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Biosorption of Cr(VI) by Metal Resistant Bacteria from Industrial Effluent

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Abstract: Chromium biosorption was found to be influenced by the pH of the solution, initial metal concentration, amount of the dried powdered cells and contact time. Batch biosorption of chromium(VI) from an aqueous solution was studied using dry biomass of inactivated bacteria isolated from the industrial effluent. At the initial metal ion concentration of 50 mg L^{-1}, the Cr(VI) adsorbed was 7.14 mg g^{-1} of adsorbent. At 300 mg L^{-1} initial Cr(VI) concentration, removal of 47.02 mg g^{-1} of Cr(VI) was recorded. The Cr(VI) adsorbed increased with increasing initial metal ion concentration up to 300 mg L^{-1}. Optimum biosorption was recorded at pH 3. At all the concentrations, the adsorption equilibrium was obtained within 6 h. The adsorption equilibrium constants were obtained from both Freundlich and Langmuir adsorption isotherms. The organism could remove 60 mg of Cr(VI) per gram of adsorbent from an effluent (300 mg L^{-1}) at pH 3. Several desorping agents like EDTA, oxalic acid, citric acid etc. were used for desorption process of which citric acid was found to be better desorping agent. Immobilization with alginate preparation was found to maintain the biosorption potential.

Key words: Biosorption, desorption, chromium, immobilization.

Introduction

Chromium(VI) has been recognized as one of the most serious pollutants among heavy metals in the environment, thus the remediation of chromate(VI) pollution receives much more concern. Sources of chromium pollution are reported to be electroplating, leather tanning, textile dyeing, and metal finishing industries (Ziagova et al., 2007). Chromium(VI) can diffuse as CrO_4^{-2} or $HCrO_4^-$ through cell membranes and oxidize biological molecules resulting in toxicity. It leads to liver damage pulmonary congestion and causes skin irradiation resulting in ulcer formation **(**Arica and Bayramoglu, 2005)**.**

Current technologies for removal and recovery of both toxic and industrial interest metals usually produce wastes with high concentrations of metals. These wastes are an important source of environmental pollution. So far, there have been a number of studies considering the possibility of removal and recovery of heavy metals from diluted solutions. These are principally due to the commercial value of some metals as well as to the environmental impact caused by them. Chemical oxidation, reduction, precipitation, adsorption, solidification, electrolytic recovery, and ion exchange are some of the physicochemical wastewater treatment processes which are being used for metal removal. However, the application of these treatment processes has been found to be sometimes restricted, because of expensive investment, operational costs and the potential generation of secondary pollution. Furthermore, such processes may be ineffective or extremely expensive when the initial heavy metal concentrations are in the range of 10–100 mg L^{-1} (Zhou et al., 2007). Alternative methods of metal removal and recovery based on biological materials have been considered. Certain types of microbial biomass can

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Abbreviations

GIDC	Gujarat Industrial Development Corporation
GESCSL	Green Environment Services Co-operative Society Limited
EDTA	Ethylene diammine tetra acetate
Q_{max}	Maximum adsorption capacity (mg g^{-1})
b	Langmuir adsorption constant
K_f	Freundlich adsorption constant
C_e	Residual metal concentration (mg L^{-1})
C_i	Initial metal concentration (mg L^{-1})
q	Specific metal uptake (mg g^{-1})
R	Correlation coefficient
Cr	Chromium
h	hour

retain relatively high quantities of metals by means of passive processes known as biosorption, which is dependent on the affinity between the metallic species or its ionic forms and the binding sites on the molecular structure of the cellular wall. The process is relatively fast and the fact that it is a surface phenomenon facilitates the removal of the metal and the subsequent use of the material as biosorbent. An important aspect of the operation is that it can be carried out when the cell is metabolically inactive or even dead. One advantage of using microorganisms in an inactive state is that its propagation and use can be separate processes (Manriquez et al., 1997).

