

FINAL REPORT

A New Concept of “Release-Capture-Destruction” to Enable Remediation of PFAS in Source Zone Soils

Kung-Hui (Bella) Chu
Texas A&M University

Jennifer Field
Oregon State University

Timothy Strathmann
Colorado School of Mines

June 2023

This report was prepared under contract to the Department of Defense Strategic Environmental Research and Development Program (SERDP). The publication of this report does not indicate endorsement by the Department of Defense, nor should the contents be construed as reflecting the official policy or position of the Department of Defense. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the Department of Defense.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.				
1. REPORT DATE (DD-MM-YYYY) 09-06-2023		2. REPORT TYPE SERDP Final Report		3. DATES COVERED (From - To) 30/09/2022-30/03/2023
4. TITLE AND SUBTITLE A New Concept of "Release-Capture-Destruction" to Enable Remediation of PFAS in Source Zone Soils			5a. CONTRACT NUMBER W912HQ21P0099	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Kung-Hui (Bella) Chu, Texas A&M University Jennifer Field, Oregon State University Timothy Strathmann, Colorado School of Mines			5d. PROJECT NUMBER ER22-3124	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Texas A&M University Civil & Environ Engineering College Station, TX 77843			8. PERFORMING ORGANIZATION REPORT ER22-3124	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of the Deputy Assistant Secretary of Defense (Energy Resilience & Optimization) 3500 Defense Pentagon, RM 5C646 Washington, DC 20301-3500			10. SPONSOR/MONITOR'S ACRONYM(S) SERDP	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) ER22-3124	
12. DISTRIBUTION / AVAILABILITY STATEMENT Distribution A: Approved for public release; distribution is limited				
13. SUPPLEMENTARY NOTES				
14. ABSTRACT The overarching goal of this project was to develop an integrated treatment system for the effective treatment of poly- and perfluoroalkyl substances (PFAS)-laden soils in source zones. Specifically, this project aimed to test the treatment strategy that was based on the "Release-Capture-Destruction" concept. The technical objectives were to determine factors affecting biodegradation of soil-bound precursors, to accelerate biodegradation of precursors in soil via bioaugmentation, to determine the efficiency of PFAS removal by magnetic activated carbon (MAC) in soil, and to assess whether hydrothermal alkaline treatment (HALT) technology can destroy PFAS in the spent MAC.				
15. SUBJECT TERMS PFAS, Per- and polyfluoroalkyl substances, Precursor, Bioaugmentation, Biodegradation, Magnetic Activated Carbon (MAC), hydrothermal alkaline treatment (HALT)				
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
A. REPORT UNCLASS	b. ABSTRACT UNCLASS	c. THIS PAGE UNCLASS	UNCLASS	65
			19a. NAME OF RESPONSIBLE PERSON Kung-Hui (Bella) Chu	
			19b. TELEPHONE NUMBER (Include area code) 979-845-1403	

TABLE OF CONTENTS

LIST OF TABLES.....	iii
LIST OF FIGURES	iv
LIST OF ACRONYMS	vii
Abstract	1
Executive Summary	2
1.Objective	8
2. Background	9
2.1 Remediation of PFAS in AFFF-impacted soils	9
2.2 Magnetic activated carbon (MAC)	9
2.3 Hydrothermal alkaline treatment (HALT-PFAS)	10
3. Materials and Methods	11
3.1 Methods for Task 1	11
Method development for Capstone analysis and validation of a close mass balance of PFAS.	11
Biodegradation of Capstone by <i>Rhodococcus jostii</i> RHA1 under different nutrient conditions.	11
Effect of hydrocarbon surfactants on defluorination of 6:2 FtS by 1-butanolgrown <i>Rhodococcus jostii</i> RHA1.	12
3.2 Methods for Task 2	12
Enrichment of soil cultures with different carbon sources and establishment of stable enrichment cultures in sequencing batch reactors (SBRs).	12
Biodegradation of precursors by C2 enrichment cultures under different nutrient conditions.	12
3.3 Methods for Task 3	14
Synthesis of magnetic activated carbon (MAC).	14
Applications of MAC for remediating PFAS-contaminated soil	14
3.4 Methods for Task 4.....	15
Mass balance analysis of PFASs and fluorine during application of HALT-PFAS to MAC.	15
Evaluation of HALT-PFAS for MAC recovered from PFAS-contaminated soil	16
4. Results and Discussion	17
Task 1: Determine factors affecting the biodegradation of soil-bound precursors	17
(i) Capstone Characterization	17
(ii) Microcosm Setup for mass balance of PFAS.	20
(ii) Biodegradation potential of Capstone by RHA1 strain under different nutrient conditions.	24
(iii) Negative and positive effects of hydrocarbon surfactants on 6:2 FtS degradation by RHA1 strain.....	26
Task 2: Accelerate biodegradation of precursors via bioaugmentation	27
(i) Establishment and characterization of stable enrichment cultures in SBRs.	27
(ii) Microbial community of the C2 soil enrichment cultures.	27
(iii) FTOH biodegradation by the cultures enriched with different carbon sources...29	
(iv) Biodegradation of 6:2 FTOH by dual enrichments	32
(v) 6:2 FtS biodegradation by the mixed cultures enriched with different carbon sources under S-free conditions.	34

(vi) 6:2 FtSaB biodegradation by the mixed cultures enriched with different carbon sources.	36
(vii) Potential suspects found in 6:2 FtS and 6:2 FtSaB microcosm (containing C2 enrichment cultures) time course studies	36
Task 3: Determine the efficiency of PFAS removal by MAC.....	41
(i) Soil properties.	41
(ii) MAC removed PFAS from PFAS-spiked clean soils	42
(iii) MAC removed PFAS from AFFF-impacted and PFAS-contaminated garden soils	42
Task 4: Assess if HALT-PFAS technology can destroy PFAS in spent MAC	45
(i) Mass balance analysis of PFASs and fluorine during application of HALTPFAS to MAC.	45
(ii) Evaluation of HALT-PFAS for MAC recovered from PFAS-contaminated soil.	47
5. Conclusions and Implications for Future Research	50
5.1 Summary and Conclusions of Present Study	50
5.2 Potential Next Steps and Proposed Follow-On Research	52
6. Literature Cited	54

LIST OF TABLES

Table 1.1A Native matching for 1:1 known suspect quantification.

Table 1.1B Target (T) and suspect (S) PFAS concentrations in Capstone 1157.

Table 1.2 Recovery of 6:2 FTOH and 5:2 sFTOH standards from C18 cartridge.

Table 1.3 5:2 sFTOH tandem C18 cartridge stacking recovery.

Table 1.4 Average (from duplicates) percent recovery of mass-labelled surrogates of volatile PFAS from cartridges installed on microcosm with and without soil.

Table 2.1 Alpha diversity indices at sequence depth 33,000 of respective enrichment cultures and original C2 soil microbial communities

Table 2.2 Potential suspects found in 6:2 FtS microcosm time course study. One suspect found in 6:2 FtSaB microcosm with 1-butanol enrichment culture on day 9 (Blue shaded).

Table 2.3 Statistically significant ($p < 0.05$) non-target chemical features that were identified using the Mann-Kendall test.

Table 3.1 Properties of soils used in Task 3

Table 4.1 Concentrations of PFASs measured in untreated and HALT-treated MAC samples incubated with AFFF-impacted San Antonio, TX soil. Reaction conditions: 350°C, 1 M NaOH, 240 min, 67 g L⁻¹ MAC. LOQ = limit of quantification, ND = no detection

LIST OF FIGURES

Figure ES-1 (a) Cultures enriched with different chain-length alcohols showed a chain-length dependent defluorination pattern toward FTOHs. However, alkanes-, phenol- or CPB-enrichment cultures showed similar defluorination ability toward 4:2 FTOH and 6:2 FTOH.

Figure ES-1 (b) Enhanced defluorination of 6:2 FTOH was observed when dual enrichment cultures were used, with the highest amount of metabolites produced in the combination of phenol- and octane-enrichment cultures.

Figure 1.1 GC-MS chromatogram indicating Capstone 1157 contains 6:2 -12:2 FTOHs.

