

## Stable Isotope Probing (SIP) of Rhizosphere Bacteria in 6:2 Fluorotelomer Sulfonic Acid (6:2 FTSA)-Contaminated Soil

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**Background/Objectives.** 6:2 Fluorotelomer sulfonic acid (6:2 FTSA,  $F(CF_2)_6CH_2CH_2SO_3H$ ) is one of the per- and poly-fluoroalkyl substances commonly detected in the environment. 6:2 FTSA has been widely used in many industries including metal plating, plastics, and fluoropolymers manufacturing, and present in aqueous film-forming foams (AFFF). Exposure to 6:2 FTSA has been linked to liver and kidney damage in rat studies. Despite factors affecting the biotransformation and phytoremediation of 6:2 FTSA in soil having been recently reported, little is known about the role of rhizosphere bacteria and the impacts of different carbon sources or non-PFAS surfactant in AFFF formula on fate of 6:2 FTSA in the plant-soil environment.

**Approach/Activities.** In this study, stable isotope probing (SIP) with  $^{13}CO_2$  was used to identify active 6:2 FTSA degraders in the rhizosphere of *Arabidopsis thaliana*. Pot experiments containing 6:2 FTSA and/or different carbon sources, and AFFF surfactants were setup. A parallel set of pot experiments were set up similarly with additional amendment of strain RHA1 as bioaugmenting agents.  $^{13}CO_2$  were provided to plants to produce  $^{13}C$ -labeled root exudates for labeling the active rhizosphere bacteria. The  $^{13}C$ -labeled DNA were then fractionated and analyzed using next generation sequencing. The biotransformation of 6:2 FTSA were determined using LC/MS/MS analysis.

**Results/Lessons Learned.** Our results showed that bioaugmentation of *Rhodococcus jostii* RHA1 and/or carbon source and AFFF-surfactant amendment enhanced biodegradation of 6:2 FTSA. SIP results suggested that a known 6:2 FTOH/6:2 FTSA degrader, *Pseudomonas*, was the most predominant active rhizosphere bacteria. However, the spiked RHA1 was less competitive in rhizosphere soil compared to bulk soil. Carbon sources or non-PFAS surfactant in AFFF formula is an important driver to cause changes of microbial community composition. This is the first study to unveil the interactions between rhizosphere bacteria and 6:2 FTSA. Amendment of carbon sources, non-PFAS surfactant in AFFF formula, and the bioaugmenting agent RHA1 could serve as possible strategies for remediation of 6:2 FTSA-impacted soil.