Characterizing the Biological Mechanism of Uranium Reduction Using Novel Voltammetric Techniques

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Uranium bioreduction represents a unique *in situ* remediation strategy which harnesses the ability of native microbial communities from contaminated sediments to utilize U(VI) as a terminal electron acceptor during anaerobic respiration. In this process, soluble U(VI) is reduced to sparingly soluble U(IV) followed by precipitation of U(IV) minerals to limit the mobility of this contaminant. Thus far, the biological mechanism of this bioremediation strategy has not been characterized in detail in a single organism. A recent study detected U(V) as a transient intermediate during uranium bioreduction, suggesting that uranium reduction proceeds via an initial one-electron transfer step from U(VI) to U(V) followed by either 1) a second biotic reduction of U(V) to U(IV) or 2) abiotic disproportionation of U(V) to regenerate U(VI) and precipitate U(IV). This mechanism opposes previously proposed pathways which describe uranium bioreduction as a single two-electron transfer step. Understanding all biotic and abiotic transformations of this radionuclide, including any chemical intermediates in these processes, is imperative to assess and implement this remediation strategy at contaminated sites. Unfortunately, the mechanism of U(VI) bioreduction continues to remain elusive due in part to the lack of analytical techniques able to measure the speciation of uranium in solution across multiple oxidation states at a high frequency.

To address these limitations, a voltammetric technique was developed using a hanging mercury drop electrode (HMDE) to quantify aqueous U(VI) and U(V), and qualitatively detect U(IV) species. Linear calibration curves were obtained for $U^{VI}O_2^{2+}$, $U^{VI}O_2(CO_3)_3^{4-}$, and $U^{V}O_2(CO_3)_3^{5-}$ with limits of detection of 1.2, 1.9, and 2.0 μM, respectively. In a medium composed of >99% $UO_2(CO_3)_3^{4-}$ species, *Shewanella putrefaciens* strain 200 reductively precipitated 2 mM U(VI) from solution at an initial rate of 7.54×10^{-10} mM/hr/cell. Au/Hg microelectrode measurements of $UO_2(CO_3)_3^{4-}$ correlated with total dissolved uranium measurements by ICP-MS thereby validating the newly developed voltammetric technique for quantifying U(VI) speciation during uranium bioreduction incubations. To assess the reactivity of a U(V) intermediate, abiotic U(V) disproportionation was investigated using kinetic isolation experiments in bicarbonate media at environmentally relevant pH. U(V) disproportionation was demonstrated to be first order with respect to $U^{V}O_2(CO_3)_3^{5-}$ with an observed rate constant of $0.174 \pm 0.025\ s^{-1}$. This finding supports a previously proposed mechanism involving two unique U(V)-carbonate species and sheds light on the behavior of the U(V) bioreduction intermediate in relevant geochemical conditions.

Overall, these results demonstrate that voltammetry can be used to rapidly quantify both U(VI) and U(V) as a function of time. This technique will be used to test the hypothesis that a pentavalent U(V) intermediate is formed during the bioreduction of U(VI). Simultaneously, fundamental rate laws for U(VI) bioreduction and U(V) disproportionation will be developed and incorporated into reactive transport models to predict the fate of uranium in diverse geochemical conditions, a crucial step in implementing U(VI) bioreduction as an effective bioremediation strategy.