Figure 1.2 ¹⁷F NMR Capstone 1157 at a 1:100 dilution.

Figure 1.3 Concentrations of a) 6:2 FtSaB and b) FtSaAm in blank microcosms over 60 min period.

Figure 1.4 Oxygen consumption in each treatment over 21 days.

Figure 1.5 Carbon dioxide production in each treatment over 21 days.

Figure 1.6 6:2 FtS (moles) and two degradation products 6:2 UFTCA and 5:3FTA (at LOQ) in S- free microcosms; no decrease observed for other microcosm conditions (see Day 21).

Figure 1.7 Consumption of oxygen and production of CO₂ by 1-butanol-grown RHA1 in the presence of seven different surfactants at two different concentrations.

Figure 2.1 Microbial community composition of respective enrichment cultures used for the fluorotelomer-based PFAS degradation resting cell and growth experiments.

Figure 2.2 Released fluoride concentrations from 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH degraded by the enrichments resting cell experiments.

Figure 2.3 Biodegradation metabolites of 4:2 FTOH by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures.

Figure 2.4 (A) PFAS metabolites of 6:2 FTOH degradation by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures, and (B) minor PFAS metabolite concentrations excluding 6:2 UFTCA and 6:2 FTCA from 6:2 FTOH degradation by the enrichment cultures.

Figure 2.5 Biodegradation metabolites of 8:2 FTOH by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures.

Figure 2.6 (A) All Biodegradation metabolites of 6:2 FTOH by two enrichment cultures, and (B) minor metabolites (excluding 6:2 UFTCA and 6:2 FTCA) of 6:2 FTOH biodegradation by two enrichments.

Figure 2.7 Released fluoride from 6:2 FtS when used as S-source for ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures.

Figure 2.8 (A) All PFAS detected from 6:2 FtS degradation by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures grown in S-free medium. (B) Metabolites (excluding 6:2 FtS) detected during 6:2 FtS degradation by the cultures.

Figure 2.9 A metabolite, 6:2 UFTCA, was detected from biodegradation of 6:2 FtSaB by butanol (BuOH)- and phenol-enrichment cultures in S-limited medium. When received extra spikes of respective carbon sources, higher 6:2 UFTCA was observed in BuOH- and phenol-enrichment cultures, and 5:3 FTCA was detected only in the CPB-enrichment culture.

Figure 2.10 NMDS ordination of microcosm data in negative mode with sulfur-free conditions in duplicate (Bottle 1, Bottle 2). Arrows begin at Day 0, and progress to Day 3, Day 7, Day 14, and Day 21. NMDS stress of 0.08 indicates a great representation.

Figure 2.11 NMDS ordination of microcosm data in positive mode according to legend in sulfur free, sulfur and nitrogen free, carbon free, nitrogen free, and nutrient-rich conditions, respectively. Arrows begin at Day 0, and progress to Day 3, Day 7, Day 14, and Day 21. NMDS stress of 0.16 indicates a good representation.

Figure 2.12 Recalcitrant chemical features were expected to remain constant (A) or increase monotonically (B) through time, while biodegradable chemical features were expected to decrease monotonically (C) or be unimodal (D) through time. Peak areas of parent compounds (A, C) were expected to be non-zero/high initially, while peak areas of transformation products (B, D) are expected to be zero/low.

Figure 3.1 Removal of PFAS from PFAS-spiked environmental soil (C2 soil) after 3 months of incubation.

Figure 3.2 Removal of PFAS from PFAS-spiked commercial sandy soil after 3 months of incubation.

Figure 3.3 Removal of PFAS from PFAS-spiked commercial high-organic sunshine soil after 3 months of incubation.

Figure 3.4 Removal of PFAS from AFFF-impacted soil after 3 months of incubation.

Figure 3.5 Removal of PFAS from PFAS-aged high-organic garden soil after 3 months of incubation.

Figure 4.1 Degradation and defluorination of a known mass of PFOS adsorbed on MAC prior to treatment. Reaction conditions: 350°C, 1 M NaOH, 167 g L⁻¹ of virgin MAC initially loaded with 4.61 ± 0.04 mg g⁻¹ PFOS.

Figure 4.2 Attraction of MAC to a magnet before reaction and following HALT treatment for varying reaction times.

Figure 4.3 Concentrations of PFASs measured in untreated MAC samples incubated with AFFF-impacted San Antonio soil and removal following HALT. Reaction conditions: 350°C, 1 M NaOH, 240 min, 67 g L⁻¹ MAC. A “*” above the removal value indicates that the compound was treated to a value detected below the LOQ. The plotted removal is based on the LOQ, which gives a lower bound for the actual removal value.

Figure 4.4. Removal of PFASs adsorbed to MAC incubated with AFFF-impacted soil following HALT. Reaction conditions: 350°C, 1 M NaOH, 67 g L⁻¹ MAC, 240 min (orange) and 600 min (light blue). The 240 min reaction is the same as the one for which data plotted in Figure 4.3. A “*” above the removal value indicates that the compound was treated to a value detected below the LOQ. The plotted removal, based on the LOQ, gives a lower bound for the actual removal value.

LIST OF ACRONYMS

ACS	acetonitrile
AFFF	aqueous film forming foam
BuOH	butanol
CBP	cocamidopropyl betaine
DGBE	diethylene glycol butyl ether
DOD	Department of Defense
EtOH	ethanol
FTCA	fluorotelomer carboxylic acid
FTOH	fluorotelomer alcohol
FTS	fluorotelomer sulfonate
FtSaB	fluorotelomer sulfonanamido betaine
FtSaAm	fluorotelomer sulfonanamido amine
GAC	granular activated carbon
GC-MS	gas chromatography mass spectrometry
HALT	hydrothermal alkaline treatment
HDPE	high density polyethylene
HRMS	high resolution mass spectrometry
LC-QTOF	liquid chromatography quadrupole time of flight
LOD	limit of detection
LOQ	limit of quantification
NIST	National Institutes of Standards and Technology
NMDS	non-metric multidimensional scaling
NMR	nuclear magnetic resonance
MAC	magnetic activated carbon
MGAC	magnetic granular activated carbon
OSU	Oregon State University
PAC	powdered activated carbon

PCA	principal coordinates analysis
PEG	polyethylene glycol
PFAAs	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFDA	perfluorodecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PFNA	perfluorononanoic acid
PFPeA	perfluoropentanoic acid
PrOH	propanol
SBR	sequencing batch reactors
SRT	solid retention time
SPE	solid phase extraction
TAMU	Texas A&M University
UFTCA	unsaturated fluorotelomer carboxylic acid

Abstract

Introduction and Objectives

The *overarching goal* of this project was to develop an integrated treatment system for the effective treatment of poly- and perfluoroalkyl substances (PFAS)-laden soils in source zones. Specifically, this project aimed to test the treatment strategy that was based on the “Release-Capture-Destruction” concept. The technical objectives were to determine factors affecting biodegradation of soil-bound precursors, to accelerate biodegradation of precursors in soil via bioaugmentation, to determine the efficiency of PFAS removal by magnetic activated carbon (MAC) in soil, and to assess whether hydrothermal alkaline treatment (HALT-PFAS) technology can destroy PFAS in the spent MAC.

Technical Approach.

Four tasks were performed to address the project technical objectives to test associated hypotheses. These four tasks were to (i) determine factors affecting biodegradation of soil-bound precursors; (ii) accelerate biodegradation of precursors via bioaugmentation; (iii) determine the efficiency of PFAS removal by MAC in PFAS-impacted soils, and (iv) assess if HALT-PFAS technology can destroy PFAS in spent MAC.

