## Transformation of Per- and Poly-Fluoroalkyl Substances (PFAS) by Environmentally Relevant Co-Metabolic Organisms

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Background/Objectives. Many per- and poly-fluoroalkyl substances (PFAS), common components in aqueous film forming foam (AFFF), have been released into soils and aquifers. Remediation, particularly biological, has proved difficult. To date, there has not been an organism identified able to reliably, metabolically mineralize PFAS; however, degradation of both the functional head group and the fluorinated tail has been observed in soil microcosms suggesting that these processes are proceeding co-metabolically. Co-metabolism occurs when microbial enzymes, expressed for normal metabolic functions, fortuitously degrade other compounds of interest (e.g., PFAS) without gaining carbon or energy from the process. Although mineralization does not occur, parent and intermediate PFAS (precursors) can produce terminal perfluoroalkyl acids (PFAAs). The purpose of this work is to explore the potential of bacteria observed to carry out co-metabolic reactions to transform PFAA precursors. Co-metabolic transformation of larger precursors with complex functional groups can be used to simplify PFAS extraction by converting precursors to more easily extracted and treated anionic PFAAs. An improved understanding of organisms capable of carrying out precursor transformation will improve PFAS fate and transport modeling and may lead to new remediation strategies with a biotransformation component.

**Approach/Activities.** A study was undertaken in which PFAS-impacted groundwater was inoculated with pure or mixed cultures grown on propane, octane, pentane, isobutane, toluene, or methane. These cultures are known to express a variety of oxygenase enzymes capable of mediating co-metabolic reactions and be common in soils and aquifers. The inoculated bottles, and appropriate controls, were grown for 4 and 8 weeks, and fed periodically with oxygen and primary substrates (e.g., methane, toluene) based on headspace concentration. Samples were then analyzed for PFAS using high-performance liquid chromatography tandem mass spectrometry for targeted analysis and high-resolution mass spectrometry for suspect screening analysis. Pure compound studies were then conducted similarly, except with select precursors and strains, to confirm results. Additionally, full microcosms, including soils and groundwater, are currently being prepared to test the feasibility of stimulating native co-metabolic organisms from impacted site material by supplying the same primary substrates used in groundwater screening.

**Results/Lessons Learned.** From the site screening study, 6:2 and 8:2 fluorotelomer sulfonate (N:2 FTS) decreased over 90% compared to controls with all strains except for those grown on toluene. Pure compound studies are underway with 6:2 FTS to confirm this observation. Other compounds with greater than 60% changes from controls included perfluorooctane sulfonamide (FOSA), 4:2 FTS, and perfluorononanoic acid (PFNA). Suspect screening is ongoing to identify transformation products from these precursor compounds. These data indicate that cometabolic organisms may be critical in the transformation of specific PFAA precursors in the environment, including 6:2 FTS which is commonly found at high concentrations at AFFF sites. Data from ongoing studies with pure compounds and natural microbial populations, designed to confirm and extend results with site groundwater, also will be presented.