THWRC Final Report Executive Summary

THWRC Project Number: 513TAM0030H
Project Title*:* Biodegradation of Fluorotelomer-Based Surfactants Under Differernt Redox Conditions
Investigators: Kung-Hui Chu, Ph.D. P.E
Institution: Texas A&M University
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Summary

Detection of perfluorinated compounds, particularly perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), in the global environment and biota is of a great concern. Since 8:2 fluorotelomer alcohol (8:2 FTOH) is a known precursor to PFOA, the manufacturers are now moving toward 6:2 FTOH as a key raw material to manufacture FTOH-based products like surfactants and polymers. Yet, biodegradation of FTOHs and FTOH-based products is a potential source of other perfluorinated carboxylic acids (PFCAs) and the fate of FTOHs and FTOH-based products is not fully understood. In this research project, we investigated the biodegradation potential of fluorotelomer-based surfactants and factors that control the extent of the biodefluorination of 6:2 FTOH and FTOH-based surfactants, focusing on polyfluoroalkyl phosphates (PAPs).

Results of our study showed that three known FTOH-degrading bacteria (*Pseudomonas butanovora*, *Pseudomonas oleovorans*, and *Pseudomonas fluorosecens*) These strains were able to transform 6:2 PAP at different extents, from low to high, *P. butanovora* (2 mole%) < *P. oleovorans* (3 mole%) < *P. fluorescens* (3.3-4.1 mole%). Addition of co-substrate enhanced slightly the extent of 6:2 PAP mixture degradation and the distribution of different metabolites. Activated sludge degraded three times more of 6:2 PAP mixture than those by the pure strains. Microbial community structure become more diverse during the first 15 days of 6:2 PAP degradation and then decreased dramatically on Day 30. The total microbial population in activated sludge also decreased two orders of magnitude at the end of experiment (on Day 30). The results suggested that the first step of hydrolysis of 6:2 PAP mixture to 6:2 FTOH is the rate limiting factor for overall 6:2 FTOH defluorination. Anaerobic degradation of 6:2 PAP was not observed in this study.

I. Description and Objective of Research Project

Detection of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in the global environment and biota [4, 5, 12, 13, 18, 38, 46] is of great concern, due to their persistence and potentially adverse health effects [24, 33]. The risk associated with these two perfluoroalkyl acids has led to the launch of USEPA "2010/2015 PFOA Stewardship Program", in which eight major companies have committed to reduce the global contribution of PFOA and their precursors by 2015 [11]. Because 8:2 fluorotelomer alcohol (8:2 FTOH) is a known precursor to PFOA, the manufacturers are now moving toward 6:2 FTOH as a key raw material to manufacture FTOH-based products like surfactants and polymers [30]. Yet, biodegradation of FTOHs and FTOH-based products is a potential source of other perfluorinated carboxylic acids (PFCAs). *A better understanding about the fate of FTOHs, particularly through biological processes, is warranted. Thus, this proposal addresses some of the factors affecting the biodegradation of 6:2 FTOH and FTOH-based products in the environment.*

The **goal** of this proposal is to better understand the biodegradation potential of fluorotelomer-based surfactants under different environmental conditions and to elucidate factors that control the extent of the biodefluorination of FTOHs and FTOH-based surfactants. Research efforts will be also placed on isolation and identification of bacteria that can degrade FTOH transformation products. The technical objectives of this proposal were to

(i) Determine biodegradation potential of FTOH-based surfactants, particularly polyfluoroalkyl phosphates (PAPs), to FTOHs and beyond.

(ii) Elucidate factors and conditions that enhance biodefluorination of PAPs.

II. Results/Accomplishments

A. **Biodegradation of PAPs by known FTOH-degrading cultures.** In this study, experiments to examine biotransformation of 6:2 PAP mixture by each strain were performed in a series of 120-mL serum bottles containing resting cell suspension (10 mL) of one of three known FTOH-degrading cultures and 10 µL 6:2 PAP mixture stock solution with or without a co-substrate (lactate or citrate). Three known FTOH-degrading cultures used in this study were *Pseudomonas butanovora*, *Pseudomonas oleovorans*, and *Pseudomonas fluorosecens*. These FTOH degraders are known to degrade FTOH via two degradation pathways, pathway I (PFCA acid pathway), and pathway II (X:3 acid pathway). The 6:2

PAP mixture contains 11.5% equivalent of 6:2 FTOH if completely hydrolyzed based on 19F NMR analysis. Liquid samples were collected over a period of 30 days and analyzed for transformation metabolites using LC/MS/MS.

The production of 6:2 FTOH and its metabolites were biodegradation of 6:2 PAP mixture was observed by all three FTOH-degrading strains, *P. butanovora*, *P. oleovorans*, and *P. fluorescens* (Figure 1). These strains were able to transform 6:2 PAP at different extents, from low to high, *P. butanovora* (2 mole%) < *P. oleovorans* (3 mole%) < *P. fluorescens* (3.3-4.1 mole%). Eight quantifiable different transformation metabolites, 6:2 FTOH, 6:2 FTCA, 6:2 FTUCA, 5:2 ketone, 5:2 sFTOH, PFHxA, 5:3 acid [$CF_3(CF_2)_4CH_2CH_2COOH$] , 5:3 Uacid[$CF_3(CF_2)_4CH{=}CHCOOH$], were formed over 30 days of incubation. As shown in Figure 1, 5:2 ketone and 5:2 sFTOH are dominant metabolites for all tested conditions.

