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Comparison of uranium(VI) removal by *Shewanella oneidensis* MR-1 in flow and batch reactors

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ABSTRACT

To better understand the interactions among metal contaminants, nutrients, and microorganisms in subsurface fracture-flow systems, biofilms of pure culture of *Shewanella oneidensis* MR-1 were grown in six fracture-flow reactors (FFRs) of different geometries. The spatial and temporal distribution of uranium and bacteria were examined using a tracer dye (brilliant blue FCF) and microscopy. The results showed that plugging by bacterial cells was dependent on the geometry of the reactor and that biofilms grown in FFRs had a limited U(VI)-reduction capacity. To quantify the U(VI)-reduction capacity of biofilms, batch experiments for U(VI) reduction were performed with repetitive U(VI) additions. U(VI)-reduction rates of stationary phase cultures decreased after each U(VI) addition. After the fourth U(VI) addition, stationary phase cultures treated with U(VI) with and without spent medium yielded gray and black precipitates, respectively. These gray and black U precipitates were analyzed using high-resolution transmission electron microscopy, energy-dispersive X-ray spectroscopy, and X-ray diffraction. Data for randomly selected areas of black precipitates showed that reduced U particles (3–6 nm) were crystalline, whereas gray precipitates were a mixture of crystalline and amorphous solids. Results obtained in this study, including a dramatic limitation of *S. oneidensis* MR-1 and its biofilms to reduce U(VI) and plugging of FFRs, suggest that alternative organisms should be targeted for stimulation for metal immobilization in subsurface fracture-flow systems.

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1. Introduction

Uranium (U) is a toxic metal and its contamination in the subsurface has become a global problem in aquifers, water supplies, and related ecosystems. For example, throughout the United States Department of Energy (US DOE) complex, the movement of groundwater through buried waste is generally the major mechanism for transporting waste beyond its original boundaries. Methods to prevent further migration of radioactive contaminants are needed for immediate implementation. The primary goal of *in situ* bio-immobilization of U is to prevent its transport to sensitive receptors (e.g., surface or drinking water). One potential method of treating U contamination is by using natural dissimilatory metal-reducing bacteria (DMRB) to reduce soluble U(VI) to insoluble U(IV) (as uraninite, UO_2), which can lead to *in situ* U immobilization and plume stabilization (Gorby and Lovley, 1992; Lovley et al., 1991). Iron-reducing bacteria (IRB) are one group of such DMRB that are present in many contaminated subsurface sites (Stevens et al., 1993); and their activities have implications for not only natural biogeochemical processes, but also the

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fate and transport of multivalent metals and radionuclides (Fredrickson et al., 2000; Lloyd et al., 2000). It has been shown that stimulating IRB for U remediation is an alternative method to treat U present in subsurface environments (Anderson et al., 2003).

The success of any microbial treatment is largely determined by the current understanding of fundamental microbial processes that occur at a contaminated site, and by our ability to replicate and carefully study those processes in the laboratory. In general, current understanding of the relationship between field and laboratory results, in many cases, is rather dim. A significant amount of research on U reduction and detoxification has been performed in aqueous cultures (Abdelouas et al., 1998; Francis et al., 1994; Ganesh et al., 1997; Lovley and Phillips, 1992; Lovley et al., 1991; Sani et al., 2002, 2004, 2005, 2006; Liu et al., 2007; Luo et al., 2007; Reed et al., 2007; and references herein), and while this work has greatly improved our understanding of the basic geo/microbial processes involved, it is very difficult to accurately extrapolate these results to the field. Due to the fundamental nature of these studies, they were performed in sealed batch reactors or serum bottles and hydrodynamic issues were not examined. Further, there was no influx or efflux of oxidized and reduced species characteristic of a field setting. The use of flow reactors that are more representative of *in situ* conditions indicates that, in the field, groundwater hydrodynamics and a continual influx of substrate and contaminants can yield significantly different results than those obtained with closed serum bottles (Gonzalez-Gil et al., 2005; Cheng et al., 2007).

