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Biosorption of aluminum through the use of non-viable biomass of *Pseudomonas putida*

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Highlights.

- Non-living biomass of *Pseudomonas putida* for the removal of Al^{3+}
- Phosphatidylcholine of the cell membranes as binding site of Al^{3+}
- -The efficiency of adsorption of Al^{3+} is depending on the number of binding sites

Abstract

Living and non-living biomass of *Pseudomonas putida* A (ATCC 12633) was used as biosorbent for the removing of Al^{3+} from aqueous solutions. The process was stable with time, efficient at pH 4.3 and between 15 ºC and 42 ºC. Two isotherms models were applied to describe the interaction between the biosorbent and Al^{3+}. Non-living biomass of *P. putida* A (ATCC 12633) was found to be the most efficient at adsorbing Al^{3+} with a maximum sorption capacity of 0.55 mg Al^{3+}/gr adsorbent and with 36 x 10^5 binding sites of Al^{3+}/microorganisms. Infrared spectroscopy analysis shows that the biosorbent present some vibrational band of functional groups that change in presence of Al^{3+}: hydroxyl, carboxyl and phosphate. Considering that Al^{3+} binds to the phosphate group of phosphatidylcholine, non-viable biomass of *P. putida* PB01 (mutant lacking phosphatidylcholine) was used. Aluminum adsorption of the parental strain was 30 times higher than values registered in *P. putida* PB01 (36 x 10^5 sites/microorganism vs 1.2 x 10^5 sites/microorganism, respectively). This result evidenced that the absence of phosphatidylcholine significantly affected the availability of the binding sites and consequently the efficiency of the biomass to adsorb Al^{3+}.

Keywords

Biosorption; Toxic metals; Aluminum; *Pseudomonas putida*.

1. Introduction

The metals are non-biodegradable chemical species, commonly found in wastewater derived from the mining, tannery industries, pharmaceuticals, pesticides, plastics, photographic and metallurgical products (Khraisheh et al., 2002; Sekhar et al., 2004; Mohammadi et al., 2005). In spite of the fact that aluminum (Al) is not considered a major environmental pollutant within metals, high concentrations of Al have been detected in regions of mining, of smelting and metallurgy industries and in regions where there has been an excessive use of nitrogen fertilizers (Wannaz et al., 2012). Acid rain has increased the bioavailability of the metals, with predominance of harmful soluble species, such as Al^{3+} or Al-OH poorly polymerized species (Garcidueñas Piña and Cervantes, 1996). Al^{3+} has been associated with acidification of soils and water (MacDonald and Martin, 1988) and it is also known for being damaging to the ecosystems (Auger et al., 2013). Several authors have described the harmful effects of aluminum in different organisms. It was found that this metal alters in the iron metabolism in *Escherichia coli* (Davis et al., 1971); it also affects DNA replication and inhibits gene expression in *Rhizobium* nodulating strains (Johnson and Wood, 1990, Brady et al., 1993); aluminum was reported as an inducer of oxidative stress and as a disruptant of antioxidant enzymes and neurotransmitter synthesis in fishes (Fernández-Dávila et al., 2012). In humans, the accumulation of this metal in the brain increases the risk of neurological diseases, such as Alzheimer, Parkinson, encephalopathy, dementia and amyotrophic lateral sclerosis (Wong et al., 1998; Buratti et al., 2006). Given these noxious effects of the Al in most of living systems, new technologies, aimed at removing the metal from different sources, are emerging (Ozdemir

and Baysal, 2004; Tuzen and Soylak, 2008; Sari and Tuzen, 2009; Basurco Cayllahua and Torem, 2010; Tassist et al., 2010; Auger et al., 2013 and citations therein).

Removal of toxic metals by biosorption is a relatively new and an emerging technology for wastewater treatment (Vieira and Volesky, 2000, Fu and Wang, 2011). Biosorption mechanisms include ionic interaction and formation of complexes between the metal cations (adsorbate) and the ligands, contained in the surface of the biosorbent. Due to the nature of the cellular components, several functional groups can acts as ligands including carboxyl, phosphate, amine, imidazel, thioether, sulfhydryl and hydroxyl groups (Wang and Chen, 2009; Vijayaraghavan and Yun, 2008; Tassist et al., 2010; Allievi et al., 2011). Major advantages of adsorption are that it represents an eco-friendly and cheap process; it usually requires non-viable biomass, and the adsorbents can be reused after desorption of the metal, reducing the costs significantly (McEldowney, 2000; Arica *et al.*, 2001; Bréant et al., 2002; Lebeau et al., 2002, Flouty and Estephane, 2012; Musso et al., 2014; Mushtaq et al., 2016).

