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Biosorption characteristics of *Bacillus* sp. ATS-2 immobilized in silica gel for removal of Pb(II)

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Abstract

The bacterial strain *Bacillus* sp. ATS-2 isolated from Pb(II) polluted soil was immobilized with a silica matrix and Pb(II) biosorption properties of immobilized biosorbent were examined. Optimum biosorption conditions were investigated in the fixed bed column with the variation in the parameters of pH, bed lenght, flow rate and influent concentration. The Pb(II) biosorption equilibrium was attained within 60 min and the maximum biosorption yield for silica gel immobilized *Bacillus* sp. ATS-2 was determined as 91.73% at pH 4.0. The higher biosorption yields were observed at flow rates of 60 and 180 ml h^{-1}. The optimum bed length for the column was found as 10 cm. Data obtained from batch studies were evaluated by Freundlich, Langmuir and Dubinin–Radushkevich (D–R) isotherm models. The maximum monolayer capacity of *Bacillus* sp. ATS-2 for Pb(II) was 2.36×10^{-5} mol g^{-1}. The involvement of the functional groups on the surface of immobilized cells in biosorption process was also evaluated by FTIR spectral analysis.

Keywords: Bacillus; Biosorption; Immobilized cells; Pb(II); Isotherm

1. Introduction

Heavy metal contamination of industrial effluents is one of the significant environmental problems due to their toxic nature and accumulation throughout the food chain as nonbiodegradable pollutants [1–3]. Heavy metals are discharged from various industries such as electroplating, plastic manufacturing, textile, storage batteries, mining and metallurgical process. Traditional technologies for removing of heavy metals from wastewaters are include chemical precipitation, ion exchange, membrane separation, reverse osmosis, evaporation and electrolysis. However, these techniques sometimes restricted because of technical or economical constraints and the imposition of stricter regulations increases the demand for new technologies to reduce the metal levels in wastewaters to the environmentally acceptable values [4].

The use of biosorbents with microbial origin especially bacteria, algae, fungi and yeasts is a considerable alternative to the existing methods because of their good performance, low cost and large availability [1]. The physicochemical interactions between metal ions and different functional groups on the biomass surface such as carboxyl, hydroxyl, sulfhydryl and amino groups play an important role in the biosorption process. Living, dead and immobilized cells can be utilizes in this process. Immobilized form of biosorbents is ideal for use in a column applications with the advantages of improved mechanical strength, online matrix isolation in flow analysis, low resistance to fluid flow, self supporting rigidity, excellent durability, easy regeneration of biosorbent material and relatively high local cell density [5–7]. Many different natural and synthetic polymeric supports such as alginate, agar, silica, carrageenan, polyethylenimine, polyacrylonitril, polysulfone, polyvinyl alcohol and polyacrylamide have been widely used for the immobilization of biosorbents [8–17].

The objective of this study was to investigate the Pb(II) biosorption performance of *Bacillus* sp. ATS-2 biomass-immobilized silica gel beads as an alternative biosorbent in upflow fixed bed column and batch systems. *Bacillus* sp. ATS-2 was chosen as a biosorbent material because of its high Pb(II) resistance. The effects of design parameters, such as pH, bed lenght,

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flow rate and influent concentration were examined. Equilibrium biosorption data obtained from batch process were applied to Freundlich, Langmuir and Dubinin–Radushkevich isotherm models in addition to the metal–biosorbent interactions evaluated by FTIR spectral analysis.

