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Factors influencing the biosorption of gadolinium by micro-organisms and its mobilisation from sand

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Abstract The present work was devoted to the study of the biosorption capacities of various microbial species (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Ralstonia metallidurans* CH34 previously *Alcaligenes eutrophus* CH34, *Mycobacterium smegmatis*, *Saccharomyces cerevisiae*) for ions of the lanthanide gadolinium (Gd^{3+}). The uptake by sand of this element was also measured. Saturation curves and Scatchard models were established for all biosorbants used in this work. The results enabled us to determine the binding affinities and the maximum capacities for biosorption of Gd^{3+}, which ranged from 350 μmol g^{-1} for *B. subtilis* to 5.1 μmol g^{-1} for *S. cerevisiae*. This study demonstrated the usefulness of optimisation of experimental conditions in biosorption investigations. Experimental results showed that biosorption could be influenced by the growth stage and by the composition of the growth medium of microbial cells. Finally, particular attention was given to the transfer of gadolinium ions from a loaded sand to a bacterial suspension.

Introduction

In the last 15 years numerous studies have shown that micro-organisms can interact with ions such as heavy metals or radionuclides. Many kinds of phenomenon can then take place: biosorption (Volesky 1990), bioaccumulation (Gadd and White 1989), resistance/detoxification mechanisms (Silver et al. 1989), or direct or indirect use in the microbial metabolism (Lovley 1995). The rare earth elements (REE) are often used in industry for the making of glass additives, fluorescent materials, catalysts, ceramics, lighters, superconductors, magnets or condensers. They are even more widely used in agriculture, forestry and aquaculture in which they are found in micro-element fertilisers or animal food (X.R. Wang et al. 1997). Moreover, some REE have been used as analogues of actinides in separation and migration chemistry: europium and gadolinium are the counterparts respectively of americium and curium in radiochemistry studies. 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} (Y.G. Wang et al. 1997), without any apparent toxicity, while cerium can be a potent antiseptic drug for gram-negative bacteria and fungi (Hirano and Suzuki 1996). The concentration factors are related to the soil-to-plant transfer conditions. This mechanism could be submitted to microbial influence, in particular via the ectomycorrhizae (Riesen and Brunner 1996). The first step of interaction between the microbial cell and the metallic ion is biosorption, which takes place at the surface layers. This is a physico-chemical mechanism involving adsorption, ion exchange and entrapment, which can take place on living or dead biomass. Biosorption is influenced by many factors such as ionic speciation, microbial growth conditions, the pH of the medium, the nature and the structure of the microbial layers, the physiological state of the cell, and so on.

The aim of this work was, first, to quantify the biosorption of gadolinium onto several biological and non-biological surfaces. One gram-positive bacterium (*Bacillus subtilis*), two gram-negative bacteria (*Pseudomonas aeruginosa, Ralstonia metallidurans* CH34 previously *Alcaligenes eutrophus* CH34), a bacterial strain with a rich lipid cell wall (*Mycobacterium smegmatis*) and a yeast with a glycan layer (*Saccharomyces cerevi-*

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siae). Sand was chosen as a non-biological surface. In a second part, this study focused on evaluation of the capacity to transfer gadolinium between the sand and a suspension of *R. metallidurans* CH34 in a dynamic experiment in order to apply the results in bioremediation and for risk assessment purposes. This strain, isolated from a decantation tank of a zinc factory (Mergeay et al. 1985), was chosen because it was well characterised and was successfully used for the treatment of effluents contaminated with heavy metals like Zn^{2+} and Cd^{2+} (Diels et al. 1995; Nies 1999).

