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ORIGINAL PAPER

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Lanthanum biosorption by a *Pseudomonas* sp.: equilibrium studies and chemical characterization

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Abstract Lanthanum biosorption by a *Pseudomonas* sp. was characterized in terms of equilibrium metal loading, model fitting, kinetics, effect of solution pH, lanthanum–bacteria interaction mechanism and recovery of sorbed metal. Lanthanum sorption by the bacterium was rapid and optimum at pH 5.0 with equilibrium metal loading as high as 950 mg g^{-1} biomass dry wt. Scatchard model and potentiometric titration suggested the presence of at least two types of metal-binding sites, corresponding to a strong and a weak binding affinity. The chemical nature of metal–microbe interaction has been elucidated employing FTIR spectroscopy, energy dispersive X-ray analysis (EDX) and X-ray diffraction analysis (XRD). FTIR spectroscopy and XRD analysis revealed strong involvement of cellular carboxyl and phosphate groups in lanthanum binding by the bacterial biomass. EDX and the elemental analysis of the sorption solution ascertained the binding of lanthanum with the bacterial biomass via displacement of cellular potassium and calcium. Transmission electron microscopy exhibited La accumulation throughout the bacterial cell with some granular deposits in cell periphery and in cytoplasm. XRD confirmed the presence of $LaPO_4$ crystals onto the bacterial biomass after La accumulation for a long period. A combined ion-exchange–complexation–microprecipitation mechanism could be involved in lanthanum accumulation by the biomass. Almost 98% of biomass-bound La could be recovered using $CaCO_3$ as the desorbing agent.

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Introduction

The continuous build-up of a variety of heavy metals including rare-earth (RE) elements originating from different industrial and nuclear activities is of tremendous environmental concern. Among the RE elements, lanthanum has currently garnered special attention due to its increasing demands in industries for making advanced new materials and in agriculture, forestry and aquaculture as a microelement fertilizer or a mineral supplement in animal food [1, 22, 23]. Owing to their close chemical similarity, some RE elements have also been used as analogue of actinides in separation and migration chemistry; for example, lanthanum ion can be used as the 'surrogate' ion for americium, plutonium and neptunium [1, 16]. All such practices may lead to the accumulation of RE elements in the environment posing a serious threat to the biota that necessitates an effective and affordable technological solution [23, 25]. Conventional methods for the removal of metal ions have significant disadvantages including expensive process economics, incomplete metal removal, high reagents or energy and generation of large volume of sludge or other waste products that require disposal [9]. The search for an alternative technology for the removal and recovery of heavy metals/radionuclides particularly from large volumes of diluted waste-water streams has focused attention on the microorganism-based bioremediation strategies as microbes often execute tremendous potential in removing such contaminants through biosorption [12, 15, 28, 31, 32]. Biosorption is known as a potential separation and recovery process for sequestering metallic cations from aqueous solutions offering several advantages like low operating cost, minimization of disposable chemical and/or biological sludge volume

and high efficiency in detoxifying very dilute effluents [9, 32, 33, 35]. Furthermore, biosorbent materials often execute high metal-loading capacity and in some cases are highly specific for certain elements of particular interest [30]. As the conventional methods of lanthanum separation and purification are highly expensive and complex comprising several steps of extraction using organic solvents and ion-exchange resins, biosorption can be considered as a potential alternative and an economically attractive strategy for the recovery of this cation [22]. Considering the abundance and diversity of microorganisms in the natural domain, it is of immense importance to identify and characterize microbial strains with high metal-removal capacity and specificity for effective biosorption process development.

Previous investigations by our group have shown that the biomass of a *Pseudomonas* strain has a high capacity in accumulating uranium (540 mg g^{-1} biomass) and thorium (430 mg g^{-1}), and may find a potential application for the removal and recovery of such elements from effluents [25, 26, 28]. The bacterium exhibited rapid removal of actinide ions with high affinity particularly at low metal ion concentrations, which is desirable for the development of any biosorption process. Such promising observations led us to look into the lanthanum-biosorption efficiency of this bacterium because despite their strategic and economic importance, lanthanide elements have not been much evaluated in terms of their removal and recovery through biosorption. Only few reports are available on lanthanum–bacteria interactions and this is yet to studied in more detail [19]. An understanding of lanthanum biosorption will help us to devise an effective and economically feasible biosorption process for the removal/separation of this element. Such an understanding would also be useful in developing a microorganism-based removal/recovery system for the highly radioactive transuranic elements, namely, americium, plutonium and neptunium as La(III) is analogous to these elements in separation and migration chemistry [16]. Among the few reports on La biosorption, Taxier et al. [30–32] have demonstrated that the bacterium *Pseudomonas aeruginosa* strain A22 has the ability to adsorb selectively La, Eu and Yb, and Palmieri et al. [22] have reported La biosorption by *Sargassum fluitans*. In another report, Merroun et al. [19] have demonstrated La fixation by a soil bacterium *Myxococcus xanthus*. In the present study, lyophilized biomass of a *Pseudomonas* sp. [strain Microbial Type Culture Collection (MTCC) 3087], with a striking capacity for the removal of actinide elements, has been evaluated for La biosorption. The mechanism of lanthanum–biomass interaction has been elucidated by employing several analytical techniques like FTIR spectroscopy, energy dispersive X-ray analysis (EDX), X-ray diffraction analysis (XRD) and transmission electron microscopy (TEM). Application of such combined analytical techniques for the elucidation of lanthanum-biosorption mechanism in a metabolically inactivated (lyophilized) bacterial cell is a novel approach. Recovery of biomass-bound La has also been demonstrated using selected desorbing agents.

