Multi-system Analysis of Microbial Biofilms


The majority of microorganisms in natural and engineered environments live in structured communities such as biofilms. Biofilms are comprised of microbial cells and a poorly characterized organic matrix commonly referred to as extracellular polymeric substance (EPS) that may support 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 are produced during microbial metal reduction. The co-location 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 synchrotron-based X-ray and infrared (IR) microimaging techniques combined with mass spectrometry imaging and high-resolution EM at the Environmental Molecular Sciences Laboratory. The result is a spatially resolved, complex chemical imaging of a biofilm community. X-ray microtomography produced images of hydrated biofilms to reveal the complex microstructure within a biofilm. Contrasting agents enhanced biofilm visibility at the high energies used. To investigate the micrometer- to nanometer-scale chemical signatures of biofilms, a cryo-sample preparation technique produced 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 distinct differences between the cell surface and the EPS matrix. Concurrent with these studies, we used IR microimaging to produce high-megapixel datasets with IR spectral data showing the locations of key biofilm components (i.e., proteins, sugars, flavins, nucleic acids, lipids). The spatially resolved IR results were corroborated with bulk IR chemical data using chemically fractionated regions of biofilms.

In situ imaging was conducted using a novel microfluidic reactor for biofilm growth, confocal laser scanning microscopy analysis, and hydrated-state, time-of-flight secondary ion mass spectrometry imaging. We generated high-resolution two-dimensional chemical images of biofilms at their surface attachment interface and detected characteristic fatty acid fragments from microfluidic reactor-grown biofilms. These fatty acid fragments are being investigated as key factors for how biofilms attach to a surface in a microfluidic reactor.

The integration of the multiscale structural studies and in situ imaging of hydrated biofilms chemistry provides 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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