charged surface of Ca-alginate existed due to the functional group of carboxylate, as proved later with FTIR. The ICP device pointed out the existence of Ca ions in the analyzed samples (filtrate). It is evidence of the ion exchange between Ca ions in the Ca-alginate beads and chromium (III) ions in the solution. As shown in Fig. 2 below, the clarity of the solution after treatment with alginate free beads and alginate

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biomass beads for 24 hours was significant. Adsorption of chromium chloride compounds on the alginate surface in both cases changed the color of alginate beads from colorless to blue. The negatively charged surface of alkali treated biomass existed due to carbonate groups. Alkali treated biomass is a better sorbent of trivalent chromium than live fungus [12].

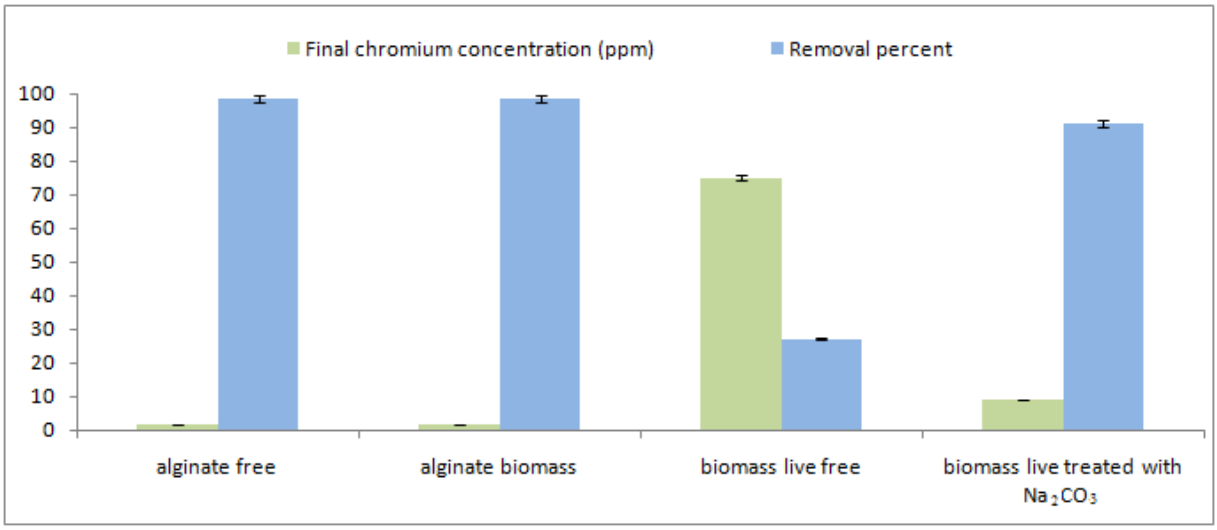

Fig. 1 Final concentration of Cr (III) and removal percentage of Cr (III) after treating 2mM of chromium chloride solution and incubation for 24 hours

Fig. 2 Bioaccumulation of Cr (III) on alginate (left) and alginate biomass beads (right)

A. FTIR Spectra of Biomass

The efficiency of metal sorption on the biomass surface is related to the functional groups present on the biosorbent [13, 14]. Thus, FTIR characterization was performed to determine the major functional groups that existed in the biosorbent. As shown from Figs. 3, 4 and 5 below, the main absorption bands in the case of live free biomass were observed at wavenumbers 3419.9, 1647.26 and 1545 cm^{-1}. The absorption band at 3419.9 cm^{-1} was assigned to (O-H) stretching or (N-H) stretching. The absorption bands at 1647.26 and 1545 cm^{-1} may be assigned to amide I and amide II (CO-NH) groups, respectively. The FTIR spectrum of alginate free beads exhibited absorption bands at 3437.26 and 1633.76 cm^{-1}, which can be assigned to (O-H) and asymmetric stretching vibrations of carboxylate (COO), respectively (Fig. 4). On the other hand, the FTIR of the immobilized biomass, Fig. 5, showed the main absorption bands at 3423.76 and 1643.4 cm^{-1}. The observed shift of the bands corresponding to the OH, amide and carboxylate groups supports the success of immobilization. Amide functional groups in the case of live free biomass caused repulsion forces with trivalent chromium cations and thus lowered the removal efficiency of the chromium ions. The carboxylate functional groups, in the case of alginate free beads and alginate biomass beads, caused attraction forces with chromium cations and thus increased the removal efficiency of chromium. This agreed with Bishnoi et al, 2007 [9], who reported that the positively charged amines of the fungal strain attracted chromate anions. Consequently, the positively charged amines created repulsive forces with cations. Abou-Shanab R. A. I. and E.E. Hafez (2006) [2] reported the same concept as well.