Materials and methods

Microorganism and growth conditions

The bacterial strain used in the present study was a *Pseudomonas* sp. (strain MTCC 3087) isolated from the garden soil and maintained in Tris-minimal medium [10, 25, 26]. The strain is deposited in the MTCC, Institute of Microbial Technology, Chandigarh, India. Mid-growth phase cells were collected by centrifugation (12,000*g*, 30 min), washed thoroughly with distilled water, lyophilized and used for the biosorption experiments.

Lanthanum-biosorption experiments

Lanthanum sorption by the bacterial biomass was followed over a wide range of metal concentrations in solutions (0–1,000 mg La l^{-1}) at pH 5.0. In each set, 50 mg of the biomass powder was mixed with 100 ml of an appropriate La solution [$La(NO_3)_3{\cdot}6H_2O$, reagent grade, Merck, Darmstadt, Germany]. The mixture was equilibrated for 24 h by continuous shaking in an orbital shaker (200 rpm, 30°C). Following equilibration, the supernatant was separated by centrifugation and used for La estimation by the Arsenazo III method [38]. For each sorption experiment, a control set (without biomass) containing only lanthanum solution of appropriate concentration was kept.

The equilibrium La-sorption capacity (q, mg metal g^{-1} dry wt.) of the bacterial biomass was calculated by the concentration difference method based on the mass balance of the metal ion expressed as:

$$q = V(C_0 - C_e)/M$$

where V is the sample volume (l), C_0, the initial metal ion concentration (mg l^{-1}), C_e, the equilibrium or final metal concentration (mg l^{-1}) and M, the biomass dry weight (g) [34]. Adsorption isotherm data were also fitted with the classical Freundlich, Langmuir or Brunauer–Emmet–Teller (BET) isotherms equations [34]. The linearized form of isotherm equations used were:

$$\text{Freundlich equation} : \ln q = \ln k + 1/n \ln C_e$$

where q and C_e indicate the same as described above. The constant 'k' is a measure of the adsorption capacity (l g^{-1}) and 'n', the intensity of adsorption.

$$\text{Langmuirequation} : 1/q = (1/q_{\max} \times b) \times (1/C_e) + (1/q_{\max})$$

The constant '$q_{\max}$' represents the maximum specific metal uptake (mg g^{-1}) and 'b' is the affinity constant (l mg^{-1}).

$$\text{BETequation}: C_e/(C_s - C_e)q = (1/bq_{\max}) + [(b-1)/bq_{\max}] \times (C_e/C_s)$$

where C_s is the saturation concentration of the solute (mg l^{-1}) and q_{max} and b indicate the same as described above.

The Scatchard model was used to evaluate the affinity constant (K) of the metal-binding site and the binding capacity (q_{max}, mg metal g^{-1} dry wt.) [1]. The linear form used is:

$$q/C_e = -Kq + Kq_{\max}$$

where K is the Scatchard affinity constant and q_{max} is the binding capacity. A plot q/C_e against q gives the K and q_{max} values.

Kinetics of lanthanum sorption

Lanthanum-sorption kinetics were followed at an initial metal concentration of 100 mg La l^{-1} solution at pH 5.0. Samples were withdrawn at selected intervals and La adsorbed by the biomass was determined as described above.

Effect of pH on Lanthanum sorption

Effect of solution pH on La sorption was investigated over a wide range of initial pH (pH 1.0–6.0) of 100 mg La l^{-1} solution. Lanthanum sorption was determined as described above.

Potentiometric titration

Potentiometric titration of the bacterial biomass was done following the methodology described by Fourest and Volesky [7]. The bacterial cells were fast rendered acidic with 0.1 M HCl and washed repeatedly with ultrapure deionized water (Millipore). To obtain constant conductance in the experimental media, the protonated biomass (100 mg dry wt.) was dispersed in 50 ml of 1 mM NaCl solution prepared in deionized water. The titration was carried out by step-wise addition of 0.05 ml of 0.01 M NaOH and the pH measurements were performed at 2-min intervals.

FTIR spectroscopy

The infrared spectra of the bacterial biomass samples (before and after lanthanum sorption at pH 5.0) were recorded within a range 400–4,000 cm^{-1}in a Thermo Nicolet NEXUS-870 FTIR Spectrometer equipped with a laser detector. The samples were prepared as KBr discs.

Energy dispersive X-ray microanalysis

Energy dispersive X-ray microanalysis of a dry powder of metal-free control and lanthanum-loaded biomass was done using an OXFORD ISIS-300 microanalytical system attached with JEOL JSM-5800 Scanning Electron Microscope.

Elemental (K and Ca) analysis

Analysis of K and Ca content of the supernatant of metal-free biomass solution or of La–biomass sorption solution was done using a Flame Photometer (Flame Photometer, 128, Systronics, Ahmedabad, India).

X-ray powder diffraction analysis

X-ray diffraction patterns of dry powder samples of metal-free control and La-sorbed biomass were recorded in a Philips X'Pert Pro Panalytical PW3040/60 powder diffractometer using a Co-source radiation ($\lambda = 1.79$) over a range of 0–100 (2θ) with a step length of 0.05° and time per step 29.8 s. The chemical nature of La crystals was determined based on comparison with the powder diffraction standard files (Search Manual for Selected Powder Diffraction Data for Metals and Alloys, JCPDS, International Centre for Diffraction Data, Newtown Square, PA, USA, 1978).

Transmission electron microscopy

Transmission electron microscopy of bacterial cells was done by using the methodology as described previously [28]. Lanthanum-free (control) and -loaded biomass was washed thoroughly with a sodium phosphate buffer (0.1 M, pH = 7.2), fixed for 2 h at room temperature in 2.5% glutaraldehyde in the same buffer, followed by osmium tetraoxide staining (1%, 1 h). The cells were dehydrated via a graded ethanol series, treated with propylene oxide and embedded in epoxy resin. Unstained ultrathin sections were cut by a Reichert Ultracut E Ultramicrotome (Reichert, Germany), loaded on a formvar carbon-coated grid and examined in a Philips CM10 transmission electron microscope at 100 kV.

