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Complexation of uranium (VI) by three eco-types of *Acidithiobacillus ferrooxidans* studied using time-resolved laser-induced fluorescence spectroscopy and infrared spectroscopy

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Abstract

Time-resolved laser-induced fluorescence spectroscopy (TRLFS) was used to study the properties of uranium complexes (emission spectra and fluorescence lifetimes) formed by the cells of the three recently described eco-types of *Acidithiobacillus ferrooxidans*. The results demonstrated that these complexes have different lifetimes which increase in the same order as the capability of the strains to accumulate uranium. The complexes built by the cells of the eco-type II were the strongest, whereas, those of the eco-types I and III were significantly weaker. The emission spectra of all *A. ferrooxidans* complexes were almost identical to those of the uranyl organic phosphate compounds. The latter finding was confirmed by infrared spectroscopic analysis.

Introduction

The intensive uranium mining and milling performed over a period of 40 years (up to 1990) have caused significant pollution with uranium and other toxic metals in the Southeast part of Germany (Saxony and Thuringia). At present a larger number of uranium mining waste piles and mill-tailings continue to be hazardous sources for environmental pollution in this region (Bernhard *et al.* 1996). These contaminated sites require a long-term stewardship in addition to remediation. For effective remediation and stewardship a better understanding is needed of the uranium environmental chemistry and also of the interactions of this metal with bacteria and other biological components present in the above mentioned environments (John *et al*. 2001). The interaction of uranium with microorganisms is well documented (Volesky & Holan 1995; Lloyd & Macaskie 2000). Microbial cells are known to reduce, oxidize, biosorb, bioaccumulate, and bioprecipitate uranium (Lovely & Phillips 1992; Fredrickson *et al.* 2000; Merroun & Selenska-Pobell 2001) and other actinides (Francis 1998). Most of the studies on uranium remediation have used biosorption by various biomasses. The biosorption mechanism can be metabolism independent a function of the microbial cell activity (metabolism dependent). Living and dead microorganisms, possess abundant functional groups, such as carboxyl, hydroxyl and phosphate that bind metal ions (Lloyd & Macaskie 2000). In regard to their influence on the biosorption process (capacities and selectivities), it is necessary to know the microbial binding sites for the metals (Klimmek *et al*. 2001), and to characterize the metal-microbial complexes. For these purposes, a variety of physical techniques have been used such as extended X-ray absorption fine structure (EXAFS) spectroscopy (Merroun *et al*. 2001; Hennig *et al*. 2001), proton-induced X-ray emission analysis (PIXE) (Jeong 1997), nuclear magnetic resonance (NMR) spectroscopy (Andres *et al*. 1994), time-resolved laser-induced fluorescence spectroscopy (TRLFS) (Panak *et al.* 1999, 2000), infrared spectroscopy (IRS) (Yun *et al.* 2001), etc.

Infrared spectroscopy was used for resolving the chemical components in biological samples, characterization of protein secondary structures, identification and classification of microorganisms (Helm et al. 1991; Naumann et al. 1991). This technique has proved to be a practical tool in the identification of microbial functional groups responsible for the binding of metals (Fourest & Volesky 1996; Pagnanelli *et al.* 2000).

TRLFS is an excellent tool for study the complex formation of uranium and other actinides with various ligands at relatively low concentrations. This method is inappropriate for metal ions which show no change in their spectroscopic properties with complexation. It is based on the fact that the measured fluorescence lifetime and intensity of the electronic transition of the excited uranyl ions are dependent on their molecular environment (Rustenholtz *et al.* 2001). TRLFS has proven to be useful in discerning sorption mechanisms such as inner-sphere complexation, outer-sphere complexation (physical sorption).

Recently, three different eco-types of *A. ferrooxidans* were recovered from different sites and depths of two uranium mining wastes (Flemming *et al.* 2000; Selenska-Pobell *et al.* 2000, 2001; Selenska-Pobell 2002). The strains of different types present different capability to accumulate and tolerate uranium (Merroun & Selenska-Pobell 2001). This research is focused on investigation whether the uranium complexes built by the three eco-types of *A. ferrooxidans* can be discriminated on the basis of their chemical nature and physical properties using IRS and TRLFS. IR spectroscopy was applied in order to identify the main *A. ferrooxidans* functional groups implicated in the interaction with uranium. By using TRLFS we determined the lifetimes and emissions fluorescence bands of these complexes.

