

Project 90258

Microbial Transformations of Tru and Mixed Wastes: Actinide Speciation and Waste Volume Reduction

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RESULTS TO DATE: Please note: Figures have been removed from this RIMS report

Work has been performed in two areas; the interaction of uranium with bacteria and the complexation of metal ions with cellulose degraded by bacteria.

Bacteria Interaction The purpose of bacteria interaction experiments was to explore the possibility of oxidative dissolution of uranium solids by the bacterium *Leptothrix discophora*. Bacteria play a large role in the geochemistry many of the environmentally significant elements, and as a group are capable of both oxidation and reduction of these elements. A majority of the work concerning bacterial interactions with uranium has involved either sorption to bacterial membranes, precipitation, or reduction of uranyl; little work has pertained to bacterial oxidation of uranium. However, a large majority of the uranium stored in the environment is in the reduced or solid UO_2 form where oxidative dissolution could lead to the spread of soluble uranium in the environment, thus it is important to determine the possibility or extent that bacteria play a role in uranium oxidation, either directly or indirectly. For this reason, we chose the manganese oxidizing bacterium *Leptothrix discophora* because with it we could investigate both direct and indirect uranium oxidation interactions.

L. discophora is a suitable candidate for direct oxidation of uranium because it is capable of oxidizing both iron and manganese. It is thought to do this utilizing at least one enzyme which belongs to a family of proteins known as multicopper oxidases. As a group, multicopper oxidases tend to be non-specific and have been shown to oxidize both organic and metallic substrates. Not only that, but manganese oxides similar to those precipitated by *L. discophora* have been shown to oxidize biogenic uranium oxides. Thus, we hypothesized that bioprecipitated manganese oxides might also lead to uranium oxidation because they are not only chemically similar, but will be less crystalline and will have a larger surface area than chemically precipitated manganese oxides.

L. discophora is also a reasonable candidate to test any indirect uranium oxidation effects. This is because in the natural environment *L. discophora* is responsible for the oxidation of $Mn(II)$ into solid $Mn(III/IV)$ oxides. These manganese oxides are strong oxidizers themselves and abiotically prepared manganese oxides similar to those precipitated by *L. discophora* have a large enough oxidation potential to oxidize $U(IV)$. This leads to the hypothesis that bioprecipitated manganese oxides might also play a role in the oxidation of uranium. A series of initial experiments were designed to test the possibility of both direct and indirect oxidation of $U(IV)$ solids by *L. discophora*.

In the first experiment, *L. discophora* was grown in rich media for six days. The bacteria were then removed by filtration and 5mL of spent media was added to approximately 9 mg of UO_2 , using fresh media as a control (see Table 1). The samples were allowed to sit overnight and soluble uranium was measured by ICP-AES. The results of the preliminary experiment indicated that approximately twice as much uranium was solubilized in the sample containing the spent media than in the control. These results suggest further investigation into the kinetics oxidative dissolution of UO_2 by spent media of *L. discophora*. In order to explore this more thoroughly it will be necessary to quantify the amount of oxidizing protein present in order to establish a relationship between the rate of oxidation and the concentration of oxidizing protein. The current challenge is the design of a sufficient defined minimal growth media which will allow for both

growth of the bacteria and measurement of protein concentration without interference from the proteins present in rich media currently being used to maintain *L. discophora*.

Table 1. Data on U oxidation by *L. discophora* spent media uM actual ppm U actual U dissolved (mg)
 Total U (mg) Control 54.45 12.96 0.052 9.4 Spent Media 127.22 30.28 0.121 9.1

Experiments were also designed to determine what influence (if any) that the manganese oxides bioprecipitated by *L. discophora* would have on the oxidation of UO₂. In this experiment approximately 5.85 g of biologically precipitated manganese oxides were collected and were contacted with 50mg of UO₂ in the presence of 30mM bicarbonate buffer. A second sample included three day old cell free spent media, 1mM Mn(II) and 50mg UO₂; in this sample the manganese oxides were allowed to precipitate in the presence of uranium. Bicarbonate buffer and 50mg UO₂ acted as a control. Samples were taken over a period of 24 hours and were measured by ICP-AES for both soluble uranium and manganese. While soluble uranium concentrations were not significantly higher than the control in either sample, the rate of MnOx precipitation was about 7 times slower in the presence of UO₂ than without. This suggests that there is some interaction is taking place between the bioprecipitated manganese oxides and the UO₂, future experiments are needed in order to better determine the effects that biogenic manganese oxides has on UO₂.

While uranium oxidation is most likely an unfavorable reaction from an environmental standpoint, it is therefore all the more important to understand the extent to which this reaction might occur in the environment. Not only will this knowledge help us to understand the complete biogeochemical cycling of uranium in the environment, but could lead to better modeling and safer long term storage of uranium. Our initial investigations do not rule out either direct or indirect bacterial oxidation of uranium and indicate the need for further study and quantification of the interactions between *L. discophora* and uranium solids.

Degraded Cellulose

The complexation of metal ions to different degrade cellulose characterized in the 1st year of the project was performed. The kinetics and thermodynamics of metal ion interaction was examined with Eu as the metal ion. Europium was chosen since it is a homolog for the trivalent actinides.

For the kinetic experiments 0.1g of the different degrade cellulose AI, AINO₃, U, and UI was placed into a 250 mL 3-neck round-bottom flask with Argon atmosphere and around 1 mL of 10⁻³ M Eu(NO₃)₃ was added with a final volume of 100 mL and the pH adjusted (see figure below). Upon addition of the Eu solution 1mL samples were then taken at timed intervals over the next thirty hours. Each 1mL sample was diluted with 9mL of 1% nitric acid and the Eu concentration evaluated by ICP-MS.

An example of the kinetics results for the sorption of Eu to AINO₃ paper at pH 5 is below. The experiments generated the kinetic constant for Eu sorption and loading capacity for Eu with the different degraded cellulose at pH 5. The loading capacity is the relative concentration of metal ion bound to the cellulose normalized to the proton exchange capacity. The data can be used to determine the conditions for the equilibrium experiments (see table below).

cellulose k (hr⁻¹) Loading AI 0.156+-0.011 0.558+-0.025 AINO₃ 0.0629+-0.0041 0.564+-0.035 U
 0.245+-0.018 0.318+-0.018 UI 0.0466+-0.0193 0.383+-0.020

The equilibrium experiment were preformed under argon atmosphere using similar conditions to the kinetic experiments except the ratio of Eu to cellulose was varied. Each solution tube was capped and allowed to site for 30 hours. After 30 hours, the tubes were removed from the glove box and centrifuged. A 1 mL sample was removed from each tube and the Eu concentration evaluated by ICP-MS. The results provided the loading data for the different cellulose for Eu as a function of pH under Ar (see table below). The results show AI and AINO₃ approach 100 % saturation while the other treated cellulose have little

change with pH. Further experiments will evaluate kinetic and loading for U and Eu under different atmosphere to see if these trends continue.

Loading of cellulose for Eu under Ar at different pH

cellulose pH 5 pH 6 pH 7 AI 0.558 \pm 0.025 0.942 \pm 0.078 0.991 \pm 0.071 AINO3 0.564 \pm 0.035 0.825 \pm 0.061 0.881 \pm 0.081 U 0.318 \pm 0.018 0.438 \pm 0.034 0.467 \pm 0.052 UI 0.363 \pm 0.020 0.328 \pm 0.029 0.330 \pm 0.025

DELIVERABLES: Kinetic data on metal ion interaction. Presentation of results at Actinides 2005 in Manchester, England in July 2005.