Mechanistic studies on the biosorption of Pb(II) by *Pseudomonas aeruginosa*
S. Vimalnath, H. Ravishankar, C. Schwandt, R. V. Kumar and S. Subramanian

**ABSTRACT**

The biosorption of Pb(II) ions from aqueous solution has been studied using both the intact and thermolyzed cells of *Pseudomonas aeruginosa*. Further, the role of the major cell wall components, namely DNA, protein, polysaccharide, and lipid, in Pb(II) binding has been assessed using an enzymatic treatment method. The Pb(II) bioremediation capability of *P. aeruginosa* cells has been investigated by varying the parameters of pH, time of interaction, amount of biomass, and concentration of Pb(II). The complete bioremoval of Pb(II) using intact cells has been achieved for an initial Pb(II) concentration of 12.4 mg L\(^{-1}\) at pH 6.2 and temperature 29 ± 1°C. The biosorption isotherm follows Langmuirian behavior with a Gibbs free energy of \(-30.7\) kJ mol\(^{-1}\), indicative of chemisorption. The biosorption kinetics is consistent with a pseudo-second-order model. The possible Pb(II) binding mechanisms of *P. aeruginosa* cells are discussed based on characterization using zeta potential measurements, Fourier transform infra-red spectroscopy, and energy dispersive X-ray spectroscopy. The results confirm that among the major cell wall components studied, polysaccharide shows the highest contribution towards Pb(II) binding, followed by DNA, lipid, and protein. Similar studies using thermolyzed cells show higher Pb(II) uptake compared to the intact cells both before and after enzymatic treatment.

**Key words** | bacteria, bioremediation, biosorption, Pb(II), *Pseudomonas aeruginosa*

**INTRODUCTION**

Technological developments in chemical, mining, metallurgical, and energy sectors, despite their massive positive economic impact on the society, have concomitantly resulted in the generation of wastes containing toxic heavy metals that are detrimental to the ecosystem. The untreated, toxic heavy metals in the waste waters can result in circulatory, skeletal, renal, and endocrine disorders in human adults and neurological disorders such as headache, convulsions, learning disorders, and hyperactive behavior in children. The treatment of waste water containing toxic heavy metals before discharging into the environment therefore has become imperative. Several conventional methods, like adsorption, precipitation, solvent extraction, and ion-exchange, as well as electrochemical methods, like electro-deposition, electro-flotation, electro-coagulation and electro-oxidation, can serve the purpose of removing toxic heavy metals. However, these have certain limitations, such as inefficiency in removing lower metal concentrations, formation of secondary sludges, and high cost of operation. Bioremediation can potentially offer an alternative cost-effective method capable of removing lower concentrations of heavy metals from waste water.

The method of bioremediation which uses microorganisms for the removal of toxic heavy metals from waste water has gained significance over time. A wide range of biological materials, mainly live and dead cells of bacteria, yeast, fungi, and algae, as well as cellular products such as polysaccharides, have been tested for their bioremediation capabilities and comprehensively reviewed (Dixit et al. 2015; Mosa et al. 2016).

