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## Chromium (VI) Biosorption by Immobilized Biomass of *Bacillus cereus* M116

### Cover Page Footnote

The authors are thankful to the University Grant Commission, Government of India, for financial support in carrying out the research work.

# CHROMIUM (VI) BIOSORPTION BY IMMOBILIZED BIOMASS OF *Bacillus cereus* M<sup>1</sup><sub>16</sub>

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## ABSTRACT

Biosorption is potentially an attractive technology for treatment of wastewater for retaining heavy metals from dilute solutions. This study investigated the feasibility of *Bacillus cereus* M<sup>1</sup><sub>16</sub> immobilized in different carriers as a biosorbent for chromium removal from aqueous solutions in batch mode; optimum conditions were determined. Experimental results showed the bacterial strain immobilized in calcium alginate gel matrix was most effective in removing Cr(VI) ion from solution. The uptake of metal was very fast initially, and equilibrium was attained within 80 mins. The overall biosorption process was best described by the pseudo second-order kinetics. Intraparticle diffusion was not the only rate-determining step. The sorption data conformed well to the Freundlich isotherm model. The highest value of Cr(VI) uptake by *Bacillus cereus* M<sup>1</sup><sub>16</sub> (6.0g/L, dry basis) immobilized in 3% calcium alginate was 92.5% at 25 °C, when initial chromium concentration was 50 mg/L.

**Key words:** Cr(VI) biosorption, *Bacillus cereus* M<sup>1</sup><sub>16</sub>, immobilization, pseudo second order, Freundlich isotherm

## INTRODUCTION

Toxic heavy metal contamination of industrial wastewater is an important environmental problem. Many industries such as electroplating, pigments, metallurgical processes, and mining and leather industries release various concentrations of heavy metals like chromium. Chromium generally exists in different forms, mostly as trivalent and hexavalent forms. Trivalent chromium is nontoxic and relatively immobile in nature; whereas hexavalent chromium is soluble in water, mobile, and is known to be highly toxic with potential carcinogenic effects (Zouboulis et al., 2004). Tannery waste contains 80-250 mg/L chromium (CIDS, 1992). Safe value in water for drinking purposes is 0.05 mg/L (U.S. EPA), and recommended value for discharge is less than 5 mg/L (Directive-98/83/EC). Conventional methods such as chemical precipitation, membrane filtration, ion exchange, and adsorption for removing heavy metals (Patterson, 1977; Sung and Ji, 1997) from wastewater have significant disadvantages including incomplete metal removal, need for expensive equipment and monitoring systems, high reagents, and energy requirements (Wilde and Benemann, 1993).

Adsorption is an alternative technique for heavy metal removal. The search for alternative and innovative treatment techniques has focused attention on use of biological materials such as algae, fungi, yeast, and bacteria for removal and recovery technologies and has gained importance during recent years because of the better performance and low

cost of these biological materials (Vegilo and Beolchini, 1997; Kratochvil and Volesky, 1998; Volesky, 2001). Commercial application of biomass as a biosorbent, however, has been hindered by problems associated with physical characteristics of these materials such as small particle size with low density, poor mechanical strength and rigidity, and solid/liquid separation (McHale and McHale, 1994; Volesky and Holan, 1995). Immobilization of the biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity, and porous characteristics to the biological material (Trujillo et al., 1995).

Many biopolymers such as calcium alginate, glutaraldehyde, agarose, and cellulose acetate are also known to absorb metals. These biopolymers are generally non-toxic, selective, efficient, and inexpensive and thus highly competitive with conventional adsorbents. Immobilization of biomass, which allows higher biomass concentration and column operation, may be well suited for non-destructive recovery (Aksu and Kutsal, 1998; Jang et al., 1991; Sag and Kutsal, 1995).

In this research, adsorption ability of immobilized *Bacillus cereus* M<sup>1</sup><sub>16</sub> was investigated for removal of chromium (VI) from aqueous solution. The effect of initial metal ion concentration, initial pH, temperature, and concentration of biomass were examined. Langmuir and Freundlich adsorption isotherms were applied to the experimental data. The pseudo first-order, pseudo second-order, and intraparticle diffusion models were used for determination of the adsorption kinetics.

## MATERIALS AND METHODS

### Organism

A mutated bacterial strain *B. cereus* M<sup>1</sup><sub>16</sub> (Bera et al., 2003) was used in the experiment and maintained by monthly sub-culturing using nutrient agar and stored at 4°C.

### Biomass Production

*Bacillus cereus* M<sup>1</sup><sub>16</sub> was grown in a 250-ml Erlenmeyer flask containing a 50-ml medium having the composition (g/L) of beef extract, 1.0; yeast extract, 2.0; peptone, 5.0; and NaCl, 5.0; and pH, 6.0 at 30°C, 120 rpm for 24 hr. After 24 hr, biomass was harvested by centrifugation at 5500 rpm (1800g) for 15 min at room temperature and washed twice with a buffer (Paul et al., 2005). After washing twice with a buffer, the required amount of wet biomass was used for immobilization.