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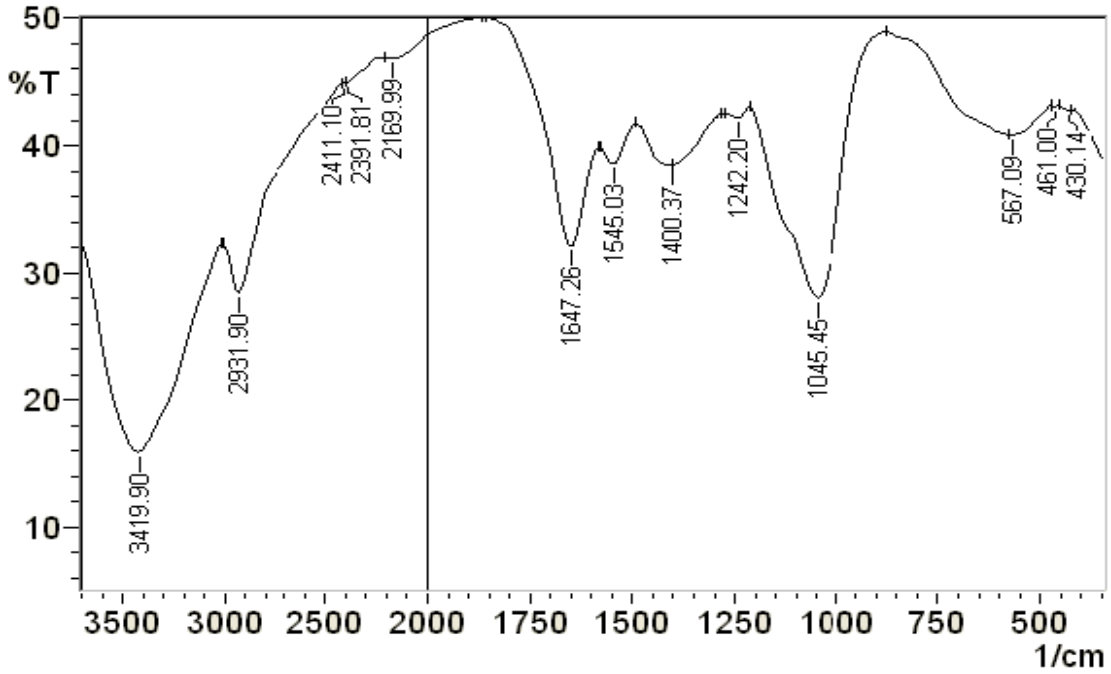

Fig. 3 FTIR spectra of live free biomass of fungal endophyte culture-collection STRI:ICBG-Panama:TK1285

