Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 5574–5581
BIORESOURCE TECHNOLOGY

Cadmium, zinc and copper biosorption mediated by *Pseudomonas veronii* 2E

Diana L. Vullo *, Helena M. Ceretti, María Alejandra Daniel, Silvana A.M. Ramírez, Anita Zalts

Área Química, Instituto de Ciencias, Universidad Nacional de General Sarmiento, J.M. Gutiérrez 1150, (B1613GSX) Los Polvorines, Buenos Aires, Argentina

Received 1 March 2007; received in revised form 25 October 2007; accepted 25 October 2007
Available online 26 December 2007

Abstract

Adsorption properties of bacterial biomass were tested for Cd removal from liquid effluents. Experimental conditions (pH, time, cellular mass, volume, metal concentration) were studied to develop an efficient biosorption process with free or immobilised cells of *Pseudomonas veronii* 2E. Surface fixation was chosen to immobilise cells on inert surfaces including teflon membranes, silicone rubber and polyurethane foam. Biosorption experiments were carried out at 32 °C and controlled pH; maximal Cd(II) retention was observed at pH 7.5. The isotherm followed the Langmuir model ($K_d = 0.17$ mM and $q_{max} = 0.48$ mmol/g cell dry weight). Small changes in the surface negative charge of cells were observed by electrophoretic mobility experiments in presence of Cd(II). In addition, biosorption of 40% Cu(II) (pH 5 and 6.2) and 50% Zn(II) and 50% Cd(II) (pH 7.5) was observed from mixtures of Cu(II), Zn(II) and Cd(II) 0.5 mM each.

Keywords: Biosorption; Heavy metals; Bioremediation; *Pseudomonas veronii*; Stripping voltammetry

1. Introduction

Heavy metal accumulation in the urban environment is a final result of industrial waste discharges. The removal and recovery of heavy metals from contaminated water and wastewater is important in the protection of the environment and human health. There are several chemical technologies used to remove Cu, Cd, Pb, Zn, Hg or Cr. These include chemical precipitation, oxidation or reduction, filtration, ion exchange, electrochemical treatment, reverse osmosis, membrane technology and evaporation recovery. Most of these are ineffective or excessively expensive when the metal concentrations are less than 100 mg/L (Ahluwalia and Goyal, 2007; Patterson et al., 1998). Biological treatment is an innovative technology available for heavy metal polluted wastewaters. Since microorganisms have developed survival strategies in heavy metal polluted habitats, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or biosorption can be applied either *ex situ* or *in situ* to design economical bioremediation processes, (Gadd, 2000; Lim et al., 2003; Lin and Lin, 2005; Lloyd and Lovley, 2001; Lovley, 2000; Malik, 2004; Munoz et al., 2006; Umrania, 2006; Valls and de Lorenzo, 2002).

In terms of biosorption, polysaccharides, proteins and lipids on bacterial cell walls offer many functional groups such as carboxylate, hydroxyl, phosphate, amine and sulphate groups which can bind metal ions. This natural affinity of biological compounds for metallic elements could contribute to the purification of metal-contaminated wastewater. Successful applications make use of either living or nonliving cells, under controlled physicochemical conditions, such as pH, temperature, sorbent mass and ionic concentration.

* Corresponding author. Tel.: +54 11 4469 7542; fax: +54 11 4469 7506.
E-mail address: dvullo@ungs.edu.ar (D.L. Vullo).

0960-8524/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2007.10.060

Commercial application of microbial biomass as a biosorbent can suffer from problems associated with the physical characteristics of this material, which can make difficult the liquid–solid separation. As a possible solution to this fact, the utilisation of immobilised microorganisms has the advantage of an easier separation of the decontaminated effluent. Entrapment, cellular aggregation or surface fixation are the most common methods for cell immobilisation (Akhtar et al., 2004; Bender and Phillips, 2004; Chang et al., 2006; Gadd, 2000; Liu et al., 2003). The choice of an adequate matrix for cell immobilisation affects the performance of the process, since the metal biosorption efficiency can be affected by using these heterogeneous systems (Han et al., 2006; Vijayaraghavan et al., 2005).

Cadmium is one of the most toxic heavy metals; its environmental appearance is a consequence of human activities, fundamentally from industrial sources. Cadmium concentration in the range 0.1–100 mg/L are typical in wastewater from several industries (chemical and metal product facilities, leather and tanning processes, electricity and gas production and sanitary industries). For cadmium, US EPA (1995) recommends a daily maximum of 0.73 mg/L and monthly average of 0.17 mg/mL, when best practicable control technology (BPT) is applied (Patterson et al., 1998).

Because of its high mobility, cadmium can be found either in water or soil of any polluted ecosystem. Cd(II) biosorption is a viable method for its immobilisation and the use of indigenous multiresistant microorganisms for that purpose is an efficient, ecofriendly and economical alternative. The retention of cadmium by bacterial biomass in industrial effluents could avoid an increase in Cd contamination of water courses.

The aim of our study is to select autochthonous microorganisms that are able to biosorb heavy metals in aqueous systems with 1–100 mg/L of Cu(II), Cd(II) and Zn(II), and can be easily immobilised in polymeric matrices for the development of a wastewater bioremediation process. Our work is structured in four stages: (1) screening of indigenous Cu(II), Cd(II) and Zn(II) multiresistant/tolerant microorganisms from natural samples, (2) selection of strains with the highest metal retention, (3) cell immobilisation studies on microorganisms with surface fixation ability and (4) cadmium biosorption studies by living biomass for future bioremediation processes.

