

# **Report for 2002WA16B: Reactive Transport of Reducible Metal Ions: Reaction Kinetics, Column Experiments, and Transport Modeling**

- Articles in Refereed Scientific Journals:
  - Viamajala, S., B. M. Peyton, and J. N. Petersen. Modeling chromate reduction in *Shewanella oneidensis* MR-1: Development of a novel dual-enzyme kinetic model. *Biotechnology and Bioengineering*. (Accepted for Publication)
  - Qiu, H.; M. Alam; S. Viamajala; B. M. Peyton; J. N. Petersen; D. Yonge. Microbially-mediated chromate reduction in 1-D soil columns: Experimental results and numerical modeling. (In preparation)
- Other Publications:
  - Qiu H., S. Viamajala, M. Alam, B.M. Peyton, J.N. Petersen, D.R. Yonge. 2002. Reactive Transport Modeling of Microbially-Mediated Chromate Reduction in 1-D Soil Columns. Poster presented at the American Geophysical Union Fall Meeting, San Francisco, California. December 6-10, 2002.

Report Follows

## **Problem and Research Objectives:**

Chromium is the second-most common inorganic contaminant at hazardous waste sites in the US. It is found at many locations in the Pacific Northwest such as the U.S. Department of Energy's Hanford Site located in Southeastern Washington State, the Pacific Sound Resources site (formerly the Wyckoff Wood Treatment Facility), the U.S. Naval Submarine Base (Subbase) at Bangor in the Puget Sound region, the Midnite Mine (located on the Colville Indian Reservation), the Silver Bow Creek / Butte Area of Montana and at Lake Coeur d'Alene. In its oxidized form, Cr(VI) presents significant health hazards and is a soluble, highly mobile species. Therefore, it is imperative that technologies be developed to remediate sites contaminated with this compound. Moreover, chromium can serve as an example of other reducible metal ions, such that knowledge gained learning how chromium interacts with the environment will provide insights into how other reducible metals, such as uranium and technetium, are transported through the environment. The primary objective of our current research was to integrate experimental and numerical tasks to gain a better understanding of the complex biogeochemical interactions that dictate the transport of Cr(VI). In particular, we proposed that we would develop general reactive transport modules to describe the reactive transport of chromium, biomass, and nutrients in bench-scale laboratory columns. We further proposed that the developed modules would be embedded into RT3D, a widely used 3-D mass transport model. RT3D is especially focused on organic contaminants, such as BTEX, PCE/TCE, and other sequential decay reactions and addition of Cr(VI) reactive transport modules would enhance the capabilities of the RT3D software package to describe reducible metal transport. The module development would be based on kinetic parameters obtained from batch experiments and would be calibrated with data from bench-scale soil columns.

## **Methodology:**

### **1. The batch tests:**

The purpose of this study was to develop and test a kinetic model that would describe Cr(VI) reduction by anaerobic stationary phase MR-1 cultures grown under different conditions. Cr(VI) reduction tests were performed with stationary phase cultures grown on fumarate as the terminal electron acceptor. The cultures were incubated for 36 hours until fumarate was consumed and the cultures reached stationary phase. After 36 hours of incubation, serum bottles containing the cultures were transferred to an anaerobic glove box (Model 1025, Forma Scientific Inc., OH; gas mix – 90% N<sub>2</sub>, 5% H<sub>2</sub> and 5% CO<sub>2</sub>) and opened where Cr(VI) reduction experiments were performed. For this purpose, batch reactors containing 2mL of culture were established in sterile 24-well tissue culture plates (Corning Inc., NY) inside the anaerobic glove box. Cr(VI) reduction was tested with five different initial Cr(VI) concentrations ranging from 0.04-0.12 mM. Required Cr(VI) concentrations were achieved in the reactors by adding K<sub>2</sub>CrO<sub>4</sub> from anaerobic sterile stock solutions that were 100 times more concentrated than the target concentration. Each initial Cr(VI) concentration was tested at least in duplicate and some tests were repeated (in duplicate) with cultures from a different serum bottle. Cell protein, cell numbers and optical density were measured at the beginning of the experiment and assumed to be constant since the cultures were at stationary phase and the duration of the experiment was short.