Materials and methods

Microbial strains

P. aeruginosa (strain CIP A 22, Institut Pasteur Collection, BP 52, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France) and *B. subtilis* (strain CIP 52.65 = ATTC 6051) were grown aerobically with agitation in a nutrient broth (tryptone 10 g l^{-1}, meat extract 5 g l^{-1} and NaCl 5 g l^{-1}) at 30 °C. *M. smegmatis* (strain CIP 73.26) was grown with agitation in a lightly modified synthetic medium [Glycerol 2.5%, L-asparagine 1 g l^{-1}, $(NH_4)_2HPO_4$ 3 g l^{-1}, KH_2PO_4 3 g l^{-1}, Na_2SO_4 1 g l^{-1}, $MnSO_4 \cdot 6H_2O$ 1.1 mg l^{-1}, $ZnSO_4 \cdot 6H_2O$ 1.9 mg l^{-1}, $MgSO_4 \cdot 6H_2O$ 418.8 mg l^{-1}] described by Hall and Ratledge (1982) at 37 °C and pH 6.5. *R. metallidurans* CH34 (VITO Belgium) was cultivated at pH 6, 30 °C, in a rich medium denoted 869 (tryptone 10 g l^{-1}, yeast extract 5 g l^{-1}, NaCl 5 g l^{-1}, D-glucose 1 g l^{-1}, $CaCl_2 \cdot 2H_2O$ 0.345 g l^{-1}, L-cysteine 0.03 g l^{-1}) and in a synthetic medium denoted 284 [Tris-HCl 6.06 mg l^{-1}, NaCl 4.68 mg l^{-1}, KCl 1.49 mg l^{-1}, NH_4Cl 1.07 mg l^{-1}, Na_2SO_4 0.43 mg l^{-1}, $MgCl_2 \cdot 6H_2O$ 0.20 mg l^{-1}, $CaCl_2 \cdot 2H_2O$ 0.03 mg l^{-1}, Na_2HPO_4 40 mg l^{-1}, $(NH_4)_2Fe(SO_4)_2$ 4.8 mg l^{-1}, sodium gluconate 2 g l^{-1} and 1 ml of a solution containing HCl 25% 1.3 ml, $ZnSO_4 \cdot 7H_2O$ 144 mg l^{-1}, $MnCl_2 \cdot 4H_2O$ 100 mg l^{-1}, H_3BO_3 62 mg l^{-1}, $CoCl_2 \cdot 6H_2O$ 190 mg l^{-1}, $CuCl_2 \cdot 2H_2O$ 17 mg l^{-1}, $NiCl_2 \cdot 6H_2O$ 24 mg l^{-1}, $Na_2MoO_4 \cdot 2H_2O$ 36 mg l^{-1}] described by Mergeay et al. (1985). *S. cerevisiae* was an industrial product (FALA, France).

Sand

Sand was heated at 550 °C for 12 h to eliminate all the organic substances initially present. Major and minor elements found in it were Si (60.1%), Ca (32%), Al (2.9%), K (2.2%), Fe (1.3%), Mg (1.2%) and Ti (0.13%). These values were measured by energy-dispersive X-ray fluorescence (EDXRF) with an Oxford ED 2000 facility and were given with 20% error. The sand size used was in the range of 0.25–2 mm.

Sorption experiments

Biosorption studies with wet micro-organisms were carried out under the following conditions. All bacterial cells used in this study were harvested after growth by centrifugation at 10,000 *g* for 15 min and washed twice with a NaCl 0.9 g l^{-1} solution at pH 5 before being used in the biosorption experiments. This biomass was added to the gadolinium solution at pH 5 and shaken for 3 h to attain a state of equilibrium. To measure the gadolinium remaining in solution the samples were centrifugated (Texier et al. 1999). In the case of sand, each batch was made up with 5 g sand sealed in a dialysis bag. The pH was adjusted to 5 by adding a dilute nitric acid or sodium hydroxide solution. The gadolinium concentrations remaining in solution were measured by EDXRF. All samples were duplicated and blank experiments were done to take into account the possible precipitation of gadolinium or its sorption onto the glassware.

Adsorption isotherms of the type Q_e vs C_e were used. The metal uptake from aqueous solutions could be fitted by the classical Langmuir or Brunauer-Emmett-Teller (BET) isotherm equations (De Rome and Gadd 1987).

