



Deliverable No. D4.2

NAME OF THE DELIVERABLE:

**CHEMICAL AND REDOX BEHAVIOR OF THE INVESTIGATED RADIONUCLIDES
IN THE DIFFERENT SYSTEMS THROUGH MICROBIOLOGICAL MEDIATED
PROCESSES**

REDOX PHENOMENA CONTROLLING SYSTEMS ReCosy

COLLABORATIVE PROJECT (CP)

Submitting organization: HZDR
Due date of deliverable: 45 Project Months
Actual submission: 45 Project Months

Grant agreement N°.: FP7-212287

Start date of the project: 01 April 2008
Duration: 48 months

Project co-funded by the European Commission under the Seventh Framework Programme of the European Atomic Energy Community (Euratom) for nuclear research and training activities (2007 to 2011)		
Dissemination Level		
PU	Public	PU
RE	Restricted to a group specified by the partners of the project	
CO	Confidential, only for partners of the project	



This deliverable is also reported as the WP 4 summary in the Proceedings of the 4th Annual Workshop

Within WP 4.2 the participating institutes are focusing their work on the study of the microbial impact (**FTMC**) and on the uranium redox state in-situ in biofilms with emphasis on biologically mediated redox processes (**HZDR**). The studies are carried out on isolated microorganisms as well as on biofilms. Biofilms are composed of bacteria, fungi, algae, protozoa, exopolymeric substances (EPS), corrosion products and 50–95% water. They are ubiquitous and have to be considered as an important factor in natural biogeochemical processes influencing the redox state of radionuclides. They show a multiplicity of interactions with metals and contribute to metal mobility or immobilization.

Work performed by partners

The activities of **HZDR (8)** during the third year of ReCosy were focused on biologically mediated redox processes of biofilms growing in the nuclear waste repository research tunnel ONKALO (Finland), which will be part of the nuclear waste repository in the future.

Introduction

Since most surface and subsurface environments are inhabited by microorganisms, natural microbial communities (= biofilms) are considered, along with minerals, as an important factor influencing the transport of radionuclides in the environment. Microsensor measurements of the oxygen concentration, redox potential and pH are helpful in interpreting in-situ microbial metabolic processes in biofilms. Since microbial processes are sensitive to metals and their speciation, the bioavailability of the metals changes with redox potential, pH, and oxygen in a complex manner. Massive biofilms are growing next to a fracture zone in a granitic rock environment. at site 777 m of the tunnel. They were described by Pedersen et al. (2008) as a pink and solid slime, consisting of *Pseudomonas anguilliseptica*, *Arthrobacter bergeri*, *Hydrogenophaga sp.*, *Methylobacter tundripaludum*, *Rhodoferrax ferrireducens*, and *Haliscomenobacter hydrossis*.

Experimental

Biofilm samples were removed from the tunnel wall at the 777 m position and exposed into a rectangular cell with an outer dimension of 121 x 42 x 15 mm. During the sensor measurements, 150 ml of the groundwater as a blank solution from the site was pumped through the cell in a closed circuit with a flow velocity of approximately 4 mL/min. For the sensor measurements a miniaturized platinum redox microelectrode, oxygen microsensor of the Clark-type and a miniaturized conventional pH electrode, each with a tip diameter of 10 µm, were used. After the values were recorded, uranium was added in ecologically relevant concentrations (4×10^{-5} M) to the groundwater solution. Microsensor measurements were recorded 22 and 42 hours after the addition of uranium. Samples of the groundwater solution were taken before and after the addition



of uranium as well as at the end of the experiments. The samples were acidified in-situ and analyzed for determination of the inorganic elements by Inductively Coupled Plasma Spectrometry. The anions were determined by Ion Chromatography. The analytical data of the groundwater sample was used for the calculation of the predominance fields of different uranium species in the pH-Eh diagram for the U-S-O-H-C system at 15°C by using the geochemical speciation code “Geochemist’s Workbench” Version 8.0.8 / ACT2 Version 8.0.8. For Energy-filtered Transmission Electron Microscopy (EF-TEM) and by electron energy loss spectroscopy (EELS) studies a biofilm sample was fixed in-situ with 1 % (vol/vol) glutardialdehyde at the end of the experiments and prepared afterwards following the routine embedding protocol with minor modifications as described by Lünsdorf et al. (2001). EF-TEM offers the possibility to systematically study and analyse the ultrastructure and elemental composition of nanoscale mater by electron energy loss spectroscopy (EELS).

