FINAL REPORT

Biogeochemistry of Uranium Under Reducing and Re-oxidizing Conditions:
An Integrated Laboratory and Field Study
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and

Acceptable Endpoints for Metals and Radionuclides: Quantifying the Stability of
Uranium and Lead Immobilized Under Sulfate Reducing Conditions
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Collaborative Research Projects between Washington State University, Montana State
University and the Pacific Northwest National Engineering Laboratory

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Biogeochemical Dynamics Research Element

by

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1. Executive Summary

Our understanding of subsurface microbiology is hindered by the inaccessibility of this environment, particularly when the hydrogeologic medium is contaminated with toxic substances. Research in our labs indicated that the composition of the growth medium (e.g., bicarbonate complexation of U(VI)) and the underlying mineral phase (e.g., hematite) significantly affects the rate and extent of U(VI) reduction and immobilization through a variety of effects. Our research was aimed at elucidating those effects to a much greater extent, while exploring the potential for U(IV) reoxidation and subsequent re-mobilization, which also appears to depend on the mineral phases present in the system. In situ coupons with a variety of mineral phases were placed in monitoring wells at the NABIR FRC. These coupons showed that the mineral phase composition significantly affected the resulting attached phase microbial community. Our comparative use of both batch and open flow reactors (more representative of field conditions) indicates that hydrodynamics and continual influx of substrate and contaminants can also yield significantly different results than those obtained with closed serum bottles.

To this end, the following overall experimental hypothesis tested was the following: On a mineral surface under anaerobic conditions, accumulations of secondary inorganic precipitates are controlled by a) the bacteria associated with the mineral surface, b) the electron acceptors available for anaerobic bacterial respiration, and c) local hydrodynamics and pH buffers govern micro- and meso-scale interaction of U in the presence of electron donors and acceptors, and nutrients.

The combined laboratory and field research extended fundamental results based on U(VI) reduction and immobilization with Desulfovibrio desulfuricans G20 as a model for the sulfate reducing bacteria (SRB) on hematite (found in FRC Nolichucky shale) and on other mineral phases. Using techniques developed to specifically probe biogeochemical processes, we expanded the current understanding of the roles played by mineral surfaces and aqueous chemistry on the reduction and immobilization of uranium by SRB. Further, we expanded this understanding into the FRC field site through the emplacement of in situ biofilm coupons. At the FRC site, surrogate geological media contained in a porous receptacle were incubated in a well within the saturated zone of a pristine region of an aquifer to capture populations from the extant communities. Minerals included micaceous, specular hematite (1-3 mm diameter) from Minas Gerais, Brazil (kindly provided by K. Rosso, Pacific Northwest National Laboratory), illite shale (Ward’s Natural Science, cat no. 46 V 0315), uncontaminated saprolite from the FRC subsurface (a gift from P. Jardine, Oak Ridge National Laboratory), and coarse quartz sand. Biofilm reactors were used to test uranium immobilization by D. desulfuricans G20 biofilms. These reactors included a flat plate flow reactor and 3 column reactors filled with hematite, dolomite and calcite. All reactors were fed with uranium in an SRB metal toxicity medium (MTM) developed in our laboratory that eliminates the formation of metal precipitates and minimizes metal complexation. The reactors were operated for approximately 4 months and were continuously monitored for microbial activity and also uranium concentration in the outlet solutions. Over the four-month period, the uranium immobilization efficiency was 88% for the flat plate reactor, 99% for the dolomite column, 99% for the calcite column, and 85 % for the hematite column.

In addition to mineral type, the effects of pH buffer on U(VI) reduction by D. desulfuricans G20 were studied using MTM, which showed that U(VI) is toxic to G20. Toxicity depended on the medium buffer and was observed in terms of longer lag times and in some cases, no measurable
The minimum inhibiting concentration (MIC=lowest concentration that utterly inhibits growth) of U(VI) in medium containing PIPES buffer was 140 μM, which is 40 times lower than previously reported for *D. desulfuricans*.

The effects of Fe(III)-(hydr)oxides (hematite, goethite, and ferrihydrite) on microbial reduction and reoxidation of uranium (U) were also evaluated under lactate-limited sulfate-reducing conditions. With lactate present, G20 reduced U(VI) in both PIPES and bicarbonate buffer. Once lactate was depleted, however, microbially reduced U served as an electron donor to reduce Fe(III) present in Fe(III)-(hydr)oxides. With the same initial amount of Fe(III) (10 mmol/L) for each Fe(III)-(hydr)oxide, reoxidation of U(IV) was greater with hematite than with goethite or ferrihydrite. As the initial mass loading of hematite increased from 0 – 20 mmol Fe(III)/L, the rate and extent of U(IV) reoxidation increased. Subsequent addition of hematite (15 mmol Fe(III)/L) to stationary phase cultures containing microbially reduced U(IV) also resulted in rapid reoxidation to U(VI). Analysis by U L3-edge XANES spectroscopy of microbially reduced U particles yielded spectra similar to that of natural uraninite. Observations by high-resolution transmission electron microscopy, selected area electron diffraction, and energy-dispersive X-ray spectroscopic analysis confirmed that precipitated U associated with cells was uraninite with particle diameters of 3 – 5 nm. Our results suggest that in the presence of Fe(III)-(hydr)oxides, Fe(III) may act as an oxidant for U(IV) under electron donor-limited conditions, whereas Fe(II) may act as a reductant for U(VI) under other conditions.

This research has made significant advances in the effort to determine the nature of iron mineral surfaces in microcosm and flow reactors. In addition, subsurface “coupons” emplaced at the FRC have presented well-defined mineral surfaces to indigenous microflora for subsequent characterization and assessment of the resulting mineral-associated bacterial communities. Data obtained from this combination of field characterization, in-well coupons, and laboratory reactors are being used to address issues connected with accumulation of surface-associated bacteria, secondary inorganic precipitates, and ultimate stability of reduced uranium. Characterizing, understanding, and finally controlling these combinations of biological and inorganic solid phases is likely the key to success for *in situ* bio-immobilization of radionuclides in fractured aquifer systems.

