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# Biosorption of Pb(II) by Bacillus cereus M1 16 Immobilized in Calcium Alginate Gel

## **Cover Page Footnote**

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# **BIOSORPTION OF Pb(II) BY** *Bacillus cereus* M<sup>1</sup><sub>16</sub> IMMOBI-LIZED IN CALCIUM ALGINATE GEL

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# ABSTRACT

Biosorption is an alternative to traditional physicochemical means for removing toxic metals from ground and wastewaters. Biosorption of Pb(II) ion from solution was studied using Bacillus cereus M116 immobilized in calcium alginate gel in batch mode, and optimum conditions were determined. The experimental results showed that the immobilized bacterial strain was effective in removing Pb(II) ion from solution. The uptake of metal was very fast initially, and equilibrium was attained within 270 min. It was found that the overall adsorption process was best described by pseudo second-order kinetics. Intra-particle diffusion was not the only rate determining step. The sorption data conformed well to the Freundlich isotherm model. Adsorption increased with an increase in pH in the pH range 3.6 – 6.0, beyond which the adsorption could not be carried out due to precipitation of metal. The highest value of lead uptake was 99%, with 5.4% biomass in 500 mg of adsorbent using 50-ml solution containing 50 mgL-1 Pb(II) ion in a 250-ml Erlenmeyer flask at 300 C and 120 rpm. **Key words:** lead biosorption, Bacillus cereus M116, immobilization, pseudo second order, Freundlich isotherm.

# INTRODUCTION

Contamination of the aquatic environment by heavy metals is a worldwide environmental problem. A variety of methods, e.g. precipitation, coagulation, ion-exchange membrane processing, and electrolytic technologies are used to remove these toxic substances from effluents and industrial wastewater. Many industries such as coating, automotive, aeronautical, and steel generate large quantities of wastewater containing various concentrations of lead. Lead poisoning in humans may cause severe damage to the kidneys, nervous system, reproductive system, liver, and brain. Severe exposure to lead is also associated with sterility, abortion, stillbirth, and neonatal deaths (Goyer and Chisolon, 1972). Permissible limits for lead in drinking water given by the U.S. Environmental Protection Agency (USEPA) is 0.015 mg/L and for wastewaters is 0.1 mg/L, given by both USEPA and Bureau of Indian Standards (BIS) (Muralikrishna, 1997). Thus it becomes mandatory to remove lead from drinking and waste waters. However, the conventional methods have some disadvantages such as incomplete removal, high reagent and energy requirements, and generation of toxic sludge or other waste products that require disposal (Wilde and Benemann, 1993).

The search for alternative and innovative treatment techniques has focused attention on use of biological materials such as algae, fungi, yeast, and bacteria for the removal and recovery technologies. This has gained importance during recent years because of the better performance and low cost of these biological materials (Veglio and Beolchini, 1997; Kratochvil and Volesky, 1998; Volesky, 2001). Commercial application of microbial 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 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).

The objective of this study was to investigate the kinetics and mechanism of lead biosorption in a batch operation using *Bacillus Cereus*  $M_{16}^1$  immobilized with calcium alginate gel.

# **MATERIALS AND METHODS**

# Organism

A mutated strain of *Bacillus cereus*  $M_{16}^1$  was used for this purpose. The bacterial strain was isolated from tannery waste from Park Circus area, Kolkata, and identified as *Bacillus cereus* following Bergey's *Manual of Determinative Bacteriology*, 1994 (Bera et al., 2003). It was maintained by monthly subculturing using nutrient agar and stored at 4°C.