### Preparation of Dry Cells

Washed wet biomass from a measured amount of culture broth was taken in a previously weighed aluminium cup and dried at 70°C overnight, then weighed again. Weight of the dry cell was calculated from the difference.

### **Immobilization of Biomass**

A 3-ml cell suspension containing a definite amount of cell was added to a 6-ml sodium alginate solution (4.5%) and mixed thoroughly so that the final concentration of sodium alginate in the mixture became 3%. The slurry was added drop-wise into a 2% calcium chloride solution using a hypodermic syringe and kept for 2 hr at 4<sup>0</sup>C. Instantaneous spherical bead formation occurred due to a cross-linkage formation by Ca<sup>2+</sup> at the interface of the drop solution. The resultant beads, which were 1.5 ± 0.2 mm in diameter, were washed thoroughly with deionized water and dried in air. For storage, the beads were dipped in normal saline (0.85%) solution (Srinath et al., 2003; Paul et al., 2005).

### **Agarose**

A 6% agarose solution was prepared and cooled to 37<sup>0</sup> C. Six ml of this solution was mixed with a 3-ml cell suspension so that the final concentration of the agarose solution became 4%. The mixture was poured into a petri plate and kept on ice. After solidification on the petri plate, 3×3×3-mm<sup>3</sup> cubes were cut and washed with deionized water (Lopez et al., 1997).

### **Agar**

The cells were immobilized by entrapping them into agar-agar (bacteriological grade) (Toda and Shoda, 1975). Two hundred seventy mg of agar was melted in 6 mL of distilled water and then cooled to 45-50<sup>0</sup>C. Three ml of cell suspension in saline water, having a definite amount of cells maintained at the same temperature, was added to 6 mL of molten agar and shaken thoroughly. This agar solution was prepared by dissolving the required amount of agar in distilled water, holding it in a boiling water bath, and then cooling it to 45<sup>0</sup>C. The biomass was added to it and mixed thoroughly. This cell-agar mixture was then cast into bead shapes by injecting it into an ice cold, toluene-chloroform (3:1) mixture. The beads were then washed repeatedly with 0.01% triton X-100 to eliminate residual phase (Uchiyama et al., 1994), air dried, and immediately used as immobilized cells or stored in a refrigerator and used later (Banerjee et al., 1982). The diameter of immobilized beads containing bacterial cells was 1.5 ± 0.2 mm.

### **Estimation of Cr(VI) Concentration**

Cr(VI) ion concentration in the solution was estimated using an atomic absorption spectrophotometer (Varian 1656).

### **Biosorption Using Immobilized Biomass**

The batch adsorption experiments were carried out to determine the biosorption of Cr(VI) by immobilized biomass of *Bacillus cereus* M<sup>1</sup>16 using a 250-ml Erlenmeyer flask containing 50 ml of aqueous Cr(VI) solution containing a pre-weighed amount of adsorbent

at definite pH, temperature, initial Cr(VI) ion concentration, and cell mass concentration for a predetermined time interval at 120 rpm speed in an orbital shaker. After adsorption, the mixture was centrifuged at 5500 rpm for 15 minutes. Residual concentration of Cr(VI) ion present in the clear supernatant was measured. The amount of metal bound was taken to be the difference between the initial and final metal ion concentrations (Gardea-Torresdey et al., 1998). All results were analyzed on a dry biomass basis. For scientific interpretations, the adsorbent material dry basis is preferred (Volesky, 2004). All experiments were performed in triplicate and mean values were used. The specific uptake of Cr(VI) was calculated using the equation

$$q_e = V(C_0 - C_e) / m \quad (1), \text{ where}$$

$q_e$  is the amount of metal ion adsorbed at equilibrium (mg/g),

$m$  is the amount of biomass (g)(dry weight),

$V$  is the volume of the reaction mixture (L),

$C_0$  is the initial Cr(VI) ion concentration (mg/L), and

$C_e$  is the metal ion concentration at equilibrium (mg/L).

### Theory

The kinetic-rate constants data were determined from Lagergren's equation (Lagergren, 1998). The selected strain *Bacillus cereus* M<sup>1</sup><sub>16</sub> biomass was collected for experiments after centrifugation at 5500 rpm for 15 minutes from a 24-hr culture broth. The residual biomass was washed with double-distilled water of pH 3.0 and centrifuged. Collected biomass was used for experiments, until the pH of the washed water showed no change.

A particular amount of biomass immobilized in the carrier was taken in a 50-ml solution of 50 mg/L metal ion concentration in a 250-ml Erlenmeyer flask. The pH of the solution was maintained at 3.0. Flasks were incubated at 25°C and 120 rpm for 8 hrs. After adsorption was over, the Cr(VI) concentration of the bulk solution was measured.

