The role of *Arthrobacter viscosus* in the removal of Pb(II) from aqueous solutions

Raluca Maria Hlihor, Mihaela Roşca, Teresa Tavares and Maria Gavrilescu

ABSTRACT

The aim of this paper was to establish the optimum parameters for the biosorption of Pb(II) by dead and living *Arthrobacter viscosus* biomass from aqueous solution. It was found that at an initial pH of 4 and 26 °C, the dead biomass was able to remove 97% of 100 mg/L Pb(II), while the living biomass removed 96% of 100 mg/L Pb(II) at an initial pH of 6 and 28 ± 2 °C. The results were modeled using various kinetic and isotherm models so as to find out the mechanism of Pb(II) removal by *A. viscosus*. The modeling results indicated that Pb(II) biosorption by *A. viscosus* was based on a chemical reaction and that sorption occurred at the functional groups on the surface of the biomass. FTIR and SEM-EDX analyses confirmed these findings. The suitability of living biomass as biosorbent in the form of a biofilm immobilized on star-shaped polyethylene supports was also demonstrated. The results suggest that the use of dead and living *A. viscosus* for the removal of Pb(II) from aqueous solutions is an effective alternative, considering that up to now it has only been used in the form of biofilms supported on different zeolites.

Key words | bacterium strain, biosorption, dead and living *A. viscosus*, lead, process efficiency

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INTRODUCTION

Although lead pollution from mining activities is considered a relatively localized problem, its magnitude is significant, particularly in terms of water pollution because it is associated to other heavy metals (Gavrilescu 2009a; Naja & Volesky 2009). Due to lead negative effects on the human health and the environment, scientists are continuously trying to solve the related pollution problems by finding economic and eco-friendly removal alternatives compared to conventional processes. The studies developed up to now on the removal of heavy metals by conventional methods highlighted their ineffectiveness for concentrations less than 100 mg/L (Gavrilescu 2004, 2009b; Hlihor & Gavrilescu 2009; Fu & Wang 2011). Therefore, biological methods such as biosorption and bioaccumulation, became an attractive alternative due to low operating costs, minimization of the sludge volume and high removal efficiency especially for lower concentrations (Gavrilescu & Chisti 2005; Gavrilescu *et al.* 2015; Roşca *et al.* 2015; Ungureanu *et al.* 2015).

Studies on the biosorption of heavy metals from liquid effluents are considering a wide range of microorganisms and not only (e.g. algae, agricultural wastes) in an attempt to find the most efficient biosorbents that could be easily used in various environmental conditions (Flouty 2015; Hlihor *et al.* 2015; Ahmady-Asbchin 2016; Khalil *et al.* 2016). From a large spectrum of microorganisms, the bacterial biomass proved a good capacity for lead removal. For example, Masoumi *et al.* (2016) studied the *Curtobacterium* sp. FM01 for lead removal. The authors found that the maximum biosorption capacity reached 186.60 mg/g. Other studies focused on the *Bacillus cereus*

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(22.1 mg/g) or the *Bacillus pumilus* (28.06 mg/g) capacity to remove lead (Çolak *et al.* 2011).

Several studies have shown the capacity of *Arthrobacter* species to remove heavy metals from liquid effluents. In their article, Banerjee *et al.* (2016) demonstrate that *Arthrobacter phenanthrenivorans* living biomass offers high efficiency for lead removal (79.91%) and good efficiency for nickel (47.62%) and cadmium (34.05%). *Arthrobacter* sp. 25 used by Jin *et al.* (2016) evidenced a maximum sorption capacity of 9.6 mg/g for an initial lead ion concentration of 108.79 mg/L, at pH value of 5.75 and 9.9 g/L biosorbent dosage. Malkoc *et al.* (2016) investigated the biosorption of Zn(II) on dead and living biomass of *Arthrobacter viscosus*. A maximum removal efficiency of living and dead *A. viscosus* cells was found as 89.4% and 90.8%, respectively. For the removal of other heavy metals from aqueous solution, literature reveals the ability of species such as *Arthrobacter globiformis* and *Arthrobacter oxidas* for Cr(III) and Cr(IV) biosorption (Gelagutashvili *et al.* 2011), *Arthrobacter viscosus* for Cr(VI) reduction (Silva *et al.* 2009; Hlihor *et al.* 2016) and *Arthrobacter protophormia*e for Cd(II) removal (Wang 2013).

The objective of this study is to evaluate the bioremoval of Pb(II) by dead and living *Arthrobacter viscosus* biomass from aqueous solutions in batch and dynamic modes. Sets of experiments were developed to quantify the biosorption efficiency and the uptake capacity of dead and living bacterial biomass. Although there are several studies performed with bacterial biofilms of *A. viscosus* employed for different heavy metals removal (Quintelas & Tavares 2002; Quintelas *et al.* 2008, 2009; Rosales *et al.* 2012), from our knowledge this is the first study performed in a batch system using only the *Arthrobacter viscosus* biomass for Pb(II) removal. Moreover, in order to assess the removal of Pb(II) in continuous mode, the *A. viscosus* bacterium was immobilized in the form of a biofilm on star-shape polyethylene supports. Kinetics and biosorption isotherms modeling together with Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX) were further used to identify the mechanisms of Pb(II) removal from aqueous solutions by *A. viscosus* biomass. Therefore, the analysis of this kind of systems performed within our work may contribute to the improvement of knowledge in the biotechnology area as well as to support process scale-up.

MATERIALS AND METHODS

Biomass growth and preparation

The *Arthrobacter viscosus* bacterium strain (CECT 908) was obtained and grown in a medium as indicated by Hlihor *et al.* (2016). The adjustment of the medium pH to 7 was made using 1 M NaOH solution; pH 7 is the optimum one for biomass growth as suggested by Quintelas *et al.* (2009). The medium was further sterilized at 120 °C for 20 min, inoculated with *A. viscosus* and kept at 28 °C and 150 rpm for 72 h. After growth, the suspension with bacterial biomass was centrifuged at 7,000 rpm for 10 min and further dried in an oven at 55 °C for 72 h, so as to achieve the inactivation of bacterial biomass. After drying, the *A. viscosus* biomass was crushed and sieved to 125–250 μm particle size and stored in a desiccator. Regarding the use of living biomass in our experiments, after the biomass growth, the bacterial suspension was centrifuged at 7,000 rpm for 10 min, resuspended in distilled water and kept at 4 °C until use.