Materials and methods

Bacterial strains used

The bacterial strains used in this work are: *A. ferrooxidans* W1 (type I), *A. ferrooxidans* ATCC 33020 (type II), and *A. ferrooxidans* D2 (type III). The strain D2 was isolated from the depths of a uranium mine (Denison Mines Ltd, Elliot Lake, Ontario, Canada); strain W1 were kindly provided by Leo Leduc, University of Guelph, Guelph, Canada. The strain D2 is capable of oxidizing iron at 2 °C and was characterized as psychrotrophic by Leduc *et al.* (1993). *A. ferrooxidans* strains were cultured in 9K liquid medium containing 3 g of $(NH_4)_2SO_4$, 0.5 g of K_2HPO_4, 44.2 g of $FeSO_4$. $7H_2O$, 0.5 g of $MgSO_4 \times 7H_2O$, 0.1 g of KCl, and 0.014 g of $Ca(NO_3)_2 \times 4H_2O$ per liter.

Experimental procedure for IRS and TRLFS sample preparation

Bacterial cells grown to the mid-exponential phase (optical density at 600 nm, 0.4) were acidified with sulphuric acid to pH 1.3 to dissolve the Fe(III) precipitate which was produced during the growth. The cells were harvested by centrifugation (12,500 rpm for 30 min at 4 °C) and washed three times with 0.1 N sulphuric acid to remove the disturbing ingredients of the growth medium. Then the pellets were washed two times with 10 ml perchloric acid (0.01 M) using centrifugation at 12,500 rpm for 10 min for each wash.

For IRS samples, the washed cells were resuspended for 48 h in 10 ml 0.5 mM solution of uranyl (VI) in 0.01 M perchloric acid, pH 2. After the contact with uranyl, the cells were harvested and washed with 0.01 M perchloric acid. The pellet samples were dried at 30 °C for 24 h and ground.

For TRLFS measurements, the cell biomasses as described above (0.10 g dry weight/l) were contacted with 0.5 mM U(VI) in 0.1 N $HClO_4$ for 48 h.

The uranyl fluorescence was measured in biomass solution (0.10 g dry weight/l) with 0.5 mM U(VI) in 0.01 N $HClO_4$. The different uranyl species (free uranyl ion and uranyl species bound to the biomass) were deconvoluted by time resolution. Emission spectra were recorded from 408 to 634 nm using an excitation wavelength of 266 nm.

The amount of uranium removed by the cells was determined on a dry-weight basis. The dried samples were then acid hydrolyzed for analysis by Inductive Coupled Plasma-Mass Spectroscopy (ICP-MS). During these analysis a mass balance of metals was carried out by analysing all the supernatants.

Uptake of metal ions (q) was calculated from a metal mass balance yielding (Volesky 1990): q (mmol metal/g dry biomass) $= V(C_i - C_f)/mM$ where V is the sample volume (l), C_i and C_f are the initial and field metal concentrations (mg/l) respectively, m is the amount of dry biomass (g) and M is the relative molecular mass of the metal.

Three replicates consisting of three samples each were prepared, in addition to the control which consisted of cells which were not treated with any metal.

Experimental control samples with no biomass added were treated identically as blanks.

FT-IR spectroscopy

IR spectra of the untreated and uranium treated *A. ferrooxidans* biomass were obtained using a Perkin-Elmer FTIR spectrometer model 2000. Spectra from KBr pellets containing equal amounts of sample were recorded at room temperature between 500 and 4000 cm^{-1} at a resolution of 2 cm^{-1}.

