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# Selective biosorption of lanthanide (La, Eu, Yb) ions by an immobilized bacterial biomass

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**Abstract** The removal of metallic ions  $\text{La}^{3+}$ ,  $\text{Eu}^{3+}$  and  $\text{Yb}^{3+}$  from aqueous solution by immobilized biomass of *Pseudomonas aeruginosa* was investigated in batch and column reactors. Batch studies consisted in kinetic measurements for lanthanum adsorption by biomass-chitosan beads. Results did not show a significant effect of the presence of bacteria into chitosan matrix on the lanthanum uptake. Then, laboratory-scale fixed-bed column experiments were carried out using biomass-entrapped polyacrylamide gel beads, which contained approximately 48% (dry weight basis) of biomass. The lanthanum sorption was dependent on the superficial liquid velocity based on empty column in the range 0.76–2.29 m.h<sup>-1</sup>. The removal of lanthanide cations (2 mM) at pH 5.0 and 0.76 m/h was 198  $\mu\text{mol.g}^{-1}$  (dry biomass) for lanthanum, 167  $\mu\text{mol.g}^{-1}$  for europium and 192  $\mu\text{mol.g}^{-1}$  for ytterbium ( $\pm 10\%$ ). These results are of the order of 1.7–2 times lower than those observed in batch systems with free bacterial cells. Column experiments with mixed-cation solutions showed the following sequence of preferential biosorption:  $\text{Eu}^{3+} > \text{Yb}^{3+} > \text{La}^{3+}$ .

**Keywords** Chitosan; column adsorption; immobilized cells; lanthanides; *Pseudomonas aeruginosa*; selectivity

## Introduction

The search for alternative technologies for removing and/or recycling heavy metals and radionuclides from process streams has focused attention on the metal binding capacities of various microorganisms (see Texier *et al.*, 1997; Kratochvil and Volesky, 1998) and biopolymers (Jansson-Charrier *et al.*, 1996). Previous studies have shown that *Pseudomonas aeruginosa* can adsorb selectively  $\text{La}^{3+}$ ,  $\text{Eu}^{3+}$  and  $\text{Yb}^{3+}$  from aqueous solution (see Texier *et al.*, 1999). Microbial biomass in its native form is not suitable for large-scale process utilization. Techniques of cell immobilization are various. One method that has emerged as successful in the laboratory and useful in commercial applications is the encapsulation of cells in a polymer gel-matrix (Cassidy *et al.*, 1996). The aim of this work was to investigate the selective sorption of lanthanum, europium and ytterbium ions by immobilized cells of *P. aeruginosa*. Two types of carrier materials were tested: chitosan, an inexpensive and abundant biopolymer and polyacrylamide gel, an extensively used synthetic polymer with a higher mechanical strength.

## Methods

*Pseudomonas aeruginosa* (strain A 22, Institut Pasteur, Paris, France) was grown aerobically with agitation in a nutrient broth at 30°C and was harvested by centrifugation (10,000g for 15 min) at the stationary growth phase. The paste was washed three times with NaCl 9‰ solution before being dried at 37°C to constant weight and ground to a powder within a granulometric range of 500–1000  $\mu\text{m}$ .

Lanthanides solutions [ $\text{La}(\text{NO}_3)_3$ ,  $6\text{H}_2\text{O}$ ,  $\text{Eu}(\text{NO}_3)_3$ ,  $5\text{H}_2\text{O}$  and  $\text{Yb}(\text{NO}_3)_3$ ,  $5\text{H}_2\text{O}$ ; Aldrich Chemical Co.] were prepared in distilled water adjusted to pH 5.0 ( $\text{HNO}_3$ ).

### **Biomass-chitosan beads**

Flaked chitosan (0.35 g) with 80% deacetylation (France-Chitine, France) was dissolved in 30 g of 3.2% acetic acid aqueous solution previously mixed with a bacterial suspension containing 0.5 g of dried cells. Chitosan beads of approximately 3.0 mm diameter were produced by dripping the resulting viscous solution into 1 M NaOH solution. The wet chitosan gel beads were crosslinked by reaction with a 2.5 wt.% glutaraldehyde solution prior to be extensively rinsed with distilled water. The same procedure was carried out to prepare chitosan particles alone serving as a reference. For dynamic studies, approximately 50 g of chitosan particles were mixed with 200 mL of a 4 mM lanthanum ion solution. Samples of the suspension were withdrawn with a 5 mL syringe at regular time intervals and forced through a 0.2  $\mu\text{m}$  membrane microfilter. Tests were conducted to ensure that the filter apparatus did not adsorb dissolved metallic ion.

