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URANIUM BIOSORPTION BY A FILAMENTOUS FUNGUS *MUCOR MIEHEI* pH EFFECT ON MECHANISMS AND PERFORMANCES OF UPTAKE

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Abstract—This study focuses on uranium sorption mechanisms by *Mucor miehei*, a fungal biomass, used in agro-industries (enzyme synthesis). The pH plays an important part in these phenomena, mainly by its influence on metal or cell wall chemistry. Hydroxylation of uranyl, dependent on the pH and total metal concentration, influences kinetics, via the nature of the limiting phases: diffusion of metal through layers bordering or constituting the biomass, or intramembranar precipitation of uranyl initially adsorbed, and sorption mechanisms. With a moderate pH, sorption of uranylhydroxides modifies extracellular sorbent structures, consequently inducing a multilayer sorption opposed to monolayer adsorption obtained with acid pH. Uptake capacity is characterized by high values obtained even with low metal concentration in solution. Biosorbent could be a technical answer to pollution treatment and valorization of low charge waste streams and leaching solutions obtained in recovery of infra-marginal ores.

Key words—uranium, removal, waste, fungus, pH, kinetics, sorption, isotherms, hydrolysis, complexation

INTRODUCTION

Mining and refining industries produce aqueous waste streams containing low metal concentrations. However the metal extractions and treatments are not the only sources of polluted effluents. For example, in the case of uranium, large amounts of mill tailing formed as by-products of uranium mining and refining operations are accumulated in tailing ponds. Bio or chemical leaching, producing sulphuric acid, solubilizes uranium from waste ores, prior to its discharge into tailing ponds or its release into the environment.

The treatment of low charge effluents, conciliating economic and technical constraints is impossible with traditional physico-chemical processes. For example non-selective precipitation generates metallic sludges containing various metals difficult to valorize, ion-exchange techniques require large volumes of resins for treatment of high delivery of low metallic concentration flows and generate a high concentration of salt residue. Uranium concentration in waste streams is too low for a classical treatment and too high to allow its discharge into the environment. It has been suggested that biomass could be used to decontaminate these wastes and to concentrate metals. The capability of some micro-organisms to accumulate metallic elements has been known for some time. In the past, studies concerned with metal sorption biomass were conducted from a toxicological point of view: understanding microbial tolerance and resistance to heavy metals (Wood and Wang, 1983; Jernelöv and Martin, 1975) by identification of uptake mechanisms. Nowadays interest is focused on metal recovery (Tsezos and Volesky, 1982; Strandberg and Shumate, 1982; Nakajima and Sakaguchi, 1986). Various processes can influence metal sorption on the cell wall. Certain ones involve metabolic dependent mechanisms: synthesis of exo-polysaccharides which adsorb or complex ions, production of inorganic phosphate, via an enzymatic system, which precipitates metal, or active transport of ions using a proton pump mechanism. Others involve the non-specific or non-metabolic binding of the metal to the cell surface or extracellular matrice, via physico-chemical mechanisms as the complexation, the ion exchange, the adsorption or the precipitation on cell wall sites. A large extent of bacterial cells are employed (Nakajima and Sakaguchi, 1986). Fungi from the Mucorale order were chosen for our studies.

The purpose of this research is to test *Mucor miehei* biomass for its ability to adsorb uranyl ion, to determine possible functional sites which contribute to the sorption and to select the various phases which take place in the sorption phenomenon. The performances of these fungi to remove metals (adsorption capacity, kinetics) will be studied.

MATERIALS AND METHODS

Material

Mucor miehei (Mucorale order, Phycomycete class, Zygomycete sub-class) is provided by Gist-Brocades, France, freeze-dried as a by-product removed from the fermentation process without pre-treatment: this waste material can be considered as a pure organic product. These

Table 1. Equilibrium concentration of uranium in solution (after filtration): effect of pH and total metal concentration

	pH 2	pH 3	pH 4	pH 5	pH 6
$C_o = 10$ ppm C_{eq} (mg/l)	6	1.1	0.5	1.7	2.9
$C_o = 100$ ppm C_{eq} (mg/l)	86	64	35	10	38.5

biomass samples are sterilized by freeze drying, consequently no biological activity is noticeable. Biomass is sieved to a size of 500 μm. Uranium is provided as uranyl nitrate hexahydrate (R. P. Prolabo).

