Journal of Hazardous Substance Research

Volume 5 Article 1

1-1-2006

Bioaccumulation of Pb(II) From Aqueous Solutions by Bacillus cereus M1 16

L. Ray
Jadavpur University

S. Paul
Jadavpur University

D. Bera
Jadavpur University

See next page for additional authors

Recommended Citation

Ray, L.; Paul, S.; Bera, D.; and Chattopadhyay, P. (2006) "Bioaccumulation of Pb(II) From Aqueous Solutions by Bacillus cereus M1 16," *Journal of Hazardous Substance Research*: Vol. 5. https://doi.org/10.4148/1090-7025.1031

Bioaccumulation of Pb(II) From Aqueous Solutions by Bacillus cereus M1 16

Cover Page Footnote

The authors gratefully acknowledge the U. G. C., Government of India, for financial support to carry out the research work.

Authors

L. Ray, S. Paul, D. Bera, and P. Chattopadhyay

BIOACCUMULATION OF Pb(II) FROM AQUEOUS SOLUTIONS BY *Bacillus cereus* M^1_{16}

L. Ray[1], S. Paul[2], D. Bera[3], and P. Chattopadhyay[4]

Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata-700032, India; E-mail: lgrftbe@yahoo.com.

ABSTRACT

Because of the severity of heavy metal contamination and potential adverse health impact on the public, tremendous efforts have been made to purify waters containing toxic metal ions. Biosorption is presented as an alternative to traditional physicochemical means for removing toxic metals from groundwaters and wastewaters. Removal of lead from solution was studied using growing cells and washed cells of *Bacillus cereus* M^1_{16}. The removal of Pb(II) ions with growing cells was maximum (85%) when initial lead concentration was 50 mg/L. Other process conditions were optimized. These were volume of medium: 40 ml in a 250-ml Erlenmeyer flask, temperature: 30°C, pH: 6.0, fermentation time: 30 hours, and inoculum concentration (24-hour cell growth): 4%. Biosorption of Pb(II) on washed biomass of the selected strain was investigated in batch mode and optimum conditions were determined. The uptake of metal was very fast, and equilibrium was attained within 30 minutes. It was found that the overall adsorption process was best described by pseudo second-order kinetics. Both Langmuir and Freundlich isotherms were tested, and it was found that the latter had a better fit with the data. The adsorption continuously increased in the pH range of 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 96%, with 1.8 g/L washed biomass (dry basis) at 20°C and 92% at 30°C.

Key words: lead biosorption, *Bacillus* sp., adsorption, isotherm.

INTRODUCTION

With progress in technology, the natural environment often suffers from detrimental effects of industrial pollution. The natural process of transportation of metal between the soil and water consolidates metal contamination that affects the areas of natural ecosystems (Runnells et al., 1992). Many industries such as coating, electric battery manufacturing, paint, lead smelting, internal combustion engines, fueled aeronautical engines, and mining generate large quantities of wastewater containing various concentrations of lead. Lead poisoning in humans causes severe damage to the kidneys, nervous system, reproductive system, liver, and brain, and can cause sickness or death. Severe exposure to lead has been 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 (U.S. EPA) is 0.015 mg/L and for wastewaters is 0.1 mg/L, given by both the U.S. EPA and Bureau of Indian Standards (BIS) (Muralikrishna, 1997). Thus it becomes mandatory for removal of lead from drinking and wastewaters. These concentration are usually too low to be treated by standard methods. Chemical precipitation leads to production of toxic sludge. Solvent extraction techniques are not suitable for effluents containing less than 1 g/L of targeted heavy metals. On the other hand, ion exchange processes are too expensive due to the high cost of

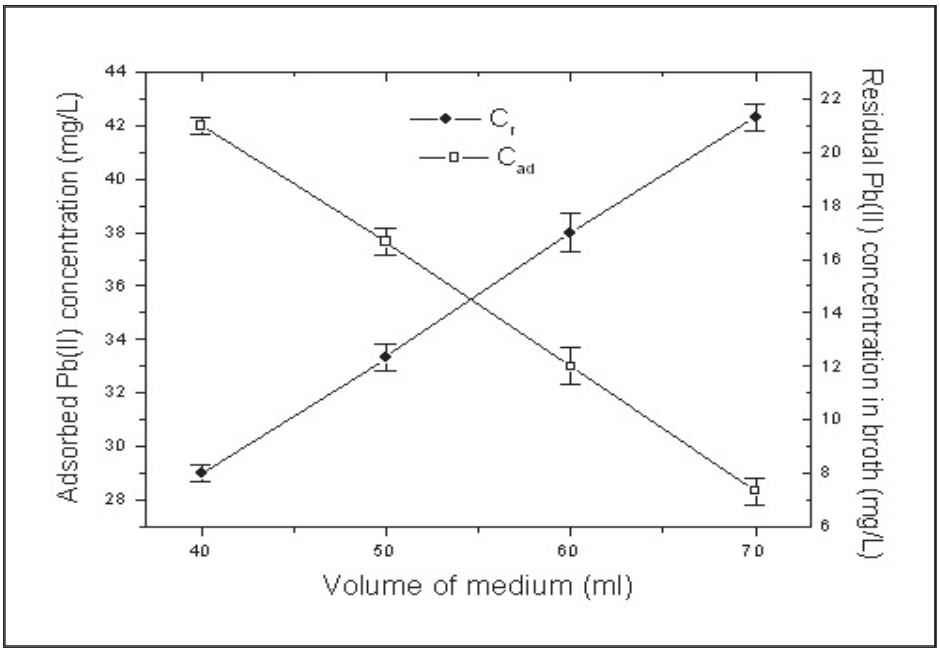

Figure 1. Consumption of Pb(II) at different medium volumes by *B. Cereus* M^{1}_{16} (initial lead concentration: 50 mg/L, temperature: 30°C).

synthetic resins.

Biosorption has distinct advantages over conventional methods, viz., the process does not produce chemical sludges. It can be highly selective, more efficient, easy to operate, and hence cost effective for the treatment of large volumes of wastewaters containing low metal concentrations (Deans and Dixon, 1992; Puranik and Paknikar, 1997). Bacteria, algae, yeasts, and other fungi have been used successfully as adsorbing agents for heavy metals. Interaction between metals and microbial cells can occur through adsorption to cell surfaces through metabolically assisted accumulation within the cell or as metal complexes with extra-cellular microbial metabolites (Norberg and Rydin, 1984). Technological applications of microbial metal accumulation may depend on the ease of metal recovery either for subsequent reclamation or further containment of toxic/radioactive waste. Nondestructive recovery may also be required for regeneration of the biomass for reuse in multiple biosorption-desorption cycles (Tsezos, 1984). Destructive recovery may be accomplished by pyrometallurgical treatment of the biomass of dissolution in strong acids or alkalies (Brierly et al., 1985).

Modak et al., (1996) showed that nonliving, *A. niger* biomass attached to wheat bran was selective for the extraction of copper and zinc. Sag et al., (1995) studied the comparative biosorption of lead by *Z. ramigera* and *R. arrhizus*, and Gardea-Torresdey et al., (1996a) performed batch experiments with inactivated cells of *Mucor rouxii* for Cu^{2+} binding. Other studies were performed with different biomaterials as marine biomass (Kuyucak and Volesky, 1989; Hao et al., 1994; Leusch and Volesky, 1995; Chong and Volesky, 1996; Khoshmanesh