### **2. The column tests**

Soil column experiments were performed to provide data for calibration of the numerical model and for validation of modeling results. Stainless steel high pressure liquid chromatography columns (2.5 cm (ID) x 15 cm), containing quartz sand as the porous media matrix, were used for the

experiments. The columns were inoculated with a well-known metal reducing bacterium, *Shewanella oneidensis* MR-1, which served as a model microorganism in these tests. Before inoculation of the columns, MR-1 cultures were grown aerobically in simulated groundwater (SGW) amended with lactate. To ensure a uniform initial biomass distribution in the column, quartz sand was mixed with log phase culture broth ( $\sim 10^8$  CFUs/mL) and the resulting slurry was loaded and packed into the column. After inoculation, the column was flushed overnight with substrate-free SGW at a flow rate of 1mL/h (Darcy velocity  $\sim 1$ ft/day), to wash out unattached biomass. This flow rate approximates natural groundwater flow velocity. Thereafter, Cr(VI) and substrate were added simultaneously to the column to stimulate both bacterial growth and Cr(VI) reduction to Cr(III). Concentration of Cr(VI), lactate and fumarate in the feed stream were 2mg/L, 15mM and 2mM respectively. The parameters measured in these experiments include 1) concentrations of nutrients (lactate and fumarate) in the column influent and effluent, 2) Cr(VI) and Cr(III) concentrations in both the column feed and effluent, 3) effluent biomass concentration (cell count and protein), 4) hydraulic conductivity (pressure drop), and 5) pH. Each column experiment was replicated to insure repeatability. Un-inoculated columns were used as abiotic controls and for residence time distribution studies using a bromide tracer. Column feed was delivered with syringe pumps.

At the completion of the test, the column was cored, and the attached biomass concentration was determined by performing protein assays using the Pierce Micro Bradford Protein Assay Kit. Data from these soil column experiments was compared to results predicted using the modified RT3D code described below. Comparing observed data to reactive flow and transport model predictions has allowed the evaluation and verification of numerical processes descriptions developed in this project.

### 3. Numerical module development and coding

To describe results from columns experiments, numerical models were developed using RT3D, a commercially available and widely-used flow and transport code. Modules were developed to describe kinetics of biotransformation of Cr(VI), biomass growth, and substrate utilization and incorporated into RT3D. Thus, the numerical code could simulate Cr(VI) reduction results in the column by taking into account convection, dispersion and microbial reduction.

For numerical modeling purposes, the column was considered to be a one-dimensional system with constant flow. For such a system, the mass balance equation for a transported species is written as:

$$\frac{\partial}{\partial t}(nc) = \frac{\partial}{\partial z}(\alpha q \frac{\partial c}{\partial z}) - \frac{\partial}{\partial z}(qc) - R_{ba} - R_{bs} \quad (1)$$

where  $c$  is the aqueous phase macroscopic averaged concentration of the transported species [ $\text{ML}^{-3}$ ],  $n$  is the porosity,  $\alpha$  is the dispersivity [L],  $q$  is the Darcy flux [ $\text{MT}^{-1}$ ],  $R_{ba}$  is the microbial reaction rate that describes the mass of the contaminant biotransformed per unit of bulk porous media volume per unit time [ $\text{ML}^{-3}\text{T}^{-1}$ ], and  $R_{bs}$  is the volume-specific rate of contaminant removal that results from surface-associated biotransformation [ $\text{ML}^{-3}\text{T}^{-1}$ ].

This model ignores variations in porosity values due to changes in biomass concentration because numerical simulations in a variable porosity model indicated that accounting for porosity changes had little effect on nutrient and biomass profiles in one-dimensional constant flow systems.

Assuming constant porosity and dispersivity values, Equation (1) can be rewritten as:

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial z^2} - v \frac{\partial c}{\partial z} - r_{ba} - r_{bs} \quad (2)$$

where  $v = q/n$  is the pore velocity [ $LT^{-1}$ ],  $D = \alpha q/n$  is the dispersion coefficient [ $L^2T^{-1}$ ], and the  $r_i$  are the various reaction rates that describe the mass of contaminant reacted or sorbed/desorbed per unit liquid volume per unit time [ $ML^{-3}T^{-1}$ ].