Metal uptake experiments

This physico-chemical study consists of two parts: the first deals with kinetic results, the second with isotherm characterization and determination of various steps involved in sorption mechanisms.

Kinetic experiments were carried out at two fields of metal concentration: 10 and 100 ppm in uranium, and at five pH values: pH 2, 3, 4, 5 and 6. At least four sets of data were obtained for each experimental condition. No metal adsorption by the filter membrane is noticeable. Sorption experiments were performed at pH 3, 4 and 5.

For each experiment (adsorption isotherms, kinetics study) series of flasks were prepared with known volumes of serial dilutions of standardized uranyl salt solutions. The pH was adjusted with 1 N HNO_3 and 1 N NaOH, before and after the addition of weighed quantities of dried biomass; and biomass concentration was set at 500 mg/l. The experiments were performed at room temperature ($\approx 20°C$). Equilibrium was reached after 6 h of contact. Biomass was removed by filtration through a 0.45 μm Millipore membrane filter, and filtrates analysed for metal concentration by the arsenazo III method (Shumate *et al.* 1978).

Sorption capacity is calculated by $q = (C_o - C_e)V/m$; where q is the sorption capacity (mg U/g of biomass), C_o the initial uranium concentration, C_e the residual uranium concentration in solution (mg U/l), V the volume of solution (l) and m the sorbent mass (g).

RESULTS

Sorption kinetics

Table 1 represents uranium residual concentration in uranium at equilibrium (mg U/l).

Whatever the initial concentration, the kinetics are largely influenced by pH. At equilibrium with a drastic pH (e.g. pH 2) sorption efficiency is reduced: hardly 40% (10 ppm) and 14% (100 ppm) against 90% with optimum conditions which depend on initial metal concentration, at pH 4 for 10 ppm and pH 5 for 100 ppm. The effect of pH on sorption equilibrium is shown by a larger dispersion of these values at a higher initial concentration. Equilibrium concentration is included between 35 and 90% of initial concentration for a 100 ppm initial content, and between 70 and 90% for a 10 ppm solution. pH induces a larger range of efficiency dispersion with a higher initial metal concentration.

Figures 1 and 2 illustrate the effects of pH on sorption kinetics: evolution of percentage of uranium removed at t (min) by uranium removed at equilibrium [$\% = (C_o - C_{(t)})/(C_o - C_e)$]. These figures

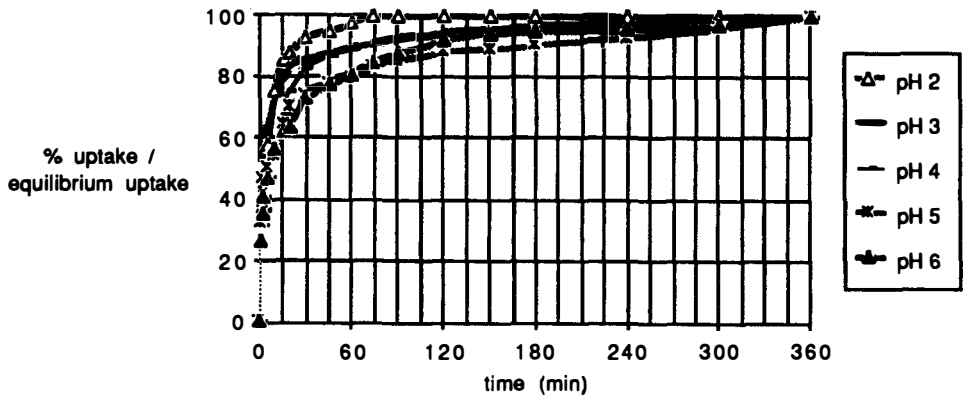


Fig. 1. Effect of pH and time on uranium removed, expressed as a percentage of uranium removed at equilibrium (initial metal concentration 10 ppm).

