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Project title: Microscale Metabolic, Redox and Abiotic Reactions in Hanford 300 Area Subsurface Sediments

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Summary

The Hanford 300 Area is a unique site due to periodic hydrologic influence of river water resulting in changes in groundwater elevation and flow direction. This area is also highly subject to uranium remobilization, the source of which is currently believed to be the region at the base of the vadose zone that is subject to period saturation due to the changes in the water levels in the Columbia River. We found that microbial processes and redox and abiotic reactions which operate at the microscale were critical to understanding factors controlling the macroscopic fate and transport of contaminants in the subsurface. The combined laboratory and field research showed how microscale conditions control uranium mobility and how biotic, abiotic and redox reactions relate to each other. Our findings extended the current knowledge to examine U(VI) reduction and immobilization using natural 300 Area communities as well as selected model organisms on redox-sensitive and redox-insensitive minerals. Using innovative techniques developed specifically to probe biogeochemical processes at the microscale, our research expanded our current understanding of the roles played by mineral surfaces, bacterial competition, and local biotic, abiotic and redox reaction rates on the reduction and immobilization of uranium.

Accomplishments

Microscale chemistry changes in response to uranium addition using microelectrodes

The presence and importance of microenvironments in the subsurface at contaminated sites were suggested by previous geochemical studies. However, no direct quantitative characterization of the geochemical microenvironments had been reported. We quantitatively characterized microscale geochemical gradients (dissolved oxygen (DO), H_2, pH, and redox potential) in Hanford 300A subsurface sediment biofilms. Our results revealed significant differences in geochemical parameters across the sediment biofilm/water interface in the presence and absence of U(VI) under oxic and anoxic conditions. While the pH was relatively constant within the sediment biofilm, the redox potential and the DO and H_2 concentrations were heterogeneous at the microscale (<500-1000 μm). We found microenvironments with high DO levels (DO

hotspots) when the sediment biofilm was exposed to U(VI). On the other hand, we found hotspots (high concentrations) of H_2 under anoxic conditions both in the presence and in the absence of U(VI). The presence of anoxic microenvironments inside the sediment biofilms suggested that U(VI) reduction proceeds under bulk oxic conditions. To test this, we operated our biofilm reactor under air-saturated conditions in the presence of U(VI) and characterized U speciation in the sediment biofilm. U L_{III}-edge X-ray absorption spectroscopy (XANES and EXAFS) showed that 80-85% of the U was in the U(IV) valence state.

Immobilization of U(VI) from oxic groundwater by Hanford 300 Area sediments and effects of Columbia River water

A high dissolved oxygen (DO) concentration usually leads to U remobilization through reoxidation. However, microorganisms in Hanford 300 Area (300A) are commonly found in the form of biofilms, and the heterogeneity of geochemical gradients in biofilms in subsurface sediments (also known as sediment biofilms) play an important role in controlling U mobility. Based on previous studies on microenvironments in model biofilms, we hypothesized that under bulk aerobic conditions, with O_2 limited or even depleted inside the sediment biofilms because of microbial O_2 consumption and diffusion limitation, U(VI) can be immobilized within the sediment biofilms as U(IV) and remain resistant to the O_2 present in the bulk aqueous phase as long as the active biofilms are maintained. The U.S. Department of Energy Hanford 300 Area (300A) site experiences periodic hydrologic influences from the nearby Columbia River as a result of changing river stage, which causes changes in groundwater elevation, flow direction and water chemistry. An important question to ask is to what extent the mixing of Columbia River water and groundwater impacts the speciation and mobility of U. We designed experiments to mimic interactions among U, oxic groundwater or Columbia River water, and sediments in the subsurface environment of Hanford 300A. The goals were to investigate mechanisms of: 1) U immobilization in 300A sediments under bulk oxic conditions and 2) U remobilization from U-immobilized 300A sediments exposed to oxic Columbia River water. Initially, 300A sediments in column reactors were fed with U(VI)-containing oxic 1) synthetic groundwater (SGW), 2) organic-amended SGW (OA-SGW), and 3) DI water to immobilize U. After that, the sediments were exposed to oxic Columbia River water for U remobilization studies. The results revealed that U was immobilized by 300A sediments predominantly through reduction (80-85%) when the column reactor was fed with oxic OA-SGW. However, U was immobilized by 300A sediments through adsorption (100%) when the column reactors were fed with oxic SGW or DI water. The reduced U in the 300A sediments fed with OA-SGW was relatively resistant to remobilization by oxic Columbia River water. Oxic Columbia River water resulted in U remobilization (~7%) through desorption, and most of the U that remained in the 300A sediments fed with OA-SGW (~93%) was in the form of uraninite nanoparticles. These results reveal that: 1) the reductive immobilization of U through OA-SGW stimulation of indigenous 300A sediment microorganisms may be viable in the relatively oxic Hanford 300A subsurface environments and 2) with the intrusion of Columbia River water, desorption may be the primary process resulting in U remobilization from OA-SGW-stimulated 300A sediments at the subsurface of the Hanford 300A site.

