

PROJECT SUMMARY

Title: Microbiological-enhanced mixing across scales during *in-situ* bioreduction of metals and radionuclides at Department of Energy Sites

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Several recent reports by the National Research Council have documented the monumental technical challenges faced by DOE in restoring and managing groundwater contamination at sites that were part of the US nuclear weapons production complex. Among the most problematic contaminants are metals and radionuclides, such as uranium, because they are widely distributed, difficult to remove, and pose significant risk to human and ecological receptors. DOE has supported fundamental research into innovative remediation technologies, and bioremediation is considered among the most promising for uranium. This technology requires manipulation of the subsurface through injection of chemicals which mix with the contaminant to stimulate the growth of bacteria that convert uranium to a different chemical form that precipitates as an immobile solid. This process is termed bioreduction since the bacteria catalyze an oxidation-reduction reaction that transfers electrons from the donor (often the injected chemical substrate) to the acceptor (i.e., uranium). This changes the valence state of uranium from the mobile U(VI) to the less mobile U(IV).

The extent of bioremediation depends upon coupled microbiological, geochemical and physical mixing processes. Current knowledge holds that biomass growth and uranium reduction is limited by physical mixing. However, a growing body of literature suggests that cell-to-cell interaction occurs over length scales extending tens to thousands of microns. We hypothesize two potential mechanisms involved in electron transfer to metal reducing bacteria will enhance reaction rates between substrates in physical mixing zones. The first is the formation of single- or multiple-species biofilms that transport electrons via direct electrical connection such as nanowires through biofilms to where the electron acceptor is available. The second is through diffusion of electron carriers from syntrophic bacteria to dissimilatory metal reducing bacteria (DMRB). The specific objectives of this work are i) to quantify the extent and rate that electrons are transported between microorganisms along physical mixing zones between an electron donor and electron acceptor (e.g. U(VI)), ii) to quantify the extent that biomass growth and reaction are enhanced by interspecies electron transport, and iii) to synthesize this fundamental knowledge about mixing across scales into an integrated numerical model to quantify these mechanisms on overall U(VI) reduction rates.

We propose to test these hypotheses with five tasks that integrate microbiological experiments, unique micro-fluidics experiments, intermediate-scale flow cell experiments, and multi-scale hybrid models. Continuous fed-batch reactors will be used to derive kinetic parameters for DMRB, and for syntrophic associations between syntrophs and DMRB. They will also be used to develop an enrichment culture for elucidation of syntrophic relationships in a complex microbial community. Pore and continuum scale experiments using microfluidic and bench top flow cells will be used to evaluate the

impact of cell-to-cell and microbial interactions on reaction enhancement in mixing-limited bioactive zones, and the mechanisms of this interaction. The microfluidic experiments will serve as a platform to develop and test a pore scale model that considers direct cell-to-cell interactions during U(VI) reduction. The pore scale model will be incorporated into a multi-scale hybrid numerical model that combines pore scale modeling at the reaction interface with conventional continuum modelling that averages over hundreds to thousands of pores away from reaction zones. We plan to validate the multi-scale model by comparison with meter-scale bench top flow cell experimental results, and then explore opportunities to extend this model to larger systems and eventually to field sites.

The proposed work will provide mechanistic insights and new knowledge on U(VI) reaction enhancement in physical mixing zones due to microbial facilitated electron transport. This will lead to improved conceptual models and better predictions of U(VI) bioremediation at DOE sites, and to new strategies that reduce cleanup times. For example, in cases where physical mixing limits reaction between an amended electron donor and U(VI), microbes identified to facilitate electron transport in this work can be used to inoculate a site, or be stimulated through electron donor conditions. Efforts to improve model predictions and improve remediation strategies directly contribute to DOE's long term stewardship of metal contaminated sites.