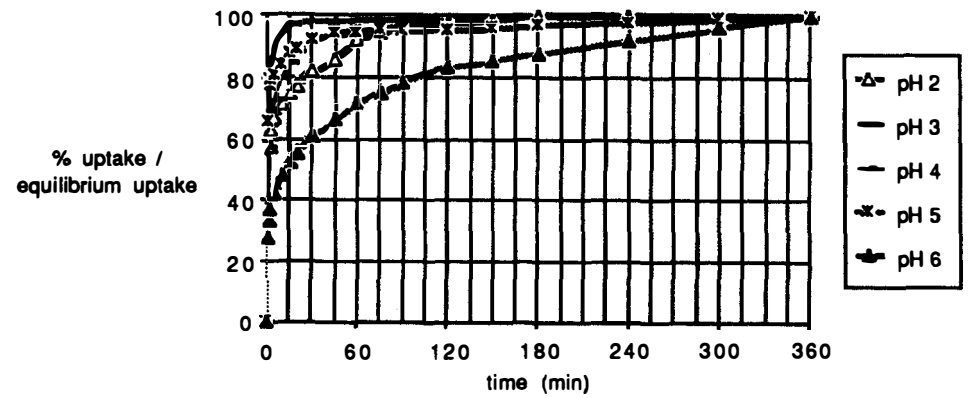


Fig. 2. Effect of pH and time on uranium removed, expressed as a percentage of uranium removed at equilibrium (initial metal concentration 100 ppm).

Table 2. Initial sorption rate (mg U/g.s) Initial sorption rate is calculated according to the determination of $dq(t)/dt$ with $t > 0$, with the approximation that: $\Delta q/\Delta t$ (0–1 min)

	pH 2	pH 3	pH 4	pH 5	pH 6
C_o = 10 ppm rate	0.036	0.126	0.1	0.09	0.06
C_o = 100 ppm rate	0.134	0.766	1.234	1.984	0.563

show that the initial uranium concentration plays an important part on pH effect. Indeed at a low initial concentration we cannot see many differences between the curves obtained at pH 4, 5 or 6. Quasi equilibrium is reached within 20 or 30 min with pH 2, and needs 2 h with pH 6 (instead of 1 h with other pHs). Table 2 represents the initial sorption rate (mg U/g.s: increase of uranium concentration in biomass during the first minute of contact). For a low metal concentration; pH effect on the initial rate of sorption is limited, but with higher concentrations, pH strongly influences the initial rate. It increases with pH between 3 and 5, showing the affinity of biomass to hydrolysed soluble species. Around pH 6 solubility is at a minimum in the concentration field studied and the initial rate decreases. With pH 2 the initial rate is the lowest, although the equilibrium is reached within 30 min.

To explain these effects, the pH acts on the cell wall chemistry and metal chemistry in solution. The cell wall contains amines, amides and carboxylic surface functional groups which are protonated or deprotonated, depending on the pH of the aqueous medium. Uranyl chemistry is complicated by a hydrolysis phenomenon: when pH increases from an acidic value to a neutral value, various hydrolysed species exist, the repartition of which is conditioned by the pH and the total uranium concentration (Baes and Mesmer, 1976). The major form of hydrolysed species is $(UO_2)_3(OH)_5^+$: at 10 ppm total uranium concentration $(UO_2)_3(OH)_5^+$ appears between pH 4.5 and 5, instead of pH 4 and 4.5 when the total metal concentration reaches 100 ppm (Figs 3 and 4). Ion species repartition is determined according to the following reactions and equilibrium constants:

$$UO_2^{2+} + H_2O \leftrightharpoons UO_2(OH)^+ + H_3O^+ \quad pK = 5.8$$

$$2\,UO_2^{2+} + 2\,H_2O \leftrightharpoons (UO_2)_2(OH)_2^{2+} + 2\,H_3O^+ \quad pK = 5.62$$

$$3\,UO_2^{2+} + 3\,H_2O \leftrightharpoons (UO_2)_3(OH)_5^+ + 3\,H_3O^+ \quad pK = 15.63.$$