Bacteria have been widely studied as biosorbent for Pb(II) removal from aqueous solution. For example, Hlihor et al. (2017) have studied *Arthrobacter viscosus* for the removal of Pb(II) from aqueous solutions. Muñoz et al. (2015) have studied kinetics and mechanisms of Pb(II) biosorption using *Klebsiella* sp. 3S1 isolated from a waste water treatment plant. The biosorption of nickel and lead have been investigated using *Curtobacterium* sp. FM01, an indigenous bacterium isolated from farmland soils of northeast Iran.
(Masoumi et al. 2016). Choin’ska-Pulit et al. (2018) isolated 51 microbial strains from heavy metal contaminated water and soil and demonstrated that Pseudomonas azotoformans (JAW1) strain yielded higher biosorption of Pb compared to Cd and Cu in aqueous medium. The isolated Bacillus sp. PZ-1 showed high biosorption rate and capacity for Pb(II) from waste water at 15 °C (Ren et al. 2015). Jiang et al. (2017) studied the biosorption onto marine bacterium Pseudoalteromonas sp. SCSE709-6 in multi-element systems. Pseudoalteromonas sp. showed higher affinity to Pb(II) in a binary system, whereas it showed the least sensitivity in a multi-metal system. Bacillus badius AK strain originating from rotary drum compost of water hyacinth was found to be efficient for the removal of Pb(II) (Vishan et al. 2017). Black et al. (2014) studied the biosorption capabilities of the naturally occurring heterogeneous mix of bacteria in suspended and fixed morphologies for removing Pb(II) and Cu(II) ions and observed that the fixed bacterial mixture showed a higher metal binding capability for both the metals. In another study (Lei et al. 2014), the biosorption of copper, lead and nickel was explored onto immobilized Bacillus coagulans (IBC) from waste water, in single and multi-metal systems, and the preferential adsorption of the biosorbent followed the order: Pb(II) > Cu(II) > Ni(II). Although considerable research has been done on bioremediation of toxic metals using bacteria, understanding the roles played by the individual cell wall components in the metal binding mechanism remains relatively unexplored.

The present work addresses three objectives. First, the biosorption of Pb(II) ions from aqueous solutions using intact cells of Pseudomonas aeruginosa has been investigated by optimizing pH, time of interaction, and amount of biomass for maximizing Pb(II) uptake for a given concentration of Pb(II) ions. Second, the Pb(II) binding capability of thermolyzed cells of P. aeruginosa has been studied under the obtained optimal conditions for intact cells. Third, the roles of individual cell wall components in the binding of Pb(II) ions have been explored and possible binding mechanisms deduced.

** MATERIALS AND METHODS **

** Biomass and chemicals **

P. aeruginosa (NCIM 2945) used in this study is a Gram-negative, rod-shaped bacterium collected from the National Chemical Laboratory (NCL), Pune. Nutrient broth medium was used to grow P. aeruginosa at around pH 7.0. The medium consisted of beef extract of 1.0 g L⁻¹ (sd Fine Chemicals), yeast extract of 2.0 g L⁻¹ (SRL), peptone of 5.0 g L⁻¹ (Merck), and NaCl of 5.0 g L⁻¹ (Fisher Scientific, Exclera), all dissolved in 1 L of water at 29 ± 1 °C.

The fully grown P. aeruginosa cells were harvested from the metabolite after 12 h. The cells were separated out from a desired volume of the culture using a refrigerated centrifuge (Remi) by centrifuging the culture at 10,000 rpm for 10 min at 4 °C. After discarding the supernatant solution, the cell pellet was washed with water and subsequently used for various experiments. The chemical reagents used for the experiments were all analytical reagent grade. High-purity Millipore water was used in all experiments.

** Solubility of Pb(II) ions as a function of pH **

Experiments were carried out to investigate the solubility of Pb(II) species in water as a function of pH. Suitable quantities of Pb(NO₃)₂ and KCl were mixed in water in Erlenmeyer flasks such that the final concentration of Pb(II) corresponded to 206 mg L⁻¹ and that of KCl was 1 × 10⁻² M. The pH was adjusted to different values in the range of 2.0 to 12.0 using HNO₃ or KOH. The Erlenmeyer flasks with the pH-adjusted solutions were agitated in a rotary incubator shaker at 200 rpm for 1 h at 29 ± 1 °C. The solutions were then centrifuged at 5,700 rpm for 10 min. After centrifugation, the clear supernatant solutions of different pH were analyzed for Pb(II) using an atomic absorption spectrophotometer (AAS) (Thermo Electron Corporation Ltd). The residual concentration of Pb(II) in solution was plotted as a function of pH. The pH range in which the Pb(II) species was most abundant was selected for the biosorption experiments.