A large quantity of materials has been investigated as biosorbent for the removal of metals, including viable and non-viable biomass of bacteria, fungi, yeast, algae and industrial and agricultural wastes (Ozdemir and Baysal, 2004; Gabr et al, 2008; Sari and Tuzen, 2009; Basurco Cayllahua and Torem, 2010; Tassist et al., 2010; Allievi et al., 2011; Hassan and El-kassas, 2012; Flouty and Estephane, 2012; Musso et al., 2014; Mushtaq et al., 2016). However, bacteria have a number of advantages over other bioadsorbentes: their ubiquity, their ability to grow under controlled conditions, their resistance to a wide range of environmental changes and their small size (Wang and Chen, 2009; Basurco Cayllahua and Torem, 2010).

We have demonstrated that *Pseudomonas putida* A (ATCC 12633), a ubiquitous Gram-negative bacterium, is able to grow in the presence of Al^{3+} 0.1 mM and that this bacteria binds the metal to the membrane phosphatidylcholine (PC) (Boeris et al., 2009, Boeris and Lucchesi, 2012). Studies carried out with the mutant *P. putida* PB01 lacking PC, showed that PC binds Al^{3+} through the formation of Al^{3+}- PC complexes, since the lack of this phospholipid implied a lower amount of aluminum bound to the membrane (Boeris and Lucchesi, 2012). The ability of *P. putida* to sequester Al^{3+} through the formation of Al^{3+}-PC complexes led us to propose *P. putida* A (ATCC 12633) as a potential biosorbent of Al^{3+}. In the present work we evaluated the ability of viable and non-viable biomass of *P. putida* to act as adsorbent of Al^{3+}. We also assessed the importance of PC in the removal of the metal.

2. Materials and methods

2.1. Microorganisms and culture conditions

Pseudomonas putida A (ATCC 12633) and *Pseudomonas putida* PB01 (*pcs : : aacC,* a mutant lacking PC (Boeris and Lucchesi, 2012) were grown aerobically at 30 ºC, with shaking, in Luria–Bertani (LB) medium until late exponential phase (OD_{660} 0.8–1.00). Growth was evaluated through the lecture of absorbances at 660 nm by using a light visible Beckman DU 640 spectrophotometer.

2.2. Preparation of biosorbents

Fractions of *P. putida* A (ATCC 12633) and *P. putida* PB01 cultures, grown as previously described, were autoclaved, separately, at 121 °C, 1.2 atm for 30 min (non-viable biomass of each strain); while the remaining cultures were maintained viable. The cells were harvested by centrifugation at 8,000×*g* for 10 min at 4 °C in a Sorvall RC5C refrigerated, washed twice and resuspended in 0.9% NaCl (w/v) for viable cells, or in deionized water, for non-viable cells. Cells were stored at -20 °C and used for further studies.

2.3. Adsorption experiments

The ability of the viable and non-viable biosorbents to bond Al^{3+} (adsorbate) was evaluated varying different parameters such as pH, biosorbent and adsorbate concentrations. Assays were conducted in a final volume of 1 mL. Unless indicated, the metal was added as $AlCl_3$ at a concentration of 100 nmol/mL. The Al^{3+} free was measured by the colorimetric method described by Hejri et al., (2011). The experiments were performed three times; errors in experiments were less than 2 % of each value.

2.3.1. Effect of pH

8 mg/mL of viable and non-viable biomass of each bacterial strain resuspended in different pH solutions (pH range of 2-12) were exposed to Al^{3+} and incubated in an orbital shaker at 30 °C and 100 rpm for 15 min. When using viable biosorbents, the assays were

carried out in 0.9% NaCl, pH 7.0; 25 mM acetic acid-sodium acetate buffer, pH 5.5; the same buffer pH 4.3 and 15 mM Tris-HCl buffer, pH 8.0. On the other hand, aqueous solutions adjusted to desired pH values with HCl or NaOH were used for non-viable cells.

2.3.2. Biosorbent concentration

Fractions from 6 to 60 mg/mL of viable biosorbents were resuspended in 25 mM acetic acid-sodium acetate buffer, pH 4.3. Non-viable biosorbents were resuspended in aqueous HCl solutions, pH 4.3. Later, 100 nmol/mL of Al^{3+} were added and, after 15 min of incubation at in an orbital shaker at 30 °C and 100 rpm, the free metal was quantified.

2.3.3. Influence of ions on the process of adsorption

8 mg/mL of *P. putida* A (ATCC 12633) non-viable biomass resuspended in aqueous solution of HCl, pH 4.3, were exposed to 100 nmol/mL of Al^{3+} both in the presence and absence of different ions: Na^{+}, Cl^{-}, K^{+}, Ca^{2+}, F^{-}, NO_3^{-}, SO_4^{2-} and HCO_3^{-} . After 1 min of incubation at room temperature, samples were taken and centrifuged and the unbound Al^{3+} was quantified as described above.

