Biosorption of Pb(II) by Bacillus badius AK strain originating from rotary drum compost of water hyacinth

Isha Vishan, Avishek Laha and Ajay Kalamdhad

ABSTRACT

The presence of heavy metals in the environment due to industrial activities is of serious concern because of their toxic behaviour towards humans and other forms of life. Biosorption of Pb(II) using dry bacterial biomass of Bacillus badius AK, previously isolated from water hyacinth compost, has been undertaken in batch system. The optimum conditions of biosorption were determined by investigating the initial pH, contact time, initial biomass dosage at constant temperature of 40°C, initial metal concentration of 100 mg/L and rotational speed of 150 rpm. The optimum pH was found to be 5 and equilibrium contact time was 2.5 h. The maximum biosorption capacity of Pb(II) on Bacillus badius AK was 138.8 mg/g at an initial concentration of 100 mg/L. A kinetics study revealed that the adsorption process followed pseudo second order rate kinetics. The experimental data were fitted to the Langmuir isotherm. Characterization of the biomass indicated the presence of several functional groups. The results indicated that the bacterium Bacillus badius AK is efficient for the removal of Pb(II).

Key words | Bacillus badius AK, biosorption, kinetics, toxic

INTRODUCTION

Heavy metals discharged from various industries such as mining, smelting, metal plating, and ore processing cause hazardous effects on humans, animals and the environment (Volesky & Holan 1995; Duffus 2002). At very low concentrations, heavy metals are essential to all living organisms but can cause severe toxic effects in humans, plants and animals at higher concentrations (Lo et al. 1999). Mercury, lead and cadmium, called ‘the big three’, have a major impact on the environment among all the toxic heavy metals (Volesky 1994). Lead is one of the most prevalent heavy metal contaminants. Lead is used in various industrial activities, such as lead acid batteries, soldering, paints, ammunition, pipes and plastic stabilizers (WHO 1989). Routes of exposure to lead include contaminated air, water, soil, and food and consumer products. Occupational exposure is a common cause of lead poisoning in adults (Staudinger & Roth 1998). Lead is a cumulative general poison, infants and pregnant women being most susceptible to adverse health effects; it deteriorates the central nervous, kidney and the reproductive systems (Sheng et al. 2004). An average daily intake of 5 μg/L lead in water will contribute a relatively small proportion of the daily lead intake for adults and children, but for bottle-fed infants it would be a significant effect (Levin et al. 1989). The World Health Organization (WHO) and the Bureau of Indian Standards recommend in their guidelines 10 μg/L of lead as the threshold limit for drinking water quality (WHO 1989; Singh et al. 2012).

There are several physico-chemical techniques applied to remove heavy metals from aqueous solutions, which include precipitation, ion exchange, evaporation, electroplating and membrane process. However, these methods have been found uneconomical and inefficient at low metal concentrations, and they generate secondary wastes (Kuyucak & Volesky 1988). Biosorption of heavy metals using bacterial, fungal and algal biomass (living or dead cells) and agricultural waste biomass has been found to be a potential alternative to conventional techniques. Their usage is advantageous on grounds of no secondary sludge production, higher selectivity, easy to operate and economically sound for handling huge volumes of wastewater (Sheng et al. 2004; Gong et al. 2005; Tunali et al. 2006; Amarasinghe & Williams 2007).

The process of biosorption involves physico-chemical interactions between metal ions and several anionic ligands like carboxyl, phosphoryl, carbonyl and sulphydryl which...
are present on the surface of the biomass (Volesky 1994). The efficiency of the biosorption process depends on the type of metal ion being studied and the type of biosorbent (Tunali et al. 2006). Biosorbent materials, such as the green algae Spirogyra species (Gupta & Rastogi 2008), Azadirachta indica bark (King et al. 2008), Rhizopus oryzae (Bhainsa & D’Souza 2008), and Bacillus jeotgali, have been reported in biosorption studies (Green-Ruiz et al. 2008). Both dead and living microbial cells for biosorption have been studied and the results showed that dead microbial cells are more advantageous in water treatment due to the fact that dead organisms are not affected by toxic wastes, do not require a continuous supply of nutrients and can be regenerated and reused for many cycles (Tunali et al. 2006). Both dead and living microbial cells for biosorption have been studied and the results showed that dead microbial cells are more advantageous in water treatment due to the fact that dead organisms are not affected by toxic wastes, do not require a continuous supply of nutrients and can be regenerated and reused for many cycles (Aksu 2005). The maximum adsorption of Pb(II) by Bacillus cereus and Bacillus pumilus was 28.06 mg/g and 22.1 mg/g, respectively (Çolak et al. 2011), whereas for Bacillus licheniformis, removal of Cr(VI) was tested and maximum biosorption capacity was obtained to be 60.5 mg/g (Zhou et al. 2007). In the case of the Citrobacter strain MCM B-181 a maximum of 70.8 mg/g of uptake was observed, but also a decrease of 13–68% of Pb(II) uptake was noticed when the biomass was treated with heat and autoclaving (Puranik & Paknikar 1999). In every study not more than 90% of metal removal was achieved by the bacterial species. Additionally, study of metal removal by a bacterial strain originating from water hyacinth compost has not yet been performed. Therefore, for the current study, a bacterial species was previously isolated from water hyacinth compost, which is loaded with huge metal content. The Bacillus badius AK strain was most robust and vigorous, surviving in the unstable conditions of composting. However, investigations on the kinetics, isothermal study, desorption and reusability of the biomass are limited. In the current study, process parameters influencing the biosorption were standardized in anticipation of utilization of biomass in large-scale metal recovery systems in future. The aim of this study is to evaluate the metallic sorption potential of bacterial strain Bacillus badius AK.