** Biosorption of Pb(II) ions onto intact P. aeruginosa cells **

To study the biosorption of Pb(II) onto P. aeruginosa cells in aqueous solution, parameters, namely pH, time of interaction, amount of biomass, and concentration of Pb(II) ions were optimized for maximum Pb(II) uptake. For all biosorption experiments, KNO₃ of concentration 1 × 10⁻² M was used as the background electrolyte.

First, the biosorption of Pb(II) was studied as a function of pH by varying the pH of the Pb(II) solution from 2.0 to 6.0, in the presence and absence of P. aeruginosa, while all the other parameters were kept constant. The interaction time was maintained at 1 h and the initial Pb(II) concentration was set at 13.5 mg L⁻¹. Cells of P. aeruginosa were obtained by centrifugation of 150 mL of fully grown culture and were added to 50 mL of the Pb(II) solutions in 250 mL
Erlenmeyer flasks, providing a cell concentration of 2.7–3.2 $\times 10^{10}$ cells mL$^{-1}$. The flasks were agitated in a rotary incubator shaker at 200 rpm for 1 h at 29 ± 1 °C. For each pH value studied, the Pb(II)-containing solution with *P. aeruginosa* cells was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatant was analyzed using AAS to determine the residual Pb(II) concentration.

For the kinetic studies, the biosorption of Pb(II) was observed by varying the interaction time between the Pb(II) ions and the *P. aeruginosa* cells from 10 to 90 min. The initial Pb(II) concentration, the cell concentration, and the pH were kept constant at 11.8 mg L$^{-1}$, 2.7–3.4 $\times 10^{10}$ cells mL$^{-1}$, and 6.1 ± 0.1, respectively. At the end of each time period, the solution was centrifuged and the supernatant was analyzed for Pb(II) ions. Analogous experiments were carried out for the intact cells, and the Pb(II) uptake capacities of the thermolyzed and the intact cells were compared.

**Biosorption of Pb(II) ions onto thermolyzed *P. aeruginosa* cells**

**Thermolysis of bacterial cells**

To achieve thermolysis of the *P. aeruginosa* cells, 100 mL of fully grown culture was centrifuged at 10,000 rpm for 10 min at 4 °C. The pellet was washed with $1 \times 10^{-2}$ M KNO$_3$ and redispersed in 10 mL of water. The redispersed pellet was boiled in water for 30 min, cooled to room temperature, and then used for Pb(II) binding studies.

**Biosorption experiments using thermolyzed cells**

Biosorption of Pb(II) using thermolyzed bacterial cells was carried out in two steps, applying the optimal conditions obtained from the biosorption studies with the intact cells. First, the thermolyzed cells were redispersed in 20 mL of solution giving a cell concentration of 4.6–5.1 $\times 10^{10}$ cells mL$^{-1}$. The solutions contained different concentrations of Pb(II) ranging from 100 to 500 mg L$^{-1}$. Each solution was adjusted to pH 5.6 and incubated at 29 ± 1 °C for 1 h in an orbital shaker at 200 rpm. Second, the incubated solution was centrifuged at 13,000 rpm for 15 min at 29 ± 1 °C and the supernatant was analyzed for concentration of Pb(II) ions. Analogous experiments were carried out for the intact cells, and the Pb(II) uptake capacities of the thermolyzed and the intact cells were compared.

**Enzymatic digestion of intact and thermolyzed *P. aeruginosa* cells**

For each experiment 100 mL of fully grown culture of *P. aeruginosa* was centrifuged at 10,000 rpm for 10 min at 4 °C. The pelleted cells were enzymatically digested in both their intact and thermolysed forms. For DNase-I, proteinase K, and lysozyme digestions, the pelleted cells were washed twice with 0.1 M sodium phosphate buffer of pH 8 and centrifuged. For lipase digestion, the pelleted cells were washed twice with 29.1 mM potassium phosphate buffer of pH 7.4 and centrifuged. The washed pellets were then redispersed in 0.1 M sodium phosphate buffer of pH 8, containing 2.5 mM of MgCl$_2$ and 0.5 mM of CaCl$_2$ in the case of DNase-I digestion, and 0.2 mM of CaCl$_2$ in the case of proteinase K digestion.