2.3.4. Adsorbate concentration

6 mg/mL of biomass from each *Pseudomonas* strain were resuspended in 25 mM acetic acid-sodium acetate buffer, pH 4.3 (for viable biomass) or in aqueous solutions of HCl, pH

4.3 (for non-viable biomass). The biosorbents were exposed to different Al^{3+} concentrations (from 0 to 300 nmol/mL) and, after 1 min of incubation at room temperature, samples were taken, centrifuged and the free metal was quantified.

2.3.5. Adsorption isotherm

The interaction between the biosorbent and Al^{3+} was analyzed using two isotherm models: Langmuir (1918) and Bueno et al., (2007). In the Langmuir model, the isotherm, in its non-linear form, it is described by the following equation: $q_e=q_{max}$ ($K_LC_e/1 + K_LC_e$), where q_e is amount of metal adsorbed, q_{max} is the maximum amount of metal per gr of biomass, C_e is concentration of the metal in the equilibrium and K_L is a coefficient related to the affinity between the metals and biomass. According to this model, the values of K_L and q_{max} correspond, respectively, to the slope and intercept of the resulting line from the double-reciprocal plot of the saturation curve.

In the model described by Bueno et al., (2007) the isotherm, in its non-linear form, is described by the equation: Adsorption = M [adsorbate] x K_{eq}/ 1 + [adsorbate] K_{eq}; where M is the maximum number of adsorption sites per microorganism, and K_{eq} (expressed in litres per mole) is equivalent to the affinity of adsorbate molecules for the adsorption sites. According to this model, the values of K_{eq} and M correspond, respectively, to the slope and intercept of the resulting line from the double-reciprocal plot of the saturation curve.

For both isotherms, the saturation curve was constructed by plotting the adsorption (calculated as the ratio between the amount of Al^{3+} bound to biosorbents and the amount of

biosorbent in the incubation medium), versus the Al^{3+} concentration in the equilibrium (equivalent to the free Al^{3+}).

2.4. Desorption and reuse of biosorbent

The non-viable biosorbent was subjected to four cycles of successive biosorption/desorption. The adsorption was carried out with 8 mg/ml of non-viable biomass and 100 nmol/mL of Al^{3+}, with 1 min of incubation at room temperature. The desorption of Al^{3+} attached to the non-viable biosorbent was conducted through washes with 100 µl of HCl 0.01 N. After each step of desorption and before a new adsorption one, the adsorbent was washed three times with 1 ml of water. The experiments were performed three times.

2.5. Scanning electron microscopy (SEM)

6 mg/mL of non-viable biosorbents were resuspended in aqueous HCl solutions, pH 4.3 and were treated and not-treated with 100 nmol/mL of Al^{3+}. After 1 min of incubation at room temperature, the adsorbents were centrifugated at 8,000×*g* for 10 min, washed twice with deionized water, immersed in glutaraldehyde for 1 h and finally dehydrated with alchohol (50-100%) for 30 min. The pre-treated samples were coated with Al via vapor deposition prior to being introduced to SEM (JEOL modelo JSM 5480 LV) for analysis.

2.6. Fourier transform infrared spectroscopy (FTIR) analysis

8 mg/mL of non-viable biomass of *P. putida* A (ATCC 12633) was resuspended in aqueous HCl solutions pH 4.3. The biosorbent was exposed or not at 100 nmol/mL of Al^{3+} and after 1 min of incubation at room temperature, the suspension was centrifugated at 8000 g for 10 min (Sorvall Dupont, Wilmington, DE, USA), washed with distilled water and lyophilized. Then, the lyophilized biomass with or without Al^{+3} loaded, were ground into fine power and translucent sample disk FITR spectra were obtained using a Bruker Tensor 27 FTIR spectrometer with a resolution of 2 cm^{-1} and recorded between 600 cm^{-1} and 4000 cm^{-1}.

3. Results and discussion

3.1. Effect of pH

It is widely known that the pH of the solution influences the degree of ionization of the functional groups of the adsorbent and the solubility of metals and, as a result, directly influences the nature of the adsorption event. With respect to functional groups, it is also known that under acidic pH values, carboxyl and phosphate groups carry negative charges (pKa=3-5) that make the cell walls potent scavengers of cations (Ozdemir and Bayzal, 2004; Vijayaraghavan and Yun, 2008). In addition, under low pH values some of the impurities and ions blocking the binding sites can easily be eliminated (Vijayaraghavan and Yun, 2008). On the other hand, the solubility and ionic nature of aluminum strongly depend on pH. The ionic species found between pH 3 and 7 are: Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^{+}$ and