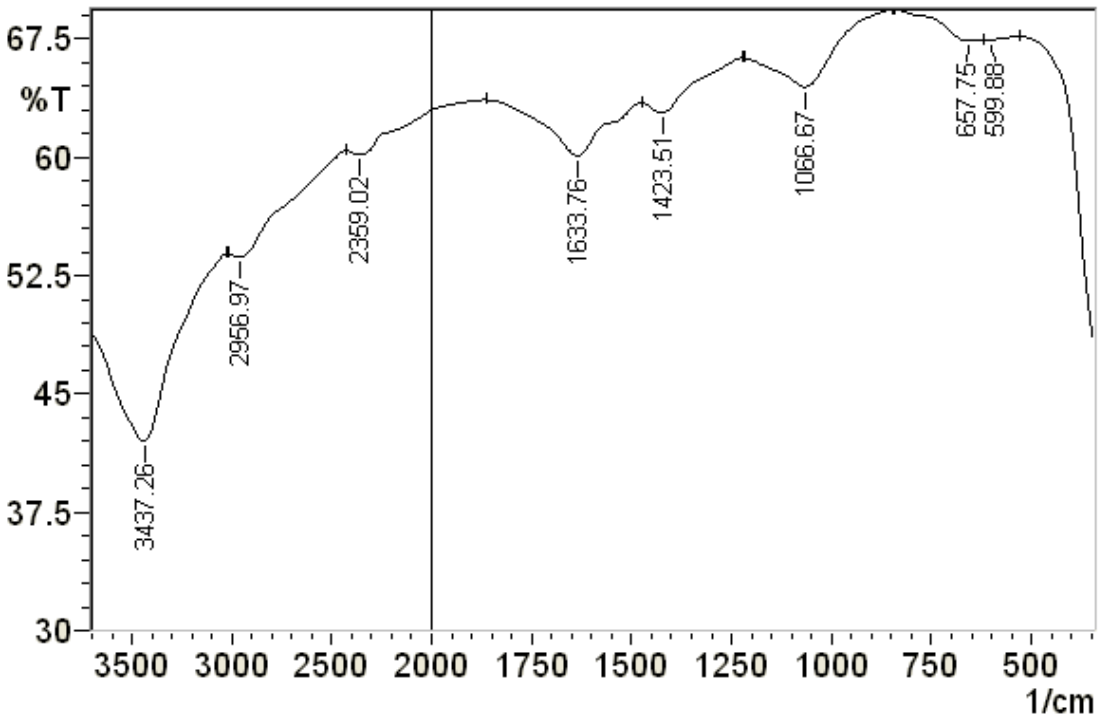

Fig. 4 FTIR spectra of alginate free beads

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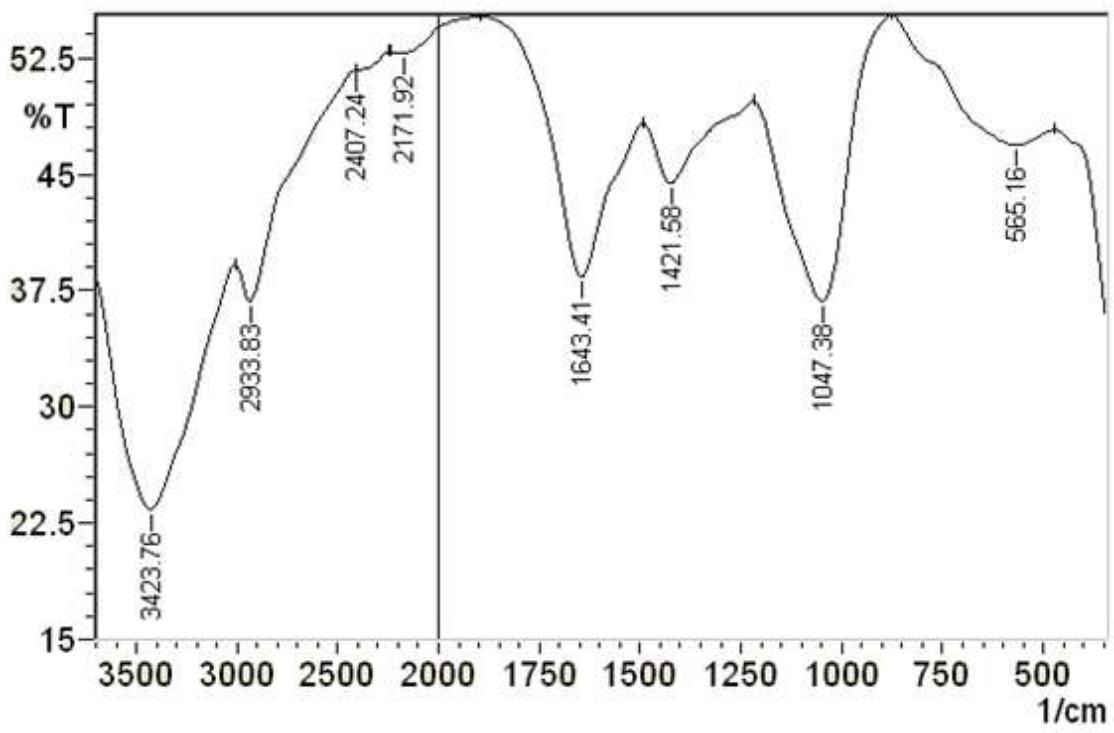

Fig. 5 FTIR spectra of biomass immobilized in Ca-alginate

IV. CONCLUSION

Immobilization and alkali treatment of the biomass of Fungal endophyte culture-collection STRI:ICBG-Panama:TK1285 improved its potential to sorb trivalent chromium. Ca-alginate biomass showed a higher affinity towards trivalent chromium, where the removal percent reached 98.5%. Moreover, its ease of use and separation from the solution after treatment made it more applicable in real-world situations. Fungi exhibited a high reduction potential towards hexavalent chromium where fungi reduced hexavalent chromium to a less toxic form, while Ca-alginate beads exhibited a high biosorption potential towards trivalent chromium.

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