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Metal ion accumulation by immobilised cells of *Brevibacterium* sp

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This paper explores the use of an experimental system based on polyacrylamide-entrapped cells of *Brevibacterium* sp strain PBZ for the removal of metal ions from solutions. Experiments were performed in columns filled with the immobilised cells and challenged with influents containing 20 mg L^{-1} of lead and 10 mg L^{-1} of cadmium. The cells were able to accumulate lead (about 40 mg g^{-1} dry biomass) and, to a lesser extent, cadmium (about 13 mg g^{-1} dry biomass) from solutions. In the presence of 0.4 g L^{-1} of glucose, the cells removed up to 53% of lead. Lead competed with cadmium for attachment to the binding sites when a solution containing both the metals was applied. Lead removal occurred by a combination of fast physico-chemical adsorption and prolonged low rate accumulation mediated by cell metabolism. The biosorptive capacity of the cells was sensitive to pH. Desorption of the metal with EDTA restored the binding capability of the cells.

Keywords: lead; cadmium; bioaccumulation; immobilised cells; *Brevibacterium* sp

Introduction

Many industrial effluents from the mining, metal plating, smelting and petroleum industries contain various undesirable toxic heavy metals and can generate significant environmental problems. During the last decade a growing number of studies have been carried out on heavy metal removal from waste waters using various biosorbents [5,9,23–25]. The use of microorganisms, selected for their metal sorptive capacity, in water treatment may represent an advantage over conventional physico-chemical waste treatments and metal recovery systems [7,8,13,22,26,27].

The high surface-volume ratio of the microbial cells allows a better contact between the biosorbent surface and soluble ions and molecules [6,8,20]. On the other hand, because of the difficulty of separating microbial cells from the liquid phase, cell immobilisation procedures may be required for the development of large-scale recovery processes. The most common strategies employed to attain such a goal, both on laboratory and industrial scales, include the colonization of cells on a solid substrate (biofilm), or their entrapment in a polymeric or porous matrix [14,16,18,19].

The biological effects of lead and cadmium are of great concern: these metals are discharged from a variety of industrial activities and their concentrations are often well over the water quality standards for industrial waste waters.

In previous studies the *Brevibacterium* sp strain PBZ, isolated from a lead-polluted soil, was characterised for metal resistance and bioaccumulative capacity for lead [1,2]. The aim of this work was to explore the feasibility of using immobilised cells of this microorganism in a laboratory-column system for the removal of lead and cadmium from solutions.

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Materials and methods

Organism and growth conditions

Experiments were carried out with *Brevibacterium* strain PBZ [2]. The medium (BTG) employed for cell cultures contained 10 g L^{-1} Bacto Tryptone (Difco Laboratories, Detroit, MI, USA) supplemented with 2 g L^{-1} glucose; the microorganism was maintained on the same solidified medium. In this medium, *Brevibacterium* strain PBZ tolerated lead up to 7 mmol L^{-1} and cadmium up to 0.5 mmol L^{-1}. Bacterial cells for immobilisation were grown aerobically in 500 ml conical flasks containing 200 ml of BTG broth on a rotary shaker (100 revolutions per min) at 30°C. Cells were harvested during the late exponential growth phase (18 h) by centrifugation (10 min at 10 000 × *g*), and washed twice with bidistilled water. About 2.5 g of cell pellet were resuspended in 20 ml of physiological solution (NaCl 8.5 g L^{-1}). An aliquot of this suspension was dried at 105°C for the determination of dry cell weight. Metal ion accumulation experiments were performed with both whole cells and a cell envelope fraction which included cell walls and cytoplasmic membranes.

Cell envelope fractionation

A cell suspension (6 ml) was twice disrupted in a French press (American Instrument Co, Washington DC, USA) at 20 000 p.s.i. The resulting lysate was quantitatively recovered and fractionated by centrifugation at 50 000 × *g* (20 min, 4°C) to separate the cell envelope fraction from soluble cytoplasmic constituents. The cell envelope fraction was washed with bidistilled water and resuspended in a final volume of 6 ml with physiological solution.

