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REMOVAL OF LEAD AND CHROMIUM FROM AQUEOUS SOLUTION BY *BACILLUS CIRCULANS* BIOFILM

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ABSTRACT

The different methods are used for the removal of heavy metals as important contaminants in water and wastewater. Biosorption is an alternative to traditional physicochemical in removing toxic metals from wastewaters and groundwater resources. In this study biosorption of lead and chromium ions from solution was studied using Bacillus circulans isolated from Anzali wetland in batch and biofilter modes and optimum conditions were determined. The experimental results showed 900-950 mg/L and 1050-1100 mg/L, for minimum bactericidal concentration and minimum inhibitory concenteration for lead and chromium, respectively. Results of metal concentration in solution containing 500 mg/L in batch culture showed a reduction about 65% and 48% in five and four days for lead and chromium, respectively. The highest value of lead and chromium uptake in solution with 500 mg/L was 78% and 40% in biofilter mode, respectively. The biosorption of lead and chromium were increased up to pH=5.5, 6, 5.5 and 7, respectively. In the other hand, maximum sorption occurred at neutral pH. There was a significant decreasing of biosorption levels by lowering pH fewer than 3. Accumulation of lead and chromium was determined by scanning electron microscopy analysis of the biofilm exposed to 500 mg/L metal concentration. Based on this analysis, the highest metal concentrations were observed in regions with including bacteria.

Key words: Lead, chromium, biosorption, biofilter, *bacillus circulans*

INTRODUCTION

Contamination of the aquatic environment by heavy metals is a worldwide environmental problem. The pollutants of serious concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper and nickel, due to their carcinogenic and mutagenic nature (Leung *et al.*, 2000). Many industries such as coating, automotive, aeronautical, steel, textile dyeing, leather tanning, electroplating and metal finishing generate large quantities of wastewater containing various concentrations of lead and chromium.

Lead poisoning in humans may cause severe damage to the kidneys, nervous system, and reproductive systems, liver and brain (Leung *et al.*, 2000). Severe exposure to lead is also associated with sterility, abortion, stillbirth and neonatal deaths. The hexavalent form of chromium is considered to be a group "A" human carcinogen because of its mutagenic and carcinogenic properties (Cieslak Golonka, 1996). Permissible limits for lead and chromium in drinking water given by Institute of Standards and Industrial Research of Iran (ISIRI) is 0.1 mg/L and 0.01 mg/L, respectively (ISIRI, 1053). These limits for water packaged (bottled) reported by this institute is 0.005 and 0.05 mg/L, respectively (ISIRI, 6694).

In recent years, cancer rate has increased because of the precence of lead in drinking and natural waters. The international public health associations have reduced the permitted amount of lead in drinking water. For example, the European Community (EC) recommends a limit of 50 μg/L of lead in potable waters (Talebi and Safigholi, 2007). Thus it becomes mandatory to remove lead and chromium from drinking water and wastewaters.

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A variety of heavy metals removal methods, such as precipitation, coagulation, ion-exchange, membrane processing and electrolytic technologies are used (Juang and Shiau, 2000; Yan and Viraraghavan, 2001). These techniques apart from being economically expensive have disadvantages like incomplete metal removal, high reagent and energy requirements, and generation of toxic sludge or other waste products that require specific disposal. Efficient and environment friendly methods are thus needed to be developed to reduce heavy metal content. In this context, considerable attention has been focused in recent years in the field of biosorption for the removal of heavy metal ions from aqueous effluents (Volesky, 2001).

Various biosorbents have been tested, which include seaweeds, moulds, yeast, bacteria, crab shells, agricultural products such as wool, rice, straw, coconut husks, peat moss and exhausted coffee (Dakiky *et al.*, 2002).

Several technologies such as immobilized cell systems have been developed with the aim of reducing or removing the presence of heavy metals in contaminated media. Among these technologies, those based on the use of microorganisms are of particular interest (Cabrera *et al.*, 2005).