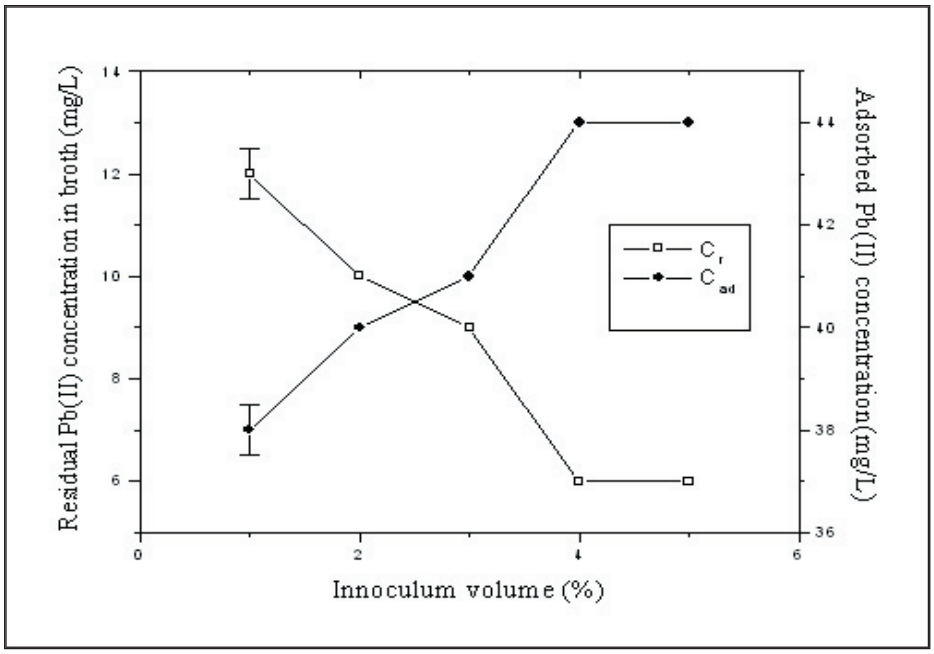

Figure 2. Accumulation of Pb(II) at different inoculum volumes by *B. Cereus* M^1_{16} (initial lead concentration: 50 mg/L, temperature: 30°C).

et al., 1996), bacteria (Urrutia and Beveridge, 1993; Mago and Srivastava, 1994; Sag and Kutsal, 1995; Mishra et al., 1996; Singleton and Simmons, 1996; Nourbakhsh et al., 2002), chitosan (Jaanson-Cherrier et al., 1996), humic substances (Gardea-Torresdey et al., 1996b), and sewage sludge (Solari et al., 1996). All these studies were done to remove and recover heavy metals from dilute aqueous streams by biosorption. The metal uptake process; however, is complex and dependent on the chemistry of the metal ions, specific surface properties of the organisms, cell physiology, and the physico-chemical influence of the environment, for example, pH, temperature, and metal concentration. The uptake of metal ions by microorganisms in batch systems has been shown to occur in two stages: an initial rapid uptake, followed by a much slower process (Ting et al., 1991).

In the present work, growing and washed cells of *Bacillus cereus* M^1_{16} were used to remove Pb(II) from aqueous solutions. Environmental factors for this removal and the adsorption isotherm of the selected organism on the biosorption of lead were studied. The mechanism and kinetics of the lead binding were determined. The main advantage of using growing cultures in bio-removal is avoiding the need for a separate biomass production process—for instance, cultivation, harvesting, drying, processing, and storage prior to use.

PROCEDURES

Organism

The strain *Bacillus cereus* M^1_{16} was used for this purpose (Bera et al., 2005). It was maintained by monthly sub-culturing using nutrient agar and stored at 4°C.

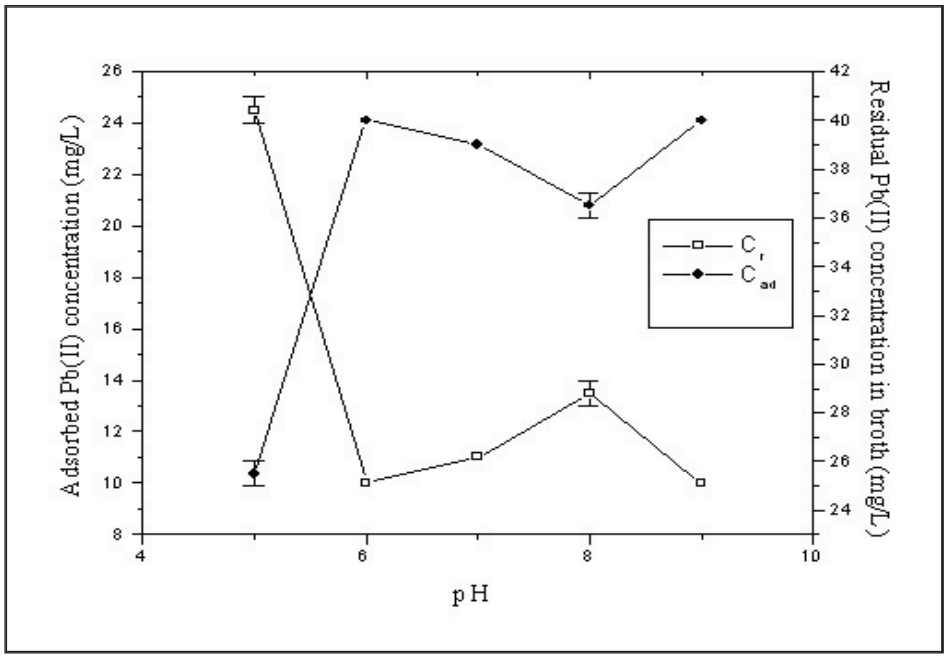

Figure 3. Effect of initial pH on adsorption of Pb(II) by the selected strain (initial lead concentration: 50 mg/L, temperature: 30°C).

Removal of Lead Using Growing Cells of the Selected Strain

Composition of the inoculum medium and fermentation medium was as follows: (g/L), beef extract, 1.0; yeast extract, 2.0; peptone, 5.0; NaCl, 5.0; and pH 6.0. Inoculum was prepared by transferring one loop-full of cells from a slant culture to 50-ml medium in a 250-ml Erlenmeyer flask, and incubating it at 30 ± 1°C and 120 rpm for 24 hours. Fermentation medium (50-ml/250-ml Erlenmeyer flask) containing 50 mg/L Pb(II) ion was inoculated with 4% (v/v) inoculum and incubated at 30°C for 48 hours under the same conditions. Samples were collected at 24 hours and 48 hours. Culture fluid was centrifuged at 5500 rpm for 15 minutes. Residual concentration of lead present in the clear supernatant was estimated. The percent metal bound was taken to be the difference between the control and the final concentration of metal in the supernatant (Gardea-Torresdey et al., 1998).

Estimation of Lead

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

Preparation of Resting Cells

The same fermentation medium containing no Pb(II) ion was used for the production of *Bacillus cereus* M^{1}_{16} biomass, and the same environmental conditions were used to grow the selected strain. After fermentation, viable biomass was harvested by centrifugation at 5500 rpm for 10 minutes at room temperature and washed twice with normal saline (0.85%

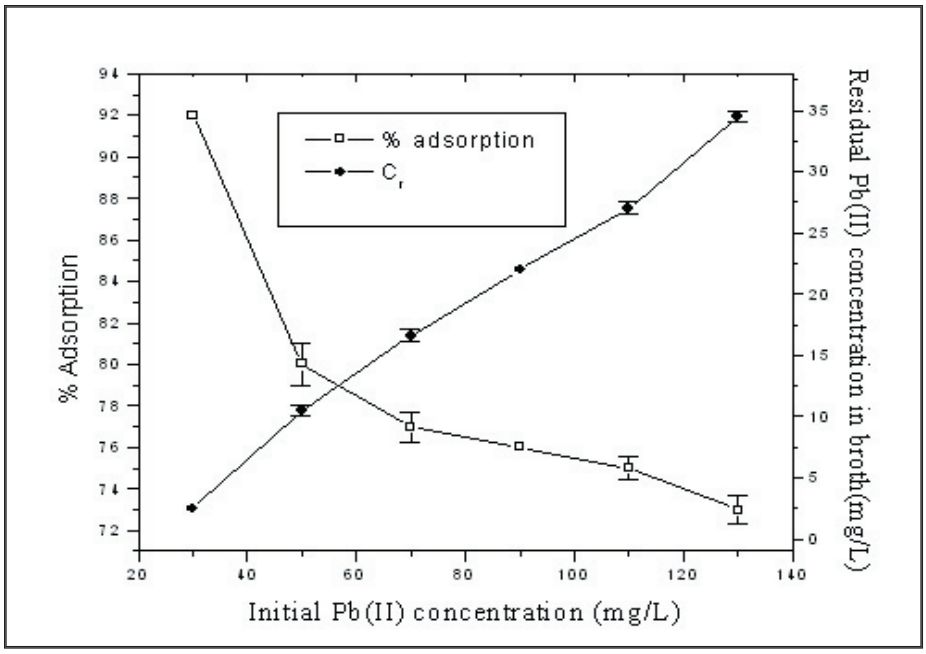

Figure 4. Influence of initial metal concentration on adsorption of Pb(II) by *B. Cereus* M^{1}_{16} (pH: 6.0, temperature: 30°C).

sodium chloride). Washed cells were transferred to a 50-ml normal saline solution in a 250-ml Erlenmeyer flask. The experiments were carried out with wet biomass of the selected strain, but results were calculated using a dry biomass basis. According to Volesky, for scientific interpretations, the sorbent material dry-weight basis is preferred (Volesky, 2004).