# **Biomass immobilization**

*Bacillus cereus*  $M_{16}^1$  was grown in a 250-ml Erlenmeyer flask containing 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 1800 × g for 10 min at room temperature and washed twice with normal saline (0.85% NaCl) (Ray et al., 2005). Three ml of cell suspension, containing definite amounts of cells, was added to 6 ml of solution containing 270 mg of sodium alginate mixed thoroughly so that the final concentration of sodium alginate in the solution became 3%. The alginate-biomass mixture was then extruded through a 20-ml hypodermic syringe into 2% CaCl<sub>2</sub> solution and kept for 2 hr at 4° C for bead formation. The resultant beads were 1.5 mm  $\pm$  0.2 mm diameter. The spherical beads were then rinsed thoroughly with deionized water and air dried. For storage, the beads were dipped in 0.2M of Tris-HCl buffer (pH 7.2) and stored at 4 °C until further use. In the same way, calcium alginate beads were prepared without adding cell biomass and used as the control to determine the adsorption capacity of the calcium alginate for Pb(II) ion.

# **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 nitrate solution at 32 °C for a pre-determined time interval at 120 rpm speed in an orbital shaker. After adsorption, the mixture was centrifuged at  $1800 \times g$  for 15 min. Residual concentration of lead present 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). All results were analyzed on dry biomass basis. For scientific interpretations, the adsorbent material expression on dry basis is preferred (Volesky, 2004). The biosorption tests were replicated twice, and the standard error values ranged from 0.5 to 4%. The mean values were used.

# Estimation of lead

Concentration of lead in the solution was estimated using an atomic absorption spectrophotometer (Varian-1656).

# Kinetics of adsorption

Pseudo first-order and pseudo second-order equations were employed to model the adsorption data over the entire time range. The first-order rate expression of Lagergren (1898), and Aksu (2001), based on solid capacity, is generally expressed as follows:

$$\log\left(q_e - q_t\right) = \log q_e - \left(\frac{k_1}{2.3}\right)t \tag{1}$$

Where  $q_e$  and  $q_t$  are metal uptake at equilibrium (mgg<sup>-1</sup>) and metal uptake at any time t (mgg<sup>-1</sup>).  $k_1$  is the rate constant of first-order biosorption (min<sup>-1</sup>).

The pseudo second-order equation is also based on the sorption capacity of the solid phase (Aksu, 2001; Ho and McKay, 1999; Arica et al., 2001). The integrated form of the equation is

$$\frac{1}{q_e - q_t} = \frac{1}{q_e} + k_2 t$$
(2)

Here  $k_2$  is the second-order rate constant (g mg<sup>-1</sup> min<sup>-1</sup>). The linear form of the Eq.(2) is

$$\frac{t}{q_t} = \frac{1}{h} + \left(\frac{1}{q_e}\right)t \tag{3}$$

where  $h = k_2 q_e^2$  (Aksu et al., 2001) can be regarded as the initial sorption rate as  $t \to 0$ . If the pseudo second-order kinetics is applicable, the plot of  $t/q_t$  versus t gives a linear relationship, which allows computation of  $q_e$  and  $k_2$  values.

# Intra-particle diffusion studies

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 intra-particle diffusion. Boundary layer diffusion is characterized by the initial rate of metal ion adsorption.

Intra-particle diffusion will be a rate-limiting step in many cases and can be determined using the following equation (Weber and Morris, 1963):

$$k_P = \frac{q_t}{t^{1/2}} \tag{4}$$

where  $k_P$  is the intra-particle rate constant (mg g<sup>-1</sup> min<sup>1/2</sup>).

The slope of  $q_t$  vs.  $t^{1/2}$  plot will give the value of  $k_P$ .

# Adsorption equilibrium isotherm

Due to the high affinity of the adsorbent to the ionic species, they are attracted and bound to the adsorbent surface. If both adsorbate [Pb(II) ion)] and adsorbent (calcium alginate or functional groups

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of cell surface) are ionic, they may be bound by electrostatic force or by ionic bond. This process takes place until the equilibrium between the amount of solid-bound ions and the concentration in solution is reached. The Freundlich model has been used here to describe equilibrium between adsorbed ionic

species on the solid  $({}^{q_e})$  and metal ions in solution  $({}^{C_e})$  at a constant temperature. The Freundlich model equation is of the form

 $q_e = kC_e^{1/n} \tag{5}$ 

where k and n are the Freundlich constant characteristics of the system (Selatnia et al., 2004). The parameter k is the relative indicator of adsorption capacity (L  $g^{-1}$ ), and n indicates the intensity of adsorption.

