

Microorganisms effects on radionuclides migration

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Abstract. In natural systems, speciation and migration of heavy metals and radionuclides depend on many factors such as pH, Eh and the presence of microorganisms. Microorganisms (bacteria, yeast, fungi) may have a direct action on the fate of metal ions through biosorption, bioaccumulation or resistance/detoxification processes. Microorganisms may influence the environment by producing mineral acids, chelating agents such as siderophore, or by-products from the metabolism (organic acids ...). In this paper, we report on microbial interaction processes including bioaccumulation, the indirect metal use for microbial life, the interaction with chelating agents, the resistance/detoxification mechanisms and some indirect influences of microorganisms on the speciation of metals. Also, we present some results of biosorption experiments with caesium, technetium, uranium, nickel and gadolinium using a variety of microbial strains. Saturation curves and Scatchard model were established for all biosorbents used in this work. For instance, the biosorption capacity for Gd³⁺ ranged from 350 $\mu\text{mol.g}^{-1}$ for *B. subtilis* to 5.1 $\mu\text{mol.g}^{-1}$ for *S. cerevisiae*. We found that biosorption could be influenced by the growth stage and by the composition of the growth medium of microbial cells. Finally, we will present some results of uranium reduction by subsurface sulfate-reducing bacteria. The reduced uranium precipitated as uraninite.

1. INTRODUCTION

In natural surface and groundwater systems, the speciation and the migration of heavy metals and radionuclides depend on many factors such as the pH, the Eh and the microbiological activity.

Microorganisms (bacteria, yeast, fungi) may have a direct action on metal mobility through biosorption, bioaccumulation or resistance/detoxification processes [1]. In addition, they may influence the environment by producing mineral acids, chelating agents such as siderophore, or by-products from the metabolism (organic acids ...). In this paper, we present, some examples of the microbial interaction processes including biosorption, the interaction with chelating agents, the indirect metal use for microbial life, bioaccumulation and the resistance/detoxification mechanisms and some indirect influences of microorganisms on the speciation of metals and / or radionuclides.

Biosorption is a physico-chemical mechanism including sorption, surface complexation, ion exchange and entrapment which are relevant for living and dead biomass as well as derived products. The solubilisation of metal oxides could occur by an interaction with chelators like siderophores [2] or with organic compounds such as acetate, gluconate and citrate [3]. Modification of the chemistry and redox conditions of environment could be caused by sulfide-oxidizing or sulfate-reducing bacteria. In fact, oxidation of sulfides such as pyrite (FeS₂) by *Thiobacillus* sp. under aerobic conditions leads to acidification of medium via sulfuric acid production, while sulfide produced by sulfate-reducing bacteria such as *Desulfovibrio* species can react with metallic ions (Fe, Zn, Ni, Cd, Pb...) to produce sparingly soluble minerals [4]. Bioaccumulation which needs the microbial viability, corresponds to a passive or active uptake of ions by ionic channels or by specific carriers located in the cellular layers.

2. DISCUSSION

2.1. Biosorption

Biosorption can be considered as the first step in the microorganism-metal interaction. It encompasses the uptake of metals by the whole biomass (living or dead) through physico-chemical mechanisms such as sorption, surface complexation, ion exchange or surface precipitation. These mechanisms take place on the cell wall [5] which is a rigid layer around the cell and they have fast kinetics.

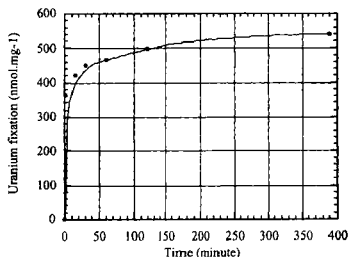


Figure 1: Kinetics of adsorption of uranium at pH 7 by *Thiobacillus intermedius* (experimental conditions: 1 % biomass and initial uranium concentration of 68 mmol.L⁻¹). The major uranium species in solution are (UO₂)₃(OH)₃⁺ and (UO₂)₂(OH)₂⁺.

Figure 1 shows that the equilibrium state is obtained within a few minutes, for the adsorption of uranium by *Thiobacillus intermedius* at pH 7. One dominant factor affecting the capacity of the microbial cell wall to "trap" the metal ion is the composition of this outer layer. The cell wall structure of microorganisms shows the presence of some free moieties, like amine, carboxylic, phosphates functions, which could be involved in the biosorption mechanisms of metallic ions [6].