$Al(OH)_4$ (Garcidueñas Piña and Cervantes 1996). At pH values ~4, most Al exists as Al^{3+} (free Al) allowing binding to negatively charged ligands; while under alkaline pH values, the metal starts to form hydroxide compounds which are insoluble (Garcidueña Piña and Cervantes, 1996). Therefore, the experiments of Al^{3+} adsorption were performed under different pH values (pH range 2-12) using viable and non-viable biomass of *P. putida* A (ATCC 12633). For both kinds of biomass, the greatest capacity of biosorption (95% approximately) was obtained at pH 4.3 (Fig. 1). For viable biomass, the adsorption of Al^{3+} decreased with the increase of pH, reaching 50% of adsorption at pH 8. For non-viable biomass, the increase of pH values from 4 to 8 did not change the degree of adsorption, keeping it at 90-95%. However, at pH 12, the percentage of adsorption significantly decreased. Therefore, taking into account the obtained results for both types of biomass, the remaining experiments were conducted at pH 4.3.

3.2. Influence of contact time, temperature, agitation and adsorbent concentration

Different concentrations (0 to 60 mg/mL) of both living and non-living microbial biomass were used to evaluate the Al^{3+} adsorption process (Fig. 2). As it can be seen, approximately 100% of the Al^{3+} added (100 nmol/mL) was removed after treatment with 8 mg/mL of non-living biomass. On the other hand, to obtain 100% of adsorption using living biomass, 40 mg/mL of the bioadsorbent were needed. It is known that different chemical and physical pretreatments (heating/boiling, autoclaving freezing/thawing, lyophilization, washes with acid, alkaline, detergents, organic solvents or salts) can modify

the surface of adsorbents either by removing or masking the groups or by exposing more metal-binding sites (Vieira and Volesky, 2000; Vijayaraghavan and Yun, 2008; Wang and Chen, 2009, Gabr, et al, 2008). The fact that the non-viable biomass of *P. putida* A (ATCC 12633) was obtained by autoclaving may explain why a fewer amount of adsorbent was needed to remove the Al^{3+}. The biosorption of Al^{3+} may be enhanced by autoclaving the biomass since the destroyed cells provide a larger available surface area and more surface binding sites (Errasquin and Vazquez, 2003).

When we evaluated the adsorption of 100 nmol/mL Al^{3+} using viable biosorbent (40 mg/mL) and non-viable biosorbent (8 mg/mL), varying the contact time between 1 and 15 min, we found that the binding of Al^{3+} was a rapid and stable process over time, for both kinds of adsorbents. After 1 min both kinds of biomass removed all the Al^{3+} added (data not shown). The rapid adsorption is likely due to the high initial concentration of Al^{3+} and the presence of free binding sites for the metal on the biosorbents. Furthermore, these results are consistent with those described by Vijayaraghavan and Yun (2008), who suggested that very short contact times between the microbial adsorbent and the adsorbate are required; since, due to its small size, the resistance to mass transfer is negligible if the cells are in solution.

In order to evaluate the effect of the temperature and agitation on the adsorption process of 100 nmol/mL of Al^{3+}, viable and non-viable biomass were treated with and without incubation in an orbital shaker (100-150 rpm) during 1 min at a temperature that ranged from 15 ºC to 42 ºC. For all evaluated conditions, no significant differences were detected in the adsorption process and a 97 % of the metal was removed (not shown).

3.3. SEM and FTIR analysis

SEM was used to analyze the morphology of the surface of the adsorbent in presence or absence of Al^{+3}. The Fig. 3 shows the SEM photomicrographs of *P. putida* A (ATCC 12633 non-living biomass taken before (a) and after (b) Al^{3+} biosorption. After treatment with Al^{3+} the presence of particles in the form of irregular globules was observed all over the cell surface which indicating the presence of metal.

It is known that the FTIR analysis provides information about of the functional groups of adsorbent responsible for the adsorption of metals. The FTIR spectra of unloaded and Al^{3+} loaded *P. putida* A (ATCC 12633) non-viable biomass and the functional groups identified are shown in Fig. 4. After Al^{3+} adsorption, several changes in the infrared bands were observed: new peaks at c.a. 3500 cm^{-1}, 2400 cm^{-1} and 630 cm^{-1} and decrease in the intensity of the 1150 cm^{-1} and 1010 cm^{-1} bands. All of these bands are presents in Al^{3+} compounds (Fripiat et al., 1965; Riesgraf and May, 1978). The band at c.a. 3500 cm^{-1} could be assigned to the stretch of OH bound to two aluminium atoms (Fripiat et al., 1965). The band at 2400 cm^{-1} is attributed to Al-O-Al unsymmetrical stretching, and the band c.a. 1010 cm^{-1} could be attributed to the Al-O-H or Al-OH-Al deformation (Fripiat et al., 1965). The change of the peak present at 1150 cm^{-1} may be assigned to complexation of aluminum ion with the phosphate esters group (Muñoz et al., 2015). The band at 630 cm^{-1} is characteristic to the aluminum compound, however is not assigned but it suggests that the band could be due to the aluminium water complex (Riesgraf and May, 1978). Although it is difficult to confirm the exact interaction of metal ions onto *P. putida* A (ATCC 12633) non-viable biomass, the changes of the FTIR spectrophotometry clearly show the participation of the