The Langmuir equation has the following form:

$$Q_e = \frac{Q_{max} \cdot b \cdot C_e}{1 + b \cdot C_e}$$

where Q_e is the adsorption capacity at equilibrium (μmol g^{-1}), C_e is the solution concentration at equilibrium (μmol l^{-1}), Q_{max} is the maximum adsorption capacity (μmol g^{-1}) and b is a constant relating to the energy of interaction with the surface. On rearrangement to a linear form, this becomes

$$\frac{1}{Q_e} = \frac{1}{Q_{max}} + \left(\frac{1}{bQ_{max}}\right) \cdot \left(\frac{1}{C_e}\right)$$

A plot of $1/Q_e$ against $1/C_e$ gives a straight line.

The BET equation has the following form:

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

where C_s is the saturation concentration of the solute (μmol l^{-1}). On rearrangement to a linear form, this becomes

$$\frac{C_e}{(C_s - C_e)} = \frac{1}{b \cdot Q_{max}} + \left(\frac{b-1}{b \cdot Q_{max}}\right) \cdot \left(\frac{C_e}{C_s}\right)$$

A plot of $C_e/[(C_s - C_e)Q_e]$ against C_e/C_s gives a straight line of slope $(b-1)/(bQ_{max})$ and intercept $1/(bQ_{max})$.

The Scatchard (1949) model was used to evaluate the affinity constants (K) of the binding sites for the gadolinium ion and the binding capacities. The linear form used is

$$\frac{Q_e}{C_e} = -K \cdot Q_e + K \cdot Q_{max}$$

where K is the Scatchard affinity constant. A plot Q_e/C_e against Q_e gives the K and Q_{max} values.

Sand-to-bacteria transfer experiment

The transfer of gadolinium from a sand to bacteria was tested with the following protocol. Three glass columns containing 62 g of the sand described above were loaded with 45.53 mg gadolinium. For each column the flow rate was 20 ml h^{-1} (Watson Marlow 302 S) with 3 l of one of the solutions below, adjusted to pH 5, and corresponded to one experiment:

- In one column NaCl 9‰ was used (control column).
- In one column *R. metallidurans* CH34 6.7 g $l^{-1}$was used. The strain was previously grown in medium 869 up to the stationary phase, centrifugated (10,000 *g*, 10 min) and washed five times in NaCl 0.9 g l^{-1}, pH 5. Twenty grams of the washed biomass were then suspended in 3 l NaCl 0.9 g l^{-1}, pH 5, for the sand-to-bacteria transfer experiment. The bacterial mass was chosen in accordance with the capacity of the cell to absorb gadolium in solution. The biomass was suspended in 3 l NaCl 0.9 g l^{-1} to avoid sealing the sand.
- In one column EDTA 1.37 mmol $l^{-1}$was used. The concentration of EDTA was ten times the complexation capacity of the gadolinium in order to take into account the complexation of EDTA with the Ca^{2+} ions of the sand.

The effluent of each column was harvested twice a day in 500 ml Erlenmayer flasks for 5 days. The gadolinium concentration was measured by EDXRF in the effluent, in the biomass after centrifugation (10,000 *g*, 10 min) every day and in the sand at the end of the experiment.

Results

Kinetics of sorption of gadolinium

The first stage of the experiment was to determine the kinetics of the sorption of gadolinium (Gd^{3+}) by the described sorbents. In this test, the appropriate time could be determined when the samples had reached the state of balance. All microbial species used in this work exhibited a relatively fast rate of Gd^{3+} sorption: the balance state was observed after 2 h shaking. On the other hand, using sand a balance state was attained in 24 h. As an example, Fig. 1 shows the curve in the case of the biosorption of Gd^{3+} by *S. cerevisiae*.