Results

Capstone 1157 is a mixture of n:2 fluorotelomer sulfonamidoalkyl betaine (FtSaB) (n= 6, 8, 12), n:2 fluorotelomer sulfonamido amine (FtSaAm) (n= 6, 8), 6:2 fluorotelomer sulfonate (FtS), and 6:2 fluorotelomer alcohols (FTOH). Strain RHA1 degraded 6:2 FtS in Capstone only under sulfur-limited conditions. The 6:2 FtS defluorination ability of RHA1 was inhibited by four different hydrocarbon surfactants but promoted by three other hydrocarbon surfactants. Soil cultures enriched with alcohols (ethanol, 1-propanol, and 1-butanol), alkane (hexane and octane), aromatics (phenol), and cocamidopropyl betaine (CPB) showed ability to degrade different precursors: FTOHs, 6:2 FtS, and 6:2 FtSaB. Potential suspects were found in 6:2 FtS and 6:2 FtSaB microcosms containing enrichment cultures in S-limited media. MAC effectively removed PFAS from PFAS-spiked soils and AFFF-impacted soils, but the removal efficiencies were affected by high organic contents in soils. Spent MAC was quickly separated from treated soils. Application of HALT (350 °C, 1 M NaOH) led to near-complete destruction of perfluorooctane sulfonate (PFOS) adsorbed onto MAC and stoichiometric generation of fluoride ion without detection of any fluoro-organic intermediates. Removal of PFOS was significantly lower for the AFFF-impacted soil-derived MAC sample (81% as opposed to >97% in the PFOS-loaded MAC) at the same treatment residence time and alkali dose.

Benefits

Results of this study filled the knowledge gaps on the poorly understood effects of co-contaminants hydrocarbon surfactants on the fate and biotransformation of PFAS precursors, and the factors controlling biodegradation of the soil-bound precursors into more mobile perfluoroalkyl acids (PFAAs) for subsequent treatments (i.e., capture and destruction technologies). The new knowledge on promoting biodegradation of precursors can lead to the success of the proposed treatment train of PFAS in soils, and provides a foundation for the future development of precursor bioremediation. The successful application of MAC to sorb mobile PFAAs produced from precursor biodegradation, followed by using hydrothermal liquefaction for the destruction of PFAS-laden MAC, provided strong proof for the proposed “Release-Capture-Destruction” remediation strategy for PFAS-laden soils which are present in numerous DoD sites.

Executive Summary

Introduction

Aqueous film forming foams (AFFFs) used in the US military sites contain zwitterionic, cationic, and anionic poly- and perfluoroalkyl substances (PFAS). Long-term, repeated applications of AFFFs have resulted in widespread PFAS contamination in soils, sediments, and groundwater at numerous DOD sites. Recent studies revealed that zwitterionic, and to a lesser extent cationic, PFAS contribute to a significant fraction (up to 97%) of total PFAS in firefighter training area (FTA) soil¹, and approximately 52% of the PFAS transported downgradient was associated with polyfluorinated precursors.² Substantial retention of PFAS found in the source zone highlights the need for effective remediation of precursors in source zones to mitigate long-term transport/transformation of PFAS to the downgradient areas.

Several classes of hydrocarbon surfactants were recently identified in AFFF and in AFFF-impacted groundwater.³ Compared to PFAS that comprise only 0.9–1.5% (w/w) in AFFFs,^{4,6} hydrocarbon surfactants account for 5-10% of AFFF formula.⁷ These hydrocarbon surfactants are biodegradable; however, their presence in the AFFF-impacted matrices raises several concerns. For example, octylphenol as a metabolite of incomplete degradation of octylphenol polyethylates (a hydrocarbon surfactant class found in AFFFs and in AFFF-impacted matrices), is a known endocrine-disrupting compound.⁸ Precursors such as fluorotelomer sulfonates are also biodegradable under aerobic conditions yet they are found persist in groundwater at inactive military sites even for decades.⁹ The presence and the degradation of hydrocarbon surfactants might potentially affect the fate of the precursors in source zone soils, an important research question that has not been investigated. Together, the soil-bound precursors and the co-contaminant hydrocarbon surfactants point out a large knowledge gap on factors controlling the fate of these biodegradable precursors in the source zone soils, and the influence of hydrocarbon surfactants might have on the precursors in the source zones. This proposed research is intended to bridge this knowledge gap and provide information that is critical for future development of a cost-effective treatment strategy for PFAS in source zone soils.

Objective

The **overarching goal** of this project is to develop an integrated treatment system for the effective treatment of PFAS-laden soils in source zones. The treatment strategy is based on the “**Release-Capture-Destruction**” concept. It was hypothesized that effective conversion of soil-bound precursors to PFAAs is the key to the success of the proposed treatment strategy for PFAS in the source zone soils, and the PFAAs can be further captured by sorbents like magnetic activate carbon (MAC) and the spent MAC can be separated from the treated soils for further destructive treatment such as HALT-PFAS technology for final disposal. Our **overall technical objectives** were to address the following questions.

- Are the sorbed zwitterionic and cationic precursors in the soils biodegradable when salt-tolerant precursor degraders, and/or nutrients, and/or oxygen are amended?
- Are factors such as nutrients and oxygen, presence of co-contaminants like hydrocarbon surfactants, and microbial precursor degraders controlling the extent of precursors biodegradation?

- Are indigenous microorganisms capable of biodegrading precursors at the source zones? If not, will bioaugmentation promote rapid bioconversion of the precursors into PFAAs?
- Can precursors and their metabolites be effectively captured by MAC sorbents that can be later separated easily from the treated soils?
- Can the PFAS in the spent MAC be destroyed via the HALT-PFAS technology developed at the CSM, potentially enabling spent MAC regeneration or allow for economical disposal of MAC residuals as non-hazardous materials?

Technical Approach

In this limited scope proposal, we can only perform selected key tasks that can be realistically accomplished within one year, and the obtained results will serve as “proof-of-concept” of the proposed research. Four tasks conducted are

Task 1: Determine factors affecting biodegradation of soil-bound precursors.

- ⇒ The goal of Task 1 was to collect proof-of-concept data to determine effects of nutrients and co-contaminants of PFAS (such as hydrocarbon surfactants) on precursor biodegradation. In Task 1, we first characterized Capstone composition and validated of PFAS mass in microcosm setup. A known 6:2 FtS degrader, *Rhodococcus jostii* RHA1, was used to assess biodegradation potential of Capstone and to determine the effects of seven different hydrocarbon surfactants (common co-contaminants in AFFF-impacted soil) at different concentrations on 6:2 FtS defluorination.

Task 2: Accelerate biodegradation of precursors in soil via bioaugmentation.

- ⇒ The goal of Task 2 in this limited-scope study was to collect data to determine if three different types of precursors, FTOHs, 6:2 FtS, and 6:2 FtSaB, could be biodegraded by bioaugmenting with soil cultures that were enriched under different nutrient conditions. In Task 2, soil enrichment cultures with different carbon sources were developed and used to accelerate biodegradation of precursors. The microbial communities of the established enrichment cultures were characterized via next generation sequencing. Different precursors: FTOHs, 6:2 FtS, 6:2 FtSaB, and Capstone were used. To assess the possibility of enhancing biodegradation, experiments were also conducted with dual enrichment cultures under different nutrient conditions. Target and suspect metabolites from the precursor degradation were conducted.

Task 3: Determine the efficiency of PFAS removal by MAC in soil.

- ⇒ The goal of Task 3 was to determine whether magnetic activated carbon (MAC) can be used to remediate PFAS-laden soils. In Task 3, MAC were synthesized and applied to assess PFAS removal efficiency from PFAS-impacted soils, including both PFAS-spiked soils and AFFF-impacted soils. Clean soils with low and high organic contents were used. Aged PFAS-contaminated soils were also selected for study.

Task 4: Assess if HALT-PFAS technology can destroy PFAS in spent MAC.

- ⇒ The goal of Task 4 was to provide initial proof-of-concept data for the treatment of PFAS-laden MAC using the hydrothermal alkaline treatment (HALT) technology pioneered at

the Colorado School of Mines (CSM). In Task 4, (A) a fluorine mass balance analysis for destruction and defluorination of PFOS adsorbed on virgin MAC; and (B) evaluating the application of HALT for treatment of MAC recovered from PFAS-contaminated soil from Task 3 were conducted.