Desorption of biomass bound lanthanum

Desorption studies were conducted immediately after the adsorption of La (3 h) by the bacterial biomass. The metal-loaded cells were collected by centrifugation, washed in deionized water and resuspended in the respective desorbing agents (0.5 mg biomass ml^{-1}). The desorbing agents used were HCl, HNO_3, H_2SO_4, Na_2CO_3, $NaHCO_3$, sodium citrate (1 and 1.5 M each), $CaCO_3$ and EDTA (0.5 and 1 M each). All other

conditions were the same as those of the sorption experiments.

All data represent the mean of three independent experiments. Standard deviations and error bars are indicated wherever necessary. All statistical analyses were done using Microcal™ Origin, Version 6.0.

Results and discussion

Effect of pH on Lanthanum sorption

Initial solution pH plays a critical role in metal sorption as this can influence the chemistry both of the biosorbent and the metal in solution. Lanthanum sorption onto the bacterial biomass was investigated over the initial pH range of 1.0–6.0 (Fig. 1). It was observed that, in the lower pH range (1.0–3.0), La biosorption by the biomass was low as the average metal loading was around 73 mg g^{-1} biomass, while further rise in the pH level resulted in a sharp increment in metal binding, reaching the maximum at pH 5.0 (120 mg g^{-1}). Compared to the previous reports on lanthanum biosorption [22, 30], the present work shows high amounts of La accumulation by the bacterial biomass even from acidic solutions, which will make it useful practically for the removal of lanthanum from acidic effluents. Values higher than pH 5 showed La precipitation and thus reduced metal sorption by the biomass (95 mg g^{-1}). The trend of pH-dependent La sorption by the test biomass, however, corroborates well with the previous reports on lanthanum biosorption using *P. aeruginosa* or *S. fluitans* [22, 30]. The observed trend of reduced La sorption in the acidic pH range may be due to the high solubility of lanthanum ions and the competition of protons with the La cations for the same binding sites, thus inducing a weak complexation affinity between the biomass and the cations [30]. The enhanced La sorption with increasing pH is attributable to the gradual deprotonation of the metal-binding functional groups like carboxyl, phosphoryl, hydroxyl, etc. present in the biomass, producing more free sites for metal biosorption [8, 22]. Furthermore, with increasing the pH level, metal ions gradually become hydrolyzed with a lesser degree of hydration and therefore, less energy is necessary for the removal or reorientation of the hydrated water molecules upon binding, thus facilitating metal sorption [36].

Kinetics of lanthanum sorption

The kinetics of La sorption by the test biomass (Fig. 2) was followed at pH 5.0 as maximum La binding was observed at this pH. Lanthanum binding to the biomass was rapid. More than 80% of the maximum loading (120 mg g^{-1}) was obtained within 30 min of contact. After that, the biosorption rate slowed down. reaching equilibrium in around 3 h. Rapid metal binding is typical both of chemical complexation and adsorption processes [25–27, 30, 35]. Such a fast kinetics is considered as an essential criterion of good biosorbent. allowing a short contact period between the waste solution and the sorbent, resulting in the desired much shallower contact beds of the sorbent materials in process application [25].

Lanthanum-biosorption isotherm and model fitting

Lanthanum-binding capacity of the bacterial biomass was quantitatively evaluated by the sorption isotherm over a wide range of initial metal concentrations (0–1,000 mg La l^{-1}) at optimum solution pH (pH 5.0, Fig. 3). A typical isotherm curve indicates a desirable high affinity of the biosorbent for the lanthanum ion with equilibrium metal loading as high as 950 mg La g^{-1}

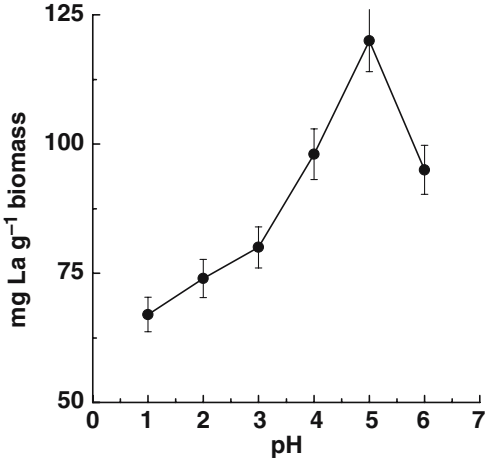

Fig. 1 Effect of pH on lanthanum biosorption (100 mg La l^{-1}) by *Pseudomonas* sp.

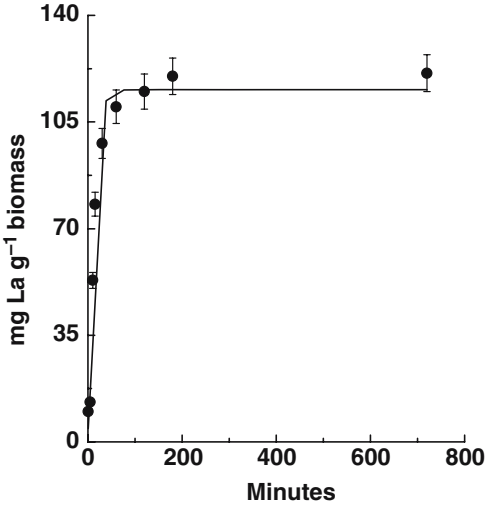

Fig. 2 Time course of lanthanum biosorption (100 mg La l^{-1}, pH 5.0) by *Pseudomonas* sp.