Spatiotemporal metabolic responses of *Shewanella oneidensis* MR-1 biofilms to U(VI) and Cr(VI) exposure

Using an in-house developed NMR-compatible flow through reactor in PNNL, NMR was used to monitor spatial and temporal metabolite concentrations and material-transport rates within live biofilms. The objective of this part of our work was to elucidate the spatiotemporal responses of live *S. oneidensis* MR-1 biofilms to U(VI) (uranyl, UO_2^{2+}) and Cr(VI) (chromate, CrO_4^{2-}), important environmental contaminants at DOE contaminated sites. Toward this goal, we applied noninvasive nuclear magnetic resonance (NMR) imaging, diffusion, relaxation, and spectroscopy techniques to monitor *in situ* spatiotemporal responses of *S. oneidensis* biofilms to U(VI) and Cr(VI) exposure in terms of changes in biofilm structures, diffusion properties, and cellular metabolism. Exposure to U(VI) or Cr(VI) did not appear to change the overall biomass distribution but did cause changes in the physicochemical microenvironments inside the biofilm as indicated by the diffusion measurements. Changes in the diffusion properties of the biofilms in response to U(VI) and Cr(VI) exposure imply a novel function of the extracellular polymeric substances (EPS) affecting the biotransformation and transport of contaminants in the environment. In the presence of U(VI) or Cr(VI), the anaerobic metabolism of lactate was inhibited significantly, although the biofilms were still capable of reducing U(VI) and Cr(VI). Local concentrations of $Cr(III)_{aq}$ in the biofilm suggested relatively high Cr(VI) reduction activities at the top of the biofilm, near the medium-biofilm interface. The depth-resolved metabolic activities of the biofilm suggested higher diversion effects of gluconeogenesis and C1 metabolism pathways at the bottom of the biofilm and in the presence of U(VI). This study provided a noninvasive means to investigate spatiotemporal responses of biofilms, including surface-associated microbial communities in engineering, natural and medical settings, and investigations of various environmental perturbations including exposure to environmental contaminants and antimicrobials.

The role of extracellular polymeric substances in uranium immobilization and remobilization

The first part of this work focused on the characterization of polymeric substances from *Shewanella* sp. HRCR-1 biofilms using infrared spectroscopy and proteomics. The composition of extracellular polymeric substances (EPS) from *Shewanella* sp. HRCR-1 biofilms was investigated using infrared spectroscopy and proteomics to provide insight into potential ecophysiological functions and redox activity of the EPS. Both bound and loosely associated EPS were extracted from *Shewanella* sp. HRCR-1 biofilms prepared using a hollow-fiber membrane biofilm reactor. Fourier transform infrared spectroscopy (FTIR) spectra revealed the presence of proteins, polysaccharides, nucleic acids, membrane lipids, and fatty acids in the EPS fractions. Using a global proteomic approach, a total of 58 extracellular and outer membrane proteins were identified in the EPS. These included homologues of multiple *S. oneidensis* MR-1 proteins that potentially contribute to key physiological biofilm processes, such as biofilm-promoting protein BpfA, surface-associated serine protease, nucleotidases (CpdB and UshA), an extracellular lipase, and oligopeptidases (PtrB and a M13 family oligopeptidase lipoprotein). In addition, 20 redox proteins were found in extracted EPS. Among the detected redox proteins were the homologues of two *S. oneidensis* MR-1 *c*-type cytochromes, MtrC and OmcA, which have been implicated in extracellular electron transfer. Given their detection in the EPS of *Shewanella* sp. HRCR-1 biofilms, *c*-type cytochromes may contribute to the possible redox activity of the biofilm matrix and play important roles in extracellular electron transfer reactions.