**MATERIALS AND METHODS**

The biosorption study of Pb(II) removal by Bacillus badius AK was performed in batch mode. Preparation of biosorbent was done by drying and powdering after the lyophilization of bacterial biomass. Parameters such as pH, temperature, thermodynamics, initial metal concentration, biomass dosage and contact time affect the ability of biosorption. Optimization of these parameters for Pb(II) removal by Bacillus badius AK was performed in detail. Adsorption kinetics, equilibrium, desorption and reuse studies performed to detect the predictability of the rate of biosorption operation. All the experiments were performed in triplicate, yielding an experimental error of less than 2%.

**Preparation of the bacterial adsorbent**

*Bacillus badius* AK (KP216715), isolated from the water hyacinth compost, was used in the study. The 16S rRNA gene sequence of *Bacillus badius* AK was amplified with primers 8F (5′-AGAGTTGTATCCTGGCTCAG-3′) (forward) and 1492R (5′CGGTACCTTGTTACGACTT-3′) (reverse). The amplification products were cleaned using the GenElute PCR Clean-Up kit (Sigma-Aldrich, St Louis, MO, USA). PCR (polymerase chain reaction) samples were sent to Xcelris Labs Ltd for 16S RNA gene sequencing; thereafter the gene sequence was submitted to the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank). The sequence was assigned accession no. (KP216715) by NCBI. The sequence showed a close relatedness with 16S rRNA sequence of *Bacillus badius* strain F (KJ000879) (99% similarity). Thus, *Bacillus badius* strain AK was assigned to the isolated strain (https://www.ncbi.nlm.nih.gov/nuccore/KP216715.1). The bacterium was cultured in agitated glucose minimal medium (glucose (1%), (NH₄)₂SO₄ (0.1%), K₂HPO₄ (0.05%), MgSO₄ (0.05%), NaCl (0.01%), yeast extract (0.05%), CaCO₃ (0.05%), agar (20 g/L)) at 28 °C. Cycloheximide (HiMedia) (0.2 g/L) was added to prevent any fungal contamination. After 24 h of maximum growth, the bacterium was separated from the media by centrifuging at 5,000 rpm. Pellets were washed with saline water and phosphate buffer to wash away the medium, and then lyophilized. The powdered form of dried biomass was used for the study. Further information about the isolation and identification of the bacterial isolate is given in the supplementary information (available with the online version of this paper).

**Preparation of lead stock solution**

Lead metal stock of 1,000 mg/L was prepared by dissolving lead nitrate (Pb(NO₃)₂) salt in distilled water. The stock was allowed to stand for 24 h before being used. Pb(II) solutions of different concentrations were obtained by diluting the stock solutions. Standards required for atomic absorption spectroscopy were prepared from pure Certipur lead standard
solution (Merck). To adjust the pH, 0.1 N HCl and NaOH solutions were used and then pH was not further controlled.

Lead metal biosorption study

A batch equilibrium method was used to determine the adsorption of Pb(II) with dry biomass of Bacillus badius AK at varying parameters. Desired bacterial dry biomass was added in 250 mL Erlenmeyer flasks containing 50 mL of the metal solution and varying the required parameters. The flasks were then transferred to an isothermal rotary shaker and agitated at the required rpm. After the desired time interval, centrifugation at 7,500 rpm for 10 min was performed. The supernatant was then filtered using Whatman 42 filter paper. The samples were stored in specimen tubes and supernatants were analysed for residual concentration on an atomic absorption spectrometer (Spectra AA Varian 55B, Australia).