In the following equations,  $q_e$  is the metal ion uptake by the immobilized bead at equilibrium (mg/g),  $V$  is the volume of the solution (L),  $C_0$  is the initial metal ion concentration (mg/L),  $C_e$  is the residual metal ion concentration at equilibrium (mg/L), and  $m$  is the amount of adsorbent (biomass only) (g).

$$dq/dt = k(q_e - q) \quad (2)$$

Considering boundary conditions  $t=0$  to  $t = t$  and  $q=0$  to  $q = q_t$ , integrating results gives

$$\ln[q_e/(q_e - q_t)] = kt \quad (3), \text{ where}$$

$q_t$  is the amount of metal ion adsorbed at time  $t$  (mg/g) and  $k$  is the pseudo first-order rate constant ( $\text{min}^{-1}$ ).

The rate constant  $k$  is obtained from the slope of  $\ln (q_e - q_t)$  vs  $t$  straight line.

The sorption data was also analysed in terms of a pseudo second-order (Lagergren, 1998) mechanism, described by

$$dq/dt = k_2 (q_e - q)^2 \quad (4), \text{ where}$$

$k_2$  is the pseudo second-order rate constant (g/mg./min), integrating and applying boundary conditions as  $t=0$  and  $q=0$  to  $t=t$  and  $q=q_t$

$$t/q_t = 1/q_e^2 k_2 + t/q_e \quad (5)$$

$$= 1/h + t/q_e \quad (6), \text{ where}$$

$$h = q_e^2 k_2$$

$t/q_t$  vs  $t$  plot gives a straight line with slope  $1/q_e$  and  $1/h$  as intercepts.

#### *Adsorption isotherm study*

The Langmuir isotherm (Langmuir, 1916) has been applied to many pollutants. The Langmuir sorption isotherm for a solute in a liquid solution is expressed as

$$q_e = q_m b C_e / (1 + b C_e) \quad (7)$$

The linearized form of this equation is

$$1/q_e = 1/q_m b \cdot 1/C_e + 1/q_m \quad (8)$$

$1/q_e$  vs  $1/C_e$  gives the straight line with slope  $1/q_m b$  and  $1/q_m$  as intercepts.  $b$  is the sorption isotherm constant (L/mg).

The generalized form of the Freundlich (Freundlich, 1906) equation is

$$q_e = K_F C_e^{1/n} \quad (9), \text{ where}$$

$K_F$  is the Freundlich isotherm constant related to sorption capacity;  $n$  is the constant related to affinity of the Cr(VI) ion on immobilized beads.

A logarithmic plot of linearized equation (9), i.e.  $\ln q_e$  vs  $\ln C_e$  gives the straight line with slope  $1/n$  and  $\ln K_F$  as intercepts.

#### *Intraparticle mass transfer coefficient*

If movement of the metal ion from the bulk liquid film surrounding the particle is ignored, the adsorption process can be divided into boundary-layer diffusion, sorption of ions onto sites, and intraparticle diffusion. Boundary-layer diffusion is characterized by the initial rate of metal ion adsorption. The initial rate of intraparticle diffusion will be a rate-limiting step in many cases and can be determined using the following equation (Weber and Morris, 1963; Paul et al., 2005).

$$q = K_i t^{0.5} \quad (10), \text{ where}$$

$K_i$  = intraparticle mass transfer coefficient in sorbent ( $\text{mg/gmin}^{0.5}$ ).

The slope of the  $q$  vs  $t^{0.5}$  gives the value of the intraparticle mass transfer coefficient ( $K_i$ ).

## RESULTS AND DISCUSSION

### *Screening of suitable matrix for immobilization*

Cr(VI) biosorption capacities of *Bacillus cereus* M<sup>1</sup><sub>16</sub> biomass immobilized in different matrices were determined and compared.

### **Effect of pH**

Cr(VI) biosorption was studied using *Bacillus cereus* M<sup>1</sup><sub>16</sub> immobilized in calcium alginate, agar, and agarose using a 50-ml solution containing 50 mg/L Cr(VI) ion in a 250-ml Erlenmeyer flask at 120 rpm and 25 °C for 8 hr. From batch studies on Cr(VI) biosorption with biomass of *Bacillus cereus* M<sup>1</sup><sub>16</sub> immobilized on different carriers, it was observed that *q* values increased with an increase in pH from 2.0 to 3.0 and then decreased with further increase in pH. Figure 1 shows that calcium-alginate-entrapped biomass was found to be superior. Maximum Cr(VI) uptake capacity was determined as 13.88, 9.25, and 12.45 mg/g using *B.cereus* M<sup>1</sup><sub>16</sub> immobilized on calcium alginate, agar, and agarose, respectively, when initial Cr(VI) ion concentration and cell mass concentration were 50 mg/L and 1.45 g/L, respectively. When free cells (washed biomass) were used for biosorption of Cr(VI) ion, *q* value was found to be 9.46 mg/g. In the case of free cells in functional groups, which are responsible for adsorption, these are free for adsorption. But in the case of immobilized cells, there is an extra permeability barrier due to the presence of a carrier.

