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In situ kinetics of microbial manganese removal in a porous medium

ELEANOR FADELY[1], GAITAN GEHIN[1], VERONICA MORALES[1] AND JASQUELIN PEÑA[2]

[1]University of California Davis

[2]Lawrence Berkeley National Laboratory

Presenting Author: ecfadely@ucdavis.edu

Microbial activity facilitates manganese (Mn) immobilization in metal-impacted environments, including mining and industrial sites. The enzymatic oxidation of aqueous Mn(II) to solid-phase Mn(III, IV) by bacteria and fungi, a process known as Mn biomineralization, produces reactive nanoparticulate Mn oxides that accumulate as biofilm-biomineral assemblages. While the performance of Mn biomineralization for Mn removal has been evaluated in packed bed bioreactors and passive treatment beds, studies that quantify pore-scale drivers of reaction efficiency are lacking. Here, we investigate Mn removal in a saturated porous medium under constant flow. We evaluate the kinetics of Mn biomineralization by the model bacterium *Pseudomonas putida* GB-1 (*P. putida* GB-1) in a pseudo two-dimensional microfluidic system modeled after a sandy soil. We combine optical time-lapse microscopy and image analysis with inductively coupled plasma mass spectrometry (ICP-MS) to quantify (i) the mass of Mn oxides and rate of Mn oxide precipitation in the reactor pore space and (ii) removal of Mn(II) from the reactor effluent. Our results showed that Mn oxides accumulated over time at a variable rate during a period of low nutrient input. At early times, we observed both a rapid Mn oxide precipitation rate and rapid Mn(II) removal, while at later times, the rate of Mn oxide precipitation decelerated and effluent [Mn(II)] decreased to less than 5% of the influent concentration. These non-monotonic global kinetic trends appear to be driven by the development of a spatial gradient in mineral precipitation away from the reactor inlet. Biofilms close to the Mn(II) source precipitated more Mn oxides at a faster rate than those near the outlet, implying a mechanism whereby both *P. putida* GB-1 and nascent mineral surfaces perpetuate Mn oxide precipitation and draw down Mn(II) in the reactor pore fluid. Our spatially and temporally resolved approach provides new insights into biogeochemical transformations in structurally complex environments and offers opportunities to optimize Mn biomineralization efficiency for upscaled metal remediation.