The second part of this work focused on quantifying the contribution of EPS from *Shewanella* sp. HRCR-1 biofilms to U(VI) immobilization. The goal was to quantify the contribution of extracellular polymeric substances (EPS) to U(VI) immobilization by *Shewanella* sp. HRCR-1. Through a comparison of U(VI) immobilization using cells with bound EPS (bEPS) and cells with minimal EPS, we showed that 1) bEPS from *Shewanella* sp. HRCR-1 biofilms contribute significantly to U(VI) immobilization, especially at low initial U(VI) concentrations, through both sorption and reduction, 2) bEPS can be considered a functional extension of the cells for U(VI) immobilization and that they likely play more important roles at lower initial U(VI) concentrations, and 3) the U(VI) reduction efficiency is dependent upon the initial U(VI) concentration and decreases at lower concentrations. To quantify the relative contributions of sorption and reduction to U(VI) immobilization by EPS fractions, we isolated loosely associated EPS (laEPS) and bEPS from *Shewanella* sp. HRCR-1 biofilms grown in a hollow fiber membrane biofilm reactor and tested their reactivity with U(VI). We found that, when reduced, the isolated cell-free EPS fractions could reduce U(VI). Polysaccharides in the EPS likely contributed to U(VI) sorption and dominated the reactivity of laEPS, while redox active components (*e.g.*, outer membrane *c*-type cytochromes), especially in bEPS, possibly facilitated U(VI) reduction.

Fe(III) Reduction and U(VI) Immobilization by *Paenibacillus* sp. 300A Isolated from Hanford 300A Subsurface Sediments

A facultative iron-reducing (Fe(III)-reducing) *Paenibacillus* sp. strain was isolated from Hanford 300A subsurface sediment biofilms that was capable of reducing soluble Fe(III) complexes (Fe(III)-NTA and Fe(III)-Citrate) but unable to reduce poorly crystalline ferrihydrite (Fh). However, *Paenibacillus* sp. 300A was capable of reducing Fh in the presence of low concentrations (2 µM) of either of the electron transfer mediators (ETMs), flavin mononucleotide (FMN) or anthraquinone-2,6-disulfonate (AQDS). Maximum initial Fh reduction rates were observed at catalytic concentrations (<10 µM) of either FMN or AQDS. Higher FMN concentrations inhibited Fe(III) reduction while increased AQDS concentrations did not. We found that *Paenibacillus* sp. 300A also could reduce Fh in the presence of natural ETMs from Hanford 300A subsurface sediments. In the absence of ETMs, *Paenibacillus* sp. 300A was capable of immobilizing U(VI) through both reduction and adsorption. The relative contribution of adsorption and microbial reduction to U(VI) removal from aqueous phase was ~7:3 and ~1:4 in PIPES and bicarbonate buffer, respectively. Our study demonstrated that *Paenibacillus* sp. 300A catalyzed Fe(III) reduction and U(VI) immobilization and that these reactions benefit from externally added or naturally existing ETMs in 300A subsurface sediments..

Quantification of electron transfer rates to a solid phase electron acceptor through the stages of biofilm formation from single cells to multicellular communities

Microbial fuel cell (MFC) technology has enabled new insights into the mechanisms of electron transfer from dissimilatory metal reducing bacteria to a solid phase electron acceptor. Using solid electrodes as electron acceptors enables quantitative real-time measurements of electron transfer rates to these surfaces. We described an optically accessible, dual anode, continuous flow MFC that allowed us to do real-time microscopic imaging of anode populations as they developed

from single attached cells to a mature biofilms. We used this system to characterize how differences in external resistance affect cellular electron transfer rates on a per cell basis and overall biofilm development in *Shewanella oneidensis* strain MR-1. When a low external resistance (100 Ω) was used, estimates of current per cell reached a maximum of 204 fA/cell (1.3×10^6 e^- $cell^{-1}$ sec^{-1}), while when a higher (1MΩ) resistance was used, only 75 fA/cell (0.4×10^6 e^- $cell^{-1}$ sec^{-1}) was produced. The 1MΩ anode biomass consistently developed into a mature thick biofilm with tower morphology (>50 μm thick), whereas only a thin biofilm (<5 μm thick) was observed on the 100 Ω anode. These data suggested a link between the ability of a surface to accept electrons and biofilm structure development.