Isotherms

Saturation curves were obtained by mixing known quantities of biomass with Gd^{3+} solutions. The concentration range varied between 1 mmol l^{-1} and 5 mmol l^{-1}, except in the batch with *S. cerevisiae* and sand, where the concentrations of Gd^{3+} were between 0.5–0.8 mmol l^{-1} and 0.3–2.0 mmol l^{-1}. Figure 2 presents a typical example of the saturation curves obtained for each sorption study. This kind of plot could be considered to represent favourable adsorption. Two principal models of fitted isotherms (BET and Langmuir) are used to extrapolate Q_{max}, the maximum adsorption capacity. Table 1 shows great variability in the values of maximum biosorption capacity relative to the various adsorbants used. *P. aeruginosa* and *B. subtilis* presented a higher biosorption capacity than *M. smegmatis* and *R. metallidurans* CH34. The lowest adsorption levels were found with *S. cerevisiae* and sand. The data show that only the "best" adsorbent followed the BET model; the others followed the Langmuir model. *M. smegmatis* cells, which were starved for 15 days had a greater biosorption capacity for Gd^{3+} than cells harvested in the early stationary stage (Fig. 2).

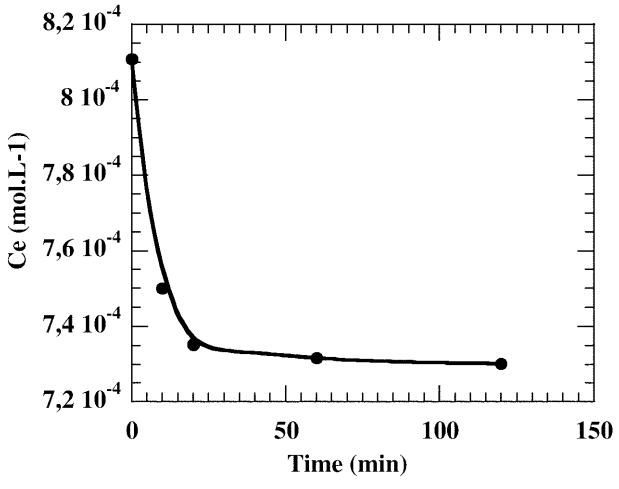


Fig. 1 Adsorption kinetic of gadolinium by *Saccharomyces cerevisiae*

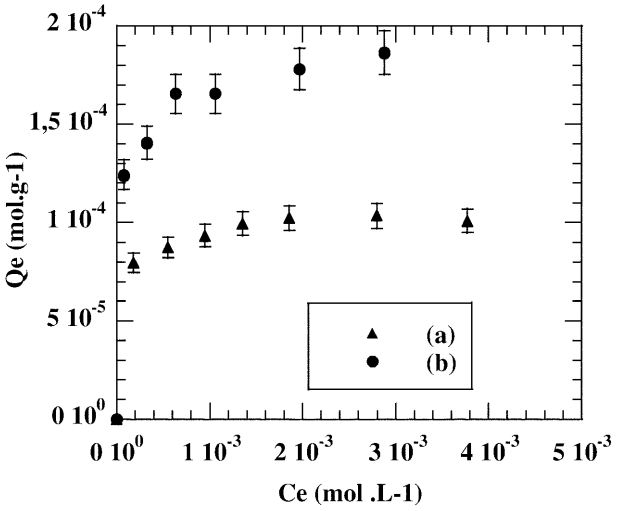


Fig. 2 Adsorption of gadolinium by *Mycobacterium smegmatis*: *a* cells harvested in the early stationary phase; *b* cells starved 15 days in stationary phase

R. metallidurans CH34 grown on 869 (rich medium) and 284 (synthetic medium) showed growth rates of 0.21 h^{-1} (4.8 h doubling time) and 0.075 h^{-1} (13.3 h doubling time) respectively at pH 6 and 30 °C. The Q_{max} value for *R. metallidurans* CH34 grown on synthetic medium was reduced by a factor of 3.7 compared to the cells harvested from the rich medium. Moreover, cells harvested from 869 medium followed the BET model and those harvested from 284 medium followed the Langmuir model.