TRLFS

A fluorescence spectrometer system based on a laser pulse from a Nd-YAG laser (GCR 230, Spectra Physics, USA) was applied for the TRLFS-measurements. A detailed description of the laser equipment and the measurement system is given in (Geipel *et al.* 1996). The excitation wavelength was 266 nm (4^{th} harmonic of the Nd-YAG laser) with a laser pulse energy between 400–600 μJ. The fluorescence signal was monitored by a 500 mm spectrograph (M1235, EG&G, USA) with a spectral resolution of 0.32 nm. The fluorescence spectra were measured by a multichannel gated diode array, cooled to −30 °C (M 1475A, EG&G, USA). The gate was controlled by a pulse generator (FPG/05, EG&G, USA) and was held constant at 1000 ns. The time resolved spectra were recorded between 0.1 and 60 μs after laser excitation in the wavelength range between 408 and 634 nm. Each time-resolved spectrum was recorded five times at each delay step, and for measurements without any time resolution each spectrum was monitored ten times at a constant delay time of 0.1 μs. Accumulation of 100 laser shots were averaged for each single spectrum. The spectra were standardized relative to the laser pulse energy.

Results

Infrared spectroscopy

The infrared spectroscopy was used in this study to determine the nature of *A. ferrooxidans* chemical groups involved in the complexation of uranium.

The FTIR spectrum (500 to 4000 cm^{-1}) of native biomass of *A. ferrooxidans* eco-type I (without uranium) is presented in Figure 1. There are distinct stretching frequencies; the band at 1532 cm^{-1} is assignable to NH bending of the secondary amide

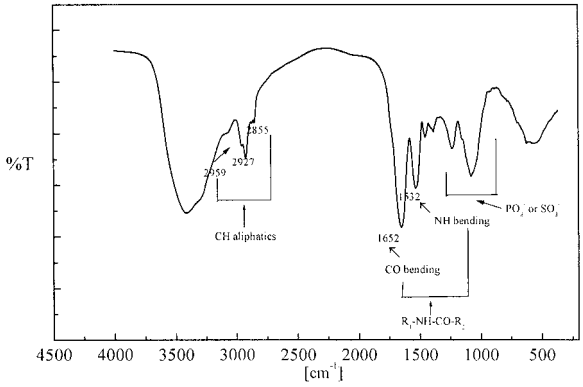

Fig. 1. IR spectrum of native cells of *A. ferrooxidans eco*-type I cells.

group CONH (amide II band), while the band between 1700 and 1600 cm^{-1} (1652 cm^{-1}) is indicative of the presence of CO groups. The spectrum also displays the absorption peak in the range from 3000 to 2800 cm^{-1} which are attributable to C-H stretching modes, indicating the presence of the alkyl groups CH_3, CH_2 and CH. In addition, the region from 1000 to 1300 cm^{-1} contains contributions from phosphate and/or sulphate groups. These groups belong to various cellular components, such as peptides, phospholipids, peptidoglycan, etc., which are able to complex metals.

The uranium treated biomass was washed, dried, and powdered under the same conditions used for the preparation of the native biomass (untreated).

Figure 2 shows the IR spectra for native and uranium treated *A. ferrooxidans* types. The absorption band at 926 cm^{-1} (eco-type I and II) and 927 cm^{-1} (eco-type III) originates from the asymmetric stretching vibration of the uranyl unit. The IR spectra of the three eco-types of *A. ferrooxidans* treated with uranium revealed a significant shift of absorption peak corresponding to phosphorus residues to higher wave numbers.

In order to validate our spectroscopic observations concerning the changes occurred after treatment of *A. ferrooxidans* cells with uranium and to investigate if there are some spectral differences between the uranium complexes formed by the 3 eco-types, we have calculated difference IR spectra between 800 and 1400 cm^{-1} (FTIR spectrum of untreated cells minus spectrum of cells exposed to uranium for each type, therefore we obtain negative values for the uranyl and phosphate absorption bands). The results shown in Figure 3 indicate the presence of three absorption

