

RARE EARTH ELEMENTS REMOVAL BY MICROBIAL BIOSORPTION: A REVIEW

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ABSTRACT

This paper reviews published work on the sorption of rare earth elements by microbial biomass. In a first part, the biosorption capacities and the various experimental conditions performed in batch reactor experiments are compared. Secondly, sorption modelling generally used in biosorption studies are described. Thirdly, the microbial cell wall characteristics of the metallic ion binding sites are considered. From these observations it seems that the important functional groups for metallic ion fixation are the carboxyl and the phosphate moieties. Moreover, the competing effect of various ions like aluminium, iron, glutamate, sulphate etc. is described. Finally, some adsorption results of the rare earth elements in dynamic reactors are presented.

Keywords: Adsorption, micro-organisms, mechanisms, cation competition, rare earth elements.

INTRODUCTION

In the last 15 years, numerous studies have shown that micro-organisms (bacteria, yeast, fungi) may interact with ions such as heavy metals or radionuclides. Many types of phenomena can take place, such as biosorption, bioaccumulation, resistance/detoxification mechanisms, and direct or indirect utilization in the microbial metabolism. It has been shown that lanthanum, europium and terbium, were accumulated during growth, in the space between the inner and outer membrane of the cell envelope (periplasmic space) of *Escherichia coli* [1]. On the other hand, they may influence the environment by producing mineral acids, chelating agents such as siderophores, or by-products of the metabolism (organic acids etc.). For example, the interactions between a mycobacterial siderophore (mycobactin) and europium [2] ions have been shown by a spectrophotometric approach. Moreover, some siderophores such as ferrioxamine B could deplete europium fixation by goethite or boehmite [3]. Biosorption encompasses the uptake of metals by the whole biomass (living or dead) through physico-chemical mechanisms such as adsorption, ion exchange or surface precipitation. The processes take place on the cell wall with rapid kinetics.

Rare Earth Elements (REE) are often used in industry for the production of glass additives, fluorescent materials,

catalysts, ceramics, lighters, supraconductors, magnets or condensers. They are even more widely used in agriculture, forestry and aqua-culture in which they are found in micro-element fertilizers or animal food. In Germany, the annual emission of gadolinium by hospitals is estimated at 484 kg [4], and up to 3400 kg yr⁻¹ are emitted from automobile catalysts. Thus, some authors [5] have shown that REE lanthanum and cerium could have a negative effect on the root elongation of corn and mungbean. A published review, on REE toxicity, has reported that cerium could be a potent antiseptic drug for Gram-negative bacteria and fungi [6]. The median lethal dose (LD₅₀) for the rat is 10 mg of cerium per kg of body weight. On the other hand, some natural plants collected from a rare earth ore area located in China contain significantly high levels of REE from 675 to 3358 µg g⁻¹ [7] without any apparent toxicity. Moreover, some lanthanide ions are produced in nuclear fission and could be dispersed in the environment like ¹⁴⁰La or ¹⁴¹Ce/¹⁴³Ce in the case of the Chernobyl accident in 1986 [8]. Finally, europium and gadolinium ions could be used as model elements in the extraction chemistry of the high α radioactive isotopes of Americium and Curium found in nuclear waste.

The aim of this paper is to compare, under different operating conditions, the biosorption capacities of various microbial species for rare earth elements, the biosorption mechanisms involved and to evaluate the potential industrial use of this process to metal ion removal.

DISCUSSION

Biosorption Capacities

Table 1 summarises some of the major published lanthanide biosorption experiments. The sorption capacities range between 2 to 1100 $\mu\text{mol g}^{-1}$ depending on the micro-organisms and experimental conditions. Besides, biosorption rate can be related to the nature and the composition of the microbial cell wall layers, to the rare earth cation chemistry, and to the medium conditions. Generally, the experiments take place at pH close to 5, below the pH of hydroxide

precipitation (for example, 7.8 for Lanthanum, 6.8 for Europium, 6.30 for Lutetium).