Cell immobilisation and determination of Pb^{2+} and Cd^{2+} uptake by immobilised cells

For cell immobilisation, 6 ml of either whole cells or cell envelope suspension were added to 11 ml of a solution containing 1.9 g of acrylamide monomer and 0.1 g of N,N′ methylenebisacrylamide. The immobilisation reaction was conducted by adding 3 ml of a 0.5% (w/v) solution of potassium persulphate and 0.1 ml of N,N,N′,N′tetramethylenediamine. The solutions were previously degassed by sonication and the polymerisation was performed on ice, within a glass cylinder, under a nitrogen atmosphere. Once set, the gel was shredded by forcing it through a 16-mesh stainless steel sieve and the fragments were repeatedly washed with physiological solution to remove the unreacted monomers. A cell-free gel was prepared by the same procedure and cell-free controls were run concurrently in all experiments. Glass columns (1.8 × 30 cm), manufactured with a thermal jacket, were packed with freshly shredded gel.

The packed columns were fed with 1 L of a solution of lead nitrate (20 mg L^{-1} of metal) containing sodium chloride (8.5 g L^{-1}) and 2 mM morpholinoethansulfonic (MES) buffer, adjusted to the desired pH. No precipitation occurred within the pH range employed in the experiments (4.6–6.8). When required, the lead solutions were supplemented with glucose at various concentrations. The removal capacity of cadmium by the immobilised whole cells was evaluated by fluxing the columns with 1 L of a cadmium chloride solution (10 mg L^{-1} of metal). To establish the existence of competing ion effects, two packed columns were loaded both with lead solution (20 mg L^{-1} of metal) and with mixed solutions at the same concentrations. The experiments were performed at 30°C and the flow rate (120 ml h^{-1}) was regulated by using a multi-channel peristaltic pump (Miniplus 2, Gilson SA, Villiers-le-Bel, France). Aliquots (20 ml) of the column outflow were collected using a fraction collector (Multirac 2111, LKB Producter AB, Bromma, Sweden). Metal content was measured by atomic absorption spectrometry (AAS) on the aliquots after acidification with nitric acid (70% v/v). Metal removal capacity was evaluated by subtracting the metal concentration in the aliquots obtained from the cell-free column with those obtained from columns containing cells. The sorptive capacity was expressed as the metal amount removed per unit of cell dry weight. At the end of the bioaccumulation process, the columns were washed with physiological solution in order to remove the residual metal solution and subsequently eluted with 150 ml of a 20-mM solution of ethylenediaminotetraacetic acid (EDTA, disodium salt), at a flow rate of 120 ml h^{-1}, to solubilise the adsorbed lead. The eluates were collected and the metal content was measured by AAS after a nitric acid digestion treatment performed according to APHA standard methods [3]. For re-use of the column the gel was washed with 150 ml of physiological solution to remove the complexing agent after the treatment with EDTA. The washed gel was then used for a second treatment of the influent.

Metal analyses

The determination of lead and cadmium was performed by using a Varian SpectrAA20 (Varian Techtron Ltd, Springvale, Australia) absorption spectrometer. All glassware was previously washed with hot 30% HNO_3, and rinsed with bidistilled water.

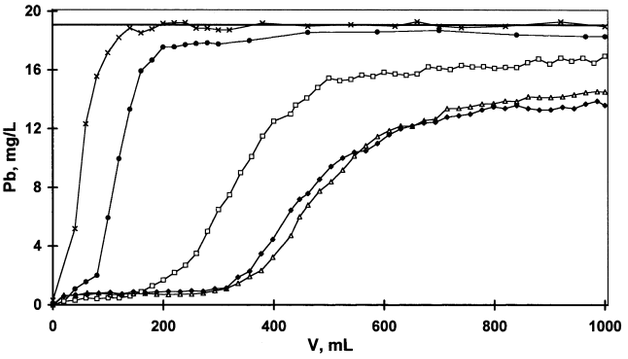

Figure 1 Effluent Pb concentration after treatment with *Brevibacterium* sp strain PBZ immobilised cells. The columns (dry biomass—referred to whole cells—179 mg) were challenged with Pb solutions, pH 6.3, at a flow rate of 120 ml h^{-1}. Pb concentration: (—) influent; effluent: (-x-) cell-free, (-●-) cell envelope fraction, (-□-) whole cells, (-◆-) 0.4 g L^{-1} glucose-supplemented whole cells, (-△-) 3.0 g L^{-1} glucose-supplemented whole cells.