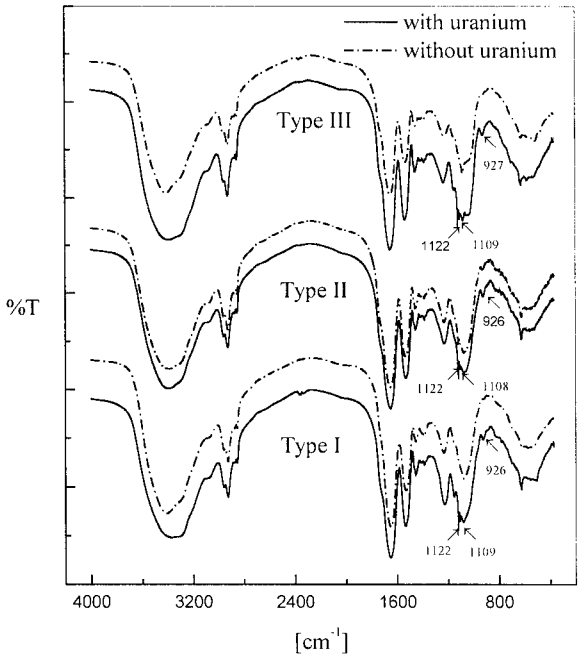

Fig. 2. IR spectra of the native and uranium treated cells of the three *A. ferrooxidans eco*-types.

peaks. The first peak at 928 cm^{-1} (type I and III); and 920 cm^{-1} (type II) is characteristic for the stretching vibration of the uranyl ion. The two other peaks at 1122 and 1109 $cm^{-1}$are attributed to phosphate groups.

TRLFS

We used time-resolved laser-induced fluorescence spectroscopy to characterize the uranium complexes built by the three eco-types of *A. ferrooxidans*. In Figure 4 the lifetimes of the *A. ferrooxidans* uranium complexes are shown. The lifetime τ_1 for uranyl ion (aq) was determined as 1.47 μs, while the lifetime τ_2 attributed to the uranyl species bound to the biomass of the *A. ferrooxidans* eco-type I was obtained as 23.6 μs. The lifetimes of the uranium complex bound to type II and III (τ_2) were determined as 44.6 and 25.8 μs, respectively. The lifetimes found in this work are in the range of the lifetime of uranyl organic phosphate compounds such as U(VI)-ATP (Geipel *et al.* in preparation).

In Table 1 the capability of the *A. ferrooxidans* to accumulate uranium and the lifetimes of the corresponding complexes are represented. The eco-type II which accumulates higher amount of uranium up to 44.6 mg uranium/g dry biomass, formed with this

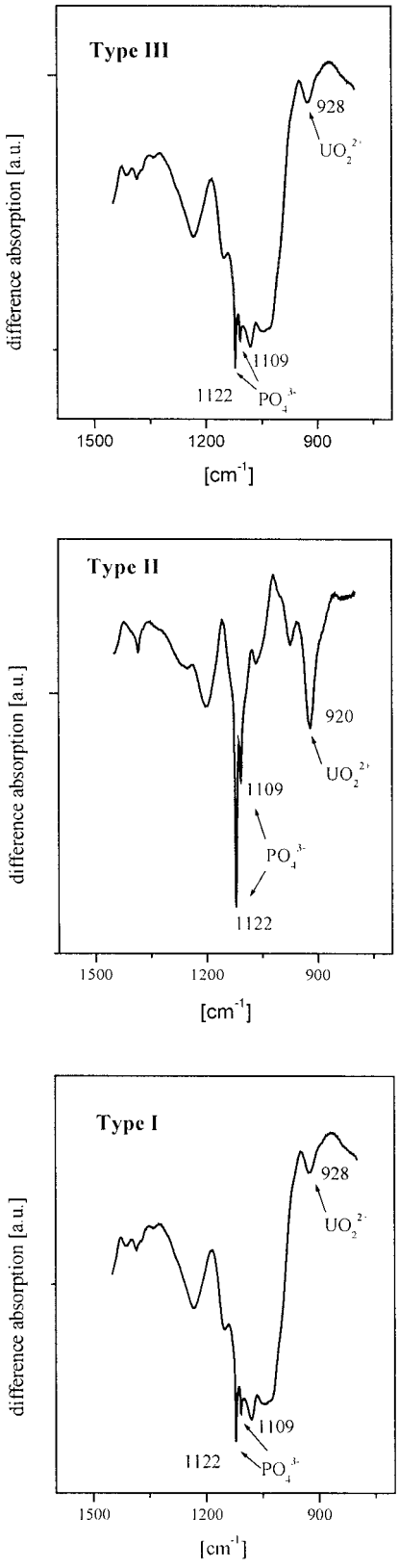

Fig. 3. IR spectra of cells exposed to uranium minus spectra of unexposed cells of *A. ferrooxidans* eco-types (difference IR spectra).