Eq.(5) is conveniently used in linear form by taking the logarithm of both sides as

$$\ln q_e = \ln k + (1/n) \ln C_e.$$
(6)

#### **RESULTS AND DISCUSSION**

#### Effect of biomass concentration

Pb(II) ion uptake by immobilized *B. cereus*  $M_{16}^1$  was studied using different biomass concentrations in the beads using 50 ml of solution (pH6.0) containing 50 mgL<sup>-1</sup> Pb(II) ion in a 250-ml Erlenmeyer flask at 30°C. The concentrations were 5.4, 10, 19, and 22.6% (dry basis), keeping the total bead volume the same in each case. Fig.1 shows that the percent adsorption remained almost the same up to 10% biomass concentration and then increased with increase in biomass concentration in the bead. The decrease in the adsorption could be due to the clogging of the biomass and hence decrease by the number of active sites responsible for Pb(II) adsorption. Calcium alginate beads without biomass adsorbed at 47.3% Pb(II) ion under the same environmental conditions (results not shown).

# Effect of pH

Effect of pH on Pb(II) uptake by the immobilized cell of the selected strain is shown in Fig. 2 with other conditions remaining the same over a pH range of 3.0 to 6.0. Pb(II) uptake capacity increased with increase in pH, and the greatest uptake capacity was obtained at pH values of 6.0. Adsorption rates of 91.25% and 99% were observed at pH 3.0 and pH 6.0, respectively. Using calcium alginate entrapped *B.cereus*  $M_{16}^1$  a high adsorption (99 %) was obtained at pH6.0. At higher pH (beyond 6.0), lead precipitation takes place. Metal biosorption depends on the protonation or deprotonation of the functional groups on the cell wall (Urrutia and Beveridge, 1993; Fourtest and Volesky, 1997; Fourtest and Roux, 1992). At low pH, the concentration of proton is high, so metal binding sites become positively charged and metal cations and protons compete for binding sites, which results in lower uptake of metal. With the increase in pH, the bifunctional groups on the cell wall with negative charge increase, due to deprotonation of the metal binding sites which promote the metal uptake. The ionic forms of the metal in solution and the electrical charge of the biomass depend on the solution pH. pH 6.0 was selected for further studies.

The same experiment was performed using calcium alginate without biomass, prepared following the

same procedure. From Fig. 2, it is evident that when calcium alginate beads having no biomass were used to biosorb Pb(II) ion, the biosorption decreased with increase in pH up to 4.5 and then remained constant with increase in pH. Adsorption of 47.3 % and 44.25 % were observed at pH 3.0 and pH 4.5, respectively. Using calcium alginate, entrapped *B.cereus*  $M_{16}^1$  high adsorption (99 %) was obtained at pH 6.0.

### Influence of initial metal concentration

Influence of initial Pb(II) ion concentration on biosorption of Pb(II) ion by the immobilized bacterial strain (amount of adsorbent 500 mg on dry basis) and calcium alginate beads without biomass were investigated using varying concentrations of Pb(II) ion. Amount of adsorption of Pb(II) ion per unit mass of biosorbent increased with an increase in initial lead ion concentration (range 50-500 mg L<sup>-1</sup>). This increase could be due to an increase in electrostatic interactions (relative to covalent interactions) involving sites of progressively lower affinity for metal ions (Al-Asheh and Duvnjak,1995). But metal removal percentage remained almost same (98-99%) with increase in initial Pb(II) ion concentration (Fig. 3 ). From Fig 3, it was revealed that specific uptake of Pb(II) ion by both biomass-immobilized beads and calcium-alginate beads without biomass gradually increased from 50 to 500 mg/L metal ion solution. In the case of immobilized beads containing biomass values of  $q_e$  were 5.2mg g<sup>-1</sup>, and 49.75mg g<sup>-1</sup>, respectively, at 50mg L<sup>-1</sup> and 500 mg L<sup>-1</sup>. When only calcium alginate beads were used for the experiment, values were 2.45 mg g<sup>-1</sup> and 22.5 mg g<sup>-1</sup>, respectively, at 50mg L<sup>-1</sup> and 500 mg L<sup>-1</sup>.