### **Column studies**

Dried cells (25% w/w) were resuspended in 10 mL of a NaCl 9‰ solution (pH 5.0). The bacterial suspension was supplemented with 8.5 mL N, N'-methylene bis-acrylamide and 6.25 mL acrylamide monomer. The polymerization reaction was initiated by adding 20  $\mu\text{L}$  N, N, N', N'-tetramethylethylenediamine (TEMED) and 0.064 g ammonium peroxodisulphate. The gel was then crushed and sieved between 500 and 1000  $\mu\text{m}$ . Experiments were also performed with polyacrylamide gel alone serving as a reference. For kinetic studies, approximately 16 g of gel beads were mixed with 500 mL of a 2 mM lanthanum ion solution. Gel particles were washed extensively with a 12 mM  $\text{NaNO}_3$  solution (pH 5.0) prior to be packed into a 1 $\times$ 30 cm column (1.5 $\pm$ 0.2 g dry weight). Solutions used for passage through the column consisted of a 2 mM lanthanum ion solution and an equimolar solution of lanthanum, europium and ytterbium, each at 1 mM.

For single-cation solutions, the final lanthanide ion concentration was determined by colorimetry using arsenazo III (Savvin, 1961), and by an energy-dispersive X-ray fluorescence apparatus (Oxford ED 2000) for the mixed-cation solutions. The metal removal capacities of immobilized cells were deduced at 50% breakthrough ( $\mu\text{mol.g}^{-1}$  dry cells).

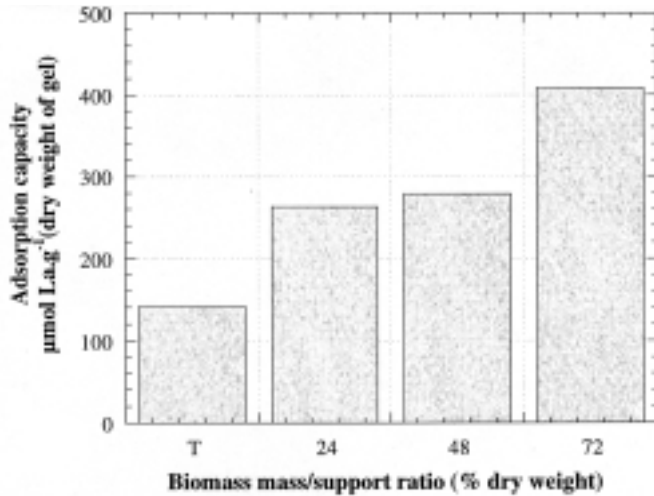
## **Results and discussion**

### **Adsorption kinetics of lanthanum on biomass-chitosan beads**

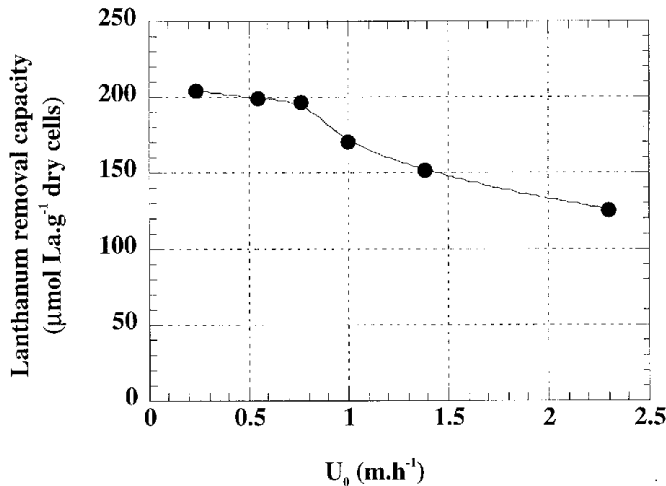
First, adsorption kinetics on chitosan matrix with and without the entrapped bacterial cells were performed at pH 5.0 for 4 mM  $\text{La}^{3+}$ . Similar adsorption dynamic curves were obtained with an uptake capacity at equilibrium (1 h) of 8.8 $\pm$ 0.7  $\mu\text{mol La.g}^{-1}$  (wet particles) for chitosan beads alone and 6.9 $\pm$ 0.7  $\mu\text{mol La.g}^{-1}$  for biomass-chitosan beads. Results do not show a significant effect of the presence of bacteria into chitosan beads on the lanthanum uptake. Consequently, preference was given to the use of polyacrylamide gel for cells immobilization.

### **Adsorption capacities of biomass/ polyacrylamide gel particles**

The first step consisted in optimization of the biomass/support ratio. Adsorption kinetics were carried out on polyacrylamide gel particles for various biomass/support mass ratios for 2 mM  $\text{La}^{3+}$ . Results for sorption capacity (1h) (expressed in  $\mu\text{mol.g}^{-1}$  dry weight of gel) are shown in Figure 1. The presence of biomass induces an increase of biosorption capacities in comparison with the gel without bacteria. The highest adsorption capacity was obtained for the biomass percentage of 72%. Meanwhile, these gel particles seemed to be less resistant than the other ones. Consequently, column experiments were conducted using a bacterial mass/support ratio of 48%.



