Visualization of hydrated bacterial structures by complementary electron microscopy techniques

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Recently, we reported that a highly hydrated extracellular polymeric substance (EPS) produced by *Shewanella oneidensis* MR-1 binds (or nucleates) nascent nanoparticles of reduced uranium (i.e. U (IV)O_2). The UO_2-EPS was found to contain polysaccharide, membrane associated uranyl reductases, and other integral outer membrane proteins. Although the discovery of UO_2-EPS could have major implications on the fate and transport of U in subsurface waters, the massive collapse of EPS that was observed during conventional electron microscopy (EM) preparations proved problematic in understanding the structure and potential importance of EPS during the bioremediation of U (VI).

Here we employ both cryo- and traditional sample preparations for transmission and scanning EM (TEM and SEM, respectively) to elucidate the high-resolution, close-to-natural, hydrated state interactions of bacterial EPS with metals, radionuclides, or even other bacteria. We investigated the use of cryoTEM/SEM as an enhanced visualization method for the fully hydrated, fine-scale interactions of *Shewanella* cells and EPS associated with the newly formed biomineral phases. Samples were imaged in their hydrated state and directly correlated with images from identical samples subjected to dehydration and drying prior to imaging. Our methodology produced extremely accurate measurements of the region exterior to the cell membrane; the location of the electron transfer proteins essential for metal reduction. Both cryo- and traditional SEM were subsequently used to visualize a mature biofilm produced during biostimulation activities at DOE's Integrated Field Research Challenge (IFRC) site near Rifle, CO to demonstrate the applicability of these techniques with natural biofilms present during *in situ* biostimulation activities. Our observations with field-relevant biofilms may influence how we perceive the pore-scale flow of subsurface waters through soils at contaminated field sites.

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Arsenic mobilization during microbial Fe(III)-reduction of bacteriogenic iron oxides

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Poorly crystalline hydrous ferric oxides (HFO) are potent sorbents of arsenic, and reductive dissolution of such phases is thought to be a major route for release of arsenic to subsurface waters. Most work assessing this mobilization pathway has used synthetic ferric (oxyhydr)oxide minerals, however, such mineral assemblages are often naturally formed with the assistance of Fe (II)-oxidizing bacteria, producing a composite of HFO intermixed with organic material termed bacteriogenic iron oxides (BIOS). BIOS have markedly different sorption properties for a range of elements over circumneutral pH compared to synthetic HFO, and limited data to date appear to indicate they are more susceptible to microbial Fe (III)-reduction than synthetic HFO. We collected two sets of As-rich BIOS (3.7 – 3.8% As, dry wt.) from circumneutral surface waters downstream of abandoned gold mine tailings in Nova Scotia, Canada. Microcosm experiments consisting of BIOS or HFO pre-sorbed with As (1.3% As, dry wt.) were incubated in chemically defined media with *Shewanella putrefaciens* CN32, a model Fe (III)-reducing bacterium, and Fe (III)/As (V) reduction were monitored in both the solution and solid phase periodically. The extent and rate of Fe (III)-reduction was greater in the BIOS (37 – 63%; 18 – 26% day^{-1}) than HFO (25%; 4% day^{-1}) microcosms, confirming the greater vulnerability of BIOS to microbial Fe (III) reduction. As (V)- and Fe (III)-reduction occurred contemporaneously within the resolution of our sampling, and arsenic mobilization to solution was tightly correlated with iron release ($R^2_{BIOS} = 0.99$; $R^2_{HFO} = 0.74$). Thus, although BIOS are able to sequester high concentrations of arsenic under oxic conditions, they may release arsenic back into solution during Fe (III) reduction upon burial. Given that BIOS appear more susceptible to Fe (III) reduction (and hence arsenic mobilization) than HFO, these data have important implications when predicting the environmental mobility of arsenic in the shallow subsurface.