Coupled Microbial and Chemical Reactions in Uranium Bioremediation

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Abstract. The chemical composition of the water and sediment affects the extent that uranium is immobilized in a microbially active environment. This paper summarizes an integrated framework of coupled microbial and chemical reactions in uranium bioremediation. The research is aimed at improving selection, design and operation of uranium biotreatment systems that use natural organic material to support the microbial consortium. Highlights of experimental and modeling efforts are presented.

Introduction

An estimated 350,000 tons of uranium (U) were produced in the United States (US) by 1998. A high proportion of the uranium ore in the US is located in the Rocky Mountain region. There are hundreds of small uranium mine sites that are not currently addressed by existing federal remediation programs. Over 1300 abandoned uranium mines have been identified on Navajo Tribal lands in Arizona and New Mexico alone. The cost of effective mitigation of numerous small and remote abandoned uranium-mining sites lends itself to passive treatment technologies. There is a need for relatively low cost and low maintenance control and treatment systems to mitigate critical and numerous sites.

Wetlands have been shown to effectively remove uranium from mining influenced water. However, the long-term stability has been variable. In some wetlands uranium retention tends to be associated with reversible sorption of U(VI). Uranium is very stable in other wetlands and requires leaching with sulfuric acid to mobilize. It is difficult to control reducing conditions in wetlands as the intrusion of the plant roots into sediments provides a pathway for oxygen to enter the sediment. Even if the lower portion of the sediment is anaerobic, a fraction of the sediment is not and thus the fraction of flow that bypasses the anaerobic environment does not allow for the reduction of uranium.

In-situ biosystems can be used to ensure anaerobic conditions are maintained in a passive remediation scheme. Permeable reactive barriers and in-situ sulfate reducing bioreactors are two biosystems that minimize oxygen intrusion and are thus able to maintain the requisite anaerobic environment needed for uranium reduction. However, ensuring anaerobic conditions is only one part of promoting successful uranium reduction and precipitation. Ultimately, the microbial functions expressed coupled with the aqueous chemistry will control the extent of uranium reduction and immobilization. Uranium fate and treatment has been a focus area since the early 1990's at the Colorado School of Mines (CSM). The efforts have centered on a theme of integration of chemical, microbial and physical processes on uranium fate, transport and remediation. Unanswered questions critical to design of successful passive remediation include understanding how solid and solution phase chemistry affects the microbial distribution, the microbial interactions with uranium, and the availability of uranium for immobilization. Understanding these effects will allow for more effective design and operation of passive biosystems.

Microbial ecology of uranium reducing biosystems

The microbial community composition in anaerobic environments is controlled by the type of biopolymer present (e.g. cellulose or protein) and the subsequent products formed by hydrolysis and fermentation. Selected bacteria from different substrate niches (e.g. glucose vs. lactate vs. acetate) are capable of uranium reduction. Most do not grow on the energy released from the uranium/organic carbon redox couple. Thus, reduction of uranium represents a competitive use of organic carbon as an electron donor for the uranium-reducing microorganism. The microorganism must also use additional organic carbon with its energy producing electron acceptor to grow and remain active. At the same substrate level there are microorganisms that do not reduce uranium and also compete for the same organic compounds. Carbon flow through non-uranium reducing microorganisms at all substrate levels could result in no uranium reduction in an apparently appropriate anaerobic environment. The structure of the active microbial ecology is also sensitive to the solution phase chemical composition of the water. For example, salinity or concentration of specific inorganic ions can affect the community structure. In addition, the solid phase organic material, microorganisms, and microbial byproducts serve as ligands or bind sites for uranium sorption and complexation that further complicate uranium reduction. Thus predicting the overall uranium reduction requires understanding the contribution of multiple microbial functions, the microbial competition at each substrate, and uranium bioavailability. Approaches available to study microbial processes in these complex biosystems include molecular methods (e.g., DNA extraction, PCR, Cloning, and DNA sequencing) to elucidate community structure, bioassays to examine inherent microbial functions, measurement of changes in microbial substrates (sequential extraction protocols and High Pressure Liquid Chromatography, HPLC) and products (Gas Chromatography and HPLC), and coupled modeling of microbial and chemical kinetics with reactive transport.