Bacteria can also be regarded as metal sorbents, because of the nature and composition of their multi-layer envelopes (membrane, cell wall and capsule). The presence, on bacterial surfaces, of polarizable groups capable of interacting with cations is responsible for their reversible metal binding capacity. Such groups ('sites') are mainly: phosphate, carboxyl, hydroxyl and amino-groups. The heterogeneity of site composition and arrangement on macromolecules and membranes confers to the bacterial biomass a wide range of metal-sorptive performances, with regard to maximum binding capacity, strength of the metal-sorbent bond, response to changes in pH and ionic strength, etc. (Tsezos et al., 1996). Biological metal removal (biosorption) has distinct advantages over conventional methods: it is non-polluting and it can be highly selective, more efficient, easy to operate, and hence cost-effective for treatment of large volumes of wastewaters containing low metal concentrations.

It has been reported that dead cells were of better efficiency for Cr(VI) biosorption than living cells (Srinath et al., 2002). Dead cells were better than live cells. This can be explained as living cells are used for metal biosorption in unbuffered condition, and the redox reactions between the cells and liquid cause an increase in final pH; however the biosorption efficiency of Cr(VI) decreased as pH increased. This motivated us to isolate new bacterial strains from metal-contaminated environment and evaluate the new strains for their ability to remove heavy metals from the polluted environment.

Materials and Methods

Bacteria and Media

Bacterial strains were isolated from equalization tank containing industrial effluent from G.E.C.S.C.L., G.I.D.C., Vatva, Ahmedabad and cultivated on Luria Bertani medium at 35°C.

Screening of Metal Resistant Bacteria

The metal solutions of $K_2Cr_2O_7$ were selected in varying concentration ranging from 50 to 500 mg L^{-1}. Stock solutions of metal salts were prepared in sterile water and added to Luria Agar in varying concentration and spot inoculated with different isolates. The plates were inoculated at 35-37°C for 24 h (Aleem et al., 2003). Bacteria showing resistance to highest concentration for given metal ion were subjected to subsequent biosorption studies.

Preparation of Bacterial Biomass

Biosorption experiments were carried out using batch equilibrium technique. After screening procedure, microorganisms were cultured on Luria Bertani medium without metals. Cells were harvested by centrifugation at 8000 rpm for 10 min. Harvested cell (biomass) were then washed twice with deionised distilled water, autoclaved and dried in an oven at 80°C for 48 h. The dried powdered biomass was used for sorption studies.

Estimation of Cr(VI) Ion

The residual Cr(VI) concentration or percentage metal removal was recorded by measuring optical density of the purple complex of Cr(VI) with 1, 5-diphenylcarbazide at 540 nm (Snell and Snell, 1959).

Biosorption Experiments

Effect of Metal Concentration

Biosorption experiments were conducted at initial Cr(VI) concentrations ranging from 50 to 300 mg L^{-1} until the equilibrium is reached. At the end of each experiment the mixture was centrifuged (8000 rpm for 5 min) and

the remaining concentration of metals in the supernatant was determined. The experimental data were processed via Langmuir and Freundlich isotherms.

Effect of Contact Time

Adsorption of metal ion by dried bacterial biomass was checked at regular interval of time. 50 ml of metal solution ($K_2Cr_2O_7$) was added with appropriate amount of bacterial biomass into each flask. Biosorption was studied for the given time interval by taking out 2 ml of solution and reading the absorbance and extrapolating biosorption using the standard curve and checked for metal biosorption. Each treatment was replicated thrice (Ikram and Malik, 2007).

Effect of pH

Several flasks for each metal ion concentration were prepared. The metal ion concentration was selected on the basis of maximum biosorption as mentioned. pH of the solution was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 by using 2 M NaOH and 1 M H_2SO_4 as required. Biosorption in each tube containing 50 ml of metal solution were studied by procedure described. Lower pH is avoided as it might cause precipitation of metal salts. For each set, a blank of metal solution without any inoculation was performed in parallel to avoid confusion between biosorption and possible metal precipitation.