Scatchard model

The Scatchard model was used in order to determine the nature of the receptor sites in their interactions with binding ions. Figure 4a shows the results obtained with *R. metallidurans* CH34 grown in the 869 medium (rich). The strains *P. aeruginosa*, *B. subtilis* and *M. smegmatis* followed the same trend. Two intersecting curve tangents representing two linear contributions of different slopes describe the adsorption system. *R. metallidurans* CH34 grown on the 284 medium (synthetic medium), *S. cerevisiae* and sand present a monophase slope, suggesting only one kind of interaction site (Fig. 4b). Table 1 summarises the Scatchard affinity constant and the binding capacities extrapolated from the plot $Q_e = f(Q_e/C_e)$ of each adsorbant used in this study. Furthermore, the B_{max} values are in a similar range to the Q_{max} previously measured (Table 1).

Sand-to-bacteria transfer

R. metallidurans CH34 was used in a sand-to-bacteria transfer experiment. A three-column experiment with sand was contaminated by gadolinium as the static phase and was eluted with a solution of NaCl

Table 1 Maximum biosorption capacity and Scatchard plot affinity constants and binding capacity (*B*) for various micro-organisms and sand (error ±6%). In the case of gadolinium all experiments were carried out at pH 5 and with 4% wet biomass (except for sand) (*BET* Brunauer-Emmett-Teller)

	Biosorption of Gd^{3+} (μmol g^{-1} dry weight)	Adsorption isotherm models	Scatchard affinity constant		Gd^{3+} binding capacity (extrapolated from Scatchard plot, μmol g^{-1} (dry weight)		
			$\log_{10} K_1$	$\log_{10} K_2$	B_1	B_2	B_{max}
Mycobacterium smegmatis[a]	110	BET	4.55	3.75	92	20	112
M. smegmatis[b]	190	BET	4.87	3.91	141	49	190
Bacillus subtilis	350	BET	4.48	3.58	289	90	379
Pseudomonas aeruginosa	322	BET	5.22	4.05	293	40	333
Ralstonias metallidurans CH34[c]	147	BET	4.35	3.83	108	33	141
R. metallidurans CH34[d]	40	Langmuir	3.41	–	–	–	42
Saccharomyces cerevisiae	5.1	Langmuir	3.86	–	–	–	5.5
Sand	7.6	Langmuir	3.24	–	–	–	7.6

[a] Cells harvested in the early stationary stage
[b] Cells starved for 15 days in the stationary phase
[c] *R. metallidurans* CH34 grown in 869 medium (rich)
[d] *R. metallidurans* CH34 grown in 284 medium (synthetic)

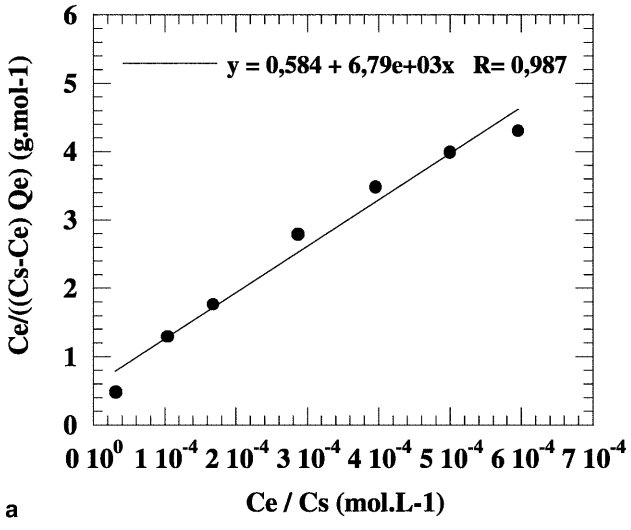
(0.15 mol l^{-1}) or EDTA (1.37 mmol l^{-1}) or a *R. metallidurans* CH34 suspension as mobile phase (6.7 g l^{-1}). Growth of bacteria was avoided during the experiments because cells were washed.