functional groups of the cell in the biosorption process. Moreover, the changes detected on the biosorbent after Al+3 treatment were similar to those described by other authors that evaluates the biosorption of this metal using another adsorbents (Sari and Tuzen, 2009; Tassit et al., 2010, Basurco Cayllahua and Torem, 2010).

3.4. Biosorption isotherm

The biosorption isotherm represents the equilibrium distribution of metal ions between the aqueous (free metal) and solid phases (metal adsorbed), and is important in determining the maximum biosorption capacity. To analyze the interaction between the biosorbent and the Al^{3+}, we chose the models of adsorption isotherms described by Langmuir (1918) ($q_e=q_{max}$ ($K_LC_e/1+ K_LC_e$)) and by Bueno et al., (2007) (Adsorption = M [adsorbate] x K_{eq}/ 1 + [adsorbate] K_{eq}). The Al^{3+} biosorption performance was assessed by measurements at initial concentrations from 0 to 300 nmol/mL of Al^{3+}, 1 min contact time, pH 4.3 and viable and non-viable biomass of *P. putida* A (ATCC 12633). In addition, to determine the contribution of PC in the binding of the Al^{3+} to its phosphate group, and also to evaluate if this PL could constitute a temporary reservoir of Al^{3+} (Boeris et al., 2009), we evaluated a mutant strain lacking PC, *P. putida* PB01, as biosorbent. Results showed that the mutant bound less Al^{3+} to its cell membranes (Boeris and Lucchesi, 2012).

The saturation curve of Al^{3+} for *P. putida* A (ATCC 12633) viable and non-viable and for *P. putida* PB01 non-viable are shown in Fig. 5 (a and b). In all cases, the isotherms became in hyperbolic when the capacity of adsorption of each biomass reached complete saturation.

Both viable and non-viable biosorbents of *P. putida* A (ATCC 12633) were saturated with a similar concentration of Al^{3+} (0.50 mg Al^{3+}/gr adsorbent (3.3 x 10^6 molecules Al^{3+}/bacteria) and 0.57 mg Al^{3+}/gr adsorbent (3.8 x 10^6 molecules Al^{3+}/bacteria), respectively). The non-viable biosorbent of *P. putida* PB01 was saturated with a lower concentration of Al^{3+} (0.27 mg Al^{3+}/gr adsorbent (1.27 x 10^6 molecules Al^{3+}/bacteria)), evidencing that the lack of PC in the bacterial membrane, significantly decreased the availability of binding sites for the cation. Previous studies have demonstrated that in multilamellar vesicles, the Al^{3+} binds to the phosphate group of PC (MacKinnon et al. 2004, 2006), but it also can bind the phosphate group of other phospholipids such as phosphatidylserine and phosphatidylethanolamine (Akeson et al., 1989, Oteiza, 1994). Although *P. putida* PB01 lacked PC, the levels of phosphatidylethanolamine in its membrane were high, representing about 70% of total phospholipids (Boeris and Lucchesi, 2012). This fact might explain why the mutant still absorbed aluminum.

The values of the constants estimated for each isotherm from double reciprocal plot of the saturation curve are summarized in Table 1. Both isotherms models showed agreement with the data obtained from the adsorption of the metal, since exhibited high regression coefficient (R^2) values. From simulation with Langmuir isotherm, the q_{max} value predicted for *P. putida* A (ATCC 12633) non-viable was higher than for biosorbent viable. The biomass of *P. putida* PB01 non-viable showed a q_{max} value considerably lower (0.27 mg Al^{3+}/gr adsorbent). From simulation with the model described by Bueno et al., (2007), the M value obtained for the non-viable biosorbent of *P. putida* A (ATCC 12633) was four times higher than the one registered for the viable biosorbent of this strain (36 x 10^5 and 9.34 x 10^5 sites/microorganism, respectively), while the K_{eq} values did not differ

significantly between both kinds of biosorbents (Table 1). Non-viable biomass of *P. putida* PB01 had a considerably lower M value (1.2 x 10^5 sites/microorganism). In addition, the efficiency of the adsorption process (E=M x Keq), was highest for *P. putida* A (ATCC 12633) non-viable mainly because the high value of M determinate.