Biosorption of Pb(II) – batch conditions

First, we have examined the removal of Pb(II) by dead *A. viscosus* biomass in a batch system by investigating the effect of different parameters on biosorption efficiency and uptake capacity: pH (3 to 7), biomass dosage (1 g/L to 8 g/L), contact time (up to 24 h) and initial concentration (25 mg/L to 500 mg/L), respectively. The influence of initial metal ions concentration on dead biomass was performed at different temperatures considering the interval 26 °C–50 °C. The solutions initial pH was adjusted by addition of 1 M H_2SO_4 and 1 M NaOH. The change in the working volume was negligible. Similar conditions were maintained for the living biomass assays, except for pH adjustments, as the pH was varied from 4 to 7. All experiments with living biomass were developed at 28 ± 2 °C, the optimum temperature for biomass growth.

All assays were performed in 250 mL Erlenmeyer flasks with 100 mL of working volume containing Pb(II) solution of known concentrations, together with a specific amount of dead or living biomass. Control experiments were considered for all set-ups. The flasks were agitated in an orbital incubator at 150 rpm. After sampling, the solutions were centrifuged at 13,000 rpm for 5 min and metal concentration in the supernatant was measured. All experiments

were performed in duplicate to assure an experimental error less than 5%. Pb(II) uptake at equilibrium and in time, and biosorption efficiency of *A. viscosus* biomass were determined as indicated by Hlihor *et al.* (2015).

Biosorption of Pb(II) – dynamic conditions

As small particles, with low density, poor mechanical strength and little rigidity, bacterial biosorbents can be the subject of several drawbacks during large scale operation (Veglio & Beolchini 1997; Vijayaraghavan & Yun 2008; Yun *et al.* 2011). Their immobilization in the form of biofilms is considered an excellent strategy in large scale-applications (Quintelas *et al.* 2008, 2009). The test developed in dynamic conditions was initiated to demonstrate the ability of *A. viscosus* in the form of a biofilm on inert solid supports for the removal of Pb(II). The influence of an initial concentration of Pb(II) on the biosorption behavior of an *A. viscosus* biofilm immobilized on star-shape polyethylene supports developed by University of Minho (17 mm external diameter, 10 mm height) (Nogueira *et al.* 2009) was assessed. The supports (38.54 g) were placed in an acrylic column (25 cm × 3.2 cm). The growth of the biofilm in column was performed as indicated by Hlihor *et al.* (2016). After the biofilm formation, a solution of 25 mg/L Pb(II) adjusted at pH 6 and in room temperature conditions (28 ± 2 °C) was passed through the column at a flow rate of 10 mL/min; this flow rate was used to prevent the break of the formed *A. viscosus* biofilm. At different time intervals, 5 mL samples were withdrawn from the effluent, centrifuged and analyzed for Pb(II). The maximum metal removal capacity for the given set of process conditions was calculated as suggested by Vijayaraghavan *et al.* (2005).

Reagents and equipment

All reagents used in our experiments were of analytical grade and were used without further purification. Lead stock solution of 1,000 mg/L was prepared by dissolving $Pb(NO_3)_2$ (Riedel) in distilled water. The concentrations of Pb(II) in liquid samples were determined by an Atomic Absorption Spectrophotometer Varian Spectra AA-400. All glassware used for experimental purposes was washed in 20% nitric acid and rinsed with distilled water to remove any possible interference by other metals.

Kinetics and isotherm modeling

In order to investigate the mechanism of biosorption of Pb(II) by *A. viscosus*, various kinetic and equilibrium models have been applied. Regarding the kinetic modeling, we used pseudo-first-order model, pseudo-second-order type 1, type 2, type 3, type 4 models and Elovich Equation. Langmuir, Freundlich, Temkin, Dubinin-Radushkevich and Dual mode models were used in modeling isotherms. These models are fully described in several papers (Zhao *et al.* 2001, 2002; Ho 2006; Hlihor *et al.* 2014, 2015; Ghosh *et al.* 2015; Liu *et al.* 2016).

Thermodynamic parameters

Thermodynamic parameters (free energy, ΔG^0, enthalpy, ΔH^0 and entropy, ΔS^0) were calculated as indicated by Hlihor *et al.* (2014, 2015).

Biomass characterization

Biomass characterization was performed using Fourier transform infrared spectroscopy (FTIR, BOMEN MB 104) and scanning electron microscopy coupled with energy dispersive X-ray microanalysis (SEM-EDX, JEOL JSM-7001F, Oxford INCA 250). Infrared spectra of loaded and unloaded Pb(II) biomass were obtained as indicated by Hlihor *et al.* (2016).

RESULTS AND DISCUSSION

Biosorption of Pb(II) by dead biomass of *Arthrobacter viscosus*

The use of dead biomass in biosorption systems appears to have several advantages in comparison with the use of living biomass. Hence, the objective of this study was to investigate the removal ability of dead bacterial cells of *A. viscosus* under different conditions in order to assess the effect of some factors on Pb(II) removal.