The effect of pH on biosorption capacity of bacterial biomass was investigated in pH range of 3 to 6 by using 2 g/L of biomass in 100 mg/L of Pb(II) solution at 150 rpm of rotation at room temperature. It was not possible to carry out the biosorption of Pb(II) at pH > 6 due to precipitation of the metals as the hydroxides, which makes interpretation of the results difficult. The effect of contact time on biosorption was investigated by observations at every 15 min in the beginning and then after every 30 min for 3 h. The optimum dosage and pH were maintained at an initial metal concentration of 100 mg/L. Investigation of the effect of biomass dosage was done in the range of 0.5 g/L to 4 g/L; other parameters were kept in optimum conditions. Metal solutions in varying concentrations of 50 mg/L to 200 mg/L were used to assess the effect of initial metal concentration on biosorption, keeping the biomass dosage of 2 g/L and a rotational speed of 150 rpm at a temperature of 40 °C. Bacillus badius AK in 2 g/L was exposed to the metal solutions (50 mL, 100 mg/L concentration) in conical flasks incubated at different temperatures ranging from 20 to 50 °C in a shaking incubator at a speed of 150 rpm for 2.5 h.

The capacity of the adsorbent was calculated using the following Equation (1)

\[
q_e = \frac{(C_i - C_f)V}{M}
\]  

where \(q_e\) is the amount of metal uptake by the biomass adsorbent (mg/g) in equilibrium, \(C_i\) and \(C_f\) are the initial and final metal ion concentration in solution respectively (mg/L), \(V\) is the volume of the metal solution (L), and \(M\) is the mass of biomass adsorbent used (g).

Adsorption kinetics

For the purpose of investigating the mechanism involved in biosorption onto the dried biomass of Bacillus badius AK, as well as potential rate of controlling steps that include mass transport and chemical reaction process, Lagergren’s pseudo first and second order kinetics models were tested to fit the kinetics experimental data. The linear form of pseudo first and second order kinetics is expressed in Equations (2) and (3), respectively (Li et al. 2010; Çolak et al. 2011).

\[
\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t
\]  

\[
\frac{1}{q_t} = \frac{t}{q_e} + \frac{1}{k_2q_e^2}
\]

where \(q_e\) and \(q_t\) (mg/g) are the adsorption capacities at equilibrium and time \(t\) (min) respectively, \(k_1\) (1/min) is the pseudo first order rate constant for the kinetic model, and \(k_2\) (g/mg min) is the rate constant for the pseudo second order equation.

Adsorption isotherms

The adsorptive metal uptake can be quantitatively evaluated by experimental equilibrium isotherms (Puranik & Paknikar 1999; Namasiyam & Kavitha 2002). The concentration of adsorbate in liquid phase and its concentration in solid phase are examined at constant temperature, to determine the equilibrium relationship. There are two widely accepted and linearized adsorption isotherm models used in the literature, namely Langmuir and Freundlich. As represented in Equations (4) and (5), respectively.

The Langmuir adsorption isotherm assumes the uniform or monolayer coverage of the adsorbate on the adsorbent’s surface, where all the adsorption sites are identical. This provides an idea about the capacity of uptake, reflecting the equilibrium process behaviour (Langmuir 1918; Duffus 2002; Mohan et al. 2006).

\[
q_e = \frac{q_{max}KLC_e}{1 + KLC_e}
\]  

where \(C_e\) (mg/L) is the equilibrium concentration of metal in solution, \(q_e\) (mg/g) the amount of metal adsorbed on the adsorbent at equilibrium, \(q_{max}\) (mg/g) the monolayer capacity of biosorbent. \(K_L\) is the Langmuir constant; it relates to the capacity and energy of the adsorption.
The Freundlich model is based on the relationship between the metal uptake capacity of adsorbent, and the residual (equilibrium) metal ion concentration. It assumes the sorbent to have a surface with a non-uniform distribution of sorption heat.

\[ q_e = K_F C_e^{1/n} \]  

(5)

where \( q_e \) (mg/g) is the concentration of metal ion on the biosorbent at equilibrium, \( C_e \) (mg/L) is the metal ion concentration in solution at equilibrium, \( K_F \) is the Freundlich constant and \( 1/n \) is the intensity of adsorption.