2. Materials and methods

2.1. Isolation and screening of microorganism

The microorganism used in this study was isolated from Pb(II) polluted soil. The soil samples were initially collected from natural environment and 10 g of soil was polluted with Pb(II) by treating with 100 ml of 1000 mg l^{-1} Pb(II) solution in 250 ml flask to select the appropriate bacterial strains having greater resistance towards Pb(II) toxicity. Soil sample was stirred with 1000 mg l^{-1} of Pb(II) solution for 48 h and then liquid phase was separated by filtration. For isolation of bacterial strains 1 g of Pb(II) treated soil sample was homogenized with 9 ml of 0.85% (w/v) sterile saline solution. Serial dilutions were prepared in 9 ml of 0.85% (w/v) sterile saline solution and plated on Nutrient Agar (NA) (Merck). From the NA plates, representative colonies of all different morphologies were chosen at random, purified by sub-culturing and maintained in slants of NA. All culture works were conducted aseptically.

For preliminary tests, bacterial strains were investigated for theirs biosorption capacities for Pb(II) in a batch system. All the batch biosorption experiments were performed at 20 °C in a magnetic stirrer at 200 rpm using 100 ml beakers containing 0.1 g biosorbent sample in 50 ml of solutions containing 100 mg l^{-1} of Pb(II). The pH of the solutions was adjusted to 4.0. After 2 h the biosorption mixture was centrifugated at 4500 rpm for 2 min and the residual Pb(II) concentrations in the solutions were analyzed. The most effective bacterial strain for the biosorption of Pb(II) was identified according to Bergey's Manual of Determinative Bacteriology and The Prokaryotes [18,19].

2.2. Preparation of the immobilized biosorbent

The bacterial isolate was inoculated into 250 ml Erlenmeyer flasks containing Nutrient Broth (Merck) and aerobically cultivated at 30 °C by the agitating at the speed of 150 rpm. The cells were harvested from the growth medium at early-stationary phase by centrifugation at 5000 rpm for 10 min. After rinsing in deionized water the cells were again centrifugated. For the immobilization of the cells, the procedure previously reported by Lopez et al. [20] was followed. About 10 g of silica gel was dissolved by heating in 100 ml of 7% (w/v) aqueous solution of KOH and sterilized by autoclaving (121 °C, 20 min). After cooling to 20 °C, 100 ml of cell suspension (20 g wet weight *Bacillus* sp. ATS-2 was suspended in 1 l of distilled water) was added and mixed. Just before bead formation, a measured amount of phosphoric acid solution (20%) was added, enough to provide a pH of 7.0. Gel beads were formed by the interphase technique.

2.3. Preparation of Pb(II) solutions

The stock solution of Pb(II) (1.0 g l^{-1}) used in this study was prepared by dissolving a weighed accurate quantity of $Pb(NO_3)_2$ (Merck) in deionized water. Other concentrations (25–300 mg l^{-1}) were prepared by dilution from this stock solution. Fresh dilutions were used for each experiment. The initial pH of the solutions was adjusted to the required values by adding 0.1N HNO_3 or 0.1N NaOH solutions for the biosorption experiments.

2.4. Column studies

The immobilized cells were stacked into glass columns with 1.5 cm internal diameter and the bed length 3, 6 and 10 cm. To avoid channel effects the Pb(II) solutions (25–300 mg l^{-1}) were continuously pumped upward through the column by a peristaltic pump (Ismatec ecoline). The flow rates of the solutions were varied from 60 to 300 ml h^{-1}. The eluents were collected to measure of the residual Pb(II) ion concentrations up to 360 min. The effect of initial pH on the Pb(II) biosorption yield of fixed bed column system was investigated in the initial pH range of 1.0–5.0.

2.5. Isotherm studies

The Freundlich, Langmuir and Dubinin–Radushkevich isotherm models for the Pb(II) biosorption by *Bacillus* sp. ATS-2 were applied to batch experimental data. Pb(II) solutions ranging from 25 to 150 mg l^{-1} were prepared and used. The initial pH of the solutions was adjusted to optimum value of 4.0 in a beaker. 0.5 g of biosorbent was added to biosorption medium and stirred for 1 h on a magnetic stirrer at 20 °C. When the biosorption procedure completed the solutions were centrifuged at 4500 rpm for 2 min and the supernatants were then analyzed for residual Pb(II) ion concentrations.