Results

Figure 1 shows the pattern of outflow concentrations from the columns challenged with lead solutions with and without added glucose. Table 1 shows the overall removal and uptake of lead by the cell envelope fraction and by whole cells. The cells accumulated a higher amount of lead in the presence of glucose. The immobilised cell envelope fraction removed a small amount of the metal and an early saturation of the binding sites was observed. Less than 0.01 mg of metal per ml of gel were removed in the control column, thus suggesting that the polymeric matrix made a negligible contribution to metal removal.

The pH of the lead solution had a significant effect on ion uptake. Table 2 summarises both the overall removal and uptake of lead by the cells at various pH values and the recovery of the metal due to treatment with EDTA. Lead uptake by the strain PBZ increased with increasing alkalinity: pH values lower than 5 almost completely inhibited lead bioaccumulation. Cell-free columns showed no appreciable lead accumulation at pH values ranging from 4.6 to 6.8. Treatment of the columns with EDTA sol-

Table 1 Pb^{2+} bioaccumulation by immobilised cells

	Pb^{2+} removal (mg)	Pb^{2+} uptake (mg Pb g^{-1} biomass d.w.)
Cell envelope fraction	1.3 (1.03–1.54)	7.2 (5.89–8.42)
Whole cells	7.0 (6.51–7.46)	39.1 (35.57–42.63)
Whole cells in the presence of glucose, 0.4 g L^{-1}	10.6 (9.71–11.50)	59.4 (52.9–65.89)
Whole cells in the presence of glucose, 3.0 g L^{-1}	10.6 (9.90–11.31)	59.3 (53.92–64.61)

Experiments carried out at pH 6.3. Dry biomass, 179 mg. Data reported are averages of two experiments; single results in brackets.

Table 2 Effect of pH on Pb^{2+} bioaccumulation by immobilised cells

pH	Pb^{2+} removal (mg)	Pb^{2+} uptake (mg Pb g^{-1} biomass d.w.)	Pb^{2+} recovered by EDTA (mg)	R[a] (%)
4.6	1.9 (1.64–2.16)	6.5 (5.85–7.24)	0.9 (0.71–1.23)	47
5.8	7.2 (6.45–7.91)	24.9 (21.64–28.31)	5.2 (4.98–5.35)	72
6.8	10.8 (9.63–11.90)	37.4 (32.32–42.53)	7.8 (7.77–7.91)	72

[a]R = mg Pb recovered mg^{-1} Pb removed.
Dry biomass, 289 mg. Data reported are averages of two experiments; single results in brackets.

utions allowed the recovery of part of the lead sorbed by the biomass. A release of over 70% of the accumulated metal was obtained from the columns treated with lead solutions at pH 5.8 and 6.8.

The capability of immobilised cells to remove lead after a primary sorption step was checked with cells exposed to the metal after a treatment with a 20 mM EDTA solution (Figure 2). Metal recovery with EDTA did not fully restore the metal sorptive capacity of the immobilised cells. The best performance was again observed in the column supplied with glucose, where about 50% of the metal sorptive capacity was restored during the second treatment (Table 3).

Cd^{2+} removed was 3.1 mg, corresponding to an uptake of 12.6 mg g^{-1} biomass d.w., lower than that observed for lead. Lead uptake (34.9 mg g^{-1} biomass d.w.) was not affected by the presence of cadmium; however, cadmium uptake was significantly inhibited by lead (7 *vs* 12.6 mg g^{-1} biomass d.w.; Figure 3).