This fact plays an important part in the sorption mechanisms: with pH uranyl species repartition changes and influences the nature, charge, size of ions

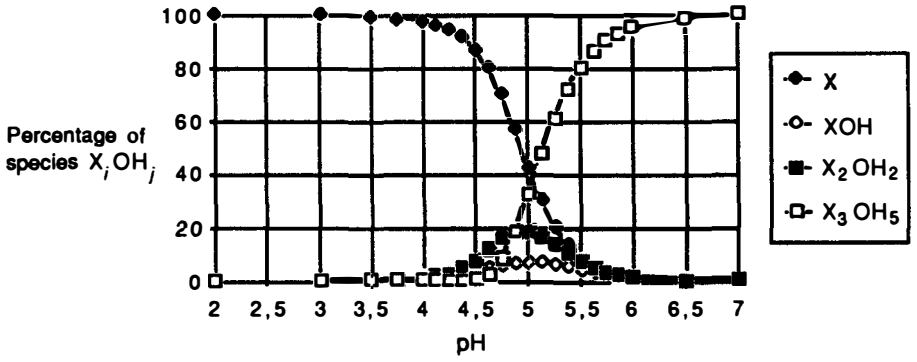


Fig. 3. Effect of pH on uranyl species repartition (10 ppm).

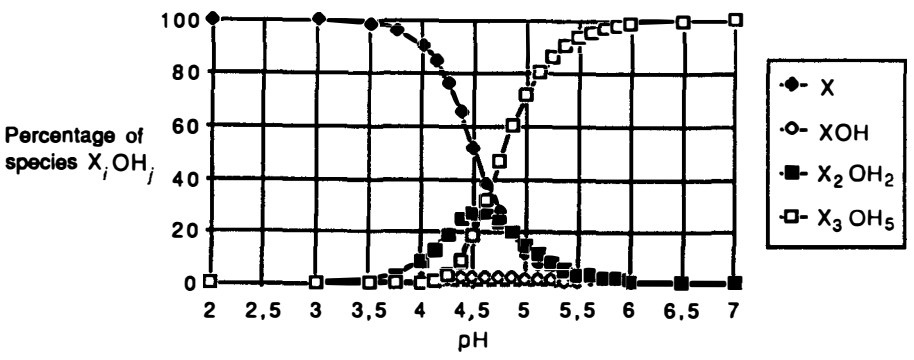


Fig. 4. Effect of pH on uranyl species repartition (100 ppm).

able to be complexed, adsorbed and accumulated by the biomass. This could explain the differences noticed in kinetic behaviour between low and high initial concentration.

Sorption isotherms

Representative sorption isotherms for UO_2^{2+} at pH 3, 4 and 5, plotted in Figs 6, 7 and 8, illustrate the evolution of metal concentration in biomass versus residual metallic concentration in solution.

The maximum uptake is largely dependent on pH: at pH 3 maximum uptake capacity (q_{max}) is equal to 70–80 mg U/g of dry fungi, this capacity is two times greater when pH reaches 4, and three times at pH 5. In an acidic medium uranyl and H_3O^+ are competing for sorption sites, and high solubility reduces the biomass ability to adsorb and complex metal, free uranyl is adsorbed via classic pathways. This effect of the solubility on the interaction of solid-molecules was shown in the case of the synthetic resin adsorption (Le Cloirec *et al.*, 1990). With a higher pH, uranyl solubility decreases, ionic size induces adsorption of hydrolysed species and biomass removes a greater quantity of metal.