Preparation of Dry Cells

Washed biomass (wet) from a measured amount of whole-cell broth was placed in a previously weighed aluminium cup and dried at 70°C overnight. It was weighed again, the weight of the dry cell mass was calculated by finding the difference.

Biosorption Isotherm

The Langmuir model (Langmuir, 1916) is described by the following equation:

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

The above equation may be rearranged to the following linear form:

$$C_e / q_e = 1/ b.q_m + C / q_m \quad (2)$$

where C_e is the equilibrium concentration (mg/L) and q_e is the adsorbed amount of metal ion per gram of biomass at equilibrium (mg/gm). q_m is the maximum amount of metal ion per unit weight of biomass to form a complete mono layer on the surface bound at high C_e (mg/L). b is a constant related to the affinity of the binding sites (L/mg). A plot of C_e/q_e versus C_e should indicate a straight line of slope $1/q_m$ and an intercept of $1/bq_m$. The Freundlich model (Freundlich, 1906) equation is of the form:

$$q_e = k.Ce^{1/n} \quad (3)$$

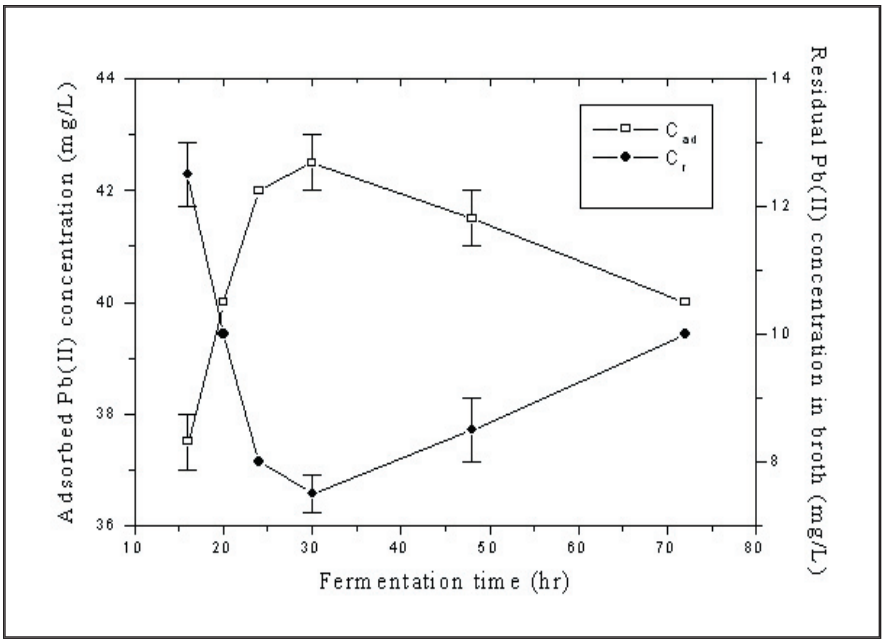

Figure 5. Effect of fermentation time on Pb(II) accumulation (initial lead concentration: 50 mg/L, temperature: 30°C).

Where k and n are the Freundlich constant characteristics of the system (Selatnia et al., 2004). k is the relative indicator of adsorption capacity (L/g), and n indicates the intensity of adsorption.

Equation (3) is conveniently used in the linear form by taking the logarithm of both sides as:

$$\ln q_e = \ln k + (l/n) \ln C_e \qquad 4$$

Kinetic Modelling

The order of adsorbate – adsorbent interactions has been described using various kinetic models. The first-order rate expression of Lagergren (1898), Ho and McKay (1999a), and Aksu (2001), based on solid capacity, is generally expressed as follows:

$$-\log_{10}(q_e - q_t)/q_e = k_1 t/2.3 \qquad 5$$

where k_1 is the rate constant of first-order biosorption (min^{-1}).

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

$$1/(q_e - q_t) = 1/q + k_2 t \qquad 6$$

Here k_2 is the second-order rate constant.

The linear form of the Eq (6) is:

$$t/q_t = 1/h + (1/q_e)t \qquad 7$$

where $h = k_2.q_e^2$ can be regarded as the initial sorption rate as t approaches 0. If the pseudo second-order kinetics is applicable, the plot of t/q_t versus t gives a linear relationship, which

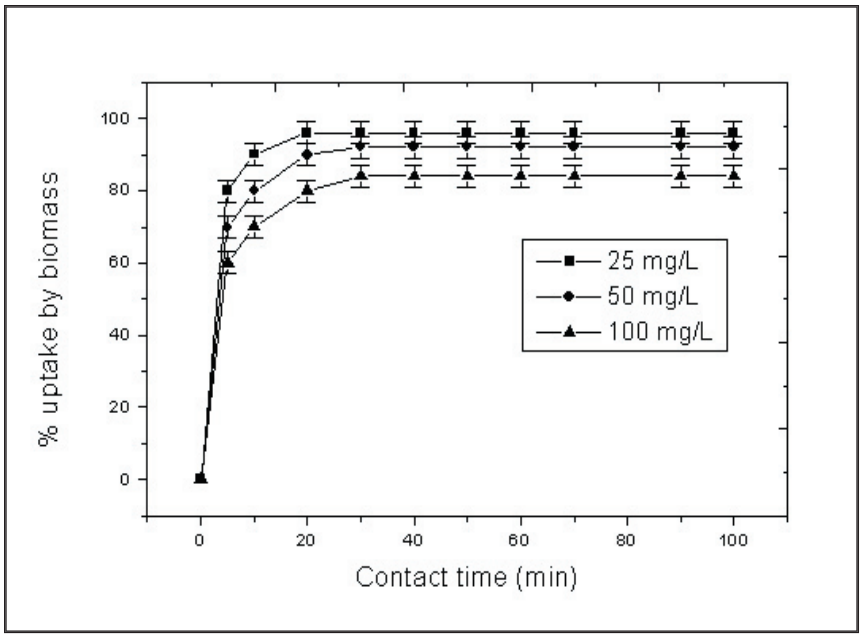

Figure 6. Rate of uptake of Pb(II) by the selected strain at different initial metal concentrations at 30°C (adsorbent concentration: 1.8 g/L).

allows computation of q_e and k_2 values.

Average Absolute Deviation

AAD% was calculated as follows:

AAD% = [Σ (experimental value – calculated value) x 100/experimental value]/number of data points.

RESULTS AND DISCUSSION

Effect of Environmental Conditions on Lead Biosorption Using *Bacillus cereus* M^1_{16}

Effect of Medium Volume

The biosorption of lead was carried out using different volumes viz., 40, 50, 60, and 70 ml of medium containing 50 mg/L Pb(II) ion at 30±1°C for 48 hours. Four percent of 24-hour cell suspension was used as inoculum. After 48 hours, the fermentation broth was centrifuged at 5500 rpm for 10 minutes, and the residual concentration of lead was estimated using an atomic absorption spectrophotometer. Accumulation was maximum (40 mg/L out of 50 mg/L) when 40-ml medium was used. Bioaccumulation decreased with an increase in medium volume (Figure 1). A 40-ml medium in a 250-ml Erlenmeyer flask was used for further studies.