This technology exploits natural tendency of cells to accumulate elements or their ability to degrade recalcitrant organic compounds. The cells with such abilities are immobilized either as entrapped biomass or as a biofilm to form a system for treating wastewater known as a bioreactor. This has gained importance during recent years because of the better performance and low cost of biological materials (Volesky, 2001). A bed of crushed rock or synthetic media could support a film of aerobic microorganisms in biofilter mode. The liquid waste is sprayed over the bed and the microorganisms act on the contaminants as they pass through the filter (Fouad *et al.*, 2001).

The objective of this study was to investigate the ability of lead and chromium biosorption by *Bacillus circulans* biofilm immobilized on crushed rock in biofilter.

MATERIALS AND METHODS

Microorganism and culture media

The bacterium strain used in this study was isolated from Anzali wetland in the north part of Iran, undergone enrichment process metals was identified as *Bacillus circulans* following Bergey's *Manual of Determinative Bacteriology*. It was maintained by monthly subculturing using Brain Heart Infusion agar (BHI; MERCK) and stored at 4°C.

Metal-tolerance levels

Bacillus circulans was tested to determine the minimal inhibitory concentrations (MICs) and minimal bacteriocidal concentrations (MBCs) for the two metals (lead and chromium). The experimental tubes were prepared by supplementing Muller-Hinton medium with metal salts for cationic concentrations of 20, 40, 60, 80, 100, 200, 400, 800, 900 and 950 mg/mL for lead and 20, 40, 60, 80, 100, 200, 400, 800, 900, 1000 and 1100 mg/mL for chromium. The compounds were lead and chromium nitrate. One milliliter of the test organism suspension (1×10^6 CFU/mL) was added to each tube. The tubes were incubated for 24h at 30°C and visual turbidity was noted. An aliquot of 0.1 mL from nonturbidal tubes was subcultured to agar for determining MBC (Forbes, 1998).

Biosorption isotherms

To determine the metal biosorption isotherms, batch biosorption experiments were conducted in media using *Bacillus circulans*.This strain (1×10^6 CFU/mL) was suspended in solutions containing different metal concentrations. After 24h of incubation at 30ºC, samples were taken from the solutions, centrifuged at 4000 rpm, decanted and the supernatant was analyzed for metal by atomic absorption spectroscopy (Leung *et al.*, 2000).

Biosorption experiments

The batch adsorption experiments were carried out in 250 mL erlenmeyer flasks by agitating a preweighed amount of adsorbent with 50 mL of aqueous lead and chromium nitrate (500 mg/L) solution at 30°C for a predetermined time interval at 120 rpm speed in an orbital shaker. After adsorption, the mixture was centrifuged at 4000 rpm for 15 min. Residual concentration of metal in the clear supernatant was measured. The amount of metal bound was taken to be the difference between the initial and final metal concentration (Gardea Torresdey *et al.*, 1998).

Metal concentration in the solution was estimated using an atomic absorption spectrometer.
Flasks were taken out on a regular basis of 15-18 i.e. after 24, 36, 48, 60, 72, 84, and 288h of inoculation, respectively, followed by lead and chromium analysis in solution. The bacterial growth was also measured for biosorption time. To assess the extent of chemical reaction, a set of experiments was carried out under sterile conditions.

Scanning electron microscopy (SEM) analysis
Harvested cells as obtained above were washed twice in NaCl 0.9% and resuspended in the same solution. The pellet was fixed in 0.2 M glutaraldehyde, washed in 0.1 M Na-cacodilate buffer and dehydrated in ascending ethanol-series (30-100%). After critical-point drying, the material was coated with gold and observed by the LEO 440i Scanning Electron Microscope (Beshay, 2003; Lopez Jimenez and Leborgne, 2003).

Effect of pH on metal (biosorption)
Bacillus circulans inoculated stock was added to the flasks containing Muller-Hinton medium with metal salts (500 mg/L). The pH of sample solutions were adjusted by using 0.1 M. HCl or 0.1 M. NaOH during the equilibrium period, at the obtained optimal values for each heavy metal as 2, 3, 4, 5, 6 and 7. The experiments were performed at 30°C.