Results and Discussion

(i) Capstone characterization and validation of PFAS mass balance in microcosm setup. Based on non-volatile PFAS by LC-QTOF, three commercial reference materials Capstone 1157, Forafac 1157, and Forafac 1157N at four dilutions (1:1K, 1:1M, 1:10M, and 1:100M) were analyzed and determined to be similar in their PFAS composition. Capstone 1157 contains n:2 FtSaB (n= 6, 8, 12), n:2 FtSaAm (n= 6, 8), 6:2 FtS, and 6:2 FTOH. Upon analysis via LC-QTOF, it was determined that there was no loss of 6:2 FtSaB or 6:2 FtSaAm onto the HDPE containers from the media.

(ii) Factors affecting precursor biodegradation. While there were no significant changes of 6:2 FtSaB and 6:2 FtSaAm, biodegradation of 6:2 FtS in Capstone by a known 6:2 FtS degrader, strain RHA1, was observed under only S-limited conditions. Two metabolites 6:2 UFTCA and 5:3 FTCA were detected. These observations might be explained by the high initial concentration of Capstone used in the experiments.

By screening the effects of seven different hydrocarbon surfactants (X-100, X102, polyethylene glycol (PEG) 400, sodium dodecyl sulfate (SDS), diethylene glycol butyl ether (DGBE), hexylene glycol and cocamidopropyl betaine (CPB)), different O₂ consumption and CO₂ production by RHA1 were observed in the presence of these hydrocarbon surfactants, indicating that RHA1 remained active but might be metabolically inhibited by the presence of these hydrocarbon surfactants. Interestingly, based on fluoride release, the defluorination ability of 6:2 FtS by the 1-butanol-grown RHA1 was inhibited by four tested hydrocarbon surfactants (PEG 400, SDS, X-100, X102, CPB), but not by hexylene glycol and 0.1% DGBE.

(iii) Soil cultures enriched with different carbon sources showed unique degradation potential toward different precursors. Environmental background soil (C2 soil) from a former air force base was a good inoculum source to enrich precursor-degrading cultures using a wide range of carbon sources. The diversity and abundance of C2 soil microbial community were higher compared to those of the enrichment cultures that received different carbon sources over time. Based on the Shannon index values of the enrichment cultures, from low to high, phenol- > ethanol- > CPB- > octane- > propanol- > hexane-> butanol-enriched cultures.

All the enrichment cultures were able to defluorinate 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH, based on fluoride ion release in the media. 1-Butanol-enriched cultures showed high defluorination capacities as high fluoride concentrations were observed for all 3 different types of FTOHs. The types and levels of metabolites from FTOH biodegradation were different among the enrichment cultures. Interestingly, cultures enriched with alcohols (i.e., ethanol, propanol, and butanol) showed a similar chain-length-dependent defluorination toward FTOHs, from high to low, 4:2 FTOH > 6:2 FTOH > 8:2 FTOH. (**Figure ES-1 (a)**). Enhanced fluoride release and metabolite concentrations

of FTOHs biodegradation were observed when dual enrichment cultures were used. The combination of phenol and octane enrichment cultures produced highest concentrations of 6:2 UFTCA (2120 µg/L) and 6:2 FTCA (1838 µg/L). (**Figure ES-1 (b)**). Interestingly, 5:3 FTCA was found in all conditions where the CPB-enriched culture was used as one of two enrichment cultures (i.e., butanol (BuOH)+CPB, phenol+CPB, and octane+CPB).

Figure ES-1 (a) Cultures enriched with different chain-length alcohols showed a chain-length dependent defluorination pattern toward FOTHs. However, alkanes-, phenol- or CPB-enrichment cultures showed similar defluorination ability toward 4:2 FTOH and 6:2 FTOH.

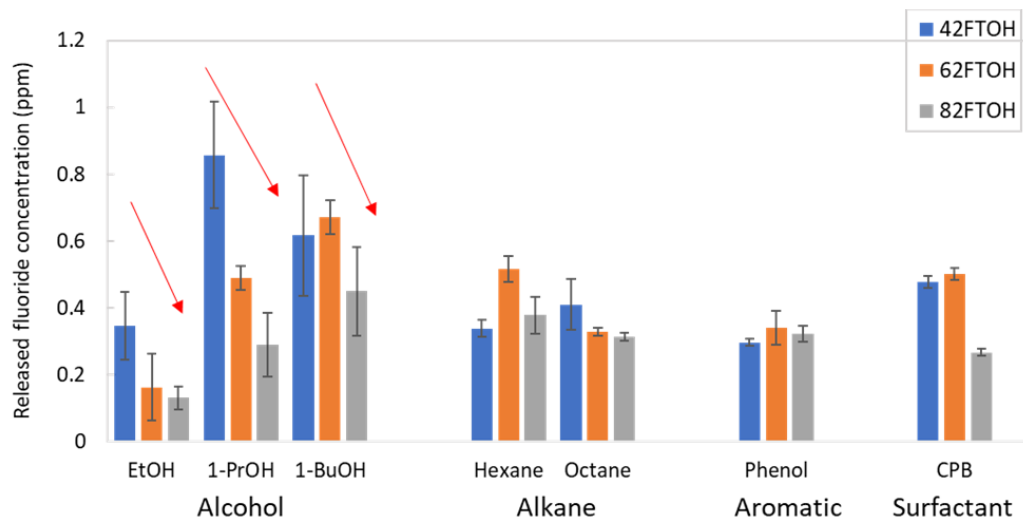
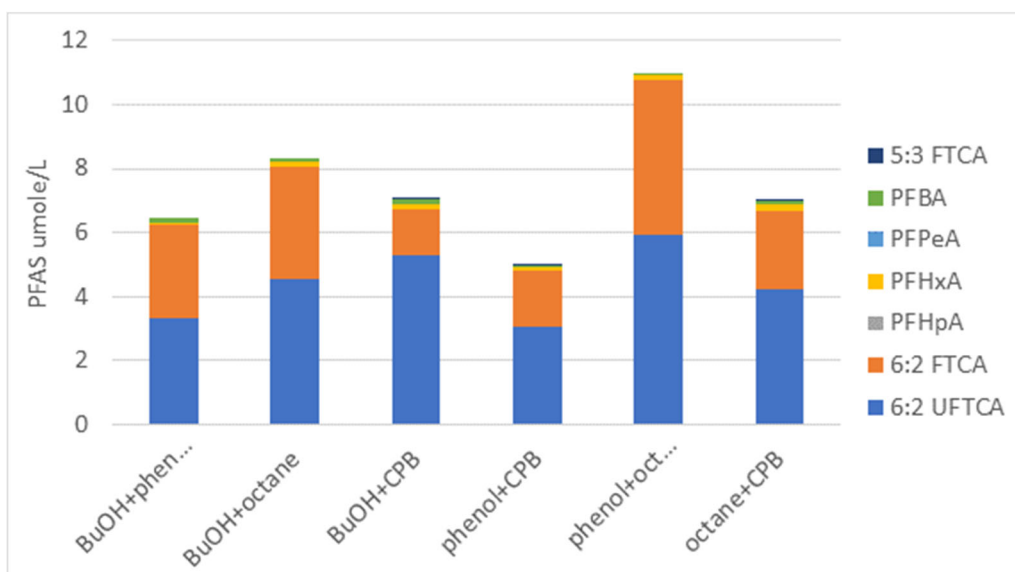


Figure ES-1 (b) Enhanced defluorination of 6:2 FTOH was observed when dual enrichment cultures were used, with the highest amount of metabolites produced in the combination of phenol- and octane-enrichment cultures.



(iv) Soil enrichments can defluorinate and utilize 6:2 FtS as a sole sulfur-source. All the enrichments could use 6:2 FtS as a sole sulfur source, with a high level of defluorination observed in the 1-butanol-enriched culture. A much slower growth was observed when hexane was provided as the carbon source compared to other carbon sources. Also, suspect metabolites from 6:2 FtS microcosms containing C2 enrichment cultures were identified.