Effect of initial pH on Pb(II) removal

Lead is known to form stable aqueous complexes with OH^-, Cl^-, CO_3^{2-}, SO_4^{2-} and HS^-. In water, lead is mainly present as Pb^{2+} below pH of about 7. With pH increasing, the species $PbOH^+$, $Pb(OH)_2^0$, and $Pb(OH)_3^-$ become dominant over Pb^{2+} (EPA 2007). Considering these aspects, the pH influence on Pb(II) biosorption efficiency (%) and uptake capacity (mg/g) of dead biomass of *A. viscosus* was investigated in the 3 to 7 pH domain. The working pH range above 7 was avoided due to possibility of metal precipitation on biomass surface. The conditions were 100 mg/L initial metal concentration, 2 g/L biomass concentration, 26 °C,

contact time of 24 h and 150 rpm agitation speed. As seen in Figure 1, the maximum biosorption efficiency of Pb(II) was found to be 97% at pH 4, while the sorption capacity of dead *A. viscosus* reached 47.6 mg/g. At pH 3, the biosorption efficiency is at ≈ 30%. At lower pH values, due to protonation of binding sites resulting from a high concentration of hydrogen ions that compete with Pb(II) ions at the sorption sites, the negative charge intensity of the sites is reduced, resulting in the reduction of metal ions binding (Kahraman *et al.* 2005). At higher pH values (from 5 to 7) the biosorption efficiency decreases to less than 60%. The corresponding uptake capacities at these pH values were between 38.1 mg/g and 17.5 mg/g. As reported by Gong *et al.* (2005) with an rise in pH, there is an increase in ligands with negative charges which results in improved binding of cations. Similar results were obtained for Pb(II) biosorption by other microorganisms as indicated by Aneja *et al.* (2010) and Wierzba (2015). Therefore, all the biosorption experiments were carried out at pH 4, as optimum pH value for Pb(II) removal by dead biomass of *A. viscosus*.

Effect of biomass dosage on Pb(II) removal

The influence of dead *A. viscosus* biomass dosage on Pb(II) biosorption is shown in Figure 2. The working conditions were pH 4, 100 mg/L Pb(II) concentration, 26 °C and 24 h of contact time. Results showed that the biosorption efficiency of the metal ions is enhanced with increasing biomass dosage from 1 g/L to 8 g/L, and it is almost constant at biomass dosages higher than 2 g/L. When the biosorbent dosage increased from 1 to 2 g/L, the biosorption

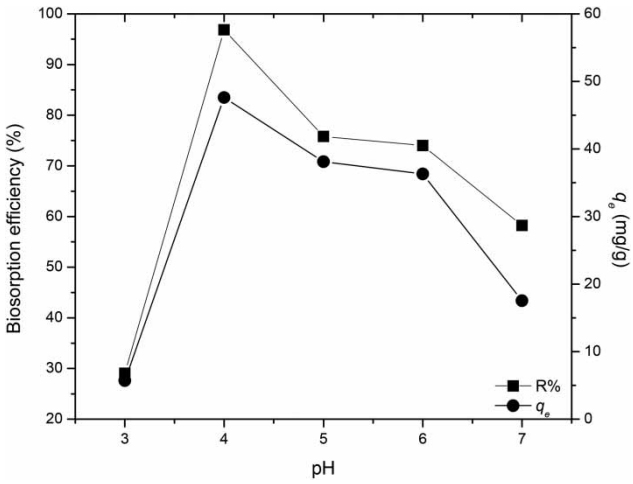

Figure 1 | Effect of solution pH on Pb(II) biosorption by dead *A. viscosus* (biomass dosage: 2 g/L; Pb(II) concentration: 100 mg/L; temperature: 26 °C; contact time: 24 h).

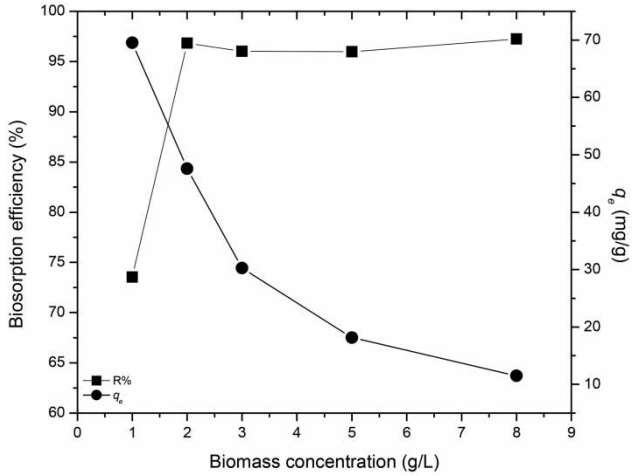

Figure 2 | Effect of biomass dosage on Pb(II) biosorption by dead *A. viscosus* (pH 4; Pb(II) concentration: 100 mg/L; temperature: 26 °C; contact time: 24 h).

of Pb(II) ions efficiency raised from 74% to 97%. However, the amount of lead(II) sorbed decreased from 69.5 mg/g to 11.5 mg/g when the biosorbent dosage increased from 1 g/L to 8 g/L. Gong *et al.* (2005) suggested various reasons to explain the decreased uptake capacity at increasing biomass including: availability of solute, electrostatic interactions, interference between binding sites and reduced mixing at higher biomass densities. A similar behavior was reported by *Streptomyces* VITSVK5 spp. biomass (Saurav & Kannabiran 2011). Therefore, the optimum biomass dosage was selected as 2 g/L in further experiments.

Effect of contact time on Pb(II) removal

Figure 3 shows the effect of contact time on the biosorption of Pb(II) ions by dead biomass of *A. viscosus*. The pH value of 4, biomass dosage of 2 g/L and temperature of 26 °C were maintained constant during the experiments, while the contact time varied for different concentrations of heavy metal (25 mg/L, 50 mg/L and 100 mg/L). The initial stage of biosorption was very rapid, Pb(II) uptake increased with rise in contact time up to 10–20 min. The equilibrium was reached after this first stage and was kept almost constant until 120 min Pb(II) samples were also taken in the time interval 120 min up to 1,440 min, but as the data followed the same behavior, they were not shown on the graph. Maximum uptake capacity was attained as 9.62 mg/g for 25 mg/L Pb(II), 21.9 mg/g for 50 mg/L Pb(II), and 47.6 mg/g for 100 mg/L Pb(II), with a biosorption efficiency of 100% for 25 mg/L and 50 mg/L Pb(II) and 97% for 100 mg/L Pb(II). The uptake of metal ions by biosorbents was often observed to occur in two subsequent stages: (i) rapid and quantitatively