Finally, the effect of Pb(II) concentration on the biosorption was studied by varying the initial concentration of Pb(II) ions from 10 to 250 mg L$^{-1}$. Cell concentration, interaction time, and pH were 2.6–3.3 $\times 10^{10}$ cells mL$^{-1}$, 1 h, and 5.8 ± 0.1, respectively. After each experiment the solution was centrifuged and analyzed for Pb(II) ions.
incubated at 29 ± 1 °C for 1 h in an orbital shaker at 200 rpm. Each incubated solution was then centrifuged at 13,000 rpm for 15 min at room temperature and the supernatant was analyzed for concentration of Pb(II).

**Characterization**

Zeta potential measurements were carried out on the *P. aeruginosa* cells using a Malvern Zetasizer ZEN3690. Fully grown *P. aeruginosa* cells were harvested and redispersed in either water or Pb(II) solution. In both cases, the pH of the solution was adjusted from 2.0 to 12.0 using HNO₃ or KOH, and 1 × 10⁻² M KNO₃ was used as background electrolyte. Mixtures containing the cells in the presence of Pb(II) were first allowed to interact at 29 ± 1 °C for 1 h in an orbital shaker at 200 rpm. The zeta potential was measured for cell dispersions in the absence and presence of Pb(II).

Fourier transform infra-red (FTIR) spectra of the *P. aeruginosa* cells were recorded using a Perkin-Elmer spectrophotometer adopting the attenuated total reflection (ATR) technique in the range of 4,000–400 cm⁻¹ wave number. Spectra were recorded for both the bare cells and the cells after interaction with Pb(II) ions after drying at 60 °C in an oven for 4 h.

Scanning electron micrographs of the *P. aeruginosa* cells were taken using an FEI Quanta 200 ESEM. The fully grown *P. aeruginosa* cells were recorded using a background electrolyte. Mixtures containing the cells in the presence of Pb(II) were first allowed to interact at 29 ± 1 °C for 1 h in an orbital shaker at 200 rpm. The zeta potential was measured for cell dispersions in the absence and presence of Pb(II).

Scanning electron microscopy of the fully grown intact cells of *P. aeruginosa* showed that the cells are rod-shaped, with an average length of 3 μm and an average diameter of 0.75 μm.

**RESULTS AND DISCUSSION**

**Solubility of Pb(II) ions as a function of pH**

The solubility of Pb(II) species in aqueous solution within the pH range from 2.0 to 12.0 is plotted in Figure 1(a). It can be seen that the highest Pb(II) concentration of 206 mg L⁻¹ is at pH 2.1. When increasing the pH, the concentration of Pb(II) decreases, first rather slowly and then more steeply until values close to zero are reached between pH 7.0 to 11.0 due to the formation of insoluble Pb(OH)₂. Beyond pH 11.0, the Pb(II) concentration increases again due to the formation of soluble anionic Pb(II) complexes such as HPbO₂ (Liu & Liu 2005). The subsequent experiments for Pb(II) biosorption with *P. aeruginosa* were carried out between pH 2.0 and 6.0.

**Biosorption of Pb(II) ions onto intact *P. aeruginosa* cells**

Scanning electron microscopy of the fully grown intact cells of *P. aeruginosa* showed that the cells are rod-shaped, with an average length of 3 μm and an average diameter of 0.75 μm.