(v) Biodegradation of 6:2 FtSaB was observed by butanol-, phenol- and CPB-enrichment culture only under S-limited conditions over 9 days of incubation. 6:2 UFCA was detected in butanol- and phenol-enrichment cultures, while 5:3 FTCA was detected in CPB-enrichment culture. One non-target suspect, $C_{13}H_{17}F_{13}N_2O_2S$, was detected in butanol-enrichment culture. The different microbial communities in the enrichment cultures and the availability of carbon sources maybe two factors affecting the extents and types of metabolites produced during 6:2 FtSaB degradation. The results suggested that the enrichment cultures might be able to degrade Capstone 1157 that contains n:2 FtSaB (n= 6, 8, 12), n:2 FtSaAm (n= 6, 8), 6:2 FtS, and 6:2 FTOH.

(vi) MAC achieved different PFAS removal efficiency from the PFAS-spiked soils, depending on PFAS types and soil properties. Over 3 months of incubation, approximately 80-90% of PFAS (PFOS, or PFOA, or 6:2 FtS) in PFAS-spiked C2 soil and commercial sandy soil were removed. However, low removal efficiency (0 to 20% PFAS removal) in the high-organic sunshine soils by MAC amendment. In addition, MAC demonstrated an ability to remove PFAS from PFAS-aged or AFFF-impacted soils, while the removal efficiency appears to be impacted by the presence of high organic contents and diverse types of PFAS in AFFF-impacted soil. Mostly, dramatic PFAS reduction was observed after one month of incubation, with marginal removal of PFAS after three months of incubation, suggesting that new MAC addition might be needed after one month of incubation to remove more PFAS.

(vii) HALT technology can defluorinate spent MAC derived from PFAS-spiked and AFFF-impacted soils. Application of HALT (350°C, 1 M NaOH) led to near-complete destruction of PFOS adsorbed onto MAC and stoichiometric generation of fluoride ion without detection of any fluoro-organic intermediates. Compared to the PFOS-loaded samples from the mass balance experiments in Task 4a, removal of PFOS was significantly lower for the AFFF-impacted soil-derived MAC sample (81% as opposed to >97% in the PFOS-loaded MAC) at the same treatment residence time and alkali dose. This could be because of the incomplete separation of MAC from the soil, leading to the presence of soil particles in the reactor, which may contain silica or other base-neutralizing compounds.

Implications for Future Research and Benefit

The promising findings from this limited scope research project are highly supportive of the proposed “Release-Capture-Destruction” concept as an effective treatment train for PFAS-containing soil that can eliminate long-term PFAS sources due to the presence of precursors in soils. To our knowledge, this was the first effort to demonstrate the fully spectrum of Capstone, to examine the poorly understood effects of co-contaminants hydrocarbon surfactants on

biotransformation of PFAS precursors, and to explore the use of multiple enrichments for enhanced biodegradation of precursors. The new knowledge on promoting biodegradation of precursors is expected not only lead to the success of the proposed treatment train of PFAS in soils but also provide a foundation for the future development of precursor bioremediation. Specifically, the knowledge can guide further development of bioremediation either through biostimulation and/or bioaugmentation.

High PFAS removal efficiency by MAC within months suggested that MAC is a promising technology for remediating PFAS-impacted soils while removing the spent MAC from the treated soils. Such a capability is particularly favorable as there is no concern of the potential of PFAS desorption from the spent sorbents back the environment. This study also demonstrated that defluorination of PFAS-laden MAC could be achieved by using HALT technology. The combination of MAC with HALT technology can be also readily applied to excavated AFFF-impacted source zone soils, which are costly to disposal or to remediate. Overall, our results provided strong proof for the proposed “Release-Capture-Destruction” remediation strategy for PFAS-laden soils which are present in numerous DoD sites.

1. Objective

This proposed research was submitted in responsive to ERSON-22-C4 and the research efforts focus on multiple objectives described in the ERSON-22-C4.

The **overarching goal** of this project is to develop an integrated treatment system for the effective treatment of PFAS-laden soils in source zones. The treatment strategy is based on the “**Release-Capture-Destruction**” concept. It was hypothesized that effective conversion of soil-bound precursors to PFAAs is the key to the success of the proposed treatment strategy for PFAS in the source zone soils, and the PFAAs can be further captured by sorbents like magnetic activate carbon (MAC) and the spent MAC can be separated from the treated soils for further destructive treatment such as HALT-PFAS technology for final disposal. Our **overall technical objectives** were to address the following questions.

- Are the sorbed zwitterionic and cationic precursors in the soils biodegradable when salt-tolerant precursor degraders, and/or nutrients, and/or oxygen are amended?
- Are factors such as nutrients and oxygen, presence of co-contaminants like hydrocarbon surfactants, and microbial precursor degraders controlling the extent of precursors biodegradation?
- Are indigenous microorganisms capable of biodegrading precursors at the source zones? If not, will bioaugmentation promote rapid bioconversion of the precursors into PFAAs?
- Can precursors and their metabolites be effectively captured by MAC sorbents that can be later separated easily from the treated soils?
- Can the PFAS in the spent MAC be destroyed via the HALT-PFAS technology developed at the CSM, potentially enabling spent MAC regeneration or allow for economical disposal of MAC residuals as non-hazardous materials?

However, in this limited scope proposal, we can only perform selected key tasks that can be realistically accomplished within one year, and the obtained results will serve as “proof-of-concept” of the proposed research. Four tasks conducted were

Task 1: Determine factors affecting biodegradation of soil-bound precursors.

Task 2: Accelerate biodegradation of precursors in soil via bioaugmentation.

Task 3: Determine the efficiency of PFAS removal by MAC in soil slurry.

Task 4: Assess if HALT-PFAS technology can destroy PFAS in spent MAC.

2. Background

2.1 Remediation of PFAS in AFFF-impacted soils

Remediation of PFAS in AFFF-impacted soils remains challenging and can be further complicated by the presence of co-contaminants such as hydrocarbon surfactants. Numerous zwitterionic, cationic, and anionic compounds in AFFF formula and in AFFF-impacted matrices,¹⁰⁻¹² and soil-bound cationic and zwitterionic precursors contribute the majority of total PFAS in source zone soils.^{1, 2, 13, 14} While some of these precursors are potentially biodegradable, they were detected at high levels in AFFF-impacted source zone soils that have not received AFFF applications since 1991 (i.e., for more than two decades).¹ The presence of these precursors is problematic because (i) soils containing strongly bound precursors can serve as a long-term PFAA contamination source due to slow and incomplete PFAS degradation, and (ii) there are no effective treatment processes for these precursors in source zone soils. Perfluoroalkyl sulfonamides (such as N-ethyl perfluorooctane sulfonamide (N-EtFOSA), anionic fluorotelomer sulfonates (n:2 FtS), and zwitterionic fluorotelomer sulfonamidoalkyl betaine (n:2 FtSaB) are dominant precursors detected in source zone soils.^{9, 15-18} Biodegradation of N-EtFOSA has been shown to produce PFOS, while biodegradation of n:2 FTSA and n:2 FtSaB and fluorotelomer alcohols (FTOHs)^{19, 20} can lead to production of various PFAAs (including PFOA, but only the linear isomer, and saturated and unsaturated fluorotelomer acids), ranging from 0.1 to 40% (molar basis) of their respective precursors.²¹ Aerobic biodegradation of 4:2, 6:2, and 8:2 fluorotelomer thioether amido sulfonate (FtTAoS) has been observed in soil microcosms.²²

As AFFFs contain not only PFAS (0.9–1.5% (w/w) in AFFFs, but also other ingredients such as hydrocarbon surfactants (5-10% in AFFFs),⁷ Garcia et al.³ detected nine classes of hydrocarbon surfactants in the achieved AFFFs and six classes of them in AFFF-impacted groundwater samples. Octylphenol polyethoxylates were the most frequently detected class of hydrocarbon surfactant and are biodegradable, particularly under aerobic conditions. However, octylphenol, a metabolite from octylphenol polyethoxylates, is a known endocrine-disrupting compound⁸. The findings of these diverse hydrocarbon surfactants in the AFFF formula and AFFF-impacted areas have also raised several important questions, i.e., *What is the role of the hydrocarbon surfactants on the fate of PFAS in source zone soils? Will the hydrocarbon surfactants facilitate the biotransformation of precursors or impede the biotransformation? Will providing oxygen and/or nutrients facilitate complete degradation of hydrocarbon surfactants and precursors?* The above questions were addressed in this limited-scope study.