The results have been published in a number of peer reviewed journal articles. The abstracts and citations to those articles make up the bulk of our final report. Our interdisciplinary team members include: Brent Peyton and Rajesh Sani (Washington State University), Gill Geesey, Zbigniew Lewandowski, and Haluk Beyenal (Montana State University), David Cummings (Idaho National Engineering and Environmental Laboratory) and James Amonette (Pacific Northwest National Laboratory).
Publications (Published or Accepted for Publication)

2.1 Publication 1

2.1.2. Abstract: The Department of Energy’s Office of Biological and Environmental Research sponsored a two-day workshop (October 12 – 13, 2000) that targeted the microbial and geochemical processes governing the interactions of bacteria with heavy metals, radionuclides, and mineral phases relevant to contaminated subsurface sediments. The objective of the workshop was to (1) provide a current state of knowledge of metal/microbe interactions, (2) to identify the knowledge gaps associated with these interactions, and (3) suggest scientific and technological approaches to address these gaps. Fifty scientists and educators from within and outside the Natural and Accelerated Bioremediation Program (NABIR) attended the workshop. Scientist with noted expertise in one of five topical areas lead general sessions (see agenda) and coordinated a series of short presentations that covered the current state of knowledge and identified gaps in our understanding of issues related to metal/microbe interactions. Breakout sessions were organized the following day to foster more detailed discussions of issues presented in the general sessions, and to provide some recommendations for research needs and approaches for meeting such needs. Implicit in these recommendations are benefits toward meeting the goals of the NABIR program while advancing our understanding of natural and accelerated bioremediation in general.
2.2 Publication 2


2.2.2. Abstract: We have identified factors limiting hydrogen sulfide production in a two-species biofilm containing sulfate-reducing bacteria (*Desulfovibrio desulfuricans*) and non-sulfate-reducing bacteria (*Pseudomonas fluorescens*). Profiles of hydrogen sulfide (H$_2$S) concentration, pH, local mass transport coefficient, local flow velocity, and local relative effective diffusivity in the biofilm were measured using microelectrodes. The biofilms had a heterogeneous structure consisting of cell clusters separated by voids. Typically, the H$_2$S concentration was lower in the voids than in the adjacent cell clusters, demonstrating that the voids acted as transport channels for removing H$_2$S from the cell clusters. The extent of biofilm heterogeneity was directly correlated with the flux of H$_2$S from the cell clusters. At flow velocities below 2 cm/s the flux of H$_2$S from the cell clusters depended on the flow velocity. We concluded that at these flow velocities the H$_2$S production rate was limited by the rate of delivery of sulfate ions to the biofilm. At flow velocities above 2 cm/s the H$_2$S production rate was nearly constant and did not depend on the flow velocity. We concluded that at high flow velocities (>2 cm/s) the H$_2$S production rate was limited by metabolic reactions in the biofilm. Local intra-biofilm flow velocity profiles were strongly influenced by biofilm heterogeneity. There was no significant pH variation within the biofilms. Surprisingly, profiles of local relative effective diffusivity indicated that the biofilm was made up of two layers, a finding that could be related to the fact that the specimen was a two-species biofilm.

2.2.3. Summary Results

- The mixed culture of *D. desulfuricans* and *P. fluorescens* biofilms had a heterogeneous structure consisting of voids and cell clusters.
- Measured H$_2$S concentration profiles in the biofilms demonstrated that the heterogeneous biofilm structure was used to remove H$_2$S from the cell clusters.
- Biofilm heterogeneity influenced the local flow velocity profiles and the rates of the intra-biofilm mass transport.
2.3 Publication 3


2.3.2. Abstract: In the presence of sulfate-reducing bacteria (Desulfovibrio desulfuricans) hematite (α-Fe$_2$O$_3$) dissolution is affected potentially by a combination of enzymatic (hydrogenase) reduction and hydrogen sulfide oxidation. As a consequence, ferrous ions are free to react with excess H$_2$S to form insoluble ferrous sulfides. X-ray photoelectron spectra indicate binding energies similar to ferrous sulfides having pyrrhotite-like structures (Fe$_{2p3/2}$ 708.4 eV; S$_{2p3/2}$ 161.5 eV). Other sulfur species identified at the surface include sulfate, sulfite and polysulfides. Thin film X-ray diffraction identifies a limited number of peaks, the principal one of which may be assigned to the hexagonal pyrrhotite (102) peak (d = 2.09 Å; 2θ = 43.22°), at the hematite surface within 3 months exposure to sulfate-reducing bacteria (SRB). High-resolution transmission electron microscopy identifies the presence of a hexagonal structure associated with observed crystallites. Although none of the analytical techniques employed provide unequivocal evidence as to the nature of the ferrous sulfide formed in the presence of SRB at hematite surfaces, we conclude from the available evidence that a pyrrhotite stoichiometry and structure is the best description of the sulfides we observe. Such ferrous sulfide production is inconsistent with previous reports in which mackinawite and greigite were products of biological sulfate reduction (Rickard 1969a; Herbert et al 1998; Benning et al 1999). The apparent differences in stoichiometry may be related to sulfide activity at the mineral surface, controlled in part by H$_2$S autooxidation in the presence of iron oxides. Due to the relative stability of pyrrhotite at low temperatures, ferrous sulfide dissolution is likely to be reduced compared to the more commonly observed products of SRB activity. Additionally, biogenic pyrrhotite formation will also have implications for geomagnetic field behavior of sediments.

2.3.3. Summary Results

- Discovery that the iron sulfide mineral pyrrhotite is formed as a secondary mineral on hematite only when sulfate reducing bacteria are growing on the hematite surface.
2.4 Publication 4


2.4.2. Abstract The bioavailability and toxicity of lead (Pb) to Desulfovibrio desulfuricans G20 is greatly influenced by aqueous phase chemical composition. Apparent Pb toxicity is reduced by precipitation and complexation with chemicals found in standard growth media for sulfate-reducing bacteria (SRB). To determine the influence of medium composition on observed Pb toxicity, a new medium was developed to more accurately assess the toxicity of Pb to D. desulfuricans. The new medium, metal toxicity medium (MTM), eliminates abiotic Pb precipitation and minimizes formation of Pb complexes in solution. Significant growth of D. desulfuricans was observed on MTM in the absence of Pb, while no measurable growth was observed at 3 mg/l Pb as PbCl2. For comparison, in Lactate C medium (Burlage et. al., 1998) abiotic Pb precipitation was apparent, and the specific growth rate at 100 mg/l Pb was only reduced 8.1% compared to the Pb-free control. Toxicity was measured in terms of longer lag times and slower growth rates (including no growth) as compared to Pb-free controls. This report describes the effects of specific medium components on Pb toxicity to D. desulfuricans and provides a better baseline for comparison of natural and industrial waters for observing heavy metal toxicity on SRB.