Two experiments have been carried out in acidic conditions (pH 1.5 and 0.6) in relation to the acidity of effluents. As described below, pH has an important role in the global surface charge of the cell wall. In the range of pH 3-6, the sorption capacities for lanthanum ions by *Pseudomonas aeruginosa* are slightly affected with an optimal fixation at pH 5 [9]. Among the results shown in Table 1, a few experiments have been carried out with imposed ionic strength [10, 12, 16]. Furthermore, a pre-treatment of the biomass, before utilization, has been proposed and could lead to an increase in the biosorption capacities.

Table 1. Comparison of maximum biosorption capacities of various micro-organisms for several rare earth metal ions.

	Element	Biosorption $\mu\text{mol g}^{-1}$ (dry weight)	Experimental medium conditions	References
<i>Pseudomonas aeruginosa</i> (CIP A 22)	La $^{3+}$	397	pH 5.0	[9]
	Eu $^{3+}$	290	pH 5.0	[9]
	Yb $^{3+}$	326	pH 5.0	[9]
	Gd $^{3+}$	322	pH 5.0	[10]
<i>Pseudomonas putida</i> (CCUG 28920)	Eu $^{3+}$	330	pH 6.4, 0.01 M KCl	[11]
<i>Pseudomonas aeruginosa</i>	La $^{3+}$	144	pH 4.0, 10 mM $\text{Ca}(\text{NO}_3)_2$	[12]
<i>Pseudomonas aeruginosa</i> (MTCC-1223)	La $^{3+}$	1000	pH 5.0 acetate buffer	[13]
	Pr $^{3+}$	940	pH 5.0 acetate buffer	[13]
	Nd $^{3+}$	1100	pH 5.0 acetate buffer	[13]
	Eu $^{3+}$	830	pH 5.0 acetate buffer	[13]
	Dy $^{3+}$	1000	pH 5.0 acetate buffer	[13]
			pH 5.0 acetate buffer	[13]
<i>Bacillus cereus</i>	La $^{3+}$	33	pH 4.0, 10 mM $\text{Ca}(\text{NO}_3)_2$	[12]
<i>Bacillus subtilis</i>	La $^{3+}$	114	pH 4.0, 10 mM $\text{Ca}(\text{NO}_3)_2$	[12]
<i>Bacillus subtilis</i> (CIP 52.65)	Gd $^{3+}$	350	pH 5.0	[10]
<i>Myxococcus xanthus</i>	La $^{3+}$	650	pH 4.5	[14]
<i>Mycobacterium smegmatis</i> (CIP 73.26)	Yb $^{3+}$	103	pH 1.5	[15]
	La $^{3+}$	57	pH 1.5	[16]
	Eu $^{3+}$	101	pH 1.5	[16]
	Gd $^{3+}$	110	pH 5.0	[10]
<i>Ralstonia metallidurans</i> CH34	Gd $^{3+}$	147	pH 5.0	[10]
(<i>Alcaligenes eutrophus</i> CH34)				
<i>Escherichia coli</i>	La $^{3+}$	70	pH 4.0, 10 mM $\text{Ca}(\text{NO}_3)_2$	[12]
<i>Saccharomyces cerevisiae</i>	Gd $^{3+}$	5.1	pH 5.0	[10]
<i>Saccharomyces cerevisiae</i> Brewery strain	La $^{3+}$	560	pH 4.5	[15]
Rh from VLB.				
<i>Saccharomyces cerevisiae</i>	Sc $^{3+}$	134	pH 0.6	[17]
<i>Candida valida</i>	Sc $^{3+}$	100	pH 0.6	[17]
<i>Rhizopus arrhizus</i>	La $^{3+}$	350	pH 3.5-4.0	[18]
<i>Rhizopus arrhizus</i>	Sc $^{3+}$	366	pH 0.6	[17]
Brewer's yeast waste	La $^{3+}$	650	pH 4.5 dry at 100° C, sieved 50-60 mesh	[19]
<i>Aspergillus niger</i>	Sc $^{3+}$	51	pH 0.6	[17]
<i>Aspergillus terreus</i>	Sc $^{3+}$	153	pH 0.6	[17]
<i>Shewanella putrefaciens</i> (CCUG-22948)	Pm $^{3+}$	2	pH 4	[20]