# Time course of biosorption

The time-dependency batch experiments were performed by varying contact time of immobilized biomass to Pb(II) ions from 0-300 min. Immobilized beads containing 500 mg immobilized biomass (dry basis) of the selected strain were taken in each of the 50-ml normal saline samples containing 350 mg L<sup>-1</sup> Pb(II) ion (pH adjusted to 6.0) in each 250-ml Erlenmeyer flask and incubated at 30°C for 300 min. Samples were collected at different time intervals to determine residual lead ion concentration. From Fig. 4, it is shown that biosorption of Pb(II) ion was rapid and occurred during the first 30 min of sorption (90% removal), but thereafter equilibrium was reached at 270 min (99% removal).

# Adsorption equilibrium isotherm

The equilibrium sorption isotherm is important in the design of biosorption systems. Equilibrium studies in biosorption indicate the capacity of the sorbent. Taking into account the fact that the pH of solution was constant (pH-6) during the biosorption process and in order to optimize the biosorption process parameters, we have modeled the equilibrium curves. The Freundlich isotherm model was able to fit Pb(II) sorption data well over an initial metal ion concentration range of 100-500 mg L<sup>-1</sup> when the equilibrium concentration range was 10.17 to 45.0 mg L<sup>-1</sup>. Fig. 5 shows the linear plot of  $\ln q_e$  versus  $\ln C_e$  (correlation coefficient 0.92), with slope 1/n and intercept ln k. The estimated values are presented in Table 1 for the Pb(II) / immobilized biomass adsorption system. Xiangliang et al. (2005) reported Pb(II) sorption by *Pleurotus ostreatus* immobilized in calcium alginate gel. They reported that k and 1/n were 0.4350 L g<sup>-1</sup> and 0.9642, respectively.

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# Kinetics of adsorption

The kinetics were investigated with a constant adsorbent amount of 500 mg (dry weight basis) at  $32^{\circ}$ C with initial Pb(II) concentration of 350 mg L<sup>-1</sup> at different time intervals up to 300 min.

The pseudo first-order equation of Lagergren (1898) has been found to describe the adsorption process in a large number of cases (Jianlong et al., 2001; Selatnia et al., 2004) despite its failure to provide a concrete mechanism of the adsorption process. Fig. 6 shows that the pseudo second-order equation was applicable to all the sorption data (correlation coefficient 0.99). Values of  $q_e$  and  $k_2$  obtained by linear regression of t/q<sub>t</sub> against t were 34.72 mg g<sup>-1</sup> dry bead and 0.01428 g mg<sup>-1</sup> min<sup>-1</sup>, respectively. Second-order kinetics were earlier reported for adsorption of Pb(II) on *Pleurotus ostreatus* (Xiangliang et al., 2005) with a rate constant of 0.04583 g mg<sup>-1</sup> min<sup>-1</sup>.

#### Intra-particle 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 (C<sub>t</sub> is the residual metal concentration in the solution at time t and C<sub>0</sub> is the initial metal concentration) curves, assuming the relationship over the first 5-15 min is linear (McKay and Ho, 1999; Keskinkan et al., 2004; Xiangliang et al., 2005). The initial rate of Pb(II) ion adsorption onto the beads was 0.0083.

Fig.7 shows that the relationship between  $q_t$  and  $t^{1/2}$  was not linear over the whole time range, and this indicates that there were several processes affecting the adsorption. Other researchers (Keskinkan et al., 2003; Fernandez et al., 1996; Keskinkan et al., 2004) have also reported this non-linear relationship and considered that there were both boundary diffusion and intra-particle diffusion. Boundary diffusion usually could be represented by the initial curved portion and intra-particle diffusion by the final curved portion. The rate constant of intra-particle diffusion ( $k_p$ ), which was calculated from the slope of the final linear portion, was 0.1337 (mg g<sup>-1</sup> min<sup>1/2</sup>). Theoretically, if the intra-particle diffusion was the only rate-determining step, the initial rate parameter ( $k_i$ ) derived by the slope of linear regression between t = 0 and  $t_{lim}$  (the first breakthrough point of the curve) should be directly related to  $C_0^{-1/2}$ . In this study, the  $k_i$  was 0.0933 and it was far less than  $C_0^{-1/2}$  (18.71). This confirmed that intra-particle diffusion was not the only rate-determining step for Pb(II) adsorption by calcium alginate immobilized *Bacillus cereus* M<sup>1</sup><sub>16</sub>.