**Temperature and thermodynamics study**

The temperature was varied to 20–50°C by keeping the samples in a shaking incubator. The pH was adjusted to 5, initial biomass dosage of 2 g/L, initial metal concentration of 100 mg/L and rotational speed of 150 rpm for 3 h. To determine the thermodynamic feasibility and spontaneous nature of the adsorption process, thermodynamic parameters such as free energy change, enthalpy change and entropy change are calculated (Namasivayam & Kavitha 2002). The free energy change can be related with enthalpy and entropy change by the following equation

\[ \Delta G = \Delta H - T \Delta S \]  

(6)

The following linear forms of equations can be used to determine the thermodynamic parameters from the experimental data (Goswami & Purkait 2022).

\[ \log \frac{q_m}{C_e} = \frac{\Delta S}{2.303R} + \frac{-\Delta H}{2.303RT} \]  

(7)

where \( q_m \) is the amount of dye adsorbed by unit mass of adsorbent (mg/g), \( C_e \) is the equilibrium concentration (mg/L), \( m \) is the adsorbent mass (g/L), \( R \) is the gas constant and \( T \) is temperature in Kelvin; \( q_m/C_e \) is called the adsorption affinity. Enthalpy of adsorption (\( \Delta H \)) and entropy of adsorption (\( \Delta S \)) can be obtained from the plot of \( \log(q_m/C_e) \) vs \( 1/T \). The value of Gibbs free energy (\( \Delta G \)) then can be calculated from Equation (6). A positive value of \( \Delta H \) will indicate endothermic behaviour of the process and negative value will indicate exothermic behaviour of the process. A positive value of \( \Delta G \) will indicate a non-spontaneous adsorption process and negative value will indicate a spontaneous adsorption process.

**Desorption, recovery and reuse**

The biosorption study was performed by using Bacillus badius AK against Pb(II) solutions with an initial metal concentration of 100 mg/L at pH 5, for 2.5 h at rotational speed of 150 rpm. The solution was then centrifuged at 6,000 rpm for 15 min to separate the biomass from the solution, and the supernatant was preserved to check the final metal concentration. The pellets were then poured into a crucible for drying at 65°C for 20 h. The dried biomass was then used for desorption.

Pb(II) was desorbed by treatment with different desorbents. The initial metal concentration was measured after biosorption and then the biosorbent was dried and contacted with desorbing solutions of 0.1 M EDTA, HCl, CH₃COOH, HNO₃, H₂SO₄ and distilled water (data not shown) (Deng et al. 2007). Desorption was carried out with 50 mL of desorbing solutions for 3 h initially. The samples were then centrifuged and filtered. The final effluent concentration was observed. The biosorbent was again recovered and kept for drying for 24 h. The initial metal concentration was measured after biosorption and then the biosorbent was dried and contacted with the above mentioned desorbents. Biosorption was again carried out at optimum conditions with initial metal concentration of 100 mg/L of Pb(II), temperature 40°C, biomass dosage of 2 g/L, pH 5 and agitation speed of 150 rpm. The dried sample was used to find out efficiency after reuse.

**Initial characterization of biomass**

To find out the characteristics of the biosorbent, different spectroscopic analyses were carried out with the sample before and after biosorption. In order to find out the chemical characteristics and functional groups of the biosorbent before and after biosorption, Fourier transform infrared (FTIR) spectroscopy was performed. The spectra were recorded in an FTIR spectrometer with the samples prepared as KBr discs. All spectra were plotted using the same scale on the transmittance axis. The crystalline structure was determined using the X-ray diffraction technique since it provides the most definitive structural information and interatomic distances.

The surface structure of biosorbent was analysed by a field emission electron microscope (FESEM) coupled with energy dispersive X-ray (EDX) analysis. The acceleration voltage was kept at 3 kV and the microprobe was focussed at different magnification. Biomass samples before and after biosorption were mounted on a stainless steel slab.
with a double-stick carbon tape followed by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs. The EDX was done under a constant voltage of 20 kV.

RESULTS AND DISCUSSION

Effect of pH

The pH is an important parameter which influences the metal microbe interaction. In the present study the pH was varied as 3, 4, 5 and 6. The change in initial concentration of Pb(II) at different pH values was observed. Many authors have reported that the surrounding pH value is one of the most important factors in biosorption efficiency using different microorganisms (Al-Garni 2007). The lower metal uptake at lower pH may be attributed to the higher concentration of H\(^+\) ions competing for metal binding sites on the biomass. However, at higher pH, solubility of lead was lowered. The increase in the adsorption at higher pH may be due to the decreased ionic competition and also the decreased solubility of the metals at higher pH, which may induce increased adsorption onto the biomass up to a particular pH beyond which the precipitation initiates (Puranik & Paknikar 1999). With the increase in pH there is an increase in ligands with negative charges which results in increased binding of the cations (Gong et al. 2005). The results from the present work are in accordance with the past studies. The efficiency of adsorption by Bacillus badius AK increased from pH 4–6 (Figure 1(a)). Other bacteria of the same genus Bacillus have also shown higher removal within this pH range of 4–6 (Çolak et al. 2011; Singh et al. 2012; Oves et al. 2013). The optimum pH for Pb removal was found to be 5. It was observed that precipitation of lead started at pH >5.65; thereby it can be concluded that pH 5.65 is the critical value for precipitation of lead, beyond which initial metal concentration started decreasing. Similar observation was also made by other authors where there was decrease in metal adsorption beyond pH 6 (Table 1). This fact is also supported by distribution of lead species at different pH (Singh et al. 2012).