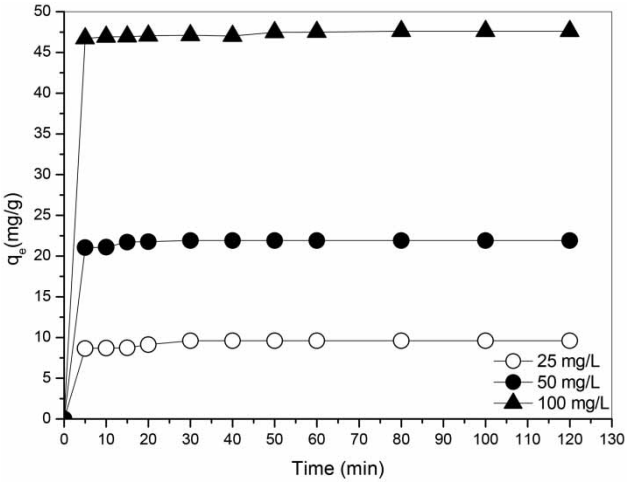

Figure 3 | Effect of contact time on Pb(II) biosorption by dead *A. viscosus* for various ion concentrations (pH 4; biomass dosage: 2 g/L; temperature: 26 °C; contact time: 24 h).

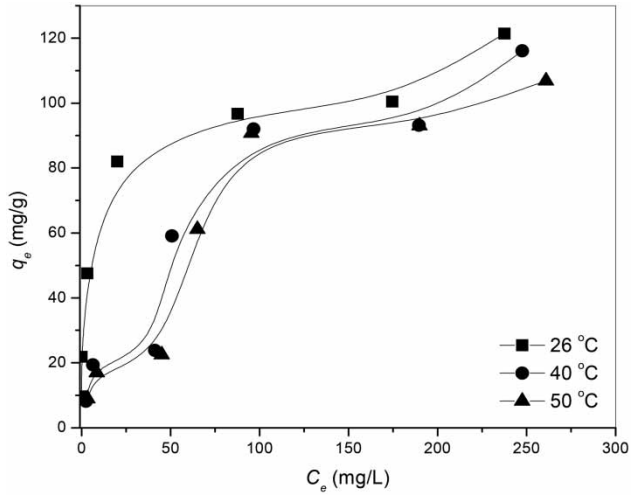

Figure 4 | Equilibrium isotherms for Pb(II) biosorption by dead *A. viscosus* at different temperatures (pH 4; biomass dosage: 2 g/L; contact time: 24 h).

predominant and (ii) slower and quantitatively insignificant. The rapid stage is probably due to abundant availability of active sites on the biomass. With gradual occupancy of these sites, sorption becomes less efficient in slower stages (Aslam *et al.* 2010).

Effect of initial concentration on Pb(II) removal

The metal uptake achieved using dead *A. viscosus* biomass corresponding to different metal concentrations from 25 to 500 mg/L at different temperatures (26, 40 and 50 °C) are shown in Figure 4. The optimum parameters used for a maximum Pb(II) removal efficiency were pH 4, 2 g/L biomass dosage and 24 h of contact time. When the concentration of Pb(II) was increased from 25 to 500 mg/L, the uptake capacity increased from 9.62 to 121.4 mg Pb(II)/ g biomass. With an increase in temperature from 26 to 50 °C, the maximum uptake capacity decreases from 121.4 to 106.8 mg/g. This indicates that, although biosorption increased rapidly with an increase in the initial concentration, the process subsequently proceeds slower until equilibrium is reached. A higher initial concentration provides an important driving force to overcome the mass transfer resistances (Calero *et al.* 2009) between the Pb(II) solution and bacterial cell wall and therefore the biosorption capacity increases.

Biosorption kinetics and isotherms

Table 1 shows the biosorption rate constant, k, the initial biosorption rate, h, and the equilibrium biosorption capacity, q_e, as functions of solution concentration for biosorption of lead(II). The pseudo-second-order kinetic model Type 1 (Ho model), with high values of correlation coefficient ($R^2 > 0.99$), showed a good agreement with the experimental values at all studied concentrations. For Pb(II) concentration variation, the initial biosorption rate, h, and the pseudo-second order rate constant, k_2 were found to increase.

Table 1 | Kinetic parameters obtained for Pb(II) biosorption by dead *A. viscosus*

		Concentration		
Kinetics	**Parameters**	**25 mg/L**	**50 mg/L**	**100 mg/L**
Pseudo-first-order	q_e (mg/g)	1.30	2.10	1.45
	k_1 10^{-5} (min^{-1})	0.0410	0.1425	0.0043
	R^2	0.6018	0.8160	0.6945
Pseudo-second-order Type 1	q_e (mg/g)	9.65	21.92	47.10
	k_2 (g/mg min)	0.1578	0.3457	0.0716
	h (mg/g min)	14.72	166.11	163.13
	R^2	0.9999	1	1
Pseudo-second-order Type 2	q_e (mg/g)	9.62	21.58	47.41
	k_2 (g/mg min)	0.1456	0.1837	0.2304
	h (mg/g min)	13.495	88.809	518.13
	R^2	0.6861	0.8163	0.5879
Pseudo-second-order Type 3	q_e (mg/g)	9.63	21.96	47.58
	k_2 (g/mg min)	0.1902	0.1902	0.1705
	h (mg/g min)	13.383	91.749	386.14
	R^2	0.7080	0.8201	0.4712
Pseudo-second-order Type 4	q_e (mg/g)	9.73	22.01	47.82
	k_2 (g/mg min)	0.1047	0.1587	0.0881
	h (mg/g min)	9.936	76.907	201.518
	R^2	0.7080	0.8201	0.4712
Elovich Equation	α·(mg/g min)	2.88×10^6	4.50×10^6	13.6×10^6
	β (mg/g min)	2.0259	2.5955	3.5713
	R^2	0.8380	0.7826	0.7450

The values of the initial biosorption rate, h, were much higher than the pseudo-second order rate constants, k_2, as predicted by the pseudo-second order kinetics. The applicability of pseudo-second order kinetic model Type 1 suggested that Pb (II) biosorption was based on a chemical reaction (Ho 2006).