Desorption Process

Several agents like EDTA, oxalic acid, citric acid (0.1M) were first used as above procedure and their effectiveness in removing metal were compared and analyzed to find out the better desorping agents. Following the metal adsorption batch experiment, metal laden pellet were taken out and suspended into 5 ml of the various eluent solutions. The metal ion is slowly released into eluent. At regular time interval, 1 ml of the sample was withdrawn to estimate metal ion released. Each experiment was continued until equilibrium condition was reached where no further change in ion concentration was observed. The unloaded biomass was regenerated, washed twice with distilled water and resuspended in new metal solution for another cycle of biosorption-desorption process. This biosorption-desorption experiment was continuously performed 2 to 3 times.

Immobilization Studies

Immobilized cell beads were prepared when the dried cells were suspended to solution containing 4% sodium alginate. The mixture was added (drop by drop) into solution containing 2% $CaCl_2$. The biosorption of Cr(VI) on the immobilized bacteria was investigated in batch biosorption experiments at a particular pH. 5 ml of sample was taken from batch sample at predetermined time interval and was analyzed to determine the residual metal concentration in the aqueous solution.

Biosorption Models

Sorption models were chosen for comparison with experimental data: Langmuir and Freundlich isotherm equations were used to describe the equilibrium state for single-ion adsorption experiments. The theoretical basis of Langmuir equation relies on the assumption that there is a finite number of binding sites which are homogeneously distributed over the adsorbent surface of the cells, having the same affinity for adsorption of a single molecular layer and there is no interaction between adsorbed molecules. The mathematical description of the equation is

$$q = \frac{Q_{\max} b C_e}{1 + b C_e}$$

where q is the amount of metal ion adsorbed, C_e is the residual metal concentration in solution (mg L^{-1}), Q_{max} the maximum specific uptake corresponding to sites saturation (mg g^{-1}), and b the biomass-metal binding affinity (Donmez and Aksu, 2002).

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. This empirical equation has the form:

$$q = K C_e^{1/n}$$

where K and n are constants indicating adsorption capacity and adsorption intensity, respectively (Bayramoglou et al., 2005).

The amount of metal ion adsorbed was obtained by using following expression:

$$q = (C_i - C_e) V/m$$

where C_i and C_e are the initial and final metal ion concentrations (mg L^{-1}), respectively. V is the volume of the medium (L) and m is the amount of the biomass (g).

Results and Discussion

Seventeen isolates were selected from serially diluted industrial effluent samples on Luria agar. Out of these, only one isolate was found to resist 500 mg L^{-1} of Cr(VI) through metal inhibition assay. Biosorption of heavy metals by microorganisms is affected by several factors.

Major factors include surface properties of micro-organisms and physicochemical parameters of solution such as pH, initial concentration and time (Sag and Kutsal, 1995).

Influence of Initial Concentration of Cr(VI) Ions

To determine the isotherms, initial metal concentration range was selected between 50-300 mg L^{-1} while the dry cell weight in each sample was kept constant at 2 g L^{-1}. The initial concentration provides an important driving force to overcome all mass transfer resistances of Cr(VI) between the aqueous and solid phases. Hence a higher initial concentration of Cr(VI) would enhance the adsorption process. Such an effect was clearly demonstrated in Figure 1.

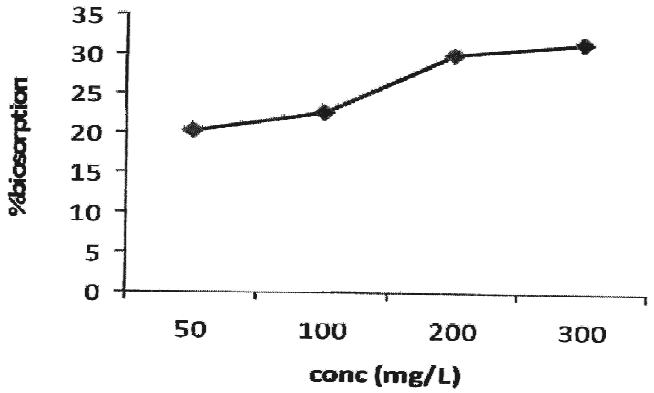

Figure 1: Effect of initial metal concentration on chromium biosorption.

