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Anaerobic Cr(VI) Bioaccumulation: Application to Industrial Wastewater and Soil Matrices in Jordan

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Chromium(VI) in the form of CrO_4^{2-} or $Cr_2O_7^{2-}$ is known to be very toxic causing cell mutation and imposing serious health and environmental impact that mandates the removal of this toxic element. In this study, an investigation of the anaerobic Cr(VI) bioaccumulation by microbial biomass originated from wastewater influent utility was conducted and optimized for different conditions and parameters. These parameters include Cr(VI) initial concentration, nutrient type and concentration, temperature and pH. The obtained results indicated that the bioaccumulation process involves an adsorption, coupled with metabolic reduction mechanism. This bioaccumulation varies inversely with pH, proportionally with temperature, proportionally with Cr(VI) initial concentration in the presence of sucrose as nutrient; and inversely with Cr(VI) initial concentration when using glycerol and sodium acetate as nutrients. The optimum conditions for best removal of Cr(VI) were 11 ppm of Cr(VI) initial concentration, 20 mM sucrose, at 60 °C and pH = 1. The optimized conditions were applied to industrial wastewater sample and soil sample from Arab Aluminum Industries plant in Jordan. The percentages of removal of Cr(VI) were 91 and 93 %, respectively over 8 days at 25 °C making it a potential efficient and low cost remediation method for Cr(VI) removal.

Keywords: Cromium(VI), Industrial wastewater, Anaerobic, Bioaccumulation, *E. coli*, Cr(VI) reduction, Biosorption.

INTRODUCTION

The release of hexavalent chromium compounds from anthropogenic sources into the environment is of great consideration. These sources cover a wide range of industries like electroplating, anodization, metal finishing and chromating[1-4]. Different methodologies have been used and proposed for the remediation of Cr(VI) contaminated industrial wastewater, soil, municipal wastewater and aquatic systems[5] including adsorption[6], ion exchange[7], precipitation[8], cementation[9] and electrocoagulation[10], where the most common used of these methods are precipitation and adsorption. All of the above methods have their own drawbacks. The precipitation methods suffer from sludge accumulation; rise in the cost and loss of specificity in the presence of other ions in case of adsorption methods and adding secondary pollutants to the environment when using the coagulation methods[11]. Biological methods have gained much of interest recently due to being effective in Cr(VI) removal, cost effective, improved selectivity and being environmentally friendly. Biosorption of Cr(VI) is a process that involves the use of inactive or dead biomass (bacteria, fungi, or algae) for Cr(VI) removal; whereas bioaccumulation is the process of intake of toxicants by living organisms[12]. A disadvantage of using the bioaccumulation is the biomass saturation and the trouble of culturing some bacterial strains free of contamination that are chromium(VI) resistant under sterile conditions[13]. In the present work, bioaccumulation of Cr(VI) is studied using wastewater influent as a natural source of microbial mass under anaerobic conditions, where *E. coli* was used as indicator of fecal bacteria. Factors affecting the bioaccumulation process, including pH, temperature, Cr(VI) concentration and type and concentration of nutrient were studied and optimized. The bioaccumulation optimized parameters were tested on real samples for industrial wastewater and soil from same location from the Arab Aluminum Industries plant in Jordan. The results indicated a high percentage of chromium(VI) sequestering.

EXPERIMENTAL

Reagents used were all of analytical grade; where phosphoric acid was supplied from Scharlu,1,5-dipheneylcarbazide from Hopkins and Williams, sodium acetate from Rasayan Laboratories and glycerol, nitric acid, acetone and sodium carbonate from Gianland chemical Co.

Chromium(VI) solutions: Standard 1000 ppm Cr(VI) stock solution was prepared by diluting Cr(VI) ampoules supplied from Riedel-de Haën in 1000 mL volumetric flask with 2 % nitric acid. Calibration curve was obtained by preparing 5, 7, 9, 11 and 13 ppm solutions by diluting appropriate volumes of the stock solution with deionized water.

