Periphyton biofilms generate methylmercury in a contaminated creek system

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Previous SFA research on mercury methylation in East Fork Poplar Creek (EFPC) in Tennessee implies key controls on net methylation occur within the stream or on the stream bed. The large diel variability in dissolved methyl mercury (MeHg) concentration appears to be correlated with the daily photocycle and the sparse wetlands (< 3% watershed area) are poorly connected to the main channel of the creek. Mercury methylation is mediated by bacteria and archaea under anaerobic conditions (Fe(III) or SO_4^{2-} reducing, fermentative, methanogenic). Methylmercury is generated in anaerobic zones within fine-grained channel margin deposits along the creek but the limited extent of these deposits, coupled with the low rates of Hg methylation, suggest these locations account for only a portion of the MeHg in the water.

This phase of our research is designed to elucidate the role of periphyton biofilms in MeHg production in the creek. Periphyton biofilms are complex assemblages of bacteria, fungi, algae, diatoms, invertebrates, detritus, mineral particles and extracellular polymers that grow on submerged surfaces in most aquatic systems. Periphyton is ubiquitous throughout the creek and redox gradients created by microbial metabolism within them support Hg-methylating microbial activity even though the bulk water is well oxygenated (mean DO = 9 mg l^{-1}). Periphyton growth surfaces deployed at upstream (closer to the historic point source of contamination) and downstream locations (~17 km apart) are collected following ~12 weeks colonization in the creek. These samples are subsequently used in laboratory assays of inorganic Hg methylation and MeHg demethylation. Enriched stable isotopes of the Hg species are employed to distinguish new activity from ambient background levels during incubations. In collaboration with Task 3 of the SFA subsamples for microbial community analysis and *hgcAB* gene abundance and phylogeny are also collected. Redox gradients within the periphyton samples are quantified using voltammetric microelectrodes.

Results to date demonstrate that both mercury methylation and MeHg demethylation occur within the periphyton biofilms. Microelectrode profiling shows steep redox gradients within these thin biofilms transitioning from microaerophilic at the biofilm-water contact to sulfate reducing conditions over a distance of 1.5 mm. Although both methylation and demethylation occur in the periphyton, the rate of methylation is greater than the rate of MeHg demethylation indicating periphyton is a net source of MeHg to the creek. Clear differences in methylation and demethylation activity exist between the upstream and downstream sites and across seasons. Samples from the downstream site support higher rates of net MeHg production, consistent with our long-term data record demonstrating increasing MeHg concentration with downstream distance. The differences in net methylation are driven by decreasing demethylation rate constants along the creek as methylation rate constants are comparable between sites. Similarly, all the three major clades of microbial methylators are present at the downstream site

(methanogenic archaea, sulfate reducing firmicutes, and sulfate reducing delta-proteobacteria). These data suggest the downstream periphyton is more complex and contains a greater diversity of redox conditions in comparison to the upstream site, which is dominated by sulfate reducing firmicutes.

Early experimental evidence indicates important environmental conditions that exert control on net MeHg production. Pseudo-first-order rate constants for methylation decrease ~2× with a 10°C decrease in temperature whereas the rate constants for demethylation did not change with temperature. Samples grown in the light but incubated in the dark and samples grown in the dark and incubated in the dark have substantially lower methylation rates suggesting a direct link between active photosynthesis in the periphyton supporting Hg methylation activity by microbes within the biofilm. Ongoing efforts seek to further understand the mechanisms underlying these connections.