Biosorption of Cr(VI) pH plays a vital role due to the nature of chemical interactions of each metal with the functional groups present on the microbial cell surface. At pH values above the iso-electric point of the cells, there is a negative charge on the cells. At low pH, overall surface charge on the cell is negative and facilitates biosorption of negatively charged Cr<sub>2</sub>O<sub>7</sub><sup>-2</sup> (Tewari et al., 2005). At pH 3.0, the negatively charged dichromate ions would interact more strongly with the positively charged functional groups of *B.cereus* M<sup>1</sup><sub>16</sub> biomass resulting in high Cr(VI) uptake. It is known that the dominant form of Cr(VI) at pH 1.0 is the acid-chromate ion species (HCrO<sub>4</sub><sup>-</sup>), and any increase in pH shifts the concentration of HCrO<sub>4</sub><sup>-</sup> to other forms, CrO<sub>4</sub><sup>2-</sup> and Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup>.

Since there is an increase in sorption of Cr(VI) as pH is increased up to 3.0, it may be suggested that CrO<sub>4</sub><sup>2-</sup> and Cr<sub>2</sub>O<sub>7</sub><sup>2-</sup> are active forms of Cr(VI), which are being sorbed by the biomass. Reduction in biosorption of Cr(VI) at pH values lower than 3.0 is probably due to the change in the chemical nature of the *B.cereus* M<sup>1</sup><sub>16</sub> biomass, due to hydrolytic activity of the acid at high concentrations. This might change the surface characteristics of *B.cereus* M<sup>1</sup><sub>16</sub>, including surface area availability (Tewari et al., 2005). Thus pH 3.0 was selected as optimum pH for biosorption of Cr(VI) ion for further studies.

Adsorption of chromium by biopolymer (viz. calcium alginate, agar, agarose) was



studied. Beads prepared from agar, agarose, and calcium alginate from a 9-ml solution (2%) without cells were separately taken in a 50-ml chromium (VI) solution (pH 3.0) in a 250-ml Erlenmeyer flask and incubated at 25°C for 8 hrs in a rotary shaker (120 rpm).

### **Effect of Biomass Concentration**

Immobilized beads prepared using calcium alginate, agar, and agarose containing 1.45, 1.8, 2.9, 3.6, 4.3, and 5.09 g/L cell mass (dry basis) were taken in a 50-ml normal saline solution containing 50 mg/L Cr(VI) ion in a 250-ml Erlenmeyer flask and incubated for 8 hr at 25°C. The residual Cr(VI) concentration of the bulk solution was measured after 8 hr. From Figure 2, it was found that removal percentage of Cr(VI) ion increased with increase in cell concentration in the immobilized biomass in different carriers from 1.45 g/L to 5.09 g/L; at the same time, the  $q$  value decreased gradually. For calcium alginate, agar, and agarose as matrices for immobilization, removal percentage increased from 73.61 to 92.59, 62.55 to 84.69, and 69.22 to 89.95, respectively. Availability of Cr(VI) adsorption sites increases with increasing cell mass concentration, but due to agglomeration of biomass, total adsorption sites are not all available and hence the  $q$  value decreases (Selatnia et al., 2004).

### **Effect of Temperature**

To determine the optimum temperature for Cr(VI) adsorption using immobilized *Bacillus cereus* M<sub>16</sub>, temperature was varied from 20°C to 35 °C with other conditions remaining the same. Figure 3 shows that specific uptake amounts gradually increased with an increase in temperature from 20°C to 25°C. At 25°C, the maximum specific uptake values were 15.85, 14.75, and 12.91, respectively for calcium alginate, agarose, and agar. With an increase in temperature from 30°C to 35°C, the  $q$  values decreased gradually. The kinetic energy of the Cr(VI) ion increased with an increase in temperature, but after a certain level, kinetic energy is much greater than weak electrostatic attraction force between metal ion and ionic functional groups on the bacterial surface. Sag and Kutsal (1996) found the same type of temperature profile for Cu(II) ion adsorption by *R. arrhizus*.

### **Effect of Initial Metal Ion Concentration**

To study the adsorption isotherm, metal ion concentration was varied from 25 mg/L to 300 mg/L in a 50-ml solution in an Erlenmeyer flask with other conditions remaining the same. Specific uptake amounts ( $q$ ) gradually increased with an increase in initial Cr(VI) ion concentrations up to a certain constant saturation level (Fig. 4) 32.45, 29.11, and 21.25 mg/g, using biomass immobilized in calcium alginate, agarose, and agar gel, respectively. Effective thrusting force (concentration gradient) increased with an increase in differences of metal ion concentrations of the cell surface and that in the bulk solution, which facilitates the

adsorption capacity (Volesky and Prasetyo, 1994). After saturation of available active sites present on the cell surface, the value of  $q$  remained constant.

### Adsorption of Cr(VI) by Only Gel Matrix Under Different Matrix Concentration

Beads prepared from agar, agarose, and calcium alginate from a 9-ml solution (final concentration 2%) without cells were separately introduced in a 50-ml solution containing a 50-mg/L Cr(VI) ion in a 250-ml Erlenmeyer flask at pH 3.0 and 25 °C and 120 rpm for 8 hrs. Under optimum conditions, the Cr(VI) removal percentages for calcium alginate, agarose, and agar were 21.63, 18.75, and 14.9, respectively (Fig. 5).