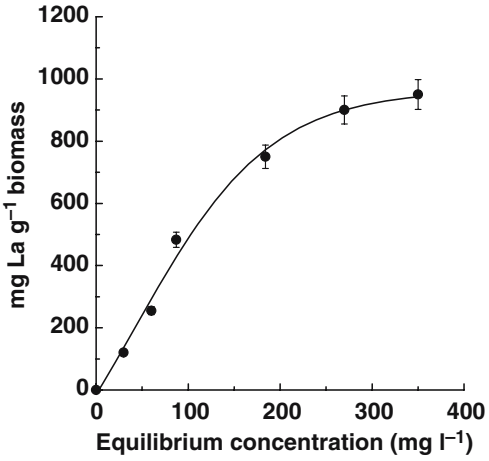

Fig. 3 Lanthanum-biosorption isotherm for *Pseudomonas* sp. at pH 5.0

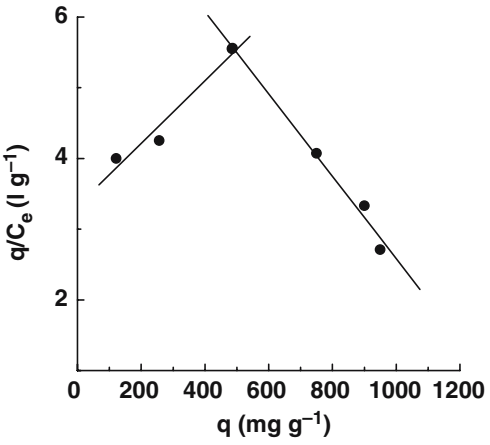

Fig. 4 Scatchard plot for lanthanum biosorption by *Pseudomonas* sp.

biomass at a low residual concentration of around 350 mg La l^{-1}. Such a high La sorption by the test biomass significantly surpasses the economic threshold value (15%) recommended for practical application of any biosorbent [27] and so also all the previous values reported for La binding by *P. aeruginosa*, *Mycobacterium smegmatis* [24, 30], *S. fluitans*, [22] or *M. xanthus* [19]. The relationship between the equilibrium metal uptake (q) and residual metal ion concentration was described employing Freundlich, Langmuir and BET model equations. The Freundlich model assumes a multilayer metal adsorption onto the binding sites with affinity distribution and heterogeneous surface energy [9]. On the contrary, the Langmuir model assumes monolayer adsorption of solutes onto a surface containing a finite number of identical sites with homogeneous adsorption energy and no transmigration of adsorbate in the surface plane [9]. The BET model, however, predicts a multilayer adsorption process in which each layer can be reduced to a Langmuir behaviour with homogeneous low surface energy [30]. The respective model parameters were derived and are presented in Table 1. The correlation coefficient value of Freundlich model ($r = 0.98$) was slightly higher over that of the Langmuir model ($r = 0.94$) or the BET model ($r = 0.93$). The Freundlich model constants 'k' and 'n' are indicators of the sorption capacity and sorption intensity and the respective values obtained in this study are 100 and 1.8. The Langmuir parameter, 'b' indicates the affinity between the biosorbent material and the metal ion and other parameter 'q_{max}' is a measure of maximum sorption capacity of the biomass. The small 'b' value (0.0027) and a very high q_{max} (1,926) obtained in this study indicate a high-affinity and high-capacity La sorption by the bacterial biomass.

The Scatchard model was also used to determine the nature of the binding sites in their interactions with binding ions [1]. Lanthanum sorption on the bacterial biomass can be described by two intersecting curve tangents representing the two linear contributions of different slopes (Fig. 4). Such a biphasic pattern indicates the presence of at least two types of metal-binding sites, corresponding to a strong and a weak binding affinity. The Scatchard affinity constants (K_1 and K_2) and metal-binding capacity (q_{max1} and q_{max2}) of each type of the binding site are extrapolated from the plot and are presented in Table 2. The Scatchard model prediction is in line with the better fitting of experimental sorption data to Freundlich model, suggesting lanthanum adsorption onto the biomass binding sites with different affinities. It is well known that a variety of potential metal-binding groups, such as carboxyl, phosphate, hydroxyl and amino functions, occur in the bacterial biomass and each functional group exhibits a different specificity toward the metal ions [24, 31]. It is also important to note the convex shape of the curve indicates a positive cooperation between the binding sites.

Table 1 Freundlich, Langmuir and Brunauer–Emmet–Teller (BET) model parameters for lanthanum sorption by *Pseudomonas* sp.

Models	Model parameters		Correlation coefficient (r)
Freundlich	k	n	
	100	1.1657	0.98
Langmuir	q_{max}	b	
	1,923	0.0027	0.94
BET	q_{max}	b	
	693	7.7	0.93

Table 2 Scatchard model parameters for lanthanum sorption by *Pseudomonas* sp.

Scatchard affinity constant	Binding capacity (q_{max}, mg La g^{-1} dry wt.)
$K_1 = 0.004$	$q_{max1} = 759$
$K_2 = 0.006$	$q_{max2} = 1{,}444$

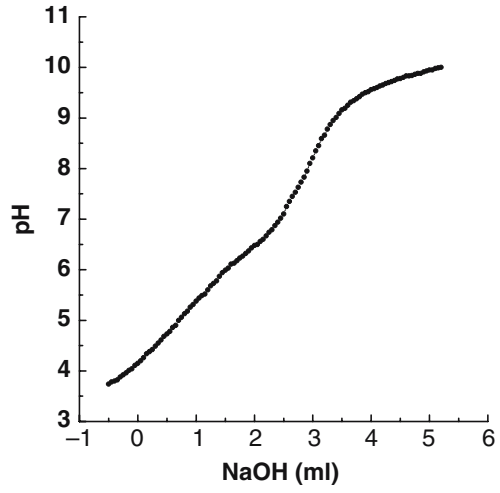

Fig. 5 Potentiometric titration of protonated biomass of *Pseudomonas* sp.