Microbial biomass source: Wastewater samples were obtained from the influent of two wastewater treatment plants which are close to the capital Amman, namely from Ma'adaba and Al-Fhees. The influent samples were screened for *Escherichia coli*[14] and found to have 1600 MPN or more. *Escherichia coli* have high Cr(VI) toxicity resistant, selectivity and considered from the best bacterial species for removal of Cr(VI) soluble anions[15].

Industrial wastewater and soil samples: Field samples for industrial wastewater were obtained from the effluent of the Arab Aluminum Industries plant in Al-Baqa's district that is 30 Km north of the capital Amman and the soil sample was taken from the surrounding block of the industrial area.

Analysis of hexavalent chromium: Chromium(VI) concentrations were determined spectrophotometrically (UV-visible Carry 100) at 540 nm in 1 cm cuvette with the 1,5-diphenyl carbazide as the colouring agent[16]. Where in a 100 mL volumetric flask, 5 drops of phosphoric acid were added to 3 mL of standard solution or sample, the pH was adjusted to 1.5 using 0.2 M H_2SO_4, then diluted to 100 mL. 2 mL of dipheneyl carbazide solution of 0.5 % (w/v) in acetone were added and mixed and then the solution was left to stand for 10 min. for the red-violet colour development.

Analysis of total chromium: Total chromium concentration was measured for different solutions using inductively coupled plasma optical emission spectrometer (ICP-OES) from Shimadzu at 267.7 nm.

Batch bioaccumulation: Bioaccumulation test solutions were prepared in 200 mL volumetric flasks, where 50 mL of wastewater (source of biomass) was mixed with the calculated volume of Cr(VI) stock solution to obtain the desired concentration of Cr(VI). Exact amounts of nutrients were added for each batch and then volume was completed to the mark with deionized water. All solutions were bubbled with N_2 (g) and closed tightly with quick-fit stopper and parafilm. Portions of the test solutions were drawn periodically from the supernatant solution under nitrogen gas and tested for Cr(VI).

Batch bioaccumulation for control samples: Two identical solutions were prepared; where 50 mL portion of wastewater was spiked with the appropriate amount of Cr(VI) stock solution to obtain 14 ppm Cr(VI) solution. Sodium acetate (20 mM) was added to the solutions as nutrient and then solutions were diluted to 200 mL. One solution was autoclaved at 120 °C for 15 min, for sterilization, then both solutions were bubbled with N_2(g) and closed tightly with quick-fit stopper and parafilm at room temperature and tested periodically for 65 days for Cr(VI).

Batch bioaccumulation for industrial wastewater samples: 180 mL portion of industrial wastewater previously tested to be free of Cr(VI) was spiked with 20 mL of 1000 ppm Cr(VI) stock solution to obtain 10 ppm Cr(VI) concentration. 20 mM sucrose and 50 mL of wastewater were added and pH was adjusted to 1 by adding concentrated HCl. Sample was bubbled with N_2 (g), closed tightly as before and tested periodically over 8 days for Cr(VI).

Batch bioaccumulation for soil sample

Arab aluminum industries plant soil: A 1 g portion of soil obtained from the Arab Aluminum Industries plant, which was previously tested to be free from Cr(VI), was spiked with 3 mL of the 1000 ppm Cr(VI) stock solution and left to dry in the fume hood. The Cr(VI) was extracted by basic digestion procedure using Na_2CO_3[17], where a 1 g of the soil in a 400 mL beaker is mixed with 100 mL of 0.1 M Na_2CO_3 and boiled on a hot plate for 10 min. After cooling down the sample was filtered and volume was completed to 100 mL with deionized water. To the extracted solution 20 mM sucrose and 50 mL of wastewater were added and mixed, then pH was adjusted to a pH value of 1. The sample was bubbled with N_2 (g), closed tightly and tested periodically over 8 days for Cr(VI).