In addition to nutrient concentrations, we were also interested in predicting the growth and accumulation of biomass in the system. Mass balance expressions that describe growth and transport of aqueous phase biomass, and growth and accumulation of attached biomass are expressed as:

$$\frac{\partial X_a}{\partial t} = \frac{\partial}{\partial x_i} (D_{ij} \frac{\partial X_a}{\partial x_j}) - \frac{\partial}{\partial x_i} (v_i X_a) + \hat{r}_X X_a - r_{att} + \frac{r_{det} \rho_k}{n} - K_{de} \cdot X_a \quad (3)$$

$$\frac{dX_s}{dt} = \hat{r}_X X_s - r_{det} + \frac{n r_{att}}{\rho_k} - K_d \cdot X_s \quad (4)$$

where  $\hat{r}_X$  is the specific bacterial growth rate [ $T^{-1}$ ],  $X_a$  is the concentration of biomass in the aqueous phase [ $ML^{-3}$ ],  $X_s$  is the solid phase biomass concentration per unit mass of porous media solids [ $MM^{-1}$ ], and  $\rho_k$  is the bulk density of porous media [ $ML^{-3}$ ].  $r_{att}$  is the rate at which suspended cells attach to the solid phase (mass of attached cells per unit liquid volume per unit time [ $ML^{-3}T^{-1}$ ]), and  $r_{det}$  is the rate at which cells detach from the solid phase (mass of detached cells per unit bulk mass of porous media solids per unit time [ $MM^{-1}T^{-1}$ ]).

Attachment and detachment of microbial cells were described as non-linear processes and the kinetic rates of attachment and detachment can be written as:

$$r_{att} = K_{att} \cdot \lambda \cdot X_a \quad (5)$$

$$r_{det} = \frac{K_{det}}{\lambda} \cdot X_s \quad (6)$$

where  $K_{att}$  is the attachment coefficient [ $T^{-1}$ ], and  $K_{det}$  is the detachment coefficient [ $T^{-1}$ ], and  $\lambda$  is the limiting factor for biomass growth and can be defined as

$$\lambda = \frac{X_{s_{max}} - X_s}{X_{s_{max}}} \quad (7)$$

This definition of  $\lambda$  is based on a maximum retention capacity such that when  $X_s$  is much less than the maximum retention capacity,  $X_{s_{max}}$ , the attachment will be high and when  $X_s$  approaches  $X_{s_{max}}$ , the attachment will approach zero.

Cr(VI) reduction kinetics by *Shewanella oneidensis* MR-1 was described by the dual enzyme kinetic model that was developed using batch kinetic data from experiments described above. The dual-enzyme kinetics is based on the hypothesis that Cr(VI) reduction in MR-1 occurs via two parallel and independent mechanisms – 1) a rapid mechanism that is susceptible to Cr(VI) and is deactivated during chromate reduction (“deactivating enzyme”) and (2) a slower mechanism that is resistant to Cr(VI) and the activity of which remains stable during chromate reduction (“stable enzyme”). Kinetic expressions for Cr(VI) reduction and substrate consumption were included in the mass balance equations (Eq. 2). A set of seven simultaneous differential equations were obtained after mass balance for biomass, substrate and Cr(VI) in the solid and aqueous phases. These equations were solved using GMS3.1 with RT3D as a modular computer code which allows for development of user defined kinetic modules by providing solvers to solve the governing equations. To solve the reactive transport portion of the model, we selected Runge-Kutta and General Gear Solver since both can give a reliable solution when proper time steps and spatial discrepancy are

adopted. Hybrid MOC method was chosen to solve the convection term. A very fine grid (300 grids for a total column length of 15 cm) was used and transport step  $\Delta t=0.001\text{h}$  was selected via precision test. The solver provide a default transport step length based on Peclet and Courant number constraints but we found this default transport step can not guarantee a reliable model precision(data not show). Trial and error method is used to calibrate the parameters on the basis of initial parameter estimation.

### **Principal Findings and Significance**

During this study, a series of batch and soil column experiments were performed in concert with reactive flow and transport numerical modeling. First, kinetic experiments conducted in batch reactors were used to develop Cr(VI) reduction kinetics for *Shewanella oneidensis* MR-1. Since Cr(VI) reduction in MR-1 could not be described using kinetics previously developed for other microorganisms, we developed a novel dual-enzyme kinetic model. This model takes into account multiple parallel Cr(VI) reduction pathways that exist in MR-1 and is therefore different from previous models that assumed a single Cr(VI) reducing enzyme. To elicit information on the complex interactions between soil, indigenous microbes, nutrients and Cr(VI), column experiments were performed. In these experiments, Cr(VI) and nutrient were fed to the column simultaneously, to simulate a nutrient-fed bioremediation system. Under these conditions it was observed that when the influent Cr(VI) concentration was less than 1mg/L, the column was able to sustain Cr(VI) reduction for more than 1 month, while when Cr(VI) feed concentration was increased to 2mg/L, the breakthrough occurred in 3-4 days. The reactive transport model developed by incorporating dual-enzyme Cr(VI) reduction kinetics was accurately able to describe the non-linear Cr(VI) breakthrough behavior in the column.