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APPLICATIONS OF SYNCHROTRON RADIATION TECHNIQUES TO MATERIALS SCIENCE

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**Dale L. Perry, Neal D. Shinn, Roger L. Stockbauer,
Kevin L. D'Amico, and Louis J. Terminello**

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EDITORS:

Dale L. Perry

Lawrence Berkeley Laboratory
Berkeley, California, U.S.A.

Neal D. Shinn

Sandia National Laboratories
Albuquerque, New Mexico, U.S.A.

Roger L. Stockbauer

Louisiana State University
Baton Rouge, Louisiana, U.S.A.

Kevin L. D'Amico

X-ray Analytics, Ltd.
Upton, New York, U.S.A.

Louis J. Terminello

Lawrence Livermore National Laboratory
Livermore, California, U.S.A.

MATERIALS RESEARCH SOCIETY
Pittsburgh, Pennsylvania

SPECIATION OF URANIUM AFTER MICROBIAL ACTION BY XANES AND XPS

CLEVELAND J. DODGE*, A.J. FRANCIS*, F. LU*, G. P. HALADA**, S.V. KAGWADE**, AND C.R. CLAYTON**

*Department of Applied Science, Brookhaven National Laboratory, Upton, NY 11973

**Department of Materials Science, State University of New York at Stony Brook, Stony Brook, NY 11794

ABSTRACT

The speciation of radionuclides and toxic metals in wastes subjected to microbial action is important in determining the extent of stabilization in a disposal environment. As part of an ongoing study, we investigated the reduction of uranium by a *Clostridium* sp. using X-ray absorption near edge spectroscopy (XANES) at the National Synchrotron Light Source (NSLS) and X-ray photoelectron spectroscopy (XPS). XPS analysis of uranyl acetate containing hexavalent uranium exhibited a binding energy of 382.0eV at the U 4f_{7/2} peak. The sample incubated in the presence of bacteria was shifted to lower binding energy (380.6eV), confirming the reduction of U⁶⁺ to U⁴⁺ at the bacterial surface. XANES analysis, using an electron yield detector, was performed at the M_V absorption edge (3d→5f). The absorption peak energy of the sample exhibited a shift from 3551.1eV to 3550.1eV which is higher than uranium metal (3549.6eV) but lower than U⁴⁺ (3550.4eV). This indicates the presence of U³⁺ which is probably located beneath the surface within the biomass. Anaerobic bacterial treatment of wastes containing uranyl ion can result in the stabilization of uranium.

INTRODUCTION

Microbial transformations of metals can affect their solubilization or immobilization as a result of electron transfer processes, production of chelating agents, or bioaccumulation by the bacterial cells [1]. Electron transfer processes, in particular, can result in the formation of highly charged metal species which can precipitate from solution or become bound to bacterial biomass. The oxidation state of the metal is therefore important in determining its potential for migration in the environment. Uranium is of particular interest because of its multiple valences and presence in nuclear waste [2]. Previous studies dealing with uranium speciation in microbial culture samples have used indirect analytical methods [3,4]. In this study we used the non-destructive analytical techniques XPS and XANES to identify the oxidation states of uranium.

MATERIALS AND METHODS

Culture Conditions

The bacterial growth medium contained the following substances (per liter): glucose, 5.0g; NH₄Cl, 0.5g; glycerolphosphate, 0.3g; MgSO₄·7H₂O, 0.2g; FeSO₄·7H₂O, 2.8mg; CaCl₂·2H₂O, 0.5g; peptone, 0.1g; yeast extract, 0.1g; pH adjusted to 6.8. The medium was prereduced by boiling for fifteen minutes while purging with ultra-high purity (UHP) nitrogen gas. Forty mL of medium was dispensed into 60mL serum bottles. Uranyl acetate (210 μM) was filter sterilized and deoxygenated before adding it to the medium. The medium was inoculated with an anaerobic, fermentative bacterium, *Clostridium* sp. (ATCC #53464) which was isolated from a coal cleaning waste. Samples were incubated in triplicate at 26°C in the dark and grown to late log phase (45h). Total gas, pH, turbidity, and organic acid metabolites were determined as described previously [5]. The mechanisms of uranium reduction also were determined [6].