Table 1. The capability of the *A. ferrooxidans* types to accumulate uranium and lifetimes of the corresponding complexes

Sample	Lifetime/μs	Uranium accumulated (mg/g dry biomass)
U(VI)- *A. ferrooxidans* type I	23.6 ± 0.8	21.5 ± 1.8
U(VI)- *A. ferrooxidans* type III	25.8 ± 5	30.0 ± 2.2
U(VI)- *A. ferrooxidans* type II	44.6 ± 3.7	44.6 ± 3.2

metal a complex with a longer lifetime (44.6 μs). Whereas, the uranium complex formed by the eco-type I which accumulates only 21.5 mg uranium/g dry biomass, has significantly shorter lifetime (23.6 μs). The results showed that the fluorescence lifetimes of the uranium bacterial complexes increase in the same order (*A. ferrooxidans* type I, type III and type II) as the capability of the strains to accumulate uranium.

The fluorescence emission bands of the uranium complexes formed by the three eco-types of *A. ferrooxidans* and those of uranyl reference solution at pH 2, organic and inorganic uranyl phosphate compounds are shown in Table 2. The shape of the *A. ferrooxidans* uranium spectra and the resolution of the vibrational levels are different from those of the reference solution (free uranyl ion, 0.5 mM, pH 2). The spectra of the uranium complexes with different *A. ferrooxidans* types showed very small differences in shape and energy of the emissions bands. The energies of the emission bands of the bacterial complexes at pH 2 (496, 517, 541, 566 nm) are almost identical with the energies obtained for the uranyl organic phosphate compounds such as (U(VI)-ATP) (495, 517, 540, 565 nm) (U(VI)-AMP) (497, 519, 542, 569 nm) as well to those of the uranium complexes formed by *B. sphaericus* (498, 519, 542, 569 mm). However, they differ from those obtained for the uranyl inorganic phosphate compounds (503, 524, 548, 574 mm) (Kopp *et al.* 2001, see also Table 2).

Discussion

The 16S rRNA genes of a large number of *A. ferrooxidans* reference and uranium waste pile isolates possess specific signatures which distinguish three types within this species (Flemming *et al.* 2000; Selenska-Pobell *et al*. 2001). These types are heterogeneously distributed in the uranium mining waste piles: The group of isolates belonging to types I and III, were predominant in more contaminated samples from greater depths, while the representatives of the type II were distributed in less contaminated areas preferably close to the surface. Recently we have demonstrated that the representatives of these types possess different capability to accumulate and tolerate uranium (Merroun & Selenska-Pobell 2001). In addition, EXAFS measurements of U(VI) accumulated by the three types of *A. ferrooxidans* demonstrated that phosphorous containing residues of this bacterium are involved in the interaction with uranium, and that the corresponding uranium bacterial complexes have the same structural parameters. Transmission electron microscope studies demonstrated that the different eco-types of *A. ferrooxidans* accumulate uranium in a similar way by phosphate compounds via biosorption at the cell surface (cell wall and extracellular polysaccharides) and intracellular accumulation (polyphosphate bodies) (Merroun *et al.* 2002). Thus, by using EXAFS, EDX and TEM techniques we could not find differences in the structural properties of the uranium complexes formed by the three eco-types. Therefore, in this work we used other physical methods such as TRLFS and IRS in order to obtain additional information about the nature of the uranium complexes formed by *A. ferrooxidans*eco-types and possibly to discriminate them at the level of eco-types.