The maximum sorption increased from 20.2 to 31% with Isolate 13 when Cr(VI) concentration was raised from 50 to 300 mg L^{-1} which indicated that increasing metal ion concentration increased the biosorption potential. However further increase in metal concentration did not have any significant effect on the sorption potential. This indicated that high metal concentration caused saturation of binding sites such that no binding sites were available for further adsorption or high metal concentration caused higher electronic repulsion which hampered further biosorption (Ziagova et al., 2007).

Effect of Time

Generally sorption increase on increasing the time of contact between biomass and metal. However after certain interval of time equilibrium is established. The equilibrium was reached within 6 h in our study (Figure 2). Maximum removal capacity of 31% was recorded with Isolate 13 in 6 h. The biosorption yields were 16.3%, 17.9% and 20.3% for Isolates 4, 14 and 15 respectively.

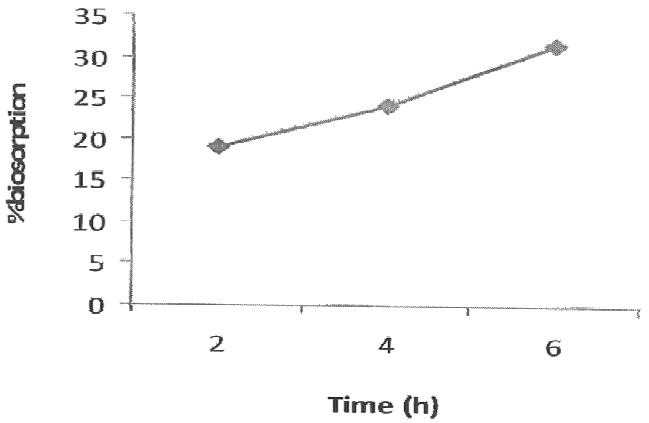

Figure 2: Effect of time on chromium biosorption.

After this period no significant increase in biosorption was observed. Saturation time beyond which no further increase in biosorption is observed may represent the time that allows complete occupation of all the binding sites on the cells which limits the cells from sequestration of any further ions due to lack of adsorption sites. The kinetics of metal uptake, assumed to be a passive physical adsorption at the cell surface, is very rapid and occurs in a very short time after the microorganisms have come into contact with metal ions (Singh et al., 2001). *Dunaliella* sp. was reported to require 72 h to remove Cr(VI) from saline wastewaters (Donmez and Aksu, 2002).

Effect of pH

Metal biosorption is critically linked with pH. Different metals show different pH optima for their biosorption. In order to define the effect of pH on the biosorption of Cr(VI) ions by Isolate 13 (selected for best biosorption among the isolates), the batch equilibrium studies at different pH values were carried out. The effect of pH was tested in the range of 3-8. Optimum pH for Cr(VI)

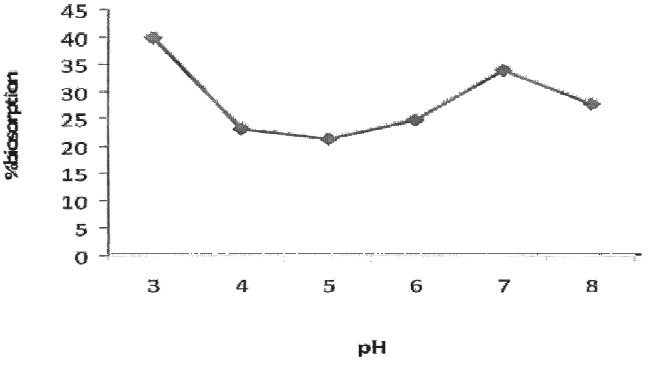

Figure 3: Effect of pH on chromium biosorption.