2.4.3. Summary Results:
- A new medium was developed that significantly increased bioavailability of toxic metals to allow more direct observation of heavy metal toxicity.
- This allowed us to show that lead was over 20 times more toxic than previously reported.
2.5 Publication 5

2.5.1. Citation

2.5.2. Abstract: The toxicity of copper (Cu(II)) to sulfate-reducing bacteria (SRB) was studied by using Desulfovibrio desulfuricans G20 in a medium (MTM) developed specifically to test metal toxicity to SRB (43). The effects of Cu(II) toxicity were observed in terms of inhibition in total cell protein, longer lag times, lower specific growth rates, and in some cases no measurable growth. At only 6 μM, Cu(II) reduced the maximum specific growth rate by 25% and the final cell protein concentration by 18% as compared to the copper-free control. Inhibition by Cu(II) of cell yield and maximum specific growth rate increased with increasing concentrations. The Cu(II) concentration causing 50% inhibition in final cell protein (IC50) was evaluated to be 16 μM. A Cu(II) concentration of 13.3 μM showed 50% inhibition in maximum specific growth rate. These results clearly show significant Cu(II) toxicity to SRB at concentrations that are 100 times lower than previously reported. No measurable growth was observed at 30 μM Cu(II) even after a prolonged incubation of 384 hours. In contrast, Zn(II) and Pb(II), at 16 and 5 μM, increased lag times by 48 and 72 h, respectively, but yielded final cell protein concentrations equivalent to those of the zinc- and lead-free controls. Live/dead staining, based on membrane integrity, indicated that while Cu(II), Zn(II), and Pb(II) inhibited growth, these metals did not cause a loss of D. desulfuricans membrane integrity. The results show that D. desulfuricans in the presence of Cu(II) follow a clearly different growth pattern than in the presence of Zn(II) or Pb(II). It is therefore likely that Cu(II) toxicity proceeds by a different mechanism than Zn(II) or Pb(II) toxicity.

2.5.3. Summary Results:
• Significant Cu(II) toxicity to SRB at concentrations that are 100 times lower than previously reported.
• Cu(II) toxicity to SRB follow a clearly different growth pattern than in the presence of Zn(II) or Pb(II).
2.6 Publication 6


2.6.2. Abstract: Over the past decade, advances in surface-sensitive spectroscopic techniques have provided the opportunity to identify many new microbiologically-mediated biogeochemical processes. Although a number of surface spectroscopic techniques require samples to be dehydrated, which precludes real-time measurement of biotransformations and generate solid phase artifacts, some now offer the opportunity to either isolate a hydrated sample within a ultra-high vacuum during analysis or utilize sources of radiation that efficiently penetrate hydrated specimens. Other non-destructive surface spectroscopic techniques permit determination of the influence of microbiological processes on the kinetics and thermodynamics of geochemical reactions. The ability to perform surface chemical analyses at micro- and nanometer scales has led to the realization that bacterial cell surfaces are active sites of mineral nucleation and propagation, resulting in the formation of both stable and transient small-scale surface chemical heterogeneities. Some surface spectroscopic instrumentation is now being modified for use in the field to permit researchers to evaluate mineral biotransformations under in situ conditions. Surface spectroscopic techniques are thus offering a variety of opportunities to yield new information on the way in which microorganisms have influenced geochemical processes on Earth over the last 4 billion years.
2.7 Publication 7


2.7.2. Abstract:
Underground waste storage at the Idaho National Engineering and Environmental Laboratories (INEEL) poses a threat to the Snake River Aquifer. Contaminants, such as organic solvents, heavy metals and transuranic wastes, may be transported beyond the repository boundaries by the subsurface flow. As a result, active research efforts are being made toward bioremediation of organic solvents and immobilization of inorganic hazardous materials at contaminated sites. In-situ immobilization of radionuclides and heavy metals can be accomplished biologically with the use of microorganisms. Dissimilatory iron reducing bacteria (DIRB) are ubiquitous and impact the environment by reducing insoluble Fe(III) on iron oxide surfaces (i.e. amorphous ferric oxide, goethite, magnetite, hematite) to soluble Fe(II). The solubilization of the Fe(III) may subsequently result in the release and mobilization of previously sorbed contaminants such as Pb and U from the mineral surface. Once released, Fe(II) may react with H₂S produced by sulfate reducing bacteria (SRB). The product, FeS, can directly reduce and hence immobilize radionuclides such as Tc(VII) and U(VI). Additionally, H₂S can react with Hg and Pb to form the corresponding sulfate precipitates. In some cases, the addition of a substrate such as lactate may be required to enhance microbial activity for the purpose of in-situ metal immobilization. However, there is limited knowledge of the redox microbial processes on mineral surfaces and the formation and stability of secondary minerals formed. The fate of the inorganic contaminants is partly dependent on the growth and activity of the two microorganisms and therefore their kinetic properties. If an in-situ metal immobilization process is initiated, it is necessary to know which microbes will dominate the process and which precipitates will be formed. The focus of this work was to monitor cell growth and determine which precipitates are formed on an iron surface (namely hematite) in the presence of a single as well as mixed population of DIRB (Shewanella/Geobacter) and SRB (Desulfovibrio). The study was conducted in a flow cell that simulates subsurface flow environments. Spatial distribution and cell growth was monitored using various fluorescent microscopy techniques. Secondary mineral formation was determined by X-ray photoelectron microscopy (XPS). Experiments were also conducted in batch reactors to obtain kinetic data of cell growth (and species relative abundance), substrate conversion, as well as hematite reduction processes. These data could eventually be compared to the results obtained under continuous flow conditions. The general information from these laboratory results could give indication of expected trends in subsurface environments in which the above organisms are widespread with respect to relative abundance and secondary precipitates.
2.8 Publication 8

2.8.1. Citation Sani RK, Peyton BM, Smith WA, Apel WA, and Petersen JN. 2002. Dissimilatory reduction of Cr(VI), Fe(III), and U(VI) by Cellulomonas isolates. Applied Microbiology and Biotechnology, 60: 192-199.

2.8.2. Abstract: The reduction of Cr(VI), Fe(III), and U(VI) was studied using three recently isolated environmental Cellulomonas sp. (WS01, WS18, and ES5) and a known Cellulomonas strain (Cellulomonas flavigena ATCC 482) under anaerobic, non-growth conditions. In all cases, these cultures were observed to reduce Cr(VI), Fe(III), and U(VI). With lactate as electron donor, in 100 h the Cellulomonas isolates (500 mg/L total cell protein) reduced nitrilotriacetic acid chelated Fe(III) {Fe(III)-NTA} from 5 mM to less than 2.2 mM, Cr(VI) from 0.2 mM to less than 0.001 mM, and U(VI) from 0.2 mM to less than 0.12 mM. All Cellulomonas isolates also reduced Cr(VI), Fe(III), and U(VI) in the absence of lactate, while no metal reduction was observed in either the cell-free or heat killed cell controls. This is the first report of Cellulomonas sp. reducing Fe(III) and U(VI). Further, this is the first report of Cellulomonas spp. coupling the oxidation of lactate, or other unknown electron donors in the absence of lactate, to the reduction of Cr(VI), Fe(III), and U(VI).