Effect of contact time

The contact time also plays a vital role in the adsorption process. The increase in the contact time had a significant effect on the biosorption of metal ions. Since a large number of vacant sites for the biosorption are available initially, the biosorption in the initial stages are rapid and increases up to 30 min and then increases slowly, becoming constant thereafter (Çolak et al. 2011). The equilibrium time for biosorption by Bacillus subtilis was found to be 100 min (Singh et al. 2012). The optimum biosorption capacity of Citrobacter freundii at pH 4 with initial lead concentration of 481.21 mg/L and biomass dosage of 2 g/L was observed to be with contact time of 100 min (Al-Garni 2005).

![Figure 1](image-url)
The equilibrium time for lead biosorption (concentration 75 mg/L) by *Bacillus cereus* and *Bacillus pumilus* was 80 min (Çolak et al. 2011). In the present study, the change in metal removal efficiency was observed for a time period of 3 h. Observations were made after every 15 min for the first 1 h and in the interval of 30 min thereafter (Table 1). Almost 90% of lead removal was achieved in the first 30 min within pH range of 4–5 (Figure 1(b)). Similarly, it was reported that the biosorption of lead by the fungus *Phanerochaete chrysosporium* was rapid in the first 15 min and equilibrium was attained after 3 h (Çeribasi & Yetis 2001). The rate of metal uptake is usually influenced by the factors affecting mass transfer from bulk solution to the binding sites in which there are three major steps. First is the bulk transport of the metal ions in the solution phase, which is rapid due to the advection flow. Second is film transport which involves diffusion of metal through a hydrodynamic boundary layer around the biosorbent surface and, in the third, the actual adsorption of the metal ions by active sites of the biomass is considered to be rapid and equivalent to an equilibrium reaction (Weber 1985).

### Effect of temperature and thermodynamics study

The temperature was varied from 20 to 50 °C by keeping samples in the temperature-controlled shaking incubator. The pH was adjusted to 5 with biomass dosage of 2 g/L at 100 mg/L of Pb(II), and rotational speed of 150 rpm for 180 min. The percentage removal was found to increase both in the case of 30 °C and 40 °C; a maximum of 98.8% removal was achieved at 40 °C, whereas 97.8% of maximum removal was observed at 30 °C. Thus, 40 °C was concluded as the optimum temperature for Pb(II) removal. At 20 °C, the maximum removal was 87% up to 1.5 h and then decreased thereafter. Much less removal was observed at 50 °C where maximum removal was 52% within 1 h and then started decreasing (Figure 2(a)). The Pb(II) metal uptake by *Bacillus badius* AK was found to increase from 20 to 40 °C, with the maximum removal at 40 °C and then was found to decrease (Figure 2(b)). The increase in metal uptake at higher temperature is due to higher affinity of sites for metal or an increase in binding sites on the biomass (Marqués et al. 1991). An increase in metal uptake was observed when the temperature was increased from 4 to 25 °C, but a decrease in metal uptake was observed when the temperature was further increased up to 50 °C, while studying Cu\(^{2+}\) biosorption on *Aspergillus carbonarius* (Al-Asheh & Duvnjak 1993); the authors attributed the increase in metal adsorption to the increase in energy of the system that facilitated attachment to the cell surface and the decrease in metal sorption due to the distortion of adsorption sites on the cell surface. The observed trend with increasing temperature suggests that the biosorption of Pb(II) with the biosorbent is kinetically controlled by an endothermic process (Zhang et al. 2013). From this study the optimum temperature was found to be 40 °C and contact time of 2.5 h.