For a better understanding of the effect of the bacterial cell wall structure on biosorption, many studies investigate the characteristics of the bacterial surfaces. The first observation is that biosorption of heavy metals is accompanied of a release of calcium and/or magnesium ions [13, 15]. A possible second step, is the use of potentiometric titrations which have shown that metal ion fixation takes place on acidic moieties. This method can be used to measure the global pKa, and be extrapolated to give the intrinsic pKa. For *Pseudomonas aeruginosa*, the pKa values are 2.8 and 6.1, which allow us to conclude that the cell wall layers present two kinds of acidic function. The acid/base properties of the cell wall of *Bacillus subtilis* have been characterized and three distinct types of surface organic acid functional groups have been shown with pKa of 4.82, 6.9 and 9.4 [21]. These various values are generally attributed to carboxyl, phosphate functions and hydroxyl moieties. For Gram negative bacteria, the functional groups are, for example, present in the lipopolysaccharide of the outer layer and in the peptidoglycan and for Gram positive bacteria in the teichoic acid. Some

electronic microscopy studies have indicated that lanthanum accumulated at the surface of *Pseudomonas aeruginosa* inducing crystalline precipitation [12]. Figure 1 shows a schematic structure for the metal ions fixation in the cell wall peptidoglycan of *Mycobacterium smegmatis*.

Time resolved laser spectrofluorimetry observations of europium ions have shown (Figure 2) two main peaks around 592 and 615 nm which correspond respectively to the transition from $^5D_0$ toward $^7F_1$ and $^7F_2$ multiplet. The europium bound onto bacterial cells, presents two different lifetimes of 254 and 677 μs indicating two distinct environments for this fixed cation. The comparison of lifetimes of standard compounds (Eu-oxalate, 239 μs , Eu(PO_4), 745 μs) suggests that this cation interacts with carboxylic or phosphate functions in the cell wall of *Pseudomonas aeruginosa* [22].

Sorption Modelling in Batch Reactor

Sorption results sometimes follow the Freundlich, the Langmuir or the Brunauer-Emmett-Teller (BET) isotherms. The Langmuir approach assumes a single-layer adsorption