2. Methods

2.1. Screening and selection of heavy metal resistant/tolerant bacteria for bioremediation purposes

2.1.1. Samples

Samples were collected from polluted environments in the Buenos Aires Metropolitan Area: (1) Estación Fluvial Tigre (34°25′S, 58°34′W) surface water and coastal sediments from four different points at a highly polluted area of Reconquista River (Olguin et al., 2004; Topalian et al., 1999a,b; Vullo et al., 2005), (2) surface water from Areco stream, downstream of a tannery effluent discharge (34°15′S, 59°28′W) and (3) two soil samples from a car washing and parking area contaminated with hydrocarbons and heavy metals.

2.1.2. Screening of Cu, Cd and Zn resistant/tolerant bacteria

A 0.1 mL of each water sample were spread on plates prepared with PCA medium (Plate Count Agar: casein peptone 5 g/L, yeast extract 2.5 g/L, glucose 1 g/L, agar 14 g/L) simultaneously spiked with 0.5 mM Cu(II), 0.5 mM Cd(II) and 0.5 mM Zn(II). When soil samples were treated, 0.1 mL of a 1:10 soil suspension in 150 mM NaCl were spread in the same medium. Plates were incubated at 32 °C for at least 1 week. Isolated colonies were purified three times in the same medium and tested for minimal inhibitory concentration (MIC) for each metal in separate assays.

2.1.3. MIC determination

MIC was determined in duplicate in PYG broth (casein peptone 2.5 g/L, yeast extract 1.25 g/L, glucose 0.5 g/L) with 0.1, 0.25, 0.5, 1, 2, 5 and 10 mM Cu(II), Zn(II) or Cd(II). Cultures were incubated at 32 °C for at least 1 week, checking bacterial development by absorbance at 600 nm every 24 h. MIC was estimated as the first dilution which completely inhibits bacterial growth in PYG medium.

2.1.4. Biochemical and molecular characterization

Biochemical characterization was performed by API multitest system (Bio Mérieux). Molecular characterization was performed by 500 bp 16S r-RNA gene sequencing (MIDI Labs, USA) or 1500 bp 16S r-RNA (MacroGen, Korea).

2.1.5. Batch culture heavy metal retention experiments

Metal retention in batch cultures was tested by inoculating 100 mL of PYG culture medium containing simultaneously Cu(II), Cd(II) and Zn(II) (0.5 mM for strains 2E, AR and M2 or 0.25 mM for strains 1P and P2) with 10 mL of a previous culture (PYG broth plus Cu(II), Cd(II) and Zn(II)). The initial metal concentrations depended on the bacterial MIC values previously obtained. Samples were taken at initial, exponential and stationary phases of growth (1 week incubation, 200 rpm and 32 °C). Growth was tested measuring absorbance at 600 nm ($A_{600\ nm}$) and pH was determined in each sample. All samples were centrifuged and supernatants analysed for total Cu, Cd and Zn to estimate total retention (%). Cell free medium (pH 5) was tested simultaneously as control and all experiments were performed in duplicates.

2.2. Biosorption experiments

Each isolate was tested for its biosorbent behaviour in the presence of a heavy metal solution.

2.2.1. Bacterial suspension preparation

Fifty milliliters early stationary phase cultures ($A_{600\ nm} = 1.1–1.2$) in PYG broth were centrifuged (3000*g*, 15 min.). Cells were washed and resuspended in an adequate volume of water (18 MΩ cm, Millipore). The resulting suspension contained living biomass at a concentration of approximately 3 g dry weight/liter.

2.2.2. Biosorption assay

A general biosorption assay was designed for testing bacterial retention of heavy metals in non growth conditions. For that purpose, the biosorption mixture [5 mL cellular suspension, 0.5 mM of each metal, 10 mM buffer and water (18 MΩ cm, Millipore) to a final volume of 10 mL] was incubated at 32 °C and 200 rpm during 24 h. Suspensions were centrifuged (3000*g*, 20 min.) and filtered through 0.45 μm-pore diameter cellulose membrane to obtain cell free supernatants. Cu, Cd or Zn concentrations in supernatants were determined in duplicates and their decrease was estimated by comparison to a cell free control biosorption mixture.

2.2.3. Influence of pH on copper, cadmium and zinc biosorption

Biosorption experiments were carried out at pH 5.5, 6.2 and 7.5. Buffer solutions of MES (2-[*N*-morpholino]ethanesulfonic acid, $pK_a = 6.1$, Sigma), PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid], $pK_a = 6.8$, Sigma) and HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid], $pK_a = 7.5$, Aldrich) were added to the biosorption mixture up to 10 mM for pH regulation.

2.3. Cell immobilisation in polymeric surfaces

Different synthetic polymers were used as matrices to evaluate bacterial adherence on their surfaces and visualize the ability of the strains to develop biofilms on them. Polyurethane foam cubes (1 cm^3), Teflon membrane squares (1 cm^2, 175 μm thickness) and silicon tubes (1.5 cm long, 5 mm internal diameter, 2 mm wall thickness) were used for surface immobilisation of microorganisms. The immobilisation was carried out by growing cells, either in batch or continuous systems, in presence of the different matrices to allow adherence to each surface. For batch systems, each material was suspended in 10 mL of PYG broth with 0.1 mM Cu, 0.1 mM Cd and 0.1 mM Zn to allow any possible activation of resistance genes and inoculated with 1 mL of the culture. The incubation was performed at 32 °C under agitation (150 rpm) or static conditions. The culture medium was replaced every 48 h in order to increase biomass. For continuous systems, each material was suspended in mini-reactors containing 10 mL of the same medium and inoculated with 2 mL of the culture. When cells reached exponential phase of growth, by incubation at 32 °C, fresh medium supply was connected at low fluxes: 0.5 mL/h. After a week, the matrices were washed four times with water and stained with safranin solution (0.1% in water) for microscopic observation (100–400×).

