NAME: Matthew Marshall
ORGANIZATION: Pacific Northwest National Laboratory
PROGRAM AFFILIATION: SBR
ABSTRACT TITLE: Multi-system Analysis of Microbial Biofilms

ABSTRACT: Direct examination of natural and engineered environments has revealed that the majority of microorganisms in these systems live in structured communities termed biofilms. In addition to microbial cells, biofilms are comprised of a poorly characterized organic matrix commonly referred to as extracellular polymeric substance (EPS) that may play roles in facilitating microbial interactions and biogeochemical reactions including extracellular electron transfer. Using high-resolution electron microscopy (EM) imaging, we have shown copious amounts of highly hydrated bacterial EPS to be produced during microbial metal reduction. The juxtaposition of extracellular electron transfer proteins and nanoparticulate reduced metal suggested that EPS played a key role in metal capture and precipitation. Here we present a multi-faceted approach to determine the composition of biofilm EPS using a combination of synchrotron-based X-ray and infrared (IR) microimaging techniques combined with high-resolution EM imaging and spectroscopies at the Environmental Molecular Sciences Laboratory (EMSL). The result is a high spatial resolution, complex chemical image of a biofilm community. To understand the pore-scale and community-scale structure of biofilms in complex, three-dimensional environments, X-ray microtomography was employed at sector 2BM of the Advanced Photon Source. We have imaged hydrated biofilms grown on multiple substrates at energies ranging from 13-18 keV and spatial scales of 0.7 and 1.4 µm/pixel to reveal the complex microstructure within a biofilm. In some tomography studies, contrasting agents were also added to enhance biofilm visibility. To investigate the micrometer- to nanometer-scale chemical signatures of biofilms, a cryo-sample preparation technique was employed to produce ultrathin biofilm sections for scanning transmission X-ray microscopy (STXM) and synchrotron IR microimaging. In STXM studies, we mapped the unique carbon signatures of biological molecules and found that several differences existed between the cell surface and the EPS matrix. Concurrent with these studies, we used synchrotron-based IR to produce high-megapixel (6.28 Mp) datasets with IR spectral data showing the localizations of key biofilm components (*i.e.*, proteins, sugars, flavins, nucleic acids, lipids) to further construct our chemical image of biofilms. The integration of the pore-scale and community-scale structural studies of hydrated biofilms with the micrometer-to nanometer-scale chemical signatures of biofilms will provide detailed, high-resolution chemical images of biofilms that will help us to better understand how a biofilm community influences local biogeochemical reactions in subsurface environments.

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