

## Multi-system Analysis of Microbial Biofilms

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Examination of natural and engineered environments reveals that the majority of microorganisms live in structured biofilms communities. Biofilms are comprised of microbial cells and an extracellular polymeric substance (EPS) matrix that supports microbial interactions and biogeochemical reactions including extracellular electron transfer (EET). Copious amounts of EPS and EET proteins are co-localized during microbial metal reduction. Their association with nanoparticulate reduced metal suggested that EPS may play key roles in local biogeochemical reactions in subsurface environments.

We employed a multi-faceted approach to determine the composition of biofilm EPS using X-ray and infrared (IR) microimaging techniques combined with electron microscopy and mass spectrometry imaging. The result is a spatially resolved chemical image of a biofilm community. X-ray microtomography produced images of hydrated biofilms to reveal the complex internal microstructure. To investigate the micrometer- to nanometer-scale chemical signatures of biofilms, a cryo-sample preparation technique produced ultrathin biofilm sections for x-ray and IR microimaging. We mapped the unique carbon signatures of biological molecules and found distinct heterogeneities between the cell surface and the EPS matrix. Concurrent with these studies, we produced high-megapixel datasets with IR spectral data showing the locations of key biofilm components (*i.e.*, proteins, sugars, lipids). The spatially resolved IR results were corroborated with bulk IR chemical data.

*In situ* imaging was conducted using a vacuum compatible microfluidic reactor for biofilm growth, confocal laser scanning microscopy analysis, and liquid time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging. Depth profile sputtering of materials resulted in a layer-by-layer, spatially-resolved live biofilm image. We generated two-dimensional (2D) images of biofilms at their surface attachment interface and detected characteristic fatty acid (FA) fragments. 2D images were reconstructed to visualize three-dimensional (3D) images of hydrated biofilm elucidating a depth-resolved, spatial heterogeneity of key FA components in biofilms and near the biofilm-attachment interface. In particular, C12 and to a lesser degree, C16 FAs were localized throughout the biofilm depth profile while C15 FAs localized deeper into the biofilm. The observed FAs are being further investigated as key factors for how biofilms attach to mineral surfaces using the microfluidic platform.

The integration of the multiscale structural studies and *in situ* imaging of biofilm 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.

This work was funded by a U.S. DOE Office of Science Early Career Research Program Award (DOE National Laboratory Announcement 10-395).