2.4. Cadmium biosorption by Pseudomonas veronii 2E

2.4.1. Cadmium biosorption kinetics: time optimization

Cadmium biosorption was determined at different incubation times of the biosorption mixture, prepared as described above, with *P. veronii* 2E suspension and regulated at pH 7.5 with HEPES buffer. The biosorption mixtures were incubated from 0 to 30 h at 32 °C and 200 rpm. Mixtures were treated as mentioned for cadmium quantification in supernatants.

2.4.2. Biosorption isotherm construction

Experimental conditions previously established (pH 7.5, HEPES, 32 °C, 200 rpm, incubation time 24 h) were applied to obtain the biosorption isotherm. *P. veronii* 2E cells were exposed to Cd(II) concentrations in the range 0.050 to 2 mM. Cd(II) equilibrium concentration was measured in each supernatant after incubation and compared to cell free control biosorption mixtures.

2.4.3. Bacterial electrophoretic mobility

The bacterial electrophoretic mobility was evaluated in the presence and absence of Cd(II). For that purpose, a suspension containing *P. veronii* 2E at a concentration of 7.6 mg dry weight/L was incubated for 3 h at 32 °C with 0, 0.01, 0.025, 0.05, 0.1 and 0.5 mM Cd(II), 10 mM KCl, 10 mM HEPES (pH 7.5) in 10 mL final volume. *Z* potential was determined by scattering of laser light in a Brookhaven 90-plus zetameter as the mean value of seven measurements of the electrophoretic mobility.

2.5. Analytical procedures

Cu, Cd and Zn were determined by Anodic Stripping Voltammetry (ASV) using an Autolab PGStat10 (EcoChemie) and a Metrohm 663 VA polarographic stand (hanging mercury drop electrode mode). Samples were photoxidised (Hg-UV lamp photoreactor) during 14 h in FEP bottles (Nalgene) in order to avoid interaction between metals and organic matter previous to cadmium quantification.

3. Results and discussion

3.1. Screening and selection of heavy metal resistant/tolerant bacteria for bioremediation purposes

Eleven strains, able to grow in presence of Cu, Cd and Zn in the semisolid medium, were isolated from the polluted samples. These isolates were tested for Cu, Cd and Zn MIC estimation in PYG broth. Five of them were chosen due to their highest MIC for each heavy metal and the highest biomass yield in shortest incubation times. Results of MIC estimation are shown in Table 1. Except for M2, Cd(II) is clearly the most toxic metal of the three for each

Table 1
Cu, Cd and Zn minimal inhibitory concentration (MIC) in PYG Broth of five of the isolates

Strain	MIC (mM)		
	Cu	Cd	Zn
P. veronii 2E	2	0.5	10
R. taiwanensis M2	2	5	10
D. acidovorans AR	2	1	5
K. ornithinolytica 1P	2	0.25	0.5
K. oxytoca P2	1	0.25	0.5

strain. These results are consistent with the screening for multiresistant bacteria performed in previous studies (Vullo et al., 2005), where isolates with Cd resistance were also resistant to Cu, Zn and Pb, belonging to the lowest percentage group of culturable microorganisms found in the natural samples assayed. Two factors can be considered to account for Cd toxicity: (a) this metal is not an essential trace element for organisms and (b) the low complexing capacity of the culture medium used in this assay leaves cadmium almost completely bioavailable (Ceretti et al., 2006).

Both biochemical and molecular characterization was carried out for each strain. The 16S rRNA gene sequence of 500 bp revealed the identity of the microorganisms as follows: *P. veronii* (2E), *Ralstonia taiwanensis* (M2), *Delftia acidovorans* (AR), *Klebsiella ornithinolytica* (1P) and *Klebsiella oxytoca* (P2).

Batch culture heavy metal removal was evaluated for each isolate, to make an adequate selection based on the maximal Cu, Cd and Zn microbial uptakes in growth conditions. In this case, different mechanisms as biosorption, bioaccumulation, chemical precipitation of an insoluble compound or any combination of these metal–cell interactions can lead to metal retention. In all cases, culture pH (pH 5 at initial stage, 7 at exponential phase of growth and 8 at stationary phase of growth) were registered. Results of maximal retention, obtained in early stationary phase of growth, are summarized in Table 2.

Zn and Cd were retained by all strains; Cd(II) showed the higher uptake of the three metals assayed, with retentions from 7.5% to 47% (Table 2). However only *P. veronii* 2E retained Cu(II) significantly (35%). Low Cu(II) bioavailability could be responsible for these results, either through metal complexation with culture medium components or through the release of a bacterial metabolite to the culture medium with complexing capacity to Cu(II) (Bridge et al., 1999). PYG medium has significant concentrations of amino acids and peptides, which can act as complexing agents. Although PYG complexing capacity was not tested for Cu(II), it is well established in the literature that thermodynamic formation constants of Cu-peptide complexes are from 3 to 7 orders higher than Cd-peptide complex constants (Martell and Smith, 2001). Low copper toxicity to these bacteria, as observed in MIC estimations, could be due to Cu complexation with medium components, biological metabolites or both.