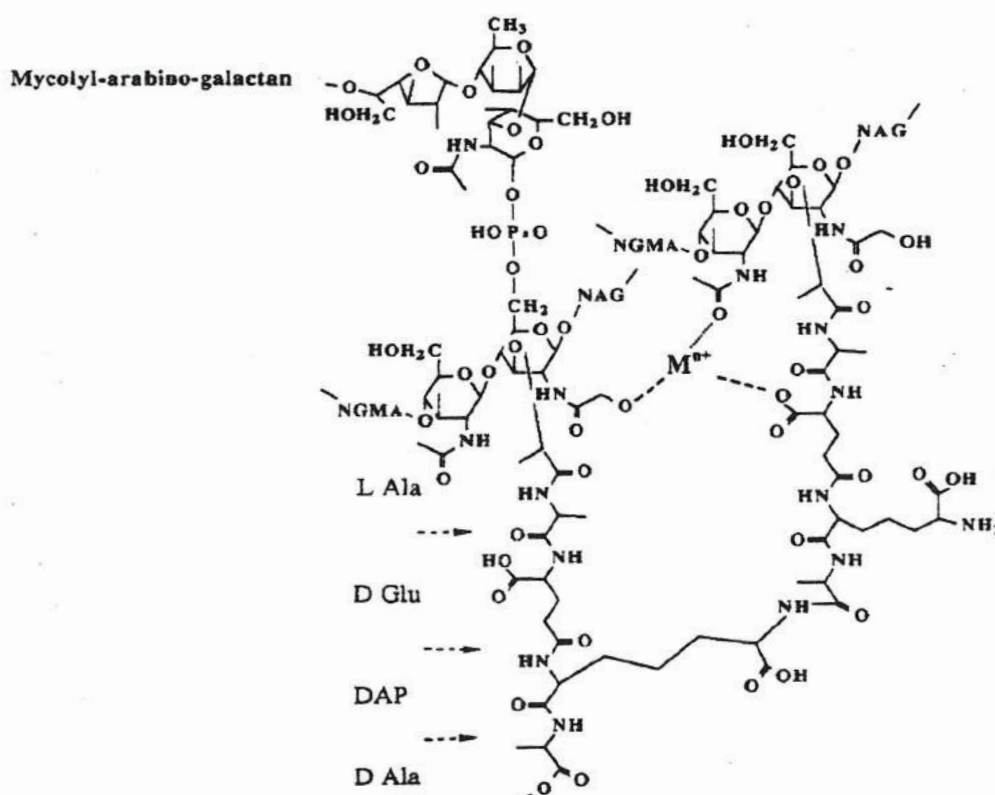


Figure 1. Schematic representation of the structure from *Mycobacterium* peptidoglycan and potential metal binding site. (NGMA : N-glycolylmuramic Acid; NAG : N-Acetylglucosamin; DAP : diaminopimelic acid; Ala : Alanine; Glu : glutamic acid).

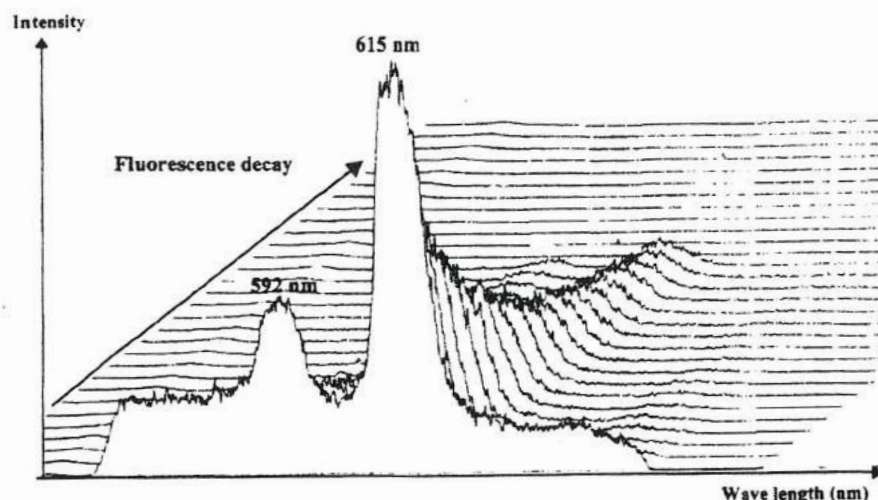


Figure 2. Fluorescence spectrum of europium, in Eu^{3+} / *Pseudomonas aeruginosa* sample prepared from $\text{Eu}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (pH 5.0) and used as dried powder at 37° C [22].

whereas the BET reflects multi-layer adsorption. Each layer of this type of model can be reduced to the Langmuir model [23]. The metal uptake from aqueous solutions can be fitted to the classical isotherm equations of Freundlich, Langmuir or Brunauer-Emmett-Teller (BET) [23].

The Freundlich equation is:

$$Q_e = K_f C_e^{1/n}$$

(i)

where K_f is the Freundlich constant ($l^{1/n} \mu\text{mol}^{1-1/n} \text{g}^{-1}$) and $1/n$: Freundlich parameter, Q_e is the adsorption capacity at equilibrium ($\mu\text{mol g}^{-1}$), C_e is the solution concentration at

equilibrium ($\mu\text{mol l}^{-1}$).

The Langmuir equation presents the following form:

$$Q_e = \frac{Q_{\max} \cdot b \cdot C_e}{1 + b \cdot C_e} \quad (\text{ii})$$

where $Q_{\max}$ is the maximum adsorption capacity ($\mu\text{mol g}^{-1}$) and b ($l \mu\text{mol}^{-1}$) is an equilibrium constant relating to the energy of interaction with the surface.

Figure 3 shows an example of the Freundlich (Figure 3a) and Langmuir (Figure 3b) equation linear fit in the case of the biosorption of La^{3+} by *Mycobacterium smegmatis*.