Factors affecting chromium(VI) bioaccumulation

Nutrient type and concentration: Chromium(VI) bioremediation with different types of nutrients as electron donors and a source of carbon and with different concentrations was studied. Nutrients used in this study were sucrose, glycerol, sodium acetate and 50 % mix of sodium acetate-sucrose. Different batches were prepared with the desired Cr(VI) concentration, with the specified nutrient with varying concentrations and mixed with 50 mL wastewater. Solutions were bubbled with nitrogen gas, closed tightly and periodically measured for Cr(VI).

Chromium(VI) initial concentration: Different sets of Cr(VI) solutions of concentrations 5, 7, 9, 11 and 13 ppm were prepared. For each set of Cr(VI) concentrations different types of nutrients at 20 mM were added, mixed with 50 mL wastewater, bubbled with the inert gas, closed tightly and measured periodically for Cr(VI).

Effect of pH: Four solutions made each of 10 ppm Cr(VI), 20 mM sodium acetate and 50 mL wastewater were prepared and adjusted to different pH values of 1, 4, 7 and 10 at 25 °C and under inert conditions solutions were periodically measured over a period of time for Cr(VI) and total chromium.

Effect of temperature: Three identical solutions each made of 7 ppm Cr(VI), 20 mM sodium acetate and 50 mL wastewater. The three solutions were kept at three different temperatures *i.e.*, 4, 25 and 60 °C. Chromium(VI) concentrations were monitored periodically over 54 days for solution kept at 4 °C and over 24 days for solutions kept at 25 and 60 °C.

RESULTS AND DISCUSSION

Calibration graph: A linear calibration curve for the spectrophotometric determination of Cr(VI) was obtained as indicated in Fig. 1 with $R^2 = 0.9981$. This curve was used to determine the Cr(VI) concentration from different bioremediation batches.

Chromium(VI) removal from control samples: Fig. 2 shows an overlay of Cr(VI) concentration decrease over time for the controlled autoclaved and non-autoclaved samples with initial Cr(VI) concentration of 14 ppm. Calculations indicated 22.97 % Cr(VI) removal for the autoclaved and 76.42 % for the non-autoclaved sample indicating that the active bacteria cells use intracellular metabolic mechanism to remove the Cr(VI), in addition to the surface processes that is used mainly by the inactive cells[18].

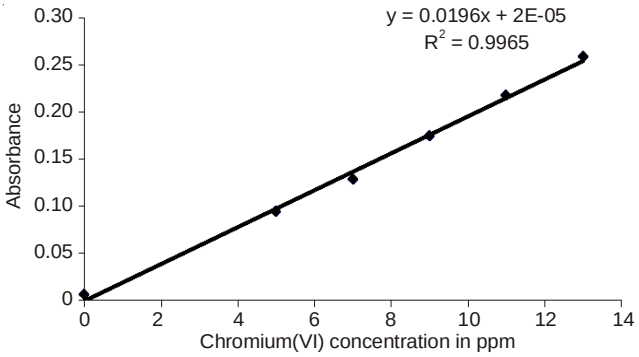

Fig. 1. Spectrophotometric linear calibration curve of Cr(VI)

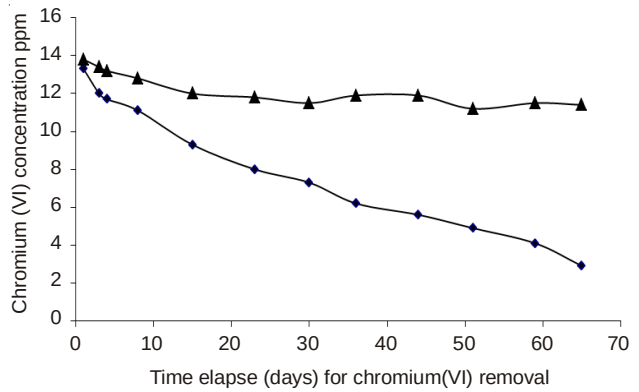

Fig. 2. An overlay of Cr(VI) concentration decrease over time for the autoclaved control sample (▲), and non-autoclaved control sample (◆)