Pb(II) equilibrium data were analyzed by Langmuir, Freundlich, Temkin, Dubinin-Radushkevich (D-R) and Dual mode isotherm models. The models parameters are included in Table 2. The value of the correlation coefficient at 26 °C, higher than 0.98 strongly supports the fact that Pb(II) - biomass interaction closely follows the Langmuir model which indicates that sorption occurred at the functional groups/binding sites on the surface of the biomass, which is regarded as monolayer biosorption (Uluozlu *et al.* 2008). At higher temperatures D-R model has slightly higher values for the correlation coefficients than the Langmuir model suggesting a better fit to the experimental data. On the other side, when carefully analyzing the values of the uptake capacity, it can be suggested that the values from Langmuir model are closer to the experimental data than the values resulted from D-R model. This indicates that the modeling of Langmuir isotherm is more suitable than D-R isotherm.

Thermodynamic parameters

In order to describe the thermodynamic behavior of Pb(II) biosorption by dead *A. viscosus* biomass, the thermodynamic parameters including the change in free energy (ΔG^0), enthalpy (ΔH^0) and entropy (ΔS^0) were calculated from the slope and intercept of the plot of $\ln K_d$ vs 1/T yields (Figure not shown). Gibbs free energy change was calculated as: −25.96, −22.12 and −22.29 kJ/mol for biosorption of Pb (II) at 26, 40 and 50 °C, respectively. The negative ΔG^0 values indicated the thermodynamically feasible and spontaneous nature of biosorption. The ΔH^0 parameter was found to be −75.58 kJ/mol for Pb(II) biosorption which indicates the exothermic nature of the biosorption process. The ΔS^0 parameter was found to be −163.96 J/molK; this value suggests a decrease in the randomness at the solid/solution interface during biosorption process (Hlihor *et al.* 2015).

Biosorption of Pb(II) by living biomass of *Arthrobacter viscosus*

The metal *biosorption* process by *living cells* is a *two-step* process. In the first step, metal ions are adsorbed onto the surface of *cells* by interactions between sorbates and functional groups displayed on the surface of the cells. All the metal ions are crossing the cell wall before gaining access to the cell membrane and cell cytoplasm. The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions (Majumdar *et al.* 2008).

Based on the above mentioned considerations, the objective of this experimental program is to assess the

Table 2 | Isotherm parameters for Pb(II) biosorption by dead *A. viscosus* at different temperatures

Langmuir isotherm	***Temperature (° C)***	q_m ***(mg/g)***	K_L ***(L/mg)***		R^2
	26	115.07	0.1651		0.9808
	40	126.58	0.0238		0.9658
	50	124.68	0.0194		0.9808
Freundlich isotherm	***Temperature (° C)***	***1/n***	K_F ***(mg/g(L/mg)$^{1/n}$)***		R^2
	26	0.8377	40.62		0.9230
	40	0.6131	21.17		0.8240
	50	0.7007	24.40		0.8324
Temkin isotherm	***Temperature (° C)***	K_T ***(L/mol)***	B^*10^7		R^2
	26	1.0010	3.4610		0.9249
	40	1.0012	2.3907		0.9494
	50	1.0012	2.3992		0.9521
Dubinin-Radushkevich isotherm	***Temperature (° C)***	q_{max} ***(mg/g)***	β^*10^{-9}		R^2
	26	190.03	1.7655		0.9433
	40	485.74	4.8524		0.9846
	50	516.50	4.8883		0.9845
Dual mode model	***Temperature (° C)***	***Qs (mg/g)***	***Kd (L/g)***	***b (L/g)***	R^2
	26	87.1453	0.1268	0.3895	0.9087
	40	160.7203	1.1145^*10^{-16}	0.0095	0.8665
	50	154.0075	4.8224^*10^{-16}	0.0092	0.8573

bioremoval of Pb(II) by living cells of *A. viscosus*, and to perform the experimental analysis of the optimum parameters: pH, biomass dosage and initial metal concentration in batch mode.

Effect of initial pH on Pb(II) removal

The effect of solution pH, as one of the most important parameters that has to be taken into consideration in a biosorption process was analyzed in the pH interval 4 to 7. The initial Pb(II) concentration (100 mg/L), biomass dosage (2 g/L), contact time (24 h), agitation speed (150 rpm) and temperature (28 ± 2 °C) were maintained constant during the experiments.

Figure 5 shows the biosorption efficiency and the sorption capacity of living *A. viscosus* for Pb(II) at various pH. The maximum biosorption efficiency was found to be 96% at pH 6. A small decrease in biosorption percentage could be observed at pH 4 and pH 5, the biosorption efficiency reaching 90%. At pH 7, the biosorption yield also decreased to 85%. The uptake capacity of living cells for Pb(II) was 44.72 mg/g at pH 6, and followed the same trend as the biosorption efficiency.

At low pH, cell wall ligands were closely associated with the hydronium ions H_3O^+ and restricted the approach of metal cations as a result of the repulsive force. As the pH increased, the functional groups involved in metal biosorption would be exposed and carry negative charges with subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface (Gupta & Rastogi 2008). Faroukhsamani *et al.* (2001) reported that the living bacterium *Chryseomonas Luteola* MGF-47 was able to remove Pb(II) at pH 7. On the other hand, Zn(II) and Cu (II) removal by living cells of *Pseudomonas putida* CZ1 was performed at optimum pH of 5. In these tests, the binding capacity of living cells of *Pseudomonas putida* CZ1 is significantly higher than that of nonliving cells at tested conditions. It demonstrated that about 40–50% of the metals were actively taken up by *P. putida* CZ1, with the remainder being passively bound to the bacterium (Chen *et al.* 2005).

All the biosorption experiments with living *A. viscosus* biomass were carried out considering pH 6 the optimum pH value for Pb(II) removal.