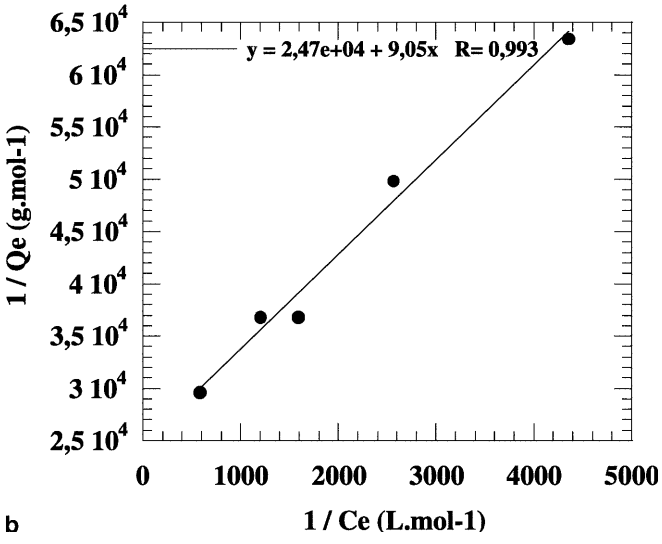
No elution was measured in the control experiment using NaCl and pH was initially adjusted to 5 for all the experiments (results not shown). Figure 5 shows the lixiviation of the sand with either the chemical (EDTA) or biological treatment (*R. metallidurans* CH34). The rate of extraction was constant with bacteria (gadolinium 0.16 mg h^{-1} or 0.013 mg ml^{-1} of the bacterial suspension), whereas four rates were observed with EDTA extraction (Fig. 5). The rates of extraction decreased from 0.54 mg h^{-1} (0.028 mg ml^{-1}) at the beginning to 0.075 mg h^{-1} (4×10^{-3} mg ml^{-1}) at the end of the extraction, corresponding to a loss of 7.2 times the extracting power.

The assessment of the balance of gadolinium between the sand and the effluent for the two different treatments (chemical or biological) showed a strong lixiviation power for the EDTA treatment (68.9% of the gadolinium was in the effluent). *R. metallidurans* CH34, for a hydraulic residence time of 42 min, recovered 36% of the initial Gd^{3+} present in the sand. Moreover, all the lixiviated Gd^{3+} was found in the bacterial fraction (not in the liquid phase) after the 5-day experiment (Fig. 6).

Although the biological treatment was less efficient than the chemical treatment, the final volume containing the complexed gadolinium was 75 times less (1.8 l EDTA solution versus 0.024 l biomass after centrifugation). Moreover, with the biological treatment, the NaCl solution was recyclable because no gadolinium was detected after the centrifugation of the bacterial biomass.

Fig. 3a, b *Ralstonia metallidurans* CH34 adsorption isotherms: **a** BET model for cells grown in 869 medium (rich); **b** Langmuir model for cells grown in 284 medium (synthetic)