biosorption by Isolate 13 was 3 (Figure 3). Metal biosorption depends on the protonation or deprotonation of the functional groups on the cell wall (Fourest and Volesky, 1997). Generally low pH causes protonation of cell wall components. Cr(VI) is adsorbed in the form of $Cr_2O_7^{2-}$; lower pH favours its maximum biosorption. However optimum pH varies among different organisms depending on the cell wall components at which become highly protonated. Higher pH values were not useful, since precipitation of the metal could occur by the formation of metal hydroxides (Pardo et al., 2003). Heavy metals have a strong affininty with proteins of the cell wall. At pH values above the isoelectric point of the cells there is a net negative charge on the cells which will inhibit binding of Cr(VI) (Sag and Kutsal, 1995).

In the pH range 2–6, $HCrO^{4-}$ and $Cr_2O_7^{2-}$ ions are in equilibrium. At the lower pH value, $Cr_3O_{10}^{2-}$ and $Cr_4O_{13}^{2-}$ species are formed (Aksu et al., 2002). Thus the decrease in solution pH causes the formation of more polymerized chromium oxide species. Removal of Cr(VI) at low pH was identical with that observed previously (Sag and Kutsal, 1989).

Desorption

Reusability of biosorbent is as important a criterion as biosorption efficiency considering their commercial viability. Desorption analysis was done using various agents like citric acid, EDTA etc. Among the tested agents, citric acid was found to be the best desorbing agent achieving 45% removal in 24 h (Figure 4). During

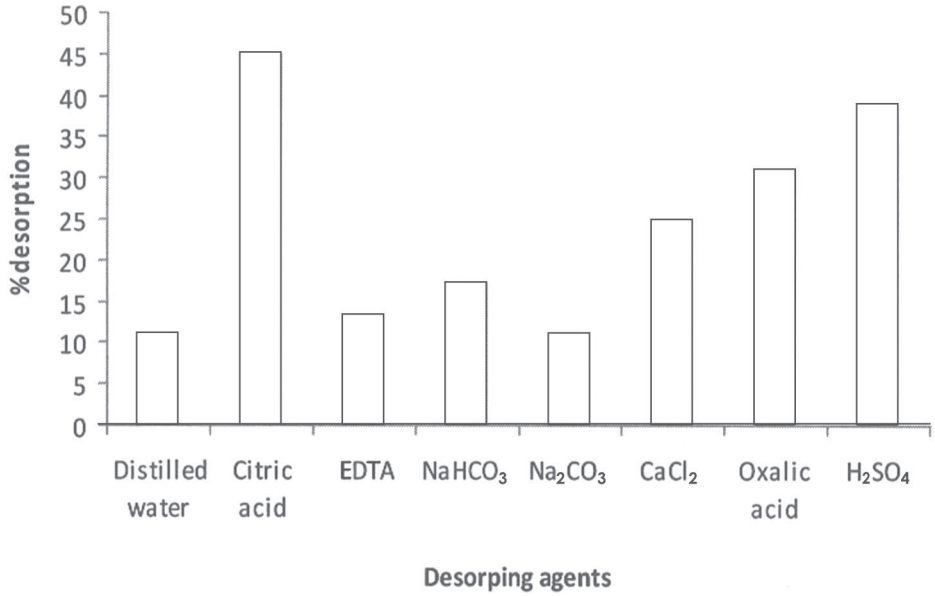

Figure 4: Comparison of desorping agents for chromium biosorption.