Effect of nutrient type and concentration: Solutions with sodium acetate as a nutrient at different concentrations of 10, 20, 40, 60 and 80 mM with Cr(VI) initial concentration of 13 ppm were observed for the Cr(VI) decline. The % efficiencies of Cr(VI) removal from these solutions over 65 days were 89.23, 86.15, 79.23, 63.84 and 48.46 %, respectively. The inversely proportional relationship could be attributed to the saturation of the nutrient and the negative charge on it that can cause repulsion affecting the number of occupied surface active sites[19]. The performance of sucrose as a carbon source at 10 and 20 mM concentrations with Cr(VI) initial concentration of 11 ppm was tested. Results showed Cr(VI) removal of 82.72 and 93.63 %, respectively. The proportionality between Cr(VI) removal % and sucrose concentration indicates that sucrose as a neutral disaccharide works better as an electron donor than sodium acetate. The open chain monosaccharide glycerin at concentrations of 10 and 20 mM was also investigated with Cr(VI) initial concentration of 11 ppm. The bioremediations of Cr(VI) over 69 days were 88.18 and 90 %, respectively. This result also indicates that glycerol works better than sodium acetate and comes second in order after sucrose.

Effect of initial Cr(VI) concentration: Results showed that the optimum Cr(VI) initial concentration with sodium acetate as nutrient was 5 ppm, for glycerol 5 ppm, whereas for sucrose it was 11 ppm, Table-1 summarizes results for optimization of Cr(VI) concentration using sucrose as nutrient. For the above three nutrients in general, the Cr(VI) removal efficiency decreases with increasing Cr(VI) over a certain limit. This behaviour is attributed to availability of sufficient number of free adsorption sites on the adsorbent (biomass) surface compared to the Cr(VI) concentration. Another possible reason is the repulsion among Cr(VI) anions at high concentrations, which makes accessing the biomass surface points' active sites difficult[12].

Effect of temperature on removal of chromium(VI): Fig. 3 demonstrates that Cr(VI) removal efficiency is best at 60 °C, compared to 4 and 25 °C. The Cr(VI) removal efficiency was 84.24 % at 60 °C over 24 days compared to 77.42 and 88.57 % at 4 and 25 °C, respectively over 53 days. This temperature dependent bioremediation could be attributed to the presence of endothermic adsorption process, where kinetic energy for both adsorbate and biomass surface groups increases; enhancing the collision frequency at the reaction sites[11]. This rise in temperature may also increase the number of binding sites as a result of cell wall components reorientation and increase enzyme activity which is involved in Cr(VI) reduction[20].

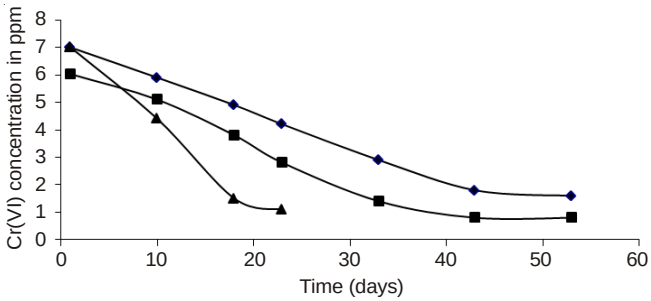

Fig. 3. Chromium(VI) decrease over time at 4 °C (◆), 25 °C (■), and 60 °C (▲) at 7 ppm Cr(VI) initial concentration, and with 20 mM sodium acetate