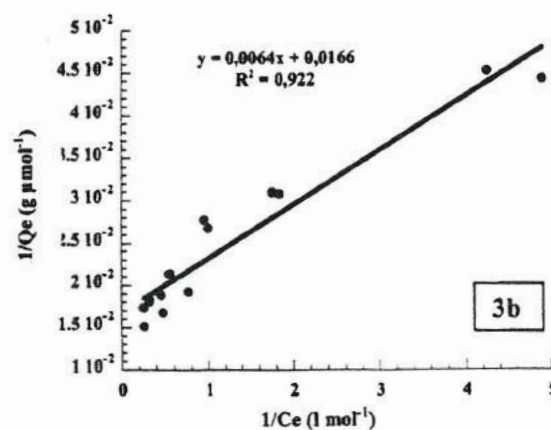
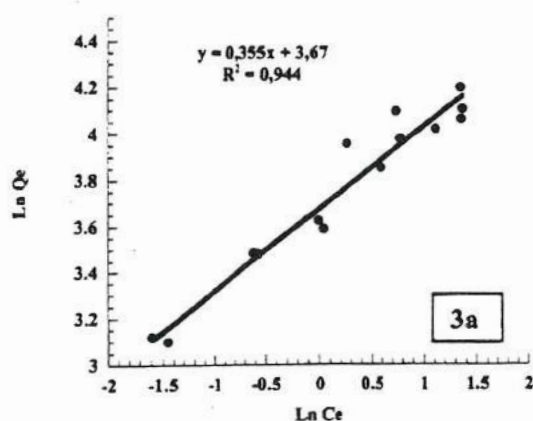


Figure 3. Freundlich (figure 3a) and the Langmuir (figure 3b) equation linear fit in the case of the biosorption of La^{3+} by *Mycobacterium smegmatis* (Lanthanum initial concentration between 0.5 and 5 mM, 5 % w/v of dry biomass, pH 1.5, for more experimental details see [15]).

The BET model which assumes a multi-layer adsorption process in which one layer need not necessarily be completely filled before another is commenced. Moreover, each adsorption layer of the BET model can be reduced to Langmuir behaviour with homogeneous surface energy.

The BET equation has the following form:

$$Q_e = \frac{Q_{\max} \cdot C_e}{(C_s - C_e) \cdot \left[1 + (b - 1) \cdot \left(\frac{C_e}{C_s} \right) \right]} \quad (\text{iii})$$

Where C_s is the solute saturation concentration ($\mu\text{mol.l}^{-1}$).

The use of the BET model leads various authors to describe a multi-layer adsorption of rare earth elements by different bacterial biomass [9,10,15].

The Scatchard model has been used [10, 13] to evaluate the affinity constants (K) of the binding sites for the gadolinium ion and the binding capacities. The linear form used is,

$$\frac{Q_e}{C_e} = K[B_{\max} - Q_e] \quad (\text{iv})$$

where K is the Scatchard affinity constant ($\text{l } \mu\text{mol}^{-1}$). A plot Q_e/C_e versus Q_e gives the K and $B_{\max}$ ($\mu\text{mol g}^{-1}$) the maximal ion binding values.

If the Scatchard plot results in a hyperbolic shape, this modelling leads to the identification of at least two types of binding sites, with high or low binding affinity (Figure 4a). If the shape is linear (Figure 4b), this approach leads to the identification of only one kind of binding site [24].

The pK values which can be calculated from this plot are near 5.22 for the binding of gadolinium by *Pseudomonas aeruginosa* for the highest affinity and 3.6 for the lowest by *Bacillus subtilis* (Figure 4a) [10]. These values are high enough to give an exchange between gadolinium fixed on sand and a non-proliferative suspension of *Ralstonia metallidurans* CH34 [10]. Another interesting result is that the medium composition for the biomass production has an influence on the biosorption capacity. For example *Ralstonia metallidurans* CH34 cultivated in a synthetic medium presents a decrease in the biosorption capacities for gadolinium of 73 %. Moreover, under these conditions, biosorption follows the Langmuir isotherm whereas in the case of the complex medium, a BET modelling is performed. On the another hand, the Scatchard plots present a hyperbolic shape corresponding, at least, for two kinds of binding site for the complex medium and a linear shape representative of a single binding site for the synthetic medium (Figure 4b). The highest affinity constant varies from 4.35 to 3.41 respectively. These observations could be correlated with the variation in the composition of the macromolecular compounds or in their quantity at the microbial surface and with the growth conditions. It has been shown [25] that the amount of the functional groups present at the cell surface could change according with the physiological state and the growth phase of the bacteria. The deprotonation constant values of these carboxyl or phosphate