Biomass characterization by potentiometric titration and FTIR spectroscopy

The unknown features of most biosorbents reduce their chance of being used as competitive products with respect to the well-known synthetic ion-exchangers [31]. The potentiometric titration of *Pseudomonas* biomass was performed to obtain an insight on biomass properties. The titration curve (Fig. 5) of protonated biomass resulting from the addition of 0.01 M NaOH clearly shows two distinct waves indicating the presence of at least two types of binding sites, corresponding respectively to strong and weaker binding affinities [31]. The two waves are characterized by the flexion points at approximately pH 4.3 and 6.5, corresponding to the pK_a values of strong and weaker acidic groups. It may be suggested that these two groups might be carboxylic and phosphate. These types of acidic sites are capable of removing metal cations from aqueous solutions through different mechanisms, such as complexation, surface precipitation, etc. [14].

FTIR spectra for the control (lanthanum-free) and the lanthanum-loaded biomass were recorded to elucidate the chemical groups involved in La binding onto the bacterial biomass (Fig. 6). Analyzing the highly complex IR spectra, certain characteristic peaks can be assigned to the involvement of main functional groups present in the bacterial biomass. The spectrum of the native biomass showed a characteristic peak at 3,787 cm^{-1} region due to the stretching of the O–H bond of carboxylic acid present in the biomass [29]. In the spectrum of the lanthanum-loaded cells, this peak was shifted to the 3,784-cm^{-1} region, which is attributable to the role of carboxylic acid in metal binding. In

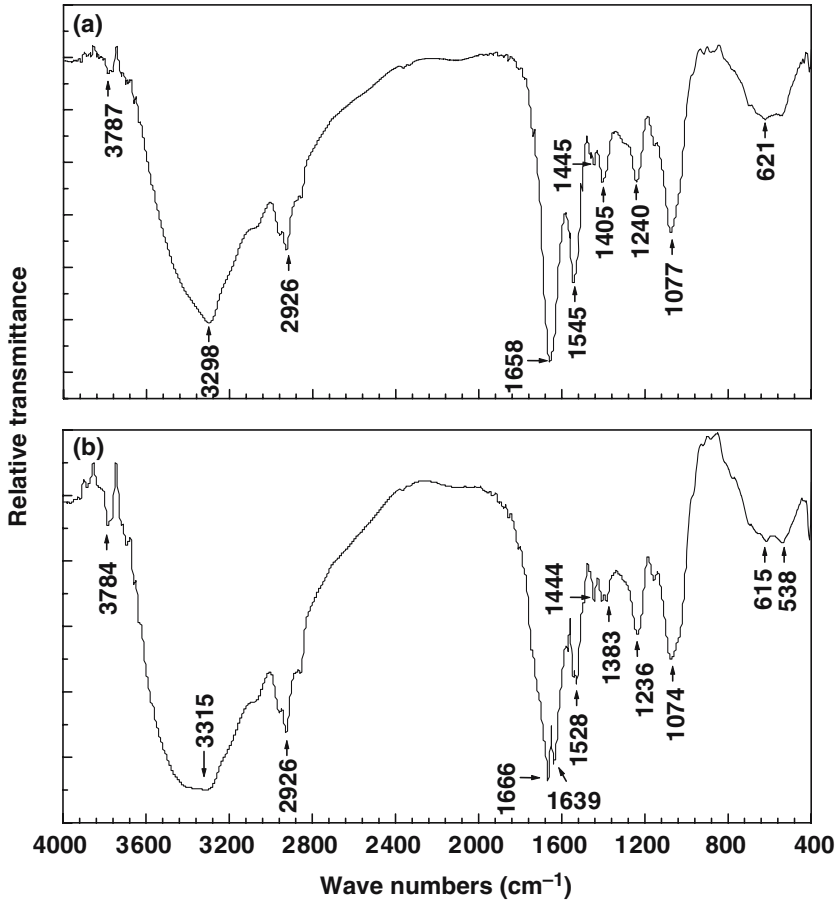

Fig. 6 FTIR spectra of *Pseudomonas* sp. biomass: **a** before and **b** after lanthanum sorption

the control spectrum, a peak at 3,500–3,200 cm^{-1} region is due to the stretching of the N–H bond of amino groups. This N–H stretching peak lies in the spectrum region occupied by a broad and strong band (3,200–3,600 cm^{-1}), which is due to the presence of γO–H of the hydroxyl groups [21]. A change in peak position in the spectrum of the lanthanum-loaded sample indicates the binding of lanthanum with amino and hydroxyl groups. In the control spectrum, the complex absorption peaks at 2,900–3,000 $cm^{-1}$are ascribed to the asymmetric stretching of γC–H bond of the $–CH_2$ groups combined with that of the CH_3 groups [3]. In the control spectrum, the $\gamma C=O$ of amide I and $\delta NH/\gamma C=O$ combination of the amide II bond were present at 1,658 and 1,545 cm^{-1}, respectively, indicating the presence of carboxyl groups [3]. Interestingly, in the metal-loaded spectrum the 1,658 cm^{-1} peak has been split, forming two new peaks at 1,666 and 1,639 cm^{-1} regions and a marked shift of 1,545 cm^{-1} peak to 1,528 cm^{-1} region suggest a strong interaction of La with carboxyl groups. In the control spectrum, the sharp peaks in between the 1,400 and 1,500 $cm^{-1}$are due to the presence of the carboxyl groups [11]. Following La sorption, the spectra exhibited a change in the peak position indicating the strong role of carboxyl groups in La binding. In the control, the strong peaks at 1,240 and 1,077 cm^{-1} are attributable to the presence of carboxyl and phosphate groups. Following metal sorption, a clear shift of these peaks indicates a strong interaction of La with these groups [14, 18]. The strong absorption peaks in between 1,000 and 1,100 cm^{-1} also ascertained the presence of the carboxyl groups in the bacterial polysaccharide structure; after metal binding by the biomass, the prominent change occurring in the peak positions in this region strongly suggest the involvement of the carboxyl groups in lanthanum sorption [11]. An obvious change in the peak position and intensity at 800–400 cm^{-1} region could be assigned to the formation of intense δ(M–O) and δ(O–M–O) bonds (M = metal ion) [27]. The overall spectral analysis strongly supports the major role of the carboxyl and phosphate groups in lanthanum binding by the bacterial biomass.