Effect of biomass dosage on Pb(II) removal

To investigate the effect of living *A. viscosus* biomass dosage on Pb(II) biosorption efficiency and uptake capacity, 1 to 8 g/L of living biomass were subjected to these tests. As shown in Figure 6, when the biosorbent dosage was increased from 1 to 8 g/L, the removal efficiency raised from 69 to 96% for Pb(II) ions. The maximum biosorption efficiency was attained at a biomass dosage of 2 g/L and it is almost the same at higher biomass dosages. A similar behavior was noticed above in the case of dead biomass of *A. viscosus*. The maximum uptake capacity achieved at pH 6 for living *A. viscosus* biomass was 44.72 mg/g. A small difference was noticed for the maximum uptake capacity of dead cells. The dead *A. viscosus* biomass removed 47.6 mgPb (II)/$g_{biomass}$, with a biosorption efficiency of 97% at pH 4. Consequently, the optimum biomass dosage in further tests was selected as 2 g/L of living *A. viscosus* for Pb(II) removal from aqueous solution.

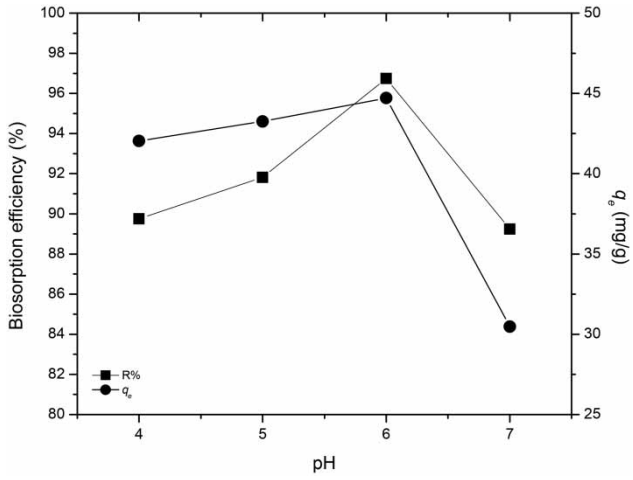

Figure 5 | Effect of solution pH on Pb(II) biosorption by living *A. viscosus* (biomass dosage: 2 g/L; Pb(II) concentration: 100 mg/L; temperature: 28 ± 2 °C; contact time: 24 h).

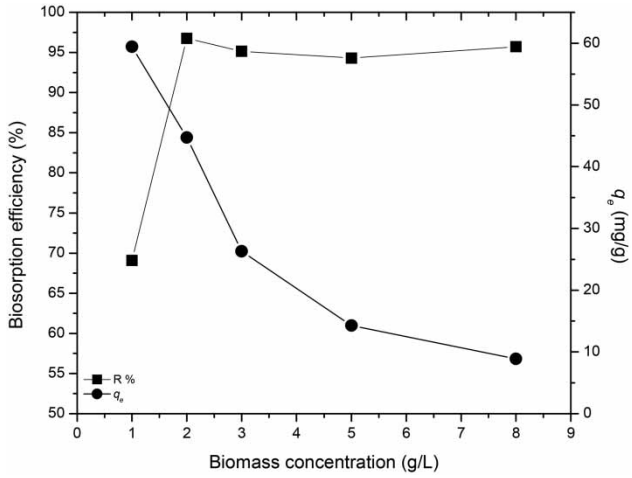

Figure 6 | Effect of biomass dosage on Pb(II) biosorption by living *A. viscosus* (pH 6; Pb(II) concentration: 100 mg/L; temperature: 28 ± 2 °C; contact time: 24 h).

Effect of initial Pb(II) concentration and biosorption isotherms

Preliminary tests performed with living biomass for Pb(II) biosorption established the equilibrium at 24 h (data not shown). Hence, the effect of Pb(II) initial concentrations was investigated a contact time of 24 h, biomass concentration of 2 g/L, pH 6, agitation speed of 150 rpm and at the concentration range of 25–500 mg/L Pb(II) (Figure 7). As observed when the concentration was raised from 25 to 500 mg/L, the biosorption capacity increased from 5.88 to 108.75 mg/g. The metal uptake mechanism is particularly dependent on the initial metal concentration: at low concentrations, metals are sorbed by specific active sites, while at higher concentrations, the lower biosorption efficiency is due to the saturation of adsorption sites (El-Sayed *et al.* 2010). In the living cells, the biosorption mechanisms include both metabolism dependent and independent processes. Metabolism independent uptake process essentially involves cell surface binding through ionic and chemical interaction, while dependent process deals with the binding of both the surfaces followed by intracellular accumulation (Das & Guha 2007).

As in the case of dead cells, five isotherm models were applied for Pb(II) biosorption by living *A. viscosus* biomass, Langmuir, Freundlich, Temkin, Dubinin-Radushkevich and Dual mode models. The isotherms constants are listed in Table 3.

The adsorption pattern of Pb(II) on living *A. viscosus* biomass was well fitted by Langmuir isotherm with a correlation coefficient, R^2 of 0.9957. The low values of correlation coefficients, R^2 ranging from 0.65 to 0.80, indicated that Freundlich, D-R, Temkin and Dual mode models did not fit well the biosorption data. The maximum biosorption capacity of living *A. viscosus* from Langmuir isotherm model was found to be 114.94 mg/g for Pb(II) ion. These values are very close to those obtained from the experimental data, 108.75 mg/g for Pb(II) biosorption by living biomass.

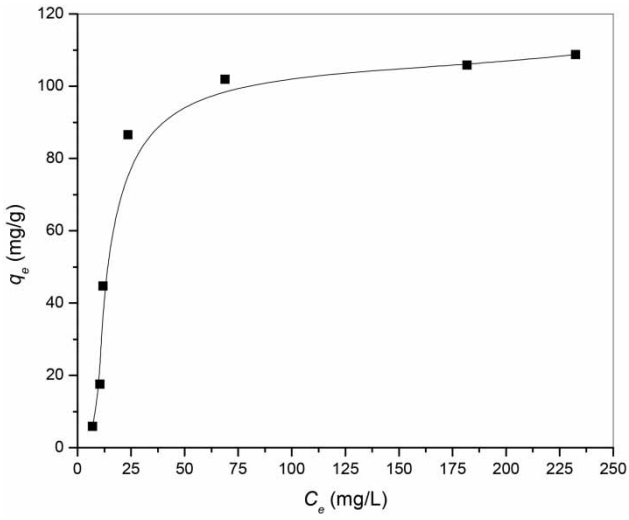

Figure 7 | Equilibrium isotherm for Pb(II) biosorption by living *A. viscosus* (pH 6; biomass dosage: 2 g/L; temperature: 28 ± 2 °C; contact time: 24 h).