Filter matrix production: Biofilm
Filter matrix was fashioned from a bed of crushed rock supporting a film of *Bacillus circulans*. The surface of crushed rock conduit was provided with grooves for flexibility, a characteristic that was expected to favor bacterial attachment.
The culture medium was prepared in two parts and autoclaved separately (at 121°C for 15 min). Part 1 consisted of inoculum of *Bacillus circulans* in BHI broth medium (with the pH adjusted to 7.0). *Bacillus circulans* was produced aerobically in 1 liter of stirred-tank chemostat during continuous culture at 30°C for 120h. Part 2 consisted of synthetic wastewater used for medium preparation and modeled after wastewater of metal mining operations and included 500 mg/L lead and chromium solution (Stanbury, 1997).
Statistical analysis
All determinations were carried out in triplicate. All data are expressed as mean±SD. Data were analyzed by an analysis of variance ($P<0.05$).

RESULTS

MICs and MBCs results were obtained as 900-950 mg/L and 1050-1100 mg/L, for lead and chromium, respectively.
Results of metal concentration in solution (containing 500 mg/L) showed a reduction about 65% and 48% in five and four days for lead and chromium in batch culture. The fraction of lead and chromium ion biosorption and bacterial growth curve are shown in Figs. 1 and 2, respectively. The bacterium growth curve at the presence of lead and chromium showed the first 10 and 8h as lag phase, from 10 to 120 and 8 to 96h as log phase and from 120 to 216 and 96 to 120h, as stationary phase, and the latter phase was known as death

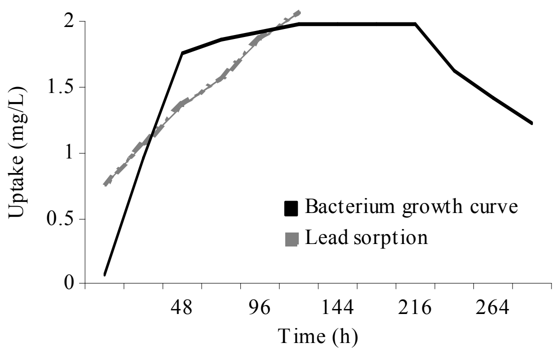

Fig. 1: Graph of metal uptake and bacterium growth curve equilibrium solution 500 mg/L concentration for lead adsorption onto 288h. The highest amount of metal uptake is 65% in occurred at 72h

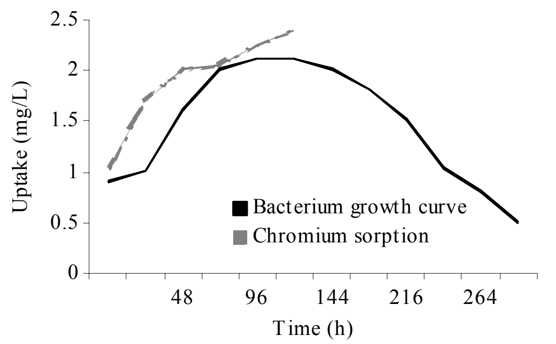

Fig. 2: Graph of metal uptake and bacterium growth curve equilibrium solution 500 mg/L concentration for chromium adsorption onto 288h. The highest amount of metal uptake is 48% in occurred at 96h

phase respectively (Figs. 1 and 2). Maximum adsorption occurred at 72 and 96h of incubation (Figs. 1 and 2).
Fig. 3 shows the biosorption trend for lead and chromium with pH variations. It was decreased by acidic pH. In Fig. 4 the adsorption of lead by immobilized biomas is shown. It was 78% for lead in the first two days and 40% in the early five days for chromium. In Fig. 5 the SEM results of *Bacillus circulans* showed that the cells were randomly distributed in biofilm matrix. The highest metal concentrations were observed in regions of included bacteria.

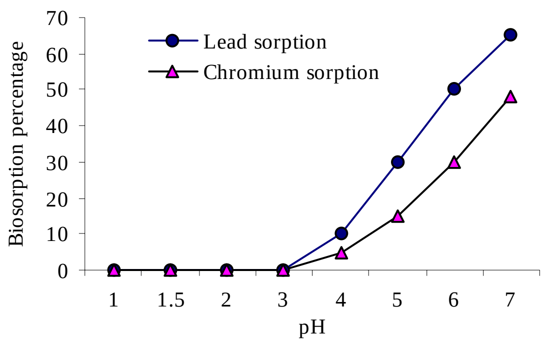

Fig. 3: Effect of process pH on the removal percentages of lead and chromium from 500 mg/L solution. The highest amount of metal uptake is obtained at pH=7