Chemistry of uranium immobilization

Uranium immobilization in anaerobic environments occurs for uranium species in both the U(VI) and U(IV) oxidation state. U(VI) species are typically immobilized by sorption processes while U(IV) immobilization is as insoluble $UO_{2(S)}$. The reduction of U(VI) to U(IV) and subsequent precipitation as $UO_{2(S)}$ is the goal of anaerobic passive treatment schemes. However, the presence of ligands or solids tends to reduce uranium reduction and the formation of $UO_{2(S)}$ in the presence of U(IV). While the inorganic solution phase chemical reactions for uranium are relatively well understood, the interactions with organic ligands, colloids and particles are not well understood. Part of the difficulty with colloids and particles is the broad range of chemical characteristics exhibited. Colloids may be made up of extracelluar polysaccharides, enzymes, and other functional exudates from microbial growth or a broad range of humic materials resulting from the microbial degradation of lignocellulose materials. Therefore, it is important to understand the additional effect of microbially related organic compounds on uranium bioavailability and precipitation availability. Approaches to elucidate the chemical dependent aspects of these complex uranium biosystems include characterization of uranium partitioning between solution and solid phase complexes by field flow fractionation (FFF) coupled with size exclusion (SEC), ultraviolet absorbance (UVA) or inductively coupled plasma - mass spectrometry (ICP-MS), measurement of uranium oxidation state and surface complexes using Extended X-ray Absorption Fine Structure (EXAFS) and X-ray Absorption Near-Edge Spectroscopy (XANES), quantification of surface chemical characteristics and surface complexation modeling, and integration of surface complex modeling into a reactive transport model.

CSM uranium bioremediation

Microbial ecology efforts

There are many elements of the microbial ecology of uranium bioremediation that require more research to improve design and operation of biotreatment systems. A conceptual model of the microbial ecology of uranium reduction is presented in Figure 1. This conceptual model provides a useful framework to direct needed research efforts to improve uranium bioremediation. One element is the direction of the carbon flow to the desired substrates and microbial functions. Sulfate reduction and fermentation are model microbial functions that also facilitate uranium reduction. Examples of CSM work in this area include the effect of initial solid phase organic matter composition on sulfate reduction (Figueroa et al. 2004); monitoring changes in solid phase organic substrates over time (Place et al. 2005); examining metal toxicity on cellulose degradation and thus on overall sulfate reduction (Logan 2003); modeling concurrent kinetics of uranium and sulfate reduction (Spear 2000); and modeling decomposition of biopolymers coupled to sulfate reduction (Hemsi et al. 2005).

The microbial community structure and long-term sustainability of anaerobic biozones can be directed by the types of organic matters used for construction of wetlands and bioreactors. However, there has not been an effective correlation developed between microbial community structure/function and initial solid phase substrate composition particularly temporally. Initial reactivity of treatment sys-

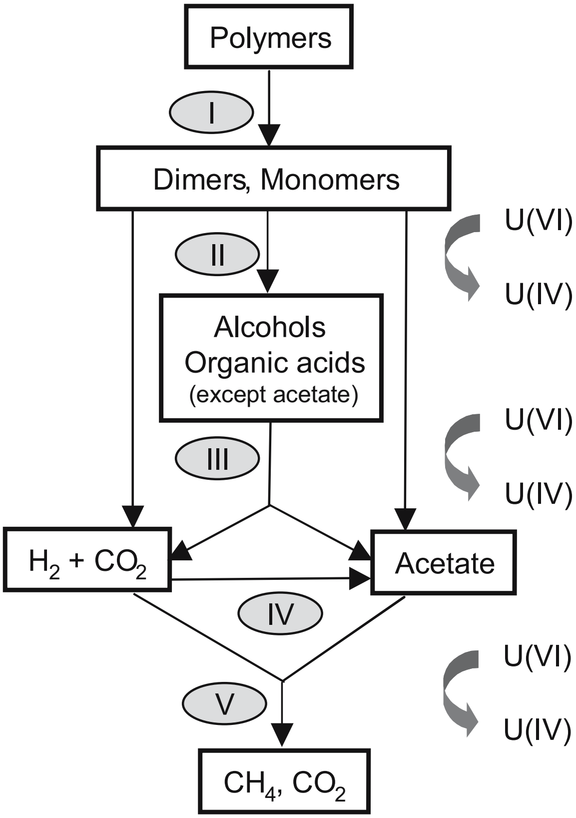

Fig.1. Conceptual model of the microbial ecology of uranium reduction. I=extracellular hydrolysis; II=primary fermenters, and acidogens; III=secondary fermenters, acidogens, iron- and sulfate -reducers; IV=acidogens, V=acetate or hydrogen consumption by methanogens, and iron- or sulfate-reducers.

tems tends to be high but after one year rates of sulfate reduction are significantly lower than initial rates. In some systems these rates are sustained for at least 5 years, in other systems significant activity may cease in less than a year. More information is needed on both the microbial community structure and function in conjunction with initial organic matter composition and changes in both with time. The elucidation of the microbial community structure of anaerobic treatment zones is only recently being monitored (e.g., Pruden et al. 2005). Similarly, quantification of the composition of the solid phase organic substrates is a relatively recent effort (Figueroa et al. 2004, Place et al. 2005). The nutritional composition of organic substrates can be characterized by applying sequential extractions methods commonly used in food science, forestry and agriculture fields. Sustainability of sulfate reduction as a function of initial substrate composition was evaluated for oak wood, pine wood, alfalfa and corn stover. A high percentage of cellulose in conjunction with a high cellulose-to-lignin ratio was related to substrate sustainability. Organic substrate composition and microbial community structure and activity over time are currently being assessed in several field-scale sulfate-reducing bioreactors. The expectation is to gain greater insight into how substrate composition affects sulfate reduction and how to design substrate mixtures to optimize desired microbial function. A biomodule for modeling sulfate reduction controlled by decomposing organic solids (Hemsi et al. 2005) was also developed. The module is design to integrate into reactor models and reactive transport codes such as MODFLOW. The field data collected will be used to validate the coupled microbial process and transport model.