2.8.3. Summary Results

- First report of Cellulomonas isolate capable of reducing Fe(III) or U(VI).
- Substrate-free reduction of metals may help build longer lasting in situ biobarriers
2.9 Publication 9

2.9.1. Citation: J. E. Amonette,* C. K. Russell, K. A. Carosino, N. L. Robinson, and J. T. Ho, Toxicity of Al to Desulfovibrio desulfuricans, Applied and Environmental Microbiology, July 2003, p. 4057–4066

2.9.2. Abstract: The toxicity of Al to Desulfovibrio desulfuricans G20 was assessed over a period of 8 weeks in a modified lactate C medium buffered at four initial pHs (5.0, 6.5, 7.2, and 8.3) and treated with five levels of added Al (0, 0.01, 0.1, 1.0, and 10 mM). At pH 5, cell population densities decreased significantly and any effect of Al was negligible compared to that of the pH. At pHs 6.5 and 7.2, the cell population densities increased by 30-fold during the first few days and then remained stable for soluble-Al concentrations of <5 x10^{-5} M. In treatments having total-Al concentrations of >1 mM, soluble-Al concentrations exceeded 5 x10^{-5} M and limited cell population growth substantially and proportionally. At pH 8.3, soluble-Al concentrations were below the 5 x10^{-5} M toxicity threshold and cell population density increases of 20- to 40-fold were observed. An apparent cell population response to added Al at pH 8.3 was attributed to the presence of large, spirilloidal bacteria (accounting for as much as 80% of the cells at the 10 mM added Al level). Calculations of soluble-Al speciation for the pH 6.5 and 7.2 treatments that showed Al toxicity suggested the possible presence of the Al_{13}O_{6}(OH)_{24}(H_{2}O)_{12}^{7+} “tridecamer” cation and an inverse correlation of the tridecamer concentration and the cell population density. Analysis by \textsuperscript{27}Al nuclear magnetic resonance spectroscopy, however, yielded no evidence of this species in freshly prepared samples or those taken 800 days after inoculation. Exclusion of the tridecamer species from the aqueous speciation calculations at pHs 6.5 and 7.2 yielded inverse correlations of the neutral Al(OH)_{3} and anionic Al(OH)_{4}^{-} monomeric species with cell population density, suggesting that one or both of these ions bear primary responsibility for the toxicity observed.
2.10 Publication 10

2.10.1 Citation: Amonette, J. E., S. M. Heald, and C. K. Russell. 2003 “Imaging the heterogeneity of mineral surface reactivity using Ag(I) and synchrotron X-ray microscopy.” *Phys. Chem. Miner.* 30:559-569.

2.10.2 Abstract: Microscopic-scale imaging of reduced zones on the surfaces of minerals can be achieved by reaction with dilute Ag(I) solutions and subsequent analysis using synchrotron X-ray microscopy (XRM) above the Ag K-edge (25.5 keV). The principal reductant is Fe(II), but other reductants such as sulfide may contribute. Reduced zones may exist intrinsically, as in the structure of biotite and augite, or may be generated by reaction with chemical agents such as dithionite or treatment with sulfate reducing bacteria (SRB). We demonstrate the method on flakes of specular hematite and biotite, as well as on thin sections of different rocks (arfvedsonitic granite, oolitic hematite, diabase, and quartz conglomerate) treated with SRB, and discuss possible artifacts that can occur. To our knowledge, this is the only microscopic technique that can image Fe(II) zones on the surface of an Fe-bearing mineral with monolayer sensitivity.
2.11 Publication


2.11.2. Abstract: The atomic and electronic structure of mineral surfaces affects many environmentally important processes such as adsorption phenomena. They are however rarely considered relevant to dissimilatory bacterial reduction of iron and manganese minerals. In this regard, surface area and thermodynamics are more commonly considered. Here we take a first step towards understanding the nature of the influence of mineral surface structure upon the rate of electron transfer from *Shewanella oneidensis* strain MR-1 outer membrane proteins to the mineral surface and the subsequent effect upon cell “activity.” Cell accumulation has been used as a proxy for cell activity at three iron oxide single crystal faces; hematite (001), magnetite (111) and magnetite (100). Clear differences in cell accumulation at, and release from the surfaces are observed, with significantly more cells accumulating at hematite (001) compared to either magnetite face whilst relatively more cells are released into the overlying aqueous phase from the two magnetite faces than hematite. Modeling of the electron transfer process to the different mineral surfaces from a decaheme (protoporphyrin rings containing a central hexacoordinate iron atom), outer membrane-bound cytochrome of *S. oneidensis* has been accomplished by employing both Marcus and ab initio density functional theories. The resultant model of electron transfer to the three oxide faces predicts that over the entire range of expected electron transfer distances the highest electron transfer rates occur at the hematite (001) surface, mirroring the observed cell accumulation data. Electron transfer rates to either of the two magnetite surfaces are slower, with magnetite (111) slower than hematite (001) by approximately two orders of magnitude. A lack of knowledge regarding the structural details of the heme-mineral interface, especially in regards to atomic distances and relative orientations of hemes and surface iron atoms and the conformation of the protein envelope, precludes a more thorough analysis. However, the results of the modeling concur with the empirical observation that mineral surface structure has a clear influence on mineral surface-associated cell activity. Thus surface structure effects must be accounted for in future studies of cell-mineral interactions.

2.11.3. Summary Results

- Model predictions of the rate of transfer of electrons from the dissimilatory iron reducing bacterium *Shewanella oneidensis* MR-1 to Fe(III) in various crystalline states were not supported by experimental results.
- The experimental results better fit a model that is based on the site density of Fe atoms in the crystal.
2.12 Publication 12


2.12.2. Abstract: Gill Geesey, Professor of Microbiology at Montana State University, Bozeman, Ph.D. student Catherine Reardon from Burley, Idaho and INEEL Principal Scientist David Cummings are evaluating the use of biofilm coupons to sample mineral surface-associated microbial communities at a pristine subsurface site and a uranium-contaminated site at the Department of Energy’s Oak Ridge Tennessee Reservation as part of a collaboration with Professor Brent Peyton at Washington State University. The coupons contain representative minerals from these sites, which, when deployed and incubated in the saturated zone of wells at the sites, become colonized by different populations of bacteria present in the groundwater. The mineral surfaces in the biofilm coupons are intended to serve as a surrogate for mineral surfaces of the surrounding geological matrix, but are easier and less costly to recover than core material from the formation. Colonization of the mineral surfaces results in the concentration of those bacterial populations in the groundwater that are able to coexist in close proximity to each other and, in some instances, develop synergistic interactions to promote their survival, some of which may promote degradation or biotransformation of contaminants that may be present. The INRA research team has also developed a technique referred to as Gradient Intact Biofilm Polymerase Chain Reaction (GIB-PCR) to facilitate characterization of the microbial communities that develop on the mineral surfaces in the biofilm coupons. This technique eliminates the bias associated with a separate DNA extraction step and a single annealing temperature for amplification of a fragment of the 16S rRNA gene carried by the different populations of bacteria, and used to construct a 16S rDNA clone library that is then used to identify and relate the different types of bacteria that are detected by this molecular biological approach.
2.13 Publication 13