TABLE-1
CHROMIUM(VI) DIFFERENT INITIAL CONCENTRATIONS DECREASE OVER TIME WITH 20 mM SUCROSE AS NUTRIENT

Day	5 (ppm) Cr(VI)	7 (ppm) Cr(VI)	9 (ppm) Cr(VI)	11 (ppm) Cr(VI)	13 (ppm) Cr(VI
1	5.00	6.20	8.50	9.50	11.70
11	3.30	4.60	5.50	5.80	8.00
18	2.20	3.30	2.70	4.30	6.80
29	1.80	1.10	1.10	3.40	6.00
35	1.40	1.50	1.40	2.70	4.30
42	1.20	1.50	1.10	1.70	3.00
56	1.10	1.00	1.20	0.90	1.40
63	1.00	1.20	1.10	1.00	1.40
Cr (VI)	4.00	5.80	7.90	10.0	11.60
Removal (%)	80.00	82.85	87.77	90.90	89.23

Effect of pH on Cr(VI) removal: Results showed that Cr(VI) is best removed at pH = 1 with % removal efficiency of 92 % over 45 days. Fig. 4 is a demonstration of the Cr(VI) removal at the indicated pH values of 1, 4, 7 and 10, where the lower the pH of the solution the better the removal efficiency. This trend is best explained by the presence of surface functional groups at the biomass, where lowering the pH makes these functional groups positively charged attracting the Cr(VI) anions by electrostatic forces. At high pH the functional surface groups of the biomass become negatively charged or less positive causing repulsion effect for the Cr(VI) anions. The control of the pH also plays a role in the solution chemistry and metal speciation[12].

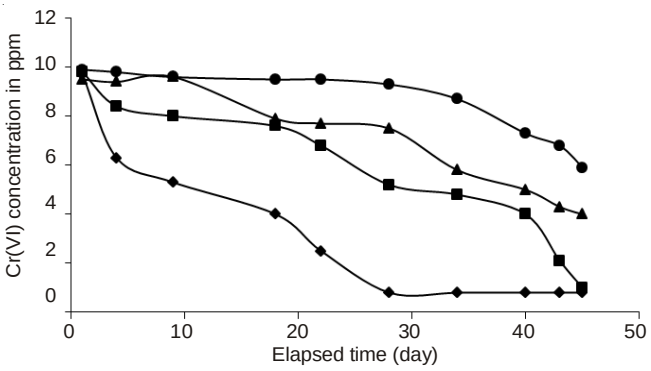

Fig. 4. An overlay of Cr(VI) concentration decrease with time at pH = 10 (●), pH = 7 (▲), pH = 4 (■), and pH = 1 (◆). The initial Cr(VI) concentration was 10 ppm with 20 mM sodium acetate

Industrial wastewater sample: The percentage of Cr(VI) removal was 91 % over 8 days for Cr(VI) initial concentration of 10 ppm under the optimized bioaccumulation conditions of pH = 1, 20 mM sucrose and 25 °C. For the industrial soil sample: Cr(VI) removal efficiency was 93.3 % over 8 days under the optimized bioaccumulation conditions. This sharp drop in time in achieving recovery over 90 % is attributed to combining the pH effect and best nutrient effect in the same solution.

Conclusion

The removal of hexavalent chromium by bioaccumulation using microbial biomass originated from the influents of wastewater plant can be applied successfully for industrial waste water samples and soils from the surrounding locations under anaerobic conditions. Simple organic compounds as carbon sources and electron donors are used to facilitate the anaerobic cycle, where in our case neutral molecules as sucrose works better since the target species for the bioremediation are soluble anions of Cr(VI). The best results for Cr(VI) removal are obtained under the optimized conditions at pH = 1, 20 mM sucrose as a nutrient, Cr(VI) initial concentration of 11 ppm and at 60 °C. The bioaccumulation process in this study involves a cell surface process, coupled with metabolic reduction mechanism as concluded from the total chromium analysis and supported by other investigations in literature.

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