**Experiment title:**
Uranium binding of sol-gel immobilized cells and S-layers of *Bacillus sphaericus* JG-A12 and NCTC 9602

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**Report:**

**Introduction.** Vegetative cells of the natural *Bacillus sphaericus* isolate JG-A12, recovered from a uranium mining waste pile, accumulate selectively large amounts of U, Cu, Pb, Al, and Cd /1/. In addition, *B. sphaericus* JG-A12 possesses a surface layer (S-layer) as outermost cell wall component, which differs at its N-terminal domain significantly from all other S-layers studied up to date /2/. The geographic origin of *B. sphaericus* JG-A12 and the ability to interact with heavy metals makes it a good candidate for preparation of bacteria-based ceramics (biocers) for *in situ* bioremediation of uranium contaminated waste wasters. Vegetative cells of *B. sphaericus* JG-A12 were embedded in a porous SiO₂ matrix using sol-gel techniques /3/. The aim of this experiment is to investigate the sorption of uranium by the biocer, the single biocomponent and the SiO₂ matrix itself.

**Experimental.** Sol-gel ceramics were prepared by dispersing vegetative cells in an aqueous silica nanosol, gelling and drying. 100 mg biocer consists of 81.8 mg SiO₂ and 18.2 mg cells. Before the experiments the pH of the biocer was adjusted to pH 4.5 by washing several times with 0.9% M NaClO₄, pH 4.5. For uranium sorption, all components were shaken 48 h in 30 ml 9x10⁻⁴ M uranium nitrate in 0.9% M NaClO₄, pH 4.5 at 30 °C and under air. After incubation, samples were washed with 30 ml 0.9% M NaClO₄ pH 4.5 and with 30 ml aqua dest., pH 4.5. The particles were dried in a vacuum incubator at 30 °C and powdered. Uranium L₃-edge (17185 eV) X-ray absorption spectra were collected at room temperature in fluorescence mode at the Rossendorf Beamline (ROBL) using the Si(111) double-crystal monochromator.

**Results and discussion.** The Fourier transforms (FT) of the EXAFS spectra of the JG-A12 cell-uranium complex show five significant peaks (Fig. 1). The most prominent peak is at 1.3 Å. It arises from the backscattering caused by oxygen atoms nearest to the uranium, which can be identified as the axial oxygens of the linear uranyl
groups (U-O\text{ax}) with a distance of 1.76 ± 0.02 Å.

The second and third peak was modeled to 2 equatorial oxygen shells, which observed to have a radial distances of 2.26 and 2.41 ± 0.02 Å, respectively. All FTs contain a peak at about 2.3 Å, which can be well fit by C neighbours at 2.91 Å. This distance is typical for carbonate groups coordinated to U(VI) in a bidentate fashion. The FT peak between 2.7 and 3.9 Å could arise from a phosphorus and the twofold degenerated 3-legged multi scattering path U-O\text{eq1}-P\text{1} or from a phosphorus and an additional single scattering path U-P\text{2} instead of the multi scattering path U-O\text{eq1}-P\text{1}.

In the case of the SiO\textsubscript{2} matrix, the uranium is coordinated to 2 axial oxygen atoms at a distance of 1.77 ± 0.02 Å. The second shell corresponds to the coordination of uranium to 3 equatorial oxygen atoms at a distance of 2.28 ± 0.02 Å. The broad shell (third peak) could be splitted in two individual peaks, one could be modeled to interaction between uranium and a second equatorial oxygen (2.44 ± 0.02 Å). This oxygen atom arises from water molecules. The second peak could be modeled to interaction between uranium and silicon at distance of 3.10 Å.

Using cell biocers uranium is coordinated to 2 axial oxygen atoms at a distance of 1.77 ± 0.02 Å. The second shell corresponds to the coordination of uranium to 5 equatorial oxygen atoms at a distance of 2.26 ± 0.02 Å. The third peak corresponds to interaction of uranium with silicon atom at a distance of 3.10 Å. The fourth peak is modelled to interaction between uranium and phosphorous atom at distance of 3.59 Å.

In summary, EXAFS analysis of the uranium complexes formed by B. sphaericus JG-A12 cells demonstrated the implication of carboxyl and phosphate groups and for the uranium complexes formed by the SiO\textsubscript{2} matrix the implication of silicate and hydroxyl (water molecules) groups in the interaction with uranium.

References
/1/ Selenska-Pobell et al., FEMS Microbial. Ecol. 29, 59-67 (1999)