**Effect of pH on the biosorption of Pb(II) ions**

The results of the Pb(II) biosorption studies using intact cells of *P. aeruginosa* as biosorbent, and the control without bacterial cells, at different pH values are depicted in Figure 1(b). All other parameters were kept constant. The figure shows that in the presence of *P. aeruginosa* the equilibrium concentration of Pb(II) ions in solution steeply decreases from 12.4 mg L⁻¹ to zero as the pH increases from 2.0 to 6.2. In contrast, in the control sample only a marginal decrease in concentration of Pb(II) ions from 13.5 to 12.4 mg L⁻¹ is observed. The steep decrease in Pb(II) concentration in the presence of *P. aeruginosa* confirms the bioremoval of Pb(II) by *P. aeruginosa*.

The amount of Pb(II) biosorbed by intact *P. aeruginosa* cells at different pH values is portrayed in Figure 1(c). It is noteworthy that virtually 100% biosorption of Pb(II) ions can be achieved in the presence of *P. aeruginosa*. The complete uptake of Pb(II) ions corresponding to 4 × 10⁻¹³ mg cell⁻¹ occurs at pH 6.2. In terms of the number of Pb(II) ions per cell and per g of dry cells, the Pb(II) ions biosorbed is 1.16 × 10⁶ (1.16 million Pb(II) ions) and 1.45 × 10¹⁶ (14.3 quintillion), respectively.

**Effect of time of interaction on the biosorption of Pb(II) ions**

The results for the time-dependent studies with *P. aeruginosa* cells as Pb(II) biosorbent are depicted in Figure 1(d). The figure shows that the kinetics of uptake of Pb(II) ions is quite rapid, and almost complete Pb(II) uptake is achieved within the first 10 min of interaction, followed by saturation.
The maximum amount of Pb(II) biosorbed is about $4.2 \times 10^{-13}$ mg cell$^{-1}$. The numbers of Pb(II) ions biosorbed per cell and per g of cells are $1.22 \times 10^6$ (1.22 million) and $1.36 \times 10^{19}$ (13.6 quintillion), respectively.

The present biosorption process using *P. aeruginosa* shows a short reaction time on the order of minutes for maximum Pb(II) biosorption, which indicates a strong affinity of the bacterial cells towards Pb(II) ions.

**Effect of amount of biomass on the biosorption of Pb(II) ions**

The effect of amount of *P. aeruginosa* biomass on the biosorption of Pb(II) is shown in Figure 2(a). When the cell number is increased from $1.5 \times 10^9$ to $30.2 \times 10^9$ cells mL$^{-1}$, the percentage uptake of Pb(II) ions is found to increase from 88% to 100%. The specific uptake of Pb(II) per individual cell of *P. aeruginosa*, however, decreases from $77.2 \times 10^{-13}$ to $4.4 \times 10^{-13}$ mg cell$^{-1}$ (31.7 mg g$^{-1}$ of dry cells to 5.3 mg/g of dry cells).

**Biosorption isotherm**

The Pb(II) biosorption capacity of *P. aeruginosa* cells for varying concentrations of Pb(II) ions is depicted in Figure 2(b). When the amount of Pb(II) ions is increased, enhanced uptake of Pb(II) is observed at lower concentrations, followed by a more gradual rise in uptake, and then a plateau at higher concentrations. *P. aeruginosa* cells show a maximum Pb(II) uptake of $39.2 \times 10^{-13}$ mg cell$^{-1}$ (48.1 mg/g of dry cells) when the equilibrium concentration reaches 12.4 mg L$^{-1}$. The maximum Pb(II) ions biosorbed is $1.14 \times 10^7$ (11.4 million) Pb(II) ions per cell or $1.4 \times 10^{20}$ (140 quintillion) Pb(II) ions per g of cells.

The increase in Pb(II) uptake at higher concentrations of Pb(II) ions for a given cell number could possibly be explained with the help of high and low affinity functional groups present on the cell surface. As Basha & Murthy (2007) highlighted, at lower metal ion concentration, the high affinity functional groups are mainly involved in metal binding. However, when the metal ion concentration...
increases, the low affinity functional groups also contribute to metal binding, thereby increasing the overall metal uptake.