The thermodynamic parameters of free energy change, enthalpy change and entropy change were calculated (Equations (6) and (7)). Table 2 summarizes the thermodynamic parameter of Pb(II) solution with initial concentration of 100 mg/L and biomass dosage of 2 g/L. The positive value of Δ\(H^\circ\) indicated the endothermic behaviour of adsorption and this was supported by the increase in the uptake with increase in temperature; the negative Δ\(G^\circ\) value indicated the process is spontaneous and feasible. The low value of Δ\(S^\circ\) implied that no remarkable change in entropy occurred during the adsorption process but there is randomness at the solid–solution interface. The biosorption heat for Pb(II) was found to be 0.102 kJ/mol. The magnitude of the heat, Δ\(H^\circ\) value, gives an indication of the type of adsorption, which can either be physical or

<table>
<thead>
<tr>
<th>Type of Biomass</th>
<th>pH</th>
<th>Contact time (min)</th>
<th>Lead uptake (mg/g)</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces cinnamomeum</em></td>
<td>3.5–4.5</td>
<td>75</td>
<td>57.7</td>
<td>Puranik &amp; Paknikar (1997)</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> and <em>Bacillus pumilus</em></td>
<td>6</td>
<td>80</td>
<td>22.1, 28.06</td>
<td>Çolak et al. (2011)</td>
</tr>
<tr>
<td>Citrobacter sp.</td>
<td>4.5</td>
<td>80</td>
<td>70.8</td>
<td>Puranik &amp; Paknikar (1999)</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>5.5–6</td>
<td>100</td>
<td>142.86</td>
<td>Singh et al. (2012)</td>
</tr>
<tr>
<td><em>Delftia tsuruhatensis</em></td>
<td>6</td>
<td>20</td>
<td>22.38</td>
<td>Bautista-Hernández et al. (2012)</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>4</td>
<td>100</td>
<td>58.5</td>
<td>Al-Garni (2005)</td>
</tr>
<tr>
<td><em>Rhizopus arrhizus</em></td>
<td>5–7</td>
<td>–</td>
<td>85.6</td>
<td>Fourest &amp; Roux (1992)</td>
</tr>
<tr>
<td><em>Bacillus badius</em> AK</td>
<td>5</td>
<td>150</td>
<td>138.88</td>
<td>Present study</td>
</tr>
</tbody>
</table>
chemical. If the heat of adsorption is less than 20.9 kJ/mol, then it is physical adsorption; the heat of chemisorption falls in the range of 20–200 kJ/mol, which is similar to the heat of chemical reactions (Goswami & Purkait 2012). The results suggest that physical adsorption was dominant onto *Bacillus badius* AK at a dose of 2 g/L. Similar results were found when *Bacillus gibsonii* S-2 waste biomass was used for removal of Pb(II) from aqueous solution: the ΔH and ΔS were 23.1 kJ/mol and 87.46 J/mol·K and ΔG was −2.57 kJ/mol (Zhang et al. 2015).

**Effect of initial biomass dosage**

The initial biomass dosage was varied as 0.5, 1, 2, 3 and 4 g/L with an initial metal concentration of 100 mg/L. The test was conducted at an optimum temperature of 50 °C, pH 5 and rotational speed of 150 rpm for 3 h. The optimum dosage was found to be 2 g/L (Figure 3(a)). The removal efficiency increased up to 2 g/L and then started decreasing. Similar observation was made when *Bacillus cereus* and *Bacillus pumilis* bacteria were used as a biosorbsent and Pb(II) metal uptake increased up to 1 g/L, then started decreasing (Çolak et al. 2011). The ratio of the metal concentration to the amount of biomass concentration was found to be an important factor in the biosorption of the metals (Deng et al. 2007). A reduction in zinc uptake by *Rhizopus arrhizus* was reported with increasing biomass concentration and is attributed to an insufficiency of metal ions in the solution with respect to the binding sites (Fourest & Roux 1992). A similar observation was made in study of metal sorption by *Phormidium laminosum* biomass (Sampedro et al. 1995). Higher uptake at lower biomass concentrations could be due to an increased metal-to-biosorbsent ratio, which decreases upon increase in biomass (Puranik & Paknikar 1999).

**Effect of initial metal concentration**

The initial metal concentration was varied as 50, 100, 150 and 200 mg/L with initial biomass dosage of 2 g/L, agitation speed of 150 rpm, pH 5 and temperature 40 °C for 2.5 h. The percentage removal was less at 50 mg/L, reached maximum at 100 mg/L and then decreased thereafter (Figure 3(b)). The specific metal uptake was observed to be increasing with the increase in initial metal concentration (Figure 3(c)). Similar enhancement in metal uptake was observed during the biosorption of Pb(II), Cd(II) and Zn(II) by *Citrobacter* strain MCMB-181; the observed enhancement in the metal sorption could be due to the increase in the electrostatic interaction, involving sites of lower affinity for metal ions (Puranik & Paknikar 1999).