The different values predicted by the isotherms models used allow to establish that the most efficient biosorbent was the non-viable biomass of *P. putida* A (ATCC 12633), mainly because it possessed higher number of binding sites (Table 1). This fact may be caused to the different structure of the bacterial surface or to the higher number of metal-binding sites exposed after autoclaving (Vieira and Volesky, 2000; Vijayaraghavan and Yun, 2008; Wang and Chen, 2009). Taken together, results of the present work indicate that the efficiency of adsorption is mainly a phenomenon of quantitative characteristics (depending on the number of binding sites) and not of qualitative ones (metal affinity for the adsorbent).

3.4. *Influence of ions on the adsorption process*

The ionic strength, given by the presence of ions in the medium, influences the adsorption of the metal to the adsorbent surface. The cations (Ca^{2+}, K^+, Mg^{2+}, Na^+) may lead to a competition with the metal by the binding sites present in the adsorbent, whereas some anions such as Cl^- may form salts with some metals, affecting their availability (Vijayaraghavan and Yun, 2008). We evaluated the adsorption of 100 nmol/mL of Al^{3+} in presence of different ions at concentrations commonly found in nature. As shown in Table

2, only Ca^{2+} significantly interfered in the adsorption of Al^{3+}, probably due to the density charge of the ions and the high affinity of surface binding sites by Ca^{2+} (Akeson et al., 1989).

3.5. *Desorption and reuse of the biosorbent*

The efficiency in reuse of the non-viable biomass of *P. putida* A (ATCC 12633) was tested during 4 cycles of adsorption/desorption. In each cycle, desorption of the Al^{3+} bound to the biomass was carried out through wash with HCl 0.01 N. In the first cycle, the adsorption and desorption were very effective, and a 95% of an initial concentration of 100 nmol/mL of Al^{3+} attached to biosorbent was recuperated. In the subsequent cycles, the adsorption efficiency decreased markedly; in the second cycle, the system bound a percentage lower than 50 of the initial concentration of Al^{3+} used, in the third cycle this percentage decreased to 38 and finally, in the fourth adsorption cycle an amount of 28% was obtained. However, in all evaluated cycles, the desorption continued being effective, with percentages of recuperation of the Al^{3+} adsorbed varying between 90% and 100% in each cycle. These results clearly demonstrate that in spite of the fact that the biosorbent can be reused, the acidic condition of the desorption process probably damage the biomass.

4. Conclusions

Non-viable biomass of *Pseudomonas putida* A (ATCC 12633), used as biosorbent, showed high efficiency in the removal of Al^{3+} from aqueous solutions, mainly due to the

availability of phosphatidylcholine and to the higher number of binding sites exposed after autoclaving. The adsorption was rapid, stable over time, efficient in a broad range of temperatures and pH and not affected by ions. Considering the performance of the assays and that the use of non-viable biomass significantly reduces costs and constitutes an eco-friendly system, our results support the possible use of this adsorption system for the removal of Al^{3+} from contaminated sites.

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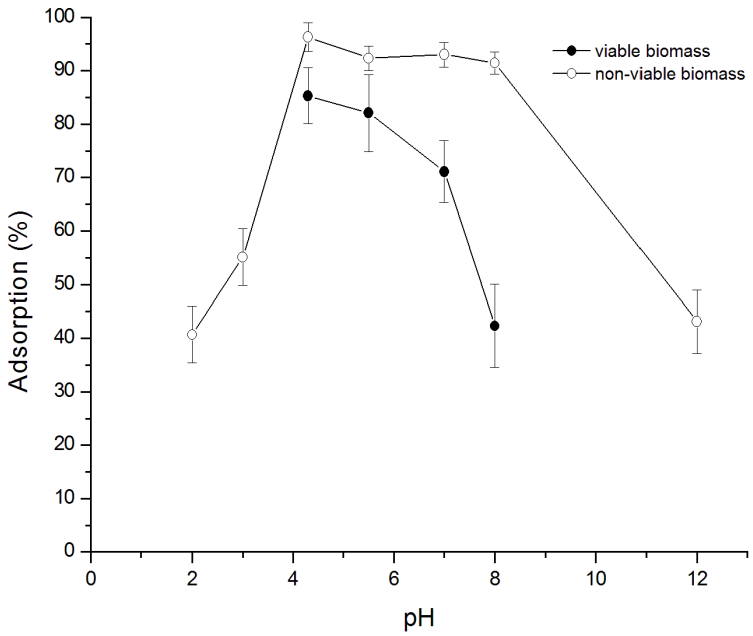

Fig. 1. Effect of pH on Al^{3+} adsorption capacity of viable (●) and non-viable (○) biomass of *P. putida* A (ATCC 12633) (pH: 2-12; biomass: 8 mg/mL; Al^{3+}: 100 nmol/mL; temperature: 30 °C; agitation rate: 100 rpm; time: 15 min). Values are means ± SD (n = 3).