2.13.2. Abstract: The era of nuclear weapons development and testing has left several DOE sites moderately to highly contaminated with metals and radionuclides. Due to partitioning of certain forms of these contaminants in the aqueous phase and subsurface fluid flow, the transport of these contaminants into aquifers threatens water sources down gradient of contamination points. In situ bioremediation is a potential treatment method since microbes are ubiquitous throughout the subsurface and have the ability to modify the solubility properties of several contaminants. The Natural and Accelerated Bioremediation Research program (NABIR) Field Research Center (FRC) was designed as a test site for biogeochemical clean-up of a contaminated DOE site. The purpose of this research is to compare the microbial diversity between two FRC sites, one pristine and one contaminated with metals, radionuclides, and volatile organic carbon compounds. To sample the indigenous population, approximately 30 g of 1-2 mm hematite particles were suspended in FRC test wells for an incubation period of 8 weeks. Upon retrieval, particle-associated microbial communities were characterized by intact biofilm PCR of the 16s rDNA followed by clone library construction and subsequent sequence analysis. Species richness and evenness data obtained from the libraries indicate there is a higher microbial diversity in the uncontaminated site as compared to the contaminated region of the aquifer. A thorough understanding these natural microbial populations and key physiologies is required for ascertaining effective remediation and management of these sites.
2.14 Publication 14
2.14.1. Citation

2.14.2. Abstract: The toxicity of Pb(II) to sulfate reducing bacteria (SRB) was studied using Desulfovibrio desulfuricans G20 in a medium specifically designed to assess metal toxicity. The effects of Pb(II) toxicity were observed in terms of longer lag times, lower specific growth rates, and in some cases, no measurable growth. With an increase in medium pH from 6 to 8, Pb(II) toxicity decreased. At all pH values, in the presence of Pb(II) concentrations ranging from 3 to 15 μM, specific growth rates decreased and lag times increased. The minimum inhibiting concentration (MIC) of Pb(II) causing a complete inhibition in growth at pH 6 was 10 μM, as compared to 15 μM at pH 7.2 and 8. These MIC values are 40 times lower than previously reported for SRB. Results also show that with increases in initial cell protein concentration (inoculum size), soluble Pb(II) removal rates increased and the degree to which Pb(II) caused increased lag times was reduced. In the presence of Pb(II), in all cases in which D. desulfuricans grew (even after a 312 h lag time), the final cell protein concentration was equivalent to that of the Pb-free control. Live/dead staining, based on membrane integrity, indicated that while Pb(II) inhibited growth, Pb(II) did not cause a loss of D. desulfuricans membrane integrity.

2.14.3. Summary Results
- This paper quantifies lead toxicity using “minimum inhibitory concentrations” (MIC) and demonstrates that lead is significantly more toxic to SRB than previously thought.
- MIC values are 40 times lower than previously reported
2.15 Publication 15


2.15.2. Abstract: We have evaluated the effects of selected minerals present in subsoil environment on the efficiency of lead removal from contaminated groundwaters using biofilms composed of sulfate-reducing microorganisms, and examined the stability of metal deposits after the biofilms had been temporarily exposed to the air. To quantify the studied effects, lead was immobilized in biofilms of Desulfovibrio desulfuricans grown anaerobically in two flat-plate flow reactors, one filled with hematite and the other with quartz. While the biofilms in both reactors were heterogeneous and consisted of voids and channels, the biofilms grown on hematite were denser, thicker, and more porous than those grown on quartz. The average H2S concentrations, measured using microelectrodes, were higher in the biofilms grown on quartz than those measured in the biofilms grown on hematite. During 18 weeks of operation, iron was continuously released from the hematite. Lead was immobilized more efficiently in the biofilms grown on quartz than it was in the biofilms grown on hematite. Lead deposits were partially reoxidized, especially in biofilms grown on hematite, and the biofilms in both reactors responded to the presence of oxygen by lowering their density and increasing the H2S production rate.

2.15.3. Summary Results
- Lead immobilization was more efficient in the biofilms grown on quartz (redox insensitive mineral) than in the biofilms grown on hematite (redox sensitive mineral).
- Sulfide reducing biofilms enhanced the rate of iron release from the hematite.
2.16 Publication 16


2.16.2. Abstract:
There is no doubt among biofilm researchers that biofilm structure is important to many biofilm processes, such as the transport of nutrients to deeper layers of the biofilm. However, biofilm structure is an elusive term, and as such it cannot be directly correlated with any measurable parameters characterizing biofilm performance. To correlate biofilm structure with the parameters characterizing biofilm performance, such as the rate of nutrient transport within the space occupied by the biofilms, biofilm structure must first be quantified and expressed numerically on an appropriate scale. The task of extracting numerical parameters quantifying biofilm structure relies on using biofilm imaging and image analysis. Although defining parameters characterizing biofilm structure is relatively straightforward, and multiple parameters have been described in the computer science literature, interpreting the results of such analyses is not trivial. Existing computer software developed by several research groups, including ours, for the sole purpose of analyzing biofilm images helps quantify parameters from biofilm images but does nothing to help interpret the results of such analyses. Although computing structural parameters from biofilm images permits correlating biofilm structure with other biofilm processes, the meaning of the results is not obvious. The first step to understanding the quantification of biofilm structure has been made by several research groups. It is now important to focus on the meaning of these analyses. This presentation reviews our research and our experience in quantifying biofilm structure and relating it to fundamental biofilm processes.

2.16.3. Summary Results
- We have demonstrated that the analysis of biofilm structure is most pertinent when used to monitor temporal variations, and it is least pertinent when used to compare biofilms accumulated in different reactors, in different laboratories, and/or grown under different environmental conditions.
- Thus, image analysis of biofilm is a useful tool for monitoring the temporal development of biofilms, but it appears to be less useful as a base for general conclusions about the nature of biofilm processes.
2.17 Publication 17

2.17.2. Abstract: Hexavalent uranium [U(VI)] was immobilized using biofilms composed of the sulfate reducing bacterium (SRB), Desulfovibrio desulfuricans G20. The biofilms were grown in flat-plate continuous-flow reactors using lactate as the electron donor and sulfate as the electron acceptor. U(VI) was continuously fed into the reactor for 32 weeks at a concentration of 126 μM. During this time, the soluble U(VI) was removed (between 88 and 96 % of feed) from solution and immobilized in the biofilms. The dynamics of U immobilization in the sulfate reducing biofilms were quantified by estimating: 1) microbial activity in the SRB biofilm, defined as the hydrogen sulfide (H₂S) production rate, and estimated from the H₂S concentration profiles measured using microelectrodes across the biofilms; 2) concentration of dissolved U in the solution; and (3) the mass of U precipitated in the biofilm. Results suggest that U was immobilized in the biofilms as a result of two parallel processes: (1) enzymatically; and (2) chemically, by reacting with microbially generated H₂S. Analytical tests showed that sulfide species and U(VI) react to produce a black precipitate. Synchrotron-based U L₃-edge x-ray absorption near edge structure (XANES) spectroscopy analysis of U precipitated abiotically by sodium sulfide indicated that U(VI) had been reduced to U(IV). Selected area electron diffraction pattern and crystallographic analysis of transmission electron microscope lattice fringes images confirmed the structure of precipitated U as being that of uraninite.