Table 2
Cu, Cd and Zn maximal retention detected in early stationary phases of batch cultures of the isolated strains

Strain	Maximal retention (%)		
	Cu	Cd	Zn
P. veronii 2E	35	47	41
R. taiwanensis M2	5	46	25
D. acidovorans AR	0	7.5	38
K. ornithinolytica 1P	13	45	29
K. oxytoca P2	0	24	10

3.2. Influence of pH on copper, cadmium and zinc biosorption

Heavy metal bioremediation processes involving biosorption do not require growing cells, so it is not necessary to add extra nutrients to the biosorption mixture. In the absence of nutrients, no complexing capacity is expected in the bacterial environment, so the metal is completely available to interact with cells. Biosorption studies were carried out to establish experimental conditions and to select the proper bacterial strain for the process design.

pH was evaluated since it affects the number of cellular surface sites available to bind cations, as well as metal speciation. Previous published data (Yan and Viraraghavan, 2003) showed significant pH changes during a biosorption process when it was carried out without pH regulation. In this work, buffered solutions are used for pH control during the biosorption assays to ensure reliable results. Simultaneous biosorption of Cu, Cd and Zn was tested for each selected strain at different pH values with proper buffer systems. 10 mM MES, 10 mM PIPES and 10 mM HEPES were used for biosorption experiments performed at pH 5.5, pH 6.2 and pH 7.5, respectively. These buffer solutions were chosen for the lack of complexing capacity for the assayed metals (Ceretti et al., 2006; Good et al., 1966; Soares et al., 1999; Soares and Conde, 2000; Vasconcelos et al., 1998). Results shown in Table 3 are mean values of Cu(II), Cd(II) and Zn(II) biosorption duplicate experiments. At pH 5.5 only Cu(II) was effectively biosorbed (22–43%, depending on the microorganism), meaning that Cu(II) adsorption could be achieved under these conditions. This fact would confirm the possible reasons for the low retention results obtained in batch cultures, except for *P. veronii* 2E where a 35% of Cu(II) retention was observed at stationary phase (pH 8). Similar results are observed at pH 6.2: besides Cu(II) biosorption (19–51%), 48% Zn was also biosorbed by *K. oxytoca* P2. Cd(II) and Zn(II) biosorption is clearly observed at pH 7.5 in all strains.

3.3. Cell immobilisation in low cost materials

Besides the high biosorption yield obtained in the isolated bacteria, the heavy metal bioremediation process requires the microorganisms to be attached to a solid

Table 3
Cu, Cd and Zn biosorption by indigenous bacteria at different pH and in low complexing capacity conditions (absence of nutrients)

Strain	% Heavy metal biosorbed							
	pH 5.5 (MES)			pH 6.2 (PIPES)			pH 7.5 (HEPES)[a]	
	Cu	Cd	Zn	Cu	Cd	Zn	Cd	Zn
P. veronii 2E	43	5	7	40	0	0	50	53
R. taiwanensis M2	25	6	3	42	0	5	33	38
D. acidovorans AR	27	0	0	51	3	4	64	78
K. ornithinolytica 1P	29	0	0	26	0	0	49	61
K. oxytoca P2	22	0	0	19	0	48	42	45

[a] Cu(II) biosorption was not evaluated at this pH due to chemical precipitation of copper compounds.

surface. Different matrices were tested for cell immobilisation. Surface fixation was chosen as immobilisation methodology instead of cell entrapment. Although cell immobilisation was successfully achieved in calcium alginate beads, this matrix was not considered for our biosorption experiments because of its high affinity for heavy metals. Studies of metal retention kinetics by calcium alginate were carried out, confirming that almost 100% of the metal assayed was retained by the beads (Vullo et al., 2003) and that it is pointless to try to improve heavy metal retention by bacterial cell entrapment in calcium alginate beads (Arica et al., 2001, 2004; Davis et al., 2003; Vullo et al., 2003). Calcium alginate is very useful for entrapping cells in its gel structure. Its advantage resides mostly in the re-utilisation of the entrapped cells. Although, the high heavy metal affinity of alginate makes it unusable for the development of continuous industrial processes as the recovery of the alginic acid would increase the final costs of the effluent treatment.

Successful bacterial immobilisation was achieved on inert surfaces such as Teflon membranes, silicone rubber and polyurethane foams. Best results on surface fixation were obtained with *P. veronii* 2E, which was able to grow on all three surfaces. This microorganism was able to develop a film over the matrix surfaces, and also to form aggregates and to adhere to glass during batch cultures. The development of the other bacteria on the same surfaces could barely be observed. Photos of cell immobilisation on different materials are shown in Fig. 1: *P. veronii* 2E red-stained cells were seen over polymeric structures of polyurethane foams (Fig. 1A), on silicone rubber surfaces (Fig. 1C) and on Teflon membranes (Fig. 1E), while *K. ornithinolytica* 1P was not able to attach successfully to any of these surfaces (Fig. 1B, D and F). Higher bacterial density