The cell pellet was collected by centrifugation then dried in a dessicator under vacuum. Uranium speciation was determined using x-ray analytical techniques. Uninoculated samples containing uranium were included as controls. The samples were manipulated under minimal light inside an anaerobic glove box filled with nitrogen.

Uranium Speciation

X-ray photoelectron spectroscopy (XPS).

X-ray spectra were obtained using a VG Scientific ESCA 3 MK II spectrometer coupled to a CLAM 100 hemispherical analyzer having an aperture of 4mm. Samples were pressed firmly onto indium foil and transferred to the analyzer under argon to minimize surface modification. Analyses were performed at a takeoff angle of 50° with respect to the sample surface using an Al K_α x-ray source operated at 400W. The samples were cooled with liquid nitrogen to minimize heating and sample decomposition. The binding energies of reference metals Au $4f_{7/2}$ and Cu $2p_{3/2}$ were 83.8eV (FWHM 1.4eV) and 932.5eV (FWHM 1.7eV), respectively. Spectra were smoothed and peak position determined after curve fitting [7,8]. A correction for charge shifting was made using the C 1s peak from adventitious hydrocarbon at 284.6eV. The oxidation states of uranium in the cell pellet and in the precipitate from the control sample were determined by comparison with binding energy shifts of uranyl acetate and uranium dioxide.

X-ray absorption near edge spectroscopy (XANES).

Measurements were made at the M_V absorption peak using an electron yield detector and a double crystal Si(111) monochromator on the X-19A beam line at the National Synchrotron Light Source (NSLS). Samples and standards were prepared for analysis by first mixing with DUCO cement (duPont de Nemours Co.) and acetone and placing the sample on the conductive surface of an aluminized Mylar foil. The dried samples then were placed in a sample chamber flushed with helium. Data points in the absorption peak region were collected with a resolution of 0.1eV and a 3s time average using a scanning program. Spectra were normalized after background subtraction and fitted by a non-linear least squares fitting program. Peak absorption values were compared to high purity uranium dioxide and uranium metal which had been cleaned in 6M nitric acid to remove the oxide coating.

All the samples were processed in nitrogen flushed glove bags to reduce exposure to oxygen.

RESULTS

Speciation of Uranium After Bacterial Action

Uranyl ion added to the bacterial growth medium immediately resulted in the formation of a colloidal precipitate due to hydrolysis and to condensation reactions with the components of the medium. At the end of the experiment, a small amount of uranium was present in solution in both the inoculated (<1%) and uninoculated control (<5%) samples.

The uranyl acetate reduced the growth of *Clostridium* sp. as evidenced by a decrease in turbidity and total gas production, and an increase in pH (Table I). This effect was enhanced with increased concentration of uranium. The organic acid metabolites, acetic and butyric acid, were decreased by 60% and 68%, respectively, in the sample containing 210 μ M uranium.

Further studies to determine the mechanisms involved in the reduction of uranyl acetate showed that it occurred only in the presence of live bacteria. Organic acid metabolites, medium components, and filtered cell-free spent medium containing extracellular products did not reduce uranium.

TABLE I.
Effect of uranyl acetate on growth of *Clostridium* sp.

Uranium (μ M)	Turbidity (600 nm)	Gas Produced (mL)	pH	Acetic acid -----mM-----	Butyric acid
0	0.84 ± 0.02	31.6 ± 0.7	3.09 ± 0.01	8.4 ± 0.3	13.7 ± 0.8
42	0.80 ± 0.00	28.2 ± 2.1	3.16 ± 0.01	7.6 ± 0.1	11.1 ± 0.4
210	0.34 ± 0.20	9.6 ± 6.1	5.04 ± 0.72	3.3 ± 1.2	4.4 ± 1.2

Values shown are $\pm$ standard error of the mean (SEM).

The cell pellet recovered from the inoculated sample at the end of the experiment was dark green indicating the presence of the reduced form of uranium, whereas the precipitate from the uninoculated control was yellow, characteristic of uranyl salts.

X-ray photoelectron spectroscopy.