2.6. Analytical methods

The residual Pb(II) ion concentrations in the solution were measured with an atomic absorption spectrophotometer (Hitachi 180-70 Model, Japan) with an air-acetylene flame. Deuterium background correction was used and the spectral slit width was 1.3 nm. The working current and wavelength were 7.5 mA and 283.3 nm, respectively. The instrument calibration was periodically checked by using standard metal solution in every 10 readings. In order to identify the functional groups responsible for the Pb(II) biosorption, immobilized biosorbents before and after Pb(II) sorption were analyzed and interpreted by FTIR spectroscopy. The spectra were recorded in a Bruker Tensor 27 Fourier transform infrared spectrometer (Bruker Optics GmbH) within the range of 400–4000 cm^{-1} with the samples prepared as KBr discs.

2.7. Mathematical equations

The breakthrough curves for the biosorption of Pb(II) ions were derived as a function of initial pH, equilibrium time, bed length, initial Pb(II) ion concentration and flow rate. The maximum (equilibrium) capacity of the column for a given feed concentration is equal to the area under the plot of the adsorbed metal ion concentration, $C_{i,ads}$ (mg l^{-1}), versus time (min) or the area behind the breakthrough curve and calculated by using the following equations. The amount of metal that remains in the effluent, $C_{i,eq}$ (mg l^{-1}), is the area under the breakthrough curve [21,22]:

$$C_{i,eq} = \frac{C_i t - \int_0^a C_{i,ads} dt}{t} \quad \text{or} \quad C_{i,eq} = \frac{W_i - q_{i,eq} X}{Qt}, \tag{1}$$

$$C_{i,max} = Q \int_0^a C_{i,ads} dt, \tag{2}$$

where $C_{i,max}$ is the maximum (equilibrium) capacity of the column (mg); $q_{i,eq}$, the amount of Pb(II) loading on *Bacillus* sp. ATS-2 (mg g^{-1}); C_i, initial Pb(II) ion concentration (mg l^{-1}); t, contact time (min); W_i, amount of Pb(II) loading into the column (mg); a, maximum value of time; X, amount of biosorbent filled in the column (mg); Q, volumetric flow rate (ml min^{-1}).

The amount of Pb(II) loading onto *Bacillus* sp. ATS-2, is calculated by Eq. (3).

$$q_{i,eq} = \frac{C_{i,max}}{X}, \tag{3}$$

The biosorption yield, Y_i% (percentage of biosorbed Pb(II)) is the ratio of the maximum capacity of the column to the amount of Pb(II) loading into the column (Eqs. (4) and (5)).

$$W_i = C_i Q t, \tag{4}$$

$$Y_i = \frac{C_{i,max}}{W_i} \times 100, \tag{5}$$

3. Results and discussion

In this study, the bacterial strain was identified according to Bergey's Manual of Determinative Bacteriology and The Prokaryotes [18,19] and the results are presented in Table 1. According to these results the strain was identified as *Bacillus* sp. and called as *Bacillus* sp. ATS-2 in this article. The isolated strain was compared with *Bacillus pasteurii* and showed the similar characteristics of these strains. But identification of the isolated bacteria should be continue with detail for entire description.

3.1. Effect of initial pH

The effect of initial pH on the biosorption of Pb(II) onto silica gel-immobilized *Bacillus* sp. ATS-2 was investigated at a constant flow rate of 180 ml h^{-1}, fixed bed column with the bed length of 10 cm and the initial Pb(II) concentration of 100 mg l^{-1}. The results were given in Fig. 1. As seen in this figure initial pH played a significant role in the biosorption process. The lower biosorption yield under the initial pH values of 4.0 has been attributed to the competition of the metal ions with the protons for the available binding sites on the immobilized biosorbent. In the other words at pH values under the isoelectric point of the cells, the surface area of the fungal cells is surrounded by the protons, which competes with positively