DISCUSSION

Sorption kinetics

For a drastic pH (e.g. pH 2 or 3) the cell wall is protonated, inducing a weak complexation affinity between the cell wall and uranyl ions, furthermore uranyl is highly soluble in an acid medium decreasing adsorption efficiency. When the pH increases, uranium solubility decreases promoting adsorption, electrostatic repulsion between metal and cell wall decreases favouring complexation, and the apparition of hydrolysed species is followed by an increase of ionic size which facilitates contact between functional sites and metal. When the initial uranium concentration increases the same mechanisms are observed. But hydrolysed species appear at a lower pH, inducing a preponderant part of the mechanism of hydrolysed metal sorption on total sorption.

According to Tsezos (1980) various steps in the sorption mechanism are proposed which deal with metal transfer through layers constituting or bordering the cell wall (Fig. 5):

(1) uranium transport from the bulk solution to the boundary film present around the cell wall [Fig. 5(a)]
(2) transport of uranium from the boundary film to the cell surface [Fig. 5(a)]
(3) transfer of uranium from the cell surface to active sites of uptake [Fig. 5(b)]
(4) uptake phases: complexation, adsorption and intramembranar precipitation [Fig. 5(c)].

A well mixed tank reactor will enable us to suppress kinetics limitation due to the first step. Figures 1 and 2 show that sorption is very rapid: biosorption, represented by step (4), is not the limiting phase of the process, kinetics seem to be more seriously influenced by the transfer of metal from solution to active sites through the boundary film and moreover through the biosorbent surface: external cell wall. In an acid medium, uranyl is free in solution, its ionic size allows its diffusion through various layers (physical, biological...), competition between H_3O^+ and UO_2^{2+} for sorption site attribution imposes its own limiting rate to global kinetics. When pH is approx. 6, metal is hydrolysed in solution, diffusion of ions is limited by steric constraints, inducing an equilibrium reached after a longer contact time than with other experimental conditions. Initial sorption rate decreases strongly, due to a solubility limitation effect. With intermediary pH, kinetic influence is conditioned by metal concentration, via ionic species repartition. Sorption rate seems to be increased by soluble species, but hydrolysed ions favour this rate as confirmed by the evolution rate with pH and total metal concentration (see Table 2).

Sorption isotherms

Maximum uptake capacity is influenced by pH, but aspects of these isotherms are also affected by the pH: at pH 3 and 4 we are able to assimilate the plots to represent Freundlich and Langmuir isotherms (see Table 3). The Freundlich model seems to be a better model for describing uranium sorption by *Mucor miehei* at pH 3, this conclusion is not so obvious at pH 4. In the first instance, sorption is described as a monolayer sorption model with lateral interaction between sorbed metal, instead of the Langmuir

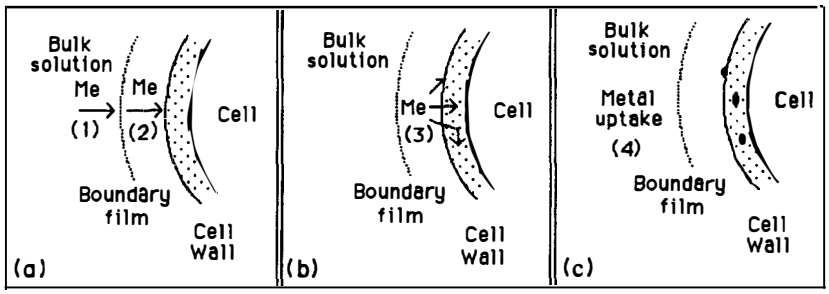


Fig. 5. Various steps involved in sorption kinetics.