Results

Several microprofilings of the redox potential and pH were performed in the biofilm sample, placed in the flow cell. Before the addition of uranium to the groundwater, which was pumped through the biofilm in a closed circle, the results showed an average value of $+70 \pm 2$ mV, including a correction factor of + 239 mV after Stumm and Morgan (1996) and a pH of 5.37. The values measured in the groundwater differed significantly. The results showed a pH of 8.68 and a redox potential of +491 mV. The redox potential of the groundwater is approximately 420 mV lower than in the biofilm. The difference of the pH amounts more than 3.5 units. These results clearly demonstrate that the geochemistry inside a biofilm is totally different compared to the surrounding water/environment. With regard to the migration and the retention of radionuclides these differences are of great importance. In uranium contaminated waters the formation of possible uranium species are dependent on the redox potential and the pH. Changes of these parameters will have the consequence of the formation of solid uranium(IV) and uranium(VI) species as precipitates in the determined biofilm. In our experiments microsensors measurements were performed 42 hours after the addition of uranium, showing a significant change of the parameters. The redox potential within the biofilm decreased to -164 mV with an increase of the pH at the same time to 7.27. The low redox potential indicates that reducing conditions are present, probably catalyzed by microorganisms. For a better interpretation a pH-Eh diagram for the U-S-O-H-C system at 15°C was constructed by using the geochemical speciation code “Geochemist’s Workbench” Version 8.0.8 / ACT2 Version 8.0.8. The analytical data of the uranium contaminated groundwater was used for the calculation of the predominance fields of different uranium species. The default data base used was the thermo.dat accompanying code, supplemented by the most recent NEA database for uranium (Guillaumont et al., 2003), and by solubility data for Bayleyite from Gourman-Lewis et al. (2008). The theoretical predominance fields of solid uranium species under the ambient condition found in the contaminated groundwater are defined clearly in geochemically different areas: The first area is characterized by a redox potential of approximately $< 1V$ and a $pH > 4.0$. This area is dominated by the solid uranyl carbonate Rutherfordine (UO_2CO_3) and the magnesia bearing uranyl carbonate mineral Bayleyite [$Mg_2(UO_2)(CO_3)3 \cdot 18(H_2O)$] at a $pH > 4.2$. The second area is characterized by a redox potential of approximately $< +320$ mV and a pH which varies between 0 and 5.8.

Assuming reducing conditions, the formation of Uraninite, a uranium(IV) oxide (UO_2) mineral, was predicted. The results of the redox potential measurements of the biofilm 42 hours after the addition of uranium to the groundwater were plotted together with the pH into the calculated pH-Eh diagram for the U-S-O-H-C system. As shown in Fig. 3, the plots appear in the area of Bayleyite, indicating that an uranium(VI) solid mineral may have been formed in the contaminated groundwater. For great importance is the localization of the measured biofilm plotted into the eh-pH diagram. Due to the fact that after the addition of uranium the redox potential inside the biofilm decreased and the pH increased the formation of a solid uranium(IV) mineral within the biofilm seems possible after thermodynamically calculations.

The retardation of uranium in the biofilm was determined by Energy-filtered Transmission Electron Microscopy (EF-TEM) and Electron Energy Loss Spectroscopy (EELS). Elongated particles of high electron density were observed in the cytoplasm of some rod shaped gram negative bacteria, which were often found associated with large rod shaped bacteria. Analysis of the elongated particles by EELS provided spectroscopic evidence for the presence of uranium immobilization, showing unequivocally uranium ionization intensity peaks of O4,5- and N6,7-edges. Distribution analysis of uranium, phosphorus and calcium clearly showed, that a solid uranium mineral has formed intracellular, which indicates the presence of a solid U-phosphate mineral similar to Autunite ($\text{Ca}[\text{UO}_2]_2[\text{PO}_4]_2 \cdot 10\text{-}12\text{H}_2\text{O}$).

