# A Novel Biodefluorination Pathway of Fluorotelomer Carboxylic Acids (FTCAs) by Municipal Activated Sludge

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**Background/Objectives.** Fluorotelomer carboxylic acids (FTCAs), including 6:2 FTCA and 5:3 FTCA, are important precursors for perfluoroalkyl carboxylic acids (PFCAs). They are also reported as critical biotransformation intermediates of many other PFCAs precursors (e.g., fluorotelomer alcohols [FTOHs], fluorotelomer sulfonates [FTSs], and fluoroalkyl phosphates [PAPs]) leading to PFCA accumulation. FTCAs have been widely detected in landfill leachates, eliciting a higher bioaccumulating potential and toxicity than their corresponding PFCAs. The discharge of landfill leachates into municipal wastewater treatment plants (WWTPs) is one major path for FTCAs to enter aquatic environments. In this study, we conducted batch experiments to investigate the biotransformation and biodefluorination potentials and pathways of 6:2 FTCA and 5:3 FTCA by activated sludge from four municipal WWTPs in the New York Metropolitan Area. Microbial species that may contribute to the FTCA biotransformation were characterized by the combination of the next-generation sequencing and bioinformatics.

**Approach/Activities.** Sludge samples were centrifuged at 12,000 rpm and then washed three times with phosphate buffer saline (PBS). For each sludge, 1 g was inoculated into serum bottles (160 mL as the total volume) containing 50 mL of synthetic wastewater. 5:3 FTCA or 6:2 FTCA was spiked at an initial concentration of 80 μM. Microcosms were incubated at room temperature (24±3°C) while being shaken at 130 rpm. Killed controls were prepared with autoclaved sludge to distinguish the abiotic loss of FTCAs, while analytical controls were prepared with sterile Mili-Q water and FTCAs. Sampling was operated on 0, 1, 2, 3, 5, and 7 days. Quantification analysis of FTCAs and their legacy metabolites was achieved by nano electrospray ionization high-resolution mass spectrometry (Nano-ESI-HRMS), operated by a high-resolution Q Exactive hybrid quadrupole−Orbitrap mass spectrometer (Thermo Fisher Scientific, San Jose, CA). Fluoride concentration was determined using the Orion Star Meter equipped with fluoride electrodes (Thermo-Fisher Scientific). Suspect PFAS mass features were screened when their relative abundances exhibited an increasing or increasing-then-decreasing trend. Collision-induced dissociation was employed to identify fragmentation patterns and moieties and make predictions on the formula and structure of the suspect mass features, which were further validated using commercially available structural analogs and by matching mass spectra with those in our local database based on a mass error threshold of 5 ppm. Metabolite identification was synergized to build a comprehensive profile of FTCA biotransformation products by activated sludge, elucidating the pathways by which FTCAs may be degraded.

**Results/Lessons Learned.** After 7-day incubation, 40.0~62.4 μM of 6:2 FTCA was removed with significant defluorination (12 ~37 μM F-), and 25% to 37% of 6:2 FTCA removal was contributed by abiotic process (i.e., adsorption). An average of 0.5~1.7 fluorine was released per biotic removed 6:2 FTCA molecule. In contrast, 16.7~50.2 μM of 5:3 FTCA was removed with negligible abiotic removal (<1%) or fluoride release (< 4 μM). Legacy metabolites based on one-carbon removal pathway were identified and quantified. However, the molar discrepancy between removed FTCA and the sum of detected metabolites for 6:2 FTCA (20%-51%) and 5:3 FTCA (45%-87%) indicated the existence of metabolites that have not been characterized. These results also revealed significant contribution of non-fluoride-releasing pathways to 5:3 FTCA biotransformation by activated sludge. Non-target analysis revealed several novel PFAS features are generated dominantly during the biotransformation of 5:3

FTCA, but minorly for 6:2 FTCA. 16S rRNA analysis implied that the genera of *Prosthecobacter*, *Sediminibacterium*, and *Dechloromonas* might contribute to the aerobic FTCA biotransformation. We are in the process of identifying the unknown biotransformation metabolites for both FTCAs using non-target and suspect screening workflow. Our research is of fundamental scientific value to advance our understanding of the diversity of PFAS biotransformation and biodefluorination pathways, as well as their impacts to the environment and human health.