Effect of Inoculum Volume

The effect of inoculum concentration on Pb(II) ion uptake by the selected strain was carried out using different concentrations of inoculum (range 1-5%), with other conditions

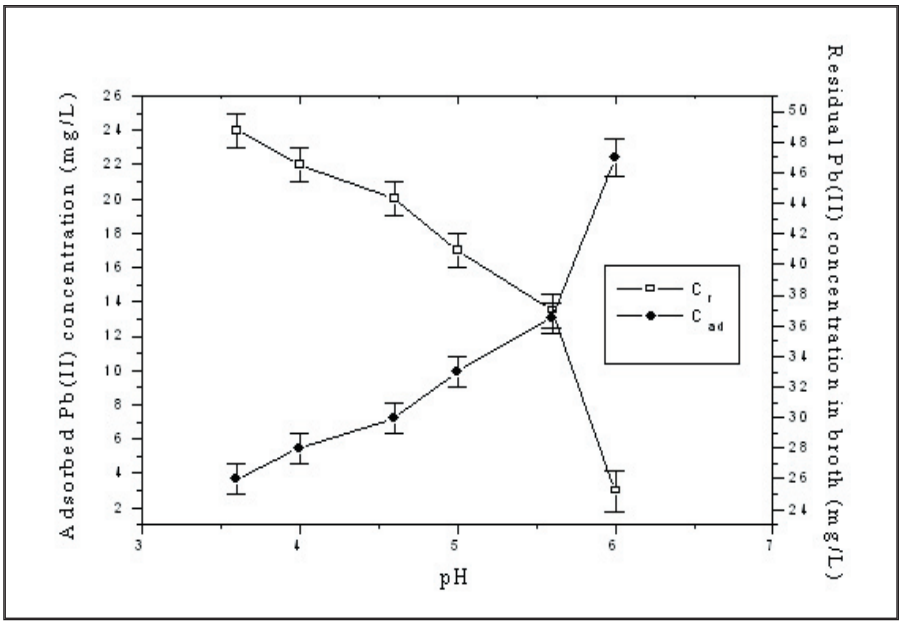

Figure 7. Effect of initial pH on Pb(II) biosorption on the washed biomass at 30°C (initial lead concentration: 50 mg/L, biomass concentration: 1.8 g/L, contact time: 30 minutes).

remaining the same. Consumption of Pb(II) ion increased with increase in inoculum concentration up to 4% and then Pb(II) ion uptake remained constant with increase in inoculum concentration. 4% inoculum volume was chosen for future studies. Pb(II) ion accumulation depends on the amount of biomass produced. Biomass production may be increased with increase in inoculum volume up to a limit due to the limiting amount of the nutrient present in the medium.

Effect of pH

In order to evaluate the effect of pH on Pb(II) uptake, the pH of the fermentation medium was adjusted to be in the range between 5 and 9, other conditions remaining the same. After 48 hours, culture fluid was tested for the presence of residual Pb(II) concentrations as usual. Figure 3 shows accumulation of Pb(II) ions was increased with an increase in pH up to 6.0, then it decreased with an increase in pH. pH plays an important role in Pb(II) ion bio-accumulation properties of the selected strain. At higher pH values (pH > 6.0), lead ions precipitated due to the high hydroxyl ion consumption in the medium. A decrease in metal uptake at low pH levels suggests cations and protons compete for the same site. Consumption of lead ions by the strain was maximum (80%) at pH 6.0 (Figure 3). pH 6.0 was selected for further studies on biosorption.

Effect of Lead Concentration

Pb(II) ion accumulation by *Bacillus cereus* M^{1}_{16} was carried out varying the concentrations of Pb(II) ions (range 30-130 mg/L), other conditions remaining the same. It was found from Figure 4 that percentage of consumption decreased with an increase in initial lead

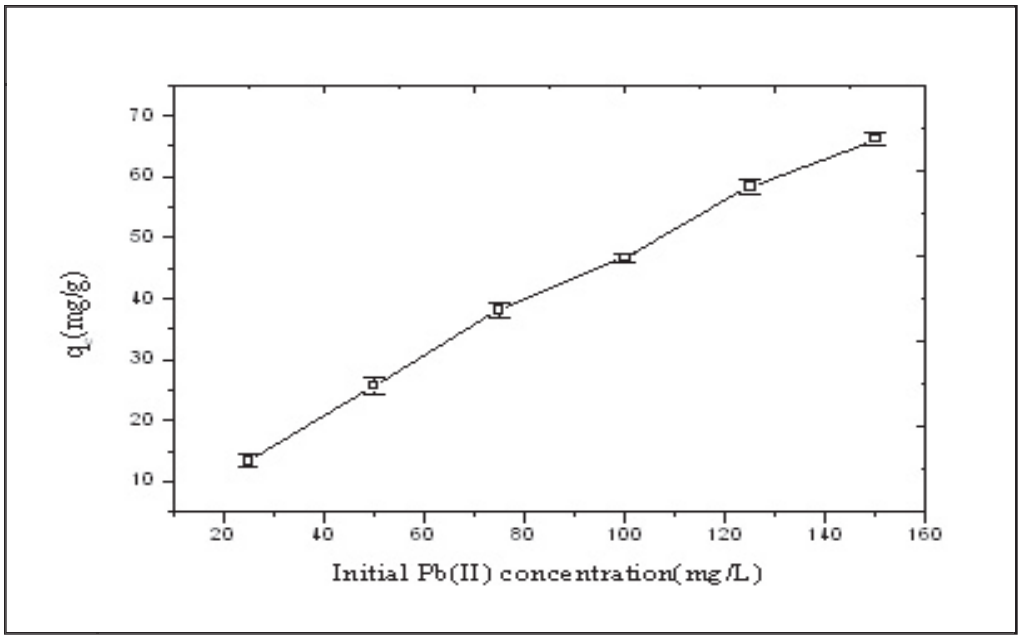

Figure 8. Adsorption of Pb(II) by washed biomass at different initial metal concentrations (adsorbent concentration: 1.8 g/L; temperature: 30°C, contact time: 30 minutes).

concentration. 50 mg/L lead was used in the medium for further studies.

Time course of Lead Bio-accumulation

Pb(II) accumulation by *Bacillus cereus* M^{1}_{16} was carried out in 40-ml medium containing 5 0 mg/L Pb(II) ion in a 250-ml Erlenmeyer flask at 30°C, pH 6.0, shaker speed 120 rpm for 72 hours. Figure 5 shows an increase in lead consumption with time (range 16 to 72 hour), other conditions remaining constant. Maximum adsorption occurred at 30 hours of incubation. It may be concluded that metal binding sites became saturated during the initial 30 hours.

Experiments with Washed Cells

Relations between washed biomass and dry biomass were calculated. The ratio was found to be wet cell; dry cell = 5.5:1. All experiments were carried out with washed cells (wet), but interpretations were cited on a dry-cell weight basis. For scientific interpretations, the sorbent material dry-weight basis is preferred (Volesky, 1999).

Time Course of Biosorption

Time-dependency batch experiments were performed with varying contact times of biomass to Pb(II) ions from 0-100 minutes. 500 mg of washed (wet) biomass (90-mg dry-cell basis, i.e., concentration–1.8g/L) of the selected strain were added to each of the 50-ml normal saline solutions containing 25, 50, and 100 mg/L Pb(II) ions (pH adjusted to 6.0) in each of the 250-ml Erlenmeyer flasks and incubated at 30°C for 100 minutes. Samples were

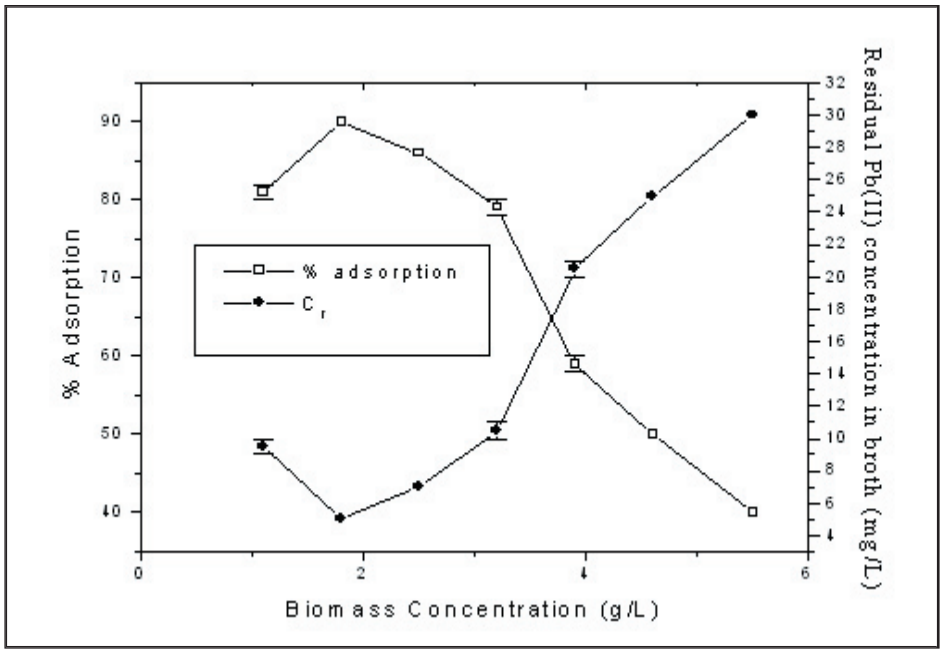

Figure 9. Effect of biomass concentration on the Pb(II) adsorption capacity of the washed biomass at 30°C (initial lead concentration: 50 ppm, contact time: 30 minutes).

collected at different time intervals and tested for residual lead-ion concentration. From Figure 6, it is found that in each case, maximum lead-ion uptake was observed at 30 minutes of incubation. Adsorptions of 96%, 92%, and 84% were observed for 25, 50, and 100 mg/L in initial Pb(II) ion concentrations. Incubation time was fixed at 30 minutes for further studies. Biosorption of Pb(II) ion was rapid during the first 30 minutes of sorption; thereafter, it remained constant. Within the first 10 minutes, 95% of the total Pb(II) accumulation was observed. Metal binding sites were saturated within 30 min. Chandra Sekhar et al., in 2004 reported the rapid binding of Pb(II) ions in 15 minutes using plant biomass as sorbent.