The scientific activities of **FTMC (21)** during the third year within ReCosy comprised the behaviour of $^{99}\text{Tc(VII)}$ in the system iron-bearing mineral-Na brine-microorganism under aerobic conditions. In order to estimate the effect of minerals/mineral surface on redox, sensitive radionuclide (technecium) sorption has been studied. To explore the interactions of Tc with iron-bearing minerals, series of laboratory batch-type experiments were carried out. The effect of pH on the radionuclide transport/retention was taken into consideration as well as the exposure time and the microbial activity. The composition of two powdered iron oxides (purchased from FSU) was verified using Mössbauer spectroscopy. Mössbauer spectra indicated the presence of hematite (Fe_2O_3) (first sample) and wustite and magnetite (second sample). For wustite, Fe(II) and Fe(III) components of Mössbauer spectra in the ratio 18:82 were detected in agreement with wustite formula $\text{Fe}(1-x)\text{O}$. Two subspectra, which are attributed to tetrahedral and octahedral sublattices are characteristic of magnetite. The ratio of area of magnetite subspectra is 45:55. Tetrahedral sublattice of magnetite is occupied by Fe(III) ions while octahedral is occupied by Fe(II)+Fe(III) ions. Thus, Fe(II) may be found either in magnetite and wustite.

For the batch experiments under ambient conditions, 0.1 g of mineral hematite (ms) and 0.2 g of mixture of wustite and magnetite (resuspended in 0.08 M Na brine solution) were used. The colloidal mineral suspensions were obtained by adding the volume (V) of 5 mL of Na-brine solution to hematite. The volume to mass ratio was 50 in the series of experiments ($V/\text{ms}=50$) with hematite. The solution volume to mass ratio ($V/\text{ms}=250$) was 250 in the experiments with mineral wustite/magnetite. After addition of ammonium pertechnetate to the suspension pH values reached 7.5 ± 0.5 . Bacteria and fungi, isolated from groundwater of two different boreholes and from soil of known physical-chemical properties, were tested under different conditions. Microbes (*Streptomyces sp.*, *Aspergillus niger*, *Arthrobacter globiformis*, *Cellulomonas cellulans*, *Bacillus mycoides*, *Fusarium oxysporum*, *Penicillium sp.*, *Rhodococcus sp.*, *Spicaria*

sp.) were selected for batch-type experiments because of their more pronounced peculiarities: ability to reduce nitrate, H₂S formation, organic acid production and resistance to different pH values. The ability of selected microorganisms to participate in adsorption processes of ⁹⁹Tc(VII) on hematite and wustite/magnetite was investigated by batch experiments. Results of the combined effect of microorganisms and iron-bearing minerals on Tc(VII) sorption peculiarities using batch-type experiments have shown that bacteria isolated from fluided soil *Arthrobacter globiformis* and *Cellulomonas cellulans* practically did not have any influence on Tc(VII) sorption onto hematite under aerobic conditions, while *Micromicete Fusarium Oxysporum* of the same substrate altered sorption to approximately 85% compared to that in the system without microorganisms. Differences in Tc(VII) sorption processes onto hematite due to microbial activity of microorganisms isolated from the groundwater borehole were observed as well. Presence of microorganisms *Penicillium sp.*, *Rhodococcus sp.* and *Streptomyces sp.* in the tested system induced Tc(VII) sorption onto hematite up to 80-85%. The effect of microbial activity of *Bacillus mycoides*, *Aspergillus niger* and *Spicaria sp.* on Tc(VII) sorption onto hematite was lower 60%, 27% and 17%, respectively. Thus, we can state that mineral hematite at neutral or slightly alkaline pH under aerobic conditions is attributed to minerals which do not adsorb Tc(VII). Stimulation of Tc(VII) sorption onto hematite is achieved because of presence of specific microorganisms.

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