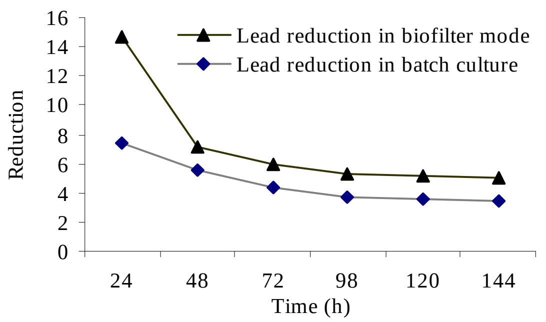

Fig. 4: Adsorption of lead by immobilized biomass in biofilm and batch culture mode (metal concentration=500 mg/L, contact time=144h, pH=7.0, temperature=30°C)

DISCUSSION

Bacillus circulans isolated from Anzali wetland revealed the biosorption capacities and metal removal efficiencies for lead and chromium in 500 mg/L solution in batch and biofilter modes. The maximum rate of lead and chromium accumulation by *Bacillus circulans* was achieved when bacterium strain was in the end of log and initial of stationary phases of growth (Figs. 1 and 2). Pongratz and Klaus (1999), Malekzadeh *et al.*, (2002) and Shirdam *et al.*, (2006) has been reported that, meny kind of microorganisms, are ability to produce a highly diverse metabolites in late of logarithmic phase which can be reduce or removal a wide range of pollutants. Biosorption of the metals in their divdual by *Bacillus circulans* was determined to be Pb^{2+}>>Cr^{6+}. The highest amount of metal uptake was 65% and 48% in occurred at 72 and 96h of incubation for lead and chromium, respectivly in batch culture (Figs. 1 and 2).
The walls of Gram positive bacteria are efficient metal chelators and in *Bacillus* sp. The carboxyle group of glutamic acid of peptidoglycan was the major site of metal deposition. Teichoic and teichronic acids were important binding sites in *Bacillus* spp. (Ilhan *et al.,* 2004). *Bacillus circulans* is a Gram positive bacterium and it has similar cell wall properties as other Gram positive bacteria. Reduction of metal sorption after these times, may be concluded that metal binding sites became saturated during the initial 72 and 96h. The experimental results showed that the immobilized bacterium strain was effective in removing lead ion from solution (Fig. 4). The uptake of metal was very fast initially, and equilibrium was attained within 48h. From Fig. 4, it is shown that biosorption of lead ion was rapid and occurred during the first 48h of sorption (78% removal) in biofilter mode. There was no significant difference between chromium removal in batch and biofilter mode ($P<0.05$). In this study, a high concentration of metal ions (500 mg/L) was used, comparing with other researchers (max 100 mg/L) (Costa *et al.*, 2001; Ilhan *et al.*, 2004; Lyer *et al.*, 2004 and Hussein *et al.*, 2005). In a biofilm process, dissolved organic materials and nutrients are directly absorbed from bulk phase to the biofilm by means of concentration gradient, whereas dissolved heavy metals are generally adsorbed onto the biofilm surface as a result of interactions between metal ions and the negatively charged microbial surfaces, gradually reducing the aqueous