According to the distribution of metabolites detected on Day 30, 6:2 PAP mixture was degraded by the FTOH-degrading bacteria via two different pathways, pathway I (PFCA acid pathway), and pathway II (X:3 acid pathway). The pathway I was the preferred pathway that produce perfluorinated carboxylic acids. The results also suggested that only a small fraction of 6:2 FTOH was released from 6:2 PAP mixture.

B. **Biodegradation of PAPs by Activated sludge.** Experiments were also conducted to determine if 6:2 PAP mixture can be degraded by activated sludge. No anaerobic degradation of 6:2 PAP mixture by activated sludge was observed. Compared to three FTOH-degrading strains, activted sludge showed the highest total transformation of 6:2 PAP mixture, with a yield of 10-12 mole % of the intially applied 6:2 PAP mixture. Different from the degradation of 6:2 PAP mixture by the three FTOH-degrading cultures, 5:2 sFTOH was the major transformation metabolite, accounting for approximately 65% of total transfromation metabolites detected on Day 30.

The changes of microbial poluation and community struction were also monitored during the degradation of 6:2 PAP mixture by activated sludge (**Figure 2**). Microbial population based on total number of 16S rRNA gene copies decreased from $3x10^7$ on Day 1 to $6.5x10^5$ on Day 30, a trend similar to slow down of biotrasnformation of 6:2 PAP mixture on Day 30. Similarly, the diversity of microbial community structure increased from Day 0 to Day 7 and

Day 15 (from 9 ribotypes on Day 0 to 13-14 ribotypes on Day 7 to Day15) and then declined dramatically on Day 30 (6 ribotypes).

C. **Factors affecting 6:2 PAP mixture degradation.** Experiments were also conducted to enhance 6:2 PAP mixture degradation by the three known FTOH-degrading cultures. Addition of co-substrate exerted slight effects on 6:2 PAP mixture by *P. butanovora* and *P. oleovorans.* For *P. butanovora*, the co-substrate addition slightly shifted the degradation toward pathway II, X:3 acid pathway, but did not enhance the overall % FTOH degradation. For *P. oleovorans*, the addition of co-substrate did both, shifting the degradation toward pathway II and increasing the overall % FTOH degradation.

III. Publications/Presentations

The results of this study have been presented in SETAC North America 36th Annual Meeting in Salt Lake City, Utah, November 2, 2015, and the 28th Symposium in Groundwater Resource Association of California Series on Groundwater Contaminants. "Emerging Contaminants" Symposium, Concord, California, February 4-5, 2014.

IV. Future Activities

We are in the process of preparing a manuscript for journal publication in the near future.

V. Supplemental Keywords

Flurotelemer Alcohol, FTOH, polyfluoroalkyl phosphates (PAPs), Biodegradation.

VI. Relevant Web Sites

None applicable.

Figure 1. Biodegradation of 6:2 PAP mixture by three known FTOH-degrading bacteria: *Pseudomonas butanovora (a), Pseudomonas oleovorans (b), and Pseudomonas fluorencens (c).*

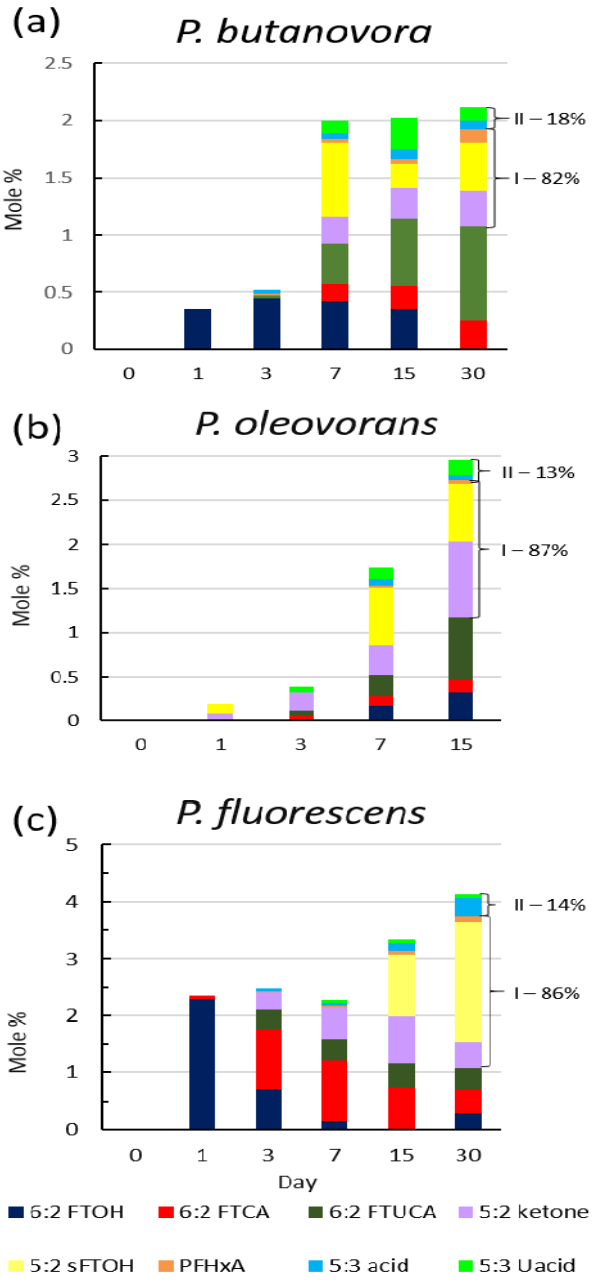

Figure 2. Changes of microbial community structure of activated sludge during 6:2 PAP mixture biodegradation.