Table 3 | Isotherm parameters for Pb(II) biosorption by living *A. viscosus* biomass

Equilibrium isotherm	Parameters	Pb(II)
Langmuir isotherm	q (mg/g)	114.94
	k_L (L/mg)	0.0748
	R^2	0.9957
Freundlich isotherm	$1/n$	0.8807
	k_F (mg/g(L/mg)$^{1/n}$)	1.3116
	R^2	0.6072
Temkin isotherm	k_T (L/mol)	1.0015
	B 10^5	180.447
	R^2	0.8072
D-R isotherm	q_{max} (mol/g)	0.0046
	$\beta \cdot 10^{-9}$(mol^2 /J^2)	6.3955
	E (kJ/mol)	8.841
	R^2	0.6533
Dual mode model	Qs (mg/g)	126.89
	Kd (L/g)	2.0637*10^{-17}
	b (L/g)	0.0388
	R^2	0.7912

Column assays

The experiments performed in the column with the bacterial biomass attached as biofilm on solid supports were conducted with 25 mg/L Pb(II) and pH 6 at a constant flow rate of 10 mL/min (Figure 8). The breakthrough times and the exhaust times for Pb(II) correspond to $C/C_0 = 0.56$ and 0.70, respectively. The amount of *A. viscosus* biofilm produced could be quantified as 5.96 g/L. As the operation in the column proceeded, the amount of metal ions bound to the biomass increased to 6.21 mg Pb(II)/$g_{biomass}$ at the end of contact time. Considering the statement of Vieira *et al.* (2008), as the ratio C/C_0 did not reach the value of 1.0, we can agree that the biomass was not completely saturated. As a consequence, Pb(II) removal from aqueous solution requires more than 8 h for a complete saturation of the column. Hence, further investigations should be made with respect to Pb(II) removal by *A. viscosus* biofilm in continuous mode. The removal of Pb(II) shows an initial rapid accumulation

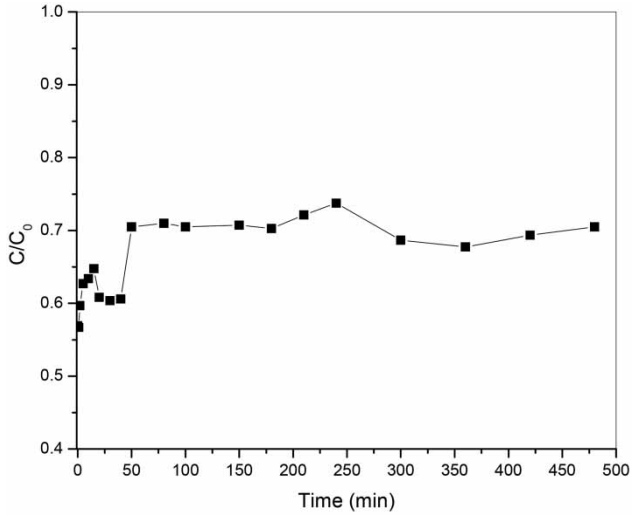

Figure 8 | Breakthrough curves for Pb(II) biosorption by *A. viscosus* biofilm.

step that is designated as independent of metabolism and temperature, involving cation binding to the surface, biosorption itself. This step is followed by a second process that appears to be metabolism – dependent (Quintelas & Tavares 2002).

Mechanism of Pb(II) removal by dead and living biomass of *Arthrobacter viscosus*

The FTIR spectra of unloaded and metal loaded *A. viscosus* biomass were performed in the range of 500–4,000 cm^{-1} in order to investigate the functional groups that may be involved in the biosorption and the possible mechanism underneath the process. The infrared spectrum of the *A. viscosus* bacterium (Figure 9) is typical for bacterial extracellular polymeric substances as suggested by Figueiredo *et al.* (2006). As seen in the Figure, the unloaded and metal loaded biomass displays a number of absorption peaks, reflecting the complex nature of the biomass.

The FTIR spectrum of *A. viscosus* shows one broad band in 3,417.61 cm^{-1} due to the vibrations of hydroxyl (OH stretching) and amino (NH stretching) groups; the small bands in the range between 2,975 and 2,840 cm^{-1} are attributed to C–H stretching of the groups CH_2 and CH_3; the band in 1,539.08 cm^{-1} is indicative of C–N stretching and N–H deformation; the characteristic region of the bands between 1,660 and 1,400 cm^{-1} are attributed to the vibrations of the functional groups such as carboxyl, phosphoric, amine and