Energy dispersive X-ray analysis

As X-ray microanalysis can provide the information regarding the elemental characteristic of the biomass, the lyophilized bacterial biomass was subjected to EDX analysis before and after La sorption (Fig. 7). The EDX spectrum of the biosorbent before metal uptake (Fig. 7a) exhibited distinct peaks of carbon, oxygen, calcium, phosphorous, potassium and sulphur, indicating substantial presence of these elements in the biomass. Following La sorption, the spectrum (Fig. 7b) showed distinct peaks for La, but no peak for potassium and a very small peak for calcium. This observation is supported by the relative abundance values of Ca and K, obtained from the microanalysis of elements present in the La-exposed sample (Ca, 0.395% and K, not detected) compared to the unexposed (control) biomass (Ca, 1.64% and K, 18.5%). To check the possible release of cellular K and Ca following La sorption, elemental analysis of the sorption solution was performed after the sorption process was over. Compared to the lanthanum-free biomass solution (control), the La-biomass sorption solution showed presence of substantial amounts of K and Ca (9 and 1.3 mg l^{-1}, respectively), clearly indicating the release of these ions from the biomass following La sorption. Concomitant with metal uptake, ion (K, Ca, Mg, Na or H) release from the biomass is a frequently observed event that has significance in deciphering the mechanism of metal biosorption [4, 36]. It has been suggested that ion-exchange rather than sorption to free sites could be a relevant mechanism for the binding of metal ions onto the biomass; since the charge of the biomass particle has to be neutral, any binding of one cation must be accompanied by either a stoichiometric release of other cations or by the binding of anions [36]. Several previous reports have demonstrated extensive K release from biomass following metal uptake, suggesting K release to maintain ionic balance across the membrane. Many of these reports have shown the absence of a simple stoichiometric relationship between metal uptake and K release [4]. La can also replace cellular Ca through ion-exchange from the binding sites because of their close ionic radii and similar ligand specificities [23]. In the present case, the overall observation strongly indicates the possibility of lanthanum binding with the biomass by displacing the cellular potassium and calcium through an ion-exchange mechanism.

X-ray powder diffraction analysis

To elucidate the chemical nature of bacterial cell-bound lanthanum, the test biomass was also subjected to XRD before (control) and after lanthanum sorption for 24 h (Fig. 8). The spectrum for the control biomass (before metal exposure) showed no peak suggesting the amorphous nature of the bacterial cells. Following La sorption, the XRD pattern of bacterial biomass showed several distinct peaks at different positions ($2\theta = 22.8151°$, 33.0980°, 36.1192°, 47.1268° and 48.7504°). Based on the d-spacing values (4.52586, 3.1427, 2.88753, 2.23921 and 2.16739 Å), these peaks are attributable to the presence of crystalline lanthanum phosphate ($LaPO_4$) onto the bacterial biomass. However, no peak for crystalline $LaPO_4$ was observed after La sorption by the biomass for shorter period (3 h) (data not shown). The crystalline lanthanum phosphate formation following long period of metal accumulation could be due to the complexation of La with the cellular phosphate groups facilitating metal nucleation and metal precipitation in crystalline state [13, 34, 36]. FTIR spectroscopic analysis of the test biomass also indicated involvement of cellular phosphate groups in lanthanum