From the above experiments, it was established that calcium alginate is superior to agar or agarose as a matrix for immobilization of *B.cereus* M<sup>1</sup><sub>16</sub> biomass. Calcium alginate is also cheaper than other matrices. Henceforth, calcium alginate immobilized *B.cereus* M<sup>1</sup><sub>16</sub> was used for the kinetic study as well as the isotherm study.

### Equilibrium Study

The equilibrium isotherm is important in the design of any biosorption system. The equilibrium study indicates the maximum sorption capacity of the sorbent. Taking into account the factors of pH of the solution, temperature, and volume of the reaction mixture during the biosorption process, the equilibrium curves were fitted. The isotherm models were fitted using initial Cr(VI) ion concentration from 25 mg/L to 250 mg/L. The linear plot of the Langmuir and Freundlich isotherm models for sorption of Cr(VI) on *Bacillus cereus* M<sup>1</sup><sub>16</sub> are presented in Figures 6 and 7. From linear regression coefficients of the adsorption isotherm, it is noted that the Freundlich isotherm model exhibits better fit to the sorption data of Cr(VI) than the Langmuir isotherm model (Table. 1). This phenomenon suggests that multilayer sorption takes place on the surface of bacteria.

### Kinetic Study of Cr(VI) Sorption by Microbial Biomass

The kinetics were studied with a constant adsorbent amount of 300 mg (dry basis) at 25°C at different time intervals up to 8 hrs. The pseudo second-order equation has been found to describe the adsorption process in a large number of cases, despite its failure to provide a concrete mechanism of adsorption. It was found that in the present study, the curve obtained by plotting  $\log(q_e - q_t)$  versus time ( $t$ ) is not linear over the entire time range, indicating that more than one mechanism was involved in adsorption. However, from the regression values of first-order and pseudo second-order kinetics, it was established that for removal of Cr(VI) by *Bacillus cereus* M<sup>1</sup><sub>16</sub>, pseudo second-order kinetics provided a better fit (Fig. 8, and Table 2). The kinetic parameters of the adsorption process strongly depend on the initial Cr(VI) ion concentration. With an increase in initial Cr(VI) concentrations from 50 mg/L to 300 mg/L, values of equilibrium metal ion uptake capacity ( $q_e$ ) and initial sorption rate ( $h$ ) increased

as expected, but the pseudo second-order rate constant decreased with an increase in initial Cr(VI) concentration. This is an indication of fact that the kinetics are strongly dependent on mass transfer phenomenon in the sorption of the component (Tewari et al., 2005). The sorption rate increased slowly at a higher metal ion concentration due to sorption-site saturation, which is indicated by the decrease in the apparent rate constant. As the initial chromium concentration increased, time to achieve equilibrium decreased (Tewari et al., 2005). When initial Cr(VI) ion concentrations varied from 50 mg/L to 300 mg/L, the  $q$  value increased from 9.12 to 25 mg/g, while the  $k_2$  value decreased from  $6.47 \times 10^{-3}$  to  $1.74 \times 10^{-3}$  g min/mg. Srinath et al. (2003) reported that pseudo second-order rate constant and  $q$  values were 0.0976 g min/mg and 35.43 mg/g for Cr(VI) removal by calcium- alginate immobilized *Bacillus coagulans* biomass. Humphries et al. (2005) reported that the best immobilization matrices for Cr(VI) biosorption were agar and agarose, where the initial rates of reduction of Cr(VI) (from 500  $\mu$ M solution) by immobilized *D. vulgaris* NCIMB8303 were 127 (agar) and 130 (agarose), respectively. Using immobilized *Microbacterium* sp NCIMB 13776 resulted in 15 (agar) and 12 (agarose) n molh<sup>-1</sup> mg dry cell wt<sup>-1</sup>, respectively.

### Intraparticle Diffusion Studies

Boundary-layer diffusion is characterized by the initial rate of metal ion adsorption. This can be represented by the initial slope of  $C_t/C_0$  vs  $t$  curves, assuming the relationship over the first 5-10 minutes is linear. The same type of results were reported by McKay and Ho (1999), Keskinan et al. (2004), and Xiangliang et al. (2005).

Figure 9 shows that the relationship between  $q_t$  and  $t^{0.5}$  was not linear over the whole time range, and this indicated there were several processes affecting the adsorption. Some authors (Fernandez et al., 1996; Keskinan et al., 2004) have also reported this nonlinear relationship and considered that there were both boundary diffusion and intraparticle diffusion. Boundary diffusion could be represented by the initial curved portion and intraparticle diffusion by the curved portion. The rate constant of intraparticle diffusion ( $K_i$ ), which is calculated from slope of the final linear portion, was  $1.536 \text{ mg/g/min}^{0.5}$ . Theoretically, if the intraparticle diffusion was the only rate-determining step, the rate parameter ( $K_i$ ) derived by the slope of linear regression between  $t=0$  and  $t_{\text{min}}$  (the first breakthrough point of the curve) should be directly related to  $C_0^{0.5}$ . In this study, the  $K_i$  was 1.536, and it was far less than  $C_0^{0.5}$ . This confirmed that intraparticle diffusion was not the only rate-determining step for Cr(VI) adsorption by calcium-alginate-immobilized *B.cereus* M<sup>1</sup><sub>16</sub>.