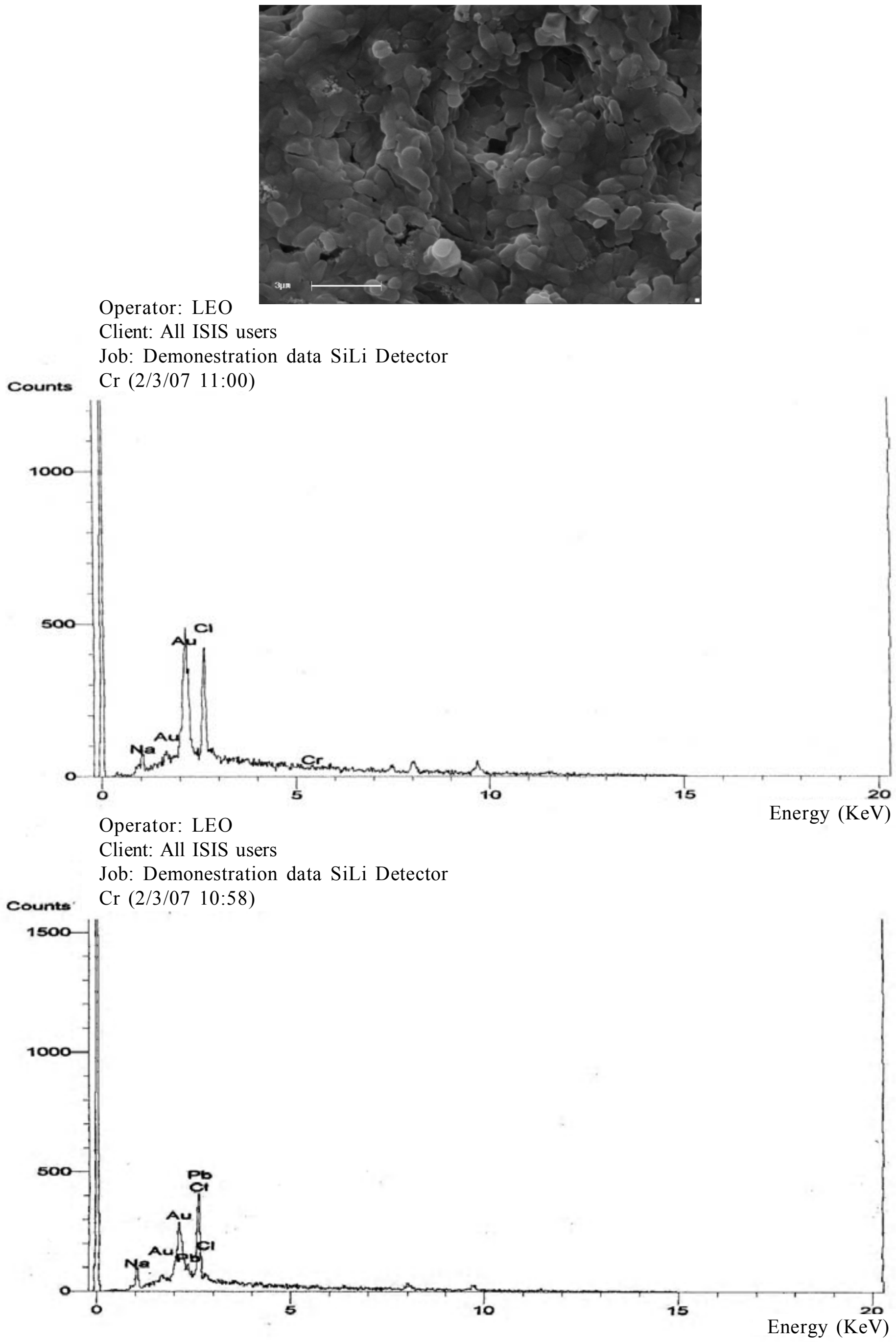

Fig. 5: Scanning electron micrograph of *Bacillus circulans* biofilm. Accumulation of lead and chromium was determined by SEM analysis of the biofilm exposed to Cr (top) and Pb (bottom). Peaks of Au are a result of gold coating of the specimen

metal concentration (Jang *et al*., 2001). In expanded bed reactor, the interaction between biofilm and heavy metals results in the adsorption of heavy metals onto biofilm, and gradually reduces the aqueous metal concentrations. The biofilm varied markedly in depth and was found more frequently in grooves on rock surfaces. Lack of biofilm on ridges may be attributed to the liquid shearing in chemostat. Fouad *et al*., (2001) showed that biofilm column was found to possess 85% Cu^{+2} removal efficiency from 15 mg/L copper solution.

The results showed that the maximum adsorption occurred at nutural pH. The decrease of biosorption levels by lowering pH can be explained due to competition between protons and metal ions for capturing same sites; at low pHs, metal ions are not successful in this regard.

The results obtained prove the acceptable potential of *Bacillus circulans* for reduction of heavy metal ions and specialy lead from contaminated effluents. Furtther studies are needed to increase the biosorption capacities of biomass and develope appropriate technologies applicable in the treatment of industrial wastewaters.

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