For intact *P. aeruginosa* cells, the biosorption isotherm of Pb(II) exhibits Langmuirian behavior as given in Equation (1).

\[
\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L q_m C_e}
\]

where, \(C_e\) is the equilibrium metal ion concentration in the solution (mg L\(^{-1}\)), \(q_e\) is the equilibrium metal ion concentration on the biosorbent (mg/g of dry cells), \(q_m\) is the maximum monolayer biosorption capacity of the biosorbent (mg/g of dry cells), and \(K_L\) is the Langmuir biosorption constant (L g\(^{-1}\)) relating to the free energy of biosorption.

The assumption for the Langmuir model is that the adsorption occurs on a sorbent surface consisting only of homogeneous sites that can be covered completely by a monolayer of sorbed molecules. When the sorbent surface is fully covered, the forces on the sorbent are chemically saturated (Langmuir 1918). The Langmuir parameters determined for the intact *P. aeruginosa* cells are given in Table 1.

The relationship for the separation factor is given in Equation (2) (Hall et al. 1966).

\[
R_L = \frac{1}{1 + K_L C_o}
\]

where, \(R_L\) is the dimensionless separation factor and \(C_o\) is the initial concentration of Pb(II) ions in the solution (mg L\(^{-1}\)). The \(R_L\) (0 < \(R_L\) < 1) values for biosorption of Pb(II) by *P. aeruginosa* are determined to be between 0.0918 and 0.0047 within the range from minimum to maximum Pb(II) concentration, indicating a favorable biosorption process.

The expression for the free energy change is given in Equation (3).

\[
\Delta G^0 = -RT \ln K_L
\]

where, \(\Delta G^0\) is the standard Gibbs free energy change of the reaction (J mol\(^{-1}\)), \(T\) is the absolute temperature (K), and \(R\) is the universal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)). The standard Gibbs free energy of biosorption of Pb(II) ions using intact *P. aeruginosa* cells is determined to be -30.7 kJ mol\(^{-1}\). The obtained negative value of free energy indicates the feasibility and spontaneity of biosorption of Pb(II) ions by *P. aeruginosa*.
Pb(II) on the *P. aeruginosa* cells with high preference (Baysal et al. 2009), and its relatively high magnitude is furthermore suggestive of chemical coordination or chemisorption of the Pb(II) ions with the *P. aeruginosa* functional groups (Bilgiç & Şahin 2001).

**Kinetic model**

In order to determine the biosorption mechanism, the biosorption rate constant, and the equilibrium biosorption capacity of Pb(II) on the intact *P. aeruginosa* cells a batch biosorption model is applied. The two batch biosorption models that are most frequently used are pseudo-first-order and pseudo-second-order models. The kinetic data of *P. aeruginosa* for Pb(II) biosorption are shown in Figure 1(d). As discussed, biosorption is occurring to a large extent within the first few minutes and then asymptotically approaches saturation. Unfortunately, it was not possible to resolve the early part of the curve any more accurately owing to experimental constraints. The observed asymptotic behavior cannot be described by a pseudo-first-order model. It is, however, possible to fit the data with a pseudo-second-order model so that an indicative value for the reaction rate constant can be obtained.

The pseudo-second-order equation is given in Equation (4).

$$
\frac{t}{q_t} = \frac{t}{q_e} + \frac{1}{k_1q_e^2}
$$

where, $t$ is time (min), $q_e$ is the amount of metal biosorbed on the surface of the biosorbent at equilibrium (mg g$^{-1}$), $q_t$ is the amount of metal biosorbed at any time $t$ (mg g$^{-1}$), and $k_1$ is the rate constant of biosorption (g mg$^{-1}$ min$^{-1}$).

The assumption for the pseudo-second-order approach is that chemical sorption or chemisorption of Pb(II) ions on the microbes may be the rate-limiting step. This chemisorption process involves sharing or exchange of electrons between biosorbent and metal ions (Ho & McKay 1999). The pseudo-second-order parameters for intact *P. aeruginosa* cells are given in Table 1.