The immobilized biomass is ideal for use in a conventional ion-exchange column or adsorption column (Kuyucak and Volesky, 1989). Information is available on use of

immobilized *Aspergillus niger* biomass (Kapoor and Viraghavan, 1998) and *Rhizopus arrhizus* biomass (Prakasham et al., 1999) for the removal of heavy metals in column experiments.

Yan and Viraraghavan (2001) studied heavy metal removal in a continuous reactor (1.2-cm dia and 40-cm in height) packed with *Mucor rouxii* immobilized in a polysulfone matrix with a bed depth of 29 cm. Metal removal capacities of the beads for Pb, Cd, Ni, and Zn were 4.06, 3.76, and 1.36 mg / g, respectively.

Volesky and Prasetyo (1994) reported that the driving force for adsorption is the concentration difference between the solute on the sorbent and the solute in the solution. A high-concentration difference provides the high-driving force for the adsorption process, and this may explain the reason that higher adsorption capacities were achieved in the column fed with a higher metal concentration than that with a lower metal concentration.

The  $q_{\max}$  values were obtained at approximately 271, 111, 71, and 60 mg/g, respectively, for removal  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $Ni^{2+}$ , and  $Zn^{2+}$  by dry *Azolla*. It was observed that an obtained adsorption isotherm by SIA produced  $q_{\max}$  values for these metal ions at approximately 186, 95, 54, and 48 mg/g (dry *Azolla*), respectively.

## CONCLUSION

*Bacillus cereus* M<sup>1</sup><sub>16</sub> immobilized in calcium alginate was found to be capable of removing Cr(VI) ion from solution efficiently. Biosorption was dependent on experimental conditions, particularly the medium pH and initial concentration of the metal ions. Intraparticle diffusion was not the only rate-determining step, and pseudo second-order kinetics were applicable to all sorptions. Equilibrium data fitted very well to the Freundlich isotherm model.

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## NOMENCLATURE

- b binding capacity of the solute on the sorbent surface (L/mg).
- C metal ion concentration at any time (mg/L)
- C<sub>0</sub> initial metal ion concentration (mg/L)
- C<sub>e</sub> metal ion concentration at equilibrium ( mg/L)
- h initial sorption rate (mg/g/min)

$k_1$	first-order rate constant ( $\text{min}^{-1}$ )
$k_2$	pseudo second-order rate constant ( $\text{g min/mg}$ )
$K_F$	Langmuir constant
$m$	amount of cell mass ( $\text{g}$ )
$n$	Fruendlich constant
$q$	amount of metal adsorbed per unit weight of biosorbent ( $\text{mg/g}$ )
$q_e$	amount of metal adsorbed per unit weight of biosorbent at equilibrium ( $\text{mg/g}$ )
$q_{\text{max}}$	maximum amount of metal adsorbed per unit weight of biosorbent ( $\text{mg/g}$ )
$r^2$	correlation coefficient
$t$	time ( $\text{min}$ )
$V$	reaction volume ( $\text{ml}$ )

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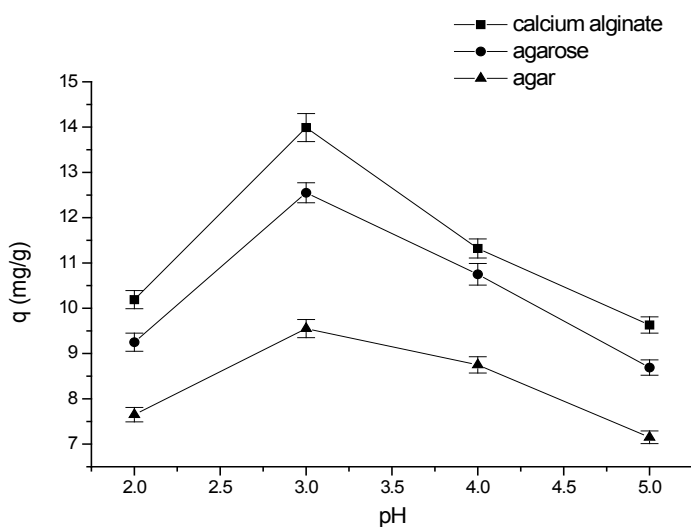
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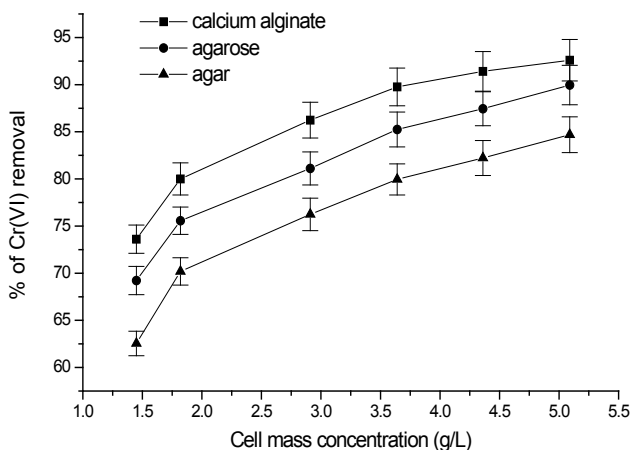
**Table 1.** Isotherm parameters for Cr(VI) sorption on *Bacillus cereus* M<sup>1</sup><sub>16</sub>.