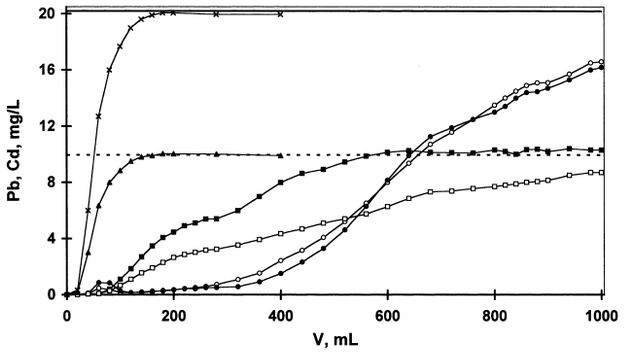

Figure 3 Effluent Pb and Cd concentrations after treatment with *Brevibacterium* sp strain PBZ immobilised cells. The columns (dry biomass, 245 mg) were challenged with an influent, pH 6.3, containing both metals at a flow rate of 120 ml h^{-1}. Influent concentration: (—) Pb, (---) Cd; effluent: (-x-) Pb cell-free, (-▲-) Cd cell-free, (-○-) Pb, (-●-) Pb in presence of Cd, (-□-) Cd, (-■-) Cd in the presence of Pb.

Discussion

The metal-affinity of microbial surfaces is mainly due to their polyanionic nature. This property imparts a high potential on microbial cells for the removal of these elements from aqueous environments [15,26]. The development of biological techniques in water treatment requires the availability of systems which afford the easy separation of the biosorbent from the liquid phase. The utilisation of immobilised microbial cells fulfils this requirement and, in addition, minimises the disadvantages associated with the use of suspended cells.

The polyacrylamide-immobilised cells of *Brevibacterium* strain PBZ were effective in the removal of lead and cadmium from a synthetic influent. By analysing the metal concentration in effluents from gel-filled columns, we detected a pattern of lead removal with similarities to a *S*-shaped curve. Such a profile is typical of a bioaccumulative process consisting of distinct steps. In the first phase, Pb removal is almost complete, as a consequence of a quick binding of lead ions, which diffuse from the bulk solution, to the cell surface. The progressive saturation of the surface-binding sites is responsible for the decreasing removal of lead from the solution. During the final phase, the lead concentration of the effluent gradually increases, asymptotically, up to the value of the influent. In this phase the con-

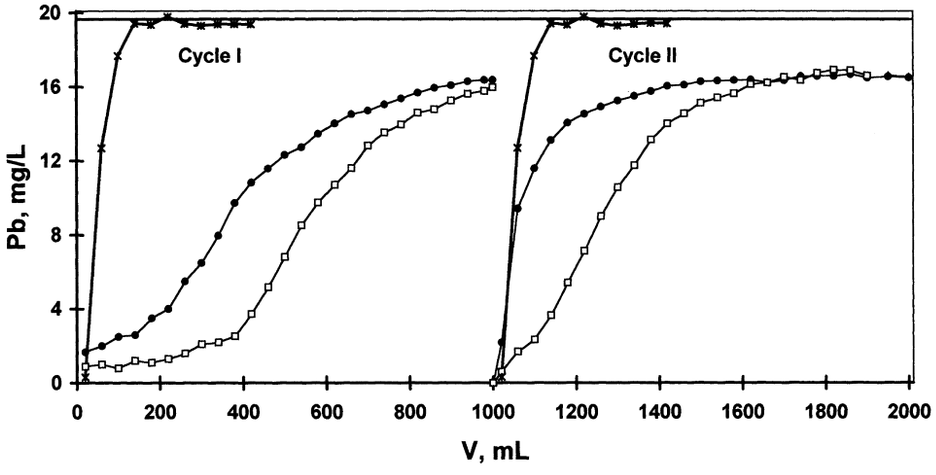

Figure 2 Effluent Pb concentration after treatment with *Brevibacterium* sp strain PBZ immobilised cells. The columns (dry biomass, 198 mg) were fed with Pb solutions, pH 6.3, at a flow rate of 120 ml h^{-1}. After treatment, the columns were washed with EDTA and rechallenged with the Pb solution. Pb concentration: (—) influent; effluent: (-x-) cell-free, (-●-) cells, (-□-) 0.4 g L^{-1} glucose-supplemented cells.