Table 1
. Biochemical and microscopic characteristics of effective isolate

Properties	Result
Gram reaction	+
Cell shape	Rod
Cell diameter >1.0 μm	+
Spores round	+
Sporangium swollen	+
Catalase	+
Anaerobic growth	−
Voges-prokauer test	−
Acid from D-glucose	+
Acid from D-xylose	−
Acid from D-mannitol	−
Hydrolysis of casein	−
Hydrolysis of gelatin	+
Hydrolysis of starch	+
Utilization of citrate	−
Formation of indole	−
Growth at pH 6.8, nutrient broth	+
Growth at pH 5.7, nutrient broth	+
Growth in NaCl 2%	+
Growth in NaCl 5%	+
Growth in NaCl 7%	+
Growth in NaCl 10%	+
Growth at 5 °C	−
Growth at 10 °C	−
Growth at 30 °C	+
Growth at 40 °C	+
Growth at 50 °C	+
Growth at 55 °C	+
Growth at 65 °C	+

+: 90% or more strain positive; −: 90% or more strain negative.

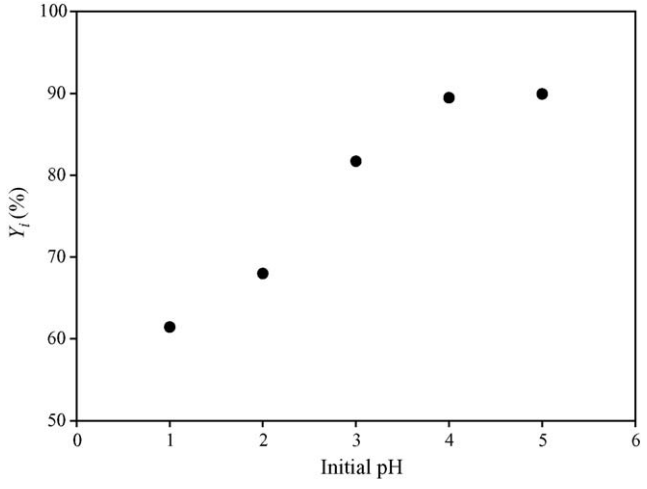

Fig. 1. The effect of the initial pH on the biosorption of Pb(II) on silica gel-immobilized *Bacillus* sp. ATS-2. (flow rate: 180 ml h^{-1}; bed length: 10 cm; inlet Pb(II) concentration: 100 mg l^{-1}).

Table 2
The effect of the bed length on the biosorption of Pb(II) on silica gel-immobilized *Bacillus* sp. ATS-2

Bed length (cm)	X (g)	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg g^{-1})	Y_i (%)
3.0	6.0	32.65	55.10	10.88	59.25
6.0	12.0	35.08	55.10	5.84	63.66
10.0	20.0	49.29	55.10	4.92	89.46

Flow rate: 180 ml h^{-1}; inlet Pb(II) concentration: 100 mg l^{-1}; initial pH: 4.0.

Table 3
The effect of the flow rate on the biosorption of Pb(II) on silica gel-immobilized *Bacillus* sp. ATS-2

Flow rate (ml h^{-1})	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg g^{-1})	Y_i (%)
60	16.75	18.36	1.67	91.23
180	49.29	55.10	4.92	89.46
300	39.48	91.83	3.94	43.00

Bed length: 10 cm; inlet Pb(II) concentration: 100 mg l^{-1}; initial pH: 4.0.

charged metal ions for binding. This prevents the approach of metal ions as a result of the repulsive forces and causes the biomass to adsorb less metal ions. The maximum biosorption yield (89.45%) was obtained at the initial pH of 4.0 .When the initial pH of the medium was adjusted to 5.0 biosorption yield stayed nearly constant. At pH values above the isoelectric point of the cells, the surface of the biomass carries negative charges, which cause an increase on the sorption capacity of biosorbents for the metal ions as a result of attractive forces. Similar observations were reported in the literature [23,24]. Experiments were not conducted higher initial pH values of 5.5 because the Pb(II) ions become precipitate due to high concentration of OH^- ions in the solution.