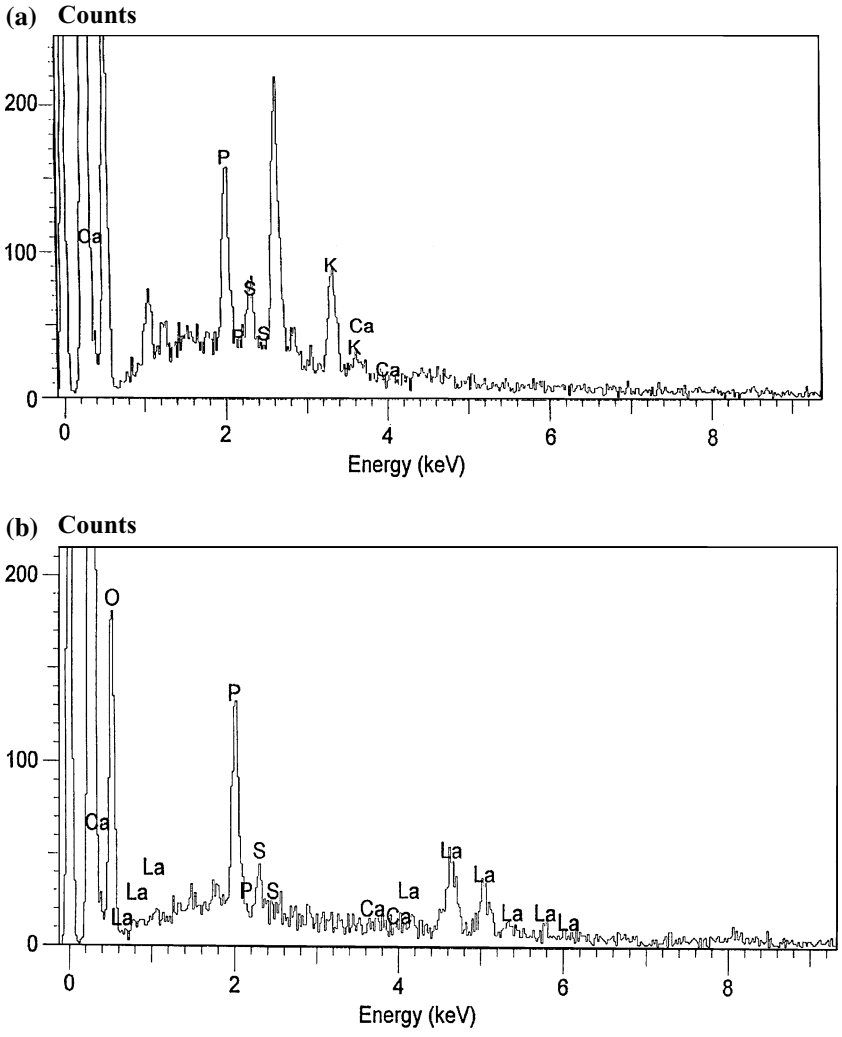

Fig. 7 Energy dispersive X-ray analyses of *Pseudomonas* sp. biomass: **a** before and **b** after lanthanum sorption

binding as described above. In Gram-negative bacteria, the phosphonate groups of the cell wall lipopolysaccharides and phospholipids were confirmed to be the primary metal binding sites as these can create a negative surface charge conductive to cation binding [18, 19]. The intracellular phosphate groups may be derived from sugar phosphates, adenosine phosphates and polyphosphates [18, 19]. The initial binding of metal ions to biomass reactive sites may serve as nucleation sites for further precipitation of metals forming large metal deposits [36]. Earlier investigators have found that La accumulates in the form of phosphate compounds in *Escherichia coli* and that microbial La accumulation was accompanied by P (phosphorous) in all parts of the cells [2]. It has been suggested that the phosphate structure is favourable to the entrance of lanthanides [19]. Precipitation of La-phosphate was observed previously in the cases of *Citrobacter* sp. and *Acinetobacter johnsonii* [5, 37], whereas non-crystalline deposition of lanthanum in *M. xanthus* was demonstrated by Merroun et al. [19]. It has been reported that phosphate containing extracellular polymers of bacteria have a potential role in metal-phosphate biomineralization through the complexation of metals with polymer phosphate groups [12].

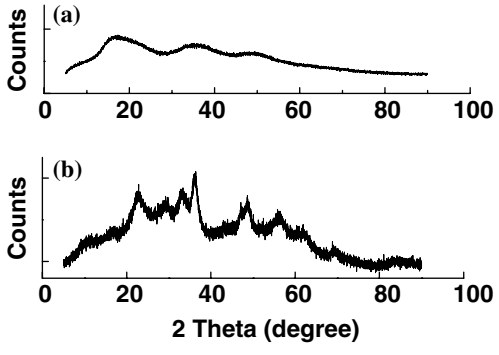

Fig. 8 X-ray powder diffraction analyses of *Pseudomonas* sp. biomass: **a** before and **b** after lanthanum sorption

Transmission electron microscopy

Lanthanum accumulation by the test *Pseudomonas* biomass was further investigated to elucidate the cellular

localization of the accumulated La by employing TEM (Fig. 9). The electron micrograph of the metal-free control cell exhibited a distinct and clear cell boundary and cytoplasm with few electron-dense areas. In contrast to this, the metal-loaded cell revealed a dark electron-opaque cytoplasm with some electron-dense deposits in the cell-wall region as well as in the cell interior indicating the homogeneous sequestration of the biosorbed lanthanum throughout the cell. This is a new observation for lanthanum accumulation by metabolically inactivated bacterial cell. The cytoplasmic La content indicates that the diffusion of the metal into the cell may be due to increased membrane permeability. Granular deposits in the cell wall and the cytoplasmic region could be attributed to the microprecipitation of crystalline lamnthanum phosphate as also confirmed by the XRD analysis. Microprecipitation of metals may be resulted in metal accumulation by the biomass several folds of cellular dry weight [36]. Previously, it has been assumed that lanthanides can bind to the external surfaces of microorganisms and that they are not transported into the cytoplasm of the bacteria, algae or yeasts [2]. Cell-surface accumulation of lanthanum in the form of large acicular deposits had been demonstrated in *P. aeruginosa* by Mullen et al. [20], whereas Merroun et al. [19] have reported substantial La accumulation in extracellular polysaccharide and cell wall and smaller amounts, in the cytoplasm of *M. xanthus*. Bayer and Bayer [2] have found periplasmic La accumulation in *E. coli* in the form of a phosphate compound.