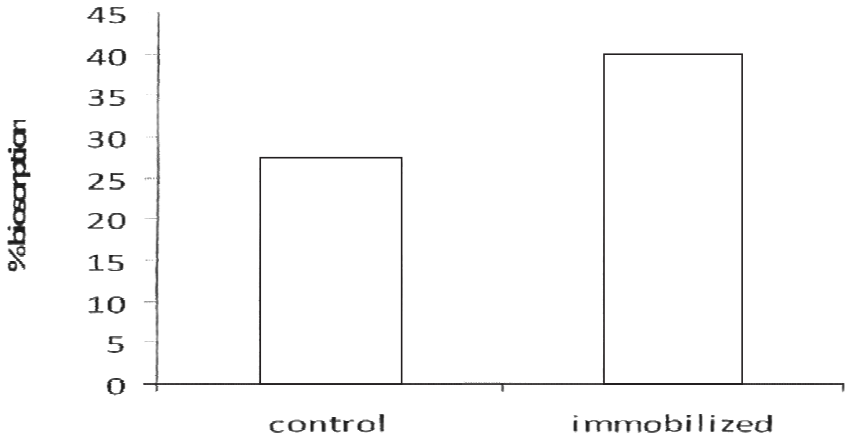

Figure 5: Biosorption of chromium by immobilized cells.

desorption studies, maximum desorption of Cr (58%) was recorded during the first cycle which significantly decreased during subsequent cycles (Figure 5). A similar trend was observed for biosorption of Cr(VI) with each cycle. The results indicated that after 3 to 4 cycles of repetitive biosorption-desorption, biosorption declined significantly. This indicated that cell components may fade away due to repeated treatment (Lu et al., 2006).

Immobilization Studies

Immobilization of the cells was done using alginate preparation (4%). For chromium, immobilized cells showed better removal capacity (39.9%) compared to control (27.1%) (Figure 6). Immobilization did not affect the biosorption efficiency of the cells. Generally immobilization decreases binding capacity of biomass for metals; however it has an advantage for increasing the reusability as cells are fragile in nature and due to repeated desorption-biosorption cycle its efficiency is bound to decrease (Paul et al., 2006).

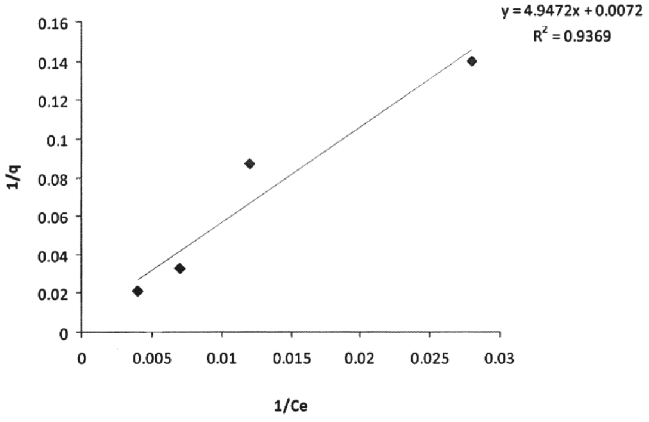

Figure 6: Langmuir isotherm for chromium biosorption.

(C_e = residual metal concentration, q = metal uptake by cells)

Biosorption Models

The equlibrium of biosorption of heavy metals were modelled using adsorption-type isotherms. The Freundlich and Langmuir models were used to describe the biosorption equilibrium (Cetinkaya et al., 1999). The linearized Langmuir adsorption isotherms of Cr(VI) ion for Isolate 13 are presented (Figure 5). After value of C_e [residual metal ion concentration at equilibrium (mg L^{-1})] and q [adsorbed metal ion quantity per gram of cell at equilibrium (mg g^{-1})] had been obtained from experimental data, the plot of $1/q$ versus $1/C_e$ was employed. The Freundlich and Langmuir adsorption constants evaluated from the isotherms with the correlation coefficients are given (Table 1). The regression correlation coefficients for metal ions-bacterium systems were high. The value of Q_{max} species [Langmiur adsorption constant (142.85 mg g^{-1})] was significantly high for the Cr(VI).