3.2. Effect of bed length of column

The biosorption performance of immobilized biosorbent was investigated with the various bed lengths at a constant flow rate (180 ml h^{-1}) and 100 mg l^{-1}of inlet Pb(II) ion concentration (Table 2). Maximum value of column capacity ($C_{i,max}$) of silica gel-immobilized *Bacillus* sp. ATS-2 for Pb(II) sorption was obtained at a bed length of 10 cm. The biosorption yields of Pb(II) ions decreased with a decrease in the bed length from 10 to 3 cm. This may be due to a relatively small amount of biosorbent in a shorter bed. However, an increase in the bed length may be caused to increase of equilibrium time of biosorption.

The increase in Y_i% values with the amount of biosorbent could be due to an increase in the surface area and the availability of more binding sites of biosorbent while the decrease in biosorption capacity may be attributed to unsaturated binding sites of the biomass during the biosorption process and reduction in the effective surface area for biosorption resulting from cell aggregation at high biosorbent concentration [25].

3.3. Effect of flow rate

When the concentration of initial Pb(II) ion (100 mg l^{-1} at initial pH 4.0) and bed length (10 cm) were kept constant, flow rates were changed from 60 to 300 ml h^{-1} and the results are presented in Table 3. The decrease in the flow rate of the column resulted in an increase in the biosorption yield (Y_i%). The higher Y_i values were obtained at flow rates of 60 and 180 ml h^{-1}. $C_{i,max}$ values were increased with flow rates up to 300 ml h^{-1} but decreased at 300 ml h^{-1} of flow rate. This could be attributed to metal solution leaved the column before the biosorption equilibrium attained when the flow rate increased [21,26,27].

The concentration of unsorbed metal ion in the effluent was high at the beginning of the column process. Sağ et al. [28] reported that, the initial decrease in the adsorption yield may be due to the diffusion limitations. This behavior was observed especially at low flow rates. After a while, concentration of metal ion in the effluent decreased in a short time. In this study, amount of biosorbed Pb(II) ion by silica gel immobilized *Bacillus* sp. ATS-2 was almost the same at the all flow rates at the beginning of the column operations. Thereafter biosorbed Pb(II) concentration increased at lower flow rates.

3.4. Effect of inlet Pb(II) ion cencentration

The effect of inlet Pb(II) ion concentration was investigated at the concentration range of 25–300 mg l^{-1}. The results are presented in Table 4. The Pb(II) biosorption capacity and $C_{i,max}$ of the immobilized biomass firstly increased with increasing of the initial concentration of Pb(II) and then reached a saturation value at 200 mg l^{-1} of Pb(II) concentration. The maximum biosorption yields for silica gel immobilized *Bacillus* sp. ATS-2 at 25 and 50 mg l^{-1} of initial Pb(II) concentration were determined as 91.72% and 91.73%, respectively.

The effect of immobilization on the Pb(II) biosorption capacity of *Bacillus* sp. ATS-2 was investigated under the determined optimum conditions. The biosorption capacity of silica gel immobilized cells was compared with the cell free silica gel matrix. As can be seen from Fig. 2, Pb(II) biosorption was 98% (C/C_i ratio 0.98) for immobilized biosorbent. For the negative control (silica gel alone with no biomass) Pb(II) was biosorbed by 41%.