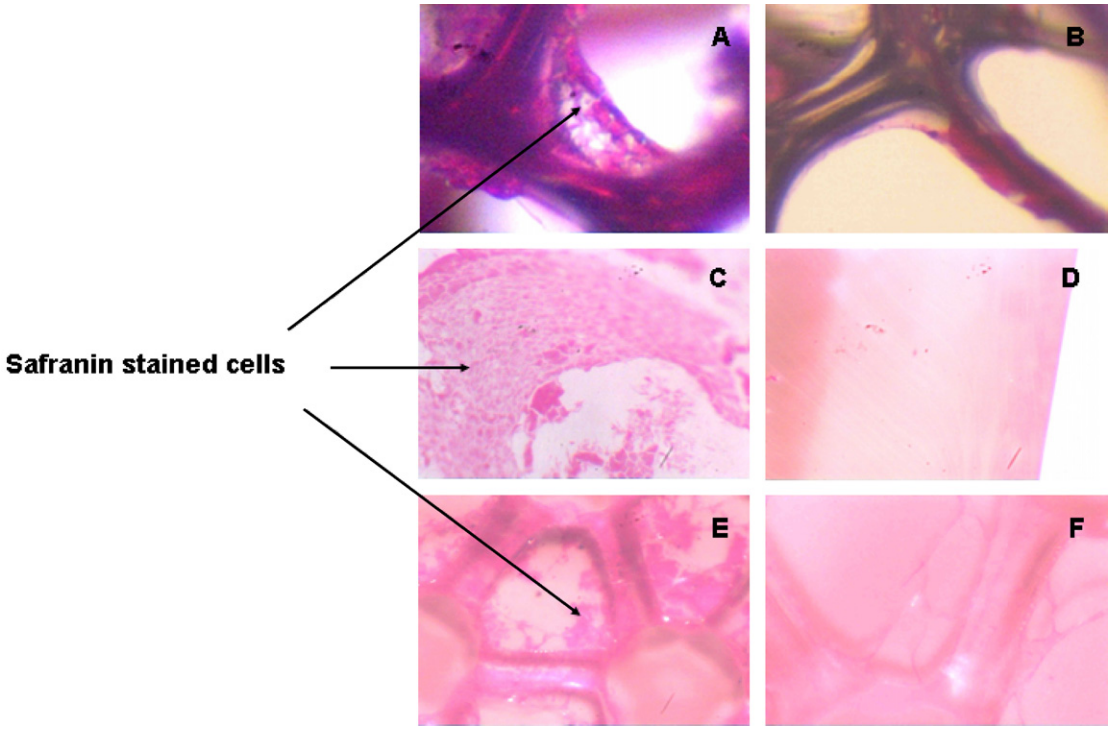

Fig. 1. Safranin stained cells immobilised on polymeric surfaces (100× magnification images): *P. veronii* 2E grown on polyurethane foam structure (A); on silicon rubber (C) and on Teflon membrane (E). *K. ornithinolytica* 1P grown on polyurethane foam structure (B); on silicon rubber (D) and on Teflon membrane (F).

was observed when the microorganisms grew in static batch culture conditions with periodic medium removal, or when very low culture medium fluxes in continuous systems were used.

3.4. Cadmium biosorption by P. veronii 2E

P. veronii 2E was selected for further studies of cadmium biosorption for the following reasons: (1) the high tolerance to cadmium and high biomass yields observed in PYG broth with low complexing capacity, (2) high cadmium biosorption yield at controlled pH (7.5) in a non-complexing mixture and (3) the ability to develop films on inert surfaces, which allow the utilisation of fixed or fluidized bed reactors.

As a first approach, biosorption kinetics were studied following Cd retention as a function of time (Fig. 2). Maximal biosorption efficiency (76.8%) was reached in 5 h and no further changes in Cd(II) concentration were observed through 30 h under these experimental conditions.

Cadmium biosorption isotherm was constructed (Fig. 3A) and data were analyzed using the classical Langmuir adsorption model. This model assumes that adsorption occurs in a monolayer, that all adsorption sites are identical and that no changes in adsorption free energy are observed at all sites. According to this, when adsorption follows Langmuir model the number of occupied sites, q, is given by

$$q = q_{max} \cdot C_f/(K_d + C_f) \quad \text{or} \quad C_f/q = K_d/q_{max} + C_f/q_{max}$$

where q_{max} is related to the total number of adsorption sites, C_f is Cd (II) final equilibrium concentration in supernatants and K_d is the equilibrium constant for the dissociation of the surface complex. The number of occupied sites, q, was calculated as follows:

$$q = (C_i - C_f) \times V_t/m_t$$

where C_i is the initial Cd(II) concentration, V_t is the total volume of the biosorption mixture assayed and m_t is the total biosorbent mass as dry weight. Cd(II) equilibrium concentration was determined as previously described.

Adsorption parameters obtained after linearization of the Langmuir model (Fig. 3A and B) showed that cadmium biosorption mediated by *P. veronii* 2E is in agreement with this model. Model parameters $K_d = (0.17 \pm 0.06)$ mM and $q_{max} = (0.48 \pm 0.09)$ mmol/g cell dry weight were obtained. Dissociation constant (K_d) is the inverse of the affinity constant of binding sites ($K_{aff} = 5.88\ mM^{-1}$) and q_{max} is the maximal amount of Cd per unit weight of cell to form a complete monolayer on the surface (theoretical saturation capacity). Comparison of these results with previous reported data on some Langmuir constants are shown in Table 4. K_d is in good agreement with the value obtained with the endospore forming Gram positive bacterium *Bacillus cereus* (Qi et al., 2006) and with the fungus *Rhizopus arrhizus* (Yin et al., 1999). Meanwhile, $q_{max} = 0.48$ mmol/g cell dry weight is similar to *Pseudomonas aeruginosa* PU21 (Chang et al., 1997), *Bacillus cereus* or *Rhizopus arrhizus* (Qi et al., 2006; Yin et al., 1999). The maximal biosorption capacity values of Langmuir parameters for