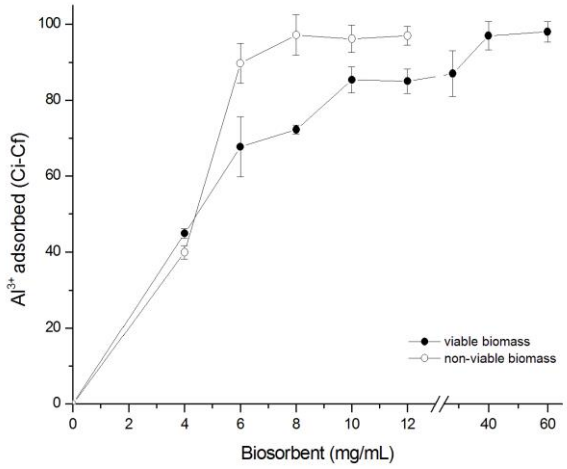

Fig. 2. Dose effect of viable (●) and non-viable (○) biosorbents of *P. putida* A (ATCC 12633) on Al^{3+} adsorption (biomass: 0-60 mg/mL; pH: 4.3, Al^{3+}: 100 nmol/mL; temperature: 30 °C; agitation rate: 100 rpm; time: 15 min; Ci and Cf: initial and final concentration of Al^{3+}, respectively). Values are means ± SD (n = 3).

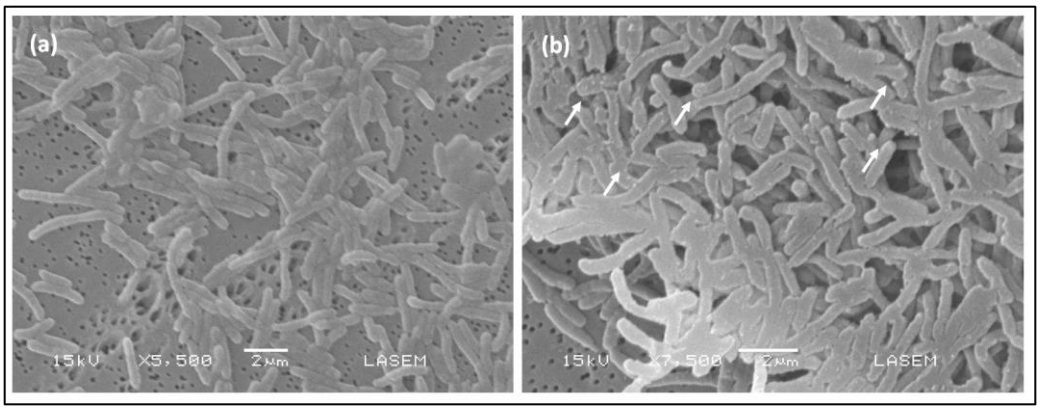

Fig. 3. SEM photomicrographs of *P. putida* A (ATCC 12633) non-viable biomass, before (a) and after (b) biosorption of Al^{3+} 100 nmol/mL. Arrows indicate irregular globules all over the cell surfaces, evidencing the presence of metal.

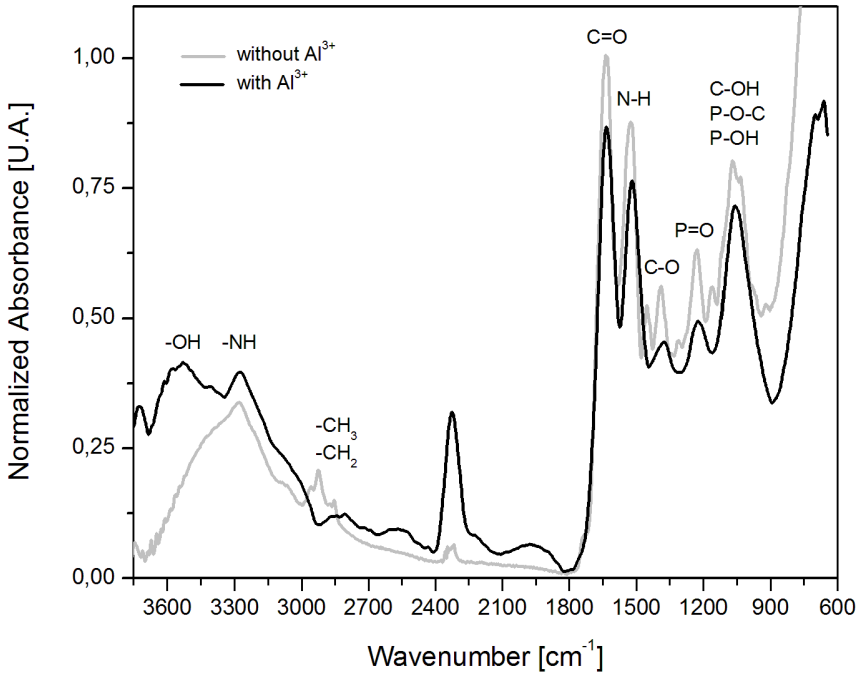

Fig. 4. FTIR spectrum for *P. putida* A (ATCC 12633) non-viable with or without Al^{+3} loaded.