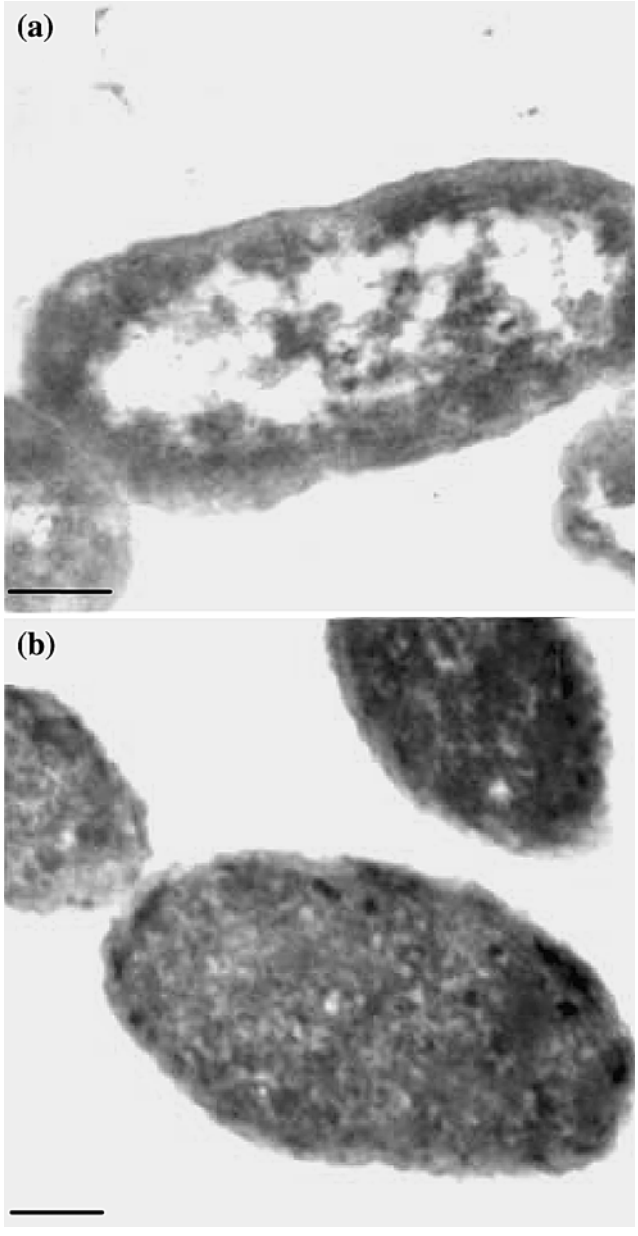

Fig. 9 Transmission electron micrographs of *Pseudomonas* sp.: **a** before and **b** after lanthanum accumulation. *Bar* indicates 200 nm

In the present study, the overall observations suggest that the high accumulation of lanthanum by the test bacterium could be due to the result of a combined ion-exchange–complexation–microprecipitation mechanism, which is a new report for La biosorption by metabolically inactivated bacterial cell. Previous investigators have demonstrated different biosorption mechanisms involved in the accumulation of other heavy metals in *Sargassum* biomass or in *P. fluorescens* [13, 36]. It has been recognized that a combination of several mechanisms, such as adsorption, ion-exchange, complexation, co-ordination, chelation or microprecipitation, each functioning independently, can contribute to the overall metal accumulation in biosorption [36].

Desorption of biomass bound lanthanum

Desorption/recovery of sorbed metal is one of the most important aspects of any successful biosorption-process development. In the present study, the extent of biomass-bound La desorption was investigated immediately after the sorption process was over (3 h) and the results are summarized in Table 3. Among the desorbing agents tested, $CaCO_3$ (1 M) was the most effective as 98% of the biomass-bound lanthanum could be recovered using this. The effectiveness of $CaCO_3$ in remobilizing the biomass-bound lanthanum is attributable to the competition of Ca ions to get sorbed with the binding sites by displacing the sorbed metal through electrostatic non-specific interactions [27]. Using HNO_3, greater than 90% desorption was achieved, while other mineral acids were not so effective: HCl (1 M) produced 58% and H_2SO_4 (1 M), only 32% desorption. However, HCl and H_2SO_4 of higher strength (1.5 M) showed enhanced La desorption. The efficiency of mineral acids in metal desorption is attributable to the increased metal mobility in acidic condition and the replacement of the sorbed metal by H^+ ions [6]. It has been suggested that due to the weak nature of the metal-binding forces, it is possible to desorb the bound metal from the biosorbent by using the desorbing solution containing another cation like H^+ or Ca^{2+}. The effectiveness of desorption depends in

Table 3 Desorption of biomass bound lanthanum by various desorbents

Desorbents	Amount of lanthanum desorbed[a] (expressed as percent of sorbed metal[b])
HCl (1 M)	58
HCl (1.5 M)	87
HNO_3 (1 M)	90
HNO_3 (1.5 M)	96
H_2SO_4 (1 M)	32
H_2SO_4 (1.5 M)	50
$CaCO_3$ (0.5 M)	87
$CaCO_3$ (1 M)	98
Na_2CO_3 (1 M)	67
Na_2CO_3 (1.5 M)	71
$NaHCO_3$ (1 M)	14
$NaHCO_3$ (1.5 M)	20
Sodium citrate (1 M)	58
Sodium citrate (1.5 M)	67
EDTA (0.5 M)	27
EDTA (1 M)	29

[a]Lanthanum-loaded biomass (120 mg La g^{-1} dry wt.) was contacted with respective desorbing agent for 24 h at 150 rpm, 30°C
[b]100% value is 120 mg La g^{-1} biomass dry wt.

this case on the binding strength of the added cation to the biosorbent [36]. The use of calcium carbonate as a desorbent seems more useful as it is less destructive to the biomass than mineral acids and therefore, will favour the overall process economics [26]. Philip et al. [24] and Palmieri et al. [22] reported that mineral acids like HCl are most effective in desorbing bound lanthanum from *P. aeruginosa* or *S. fluitans*. In a similar comparison, Na_2CO_3 and sodium citrate were able to recover around 65% of the bound metal even at higher concentrations, whereas EDTA (0.5 or 1 M) allowed only around 28% desorption. The diffusion of lanthanum into the interior of the cells and the inaccessibility of the metal-binding sites to EDTA could be the reasons for the inefficiency of EDTA in recovering the biomass-bound metal [17].

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