Uranium availability efforts

Understanding the effect of solid and solution phase composition on availability of uranium for reduction and precipitation reactions is critical to designing successful uranium bioremediation systems. A conceptual model for uranium availability is presented in Figure 2. The organic ligands and particles in bioremediation systems are complex as natural organic materials and degradation products are represented by a distribution of sizes and chemical characteristics. Examples of CSM efforts in uranium availability include the characterization of particle size distributions and composition coupled to uranium binding and mobility (Jackson et al. 2005a & 2005b, Hendry et al. submitted, Honeyman and Ranville 2002); the effect of exobiopolymers on thorium complexation (Quigley et al. 2002); surface complexation modeling of uranium sorption to bacterial surfaces (Landkamer 2003); and surface complexation modeling coupled to reactive transport of uranium (Kantar 2001).

The effects of solid and solution phase chemical constituents on uranium availability are important to developing predictive models for uranium bioremediation. Besides inorganic constituents, microorganism and their by-products provide surfaces and compounds that interact with uranium. Landkamer (2003) examined the sorption of uranium onto a gram-negative bacterium Desulfovibrio vulgaris. There were many artifacts to overcome in understanding sorption to bacteria and an experimental and modeling framework was developed. For example, the preparation protocol required the removal of a suspected iron sulfide layer precipitation on the bacterial surface during growth conditions. This layer caused difficulties in reproducibility of sorption data and interfered with uranium binding to bacterial surface function groups until it was removed. Also, the three dimensional nature of the bacterial surface required the inclusion of a three dimensional volume (using the Donnan model) in the surface complexation modeling to account for the porous nature of the bacterial surface. The model calibration included the examination of uranium sorbed onto bacterial surfaces by X-ray absorption spectroscopy to determine the distribution of function groups binding uranium to the bacterial surface and the oxidation state of the uranium. Sorption of U(VI) to bacterial surfaces may reduce its availability for uranium reduction and subsequent $UO_{2(S)}$ precipitation.

The characterization of natural organic matter coupled with uranium binding capabilities was studied using coupled analytical methods. Jackson et al. (2005a) reported cell suspension separation using FFF into two separate peaks that represent different particle sizes and distributions. One peak eluted in a time frame that suggests it was the nominally 1 micron sized bacterium. The other peak eluted earlier and is hypothesized to be bacterial exopolymer. Uranium sorption to these two size fractions significantly varied in extent as a function of pH. At pH 8 and 1 micromole uranium, the percent uranium sorption was about equal between the two fractions. However, at pH 6, 80% of the uranium was associated with the cell fraction and only 20% with the extracellular fraction. Jackson et al. (2005b) also measured uranium and nickel distribution in the dissolved fraction of contami-

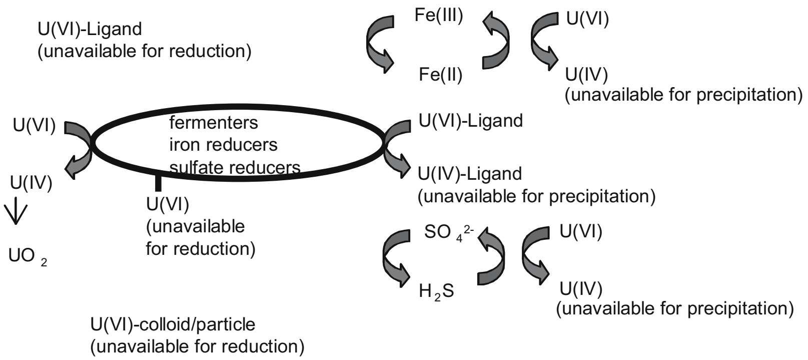

Fig. 2. Conceptual model of uranium availability for reduction and precipitation in the presence of complexing ligands and particles.

nated sediment extracts by FFF, SEC coupled to UVA and ICP-MS. Uranium was found complexed to dissolved organic matter (DOM) and a colloidal fraction. This will tend to make the uranium unavailable for bioreduction or removal by sorption. In addition, the nickel was predominately present as a free ion or labile complex that is more bioavailable. Nickel in particular has demonstrated differential toxicity to fermenters over acidogens and sulfate reducers. Thus, the uranium bioreduction ability of a microbial community could be impaired by the presence of a toxic metal such as nickel.

Summary

The complex microbial, chemical and physical interactions within anaerobic biozones require a systems approach to understanding the complex interrelationships. The framework for microbial ecology of uranium reduction coupled with the availability of uranium provides a useful way to conceptualize the key processes. Elucidation of the interactions will allow for improvements in design and operation of passive biotreatment systems such as wetlands, permeable reactive barriers and sulfate-reducing bioreactors.

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