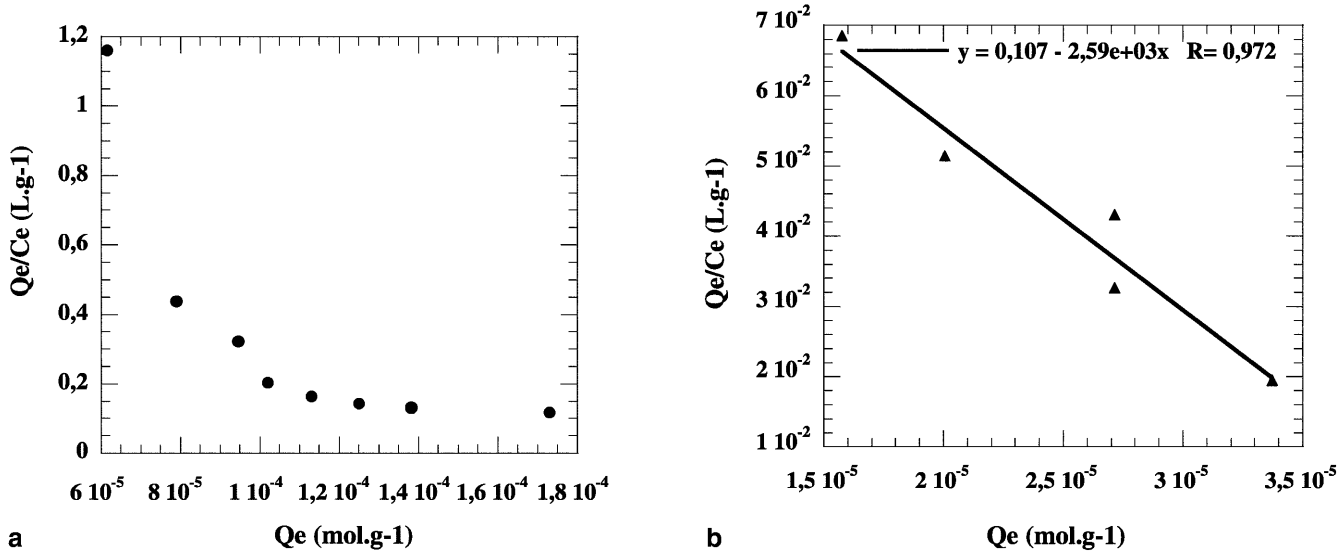


Fig. 4a, b Scatchard plots for *Ralstonia metallidurans* CH34 **a** grown in 869 medium (rich), **b** grown in 284 medium (synthetic)

Discussion

The fast kinetics of sorption and previous investigations (Andrès et al. 1994) indicate that metal binding sites are predominantly associated with the cell wall structure and the observed phenomena could be named biosorption. This mechanism can be considered as the first step in the micro-organism–metal interaction. It encompasses the uptake of metals by the whole biomass (living or dead) through physico-chemical mechanisms such as adsorption, ion exchange or micro-precipitation. Significant differences were observed in the capacities of the various micro-organisms used to take up gadolinium ions (Table 1) and no general relationship was valid for all microbial species. These differences may be related to the nature, the structure, the composition of the layers of the cell wall and the specific surface developed by the sorbents in suspension. Morley and Gadd (1995) concluded for fungal biomass that the different cell wall polymers have various functional groups and differing charge distribution that could explain different metal-binding capacities and affinities. Furthermore, in the case of *M. smegmatis* the physiological stage of the bacteria seems to be important (Fig. 2). This observation could be explained by the fact that cell starvation leads to a modification in the cell wall layers composition. Penumarti and Khuller (1983) measured effectively an increase in the total amount of mannosides with the culture age of *M. smegmatis*.

The variation in the biosorption capacity for gadolinium by *R. metallidurans* CH34 according to the composition of the medium was in agreement with the observation previously described for environmental bacteria (McEldowney and Fletcher 1986) and for fungal biomass (Volesky 1994). McEldowney and Fletcher concluded that the macromolecular compounds of bacterial surfaces varied in quantity and in composition with the growth conditions and growth rate. This phenomenon was confirmed by the adsorption isotherms