Langmuir isotherm			Fruendlich isotherm		
b	q <sub>max</sub>	r <sup>2</sup>	K <sub>F</sub>	1/n	r <sup>2</sup>
0.105	21.79	0.9252	5.01	0.3058	0.995

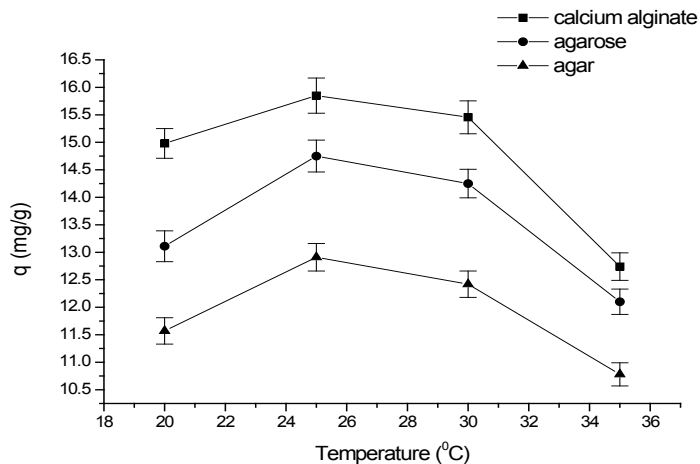
**Table 2.** Variation of rate constants at different initial Cr(VI) concentrations.

First-order model				Pseudo second-order model in linearized form		
Concentration (mg/L)	q <sub>e</sub> (mg/g)	k <sub>1</sub> (min <sup>-1</sup> )	r <sup>2</sup>	q <sub>e</sub> (mg/g)	k <sub>2</sub> (g /mg min)	r <sup>2</sup>
50	3.92	0.0064	0.9463	9.12	0.00647	0.99
100	6.07	0.0071	0.9471	14.14	0.00433	0.99
200	8.83	0.0074	0.9456	21.60	0.00249	0.99
300	14.11	0.0078	0.9463	25	0.00174	0.99

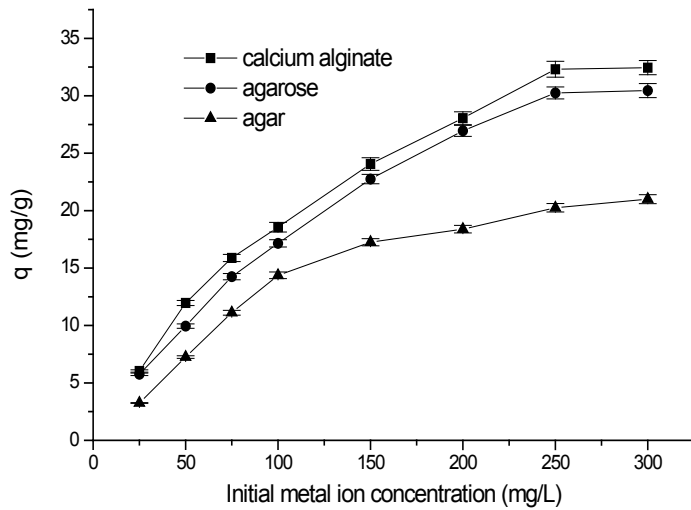
**Figure 1.** Effect of pH on Cr(VI) adsorption on *B.cereus*M<sup>1</sup><sub>16</sub> at 25°C and 50 mg/L initial Cr(VI) concentrations.



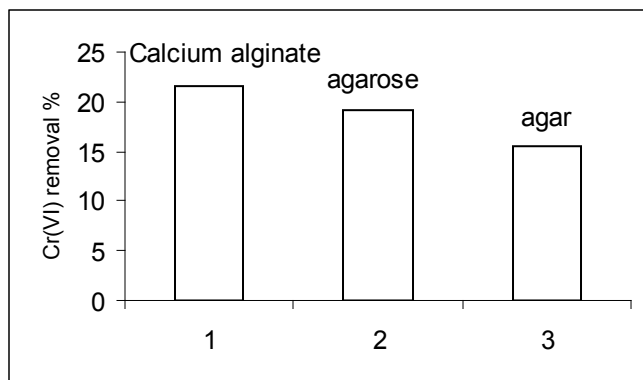
**Figure 2.** Effect of cell mass concentration on Cr(VI) adsorption on *B.cereus* M<sup>1</sup><sub>16</sub> at 25<sup>0</sup>C and pH-3.0.



