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### Title

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## Monitoring Microbe-Induced Sulfide Precipitation Under Dynamic Flow Conditions Using Multiple Geophysical Techniques

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A laboratory study was undertaken to investigate the feasibility of using minimally invasive geophysical techniques to monitor microbe-induced zinc and iron sulfide precipitation in saturated sand-packed columns during stimulated sulfate-reduction by *Desulfovibrio vulgaris*. Four inoculated columns and one non-inoculated control were operated under continuous upward flow using a nutrient- and metals-amended growth medium with the following measurements being made: multi-port fluid sampling, acoustic wave propagation, induced polarization, time domain reflectometry and saturated hydraulic conductivity. The onset and progression of sulfate reduction was monitored over seven weeks through decreasing substrate and metals concentrations, increasing biomass, and visually discernable regions of sulfide accumulation. Decreases in initial lactate and sulfate concentrations followed predicted stoichiometric relationships and soluble metals concentrations were reduced to levels below detection through sequestration as insoluble sulfide phases. The zone of active sulfide precipitation showed a shift toward the influent portion of the column as the experiment progressed, likely the result of chemotactic motility by *D. vulgaris* towards elevated substrate concentrations. Regions where sulfide precipitation and accumulation occurred resulted in significant changes in two of the three geophysical measurements. High frequency acoustic wave amplitudes were reduced by nearly one order of magnitude with only minimal changes in wave velocity. Neither the wave amplitudes nor velocities changed significantly in the downgradient portions of the column where microbial activity and sulfide precipitation were depressed. Significant changes in the imaginary conductivity were observed during the induced polarization measurements with only minimal changes in the real conductivity. No significant polarization effects were observed in the regions lacking visible precipitates. Hydraulic conductivity values for all inoculated columns decreased by nearly two orders of magnitude over the experimental period. Similar time-course measurements on the control column showed no change in acoustic wave amplitudes, complex electrical conductivity, or hydraulic conductivity. Phospholipid fatty acid analysis of the grain-affixed biomass showed a decrease of two orders of magnitude with distance from the influent end. Scanning electron microscopy revealed dense accumulations of sulfide-encrusted microbes covering grain surfaces in the regions nearest the influent end. Transmission electron micrographs revealed the biogenic sulfides to be nanoparticulate and both extra-cellular and periplasmic in nature. Evaluation of the multiple data sets suggests that microbe-induced sulfide precipitation is both directly detectable using geophysical techniques and capable of altering saturated flow conditions. The geophysical monitoring approach may prove useful at the field-scale for the time-course monitoring of contaminant metals remediation during engineered bioremediation.