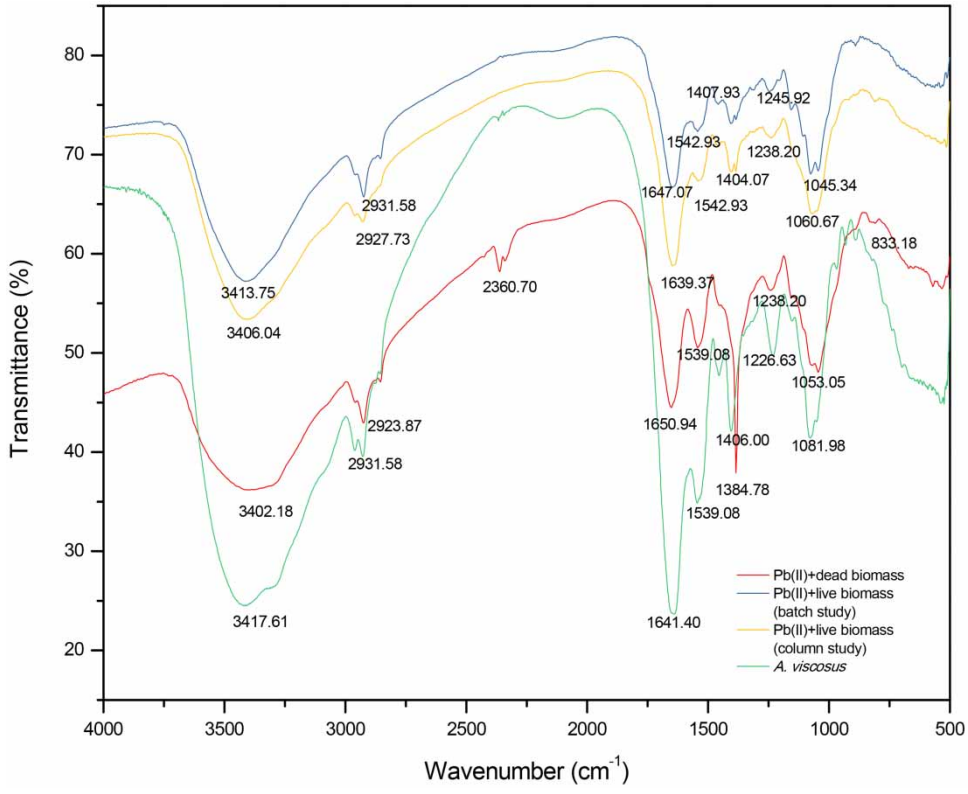

Figure 9 | FTIR spectra of living and dead biomass of *A. viscosus* before and after Pb(II) loading.

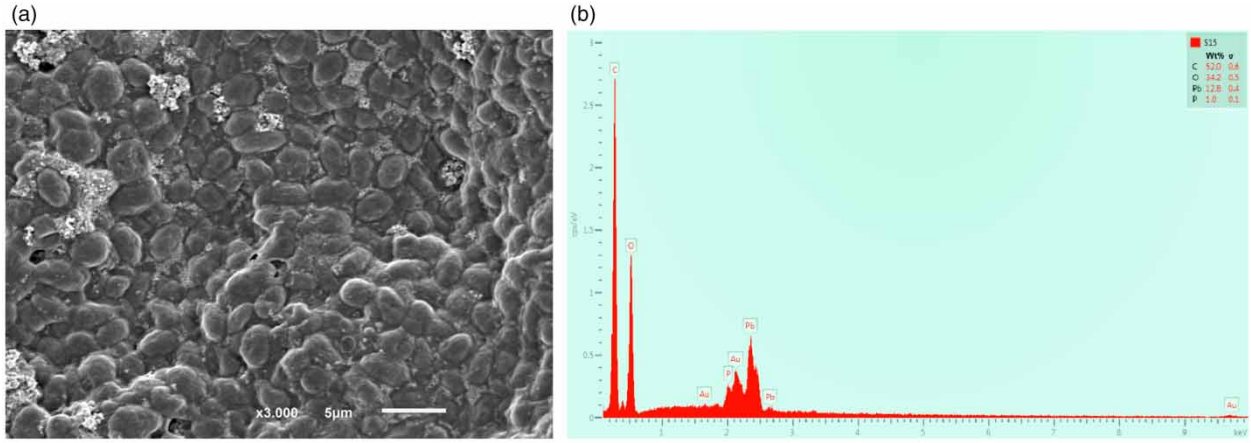

Figure 10 | (a) SEM image and (b) EDX analysis of *A. viscosus* biomass after Pb(II) loading.

the bands in the frequency range 1,250–900 cm^{-1} result from vibrations of the polysaccharides from the bacterium. The spectrum pattern of the unloaded *A. viscosus* biomass showed some changes of certain bands when compared to Pb(II)-loaded biomass. The changes in the spectrum indicated the possible involvement of those functional groups on the surface of the biomass in biosorption process. The shifting of peak 1,384.7 to 1,411.7, 1,407.93 and 1,404.07 cm^{-1} is due to COO^{-} of the carboxylate group present on the biomass (Majamdar *et al.* 2008). The spectral data of unloaded and metal loaded *A. viscosus* biomass confirms the presence of hydroxyl, amino, carboxyl and phosphate groups and suggest the interactions of these groups with metal ions. FTIR spectral analysis and experimental data confirmed the biosorption of Pb(II) by dead *A. viscosus* as chemical ion-exchange (indicated also by D-R model) and follows a monolayer coverage (Langmuir model).

The textural characteristics of *A viscosus* biomass after Pb(II) biosorption process observed by SEM analysis (3,000 × magnification) are shown in Figure 10(a). The precipitated Pb(II) ions appear on the surface of the biomass, indicating changes in the bacterial surface probably also due to linkage with the functional groups and the ion exchange process between biosorbent and Pb(II) ions (Saravanan *et al.* 2011). EDX analysis (Figure 10(b)) confirmed the sorbed Pb(II) on the biomass; the principal elements found in *A. viscosus* composition after biosorption are: C (52%), O (34.2%), P (1%) and Pb (12.8%).

CONCLUSIONS

The biosorption of Pb(II) by dead and living cells of *A. viscosus* was investigated to find the optimum conditions for a maximum removal efficiency from aqueous solution. At these conditions the maximum sorption capacity from Langmuir isotherm model was achieved as 115.07 mg/g in the case of dead biomass and as 114.94 mg/g in the case of living biomass. Dead and living cells were able to remove more than 95% of 100 mg/L Pb(II).

As suggested by the Langmuir model, with the highest correlation coefficients from all the tested models, sorption occurred at the functional groups on the surface of the biomass which is regarded as monolayer biosorption. The kinetics of the biosorption of Pb(II) by dead biomass were described by pseudo-second order type 1 model, suggesting that a chemisorption reaction or an activated biosorption between the metal ions and functional groups present on bacterial biomass surface occurs.

The entrapment ability of *A. viscosus* biomass as a biofilm was demonstrated by a capacity of 6.21 mg/g for the removal of 25 mg/L Pb(II). These results indicate that *A. viscosus* is a reliable alternative for the removal of Pb(II) from polluted wastewaters, with potential to be applied at larger scales

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