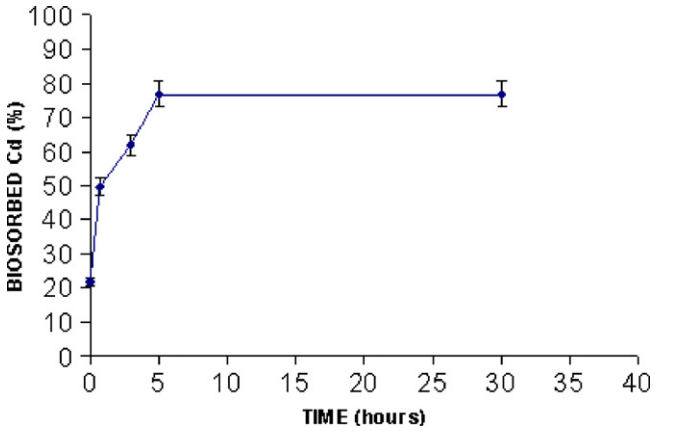

Fig. 2. Cd biosorption kinetics of *P. veronii* 2E: total Cd(II) biosorbed as % of initial concentration as a function of incubation time (32 °C, 200 rpm, pH 7.5).

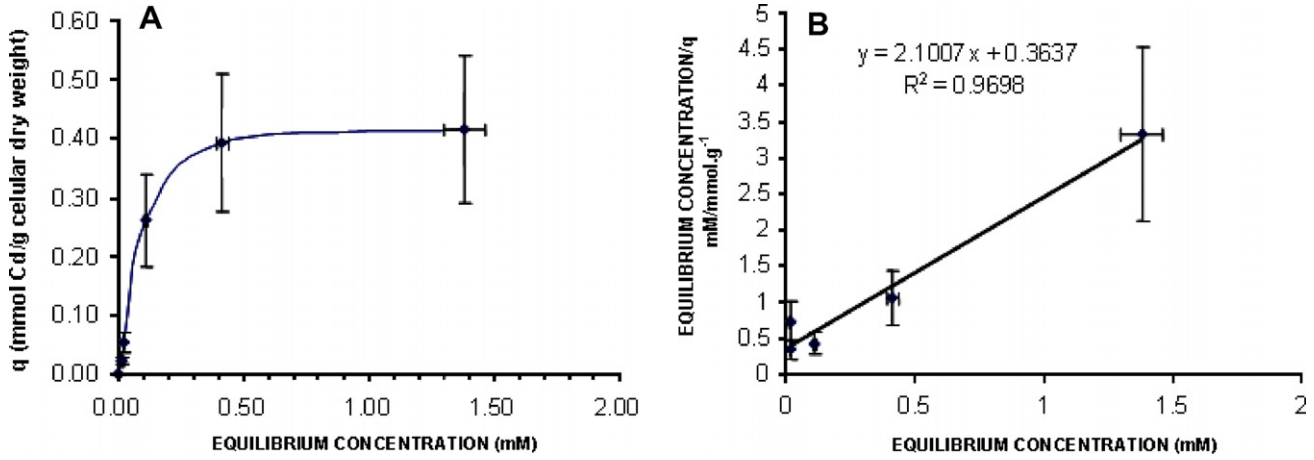

Fig. 3. Biosorption isotherm of Cd by *P. veronii* 2E: (A) q (number of occupied sites: mmol Cd biosorbed per g cellular dry weight) vs. cadmium equilibrium concentration: (◆) experimental data, (B) Langmuir model linearization.

Table 4
Comparison of Cd(II) biosorption Langmuir parameters (K_d, q_{max}) between microorganisms reported in the literature (Chang et al., 1997; Yan and Viraraghavan, 2003; Qi et al., 2006; Yin et al., 1999) and *P. veronii* 2E

Microorganism	K_d		q_{max}	
	mg/L	mM	mg/g	mmol/g
Pseudomonas aeruginosa PU21	1.25	–	57.37	–
	0.59		43.44	
	0.39		40.80	
Mucor rouxii	0.17	–	8.46	–
Bacillus cereus	20	–	44.4	–
Rhizopus arrhizus	–	0.082	–	0.56
P. veronii 2E	19	0.17	54	0.48

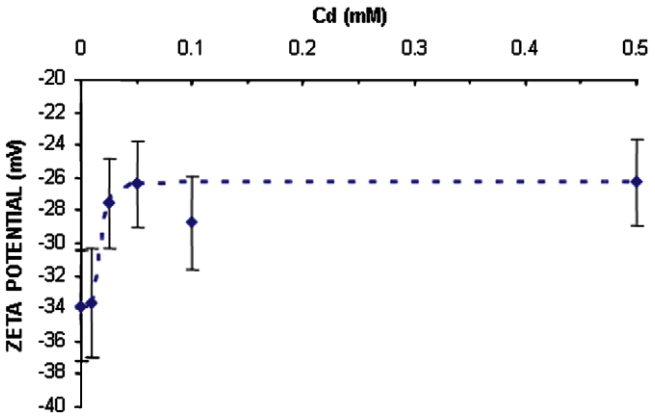

Fig. 4. Effect of increasing cadmium concentration on *P. veronii* 2E electrophoretic mobility.

different microorganisms, including *P. veronii* 2E, are in the same range.