In situ effective diffusion coefficient profiles in live biofilms using pulsed-field gradient nuclear magnetic resonance

Diffusive mass transfer in biofilms is characterized by the effective diffusion coefficient. It is well-documented that the effective diffusion coefficient can vary by location in a biofilm. The current literature is dominated by effective diffusion coefficient measurements for distinct cell clusters and stratified biofilms showing this spatial variation. Regardless of whether distinct cell clusters or surface-averaging methods are used, position-dependent measurements of the effective diffusion coefficient are currently: 1) invasive to the biofilm, 2) performed under unnatural conditions, 3) lethal to cells, and/or 4) spatially restricted to only certain regions of the biofilm. Invasive measurements can lead to inaccurate results and prohibit further (time-dependent) measurements which are important for the mathematical modeling of biofilms. In this part of our project, we: 1) measured the effective diffusion coefficient for water in live biofilms, 2) monitored how the effective diffusion coefficient changes over time under growth conditions, and 3) correlated the effective diffusion coefficient with depth in the biofilm. We measured *in situ* two-dimensional effective diffusion coefficient maps within *Shewanella oneidensis* MR-1 biofilms using pulsed-field gradient nuclear magnetic resonance methods, and used them to calculate surface-averaged relative effective diffusion coefficient (D_{rs}) profiles. We found that 1) D_{rs} decreased from the top of the biofilm to the bottom, 2) D_{rs} profiles differed for biofilms of different ages, 3) D_{rs} profiles changed over time and generally decreased with time, 4) all the biofilms showed very similar D_{rs} profiles near the top of the biofilm, and 5) the D_{rs} profile near the bottom of the biofilm was different for each biofilm. Practically, our results demonstrate that advanced biofilm models should use a variable effective diffusivity which changes with time and location in the biofilm. The data generated here was used to develop mathematical model described below.

Modeling substrate utilization, metabolite production, and uranium immobilization in *Shewanella oneidensis* MR-1 biofilm

We developed a 2-dimensional mathematical model to predict substrate utilization and metabolite production rates in *Shewanella oneidensis MR-1* biofilms in addition to predicting uranium immobilization and remobilization. In our model, lactate and fumarate were substrates used as the electron donor and electron acceptor, respectively. Acetate was the metabolite from the oxidation of lactate and succinate from the reduction of fumarate. The model also included the production of extracellular polymeric substances (EPS). The EPS bound to the cell surface and the EPS distributed in the biofilm were considered bound EPS (bEPS) and loosely-associated EPS (laEPS), respectively. COMSOL® Multiphysics finite element analysis software was used on a high performing computer to solve the model numerically. The input variables of

the model were input lactate and fumarate concentrations, cell density, EPS density, half saturation constant for fumarate, and diffusion coefficients of the substrates and metabolites. We used a custom designed biofilm reactor placed inside a nuclear magnetic resonance (NMR) micro-imaging and spectroscopy system, and monitored substrate utilization and metabolite production rates to compare the model with experimental data. We estimated yield coefficients, maximum substrate utilization rates, the half saturation constant for lactate, the stoichiometric ratio of fumarate and acetate to lactate, and the stoichiometric ratio of succinate to fumarate. These parameters were essential for the prediction of the activity of biofilms and were not available in the literature. Finally, the model was used to predict U immobilization in *S. oneidensis* MR-1 biofilms by considering reduction and adsorption processes in the cells and in the EPS.

Products

Publications

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Book Chapter

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Conference presentations

11. Cao, B., Shi, L., Brown, R., Xiong, Y., Fredrickson, J. K., Romine, M. F., Marshall, M. J., Lipton, M. S., Beyenal, H. Extracellular polymeric substances of *Shewanella* biofilms contains redox active components with potential roles in extracellular electron transfer. American Chemical Society, 2011 Spring Meeting, March 27-31, Anaheim, CA.
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