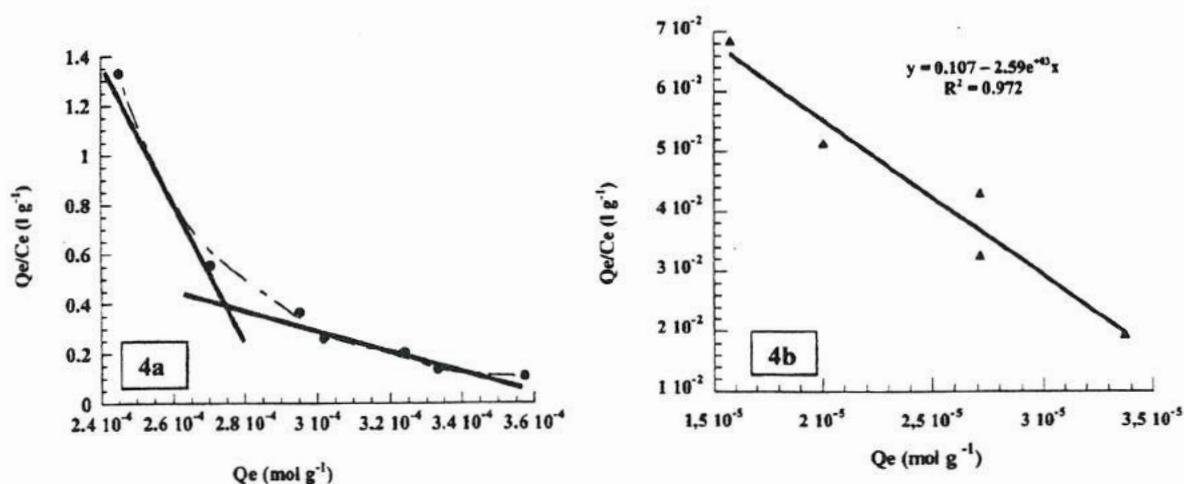


Figure 4. Scatchard plot (4a) for *Bacillus subtilis*, extrapolated affinity constants (pK) are in this case: 4.48 for the high affinity and 3.58 for the lowest (experiment carried out at pH 5, with 4 % wet biomass and a gadolinium concentration range of 2-6 mM, for more details see [10]) and (4b) for *Ralstonia metallidurans* CH34 grown in a synthetic medium [10].

functional groups also varies, with the consequence of a change in the global electronegativity of the cell wall structure of bacteria according to the growth phase.

Effects of Competing Ions

Multi-elemental studies show that in the case of lanthanum, europium and ytterbium, fixation takes place at the same cell wall site [9]. This work has pointed out a preferential adsorption for europium ions by *Pseudomonas aeruginosa* biomass. The influence of the presence of cations (Al^{3+} , Ca^{2+} , Na^+ , K^+) and anions (NO_3^- , SO_4^{2-} , Cl^-) in the solution, on biosorption performance has also been studied. Aluminium appears to be the more inhibitive ion for the fixation of europium, lanthanum and ytterbium by *Pseudomonas aeruginosa*. The presence of glutamate, sulphate, phosphate, carbonate, chloride and ethylenediaminetetraacetate (EDTA) in solution with metal ratios up to 15, affects the biosorption of lanthanum by a *Rhizopus arrhizus* biomass [26]. Furthermore, the biosorption of lanthanum by *Mycobacterium smegmatis* is greatly decreased by the presence in solution of thorium or uranyl ions at pH 1.0 [15]. Scandium biosorption by various micro-organisms could be decreased between 56 and 94 % in the presence of aluminium, iron and titanium [17]. Some authors [27] have studied the effect of competing ions on the biosorption of metals in relation to their Pearson classification (soft, hard and borderline species) [28]. In the case of the "hard-hard" pair represented by uranium and yttrium, the biosorption of the latter is strongly depressed. More generally, a significant ionic competition effect is observed for metals belonging to the same class of the hard and soft classification proposed by Pearson [27].