The oxidation states of uranium at the surface of the samples were determined by XPS analysis. (Figure 1). The presence of two peaks in the spectrum is due to multiplet splitting of electrons with unpaired spins in the atomic shells representing U $4f_{7/2}$ and U $4f_{5/2}$ spin states. In the uninoculated control sample, the binding energy of the U $4f_{7/2}$ peak (A) was at 382.0eV, which was identical to the spectrum of the uranyl acetate standard (data not shown), confirming that the uranium in the uninoculated culture medium was present in the 6+ valence state. The cell pellet obtained from the inoculated sample exhibited a marked shift of the U $4f_{7/2}$ peak to lower binding energy (B) at 380.6eV, which is in the range of the binding energy for 4+ oxidation state. However, curve fitting of the data indicated the presence of a small amount of U⁶⁺ (A) in the inoculated samples. Because the x-ray source is not monochromatic, there was an additional peak (C).

X-ray absorption near-edge spectroscopy.

The M_V absorption peak represents the transition from an initial inner shell $3d_{5/2}$ state to the unoccupied final states $5f(7/2, 5/2)$. The chemical shift of the peak reflects the change in net charge on the absorbing atom which indicates the change in oxidation state. The XANES M_V absorption peak for the uninoculated control sample was 3551.1eV (Figure 2) which was identical to the absorption peak for uranyl acetate standard (not shown) and confirmed the presence of U⁶⁺ oxidation state. In the inoculated samples, there was a shift to lower energy at 3550.1eV which was lower than the absorption peak for uranium dioxide (3550.4eV) but higher than that of uranium metal (3549.6eV) indicating the presence of a highly reduced form of uranium, probably U³⁺ valence state, in addition to U⁴⁺.

This work has led to additional information on uranium compounds. Comparison of standards containing U⁶⁺, including uranium trioxide and uranyl salts, showed no detectable change in absorption peak position. The U⁶⁺ peak envelope can be fitted with a second peak approximately 4eV higher in energy than the primary peak.

Figure 1. XPS analysis of sample inoculated with *Clostridium* sp. showed reduction of U^{6+} (A,a) to U^{4+} (B,b); C-satellite peak.

Figure 2. XANES analysis of M_V absorption peaks showed bacterial reduction of U^{6+} to U^{3+} and U^{4+} .

The ratio of these two peaks gives information on the bonding of the uranium. For example, the ratio of the two peaks for uranyl acetate is 0.31, while uranium trioxide has a value of 0.59 (data not shown). Thus, the predominance of uranyl ion type bonding is reflected in the lower ratio for uranyl acetate.

DISCUSSION

Biologically mediated processes involving the oxidation of organic matter and the concomitant reduction of electron acceptors such as oxygen, nitrate, manganese, and iron have been shown to occur according to the free energy change of the overall reaction. The *Clostridium* sp. used in this study reduces Mn^{4+} to Mn^{2+} in pyrolusite, and Fe^{3+} to Fe^{2+} in ferric hydroxide. The free energy for these reactions are -83.4 and -27.2 kcal/mol CH_2O (as glucose), respectively. Calculation of the free energy change for reduction of uranyl ion to tetravalent (-63.3 kcal/mol) and trivalent (-52.2 kcal/mol) uranium suggests that the reduction should occur in between the manganese and iron sequence [9,10]. Direct evidence for the presence of a reduced form of uranium in the solid phase confirms that bacteria are involved in uranium redox processes. The detection of tetravalent uranium by XPS, a surface-sensitive technique, suggests that it is located on the surface of the bacteria. XANES, however, determines bulk properties of materials. The detection of trivalent uranium by this technique indicates that the uranium is associated with the bacteria, although its exact association is not known. The stability of U^{3+} is most probably enhanced by the reduced conditions encountered in the culture medium prior to dehydration of the biomass. The absence of trivalent uranium on the surface samples is probably due to the fact that it is unstable in solution and is rapidly oxidized to tetravalent form in aqueous medium.

CONCLUSIONS

1. The anaerobic bacterium, *Clostridium* sp., reduced uranyl ion to its tetravalent and trivalent oxidation state.
2. Bacterial reduction of uranium was observed only in the presence of live bacteria and was due to enzymatic action.
3. X-ray photoelectron spectroscopy (XPS) confirmed presence of tetravalent uranium at the bacterial surface.
4. X-ray absorption near-edge structure (XANES) showed the presence of trivalent uranium in the bacterial biomass.

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