Similarly the linearized model of Freundlich adsorption system was obtained by plotting log q versus log C_e (Figure 7).The intercept K is an indication of the adsorption capacity of the adsorbent; the slope $1/n$ indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. The forces within the surface layer are attractive if n is less than unity and repulsive if n is greater than unity. The closer the n value of the Freundlich adsorption system is to zero, the more heterogeneous is the system (Sag and Kutsal, 1995). Both the model properly fitted the experimental data which is indicated by high regression correlation coefficients.

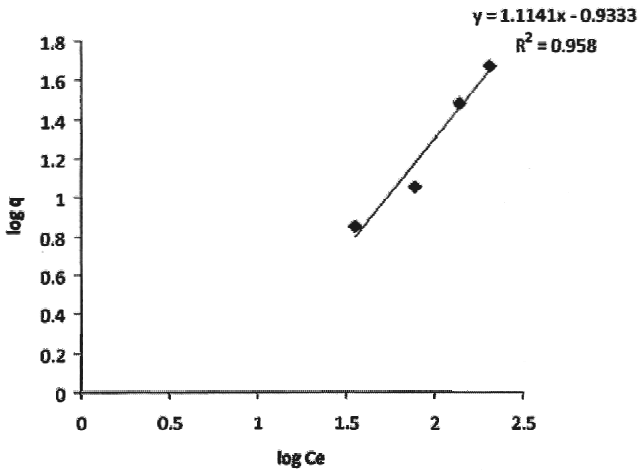

Figure 7: Freundlich isotherm for chromium biosorption.

(C_e = metal concentration, q = metal uptake by cells)

Table 1: Linear regression data for Langmuir and Freundlich isotherm for chromium biosorption

Model	*Langmuir model*			*Freundlich model*		
Parameters	Q_{max} (mg g^{-1})	b	R^2	K	$1/n$	R^2
Cr(VI)	142.85	0.0014	0.936	0.116681	1.114	0.958

Chromium biosorption has been extensively studied using a variety of bacteria or other microbial species such as algae and fungi. Q_{max} values observed for *Ochrobactrum anthropi*, a dead exopolysaccharide producing bacterium, and *Mucor hiemalis* were 86.2 mg g^{-1} and 53.5 mg g^{-1} Cr(VI), respectively (Tewari et al., 2005). Lower adsorption capacities 18.2, 25.6 and 21.2 mg Cr(VI) g^{-1} were obtained with native, heat and acid treated algal preparations of *Chlamydomonas reinhardtii* respectively (Arica et al., 2005), whereas dried activated sludge showed maximum uptake capacity 294 mg Cr(VI) g^{-1} (Aksu et al., 2002).

Conclusions

Dead cells of bacterial isolates resistant to higher concentration of Cr(VI) were used in present study as biosorbent as they don't require any growth conditions to be maintained. Various parameters like pH, metal concentration, time, and amount of dosage of biosorbent were optimized for increasing the biosorption potential on the selected metal resistant isolates. It was found that increase in contact time generally increased the biosorption potential; however, all the isolates reached equilibrium within 6 h. Variation in biosorption pattern was observed due to effect of initial metal concentration. Maximum Cr(VI) biosorption occurred at pH 3. After assessing various parameters, Isolate 13 was found to give maximum biosorption for chromium. To assess the reusability of biosorbent, several desorbing agents like EDTA, H_2SO_4, citric acid, oxalic acid etc. were used. Out of these, citric acid was found to be the best desorbing agent. Immobilization of cells with alginate was done to assess the importance of immobilization for biosorption process. Adsorption ability of isolate for Cr(VI) was examined and adsorption data obtained were applied to both Langmuir and Freundlich isotherm and they were found to describe adsorption equation adequately. The isolate obtained can be further used for studying various other parameters which affect biosorption process and thus may help in understanding the mechanism involved in biosorption and improving biosorption potential.

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