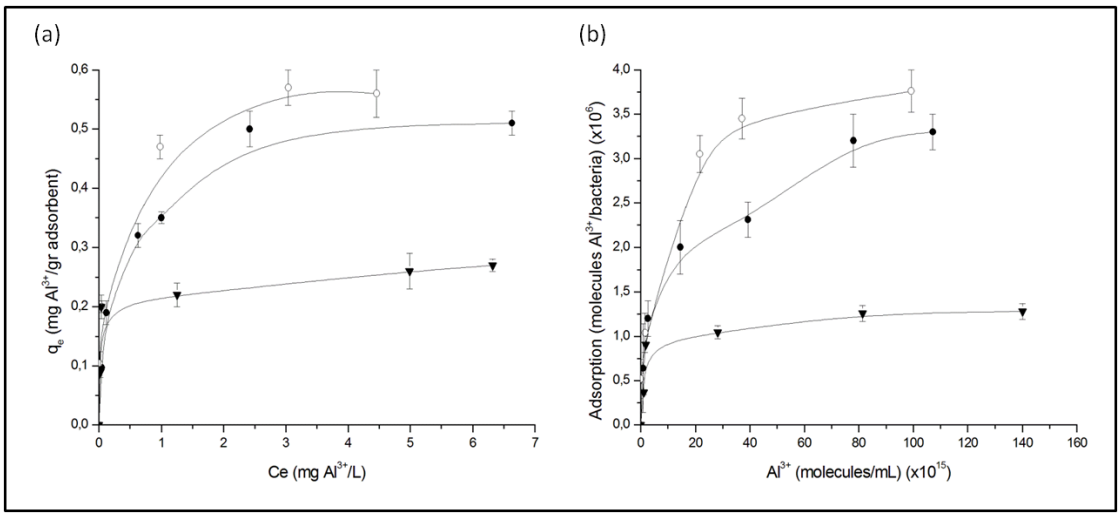

Fig. 5. Saturation curve for the biosorption of Al^{3+} by *P. putida* A (ATCC 12633) viable biomass (●); *P. putida* A (ATCC 12633) non-viable biomass (○) and *P. putida* PB01 non-viable biomass (▼) (Al^{3+}: 0-300 nmol/mL: biomass: 6 mg/mL; pH: 4.3; room temperature; time: 1 min). (a) Langmuir isotherm and (b) Bueno et al., 2007 isotherm. Values are means ± SD (n = 3).

Table 1 Adsorption isotherm constants for the biosorption of Al^{3+} with different biomass

	P. putida A (ATCC 12633) viable	*P. putida* A (ATCC 12633) non-viable	*P. putida* de PB01 non-viable
Langmuir			
q_{max} (mg/gr)	0.48	0.55	0.27
K_L ((mg Al/l)$^{-1}$)	4.35	11.36	23.14
R^2	0.98	0.98	0.98
Bueno *et al*. (2007)			
M (x10^5 sites/microorganism)	9.34	36	1.20
K_{eq} (x10^3 M^{-1})	1.16	1.24	8.10
E (x10^8 M^{-1})	10.83	44.64	9.72
R^2	0.92	0.98	0.98

q_{max}= maximum biosorption capacity (mg Al per gr adsorbent), K_L= Langmuir biosorption equilibrium constant, M= total number of binding sites per microorganism, K_{eq}= equilibrium constant, E= efficiency of the adsorption process (E=M x Keq), R^2= regression coefficient.

Table 2 Influence of ions on the adsorption of Al^{3+}

Ion	**Added as**	**nmol/mL**	**Al^{3+} adsorbed (%)**
Na^+	NaCl	0.34	97.5 ± 2.0
K^+	KCl	0.003	98.1 ± 1.2
Ca^{2+}	$CaCl_2$	0.005	85.2 ± 1.0
Cl^-	NaCl	0.22	98.0 ± 2.0
F^-	NaF	0.0003	99.0 ± 1.0
NO_3^-	NO_3K	0.006	98.5 ± 1.2
SO_4^{2-}	$MgSO_4$	0.003	99.0 ± 1.0
HCO_3^-	HCO_3K	0.049	96.2 ± 0.5

Al^{3+}: 100 nmol/mL; non-viable biomass of *P. putida* A (ATCC 12633): 6 mg/mL; pH: 4.3; room temperature; time: 1 min. Values are means ± SD (n = 3).