Bacterial cell surface properties play an important role in many physical phenomena. Metal biosorption depends upon the available surface negative charges. Cd(II) biosorption could affect these surface negative charges and thus bacterial electric properties. Electrophoretic mobility measurements are generally done to understand bacterial cell electric properties (Borrok and Fein, 2005; Hayashi et al., 2003; Hetzer et al., 2006), determining Zeta potential values. Results of bacterial electrophoretic mobility experiments in presence of Cd(II) are shown in Fig. 4. Higher values of Zeta potential were obtained with increasing Cd concentrations. Since this parameter is proportional to the electrophoretic mobility, these results mean that the number of exposed negative charges on bacterial surface decreased with concentrations of 0.01 and 0.025 mM Cd. For concentrations greater than 0.05 mM, no changes are observed in Z potential, which could be explained by the saturation of the cell surface sites available for Cd, under the experimental conditions set for this assay.

4. Conclusion

In conclusion, *P. veronii* 2E may be considered a good candidate for the biosorption of metals, in particular cadmium. When designing a reactor for water treatment, it is important to achieve the best conditions for metal retention at the lowest cost. Experimental biosorption conditions (pH 7.5, 3 g dry weight biomass/L, 32 °C) were studied in absence of microbial nutrients to ensure a low complexing capacity of medium components and high bioavailability of metals. Also, for an *ex situ* bioremediation process, costs will be lower when there is no need to include nutrients. The use of fixed or fluidized bed reactors is preferred because of easier recovery of the treated effluent, so a successful bacterial immobilisation on different matrices is required. Experiments on complexing capacity evaluation in industrial effluents are necessary to verify the metal bioavailability and improve the efficiency of the process. Our immobilisation and sorption results can be used to guide and optimize bioremediation pilot-scale experiments using this indigenous microorganism as metal biosorbent.

Acknowledgements

This work was supported by the Universidad Nacional de General Sarmiento.

We are grateful to Dr. Marcela Ferrero (PROIMI, Tucumán, Argentina) for the 16S r-RNA identification of P2 strain and to Dr. Roberto Candal (INQUIMAE, Buenos Aires, Argentina) for his help on electrophoretic mobility experiments.

References

Ahluwalia, S.S., Goyal, D., 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour. Technol. 98 (12), 2243–2257.

Akhtar, N., Iqbal, J., Iqbal, M., 2004. Removal and recovery of nickel (II) from aqueous solution by loofa sponge-immobilized biomass of *Chlorella sorokiana*: characterization studies. J. Hazard. Mater. B 108, 85–94.

Arica, M.A., Kaçar, Y., Genç, O., 2001. Entrapment of white-rot fungus *Trametes versicolor* in Ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution. Bioresour. Technol. 80, 121–129.

Arica, M.A., Bayremoglu, G., Yilmaz, M., Bektas, S., Genç, O., 2004. Biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} by Ca-alginate and immobilized wood-rotting fungus *Funalia trogii*. J. Hazard. Mater. B 109, 191–199.

Bender, J., Phillips, P., 2004. Microbial mats for multiple applications in aquaculture and bioremediation. Bioresour. Technol. 94, 229–238.

Borrok, D.M., Fein, J.B., 2005. The impact of ionic strength on the adsorption of protons, Pb, Cd and Sr onto surfaces of Gram negative bacteria: testing non-electrostatic diffuse and triple monolayer models. J. Colloid Interf. Sci. 286, 110–126.

Bridge, T.M., White, C., Gadd, G.M., 1999. Extracellular metal-binding activity of the sulphate-reducing bacterium *Desulfococcus multivorans*. Microbiology 145, 2987–2995.

Ceretti, H.M., Vullo, D.L., Zalts, A., Ramírez, S.A., 2006. Cadmium complexation in culture media. Electroanalysis 18 (5), 493–498.

Chang, J.S., Raw, R., Chang, C.C., 1997. Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. Water Res. 31 (7), 1651–1658.

Chang, W.C., Hsu, G.S., Chiang, S.M., Su, M.C., 2006. Heavy metal removal from aqueous solution by wasted biomass from a combined AS-biofilm process. Bioresour. Technol. 97 (13), 1503–1508.

Davis, T.A., Volesky, B., Mucci, A., 2003. A review of the biochemistry heavy metal biosorption by brown algae. Water Res. 37, 4311–4330.

Gadd, G.M., 2000. Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr. Opin. Biotechnol. 11, 271–279.

Good, N.E., Winget, G.D., Winter, W., Connolly, T., Izawa, S., Singh, R.M.M., 1966. Hydrogen ion buffers for biological research. Biochemistry 5 (2), 467–477.

Han, R., Zhang, J., Zou, W., Xiao, H., Shi, J., Liu, H., 2006. Biosorption of copper(II) and lead(II) from aqueous solution by chaff in a fixed-bed column. J. Hazard. Mater. 133 (1–3), 262–268.

Hayashi, H., Seiki, H., Tsuneda, S., Hirata, A., Sasaki, H., 2003. Influence of growth phase on bacterial cell electrokinetic characteristics examined by soft particle electrophoresis theory. J. Colloid Interf. Sci. 264, 565–568.

Hetzer, A., Daughney, C.J., Morgan, H.W., 2006. Cadmium ion biosorption by the thermophilic bacteria *Geobacillus stearothermophilus* and *G. thermocatenulatus*. Appl. Environ. Microbiol. 72 (6), 4020–4027.

