



## Multiple Roles of *Desulfovibrio vulgaris* to U (VI) Reduction and Long-term Stability of Uraninite ( $\text{UO}_2$ )

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Our current research is focused on assessing the *Desulfovibrio vulgaris* strain and its versatility in uranium (U) bioremediation. *D.vulgaris* reduces U(VI) to U(IV), which can be immobilized by precipitation to uraninite ( $\text{UO}_2$ ) solids. We first studied the complementary mechanisms of direct enzymatic and indirect chemical reduction of U (VI) with the help of series of batch experiments. We observed 90% removal of U (VI) by enzymatic activity within 10 hours and formation of biogenic  $\text{UO}_2$  solids. These experiments also revealed that *D. vulgaris* reduced U(VI) fastest under sulfate-reducing conditions in absence of aqueous Fe(II). When, Fe (II) is present, the rate of uraninite formation was significantly reduced; suggesting the retardation by aqueous  $\text{Fe}^{2+}$ . However, X-ray diffractometry (XRD) data indicated that biogenic uraninite was far more crystallized in presence of  $\text{Fe}^{2+}$ . This data showed that the presence of aqueous iron (II) could enhance crystallization of uraninite. These results clearly confirm the chemical reduction of U(VI) by biogenic  $\text{H}_2\text{S}$  as well as direct enzymatic U(VI) reduction by *D.vulgaris* strain.

We are also investigating the other important role of biogenic iron sulfide ( $\text{FeS}$ ) solids generated by *D.vulgaris* to long-term U (VI) bioremediation. Iron sulfide precipitates protect  $\text{UO}_2$  against oxidative dissolution of U by serving as an effective oxidant scavenger. In this regard, we first systematically studied effects of varying experimental conditions that represent real-life scenarios. In several batch experiments, we demonstrated that, over ranges of pH (6.5 to 8.6) and concentration ratios of lactate-to-sulfate (0.5:1 to 1.9:1) and iron-to-sulfate (0.11:1 to 1:1), *D. vulgaris* primarily produced mackinawite form of  $\text{FeS}$  when either soluble  $\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$  was used as the iron source. We observed that poorly crystalline mackinawite ( $(\text{Fe}_{1+x}\text{S})$  - which is a more desired type of  $\text{FeS}$ , was found when low lactate-to-sulfate ratio (0.5:1), low iron-to-sulfate ratio (0.11:1), weakly basic pH (8.6), or the concomitant occurrence of reduction of soluble  $\text{Fe}^{3+}$  was occurred. Poorly crystalline mackinawite is desired over amorphous  $\text{FeS}$ , because lower degrees of crystallinity typically result in less stable and more redox-reactive solid for retarding reoxidation/remobilization of uraninite. However, a lactate-to-sulfate ratio as high as 1.9:1 led to greigite ( $\text{Fe}_3\text{S}_4$ ) mineral – yet another undesirable form of  $\text{FeS}$  for this application. In addition, vivianite [ $\text{Fe}_3(\text{PO}_4)_2 \cdot 8(\text{H}_2\text{O})$ ] was detected when the iron-to-sulfate mole ratio was high or when additional iron was provided before or immediately after inoculation. The quantitative information obtained by formation and crystallinity of iron sulfide solids is supported by the growth and kinetics of *D.vulgaris*. Further, experimental data supported by modeling results demonstrate that, besides poorly crystalline  $\text{FeS}$ , elemental sulfur was produced when goethite, hematite, or ferrihydrite was the iron source. This underscores the importance of testing and optimizing various biogeochemical conditions for production of desired poorly-crystalline mackinawite towards long-term stability of uraninite. These results along with complementary modeling data will be presented to understand the significance and multiple roles offered by *D.vulgaris* strain in long-term U bioremediation.