Adsorption in Dynamic Reactors

The applicability of biosorption in continuous metal recovery processes has received increasing attention from researchers because of this potential industrial applications [29]. In fact, metal sorption on cell surfaces can occur with non-living micro-organisms and other biological materials. The latter can be low-cost sorbents, available in large quantities, as by-products of industrial fermentations, activated sludges, or specific micro-organisms produced using inexpensive growth media. The numerous factors which must be taken into consideration for the applications of biosorption technologies has been described [30].

Promising results from equilibrium batch studies presented in Table 1 for lanthanide ions have led many researchers to continue investigations in flow-through sorption column systems. At first, dynamic studies were performed in a laboratory-scale fixed bed reactor by using immobilized microbial cells as a biosorbent for the removal of heavy metal ions from aqueous solutions. Column experiments are a simple and reproducible method, commonly used to assess the metal removal performances of

the biosorbent material in a continuous system. An advantage of this process, using a fixed bed of immobilized micro-organisms, is to avoid the separation of the two phases: the biomass and the effluent. A successful method emerging in laboratories and that could be useful in commercial applications is the encapsulation of cells in a polymer gel-matrix [31] or by immobilization onto activated alumina [13]. The immobilisation procedure converts the biomass to a spherical shape for use like conventional adsorbents (ion exchange resins or activated carbon materials). The sizes range from 0.5 to 1.5 mm with a good external porosity, and chemical and physical resistance is generally achieved for the sorbent particles. The use of materials with lattice structures able to enclose the micro-organisms, such as polyacrylamide gels, polyurethane polymers or calcium alginate, have been described in the literature. In the case of selective biosorption, calcium alginate, commonly used for cell immobilisation, contains complexing carboxyl groups which could interfere with the metal ions. On the other hand, polyacrylamide gels have been extensively used as immobilisation materials at a laboratory scale. Their major advantage is a simple and rapid experimental protocol for encapsulation. Moreover, the small pore size and the mechanical stability of these biosorbent beads may be useful for applications in bioreactors because of the low leakage between the cells and the matrix. It has been observed [32] that the selective adsorption properties of a mycobacterial biomass were not affected by the presence of this synthetic polymer, as seen in Figure 5. This figure shows an initial stage of non-selective adsorption of lanthanum, thorium and uranyl ions. In the second stage, cation competition occurs with continuing fixation of thorium and displacement first of lanthanum and then of uranyl ions. The uranium release is followed by a concentration peak in the eluent corresponding to the sum of the displaced ions and those introduced from the feeding solution. A similar phenomenon has been described for the selective adsorption of europium ions from a solution containing lanthanum, europium and ytterbium ions, by immobilized cells of *Pseudomonas aeruginosa* [33]. The selective feature of immobilized cells has been confirmed by the following sequence of preferential adsorption: $\text{Eu}^{3+} > \text{Yb}^{3+} > \text{La}^{3+}$.

The breakthrough curves performed in a dynamic process could be successfully modelled by using classical models or a statistical approach like neural networks [34].

Numerous studies present desorption experiments with a recycling step of the biosorbent. Desorption studies with EDTA lead to a concentration factor of 6 for lanthanide ions. The immobilized biomass could be re-used after an elution step [32]. Nevertheless, with three adsorption/desorption cycles, the lanthanum adsorption capacity of the biomass decreased from $Q_{\text{max}} = 190 \mu\text{mol g}^{-1}$ (cycle 1) to about $Q_{\text{max}} = 98 \mu\text{mol g}^{-1}$ ($\pm 10\%$) (cycle 3) [34]. In the case of *Pseudomonas aeruginosa* immobilized onto activated alumina the efficiency of lanthanum adsorption was 80 % in the second cycle [13].