Lim, P.E., Mak, K.Y., Mohamed, N., Noor, A.M., 2003. Removal and speciation of heavy metals along the treatment path of wastewater in subsurface-flow constructed wetlands. Water Sci. Technol. 48 (5), 307–313.

Lin, C.C., Lin, H.L., 2005. Remediation of soil contaminated with the heavy metal (Cd2+). J. Hazard. Mater. 122 (1–2), 7–15.

Liu, Y., Xu, H., Yang, S., Tay, J., 2003. A general model for biosorption of Cd, Cu, and Zn by aerobic granules. J. Biotechnol. 102, 233–239.

Lloyd, J.R., Lovley, D.R., 2001. Microbial detoxification of metals and radionuclides. Curr. Opin. Biotechnol. 12, 248–253.

Lovley, D.R., 2000. Environmental Microbe-Metal Interactions. American Society for Microbiology, Washington, DC.

Malik, A., 2004. Metal bioremediation through growing cells. Environ. Int. 30, 261–278.

Martell, A.E., Smith, R.M., 2001. NIST Critically Selected Stability Constants of Metal Complexes Database 46 Version 6.0.

Munoz, R., Alvarez, M.T., Munoz, A., Terrazas, E., Guieysse, B., Mattiasson, B., 2006. Sequential removal of heavy metals ions and organic pollutants using an algal-bacterial consortium. Chemosphere 63 (6), 903–911.

Olguin, H.F., Puig, A., Loez, C.R., Salibian, A., Topalian, M.L., Castañe, P.M., Rovedatti, M.G., 2004. An integration of water physicochemistry, algal bioassays, phytoplankton, and zooplankton for ecotoxicological assessment in a highly polluted lowland river. Water Air Soil Pollut. 155, 355–381.

Patterson, J.W., Minear, R.A., Gasca, E., Petropoulou, C., 1998. Industrial discharges of metals to water. In: Allen, H.E., Garrison, A.W., Luther, G.W., IIIIII (Eds.), Metals in Surface Waters. Ann Arbor Press (Sleeping Bear Press), Chelsea, Michigan, USA, pp. 37–65 (Chapter 3).

Qi, H.Y., Hu, Q., Dou, M.N., Zeng, J.H., 2006. Biosorption of Cd^{2+} by a hyperresistant newly isolated *Bacillus cereus* strain. In: 11th International Symposium on Microbial Ecology. Vienna, Austria, Abstract page 79.

Soares, H.M.V.M., Conde, P.C.F.L., Almeida, A.A.N., Vasconcelos, M.T.S.D., 1999. Evaluation of *n*-substituted aminosulfonic acids pH buffers with a morpholinic ring for cadmium and lead speciation studies by electrochemical techniques. Anal. Chim. Acta 394, 325–335.

Soares, H.M.V.M., Conde, P.C.F.L., 2000. Electrochemical investigations of the effect of N-substituted aminosulfonic acids with a piperazinic ring pH buffers on heavy metal processes which may have implications on spectiation studies. Anal. Chim. Acta 421, 103–111.

Topalian, M.L., Rovedatti, M.G., Castañe, P.M., Salibian, A., 1999a. Pollution in a lowland river system. A case study: the Reconquista River (Buenos Aires, Argentina). Water Air Soil Pollut. 114, 287–302.

Topalian, M.L., Castañe, P.M., Rovedatti, M.G., Salibian, A., 1999b. Principal component analysis of dissolved heavy metals in water of the Reconquista River (Buenos Aires, Argentina). Bull. Environ. Contam. Toxicol. 63, 484–490.

U.S. EPA, 1995. Development Document for Proposed Effluent Limitations Guidelines and Standards for the Centralized Waste Treatment Industry, EPA 821/R-95-006. U.S. Environmental Protection Agency, Office of Water, Washington, DC.

Umrania, V.V., 2006. Bioremediation of toxic heavy metals using acidothermophilic autotrophes. Bioresour. Technol. 97 (10), 1237–1242.

Valls, M., de Lorenzo, V., 2002. Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. FEMS Microbiol. Rev. 26, 327–338.

Vasconcelos, M.T.S.D., Azenha, M.A.G.O., Almeida, A.A.N., 1998. Copper (II) complexation properties and surfactant activity of 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid and *N*-(2-hydroxyethyl)piperazine-*N*′-2-hydroxypropanesulfonic acid pH buffers which may affect trace metal speciation in *in vitro* studies. Anal. Biochem. 265, 193–201.

Vijayaraghavan, K., Jegan, J., Palanivelu, K., Velan, M., 2005. Biosorption of copper, cobalt and nickel by marine green alga *Ulva reticulata* in a packed column. Chemosphere 60 (3), 419–426.

Vullo, D.L., Ceretti, H.M., Ramírez, S., Zalts, A., 2003. Metal retention in calcium alginate (Retención de metales en gel de alginato de calcio). VI Society of Environmental Toxicology and Chemistry Latinoamerican Annual Meeting (SETAC), Buenos Aires, Argentina.

Vullo, D.L., Ceretti, H.M., Hughes, E.A., Ramírez, S., Zalts, A., 2005. Indigenous heavy metal multiresistant microbiota of Las Catonas stream. Environ. Monit. Assess. 105, 81–97.

Yan, G., Viraraghavan, T., 2003. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. Water Res. 37, 4486–4496.

Yin, P., Yu, Q., Jin, B., Ling, Z., 1999. Biosorption removal of cadmium from aqueous solution by using pretreated fungal biomass cultured from starch wastewater. Water Res. 33 (8), 1960–1963.