Effect of pH

The effect of pH on Pb(II) uptake by the washed cell of the selected strain is shown in Fig. 7, with other conditions remaining the same. Pb(II) uptake capacity increased with increase in pH and the greatest uptake capacity was obtained at pH 6.0. Metal biosorption depends on the protonation or deprotonation of the functional groups on the cell wall (Urrutia and Beveridge, 1993; Fourest and Volesky, 1997; Fourest 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, bifunctional groups on the cell wall with negative charges increased, due to deprotonation of the metal binding sites, which promote the metal uptake. Ionic forms of the metal in solution and the electrical charge of the biomass depend on the pH of the solution.

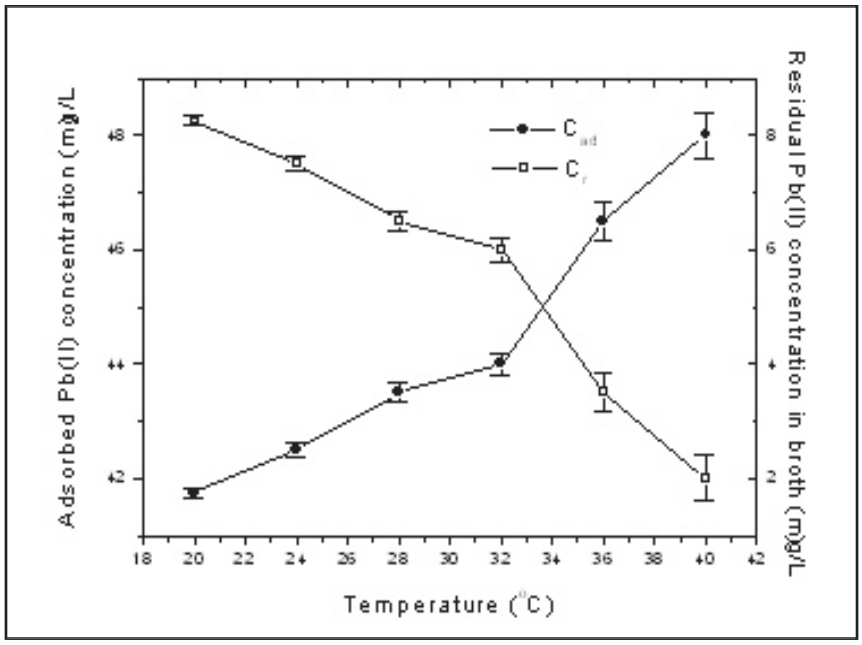

Figure 10. Effect of temperature on adsorption of Pb(II) by washed biomass at (adsorbent concentration: 1.8 g/L, initial lead concentration: 50 mg/L).

Effect of Biomass Concentration

Pb(II) ion uptake by washed cells of *Bacillus cereus* M^{1}_{16} was studied using different biomass concentrations (range 1.1 – 5.5 g/L-dry basis), other conditions remaining the same. Figure 8 shows that the percent adsorption increased with the increase in wet biomass concentration, up to a concentration of 1.8 g/L, and then decreased with an increase in biomass concentration. The decrease in rate of elimination of Pb(II) ion could be attributed to the biomass granules, which were agglomerated (Selatnia et al., 2004).

Influence of Initial Lead Concentration

Influence of initial lead ion concentration on biosorption of Pb(II) ion by the selected bacterial strain (biomass concentration in dry-cell weight basis-1.8g/L) was 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 25 – 150 mg/L), but percent adsorption decreased with increase in initial lead concentration (Figure 9) due to rapid saturation of the metal binding sites of the biosorbent.

Effect of Temperature

Biosorption of Pb(II) was carried out using washed cells of *Bacillus cereus* M^{1}_{16} at different temperatures (20^{o} – 40^{o}C), other conditions remaining the same. Figure 10 shows that maximum adsorption was observed at 20^{o}C and Pb(II) ion uptake decreased with an increase in temperature. Physical adsorption reactions are normally exothermic, thus the extent of adsorption generally increases with a decrease in temperature (Salinas et al., 2000).

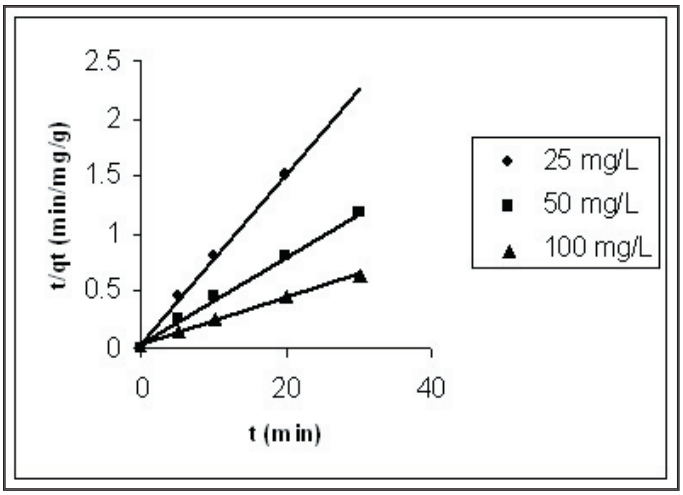

Figure 11. Pseudo second-order adsorption kinetics of Pb (II) on the washed biomass at different initial concentrations at 30°C (adsorbent concentration: 1.8 g/L).

Kinetics of Adsorption

The kinetics were investigated with a constant adsorbent concentration of 1.8 g/L (dry-weight basis) at 30°C with three different initial Pb(II) concentration of 25, 50, and 100 mg/L at different time intervals up to 100 minutes. The percent of biosorption of Pb(II) by the organism is shown in Figure 6. The sorption process was rapid and reached equilibrium within 30 minutes.

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. It was found that in the present work, although the first-order equation was suitable for some of the data, it was not applicable to all the results. Therefore, the pseudo second-order model based on Equation 6 was applied. The linear form of Equation 6, i.e., $t/q_t = 1/h + (1/q_e)t$, which considers the rate-limiting step as the formation of chemisorptive bond involving sharing or exchange of electrons between the adsorbate and the adsorbent, was therefore applied. The plot of t/q_t versus t (Figure 11) yields very good straight lines (correlation coefficient, $R^2 = 0.99$). The second-order rate constant were in the range of 1.527×10^{-2} to 1.369×10^{-1} g/mg minutes. Second order kinetics was earlier reported for adsorption of Pb(II), Cu(II), and Zn(II) on *Myriophyllum spicatum* (Keskinkan et al., 2003) with rate constants of 3.695×10^{-3}, 1.508×10^{-3}, and 3.38×10^{-2} g/mg minutes, respectively. Success with the second-order kinetics suggests chemisorption as the rate-controlling step (Ho and McKay, 2000). Second order kinetics were earlier reported for adsorption of Pb(II) on peat with rate constants of 8.69×10^{-3} to 4.59×10^{-1} g/mg minutes (Ho and McKay, 1998). Kinetics of adsorption of Pb(II) on Neem leaf powder followed the pseudo second-order equation with plots of t/q_t versus t yielding