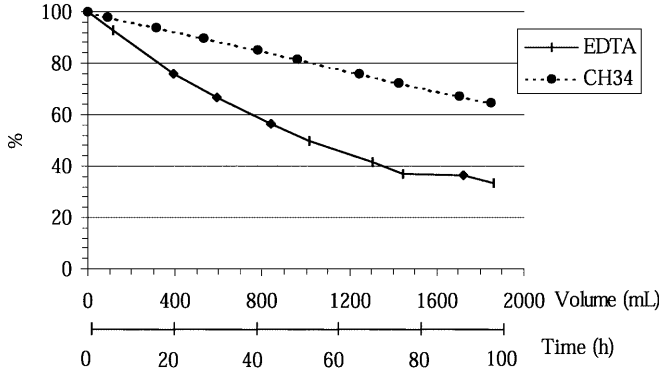


Fig. 5 Evolution of the percentage of remaining gadolinium in a sand column either with EDTA treatment or with bacteria treatment (*Ralstonia metallidurans*-like CH34). EDTA extraction rate = gadolinium 0.54 mg h^{-1} (0.028 mg ml^{-1}) from t_0 to t_{20}; 0.36 mg h^{-1} (0.019 mg ml^{-1}) from t_{20} to t_{53}; 0.24 mg h^{-1} (0.014 mg ml^{-1}) from t_{53} to t_{78} and 0.075 mg h^{-1} (4×10^{-3} mg ml^{-1}) from t_{78} to t_{100}

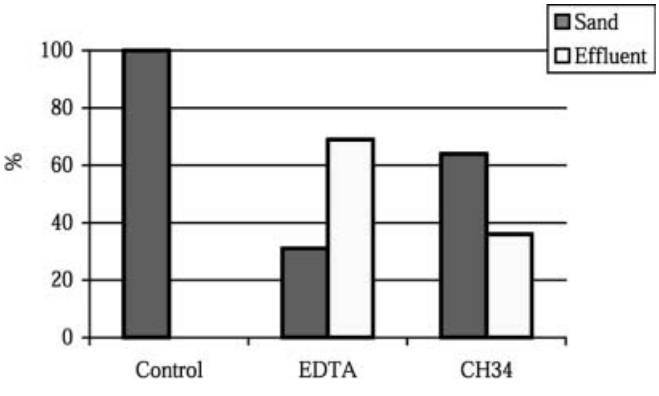


Fig. 6 Assessment of the remaining balance of gadolinium in the sand and in the effluent for the EDTA or the bacteria depollution protocols in comparison to the control (see text)

(Fig. 3) and should be carefully taken into account for bioremediation purposes. The BET model assumes a multi-layer biosorption system; in contrast, the Langmuir model assumes a mono-layer surface biosorption mechanism (De Rome and Gadd 1987). It might be possible to correlate this observation with a difference in the complexity of the cell wall layer composition, the BET model indicating adsorption to a greater number of heterogeneous exchange sites.

The shapes of the Scatchard plots (Scatchard 1949) for *P. aeruginosa*, *B. subtilis, M. smegmatis* and *R. metallidurans* CH34 grown in rich medium indicate the presence of at least two types of binding sites, corresponding to a strong and a weak binding affinity. Furthermore, concave curve shapes suggest negative co-operation, reflecting a binding priority of one type of site over that of another (Dalquist 1978). From Table 1, we can see that there are more strong affinity binding sites present in the biomass than there are weaker sites. It also seems interesting that the sorbent presenting an isotherm of the Langmuir type exhibits only one kind of binding site.

As expected, the test with the EDTA solution showed the higher lixiviation rate, correlating with its high affinity constant ($\log_{10} K = 17.37$ from Callow 1967) in solution for Gd^{3+}. The sand-to-bacteria transfer could be explained by the difference between the affinity constants for the gadolinium ions measured for *R. metallidurans* CH34 ($\log_{10} K_1 = 4.35$; $\log_{10} K_2 = 3.83$) and the sand ($\log_{10} K = 3.24$).

In this study, we have attempted to understand the diversity in the capacities and affinities of various microbial species for the sorption of gadolinium ions. Results also show that a variability must exist between the metal binding capacities determined in the laboratories and the biosorption rate in the natural environment, which relates to the effect of the growth conditions on the surface properties of the microbial cells.

Finally, we have tried to measure some remobilisation behaviours that may occur naturally with bacteria. From previous results (Flemming et al. 1990) and our contribution, it is also apparent that plans utilising fine-grain sand as buffer matrices surrounding waste facilities to immobilise leached heavy metals and radionuclides (natural and artificial) may take into account the contribution of the presence of organo-particles like micro-organisms.

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