2.1.1. Cell wall characteristics

Potentiometric titrations have shown that the metal ions fixation takes place on acidic moieties. This method can be used to measure the global pKa. For *Pseudomonas aeruginosa*, the pKa values are 2.8 and 6.1, and allow us to conclude that the cell wall layers present two kinds of acidic function. Fein *et al.* [7] have characterized the acid/base properties of the cell wall of *Bacillus subtilis* and have shown three distinct types of surface organic acid functional groups with pKa of 4.82, 6.9 and 9.4. These various values are generally attributed to carboxyl, phosphate 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. Mullen *et al.* [8] have indicated, after electronic microscopy studies, that lanthanum was accumulated at the surface of *P. aeruginosa* inducing crystalline precipitation.

Time resolved laser spectrofluorimetry observations of europium ions have shown two main peaks around 592 and 615 nm which correspond respectively to the transition from ⁵D₀ toward ⁷F₁ and ⁷F₂ 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(PO₄), 745 μs) suggests that this cation interacts with carboxylic or phosphate functions in the cell wall of *Pseudomonas aeruginosa* [9].

2.1.2. Biosorption capacities

Differences in the structures of cell walls lead to variations in the sorption capacity illustrated in table 1. For example, the biosorption capacity for Gd³⁺ ranged from 350 μmol.g⁻¹ for *Bacillus subtilis* to 5.1 μmol.g⁻¹ for *Saccharomyces cerevisiae* and the fixation of uranyl ions from 756 μmol.g⁻¹ for *Rhizopus arrhizus* to 170 μmol.g⁻¹ for *Mycobacterium smegmatis*. More over, the medium used for the microbial growth before the cells were harvested, washed, and added to the metallic ions, could affect the biosorption capacities (for example see the case of *Ralstonia metallidurans* in table 1). In addition, the physiological state in which the cells are harvested influence the fixation of gadolinium by *Mycobacterium smegmatis* (table 1).

No microbial uptake could be observed by *Pseudomonas aeruginosa* with caesium and technetium (VII), at pH 7, [10]. For this latter element the main species in solution is TeO₄⁻ and the global charge of the cell wall layer at pH 7 is negative, which suggests a repulsive mechanism between Tc and the biomass. Caesium is known to be an element which presents a poor sorption capacity [11]. We conclude that biosorption depends on cation chemistry and on composition, structure and nature of the bacterial layers.

Tableau 1 : Biosorption capacities for various metallic ions by different microbial biomass.(a) Cells harvested in the early stationary phase (b) Cells starved 15 days in stationary phase; (c) *Ralstonia metallidurans* CH34 (*Alcaligenes eutrophus* CH34) grown in 869 medium (rich) and (d) in 284 medium (synthetic)

	Element	Biosorption $\mu\text{mol. g}^{-1}$ (dry weight)	References
<i>Mycobacterium smegmatis</i> (CIP 73.26)	La ³⁺	57	[12]
	Eu ³⁺	101	
	Yb ³⁺	103	[13]
	Gd ³⁺	110 (a)	[14]
	Gd ³⁺	190 (b)	
	UO ₂ ²⁺	170	[13]
	Th ⁴⁺	187	
<i>Pseudomonas aeruginosa</i> PU 21	Hg ²⁺	969	[15]
	Pb ²⁺	531	[16]
	Cd ²⁺	362	
<i>Pseudomonas aeruginosa</i>	UO ₂ ²⁺	630	[17]
<i>Pseudomonas aeruginosa</i> (CIP A 22)	La ³⁺	397	[18]
	Eu ³⁺	290	
	Yb ³⁺	326	
	Gd ³⁺	322	[14]
<i>Saccharomyces cerevisiae</i>	Zn ²⁺	260	[19]
	Cd ²⁺	632	[20]
	UO ₂ ²⁺	630	[17]
	Th ⁴⁺	500	[21]
	Gd ³⁺	5	[14]
<i>Ralstonia metallidurans</i> CH34	Gd ³⁺	147 (c)	[14]
	Gd ³⁺	40 (d)	
<i>Bacillus subtilis</i>	Gd ³⁺	350	[14]
<i>Penicillium chrysogenum</i>	Pb ²⁺	559	[22]
	UO ₂ ²⁺	336	[23]
<i>Rhizopus nigricans</i>	Zn ²⁺	220	[19]
<i>Rhizopus arrhizus</i>	Cu ²⁺	252	[22]
	Cd ²⁺	267	
	Hg ²⁺	289	
	Pb ²⁺	502	
	UO ₂ ²⁺	756	[24]
	Th ⁴⁺	733	[25]
<i>Aspergillus niger</i>	Zn ²⁺	210	[19]
	Au ⁺	862	[22]
<i>Zoogloea ramigera</i>	Pb ²⁺	392	[26]
	Cu ²⁺	536	
	Ni ²⁺	791	
Sterile Activated sludge	Cd ²⁺	325	[27]
	Zn ²⁺	392	
	Ni ²⁺	369	

