Pore-scale hydrodynamic and biogeochemical controls on manganese biomineralization in granular media

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Manganese (Mn) biomineralization is a ubiquitous biogeochemical process with promising applications for *in situ* bioremediation of contaminated soils and sediments. This process involves the enzymatic oxidation of aqueous Mn(II) to form reactive solid-phase Mn(III)/Mn(IV) oxides that aggregate around Mn-oxidizing bacteria. This transformation can immobilize Mn from flowing groundwater, and the resulting oxide particles can sequester co-occurring toxicant metals prevalent at sites impaired by mine drainage and industrial processes. While Mn biomineralization has been investigated in well-mixed batch systems, no studies have considered the effect of incomplete fluid mixing in heterogeneous porous media on this biogeochemical process.

To enhance the ability of Mn biominerals to react with environmental contaminants, it is crucial to understand the extent to which biomineral formation can be externally controlled by tuning flow conditions. Both size and distribution of Mn oxide aggregates must be optimized to maximize the reaction between the biominerals and contaminants in flowing groundwater. Specifically, biominerals should be distributed uniformly but not be so abundant that the pore network becomes clogged. Our research investigates the pore-scale **transport and mixing mechanisms** that control **biomineral formation extent** (location, percent coverage of pore network) and **morphology** (aggregate size, shape). We use "soil-on-a-chip" **microfluidic reactors** to simulate the geometry of a sandy soil pore network and visualize biogeochemical activity at the microbe-mineral scale with brightfield and epifluorescence microscopy.

In this study, we performed experiments to i) quantify the **spatial distribution and aggregation of the microbial inoculum** (*Pseudomonas putida* GB-1, a Mn-oxidizing bacterium), and ii) characterize the **extent and morphology of the biominerals** formed after the introduction of aqueous Mn(II) into the microfluidic reactor for conditions of variable flow rate (slow, medium, high), flow continuity (intermittent or continuous), and feeding regime (nutrient-rich or minimal salts medium). Preliminary results for experiments using a continuous fluid injection at medium flow rate (0.1 mL/hr) show that the bacteria in the inoculum distribute evenly throughout the pore network and coalesce over time into large microbial aggregates in pore throats and at grain contacts. Mn oxidation occurs at microbial aggregate boundaries in contact with the pore fluid. We are currently developing image processing methods to quantify the size distribution and percent coverage of microbial aggregates across the microfluidic pore network as well as the prevalence of Mn oxides relative to grain surfaces.