2.17.3. Summary Results
- Demonstrated long-term U(VI) reduction by SRB biofilms.
- Reduction occurred by two parallel processes: 1) Enzymatic reduction and 2) reduction by hydrogen sulfide.
- Demonstrated a method to abiotically produce uraninite nanoparticles via sulfide reduction.
2.18 Publication 18


2.18.2. Abstract: In the United States, the era of nuclear weapons development has left over 3,000 sites and 6.4 billion cubic meters of soil and groundwater moderately to highly contaminated with organic and inorganic pollutants. High energy radionuclides and heavy metals are included as some of the pollutants at many of these sites. Proposed remediation methods for organic/inorganic contaminated sites include expensive chemical treatments, passive reactive barriers (underground treatment walls), and pump and treat methods. Bioremediation, as an alternative, is often inexpensive and at some sites the only monetarily feasible method. A variety of microorganisms are capable of immobilizing metals by metabolically coupling oxidation of an organic compound to the reduction of the metal from a higher, more soluble form to lower, less soluble oxidation state. Sulfate-reducing bacteria (SRB), however, are able to immobilize metals not only by direct enzymatic reduction but also by chemical reduction via the production of sulfide. One group of SRB, *Desulfovibrio* sp., are able to grow using diverse metabolic pathways including sulfate reduction, nitrate ammonification, and fermentation. Sulfate reduction couples the oxidation of an electron donor to the reduction of sulfate resulting in the formation of HS-. Sulfide is a highly reactive species that precipitates and immobilizes soluble metals such as Pb(II), Cu(II), Cd(II), Zn(II), As(III), and Hg(II) to their sulfide form. Cultures of *Desulfovibrio desulfuricans* have been found to enzymatically reduce Fe(III), U(VI), Cr(VI), Tc(VII), and Pb(II), to less soluble and often less toxic phases (excluding Fe).
2.19 Publication 19


2.19.2. Abstract: Modeling uranium (U) transport in subsurface environments requires a thorough knowledge of mechanisms likely to restrict its mobility, such as surface complexation, precipitation and colloid formation. In closed systems, sulfate-reducing bacteria (SRB) such as Desulfovibrio spp. demonstrably affect U-immobilization by enzymatic reduction of U(VI) species (primarily the uranyl ion, UO$_2^{2+}$, and its complexes) to U(IV). However our understanding of such interactions under chronic U(VI) exposure in dynamic systems is limited. As a first step to understanding such interactions, we performed bioreactor experiments under continuous flow to study the effect of a biofilm of the sulfate-reducing bacterium Desulfovibrio desulfuricans attached to specular hematite (α-Fe$_2$O$_3$) surfaces on surface-associated U(VI) complexation, transformation, and mobility. Employing real-time microscopic observation and X-ray photoelectron spectroscopy (XPS), we show that the characteristics of the U(VI) complex(es) formed at the hematite surface are influenced by the composition of the bulk aqueous phase flowing across the surface and by the presence of surface-associated SRB. The XPS data further suggest higher levels of U associated with hematite surfaces colonized by SRB than with bacteria-free surfaces. Microscopic observations indicate that at least a portion of the U(VI) that accumulates in the presence of the SRB is exterior to the cells, possibly associated with the extracellular biofilm matrix. The U$^{4f_{7/2}}$ core-region spectrum and U$^{5f^2}$ valence band spectrum provide preliminary evidence that the SRB-colonized hematite surface accumulates both U(VI) and U(IV) phases, whereas, only U(VI) phase(s) accumulates on uncolonized hematite surfaces. The results suggest that mineral surfaces exposed to a continuously replenished supply of U(VI)-containing aqueous phase will accumulate U phases that may be more representative of those that exist in U-contaminated aquifers than those which accumulate in closed experimental systems. These phases should be considered in models attempting to predict U transport through subsurface environments.

2.19.3. Summary Results

- The hematite surface-associated activities of sulfate-reducing bacteria promote the reduction of U(VI) dissolved in the bulk aqueous phase to U(IV) with subsequent precipitation of U(IV) as a secondary mineral phase on the hematite surface.
- Insoluble forms of both U(VI) and U(IV) are deposited on hematite surfaces colonized by sulfate-reducing bacteria in an open, flow-through system.
2.20 Publication 20


2.20.2. Abstract: A study was undertaken to investigate expression of a gene encoding a c-type cytochrome in cells of the dissimilatory metal reducing bacterium (DMRB) *Geobacter sulfurreducens* during association with poorly crystalline and crystalline solid phase Fe(III)-oxides. The gene encoding OmcC (outer membrane c-type cytochrome) was used as a target for PCR-based molecular detection and visualization of *omcC* gene expression by individual cells and aggregates of cells of *G. sulfurreducens* associated with ferrihydrite and hematite mineral particles. Expression of *omcC* was demonstrated in individual bacterial cells associated with these Fe-oxide surfaces by in-situ RT-PCR (IS-RT PCR) and epifluorescence microscopy. Epifluorescence microscopy also permitted visualization of total DAPI-stained cells in the same field of view to assess the fraction of the cell population expressing *omcC*. By combining reflected differential interference contrast (DIC) microscopy and epifluorescence microscopy, it was possible to determine the spatial relationship between cells expressing *omcC* and the mineral surface. Introduction of the fluorescently-labeled lectin, Concanavalin A, revealed extracellular polymeric substances (EPS) extending between aggregations of bacterial cells and the mineral surface. The results indicate that EPS mediates an association between cells of *G. sulfurreducens* and ferrihydrite particles, but that direct cell contact with the mineral surface is not required for expression of *omcC*. XPS analysis revealed forms of reduced Fe associated with areas of the mineral surface where EPS-mediated bacterial associations occurred. The results demonstrate that by combining molecular biology, reflectance microscopy and XPS, chemical transformations at a mineral surface can be related to the expression of specific genes by individual bacterial cells and cell aggregates associated with the mineral surface. The approach should be useful in establishing involvement of specific gene products in a wide variety of surface chemical processes.

2.20.3. Summary Results

- A method was developed that enabled the fluorescent microscopic visualization of gene expression at the single cell and microcolony level in a population of dissimilatory Fe-reducing bacteria colonizing an Fe oxide surface.
2.21 Publication 21


2.21.2. Abstract: Investigations of groundwater and vadose zone microbiology are hindered by various obstacles associated with our limited access to the subsurface. These challenges are compounded when the hydrogeologic medium of interest is contaminated with toxic substances such as metals or radionuclides. In this study, exogenous solid substrata were contained in a porous receptacle and incubated within the saturated zone in order to capture populations from the extant communities. After an eight-week incubation, biofilms had formed on the particle surfaces, and the coupons were recovered and analyzed for attached community structure by 16S rDNA-based methods. T-RFLP was used to compare the communities formed on various substrata with those found in the groundwater and on the native sediments in a pristine region of the aquifer. Based on Jaccard similarities and cluster analysis of the T-RFLP patterns, the groundwater and sediment communities were highly distinct from one another, and the solid substrata better represented the groundwater communities than the sediments. Specular hematite was incubated in two wells, one pristine and one acidic and contaminated, and analyzed with 16S rDNA clone libraries. The biofilm formed in the pristine area was highly diverse at the species level, with 25 distinct phylotypes identified, the majority of which (73%) were affiliated with the β-Proteobacteria. Similarly, the biofilm formed in the contaminated area was populated in large part by β-Proteobacteria (62%); however, only 13 distinct phylotypes were apparent. The three numerically dominant clones in the contaminated biofilm were affiliated with metal- and radionuclide-tolerant or acidophilic taxa, consistent with the environmental conditions. Only two populations were common to both sites.