**Figure 1** Adsorption capacities of lanthanum ( $\mu\text{mol.g}^{-1}$  gel dry weight) for various amounts of biomass in the support. T = gel without biomass serving as a reference

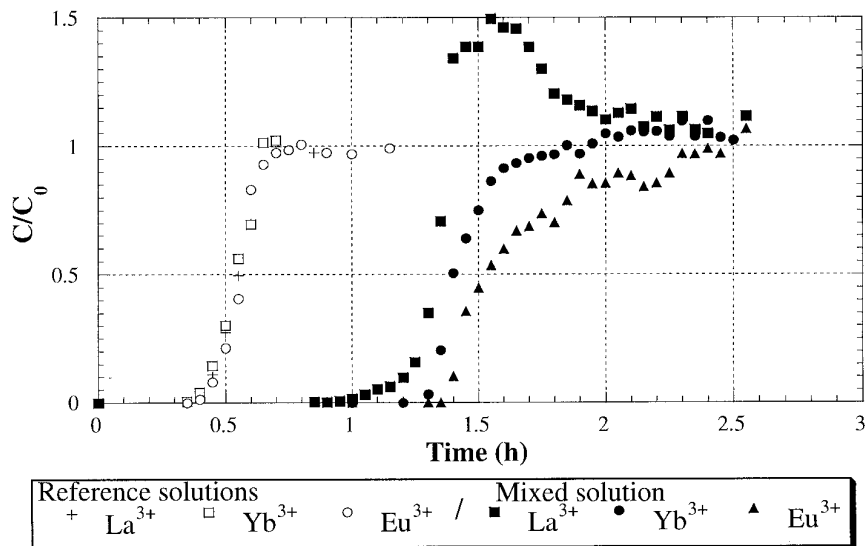


**Figure 2** Effect of the superficial liquid velocity based on empty column ( $U_0$ ) on the lanthanum (2 mM) removal capacity of polyacrylamide-gel-immobilized cells of *P. aeruginosa* (pH 5.0; support  $1.5 \pm 0.1$  g dry weight)

As shown in Figure 2, lanthanum removal was not affected by the superficial liquid velocity based on empty column ( $U_0$ ) between  $0.23$ – $0.76$   $\text{m.h}^{-1}$ .

Increasing  $U_0$  values in the range  $0.76$  to  $2.29$   $\text{m.h}^{-1}$  resulted in the decrease of the column sorption rate. For the following adsorption investigations,  $U_0$  was adjusted at  $0.76$   $\text{m.h}^{-1}$ , inducing a residence time of 24 min. Significant metal adsorption capacities of immobilized biomass were found, with  $198$   $\mu\text{mol/g}$  for lanthanum,  $167$   $\mu\text{mol.g}^{-1}$  for europium and  $192$   $\mu\text{mol.g}^{-1}$  for ytterbium ( $\pm 10\%$ ). Previous studies on free bacterial cells in batch reactors showed higher maximum adsorption capacities, with  $397$   $\mu\text{mol.g}^{-1}$  for lanthanum,  $290$   $\mu\text{mol.g}^{-1}$  for europium and  $326$   $\mu\text{mol.g}^{-1}$  for ytterbium ( $\pm 10\%$ ) (Texier *et al.*, 1999).

It was of interest to confirm the selective behaviour of *P. aeruginosa* cells when they are immobilized in polyacrylamide gel.



**Figure 3** Breakthrough curves for immobilized *P. aeruginosa* cells column fed with a solution containing lanthanum, europium and ytterbium ions, each at 1 mM (pH 5.0; support 1.7 g dry weight;  $U_0=0.76 \text{ m.h}^{-1}$ )

As shown in Figure 3, the three mixed metallic species are first sorbed non-selectively. After 1 h, cation competition for the same bacterial sites occurs.  $\text{Eu}^{3+}$  and  $\text{Yb}^{3+}$  ions are preferentially adsorbed with respect to the  $\text{La}^{3+}$  cations. Release of the lanthanum ions is characterized by a concentration peak in the eluate corresponding to the sum of the displaced lanthanum and that introduced from the feeding solution. The selective character of immobilized cells was confirmed with the following sequence of preferential adsorption:  $\text{Eu}^{3+} > \text{Yb}^{3+} > \text{La}^{3+}$  and the respective breakthrough times values : 83, 79, and 66 min.

## Conclusion

Column results have shown that *P. aeruginosa* biomass immobilized within polyacrylamide gel particles was effective and selective for removal of lanthanide ions from low-concentration wastewaters and could show promise as the basis of an immobilized-cell process for removal and separation of lanthanide ions from contaminated wastewaters.

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