Using IR spectroscopy we have found that phosphate groups are the main binding sites for uranium. Metal ions are classified by their binding preferences, specifically, whether they seek out O-, N- or S-containing ligands. Pearson (1963) separated metal ions into hard acids (O-seeking) and soft acids (N- or S-seeking). In addition, Nieboer & Richardson (1980) proposed a third class (borderline) defined by behavior intermediate between these groups. Uranium as UO_2^{2+} is categorized as a hard acids and has affinity to carboxylate, phosphate, carbonyl, alcohol, and phosphodiester groups. The implication of bacterial phosphate groups in the binding of uranium is well

Table 2. Spectroscopic parameters of the bacterial UO_2^{2+}-complexes, the UO_2^{2+}-phosphate complexes, and the UO_2^{2+}-reference solution at pH 2.

Sample	Emission maxima (nm) (Halfwidth (nm))					
UO_2^{2+}, 0.5 mM, pH 2	474.9	491.1	512.2	536	561.2	585.9
	(11.6)	(13.1)	(14.7)	(17.8)	(15.8)	–
A. ferrooxidans type I	–	496.1	517.2	540.9	566.4	–
		(12.6)	(13.0)	(13.5)	(18.4)	
A. ferrooxidans type II	–	495.4	516.8	540.8	566.4	–
		(18.4)	(13.6)	(13.8)	(17.8)	
A. ferrooxidans type III	–	496.3	517.5	541.6	566.3	–
		(14.8)	(12.8)	(13.7)	(11.4)	
U-ATP (adenosine-triphosphate)	480.9	495.3	516.5	540.2	5.64.6	593.8
$UO_2(H_2PO_4)_2$	–	503	524	548	574	–
		(6.5)	(7.1)	–	–	
B. sphaericus (decomposed cells)	–	502	524	548	574	–
		(5.9)	(7.1)	–	–	
B. sphaericus (spores)	–	498	519	542	569	–
		(11.0)	(11.7)	–	–	
B. sphaericus (vegetative cells)	–	498	519	542	569	–
		(10.8)	(11.7)	–	–	
U-AMP (adenosine – monophosphate)	–	497	519	542	569	–
		(10.7)	(11.4)	–	–	

documented. Studies using ^{31}P nuclear magnetic resonance suggested that the cellular phosphate groups play an important role in uranium biosorption (Andres *et al*. 1994, 1995). In addition to the biogenic phosphate ligand, which precipitates with uranyl ions, phosphate groups of the lipid A component of the exocellular lipopolysaccharidic material of *Citrobacter* sp. was suggested on the basis of ^{31}P NMR measurements to be involved in the formation of nucleating (priming) deposits of uranyl phosphate (Macaskie *et al*. 2000). Ferris & Beveridge (1986) demonstrated that the phosphoryl residues of the polar head of phospholipids and LPS in the outer membrane were the most probable binding sites for metal cations in the *Escherichia coli* K-12. For *Mycobacterium smegmatis*, the phosphate was hypothesized to be derived from sugar phosphates or adenosine phosphates or even from cellular polyphosphates (Andres *et al.* 1995).

The difference IR spectroscopy allows to confirm the implication of phosphate groups in the complexation of uranium and to show some spectral differences between the uranium complexes formed by the three bacterial types, particularly, in the frequency of uranium absorption band (920 cm^{-1} (type II) vs. 928 cm^{-1} (type I and III)). These results are in agreement with those found by TRLFS which demonstrate that the lifetime of the uranium complexes formed by type II are longer than those corresponding to type I and III.

The experiments performed in this work were conducted at pH 2. At this low pH values and in the presence of uranium, the phosphate groups are partially protonated.

A part of uranium is bound to the deprotonated phosphate since the complex formation constants of uranium with PO_4^{3-} are higher than those of protons with PO_4^{3-}.