2.21.3. Summary Results

- Groundwater chemistry dictates the structure of the microbial community that develops in association with solid phase mineral surfaces incubated in a pristine and contaminated aquifer.
- The surface-associated microbial populations appear to have been selected on the basis of their physiological adaptations to specific chemical properties of the surrounding groundwater.
2.22 Publication 22


2.22.2. Abstract: Department of Energy (DOE) facilities within the weapons complex face a daunting challenge of remediating huge below inventories of legacy radioactive and toxic metal waste. More often than not, the scope of the problem is massive, particularly in the high recharge, humid regions east of the Mississippi river, where the off-site migration of contaminants continues to plague soil water, groundwater, and surface water sources. As of 2002, contaminated sites are closing rapidly and many remediation strategies have chosen to leave contaminants in-place. In situ barriers, surface caps, and bioremediation are often the remedial strategies of chose. By choosing to leave contaminants in-place, we must accept the fact that the contaminants will continue to interact with subsurface and surface media. Contaminant interactions with the geosphere are complex and investigating long term changes and interactive processes is imperative to verifying risks. We must be able to understand the consequences of our action or inaction. The focus of this manuscript is to describe recent technical developments for assessing the performance of in situ bioremediation and immobilization of subsurface metals and radionuclides. Research within DOE’s NABIR and EMSP programs has been investigating the possibility of using subsurface microorganisms to convert redox sensitive toxic metals and radionuclides (e.g. Cr, U, Tc, Co) into a less soluble, less mobile forms. Much of the research is motivated by the likelihood that subsurface metal-reducing bacteria can be stimulated to effectively alter the redox state of metals and radionuclides so that they are immobilized in situ for long time periods. The approach is difficult, however, since subsurface media and waste constituents are complex with competing electron acceptors and hydrogeological conditions making biostimulation a challenge. Performance assessment of in situ biostimulation strategies is also difficult and typically requires detailed monitoring of coupled hydrological, geochemical/geophysical, and microbial processes. In the following manuscript we will (1) discuss contaminant fate and transport problems in humid regimes, (2) efforts to immobilize metals and radionuclides in situ via bioremediation, and (3) state-of-the-art techniques for assessing the performance of in situ bioreduction and immobilization of metals and radionuclides. These included (a) in situ solution and solid phase monitoring, (b) in situ and laboratory microbial community analysis, (c) noninvasive geophysical methods, and (d) solid phase speciation via high resolution spectroscopy.
2.23 Publication 23


2.23.2. Abstract: Hexavalent uranium [U(VI)] dissolved in a modified lactate-C medium was treated under anoxic conditions with a mixture of an Fe(III)-(hydr)oxide mineral (hematite, goethite, or ferrihydrite) and quartz. The mass of Fe(III)-(hydr)oxide mineral was varied to give equivalent Fe(III)-mineral surface areas. After equilibration, the U(VI)-mineral suspensions were inoculated with sulfate-reducing bacteria, *Desulfovibrio desulfuricans* G20. Inoculation of the suspensions containing sulfate-limited medium yielded significant G20 growth, along with concomitant reduction of sulfate and U(VI) from solution. With lactate-limited medium, however, some of the uranium that had been removed from solution was re-solubilized in the hematite treatments and, to a lesser extent, in the goethite treatments, once the lactate was depleted. No re-solubilization was observed in the lactate-limited ferrihydrite treatment even after a prolonged incubation of four months. Uranium re-solubilization was attributed to re-oxidation of the uraninite by Fe(III) present in the (hydr)oxide phases. Analysis by U L3-edge XANES spectroscopy of mineral specimens sampled at the end of the experiments yielded spectra similar to that of uraninite, but having distinct features, notably a much more intense and slightly broader white line consistent with precipitation of nanometer-sized particles. The XANES spectra thus provided strong evidence for SRB-promoted removal of U(VI) from solution by reductive precipitation of uraninite. Consequently, our results suggest that SRB mediate reduction of soluble U(VI) to an insoluble U(IV) oxide, so long as a suitable electron donor is available. Depletion of the electron donor may result in partial re-oxidation of the U(IV) to soluble U(VI) species when the surfaces of crystalline Fe(III)-(hydr)oxides are incompletely reduced.

2.23.3. Summary Results
- Demonstrated concomitant reduction of sulfate and U(VI) by SRB. Results indicated that U(IV) reoxidation may be possible even under strongly reduced conditions if crystalline Fe(III)-(hydr)oxides are incompletely reduced.
2.24 Publication 24


2.24.2. Abstract:
The most recent mathematical models of microbial activity in heterogeneous biofilms are based on cellular automata. The main weakness of these models is that to obtain numerical solutions the operator must specify the rules governing microbial cell behaviour in the biofilm, and these rules are difficult to establish experimentally. To avoid this difficulty, we have used an alternative approach, discretizing biofilms into layers, to include the effects of biofilm heterogeneity on biofilm activity. This procedure conceptually converts heterogeneous biofilms into a stack of stratified layers of various densities, activities, and diffusivities, and can include some effects of biofilm heterogeneity, e.g. vertical distribution of biofilm density, activity, and effective diffusivity. We present this model and selected examples of computational procedures illustrating it. We found that the activity of homogeneous biofilms can be lower, higher, or equal to the activity of stratified biofilms; since homogeneous biofilms do not exist, their properties have to be assumed. Surprisingly, stratified biofilms with high effective diffusivity gradients had lower activities than homogeneous biofilms having average effective diffusivity computed as the average effective diffusivities of the individual layers in stratified biofilms. As expected, the model predicts that the growth-limiting nutrient penetrates deeper into stratified biofilms than it does into homogeneous biofilms.