2.2 Magnetic activated carbon (MAC)

Magnetic activated carbon (MAC) can be used to remove PFAS and the spent MAC can be easily recovered from the treated matrices. Magnetic activated carbon, referred to as activated carbon (AC) with ferromagnetic metal oxides, has been recently introduced as an alternative

sorbent for removing micropollutants (such as pesticides, herbicides, and pharmaceutical residues) in drinking water and wastewater, because the spent sorbent can be easily separated from the treated matrices by applying an external magnetic field.^{23, 24, 25} Mostly, magnetic activated carbon (MAC) shows a similar adsorption capacity toward PFAS as that of granular activated carbon (GAC) /powder activated carbon (PAC).²⁶ The magnetic GAC (MGAC) with a high amount of magnetite exhibits 29 % higher PFOA sorption than that of GAC²⁷.

While GAC/PAC has been shown effective for PFAS-contaminated water, to our best knowledge, the application of AC to PFAS-laden sediments or soils has not been reported nor explored. Prior studies have applied GAC/PAC to PCB- or PAH-contaminated sediments to reduce the bioavailability to animals and plants in the environment. However, these contaminants are not destructed but simply transferred into the sorbents. We have recently observed > 50% of PFOS removal from PFOS-laden soils after amendment with PAC (5%, w/w) for 21 days. Our results suggested that PAC can be directly applied to remediate PFAS-laden soils. Thus, if MAC is used, it can be a rapid and effective method to remediate PFAS-laden soils, as the spent MAC can be recovered easily and clean MAC can be reapplied as needed until reaching the desired treatment endpoint.

2.3 Hydrothermal alkaline treatment (HALT-PFAS)

Hydrothermal alkaline treatment (HALT-PFAS) has been shown to defluorinate PFAS-laden water, soil, and wastewater sludge.²⁸⁻³⁰ Accordingly, this technology can potentially be used to destroy and mineralize PFAS in the spent MAC. Dr. Timothy Strathmann, the expert on defluorination of PFAS via HALT-PFAS, will conduct the experiments necessary to demonstrate the destruction of PFAAs in spent MAC (Task 4). Recent advances in hydrothermal conversion technologies have resulted from development of second- and third-generation biofuels from cellulosic crop residues and feedstocks like algae. Subcritical hydrothermal processing refers to reactions catalyzed in liquid water below its critical temperature and pressure (374°C, 22.1 MPa). These conditions promote chemical reactions because water can act as a solvent, a reactant, and even a catalyst as temperature approaches the critical point. The same hydrothermal characteristics can be exploited to degrade highly recalcitrant organic chemicals like PFAS. PFAS's fluorinated backbone imparts extreme chemical stability. Many of the same remedial technologies used for destruction of other legacy contaminants. Results from a previous limited scope project (ER18-1501) showed that alkaline hydrothermal treatment can rapidly destroy and liberate fluoride from the full suite of PFASs identified in different AFFF mixtures, including short-chain sulfonates. Tests with PFAS-contaminated soil collected from Department of Defense (DoD) facilities also showed that HALT-PFAS treatment can destroy PFASs adsorbed to soil surfaces.

3. Materials and Methods

3.1 Methods for Task 1

Method development for Capstone analysis and validation of a close mass balance of PFAS. Before initiate Task 1, research efforts were placed in addressing materials to be used to close mass balance of PFAS. Research efforts were also placed on method development focusing on (i) Volatile PFAS in C18 Cartridge Traps, and (ii) Capstone Characterization, and (iii) Background Analysis on HDPE Bottles and Medium Solution.

Biodegradation of Capstone by *Rhodococcus jostii* RHA1 under different nutrient conditions. Batch experiments were designed to investigate effects of nutrients on the biotransformation Capstone 1157 by *Rhodococcus jostii* RHA1. The strain *Rhodococcus jostii* RHA1 was selected for this task because this strain is known for its ability to degrade 6:2 FTOH and 6:2 FtS. The experiments were conducted in a series of microcosms containing RHA1, Capstone 1157, in one of four different mineral salt media: Nutrient rich, Sulfur free (S-free), Nitrogen free (N-free), and Nitrogen/Sulfur free (N/S-free). The recipes of the media are described in the section below. RHA1 inoculum cells were first prepared by growing in nutrient-rich medium contained 1-butanol 20 mM as carbon sources. When cell density reached optical density (OD600) 0.7-0.8, cell suspension was pelleted at 10,000g for 10 min. The pellet was washed and resuspended with N-/S-free medium and then resuspended for experimental use.

Briefly, RHA1 was spiked (to achieve an initial OD600 = 0.4) into a series of 120-mL HDPE serum bottles containing 10 mL of each medium (nutrient rich, S-free, N-free, or N-/S-free medium) and 0.5 mM of 1-butanol. One hundred microliter of capstone 1157 stock solution (3g/L in methanol) was then added to each bottle to result in an initial concentration of 0.03 g/L capstone 1157. Additional treatment contained 10 mL of nutrient rich medium and capstone 1157 without 1-butanol was also prepared to examine the effects of carbon source. All treatments were conducted in duplicate. Following inoculation, the bottles were incubated at 30 °C and 150 rpm. A parallel set was to monitor cell growth, O₂ consumption, and CO₂ production over the course of the experiments. At time zero, day 3, 7, 14, and 21, two replicate bottles were sacrificed for chemical analysis. At day 9, due to fast depletion of oxygen, 6 mL of pure oxygen were spiked to bottles of day 14 and day 21. Additional 100 µL of 1-butanol stock solution (50 mM in water) was also spiked to bottles of day 14 and day 21 except for the C-free treatment. At the end of the experiments, a C18 cartridge was attached on the top of each bottle to capture any volatile PFAS metabolites that were pursued from the headspace of the bottle. All the purged bottles and cartridges were shipped to OSU for chemical analysis.

Nutrient rich, Sulfur free (S-free), Nitrogen free (N-free), and Nitrogen/Sulfur free (N/S-free) mineral salt medium. Sulfate and ammonium were the sulfur and nitrogen source in the nutrient-rich medium consisting of K₂HPO₄ (3.5 g/L), KH₂PO₄ (1.5 g/L), K₂SO₄ (0.17 g/L), NH₄Cl (0.5g/L), MgCl₂ • 6H₂O (0.15 g/L), NaCl (0.62 g/L) and 0.1 % (v/v) S-free trace mineral solution. The S-free trace solution contains FeCl₃ • 6H₂O (0.24 g/L), MnCl₂ • 4H₂O (0.03 g/L), CoCl₂ • 6H₂O (0.04 g/L), CuCl₂ • H₂O (0.04 g/L), ZnCl₂ (0.15 g/L), and Na₂MoO₄ • 2H₂O (0.03 g/L). For S-free, N-free, and N/S-free medium, same ingredients were used except no K₂SO₄, NH₄Cl, or both included in the medium respectively.

Effect of hydrocarbon surfactants on defluorination of 6:2 FtS by 1-butanol- grown *Rhodococcus jostii* RHA1. Experiments were designed to investigate the effects of hydrocarbon surfactants on the defluorination of 6:2 FtS by 1-butanol grown *Rhodococcus jostii* RHA1. 1-Butanol-grown RHA1 were prepared by growing in a 10X-diluted S-free medium with 1-butanol (20 mM) as a carbon source and 6:2 FtS (20 mM) as sulfur source. When optical density (OD₆₀₀) reached 0.7-0.8, the RHA1 cells were centrifuged at 10,000 g for 10 min and resuspended with a new 10X diluted S-free medium. Then the cell suspension was aliquoted 5 ml into a series of 20-ml vials containing 1-butanol (5 mM), 6:2 FtS (20mM), and one of seven different hydrocarbon surfactants at two different concentrations 1% and 0.1%. These seven hydrocarbon surfactants were X-100, X-102, CPB, PEG 400, SDS, DGBE, and hexylene glycol. One set without addition of surfactant was designed as live control (negative control). All treatments were conducted in duplicate. All vials were incubated at 30°C and on a 150-rpm shaker for three days before analyzed for O₂/CO₂, fluoride release, and 1-butanol concentration.