Table 4
The effect of the inlet Pb(II) concentration on the biosorption of Pb(II) on silica gel-immobilized *Bacillus* sp. ATS-2

Inlet Pb(II) concentration (mg l^{-1})	$C_{i,max}$ (mg)	W_i (mg)	$q_{i,eq}$ (mg g^{-1})	Y_i (%)
24.40	12.08	13.17	1.20	91.72
48.60	24.07	26.24	2.40	91.73
73.30	33.30	37.43	3.33	88.96
96.60	46.54	52.16	4.65	89.22
153.80	53.03	66.89	5.30	79.27
196.90	68.11	100.90	6.81	67.50
245.30	55.68	132.46	5.58	42.10
300.30	76.51	162.17	7.65	47.17

Flow rate: 180 ml h^{-1}; bed length: 10 cm; initial pH: 4.0.

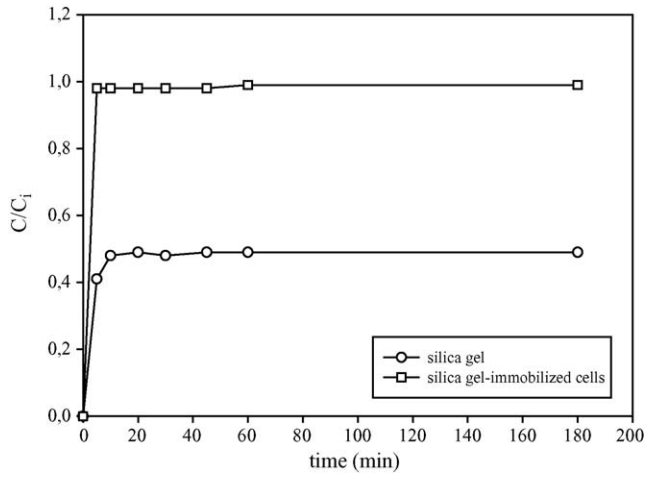

Fig. 2. Comparison of the breakthrough curves of the cell free silica gel and silica gel-immobilized *Bacillus* sp. ATS-2 for Pb(II) ions (flow rate: 60 ml h^{-1}; bed length: 10 cm; inlet Pb(II) concentration: 50 mg l^{-1}; initial pH: 4.0).

3.5. Isotherm models

The equilibrium adsorption isotherms are one of the most important data to understand the mechanism of the adsorption. Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherm models were applied to the experimental data obtained from batch system in this study. The biosorption isotherm for Pb(II) biosorption onto silica gel-immobilized biosorbent is shown in Fig. 3.

The Langmuir isotherm model assumes a monolayer sorption which takes place at specific homogeneous sites within the biosorbent. The Langmuir isotherm equation is represented by the following [29]:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e}, \tag{6}$$

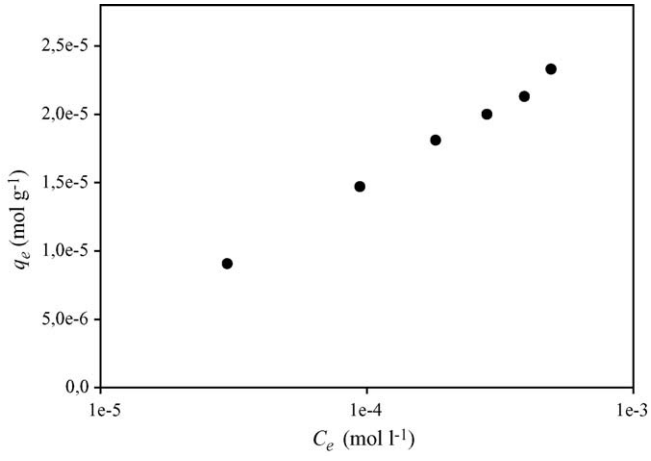

Fig. 3. Biosorption isotherm for the biosorption of Pb(II) onto silica gel-immobilized *Bacillus* sp. ATS-2 at 20 °C (initial pH: 4.0; contact time: 60 min; biosorbent concentration: 10 g l^{-1}).