2.24.3. Summary Results
- Implemented the concept of stratified biofilms, composed of layers of various densities and activities.
- Developed a novel mathematical model of heterogeneous biofilms using the concept of stratified biofilms
2.25 Publication 25


2.25.2. Abstract: Dissimilatory reduction of Fe(III) by *Shewanella oneidensis* MR-1 was evaluated using natural specular hematite as sole electron acceptor in an open system under dynamic flow conditions to obtain a better understanding of biological Fe(III) reduction in the natural environment. During initial exposure to hematite under advective flow conditions, cells exhibited a transient association with the mineral characterized by a rapid rate of attachment followed by a comparable rate of detachment before entering a phase of surface colonization that was slower but steadier than that observed initially. Accumulation of cells on the hematite surface was accompanied by the release of soluble Fe(II) into the aqueous phase when no precautions were taken to remove amorphous Fe(III) from the mineral surface prior to colonization. During the period of surface colonization following the detachment phase, cell yield was estimated at $1.5-4 \times 10^7$ cells/µmol Fe(II) produced, which is similar to that reported in studies conducted in closed systems. This yield does not take into account those cells that detached during this phase or the Fe(II) that remained associated with the hematite surface. Hematite reduction by the bacterium led to localized surface pitting and the establishment of discrete areas on the hematite surface that could reduce Ag(I), possibly sites of Fe(II) precipitation. The cleavage plane of hematite left behind after bacterial reduction, as revealed by our results, strongly suggests, that heterogeneous energetics of the mineral surface play a strong role in this bioprocess. AQDS, an electron shuttle shown to stimulate bioreduction of Fe(III) in other studies, inhibited reduction of hematite by this bacterium under the dynamic flow conditions employed in the current study.

2.25.3. Summary Results

- Colonization of the crystalline Fe oxides that serve as the sole electron acceptor for dissimilatory Fe reduction by *Shewanella oneidensis* MR-1 involves an initial reversible attachment phase, followed by a major detachment phase before the establishment of a slower cell accumulation phase that involves further cell detachment.
- Pitting of the Fe oxide surface occurs during later stages of cell accumulation on the Fe oxide.
- The Fe oxide surface contained localized areas that contained reduced forms of iron.
2.26 Publication 26  

2.26.2. Abstract: Hexavalent uranium [U(VI)] was immobilized in biofilms composed of the sulfate reducing bacteria (SRB), *Desulfovibrio desulfuricans* G20. The biofilms were grown in two flat-plate, continuous-flow reactors using lactate as the electron donor and sulfate as the electron acceptor. The growth medium contained uranium U(VI) and the pH was maintained constant using bicarbonate buffer. The reactors were operated for 5 months, and during that time biofilms activity and uranium removal were evaluated. The efficiency of uranium removal strongly depended on the concentration of uranium in the influent, and was estimated 30.4% in the reactor supplied with 3 mg/L of U(VI) and 73.9% in the reactor supplied with 30 mg/L of U(VI). TEM and SAED analysis showed that uranium in both reactors accumulated mostly on microbial cell membranes and in the periplasmic space. The deposits had amorphous or poor nanocrystalline structures.

2.26.3. Summary Results
- Sulfate-reducing biofilms showed high removal efficiencies of uranium.
- Uranium was precipitating on microbial cell membranes and in the periplasmic space regardless of the bulk uranium concentration.
2.27 Publication 27


2.27.2. Abstract: Biofilms of sulfate-reducing bacteria Desulfovibrio desulfuricans G20 were used to immobilize/reduce U(VI) in the presence of uranium-complexing carbonates. The biofilms were grown in three identically operated fixed bed reactors (columns), filled with three types of minerals: one non-carbonate-bearing mineral (hematite) and two carbonate-bearing minerals (calcite and dolomite). Lactate was the electron donor, and sulfate and uranium were the electron acceptors. The growth medium supplied to the reactors filled with calcite and dolomite was not buffered, while the growth medium supplied to the column filled with hematite was buffered with a 10-mM carbonate buffer, pH 7.2. The reactors were operated for five months at a flow rate of 0.35 ml/min using a solution of nutrients amended with 126 μM U(VI). The residence time in the reactors, based on the flow rate and the volume of the empty voids in the packing material, was 1.15 days. Deposition of uranium in the reactors reached, on average, 72.5% in the reactor filled with hematite, 82.4% in the reactor filled with dolomite and 87.2% in the reactor filled with calcite. High-resolution transmission electron microscopy (HRTEM), energy-dispersive X-ray spectrometry (EDS) and selected area electron diffraction (SAED) showed that in all biofilms U(VI) was precipitated on bacterial membranes as U(IV) material. The structure of the precipitated uranium in the biofilms deposited in the reactors filled with calcite and dolomite was amorphous, while the deposits of uranium in the biofilm deposited in the reactor filled with hematite showed crystals of U(IV) between 2 and 6 nm in size. Our five-month study demonstrated that the sulfate-reducing biofilms grown in the reactors filled with hematite, calcite, and dolomite were able to immobilize/reduce uranium efficiently, despite the presence of uranium-complexing carbonates in the solution.

2.27.3. Summary Results

- Sulfate-reducing biofilms grown in the reactors filled with hematite, calcite, and dolomite were able to immobilize/reduce uranium efficiently, despite the presence of uranium-complexing carbonates in the solution.
- The structure of the precipitated uranium in the biofilms deposited in the reactors filled with calcite and dolomite was amorphous, while the deposits of uranium in the biofilm deposited in the reactor filled with hematite showed crystals of U(IV) between 2 and 6 nm in size.
2.28 Publication 28


2.28.2. Abstract: In cultures of *Desulfovibrio desulfuricans* G20 the effects of Fe(III)-(hydr)oxides (hematite, goethite, and ferrihydrite) on microbial reduction and reoxidation of uranium (U) were evaluated under lactate-limited sulfate-reducing conditions. With lactate present, G20 reduced U(VI) in both PIPES and bicarbonate buffer. Once lactate was depleted, however, microbially reduced U served as an electron donor to reduce Fe(III) present in Fe(III)-(hydr)oxides. With the same initial amount of Fe(III) (10 mmol/L) for each Fe(III)-(hydr)oxide, reoxidation of U(IV) was greater with hematite than with goethite or ferrihydrite. As the initial mass loading of hematite increased from 0 – 20 mmol Fe(III)/L, the rate and extent of U(IV) reoxidation increased. Subsequent addition of hematite (15 mmol Fe(III)/L) to stationary phase cultures containing microbially reduced U(IV) also resulted in rapid reoxidation to U(VI). Analysis by U L3-edge XANES spectroscopy of microbially reduced U particles yielded spectra similar to that of natural uraninite. Observations by high resolution-transmission electron microscopy, selected area electron diffraction, and energy-dispersive X-ray spectroscopic analysis confirmed that precipitated U associated with cells was uraninite with particle diameters of 3 – 5 nm. Using the same techniques, iron sulfide precipitates were found to have a variable Fe and S stoichiometry, and were not associated with cells.

2.28.3. Summary Results
- Demonstrated that Fe(III)-(hydr)oxides (hematite, goethite, and ferrihydrite) were capable of reacting to allow the reoxidation of uranium.
- Showed that reoxidation was biotically influenced in that active SRB (versus heat killed) cultures gave significantly more reoxidation than the heat killed or abiotic controls.
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