**Characterization of *P. aeruginosa* cells before and after interaction with Pb(II) ions**

Figure 3(a) gives the zeta potential of intact *P. aeruginosa* cells before and after interaction with Pb(II) ions as a function of pH. The figure shows that in both cases the zeta potential becomes more negative when the pH increases from 2.0 to 12.0. As the pH is increased, the surface of *P. aeruginosa* becomes gradually more negative because of the functional groups such as carboxyl and phosphate deprotonate. After interaction with Pb(II) ions, the surface of *P. aeruginosa* is slightly less negative compared to the bare cells due to the binding of positively charged cationic Pb(II) species on the surface.

Figure 5(b) shows the wave numbers of the most important bands seen in the FTIR spectra of intact *P. aeruginosa* cells before and after interaction with Pb(II) ions. These are indicative of the presence of O–H, N–H, C–N in amide, COO$^-$, COOH, C–O–C, P–O, and phosphoryl groups. This also agrees with the assignments reported by Santhiya et al. (2001), Jiang et al. (2004), and Öjeda et al. (2008). After interaction with Pb(II) ions, the wave numbers of some of these bands shift to somewhat lower values while others remain unaffected. The observed shifts are suggestive of the involvement of the amine, carboxyl, and phosphate groups in the binding of Pb(II) ions with the bare *P. aeruginosa* cell surface and thereby the affinity of these functional groups to Pb(II) ions.

The EDAX spectra for intact *P. aeruginosa* cells after interaction with Pb(II) ions confirmed the presence of a Pb(II) peak attesting to the biosorption of Pb(II) ions on the cell surface.

**Biosorption of Pb(II) ions onto thermolyzed *P. aeruginosa* cells**

In the experiments so far, intact *P. aeruginosa* cells were investigated for their Pb(II) biosorption capacities. The results show that the uptake of Pb(II) ions is mainly due to the functional groups in cell wall components present on the external cell surface. Similar functional groups are also present in the interior cell components, but when the cells are intact these components are not accessible for metal uptake. Therefore, in order to expose the interior cell surface, the intact cells were ruptured using a thermolysis method. This resulted in the formation of insoluble cell debris as well as other soluble components, namely DNA, protein, and polysaccharide. Figure 4 shows the amount of Pb(II) biosorbed by the intact and the thermolyzed cells of *P. aeruginosa* for varying initial amounts of Pb(II) ions in solution from 2.0 to 9.7 mg.

When the initial amount of Pb(II) ions in solution is increased, both the intact and the thermolyzed *P. aeruginosa* cells show an increase in the Pb(II) uptake. The maximum uptake of Pb(II) by the intact and the thermolyzed cells are 6.1 mg (6.3 × 10$^{-12}$ mg cell$^{-1}$, 1.77 × 10$^{19}$ Pb(II) ions) and 7.2 mg (7.4 × 10$^{-12}$ mg cell$^{-1}$, 2.09 × 10$^{19}$ Pb(II) ions).
ions), respectively. The difference in the uptake of number of Pb(II) ions after thermolysis is $3.2 \times 10^{18}$. As expected, the thermolyzed cells have a higher uptake capacity than the intact cells, confirming the exposure of more binding sites through thermolysis. This is likewise evident from the high amount of unbound Pb(II) in the case of the intact cells compared to the thermolyzed cells.

**Role of individual cell wall components of *P. aeruginosa* cells in the biosorption of Pb(II) ions**

Having confirmed the Pb(II) biosorption capabilities of the intact and the thermolyzed *P. aeruginosa* cells, the roles of individual cell wall components in Pb(II) binding were investigated to understand the underlying mechanism. In general, a bacterial cell wall has a multitude of components...
in a complex arrangement. Based on that, detailed studies were carried out by selectively removing DNA, protein, polysaccharide, and lipid using an enzymatic method and evaluating their individual adsorption capacities. If a particular cell wall component is responsible for Pb(II) binding, then the removal of that component is expected to reduce the percentage of Pb(II) biosorption compared to both the undigested intact and the undigested thermolyzed cells.