The other part of this metal is bound to the protonated phosphate groups. Several lines of evidence support this conclusion: (i) EXAFS study of the uranium complexes formed by *Bacillus subtilis* at pH 1.7 demonstrated that uranium is coordinated to two different phosphate groups with different distances (3.63 and 3.90 Å) (Kelly *et al.* 2001). The larger bond distance (3.90 Å) may arise from the complexation of uranium with protonated phosphate groups. (ii) Fowle *et al*. (2000), studying uranyl adsorption onto

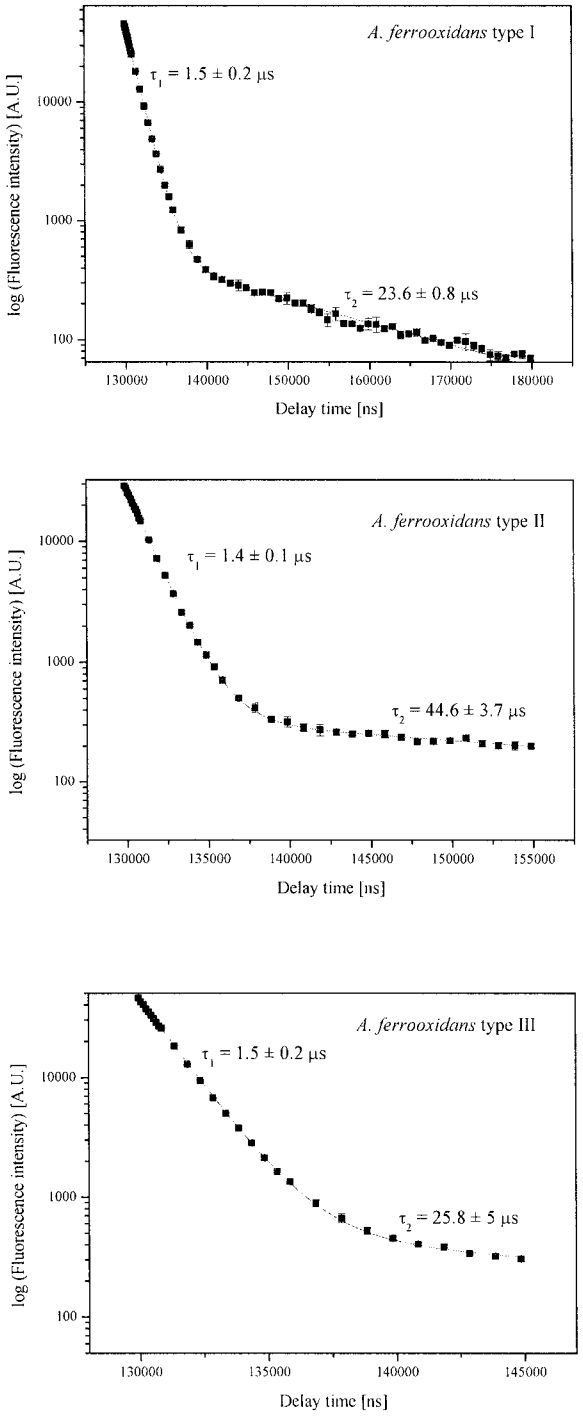

Fig. 4. Time-dependency of emission decay of U(VI)- *A. ferrooxidans* complexes.

B. subtilis, have demonstrated that the best fitting for uranium adsorption at low pH is the one involving adsorption onto protonated phosphate sites; (iii) independent ^{31}P NMR measurements have demonstrated that uranyl ion binding to phosphate sites of *M. smegmatis* occurs at pH values as low as 1.0 (Andres *et al.* 1994); (iv) metal complexes with protonated phosphate ligands are well documented (Martell & Smith 1977).

TRLFS is a very sensitive method which can give insight into complexation reaction even with poorly defined polyfunctional ligands like living bacterial cells. Using this method, different spectroscopic characteristics, i.e., energy and shape of the emission bands and the lifetime of fluorescence decay can be used to identify a particular uranyl species. Furthermore, the emission lifetime of U(VI) in the micro second range allows an efficient suppression of Rayleigh and Raman scattering and also of a short-living fluorescence of organic compounds. Therefore, the complexation of uranyl with the biomass can be conveniently investigated. Any significant background signals coming from organic substances can be discriminated by setting an appropriate time gate.