| Table 2 | Thermodynamics parameters for Pb(II) (100 mg/L) |
|---|---|---|---|---|
| Biomass dosage (g/L) | Temperature (°C) | ΔH (J/mol) | ΔS (J/mol·K) | −ΔG (J/mol) |
| 2 | 20 | 102.60 | 362.21 | 106,023.76 |
| | 30 | | | 109,643.82 |
| | 40 | | | 113,267.88 |
| | 50 | | | 117,017.20 |

The adsorption kinetics onto *Bacillus badius* AK was analysed using Lagergren’s pseudo first order and second order kinetics. The experimental data obtained were fitted into the pseudo first order kinetic model using the method of least squares and the obtained parameters are listed in Table 3. Similarly, the obtained experimental data were fitted to the pseudo second and first order model as shown in Figure 4. The initial metal concentration was kept at 100 mg/L, biomass dosage 2 g/L, temperature 40 °C and an agitation of 150 rpm. From the kinetics study it was observed that equilibrium was reached after 2.5 h. The values of rate constant, capacities, and correlation coefficient from the pseudo first and second order models are summarized in Table 3. The R² value was 0.99 for the pseudo second order model and moreover the equilibrium adsorption capacity calculated using the pseudo second order model was in close agreement with the experimentally

<table>
<thead>
<tr>
<th>Initial conc. of Pb (mg/L)</th>
<th>( q_{e,\text{exp}} ) (mg/g)</th>
<th>( k_1 ) (1/min)</th>
<th>( q_{e,\text{cal}} ) (mg/g)</th>
<th>( k_2 ) (g/mg.min)</th>
<th>( q_{e,\text{cal}} ) (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>48.94</td>
<td>0.00384</td>
<td>32.6</td>
<td>0.999</td>
<td>0.0074</td>
</tr>
</tbody>
</table>
obtained values. These results suggest that the pseudo second order kinetic model is better than the pseudo first order kinetic model in representing the kinetics of the biosorption system. The pseudo second fitted better for the biosorption system when Bacillus gibbonii S-2 waste biomass was used for removal of Pb(II) from aqueous solution (Zhang et al. 2015).

**Isotherm study**

The Langmuir and Freundlich isotherm models were adopted to predict the behaviour of metal uptake by dry Bacillus badius AK. The results are shown in Table 4. The initial metal concentration was 100 mg/L. From the results it can be seen that the Langmuir model fitted better than the Freundlich model (Figure 5), as the $R^2$ value is higher for the Langmuir model. Conformity of the data to the Langmuir isotherm indicated that biosorption of metal ions could be characterized as a monolayer, single-type phenomenon with no interaction between the metal ions. The maximum adsorption capacity $q_{\text{max}}$ was 138.88 mg/g and the constant $b$ (which is the ratio of adsorption rate constant to the desorption rate constant) showed a high value, thereby indicating high affinity of the metal ions for the binding sites on the adsorbent (Tunali et al. 2006).

The equilibrium parameter $R_L$, which is defined as $1/(1 + bC_0)$, in the range of $0 < R_L < 1$ reflects a favourable adsorption process where $b$ (L/mg) is the Langmuir constant and $C_0$ (mg/L) is the initial adsorbate concentration. The $R_L$ value was found to be 0.02, therefore indicating that the adsorption process was favourable and Langmuir's isotherm was applicable. This was in agreement with other biosorption studies by bacterial biomass, as shown in Table 5.

**FESEM and EDX analysis**

FESEM micrographs indicated the topology of Bacillus badius AK as rods and regular in shape before interacting

| Table 4 | Comparison of Langmuir and Freundlich isotherms |
|-----------------|------------------|------------------|------------------|
| **Initial concentration of Pb (mg/L)** | **Langmuir isotherm** | **Freundlich isotherm** |
| | $R^2$ | $q_{\text{max}}$ (mg/g) | $b$ (L/mg) | $R^2$ | $n$ | $K_F$ (mg/g) |
| 100 | 0.985 | 138.88 | 0.63 | 0.947 | 4.94 | 6.27 |

**Figure 4** | Plots of pseudo first order, second order and experimental data.

**Figure 5** | Plot of $q_e$ (mg/g) against the final concentration $C_0$ (mg/L) using experimental, Langmuir and Freundlich isotherms for biosorption of Pb(II) with Bacillus badius AK. (Initial conc. 100 mg/L, temp. 40°C, agitation speed 150 rpm, contact time 2.5 h.)
with Pb(II); after Pb(II) biosorption, its surface became rough and porous (Figure 6(a) and 6(b)). The observation from FESEM was confirmed by EDX spectra which revealed the presence of Pb(II) after metal loading (Figure 7(b)). This observation was confirmed by the EDX analysis, the peaks between 2 and 3 keV (Figure 7(a)), corresponding to Cl\(^{-}\), disappeared after Pb(II) biosorption; significant appearance of peaks for Pb(II) were observed within 2 to 3 keV range. The EDX analysis of the biomass loaded with Pb(II) shows a decrease in intensity of Cl\(^{-}\) bands and no
A significant decrease in intensity was observed for Mg and K; these findings indicate that the biosorption process might include ion exchange.