**Biosorption of Pb(II) ions on individual cell wall components of intact* P. aeruginosa* cells**

Figure 5(a) shows a bar graph comparing the Pb(II) biosorption capacities of the untreated and the enzymatically treated intact *P. aeruginosa* by compiling the relative contributions of the individual components in Pb(II) uptake. The untreated intact cells show a 54.2% uptake of Pb(II) ions. As expected, after removing each of the components from the cell wall, the percentage of biosorption is decreased in all four cases. More specifically, after removing DNA, protein, polysaccharide, and lipid, the percentage of biosorption is reduced to 37.3%, 41.2%, 19.2%, and 39.7%, respectively. The results confirm that DNA, protein, polysaccharide, and lipid present on the cell surface all play a major role in the uptake of Pb(II), and that polysaccharide provides the largest contribution. When the cumulative effect of the three components DNA, protein, and polysaccharide is studied by removing them at the same time, a drastic reduction in biosorption of Pb(II) down to 5% is noticed.

**Biosorption of Pb(II) ions on individual cell wall components of thermolyzed* P. aeruginosa* cells**

The Pb(II) biosorption capacities of the untreated and the enzymatically treated thermolyzed cells of *P. aeruginosa* are shown in Figure 5(b). The untreated thermolyzed cells show a Pb(II) uptake of 71.7%. A reduction in percentage of biosorption is observed after removing each individual cell wall component. When removing DNA, protein, polysaccharide, and lipid, the percentage of biosorption is reduced to 64.3%, 62.7%, 29.9%, and 52.9%, respectively. As in the case of intact cells, the results confirm that DNA, protein, polysaccharide, and lipid are also involved in Pb(II) binding in the thermolyzed cells, with polysaccharides again providing the largest contribution. When DNA, protein, and polysaccharide are all removed at the same time, the percentage of biosorption is substantially reduced to 10.3%.

**CONCLUSIONS**

Complete Pb(II) uptake from aqueous solution is achieved by intact *P. aeruginosa* cells as biosorbents at lower Pb(II) concentration (12.4 mg L^{-1}). Time-dependent studies indicate that the biosorption of Pb(II) using intact *P. aeruginosa* cells is quite rapid, proceeding on the timescale...
of minutes. At relatively higher concentrations, Pb(II) biosorption capacity of \textit{P. aeruginosa} improves when thermolyzed cells are used, with the Pb(II) uptake increasing by about 18%. Detailed enzymatic treatment studies show that all the major cell wall components studied, namely DNA, protein, polysaccharide, and lipid, participate in Pb(II) binding, with the polysaccharide component showing the largest contribution in both intact and thermolyzed cells. The major cell wall components contribute almost 90% of lead binding to what could be achieved with the whole intact cell. The finding implies the potential use of the major cell wall components to remove Pb(II) ions instead of the whole cell.

The biosorption isotherm of Pb(II) for \textit{P. aeruginosa} exhibits Langmuirian behavior, and the free energy change for biosorption of Pb(II) onto \textit{P. aeruginosa} is suggestive of a spontaneous chemisorption process. FTIR studies indicate that amine, carboxyl, and phosphate functional groups on the \textit{P. aeruginosa} cell surface have affinity towards Pb(II) ions and play an active role in Pb(II) binding.

**ACKNOWLEDGEMENTS**

The authors profusely thank the Indo-UK, UKIERI (UK-India Education and Research Initiative) programme for sponsoring this collaborative project (code SA08-005). One of the authors (SV) is grateful to the Ministry of Human Resources Development, Government of India, for a grant of a fellowship to pursue the PhD programme.

**REFERENCES**


First received 27 December 2017; accepted in revised form 20 June 2018. Available online 3 July 2018