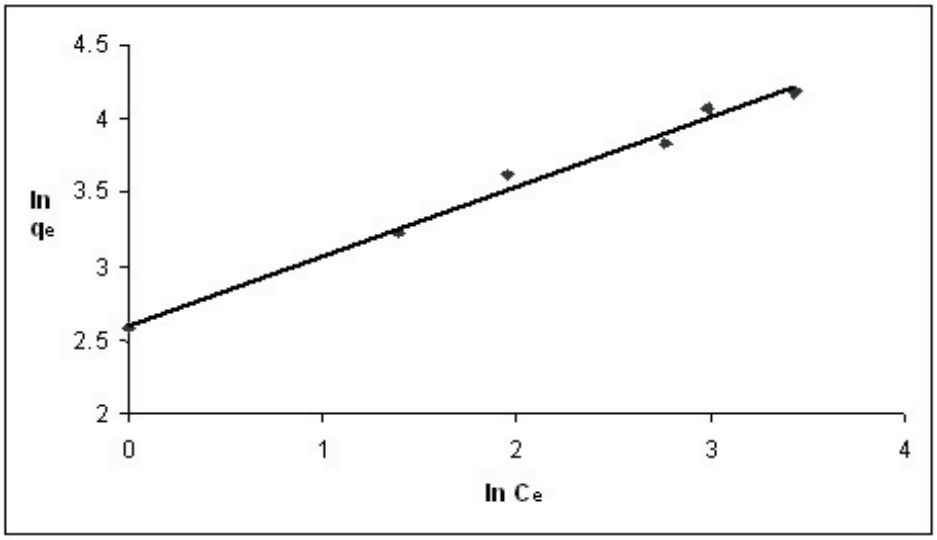

Figure 12. Application of Freundlich isotherm to the adsorption data of Pb (II) adsorbed onto the washed biomass at 30°C (adsorbent concentration: 1.8 g/L, contact time: 30 minutes).

straight lines with a high correlation co-efficient of 0.99. The second-order rate constant was in the range of 1.78 x 10^{-4} to 1.80 x10^{-3} g/mg minutes (Bhattacharyya and Sharma, 2004).

Adsorption 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, in order to optimize the biosorption process parameters, we have modelled the equilibrium curves. Figure 12 shows the linear plot of ln q_e versus ln C_e and gives a straight line having a slope 1/n and intercept ln k. The values of the Freundlich constants n and k are presented in Table 1 for the Pb (II) / unit weight of the biomass adsorption system. These values of the correlation coefficients indicate a strong positive relationship for the data, and the metal/biomass sorption data follows the Freundlich adsorption isotherm. The applicability of the Langmuir adsorption isotherm has also been analyzed by plotting C_e/q_e versus C_e (Figure 13), but data were not found in good agreement (AAD% 9.1), as in case of the Freundlich (AAD% 5.6). Table 1 shows the Langmuir and Freundlich adsorption isotherm constants and correlation coefficients. Values of co-efficients of correlation R^2 (0.988) show that the Freundlich model best fit our experimental data. Figure 14 shows plots comparing the theoretical Langmuir isotherm and the empirical Freundlich isotherm with experimental data. The equation shows an excellent agreement with the experimental data for the Freundlich isotherms. Jianlong et al., 2001 reported Pb(II) sorption from an aqueous solution by fungal biomass of *Aspergillus niger*. Freundlich constants for the system were as follows: k: 1.69, 1/n: 0.39, and R^2: 0.93.

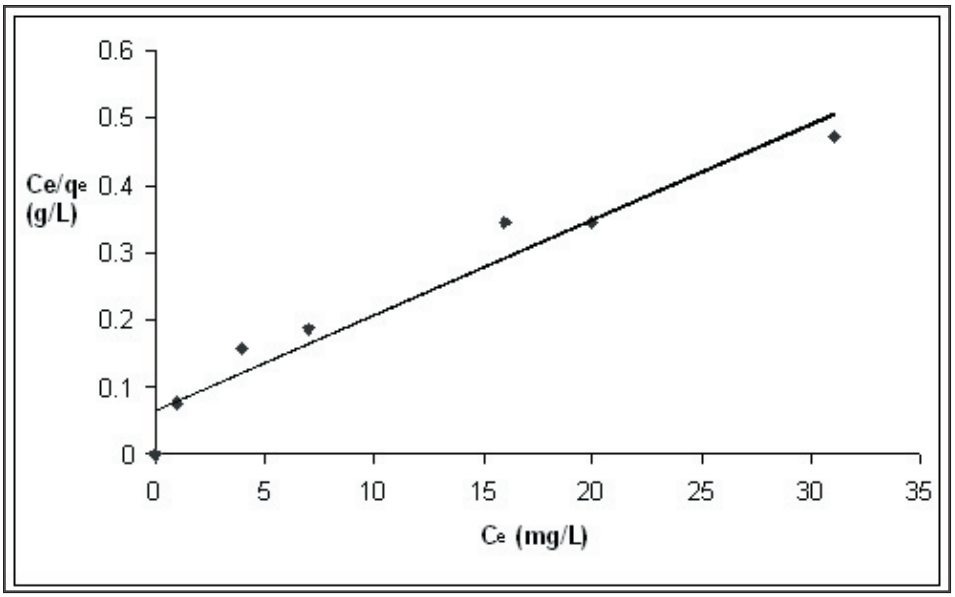

Figure 13. Application of Langmuir isotherm to the adsorption data of Pb(II) adsorbed onto the washed biomass at 30°C (adsorbent concentration: 1.8 g/L, contact time: 30 minutes).

The high value of correlation co-efficient indicated that the data conformed well to the Freundlich model (Jianlong et al., 2001).

In resting-cell experiments, all the experiments were carried out using wet cells but interpretations were on the basis of dry-cell weight. For scientific interpretations, the sorbent material dry-weight basis is preferred. Use of "wet biomass weight", unless the wet-weight/dry-weight conversion is well specified, should be discouraged (Volesky, 1999).

Gadd (1990) reported that an equilibrium was reached at 2 hours with initial lead concentration of 250 mg/L. It was also observed that the uptake reached 70% during the first 15 minutes. Uslu et al., (2003) reported optimum initial pH and temperature for growing cells of *Rhizopus arrhizus* were 4.0 and 25°C, respectively. The culture was able to remove 54.9 and 30.5% total Pb(II) ions at 50 and 75 ppm initial metal ion concentrations, respectively. Sag et al., (2000) found that the maximum Pb(II) uptake capacity of *R. arrhizus* in a continuous-flow stirred-tank reactor was 48.79 mg/g. Keskinkan et al., (2004) studied Pb(II) biosorption by *Ceratophyllum demursum* and observed that equilibrium was attained within 20 minutes with maximum adsorption capacity of 44.8 mg/g at pH just below 6. Their studies reported equilibrium according to the Langmuir model and that the overall adsorption

Table 1. Sorption isotherm coefficients of Langmuir and Freundlich models.

Freundlich			Langmuir		
1/n	k (L/g)	R^2	qm (mg/g)	b (L/mg)	R^2
0.47	13.487	0.988	70.423	0.214	0.941

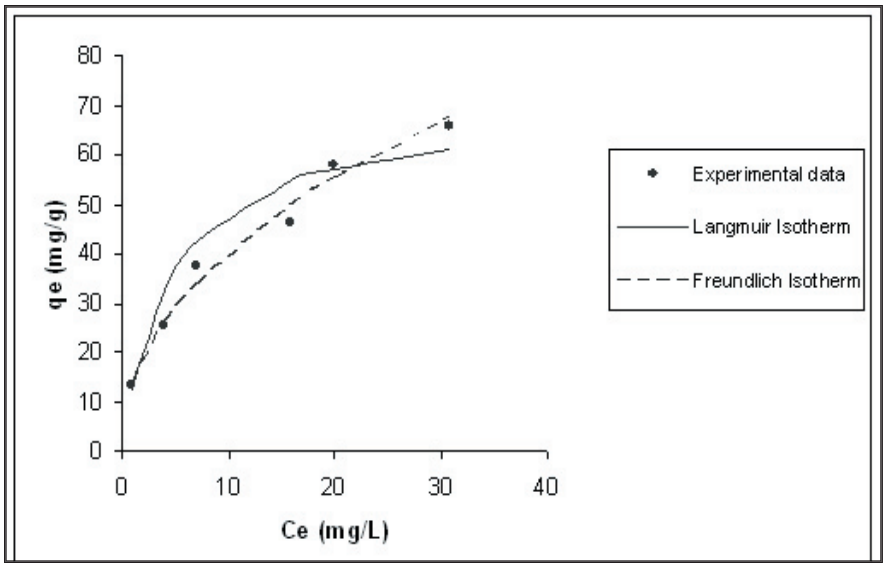

Figure 14. Isotherm of Pb(II) adsorption on the washed biomass at 30°C (adsorbent concentration: 1.8 g/L, contact time: 30 minutes).