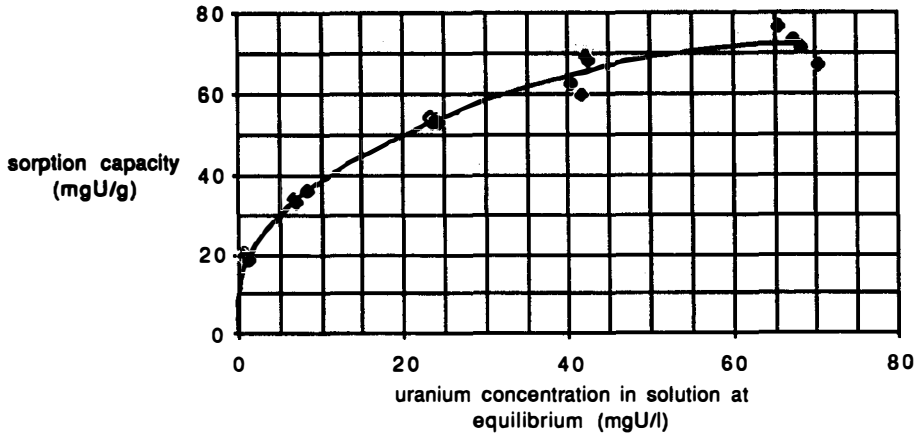


Fig. 6. Uranium sorption isotherm at pH 3.

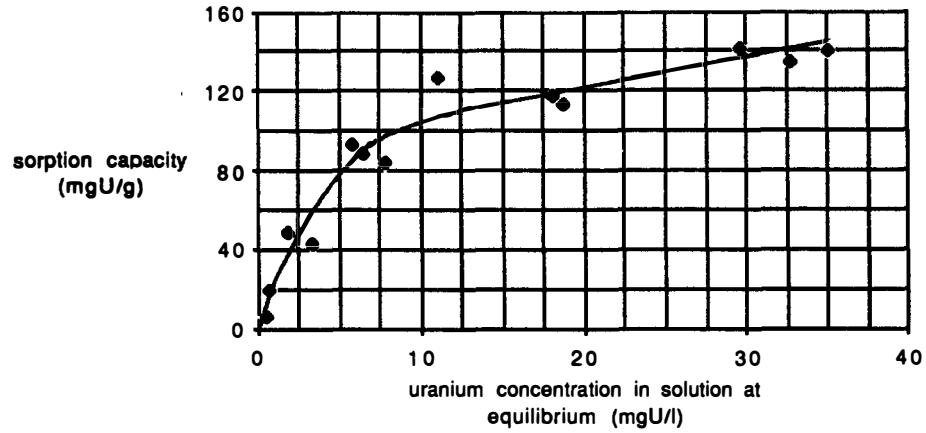


Fig. 7. Uranium sorption isotherm at pH 4.

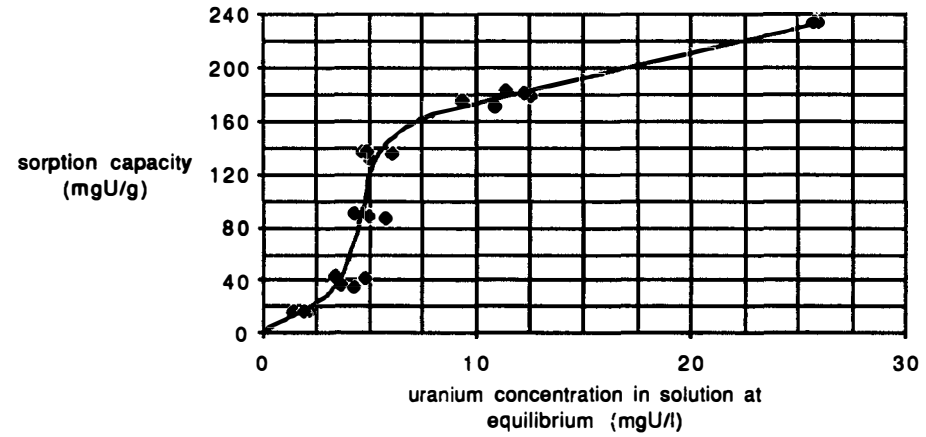


Fig. 8. Uranium sorption isotherm at pH 5.

hypothesis which predicts sorbing surface homogeneity and no interaction. At pH 5 a great difference is noticed: sigmoidal aspect with a typical behaviour for low residual concentration. A surface precipitation model for the sorption of uranyl on biomass has been proposed, by analogy of metal sorption by metal oxides, as characterized by Farley *et al.* (1985): an approach of mechanisms which presents similarities to the Brunauer Emett Teller isotherm concept (de Rome and Gadd 1987).