2.1.3. Effects of competing ions

Multi-elemental studies show that in the case of lanthanum, europium and ytterbium the fixation takes place at the same cell wall site [18]. This work has pointed out a preferential adsorption for europium ions by a *Pseudomonas aeruginosa* biomass. The influence of the presence of cations (Al³⁺, Ca²⁺, Na⁺, K⁺) and anions (NO₃⁻, SO₄²⁻, Cl⁻) on biosorption performances has also been studied. Aluminum seems to be the more competitive ion for the fixation of europium, lanthanum and ytterbium by *P. aeruginosa*. The presence of glutamate, sulphate, phosphate, carbonate, chloride and ethylenediaminetetraacetate (EDTA) in solution with metal / complexant ratios up to 15, affects the biosorption of lanthanum by a *Rhizopus arrhizus* biomass [28]. 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 [13]. Karavaiko *et al.* [29] have obtained results of the decrease (values from 56 to 94 %) of scandium biosorption by various microorganisms in the presence of aluminum, iron and titanium. Tsezos *et al.* [30] have studied the effect of competing ions on the biosorption of metals in relation to their Pearson classification (soft, hard and borderline species). 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.

2.2. Complexing substances

Bacteria and fungi can produce many complexing or chelating substances. The mobilization capacities of a bacterial and fungal iron chelating agent, for plutonium and uranium, have been studied by Brainard *et al.* [31]. They used two siderophores: the first produced by *Escherichia coli* (enterochelin) with catechol functions, and the second, by *Streptomyces pilosus* (desferrioxamin B), with hydroxamate groups. They showed that plutonium and uranium oxides could be solubilised by these molecules. A review has been published by Fogarty and Tobin [32] about the complexation between fungal melanins and metal ions (Ni, Cu, Zn, Cd, Pb etc...). These compounds are dark brown or black pigments located in the cell walls. Fungi are also able to produce small organic acids (gluconic, oxalic etc....) which can react with metallic oxides and lead to their solubilizations.

2.3. Indirect influences

Figure 2 and 3 shown the two main indirect mechanisms of interaction which are related to the change in pH or redox conditions of the medium.

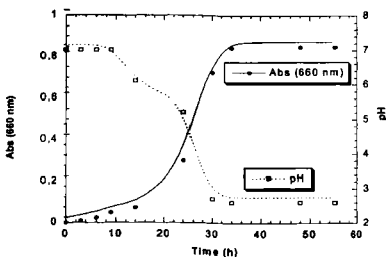


Figure 2: Medium acidification by *Thiobacillus intermedium* in relation with growth.

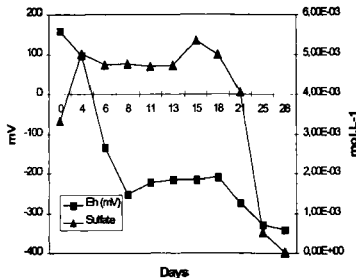
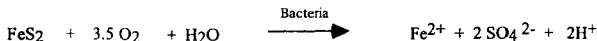
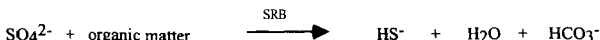


Figure 3: Sulfate consumption by subsurface sulfate reducing bacteria.

In the presence of air, sulfur oxidizing bacteria (SOB) such as *Thiobacillus* use sulfide minerals (FeS_2 , Cu_2S , PbS) for their growth. These reactions can produce mineral acids which modify the pH of the medium. The direct microbial oxidation of iron pyrite is summarized by the following reaction:

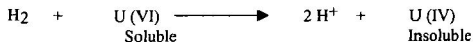


Under reducing conditions sulfate-reducing bacteria (SRB) such as *Desulfovibrio* species are capable of reduction of sulfate into sulfide, which reacts with metal ions to precipitate highly insoluble sulfides [3] : NiS , 3×10^{-21} ; Cu_2S , 2.5×10^{-50} ; CoS , 7×10^{-23} ; PbS , 1×10^{-29} ; HgS , 3×10^{-53}). Also, dissolved sulfide ions can directly reduce metals including U(VI), Cr(VI) and Tc(VII). Reduction of sulfate requires organic carbon like natural organic matter or more simple compounds such as lactate, ethanol and acetate or H_2 as electron donor:



2.4. Indirect metal use for microbial life

Some microorganisms are able to grow under anaerobic conditions by coupling the oxidation of simple organic substances with the reduction of metallic compounds. For example, the simplified equation of the reduction of uranyl ions (U(VI)) by *Shewanella putrefaciens* [34] in presence of hydrogen can be written as:



Uranium can be reduced by a variety of metal- and sulfate-reducing bacteria [35-37]. Pure or mixed cultures of these bacteria can couple the oxidation of organic compounds (lactate, formate, ethanol) or H_2 to the reduction of U(VI). The reduced U precipitates as the highly insoluble mineral uraninite (UO_2). Abdelouas *et al.* [37] showed that subsurface sulfate-reducing bacteria from a mill tailings site are capable of reducing U(VI), which precipitates at the periphery of the cell (Figures 4 and 5). Enzymatic reduction of U was shown by Lovley *et al.* [38]. The authors showed that the cytochrome c_3 enzyme, which is located in the soluble fraction of the periplasmic region of *Desulfovibrio vulgaris*, reduced U(VI) in the presence of excess of hydrogenase and H_2 . In natural reducing environments metal- and sulfate-reducing bacteria are expected to play a significant role in uranium immobilization.

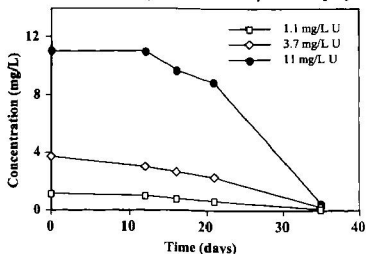


Figure 4: Reduction of U(VI) by subsurface sulfate-reducing bacteria in groundwater from a mill tailing site amended with ethanol and phosphate.

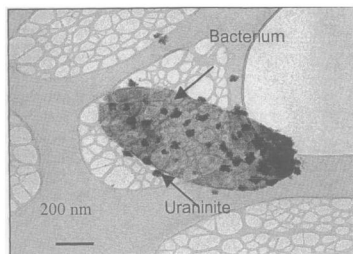


Figure 5: Uraninite particles on the surface of a sulfate-reducing bacterium in groundwater from a mill tailing site amended with ethanol and phosphate.

2.5. Bioaccumulation

Bioaccumulation is one kind of the possible interactions between microorganisms and metal ions in relation with metabolic pathways; it needs living cells. Metal ions are involved in all aspects of the microbial growth. Many metals are essential, whereas others have no known essential biological function. Accumulation of radionuclides through the pathways of their stable isotopes or of chemically homologous elements can be considered as bioaccumulation. It is well known that caesium ion can be accumulated by the channel of potassium [11].

2.6. Resistance and detoxification mechanisms

In a polluted environment, microorganisms are able to develop a great diversity of resistance and detoxification mechanisms. The most important is the transformation of toxic species into inactive forms, by reduction, methylation or precipitation. For example, the predominant redox states of selenium in natural environment are Se(VI) (SeO_4^{2-}) and Se(IV) (SeO_3^{2-}), which can be reduced into elemental selenium Se(0) by telluric (*Clostridium*, *Citobacter*, *Flavobacterium*, *Pseudomonas*...) or by bacteria in anoxic aquatic sediments bacteria [39]. The oxianion species can potentially serve as electron acceptors for the microbial metabolism. Another way of transformation is the biomethylation of inorganic selenium

compounds in dimethylselenide $[(CH_3)_2Se]$. The methylated species are generally volatile in environmental conditions [40] and may have a great influence on migration of these heavy metals.

3. CONCLUSION

Microorganisms can be considered as potential vectors for radionuclides and metals migration and retention through bioaccumulation and associated mechanisms, and through biosorption. It has been shown that their fixation capacities are more or less important, depending on the structure of the cell wall, the medium conditions and the speciation of the metal ions. Metal ions can either be extracted from a solid matrix and then dispersed in the environment by microbial actions or mobile metal ions can be immobilized by the action of microorganisms.

This presentation is not an exhaustive explanation of microorganism-metal interactions, but we have presented the phenomena which could have an implication in migration/retention of radionuclides.

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