The results of this work demonstrate that the uranium complexes built by the three eco-types of *A. ferrooxidans* have different lifetimes. These differences could be explained by the fact that the eco-type II forms very strong complexes (higher formation constants) with uranium and the lifetime of the corresponding uranyl complexes obtained are longer in comparison with the eco-types I and III which form significantly weaker uranium complexes with shorter lifetimes. In addition, the amount of the uranium bound increases with the increasing the formation constants values (mass action law). Therefore, the amount of uranium accumulated by the three bacterial eco-types increases with the increase of the lifetime of the corresponding uranium complexes. This statement (stronger complexes give rise to longer lifetimes) is supported by the followings:

(1) EXAFS study of the uranium complexes formed by these eco-types demonstrated that the uranium is coordinated to 2 different binding sites at the phosphoryl groups (at bond distances of 3.62 and 3.88 Å) (Merroun *et al.* 2002).

(2) A change in the uranium coordination shell may occur during the contact of the cells with uranium solution. This change leads to a decrease of the number of water molecules in the coordination shell of this metal. As the water acts as a quencher of the

uranyl fluorescence, a decrease in the number of water molecules leads to an increase in the fluorescence lifetimes. In aqueous solution, the lifetime of the excited state is determined in part by a non-radiative decay path according to the energy transfer from electronic states of the metal ion to vibrational states of coordinated water molecules. Therefore, the substitution of water molecules from the inner coordination sphere by strong complexing ligands causes an increase of fluorescence field and thus, an increase of the lifetime. Such an increase of the fluorescence lifetime as function of the decrease of water molecules in the coordination shell is described for curium (Kimura & Choppin 1994). Moreover, Stumpf *et al.* (2001) demonstrated that the increase in lifetime of the kaolinite- and smectite- curium complexes reflects the exclusion of water molecules of the first coordination sphere of the curium.

In summary, the formation of strong complexes is accompanied by an increase of the binding coordination between uranium and phosphorous and a decrease of the water molecules in uranium coordination shell which in turn increases the fluorescence lifetimes.

In addition, the lifetime of the uranyl ion species bound to type I, III, and II of *A. ferrooxidans* is 15.7, 17.1, 29.8 times larger than that of the aqueous uranyl ion, respectively. This fact confirms the formation of inner-sphere uranium complexes by these bacterial types.

Panak *et al.* (1999) found that the lifetime of the uranium complexes formed by *A. ferrooxidans* 33020, given as type II in this work, is 3.91 μs (44.62 μs in this work). However, the conditions of that experiments were different from ours. For instance, Panak *et al.* (1999), used $HClO_4$ at a concentration of 0.032 M for preparing uranium solution, in this work we used 0.01 M. This finding is in agreement with Rustenholtz *et al.* (2001) who demonstrated that the fluorescence lifetime of the excited uranyl ion depend considerably on the concentration of $HClO_4$. Although perchlorate ions are known not to complex with uranyl ions, increasing amounts of ClO_4^- lead to a decrease of the lifetime of the uranium fluorescence signal.

The wavelengths of the emission spectra of the uranium complexes formed by the three eco-types did not shift with the increasing of the delay time (data not shown). The latter indicates that the same chemical groups are involved in the complexation of uranium.

The emission fluorescence bands of the *A. ferrooxidans* uranium complexes studied using TRLFS are in agreement with the results found using other spectroscopic techniques such as EXAFS, and EDX which indicate that organic phosphate coordinates to the uranyl ions (Merroun *et al.* 2002). Similar results were found by Knopp *et al.* (2001), by comparing TRLFS spectra of U(VI) complexed by vegetative cells, spores and intact dead cells of *B. sphaericus* to those of AMP-U(VI) complexes. They found very similar spectra for all these complex species and concluded that uranium was bound via organic phosphate-containing cell structures like teichoic acids. The authors found also that U(VI) in contact with decomposed cells, showed a very different fluorescence spectrum nearly identical to the spectrum of $UO_2(H_2PO_4)_2$.

The current study has demonstrated the implication of organic phosphate groups of the three types of *A. ferrooxidans* in the interaction with uranium using IRS and TRLFS. These results are in agreement with those obtained by using other physical methods such as EXAFS and EDX. In addition, TRLFS and IRS can discriminate between the uranium complexes formed by the three bacterial eco-types.

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