We suggest that with a moderate pH (e.g. pH 5 or 6) and high initial concentration, the first step in the sorption mechanism consists of adsorption complexation of hydroxylated species, consequently the total metal concentration in solution decreases, repartition between the free and hydroxylated form is replaced in favour of free metal, favouring adsorption of this form. The preliminary uranylhydroxide uptake gives the cell wall a new adsorbing structure which is able to remove metal in solution: this could explain the

Table 3. Application of Langmuir and Freundlich isotherms to the results for uranium accumulation by *Mucor miehei*

	Freundlich model			Langmuir model		
	k	n	R^2	q_m	b	R^2
pH 3	19.84	3.328	0.977	53.23	0.737	0.805
pH 4	27.77	1.989	0.939	116.5	0.348	0.940

Langmuir and Freundlich models are not applied for pH 5 sorption results: correlation coefficients are not significant.
Langmuir isotherm: $q = (q_m\ b\ C_{eq})/(1 + b\ C_{eq})$
Freundlich isotherm: $q = k\ C_{eq}^{1/n}$
where q is the sorption capacity (mg/g), C_{eq} the metal concentration in solution (mg/l) at equilibrium, q_m (mg/g), b (l/mg) k and n are constants and R^2 is the correlation coefficient.

existence of two phases observed in the sorption isotherm at pH 5.

Throughout this study the major effect of pH on sorption mechanisms was obvious. Concerning the two hypotheses formulated from its influence: wall chemistry and metal solution chemistry, it seems that the second proposal is prominent. Experiments developed on other metals (Guibal 1990), such as cobalt or copper, show a significant correlation between the pH of the first apparition of the sorption mechanisms and the pH of hydroxylation of these metals. Protonation of the cell wall inhibits strong metal sorption, if the pH is too acid (pH $\approx$ 2), but its effect stays minor with an intermediate pH. On the contrary, with a moderate pH, the metal chemistry plays an important part in sorption performances: hydroxylation observed with pH 5 or 6, and high metal concentration induce a change on sorbed metal nature, on kinetic limiting phases and on the types of major mechanisms.

In conclusion the sorption mechanisms proposed can be described as:

Acidic pH (2). Proton competes with uranyl, hydroxylation phases of complexed metal [third step of sorption as characterized by Tsezos and Volesky (1982)] with *Rhizopus arrhizus* inside the cell wall is reduced, fast phenomenon.

Intermediary pH (3–4). Free metal is sorbed, the kinetic limiting step is at the hydroxylation phase of the metal initially adsorbed, when the total metal concentration is relatively high (100 ppm). The metal can exist in solution in a hydroxylated form, whose diffusion through layers bordering the active sites is reduced. The other limiting step is the hydroxylation phases of the metal sorbed during the first few minutes of contact.

Moderate pH (5–6). Metal is mainly as uranyl hydroxide in solution, its sorption gives the biosorbent a new external sorbing structure which can remove free metal in solution. Kinetic limitations are due to diffusion constraints, related to the ionic size of the hydroxylated species. Equilibrium is achieved later but the initial rate is increased by the soluble, hydrolysed metal species.

CONCLUSION

This study deals with the interpretation of sorption mechanisms. The important role played by pH on the uptake performances and the understanding of the various steps involved in this process have been demonstrated. Except for a small pH extent (acidic medium in which the pH specially influences cell wall chemistry), this parameter induces changes in uranyl chemistry such as solubility and the nature and percentages of various uranyl hydroxides (close to pH and to initial metal concentration).