Bio-immobilization of U in a fractured aquifer system is of particular importance since fractures are present in contaminated subsurface sites, for example at the Oak Ridge Reservation, USA. Fractures occur in many stratigraphic geologic units (Hatcher et al., 1992) and are often dominant mesoscopic structures controlling groundwater flow (Lee et al., 1992). Nutrient stimulation of microorganisms in a subsurface fracture-flow system would affect fracture-flow hydrodynamics. In a fracture-flow system, accumulated microorganisms can physically plug critical aquifer junctions. This could drastically change local substrate flux and redox conditions in such a system. In this study, we quantified U(VI) reduction by biofilms in fracture-flow reactors (FFRs) of different geometries under nutrient flow conditions. Results of U immobilization by biofilms and suspended cultures under fracture flow and batch conditions, respectively, were also compared. The results presented here in this study will benefit U-contaminated sites that have metals and radionuclide contamination in fractured aquifers including Oak Ridge Reservation. Due to fundamental nature of our research, we choose the well-studied bacterium, *Shewanella oneidensis* MR-1. This organism is currently being used as a model organism for basic as well as applied research (Marshall et al., 2008; Ross et al., 2007; Liu et al., 2007; Lall and Mitchell, 2007) and can grow aerobically and anaerobically on a vast array of electron acceptors including Fe(III) (given the abundance of Fe in most soils and sediments; Amonette, 2003; Fredrickson and Gorby, 1996; Fredrickson et al., 1998, 2000; Zachara et al., 2001) and solid-phase U(VI) (Liu et al., 2007).

2. Materials and methods

2.1. Microorganism, medium, and cultivation conditions

S. oneidensis MR-1 was maintained on a simulated groundwater medium as described previously by Viamajala et al. (2002a, b). Lactate (15 mM) and fumarate (12 mM) were the electron donor and electron acceptor, respectively, for anaerobic growth. The medium components were of analytic grade from Fisher Scientific (Pittsburgh, PA, USA). Brilliant Blue FCF was purchased from Seltzer Company (Carlsbad, CA) and uranium was purchased as $UO_2Cl_2 \cdot 3H_2O$ (0.00292 μCi/mg, Bodman Industries, Aston, PA). A fresh inoculum for each experiment was prepared by growing *S. oneidensis* MR-1 in 100 mL serum bottles. When late exponential growth ($A_{595\,nm} - 0.12$) was reached, the culture was used to inoculate the flow through reactors as described below.

2.2. Biofilm reactors

The six FFRs (each 5 cm long) with different geometries were constructed using 0.5 cm thick polycarbonate (Fig. 1). Due to the fundamental nature of the study, all reactors had one inlet and one outlet. The fracture zone (defined as the space where microorganisms, nutrients, and uranium interact) in each reactor was 0.2 cm in length. As shown in Fig. 1, after machining the fracture zones of different geometries in reactors (e.g., oval, triangular, square at the reactors' entrances), transparent glass plates (0.1 cm) were glued. The reactors and tubing were autoclaved, and sterile anaerobic medium containing lactate as electron donor and fumarate as electron acceptor with concentrations as given above was pumped using a syringe pump (KDS200, KD Scientific, New Hope, PA, USA) into the reactors at a constant flow rate of 1 mL/h. The reactors were inoculated with *S. oneidensis* MR-1. The *S. oneidensis* MR-1 biofilms (here after simply written as biofilms) were grown at room temperature (25 °C) in reactors.

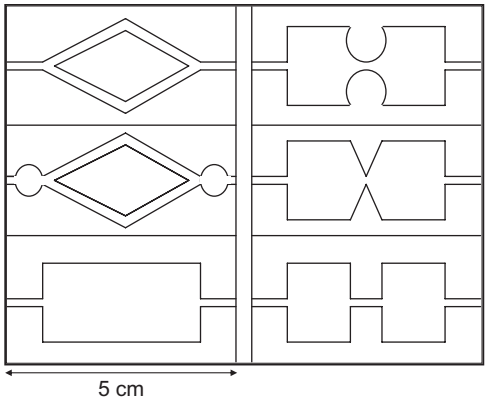

Fig. 1 – The fracture-flow reactors used to grow the biofilms of *Shewanella oneidensis* MR-1 were constructed of polycarbonate (0.5 cm thick). Each reactor was 5 cm long and fracture zone was 0.2 cm. All dimensions are in cm and figures are sketched with scale.