process was best described by pseudo second-order kinetics. Veglio et al., (1997) observed that the highest value of lead-specific uptake was 130 mg/g at initial Pb(II) ion concentration of 250 mg/L with a biomass concentration of 1.4 g/L. The Langmuir adsorption model was used to fit the experimental points. Ceribasi and Yetis (2001) reported removal efficiencies for Pb(II) generally were achieved in the first 30 minutes of contact at initial pH 5.0 and initial Pb(II) concentration of 50 mg/L. In the first 5 minutes sorption took place very rapidly, then it continued slowly and equilibrium was reached in a contact time of 3 hours. The highest sorption capacity of *Phanerochaete Chrysosoporium* for Pb(II) was 73.56 mg/g at initial Pb(II) concentration of 50 mg/L. Dursun et al., (2003) observed that *Aspergillus niger* consumed lead(II) by 86% when initial lead concentration was 50 mg/L and qm was 16.8 mg/g of dry cell at pH 4.5 and temperature 30°C.

In the present study, we observed optimum pH, temperature, and time for maximum Pb(II) adsorption (96%) were 6.0, 20°C, and 30 minutes, respectively, at initial Pb(II) ion concentration of 50 mg/L using washed biomass of *Bacillus cereus* M^{1}_{16}. At low pH, the concentration of protons is high, so metal binding sites become positively charged. Metal cations and protons then compete for binding sites, which results in a lower uptake of metal. With an increase in pH, bifunctional groups on the cell walls with negative charges increase due to deprotonation of the metal binding sites which promote metal uptake. Tran et al., (1999) reported the same trend in adsorption characteristics of Pb(II) ions, and the adsorption increased from 0-90% with an increase in the pH range from 2.0-6.0. Large discrepancies in metal removal at higher pH were described as being due to another possible mechanism such

as precipitation (Bhattacharyya and Sharma, 2004). Maximum Pb(II) ion removal (85%) using growing cells of the bacterial strain was observed at 30°C, pH 6.0, on 30 hours of fermentation, when initial lead concentration was 50 mg/L. Optimum temperature for biosorption by growing cells of the selected strain was found to be 30°C, because this temperature promotes growth of the selected strain, whereas in the case of washed cells there was no need of growth of biomass and the optimum temperature was observed as 20°C. Biosorption of Pb(II) on washed biomass of *Bacillus cereus* M^1_{16} was very fast, and equilibrium was attained within 30 minutes. The overall adsorption process was best described by pseudo second-order kinetics. The Freundlich isotherm provided the best fit. In this work, 96% lead(II) uptake was possible with 1.8 g/L (dry cell-weight basis) of adsorbent. Our results are more or less similar to the above mentioned results reported by different authors. Interaction between metal and microbial cells can occur through adsorption to cell surfaces, through metabolically assisted accumulation within the cell or as metal complexes with extra-cellular microbial metabolites (Norberg and Rydin, 1984). The walls of gram-positive bacteria are efficient metal chelators and in *B. subtilis*, the carboxylic group of glutamic acid of peptidoglycan was the major site of metal deposition (Gadd, 1990). Teichoic and teichuronic acids were important binding sites in *B. licheniformis* (Gadd, 1990). *Bacillus cereus* M^1_{16} is a gram-positive bacterium and has similar cell-wall properties as other gram-positive bacteria.

CONCLUSION

Growing and washed biomass of *Bacillus cereus* M_1^{16} was found to be efficient for adsorption of Pb(II) in dilute solutions. The characterization of lead uptake showed that the lead binding is dependent on initial pH, temperature, initial lead-ion concentration, and biomass concentration, and occurs within 30 minutes. Percentage uptake increased with an increase in pH. The percentage uptake decreased with an increase in biomass concentration above 1.8 g/L. The experimental results were analyzed using Langmuir and Freundlich equations. Table 1 shows the Langmuir and Freundlich adsorption isotherm constants. Correlation coefficients (R^2) show that the Freundlich model fitted best to our experimental data. Results from this study are extremely well described by the Freundlich isotherm. The overall adsorption rate showed that the kinetics of the adsorption of Pb(II) on the *Bacillus cereus* M^1_{16} was best described by the pseudo second-order model. The results obtained during this study showed that this method of accumulation of Pb(II) ion was very promising compared to more conventional processes.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the U. G. C., Government of India, for financial support to carry out the research work.

NOMENCLATURE

B	Langmuir constant (L/mg)
C	Concentration of metal in solution (mg/L)
C_{ad}	Adsorbed Pb(II) concentration (mg/L)
C_e	Liquid-phase concentration of metal at equilibrium (mg/L)
C_r	Residual Pb(II) concentration in the broth (mg/L)
k_1	First-order rate constant (min^{-1})
k_2	Second-order rate constant (g/mg minutes)
k	Constant, relative indicator of adsorption capacity (L/g)
1/n	Constant, intensity of the adsorption
q_e	Metal uptake at equilibrium (mg/g biomass)
q_m	Maximum theoretical metal uptake (mg/g biomass)
q_t	Metal uptake at any time (mg/g biomass)
t	Time (minutes)

REFERENCES

Aksu, Z., 2001. Equilibrium and kinetic modeling of Cadmium(II) biosorption by *C. vulgaris* in batch system: effect of temperature. Sep. Purif. Technol. 21, 285-294.

Arica, M.Y., Kacar, Y., and Genc, O., 2001. Entrapment of white rot fungus *Trametes vesicolor* in Ca-al genate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. Bioresource Technology. 80, 121-129.

Bera , D., Ray, L., and Chattopadhyay, P., 2005. Cr(VI) biosorption by growing and resting cells of *Bacillus cereus* M116. J. Bioresource Technology. In Press.

Bhattacharyya, K.G., and Sharma, Arunima, 2004. Adsorption of Pb(II) from aqueous solution by *Azadiracta indica* (Neem) leaf powder. J. Haz. Materials. 113, 97-109.

Brierley, C.L, Kelly, D.P., Seal, K.J., and Best, D.J., 1985. In Biotechnology. Higgins, I.J., Best D.J., and Jones, J(eds.), Blackwell, Oxford, 212.

Ceribasi, I.H., and Yetis, U., 2001. Biosorption of Ni(II) and Pb(II) by *Phanerochaete chrysosporium* from a binary metal system. Kinetics. 27 (1), 15-20.

Chandra Sekhar, K., Kamala, C.T., Chary, N.S., Sastry, A.K., Rao, T.N., and Vairamani, M., 2004. Removal of lead from aqueous solutions using an immobilized biomaterial

derived from a plant biomass. J. Hazd. Materials. 108, 111-117.

Chong, K.H., and Volesky, B., 1996. Metal biosorption equilibria in ternary system. Biotechnol. Bioeng. 49, 629-638.

Deans, J.R., and Dixon, B.G., 1992. Uptake of Pb2+ and Cu2+ by novel biopolymers. Water Res. 26, 469-472.

Dursun, A.Y., Uslu, G., Cuci, Y., and Aksu, Z., 2003. Bioaccumulation of copper(II), lead(II), of Chromium(VI) by growing *Aspergillus niger*. Process Biochem. 38 (12), 1647-1651.

Fourest, E., and Roux, G.C., 1992. Heavy metal biosorption by fungal mycelial by-products, mechanisms and influence of pH. Appl. Microbiol. Biotechnol. 37, 399-402.

Fourest, E., and Volesky, B., 1997. Alginate properties and heavy metal biosorption by marine algae. Biochem. Biotechnol. 67, 215-226.

Freundlich, H.M.F., 1906. Uber die adsorption in losungen. Zeitschrift fur Physikalische Chemie. 57, 385-470.

Gadd, G.M., 1990. Heavy metal accumulation by bacteria and other microorganisms. Experientia, 46, 834-839.

Gardea-Torresdey, J.L., Cano-Aguilera, A., Webb, R., Tiemann, K., and Gutierrez-Corona, F., 1996a. Copper adsorption by inactivated cells of *Mucor roxii*: effect of esterification of carboxyl groups. J. Hazard. Mat. 48, 171-180.