The linear form of the Langmuir isotherm equation is

$$\frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max} K_L}\right)\frac{1}{C_e}, \tag{7}$$

where q_e is the equilibrium Pb(II) concentration on the biosorbent (mol g^{-1}), C_e the equilibrium Pb(II) concentration in the solution (mol l^{-1}), q_{max} the monolayer biosorption capacity of the biosorbent (mol g^{-1}), and K_L is the Langmuir biosorption constant (l mol^{-1}) and is related to the free energy of biosorption. The plot of $1/q_e$ versus $1/C_e$ for the biosorption gives a straight line of slope $1/q_{max}K_L$ and intercepts $1/q_{max}$.

The Freundlich isotherm is an empirical equation employed to describe heterogeneous systems. The Freundlich equation is [30]:

$$q_e = K_F C_e^{\,1/n}, \tag{8}$$

A linear form of the Freundlich equation is

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e, \tag{9}$$

where K_F (l g^{-1}) and n are Freundlich isotherm constants, being indicative of the extent of the biosorption and the degree of nonlinearity between solution concentration and biosorption, respectively. The plot of $\ln q_e$ versus $\ln C_e$ for the biosorption was employed to generate K_F and n from the intercept and the slope values, respectively.

The Dubinin–Radushkevich (D–R) isotherm is more general than the Langmuir isotherm. It was applied to distinguish the nature of biosorption as physical or chemical [31]. The D–R isotherm equation [32] is:

$$q_e = q_m\, e^{-\beta\varepsilon^2}, \tag{10}$$

The linear form of (D–R) isotherm equation is

$$\ln q_e = \ln q_m - \beta\varepsilon^2 \tag{11}$$

where β is a constant related to the mean free energy of biosorption (mol^2 J^{-2}), q_m the theoretical saturation capacity, and ε is the Polanyi potential, which is equal to $RT\ln(1 + (1/C_e))$, where R (J mol^{-1} K^{-1}) is the gas constant, and T (K) is the absolute temperature. Hence by plotting $\ln q_e$ against ε^2 it is possible to generate the value of q_m (mol g^{-1}) from the intercept, and the value of β from the slope.

The constant β gives an idea about the mean free energy E (kJ mol^{-1}) of biosorption can be calculated using the relationship [33–35]:

$$E = \frac{1}{(2\beta)^{1/2}}, \tag{12}$$

E values give information about biosorption mechanism as chemical ion-exchange (If E values are between 8 and 16 kJ mol^{-1}) or physical adsorption. The numerical value of the mean free energy of biosorption is 12.83 kJ mol^{-1} which may correspond to chemical ion-exchange.

The Langmuir, Freundlich and D–R parameters for the biosorption of Pb(II) onto silica gel-immobilized *Bacillus* sp.

Table 5
Biosorption isotherm constants for the biosorption of Pb(II) onto silica gel-immobilized *Bacillus* sp. ATS-2 at 20 °C

Langmuir				Freundlich			Dubinin–Radushkevich (D–R)			
q_{max} (mol g^{-1})	K_L (l mol^{-1})	r_L^2	R_L	n	K_F (l g^{-1})	r_F^2	q_{max} (mol g^{-1})	β (mol^2 kJ^{-2})	r_{D-R}^2	E (kJ mol^{-1})
2.36×10^{-5}	2.05×10^{5}	0.99	0.06	3.08	2.82×10^{-4}	0.98	6.68×10^{-5}	3.05×10^{-3}	0.99	12.83

ATS-2 are listed in Table 5. It is indicated that all of the isotherm models fit very well when the r^2 values are compared.

The essential feature of the Langmuir isotherm can be expressed by means of a separation factor or equilibrium parameter, R_L, is calculated using the following equation:

$$R_L = \frac{1}{1 + K_L C_0}, \quad (13)$$

where C_0 is the highest initial Pb(II) concentration (mol l^{-1}). As the R_L values lie between 0 and 1, the biosorption process is favorable [36,37]. The R_L value for this study was 0.06, therefore, biosorption of Pb(II) was favorable.