Table 3 Lead bioaccumulation by immobilised cells in repeated cycles

	Cycle I		Cycle II	
	Pb^{2+} removal (mg)	Pb^{2+} uptake (mg Pb g^{-1} biomass d.w.)	Pb^{2+} removal (mg)	Pb^{2+} uptake (mg Pb g^{-1} biomass d.w.)
Cells	7.7 (6.95–8.54)	39.1 (35.64–42.49)	3.4 (2.97–3.80)	17.1 (14.77–19.49)
Cells in the presence of glucose, 0.4 g L^{-1}	10.8 (10.48–11.09)	54.5 (53.85–55.17)	6.0 (5.49–6.42)	30.1 (27.31–32.92)

Experiments carried out at pH 6.3. Dry biomass, 198 mg. Data reported are averages of two experiments; single results in brackets.

tinuous, but slow, lead removal could be probably attributable to its transport and accumulation inside the cells. Other authors have found similar metal-bioaccumulation features using flow-through systems containing viable biomass [10,17]. Such behaviour seems to be the result of a two-step kinetics, in which extracellular and metabolism-independent metal-surface association (biosorption) usually takes place quickly. A further, slower and more prolonged metal uptake by the cells may be controlled by metabolic processes [4]. In columns containing the cell envelope fraction, lead appeared to be removed only during the initial phase by the adsorptive mechanism. The supply of a carbon source allowed higher lead bioaccumulation. Overall lead removal increased by 52% when the influent was supplemented with glucose. In the presence of glucose the high efficiency biosorption phase lasted longer although the removal capacity of the microorganism was not affected by different glucose concentrations (0.4–3 g L^{-1}). The better performance observed when glucose was added could be explained by assuming that external binding sites, previously occupied by lead ions, became free following the transport of sorbed ions towards the cell cytoplasm by a metabolism-dependent transport system.

This dual bioaccumulation mechanism in *Brevibacterium* strain PBZ has been observed in previous investigations carried out using resting cells of this microorganism. Under such conditions, cells bioaccumulated lead by combining physico-chemical sorption, metabolism-dependent transport and accumulation in the cytoplasm, where electron dense deposits containing lead, phosphorus and potassium were observed by electron microscopy [1]. Cytoplasmic lead accumulation has been recently demonstrated also in *Staphylococcus aureus* cells as a specific response of lead-resistant cells to the presence of the metal in the growth media [12]. The internal location of part of the bioaccumulated lead could hamper the complete recovery of the metal with EDTA but should still allow the restoration of the surface-binding sites. This behaviour was observed when immobilised cells of the *Brevibacterium* strain PBZ were used for two subsequent adsorption-desorption cycles. After desorption with EDTA, the lead removal capacity of the cells decreased as a consequence of the incomplete restoration of the overall potential binding capability. Lead uptake was strongly affected by the proton activity of the influent solution. Maximum Pb accumulation was obtained at the higher pH values, suggesting that competition between H^+ ions and metal cations is a factor which severely affects the bioaccumulative capacity of the biomass.

The metal sorptive capacity of the system for cadmium was lower than that for lead. A lower affinity of the cells for cadmium has also been previously determined with suspended resting cells, where a maximum bioaccumulation of 16 mg Cd g^{-1} dry cells was determined, compared with 74 mg Pb g^{-1} dry cells removed under the same conditions. The presence of Cd did not affect the Pb removal capacity of the bacterial cells. In contrast, lead competed with cadmium for binding to the cells. Similar findings, regarding the different affinity of these two metals for microbial biomass, have previously been reported for a number of different biosorbents, such as activated sludges, streptomycetes and fungi [6,10,11,21].

The cells of this strain have the potential to bioaccumulate lead and cadmium ions and may represent a biomaterial for the treatment of polluted waters containing low metal concentrations. One limitation of such material is the irreversible binding of part of the metal within the cells. This reduces the bioaccumulative capacity in subsequent treatments. The higher bioaccumulation obtainable in the presence of a carbon source suggests that this system may be suitable for waste waters containing metabolisable organic pollutants.

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