Anabolic activity in Geobacter biofilms as a function of distance to insoluble electron acceptor

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Project Goals: To investigate the spatial patterns of cellular activity during extracellular electron transfer by a pure culture grown on an electrode and applying these results to further our understanding of electron transfer during syntrophic anaerobic oxidation of methane.

Microorganisms in the environment inhabit complex ecosystems. These ecosystems are often characterized by a high degree of spatial heterogeneity in terms of chemistry, nutrient concentrations and species distribution. This heterogeneity leads to a huge diversity of ecological niches, defined not only by abiotic factors, but also a spectrum of interspecies relationships from predation and parasitism to mutualism and symbiosis. For a complete understanding of any ecological system, we need to be able to understand the way in which ecological pr ocesses vary over different spatial scales.

A central question in microbial ecology is what is the importance of spatial structure of microbial communities, particularly when large aggregations of microorganisms create and respond to complex gradients of nutrients and waste products within the community. Questions along these lines are common in almost all subfields of microbiology including medical microbiology, wastewater treatment, and environmental microbiology. Unfortunately, it is often quite difficult to assay microbial activity at single-cell resolution within environmental communities.

The application of stable isotope probing coupled to nanometer scale secondary ion mass spectrometry (nanoSIMS) provides a tool with which to assess the assimilatory activity of a wide array of microorganisms in the environment at subcellular spatial resolution. In order to better understand the connection between stable isotope assimilation and microbial activity and cell-cell interactions, we are applying this technique to controlled *Geobacter* biofilms grown in a microbial fuel cell.

Geobacter forms thick (10's of cell layers thick) biofilms on anodes of microbial fuel cells. The electrons produced by their catabolic oxidation of acetate are transported through the conductive biofilm to the surface of the electrode. This system serves as a model for microbe-mineral interactions, as well as a growing list of natural microbial syntrophies in nature (e.g. anaerobic oxidation of methane) that are likely dependent on such direct electron transfer processes. The benefit of analyzing *Geobacter* grown on an electrode is that the metabolism is well understood, it is genetically tractable, and models aimed at capturing the important aspects of their metabolism can be simplified to 1 dimension. Although much research has been conducted on these model microorganisms, it is still not known how their cellular activity varies with distance to the electrode surface.

Our stable isotope probing experiments and nanoSIMS analysis have demonstrated the cells in the Geobacter biofilm have a negative correlation between their cellular activity and distance to the electrode, implying that a penalty exits with increasing distance to the terminal electron accepter even though the biofilms have been show to be electrically conductive. This data, coupled with future experiments varying parameters such as electrode potential, nutrient concentrations, and genetic EET mutants will allow us to make better models for this process in *Geobacter* biofilms, and hopefully begin to extend these models to more complex associations in the environment. Of particular interest are the syntrophic communities of anaerobic methane oxidizing archaea and sulfate reducing bacteria which have been suggested to be performing extracellular electron transport.