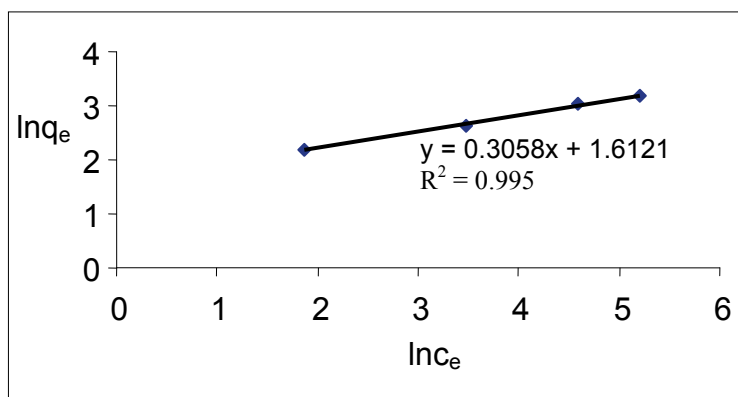
**Figure 3.** Effect of temperature on Cr(VI) adsorption on *B.cereus* M<sup>1</sup><sub>16</sub> at pH-3.0 and 50 mg/L initial Cr(VI) concentrations.



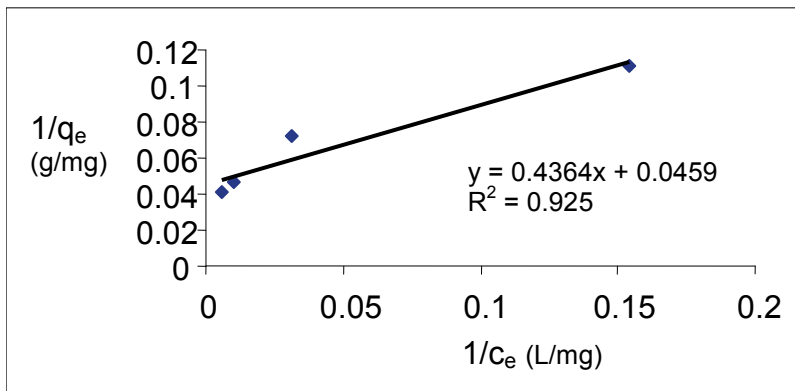
**Figure 4.** Effect of initial metal ion concentrations at 25°C and pH-3.0.



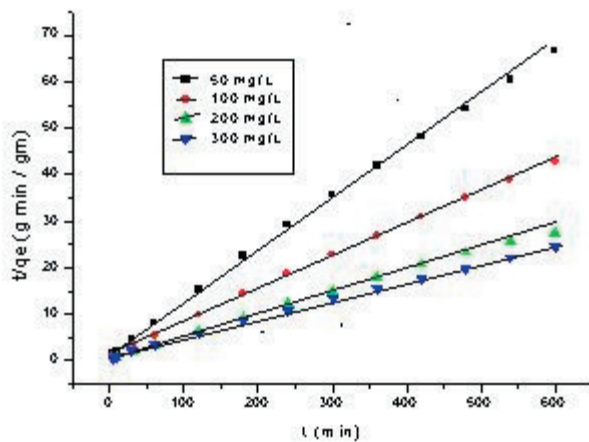
**Figure 5.** Comparison study of Cr(VI) removal by different matrices without cells ( 2% in 9 ml solution, at 25 °C, for 8 hr).



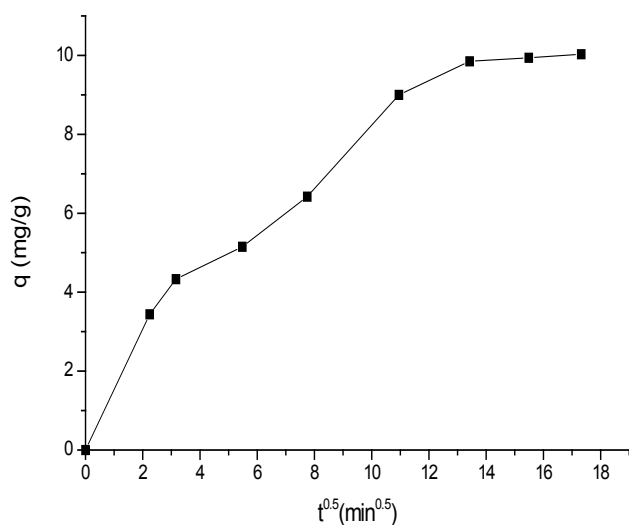
**Figure 6.** Freundlich isotherm plot for removal of Cr(VI) adsorption on *Bacillus cereus* M<sub>16</sub>.



**Figure 7.** Langmuir isotherm plot of Cr(VI) adsorption on *Bacillus cereus*  $M^1_{16}$ .



**Figure 8.** Pseudo second-order kinetics of Cr(VI) adsorption on *Bacillus cereus*  $M^1_{16}$  at 200C.



**Figure 9.** Figure for intra-particle mass transfer.

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