# CONCLUSION

*Bacillus cereus* M<sup>1</sup><sub>16</sub> immobilized in calcium alginate was capable of removing lead from solution efficiently. Biosorption was found to be dependent on experimental conditions, particularly the medium pH and the initial concentration of the metal ions. Intra-particle diffusion was not the only rate-determining step, and pseudo second-order kinetics were applicable to all the sorption data over the entire time range. The experimental equilibrium data fit very well to the Freundlich isotherm model.

# ACKNOWLEDGEMENT

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### NOMENCLATURE

- C Concentration of metal in solution (mg L<sup>-1</sup>)
- $C_{a}$  Liquid-phase concentration of metal at equilibrium (mg L<sup>-1</sup>)
- $C_t$  Residual metal concentration in the solution at time t (mg L<sup>-1</sup>)
- k Constant, relative indicator of adsorption capacity (L g<sup>-1</sup>)
- $k_1$  First-order rate constant (min<sup>-1</sup>)
- $k_2$  Second-order rate constant (g mg<sup>-1</sup> min<sup>-1</sup>)
- $k_{p}$  Intra-particle rate constant (mg g<sup>-1</sup> min<sup>1/2</sup>)
- 1/n Constant, intensity of the adsorption
- $q_e$  Metal uptake at equilibrium (mg g<sup>-1</sup>)
- $q_t$  Metal uptake at any time t (mg g<sup>-1</sup>)
- t Time (min)

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#### **FIGURES**



**Figure 1:** Effect of biomass concentration on the Pb(II) adsorption capacity of the immobilized biomass (initial lead concentration: 50 mg  $L^{-1}$ , contact time: 24 hr, temperature: 30<sup>o</sup> C, pH : 6.0)



**Figure 2:** Effect of initial pH on Pb(II) biosorption by immobilized biomass and calcium alginate bead without biomass (initial lead concentration:  $350 \text{ mg L}^{-1}$ , amount of biomass: 500 mg, contact time: 24 hr, temperature :  $30^{\circ}$  C).



**Figure 3:** Adsorption of Pb(II) by immobilized biomass and calcium alginate beads without biomass at different initial metal concentration (biomass amount: 500 mg, contact time: 24 hr, pH : 6.0, temperature :  $30^{0}$ C).



**Figure 4:** Effect of incubation time on adsorption of Pb(II) on immobilized biomass (initial lead concentration:  $350 \text{ mg L}^{-1}$ , biomass amount: 500 mg, pH: 6.0, temperature:  $30^{\circ}$  C).



**Figure 5:** Application of Freundlich isotherm to the adsorption data of Pb(II) adsorbed onto the immobilized biomass (amount of biomass: 500 mg, contact time: 24 hr).



**Figure 6:** Pseudo second-order adsorption kinetics of Pb (II) on the immobilized biomass (amount of biomass: 500 mg, initial metal concentration:  $350 \text{ mg } \text{L}^{-1}$ ,pH :6.0, temperature:  $30^{0}$ C).



**Figure 7:** Plot of intra-particle diffusion for adsorption of Pb(II) on immobilized *B. cereus*  $M_{16}^{1}$  (amount of biomass: 500 mg, initial metal concentration: 350 mg L<sup>-1</sup>).

| 1/n    | $k (Lg^{-1})$ | $R^2$ |
|--------|---------------|-------|
| 0.8953 | 8.782         | 0.92  |

 Table 1. Sorption isotherm constants of Fruendlich model