3.2 Methods for Task 2

Enrichment of soil cultures with different carbon sources and establishment of stable enrichment cultures in sequencing batch reactors (SBRs). Clean characterized background soil collected from the former Wurtsmith Air Force Base, designated as C2 soil, was shipped from co-PI Field's lab to Chu's lab. The C2 soil was used as inocula to establish soil enrichment cultures. For initial enrichment, 0.2g of C2 soil provided from Oregon State University was suspended in 2mL ammonia mineral salt (AMS) media with one of the following carbon sources: of ethanol (0.05%), 1-propanol (5mM), 1-butanol (5mM), hexane (0.05%), octane (0.05%) phenol (0.05%), and CPB (0.05%). Enrichment cultures showed signs of growth (i.e., increased of turbidity, production of CO₂ and consumption of O₂) were selected for the development of stable enrichment cultures in lab-scale SBRs. To obtain stable enrichment cultures for follow-up biotransformation experiments, SBRs were operated under specific solid retention times (SRTs). The stable conditions were determined based on optical density, CO₂ production, and/or O₂ consumption, and the microbial communities of the cultures were characterized using next generation sequencing.

Microbial community analysis. Three consecutive biomasses were collected through the 3-time SRT period operated before the start of the initial PFAS biodegradation tests. The collected biomass samples were frozen at -50°C before genomic DNA extraction. Genomic DNA was extracted using the FastDNA spin kit for soil (MP biomedical, USA). Library preparation was conducted with targeting the V3-4 region using the Nextera XY library preparation kit (Illumina, USA). The prepared library was sequenced in the MiSeq platform (Illumina, USA) using a paired-end (2x300 bp) read sequencing strategy. The obtained raw sequence reads were processed and downstream analyzed using qiime2-2022.8. The raw sequence reads were quality filtered, denoised, merged, and chimera removed using the DADA2 algorithm. Taxonomy was assigned from the SILVA 138 reference database by BLAST+ alignment.

Biodegradation of precursors by C2 enrichment cultures under different nutrient conditions. The enrichment cultures were maintained in a set of SBRs and operated at 1-day or 2-day SRT

until reaching steady-state condition. Then the stable enrichment cultures were used for a set of precursor biodegradation tests. Three different types of precursors - fluorotelomer alcohols (4:2 FTOH, 6:2 FTOH, and 8:2 FTOH), 6:2 fluorotelomer sulfonate (6:2 FtS), and 6:2 fluorotelomer sulfonamide alkylbetaine (6:2 FtSaB) - were used as model precursors in biodegradation tests. Detailed experimental setup and experimental results are presented below.

(i) Setup of precursor degradation tests using C2 enrichment cultures. Upon reaching steady state conditions (i.e., based on optical density (OD_{600}) and headspace O_2/CO_2 concentrations), the enrichment samples were harvested and used immediately for resting cell experiments to test the biodegradation of various chain length fluorotelomer alcohols (i.e., 4:2 FTOH, 6:2 FTOH, 8:2 FTOH). Briefly, the harvested cell suspensions were centrifuged, supernatant discard to remove residual carbon in the spent media, and then the pellets were resuspended in carbon-free AMS media for experimental uses. FTOH biodegradation tests were conducted in a series of glass vials containing 2 mL of cell suspension with one of three FTOHs. The glass vials were crimp sealed with a silicone septum with silicone side facing inside the vial. The sealed vials were then spiked with a known amount of FTOH stock solutions (prepared in methanol) to reach an initial 20 ppm n:2 FTOH. Following FTOH addition, the vials were then inverted to minimize loss through volatilization and incubated for 3 days at room temperature. All conditions were conducted in duplicate.

As 6:2 FtS has been known to be used as a sulfur (S) source for microbial growth and biodegradation of 6:2 FtS occurred only under S-limited conditions, biodegradation of 6:2 FtS was performed using each enrichment culture as inoculum and its respective carbon source in S-free medium. Similarly, cell suspensions were harvested, centrifuged, washed, and resuspended in a modified sulfur-free AMS media for experimental uses. In a series of glass vials, a known amount of 6:2 FtS stock solution (prepared in methanol) was first spiked and allowed methanol to be evaporated before use. The 6:2 FtS-containing vials were then allocated with cell suspension (a starting OD_{600} of 0.05). The vials were sealed with silicone septa as described above incubated for eight days at room temperature before collected for chemical analysis. The experiments were conducted with two sets, one set with an initial 6:2 FtS concentration of 20 ppm for assess fluoride release and the other set with the initial 6:2 FtS concentration of 5 ppm for metabolite identification.

Biodegradation tests for 6:2 FtSaB was conducted similarly as described for FTOH biodegradation tests, except that 20-mL HDPE vials with screw caps and silicone septa were used. For this test, 1g/L of 6:2 FtSaB stock was prepared in 10% methanol. The resting cell were suspended in 1 mL to each vial, closed with the septa, and 4 μ L of the 6:2 FtSaB stock was spiked. All vials were established in 2 sets designated to perform 1 set for pure resting cell and another set to perform a semi-resting cell by spiking twice of 10 times diluted carbon source over the course of the experiment. For ethanol (EtOH), 1-propanol (1-PrOH), 1-butanol (1-BuOH), phenol, CPB, and betaine samples, the resting cell set was incubated at room temperature for 3 days and subsequently stored in the freezer before solvent extraction. The other set was spiked with the reduced carbon at day 3 and day 6. The set was further incubated until day 10 and subsequently frozen. In case for hexane and octane conditions, the resting cell set was incubated for 7 days, and the other set spiked at day 7 and 14.

(ii) PFAS extraction, fluoride measurement, and LC/MS analysis. PFAS contents in the collected samples were extracted with acetonitrile (1:1 v/v). After adding an equal volume of

acetonitrile to the collected sample, the mixture was incubated at 50°C for 3 days on shaker. The extract was used for PFAS analysis. Fluoride present in the extract was measured using the fluoride 9609BNWP ion-selective probe (Thermo Scientific, USA). Standard curves of fluoride ions in solutions were generated, with high R^2 values (i.e., $R^2=0.9944\sim0.9962$). Two milliliter of the extracts was mixed with 30 mg ENVI-Carb (Sigma-Aldrich, USA) in polypropylene (PP) tubes to remove background organics. After centrifugation, supernatants were collected and stored in PP vials at 4°C before PFAS analysis using LC/MS. Special cares of not exposing samples to any PTFE containing components were taken during the sample preparations. The prepared samples were sent to Oregon State University for PFAS analysis using LC/MS operation.

3.3 Methods for Task 3

Synthesis of magnetic activated carbon (MAC). MAC was synthesized via chemical coprecipitation as described previously.^{31, 32} Briefly, 10 g of activated carbon, 7.32 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 13.32g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ were slowly added into 400 mL N_2 -purged water. The mixture was stirred and heated to 70 °C for 5 minutes, and then cooled down to 40 °C before adjusting pH to 10 by adding NaOH (5 M) slowly. The pH-adjusted mixture was stirred for 1 h before rested overnight. The MAC in the mixture was pelleted by centrifugation. After discarding the supernatant, the MAC was washed with deionized water, rinsed with ethanol, and then dried in an oven at 80 °C. The synthesized MAC was used in Task 3 and sent to Dr. Strathmann, Colorado School of Mines for experimental used in Task 4a.