The Freundlich constants K_F and n indicate the biosorption capacity of the biosorbent and a measure of the deviation from linearity of the biosorption, respectively. The values of K_F and n at equilibrium were 2.82×10^{-4} l g^{-1} and 3.08, respectively. The well description of the experimental results with both Langmuir and Freundlich isotherm models implies that the surface of *Bacillus* sp. ATS-2 is made up of homogeneous and heterogeneous biosorption patches. Also E value obtained from D–R isotherm indicated that biosorption takes place with chemical ion-exchange mechanism. It can be concluded that the biosorption of Pb(II) ions onto silica gel-immobilized *Bacillus* sp. ATS-2 is complex and involving more than one mechanism.

3.6. FTIR spectral analysis

The FTIR spectra of the immobilized biosorbent before and after Pb(II) sorption in the range of 400–4000 cm^{-1} were taken and compared with each other to find out which functional groups are responsible for the Pb(II) biosorption (Fig. 4). The spectra of the unloaded immobilized biosorbent indicated strong asymmetrical stretching bands at 3297 cm^{-1} (–NH and bonded –OH groups). 1656 and 1565 cm^{-1} (carbonyl stretching vibration of amide considered to be due to the combined effect of double-bond stretching vibrations) and –NH deformation band, respectively [38], 1086 cm^{-1} (C–O stretching of carboxyl groups) [39], 796 cm^{-1} (possibly belonging to di substituted aromatic protons) and under 700 cm^{-1} (N-containing bioligands) [40]. The FTIR spectra of immobilized biomass exposed to Pb(II) ions indicated the band shifts from 3297, 1086 and 541 cm^{-1} to 3424, 1076 and 572 cm^{-1}, respectively. Also after the Pb(II) biosorption appearance of the peak at 1384 cm^{-1} (amide or sulfamide band) and a small increase in the intensity of the peak at 796 cm^{-1} were observed. These results indicated that these functional groups are likely to participate in metal binding process.

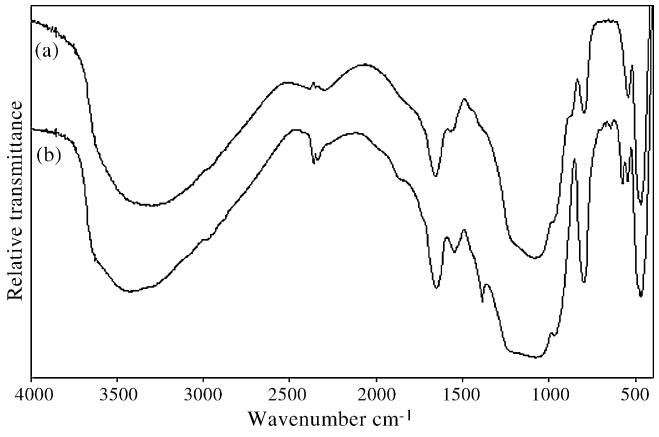

Fig. 4. FTIR spectra of immobilized *Bacillus* sp. ATS-2: (a) unloaded and (b) Pb(II) loaded.

4. Conclusion

The Pb(II) biosorption properties of immobilized *Bacillus* sp. ATS-2 cells were investigated in the fixed bed column and batch system. The maximum Pb(II) biosorption capacity of the immobilized biosorbent was obtained at the initial pH of 4.0. Column studies showed that the bed length and flow rate were affected the biosorption process. Better results were obtained with the highest bed length and 60 and 180 ml h^{-1} of flow rates. The batch experimental results fitted well to Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherm models. The possible interactions between Pb(II) ions and biosorbent surface were confirmed by the FTIR analysis. These results indicated that silica gel immobilized *Bacillus* sp. ATS-2 cells may be used as an inexpensive, effective and alternative biosorbent for the removal of Pb(II) ions from aqueous solutions.

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