**Bacterial Nanowires and Extracellular Electron Transfer to Heavy Metals and Radionuclides by Bacterial Isolates from DOE Field Research Centers**

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Research conducted at the University of Southern California, Montana State University, and at Rensselaer Polytechnic Institute in Troy, New York, continues to embrace controlled cultivation technologies for evaluating the response of signature microorganisms to changes in environmental conditions. We are particularly interested in the activity and strategies invoked by attached microbial populations within highly-integrated "biofilm" communities.

Desulfovibrio vulgaris RCH-1 is a sulfate-reducing bacterium that was isolated from chromium-contaminated groundwater at the 100H Hanford Site. This organism formed biofilms under conditions of sulfate limitation at environmentally relevant temperature of 20°C. A variety of extracellular structures were observed by a variety of microscopic techniques. Apart from some metal deposits near the bacterial cell surface, metal precipitation occurred extracellularly predominantly on thin, elongated structures that in cross section appeared as stacks of membranous structures, or as complex geometrical enclosed shapes whose inside were always devoid of bacteria. Non-osmicated biofilms that were stained with a solution of uranyl acetate revealed an unstained thin core structure, which upon osmication becomes black, indicating that the thin structure is lipid-based. We also observed membrane vesicles nearby or docking to the extracellular structures, and these observations supported a membrane lipid-based origin. Serial section lipophilic dye FM1-43 in cryostat-sections revealed that the membrane structures persist for tens of micrometers. EDS imaging revealed presence of Fe, O and P, but not sulfide, and these results suggested the metal deposits are not solely the result of inorganic chemistry interactions of metals ions with hydrogen sulfide. The biofilm and metal deposition was visualized in 3D with SBF/SEM, and showed a heterogeneous distribution of metal precipitates away from cells. Assessing metal interactions at in situ temperatures in biofilms rather than optimal growth temperatures in bulk-phase and under electron donor- and acceptor-limitation with field isolates provides relevant insight into metal reduction for respective field sites.

Methods for visualizing the growth of extracellular structures in real time and under physiologically relevant and metabolically active conditions were developed by members of our extended team at USC and initially applied those methods to the facultative metal reducing bacterium Shewanella oneidensis. Recent results provide new insights into the strategies and components invoked for efficient extracellular charge transfer within electron acceptor-limited biofilms, many of which are consistent with observations made with D. vulgaris RCH-1. These approaches are currently being applied to the field isolate Geobacter. daltonii FRC32T using...