**FTIR spectral analysis**

The FTIR spectra of unloaded and metal-loaded *Bacillus badius* AK biomass in the range of 400–4,000 cm⁻¹ were taken to find out which functional groups are responsible for the biosorption process, and are presented in Figure 8. As seen from the figure unloaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass and the presence of functional groups responsible for biosorption of Pb(II). It indicated the probable involvement of the functional group in the adsorption process. The FTIR spectra of the unloaded biomass and loaded biomass, respectively, indicated bounded hydroxyl (−OH) or amine (−NH) groups. The peak at 2,929 cm⁻¹ is related to the (CH) of cellulose and hemicelluloses (Weng & Wu 2011). The peak with a wave number of 1,648 cm⁻¹ was carboxylic groups (Mohanty et al. 2000). The peaks at 1,384 cm⁻¹ and 1,402 cm⁻¹ are CH₃ with symmetric bending. Peaks at 1,239 and 1,234 cm⁻¹ refer to SO₃ stretching. The peaks at 1,074 and 1,044 cm⁻¹ may be explained as due to O-H alcohols. Transmittance of peaks in loaded biomass was substantially lower than in the unloaded biomass. These changes suggest that bond stretching occurs due to presence of metals and therefore peak transmittance is reduced. Consequently, the formation of varying spectra followed by the adsorption of Pb(II) on bacterial biomass validated the contribution of functional groups in Pb binding. In agreement with the present work other researchers have reported similar observation (Dekhil et al. 2011; Oves et al. 2013). These changes observed in the spectrum indicated the possible involvement of functional groups in the biosorption process on the surface of the biomass.

**Desorption, recovery and reuse**

Lead was desorbed by treatment with different desorbents. The initial metal concentration was measured after biosorption, and the biosorbent had been dried and contacted with 0.1 M EDTA, HCl, HNO₃, CH₃COOH and H₂SO₄ and distilled water (Deng et al. 2007). The percentage of Pb(II) released is shown in Figure 9. The initial pH of each desorbent was noted and a total desorption time of 3.5 h was provided initially. It was found that 0.1 M HCl, H₂SO₄ and HNO₃ gave more than 98% recovery of Pb(II). But desorption with HCl was achieved within 2.5 h as compared to 3.5 h each for H₂SO₄ and HNO₃; also the recovery with HNO₃ nitrified the biosorbent. The recovery with 0.1 M CH₃COOH and EDTA was 96 and 88%, respectively, whereas negligible recovery was observed with distilled water (data not shown). The recovery rate of Pb(II) by HCl makes the biosorbent suitable for reuse.

The recovery of biosorbent after biosorption was 42% of the initial value and the recovery after desorption was 71.4% (all these values are reported after considering the losses during recovery). The biosorbent obtained after desorption was reused for studying the biosorption of *Bacillus badius* AK with initial metal concentration of 100 mg/L of Pb(II), temperature 40 °C, biomass dosage of 2 g/L, pH 5 and agitation speed of 150 rpm. Results indicated that percentage removal of Pb(II) was 60%; this might be due to the destruction of sites at the surface of the biosorbent during
desorption. Similar decrease in biosorption after desorption with acid (0.1 M HCl) was observed during biosorption of Pb(II), Cd(II) and Zn(II) by *Citrobacter* strain MCMB-181, and the researchers attributed the decrease to the deleterious effect of acid on the biomass (Puranik & Paknikar 1999).

**CONCLUSION**

This study indicated that *Bacillus badius* AK strain, which is a widely available bacterium in water hyacinth compost, can be used as an efficient biosorbent material for the removal of Pb(II) from aqueous solutions. The removal rate was rapid in the first 30 min, and further studies indicated the influence of contact time, temperature, initial biomass dosage and initial metal concentration. The biosorption followed pseudo second order kinetics. The adsorption isotherm can be described by the Langmuir isotherm model indicating the possibility of monolayer adsorption. The heat of adsorption was very low indicating the possibility of physical adsorption. The maximum adsorption capacity was 138.88 mg/g. Interactions between the metal ions and the functional groups on the surface were confirmed by FESEM and EDX spectra. The data from FTIR analysis indicated the presence of several functional groups on the surface of the biomass and their participation in the biosorption process.

**REFERENCES**


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