Gardea-Torresdey, J.L., Tang, L., and Salvador, J.M., 1996b. Copper adsorption by esterified and unesterified fractions sphagnum peat moss and its different humic acid. J. Hazard. Mat. 48, 191-206.

Gardea-Torresdey, J.L., Arenas, J.L., Francisco, N.M.C., Tiemann, K.J., and Webb, R., 1998. Ability of immobilized cyanobacteria to remove metal ions from solution and demonstration of the presence of metallothionein genes in various strains. J. Hazard. Subs. Res. 1, 2-1, 2-18.

Goyer, R. A., and Chisolon, I. J., 1972. Lead, in metallic contaminations and human health. Lee, D.H.K. (Ed). Academic Press, New York, p. 57.

Hao, Y., Zhao, Y., and Ramelow, G. J., 1994. Uptake of metal ions by non-living biomass derived from marine organisms: effect of pH and chemical treatments. J. Environ. Sci. Health. 29, 2235-2254.

21 Ho, Y.S. ,…….2001.-Reference omitted

Ho, Y.S., and McKay, G., 2000. Batch sorber design using equilibrium and contact time data for the removal of lead. Air Soil Pollut. 124 (1), 141-153..

Ho, Y.S., and McKay, G., 1999a. Comparative sorption kinetic studies of dye and aromatic compounds onto fly ash. J. Environ. Sci. Health A. 34, 1179-1204.

Ho, Y.S., and McKay, G., 1999b. Pseudo second-order model for sorption process. Process Biochem. 34, 451-459.

Ho, Y.S., and McKay, G., Trans, I., 1998. Chem. E. 76B, 332.

25 Hunt, S.,1986 …….-Reference omitted

Jaanson-Cherrier, M., Guibal, E., Roussy, J., Belanghe, B., and Lecloirec, P., 1996. Vanadium(IV) sorption by Chitosan: kinetics and equilibrium. Water Res. 30, 465-475.

Jianlong, W., Xinmin, Z., Decai, D., and Ding, Z., 2001. Biosorption of lead(II) from aqueous solution by fungal biomass of *Aspergillus niger*. J. Biotechnol. 87, 273-277.

Keskinkan, O., Goksu, M.Z.L., Yuceer, A., Basibuyuk, M., Forster, C.F., 2003. Heavy metal adsorption characteristics of a submerged aquatic plant (*Myriophyllum spicatum*). Process Biochem. 9, 179-183.

Keskinkan, O., Goksu, M. Z. L., Basibuyuk, M., and Forster, C.F., 2004. Heavy metal adsorption properties of a submerged aquatic plant (*Ceratophyllum demersum*). Bioresorce Technol, 92, 197-200.

Khoshmanesh, A., Lawson, F., and Prince, I.G., 1996. Cadmium uptake by unicellular green microalgae. Chem. Eng. J. 62, 81-88.

Kuyucak, N., and Volesky, B., 1989. Accumulation of cobalt by marine alga. Biotechnol. Bioeng. 33, 809-822.

Lagergren, S., 1898. Handlingar. 24, 1.

Langmuir, I., 1916. The constitution and fundamental properties of solids and liquids. J. American Chem. Soc. 38, 2221-2295.

Leusch, A., and Volesky, B., 1995. Influence of film diffusion on cadmium biosorption by marine biomass. J. Biotechnol. 43, 1-10.

Mago, R., and Srivastava, S., 1994. Uptake by zinc in Pseudomonas sp. Strain UD626. Appl. Environ. Microbiol. 60, 2367-2370.

36 Manju,G.N., …. –Reference omitted

Mishra, S., Chaudhury, P., and Roy, G., 1996. Kinetics of Zn+2 adsorption by *Penicillium* sp. Hydrometallurgy. 40, 11-23.

Modak, J.M., Natarajan, K.A., and Saha, B., 1996. Biosorption of copper and zinc using waste *Aspergillus niger* biomass. Miner. Metall. Process. 13, 52-57.

Muralikrishna, K.V.S.G., 1997. Chemical analysis of water and soil. A laboratory manual, Environmental Protection Society, National Institute of Ecology and Environment, Kakinada, India.

Norberg, A., and Rydin, S., 1984. Development of a continuous process for metal accumulation by *Zoogloea ramigera*. Biotech. Bioeng. 26, 265-268.

Nourbakhsh, M.N., Kilicarslan, S., Iihan, S., and Ozdag, H., 2002. Biosorption of Cr6+, Pb2+ ions in industrial wastewater on *Bacillus* sp. Chem. Eng. J. 85, 351-355.

42 Ozer,A., ……. – Reference omitted

Puranik, P.R., and Paknikar, K.M., 1997. Biosorption of lead and zinc from solutions using *Streptoverticillium cinnamoneum* waste biomass. J. Biotechnol. 55, 113-124.

Runnells, D.D., Shepherd, T.A., and Angino, E.E., 1992. Metals in determining natural background concentrations in mineralized areas waters. Environ Sci. Technol. 26, 2316-2323.

Sag, Y., and Kutsal, T., 1995. Biosorption of heavy metals by *Zoogloea ramigera*: use of adsorption isotherms and a comparison of biosorption characteristics. Chem. Eng. J. biochem. Eng. J. 60, 181-188.

Sag, Y., Ozer, D., and Kutsal, T., 1995. A comparative study of the biosorption of lead(II) ions to *Z. ramigera* and *R. arrhizus*. Process Biochem. 30, 169-174.

Sag, Y., Yalcuk, A., and Kutsal, T., 2000. Mono and multi-component biosorption of heavy metal ions on *Rhizopus arrhizus* in a CFST. Process Biochem. 35 (8), 787-799.

Salinas, E., de Orellando, M.E., Rezza, I., Martinez, L., Marchesvky, E., and Tosset, M.S., 2000. Removal of cadmium and lead from dilute aqueous solutions by *Rhodotorula rubra*. Bioresource Technol. 72, 107-112.

Selatnia, A., Boukazoula, A., Kechid, N., Bakhti, M.Z., Chergui, A., and Kerchich, Y., 2004. Biosorption of lead(II) from aqueous solution by a bacterial dead *Streptomyces rimosus* biomass. Biochem. Eng. J. 19, 127-135.

49 Segel, I.E., ……- Reference omitted

51 Sharma.D.C., ……..- Reference omitted

Singleton, I., and Simmons, P., 1996. Factors affecting silver biosorption by an industrial strain of *Saccharomyces cerevisiae*. J. Chem. Tech. Biotech. 65, 21-28.

Solari, P., Zouboulis, A.I., Matis, K.A., and Stalidis, G.A., 1996. Removal of toxic metals by biosorption onto nonliving sewage sludge. Sep. Sci. 31, 1075-1092.

Ting, Y.P., Lawson, F., and Prince, I.G., 1991. Uptake of cadmium and zinc by the alga *Chorella vulgaris*: multi-ion situation. Biotech. Bioeng. 37, 445-445.

Tran, H.H., Roddic, K.J., and O'Donnel, J.A., 1999. Comparison of chromatography and dessicant silicagels for the adsorption of metal ions-I adsorption and kinetics. Water Res. 33, 2992-3000.

Tsezos, M., 1984. Recovery of uranium from biological adsorbents-desorption equilibrium. Biotechnol. Bioeng. 26, 973 -981.

Urrutia, M., and Beveridge, T.J., 1993. Re-mobilization of heavy metals retained as oxyhydroxides or silicates by *Bacillus subtilis* cells. Appl. Environ. Microbiol. 59, 4323-4329.

Uslu, G., Dursun, A.Y., Ekiz, H.I., and Aksu, Z., 2003. The effect of Cd(II), Pb(II), and Cu(II) ions on the growth and bioaccumulation properties of *Rhizopus arrhizus*. Process Biochem. 39, 105-110.

Veglio, F., Beolchini, F., and Gasbarro, A., 1997. Biosorption of toxic metals: an equilibrium study using free cells of *Arthrobacter* sp. Process Biochem. 32 (2), 99-105.

Volesky, B., 1999. Evaluation of biosorption performance (Chapter 6) in Sorption and Biosorption. 1-10, from Internet. www.biosorption.Mcgill.ca.

Volesky, B., 2004. Sorption and Biosorption. (Chapter 6), 103-128. BV Sorbex, Inc., St. Lambert, Quebec.

Original Manuscript Received: October 18, 2005

Revised Manuscript Received: June 5, 2006