The pH influencing the appearance of the hydroxides, and ionic sizes of the metal in solution, determines the steps which impose the limiting rate of the global kinetics. In a moderate acid medium the sorption mechanisms follow an original pathway, called the surface precipitation sorption model, including a phase of sorption of hydroxylation products on the cell wall, inducing a change in this sorbing surface capable of recovering other metallic species and free ions present in solution.

This kind of biomass, by-product of the enzyme industries, traditionally considered as waste, constitutes a good sorbing material. The maximum uptake capacity depends on the pH, and the uranium concentration in solution, but this capacity can reach a value considerably higher than the values reached with a classical sorbent: activated carbon, resins ... for a low metal concentration. The major interest of this process consists of the valuable treatment of industrial flow with low metal content.

The application of this technique to an industrial effluent would provide an answer to its economic feasibility. The experiments carried out on this subject prove the ability of biosorption to treat industrial wastewater and to remove selectively low concentrations of uranium in mining effluents (Guibal and Roulph, 1990; Guibal, 1990). Metal recovery from used and charged biomass enables us to valorize the step of depollution.

Application of these processes can cover a wide range of areas such as: detoxification of metal-bearing wastewaters, recovery of precious or strategic metals diluted in mining effluents, removal of metal from ore-processing solutions (treatment of leaching solutions) and concentration of rare metals from seawater or tailing ponds.

REFERENCES

Baes C. F. Jr and Mesmer R. E. (1976) *Hydrolysis of Cations*. Wiley, New York.

Farley K. J., Dzombak D. A. and Morel F. M. M. (1985) A surface precipitation model for the sorption of cations on metal oxides. *J. Colloid Interface Sci.* **106**, 226–242.

Guibal E. (1990) Contribution à l'étude de la biofixation de l'uranium par un champignon filamenteux: *Mucor Miehei*. Thèse de Doctorat, INSA de Lyon, Villeurbanne, France.

Guibal E. and Roulph C. (1990) Fixation de l'uranium par un champignon filamenteux: application au traitement d'effluents faiblement chargés. *J. fr. Hydrol.* **21**, 229–240.

Jernelov A. and Martin A. L. (1975) Ecological implications of metal metabolism of microorganisms. *A. Rev. Microbiol.* **29**, 61–77.

Le Cloirec P., Lelacheur R. M., Johnson J. D. and Christman R. F. (1990) Resin concentration of by-products from chlorination of aquatic humic substances. *Wat. Res.* **24**, 1151–1155.

Nakajima A. and Sakaguchi T. (1986) Selective accumulation of heavy metals by microorganisms. *Appl. Microbiol. Biotechnol.* **24**, 59–64.

de Rome L. and Gadd G. M. (1987) Copper adsorption by *Rhizopus arrhizus*, *Cladosporium resinae* and *Penicillium italicum*. *Appl. Microbiol. Biotechnol.* **26**, 84–90.

Shumate S. E. II, Strandberg G. W. and Parrott J. R. Jr (1978) Biological removal of metal ions from aqueous process streams. *Biotechnol. Bioengng Symp.* **8**, 13–20.

Strandberg G. W. and Shumate S. E. II (1982) Accumulation of uranium, cesium and radium by microbial cells, bench scale studies. Oak Ridge National Laboratory, ORNL TM-7599, Contract W. 7405 eng.26, Oak Ridge, Tenn., U.S.A.

Tsezos M. (1980) Biosorption of uranium and thorium. PhD. thesis, McGill University, Montreal, Canada.

Tsezos M. and Volesky B. (1982) The mechanism of uranium biosorption by *Rhizopus arrhizus*. *Biotechnol. Bioengng* XXIV, 385–401.

Wood J. M. and Wang H-K. (1983) Microbial resistance to heavy metals. *Envir. Sci. Technol.* **17**, 582A–590A.