Desorption of lanthanum from yeast biomass has been performed using HCl , Na_2CO_3 , CaCl_2 and EDTA at 0.1 M,

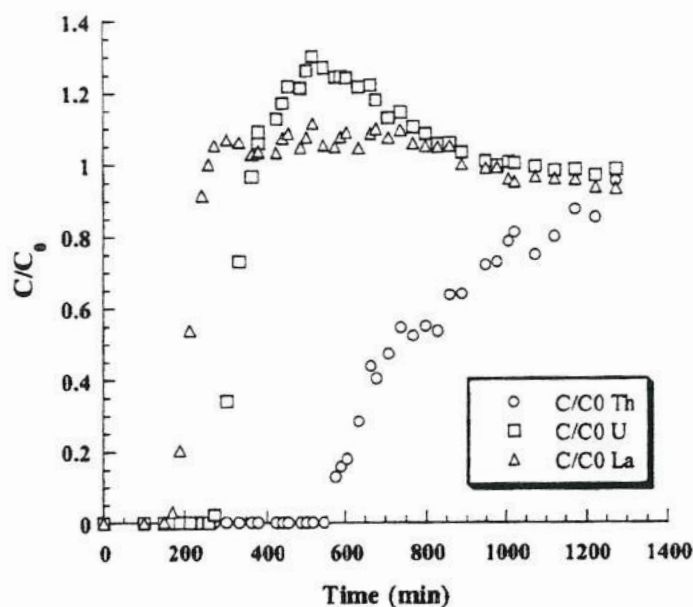


Figure 5. Breakthrough curves for immobilized *M. smegmatis* cells in a column fed with a solution containing 200 μM lanthanum, 100 μM thorium and 100 μM uranyl ions (pH 1.6, support 0.87 g dry weight, flow rate 0.2 ml min⁻¹, dead volume of the column 20 ml).

with respectively 88.3, 68.7, 11.4 and 99.4 % recovery [19]. Alternatives include the use of sodium carbonate or bicarbonate [14] or citrate buffer at pH 4.0 [13].

Pilot Scale Studies

Some pilot scale studies for the treatment of solution containing heavy metals are described in this last part. Industrial fermentation by-products composed of *Streptomyces* mycelium was used for the biosorption of uranium, iron and sulphates ions from low acidic solution in a 40 m³ batch reactor [35]. The AMT-Bioclaim process (Advanced Mineral Technologies, Colorado USA) is composed of *Bacillus subtilis* biomass produced in industrial fermentation and was used to treat solutions containing lead, copper, zinc, cadmium and silver [36]. A mixed powder of cyanobacteria (*Spirulium*) yeast and plant biomass (*Lemnu*, *Sphagnum* sp.) immobilized with xanthan was commercially proposed under Bio-Fix trademark for zinc removal with the possibility of 120 adsorption/elution cycles [37]. Some pilot scale studies were conducted using a fluidized bed reactor for the remediation of 150 l of water polluted with Zinc ions [38].

CONCLUSION

The industrial use of low cost biosorbents, like micro-organisms, has been of increasing interest in environmental

remediation. In the longer term, the use of microbial biomass as specific chemical-binding agents seems to be very promising. The optimisation of the biosorption conditions, the location of the rare earth element binding sites and the studies of the sorption capacities of immobilized cells are good arguments for using biosorption in the industrial removal of heavy metal from solutions.

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List of symbols

- b : Equilibrium constant relating to the energy of interaction with the surface ($\text{l } \mu\text{mol}^{-1}$)
- B_{max} : Maximal ion binding capacity ($\mu\text{mol g}^{-1}$)
- C_e : Ionic solution concentration at equilibrium ($\mu\text{mol l}^{-1}$)
- C_s : Solute saturation concentration ($\mu\text{mol l}^{-1}$)
- K : Scatchard affinity constant ($\text{l } \mu\text{mol}^{-1}$)
- K_f : Freundlich constant ($\text{l}^{1/n} \mu\text{mol}^{1-1/n} \text{g}^{-1}$)
- Q_e : Adsorption capacity at equilibrium ($\mu\text{mol g}^{-1}$)
- Q_{max} : Maximum adsorption capacity ($\mu\text{mol g}^{-1}$)
- $1/n$: Freundlich equation parameter

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