Applications of MAC for remediating PFAS-contaminated soil. The experiments were conducted to investigate PFAS removal efficiency of MAC from PFAS-spiked soil and PFAS-contaminated soil. Five types of soils (commercial sandy soil, C2 environmental sandy soil, commercial garden soil (Sunshine organic clean soil), San Antonio AFFF-impacted soil, and PFAS-impacted commercial garden soil), three types of PFAS (PFOS, or PFOA, or 6:2 FtS) were used. Briefly, 10 grams of each of clean soils (commercial sandy soil, environmental C2 sandy soil, and commercial garden soil) were placed in a 50-mL of Falcon Conical Centrifuge Tube. One hundred microliter of 10 mg/L stock solutions of PFAS (PFOA, PFOS, 6:2 FtS) stock solution (total mass 1 ug) was spiked into the tube and mixed by vortexing to achieve initial concentration of 0.1 ug PFAS /g of soil. After homogenization, 0.5 g MAC was added into the soil (10 grams) and then vortexed again to achieve a ratio of 5% MAC in the PFAS-spiked soil. The tubes were then incubated on a shaker at 150-rpm at 30°C. Due to different densities of soils and MAC, tubes were rotated by hands twice a week to avoid stratification and to ensure constant mixing of MAC with the soil. Two parallel sets of experiments using PFAS-impacted soils were conducted similarly, except that no PFAS was amended in the soils. San Antonio AFFF-impacted soil and PFAS-impacted garden soil were used. Samples were collected after one, and three months of incubation. The spent MAC in the collected samples were separated from the treated soil via applying magnets. The PFAS remained in the treated soil was analyzed using LC/MS. The spent MAC samples were sent to Dr. Strathmann at Colorado School of Mines for PFAS mass balance and HALT treatment as described in Task 4.

3.4 Methods for Task 4

Mass balance analysis of PFASs and fluorine during application of HALT-PFAS to MAC.

A solution containing perfluorooctane sulfonate (PFOS) was prepared by adding the solid potassium salt of PFOS to 1.0 L of Milli-Q water with 5 mM NaCl and 1 mM NaHCO₃. 100 mL of the well-mixed PFOS solution was then transferred to a Pyrex bottle, to which 5.0 g of MAC was added. The MAC was left to equilibrate with the PFOS solution for three days, after which the liquid was removed, and the spent MAC was dried in an oven at 80°C for 2 days.

All HALT reactions were carried out in reactors made of 316 Stainless Steel parts, as described previously (Hao et al., 2021). Each reactor contained 500 mg of MAC and 3.0 mL of liquid. The liquid added to the reactor was a basic solution of Milli-Q water containing NaOH. To achieve subcritical conditions, sealed reactors were immersed in a temperature-controlled fluidized sand bath set at 350°C. Following the specified reaction time, reactors were removed from the heated sand bath and reactions were quenched by submersing the reactors in a container of tap water. After cooling, reactor contents were emptied into a 50 mL polypropylene centrifuge tube. MAC remaining inside the reactor was collected by rinsing the reactor with Milli-Q water and adding the wash mixture to the centrifuge tube. Tube contents were vortexed and centrifuged, after which supernatant was transferred to a separate centrifuge tube for fluoride measurement and LC-QTOF-MS analysis of any dissolved PFASs. The MAC in the centrifuge tube was then dried at 80°C for 2 days before subjecting to PFAS extraction.

Multiple extractions with methanol and ammonium acetate were used to desorb PFASs from MAC prior to analysis following procedures adapted from Xiao and co-workers.³³ The resulting extracts were pooled and analyzed by LC-QTOF-MS. Briefly, after oven drying, 150 mg of MAC was transferred to a 50 mL centrifuge tube. 24 mL of methanol and 1 mL of 2.5 M ammonium acetate was added to the tube, which was placed on the shaker table at 120 rpm for 24 hours. Supernatant was then collected from the tube, leaving minimal liquid in contact with the MAC. The extraction process was then repeated twice more to yield 75 mL of liquid extract.

PFOS was analyzed by LC-QTOF-MS targeted analysis. A SCIEX X500R QTOF-MS system (Framingham, MA) was used to measure PFASs following electrospray ionization in ESI- mode with SWATH® Data-Independent Acquisition. Precursor ion data for m/z 100-1200 Da was collected with accumulation time = 200 ms. The ion spray voltage was set at -4500 V (ESI-) and temperature was set to 525°C with an ion source gas pressure of 60 psi, a curtain gas pressure of 35 psi, and a collision (CAD) gas pressure of 10 psi. For QTOF scanning, the collision energy was set to -5 V and the declustering potential was set to -20 V, both with a spread of 0 V. Product ion (MS/MS) scanning for m/z 50-1200 Da was conducted in the following mass m/z windows (Da): 100 – 150, 149 – 200, 199 – 250, 249 – 300, 349 – 400, 399 – 450, 449 – 550, 549 – 650, 649 – 800, 799 – 1200. For each SWATH window, the accumulation was 50 ms and collision energy was -35 V with spread of 30 V. Fluoride ion-selective electrode (ISE) analysis was used to quantify fluoride release into reactor liquid during HALT reactions of PFOS adsorbed on MAC.

The sample analyzed was the diluted reactor liquid – the liquid reactor contents diluted by the Milli-Q water used for MAC recovery.

Evaluation of HALT-PFAS for MAC recovered from PFAS-contaminated soil. After establishing that PFOS, among the most recalcitrant of PFASs during HALT treatment, can be effectively degraded and defluorinated when adsorbed on virgin MAC in closely controlled environment, experiments were undertaken to assess HALT treatment of MAC recovered from soils in Task 3. MAC was recovered from AFFF-contaminated soil recovered from a site in San Antonio, TX. These soil-derived MAC samples were subjected to hydrothermal treatment in a similar manner described in Task 4a and were extracted and analyzed for PFASs by LC-QTOF-MS as described above. No attempt was made to measure fluoride generation due to the low adsorbed PFAS concentrations. Due to sample limitations, the mass of MAC treated in individual batch reactions was reduced to 200 mg and extractions were performed on approximately 60 mg of sorbent. A smaller extract volume of 20 mL was generated following two rounds of extraction into 10 mL of the methanol-ammonium acetate mixture described in Task 4a.

4. Results and Discussion

Task 1: Determine factors affecting the biodegradation of soil-bound precursors

The goal of Task 1 was to collect proof-of-concept data to determine factors such as nutrients and co-contaminants of PFAS such as hydrocarbon surfactants, on precursor biodegradation. Research efforts were first placed on characterization of constituents of Capstones 1157 and validation of a close mass balance of PFAS. Biodegradation potential of Capstone 1157 by a known 6:2 FtS-degrading strain, RHA1, under different nutrient conditions were conducted. Meanwhile, research efforts were also placed on assessing effects of hydrocarbon surfactants on the degradation ability of 6:2 FtS by strain RHA1.

(i) Capstone Characterization

Non-volatile PFAS by LC-QTOF. Three commercial reference materials were initially characterized including Capstone 1157, Forafac 1157, and Forafac 1157N at four dilutions (1:1K, 1:1M, 1:10M, and 1:100M) to get all PFAS on scale. Upon analysis, it was determined that all three reference materials were similar in their PFAS composition. Only Capstone 1157 was used in subsequent microcosms.

Currently, there are no positive mode mass labeled surrogates available for purchase for any components in Capstone. Thus, Capstone dilutions were spiked with mass-labeled d3-prometon as a surrogate and mass labeled d10-diphenyl urea for use as an internal standard. Analyses for target and suspect PFAS were performed in positive- and negative mode-liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF) on a SCIEX X500. SciexOS processing software, a custom PFAS library, and the NIST PFAS List (<https://data.nist.gov/od/id/mds2-2387>)³⁴ were used to identify target and suspect PFAS. Calibration curves were established for the target compounds (Table 1.1B) using native standards from Wellington and d3-prometon as surrogate.

Suspects (8:2, 10:2, and 12:2 FtSaB, and 6:2 and 8:2 FtSaAm) were quantified using 1:1 matching of compounds with the same, or similar, head groups, and then a mass adjustment was made to account for differences in mass from native standard to known suspect. Below in Table 1.1A that shows the native standards used in the 1:1 matching for each known suspect.

Table 1.1A. Native matching for 1:1 known suspect quantification

Known suspect compound	Native standard used for 1:1 matching
8:2 FtSaB	6:2 FtSaB
10:2 FtSaB	6:2 FtSaB
12:2 FtSaB	6:2 FtSaB
6:2 FtSaAm	PFHxSaAm
8:2 FtSaAm	PFHxSaAm

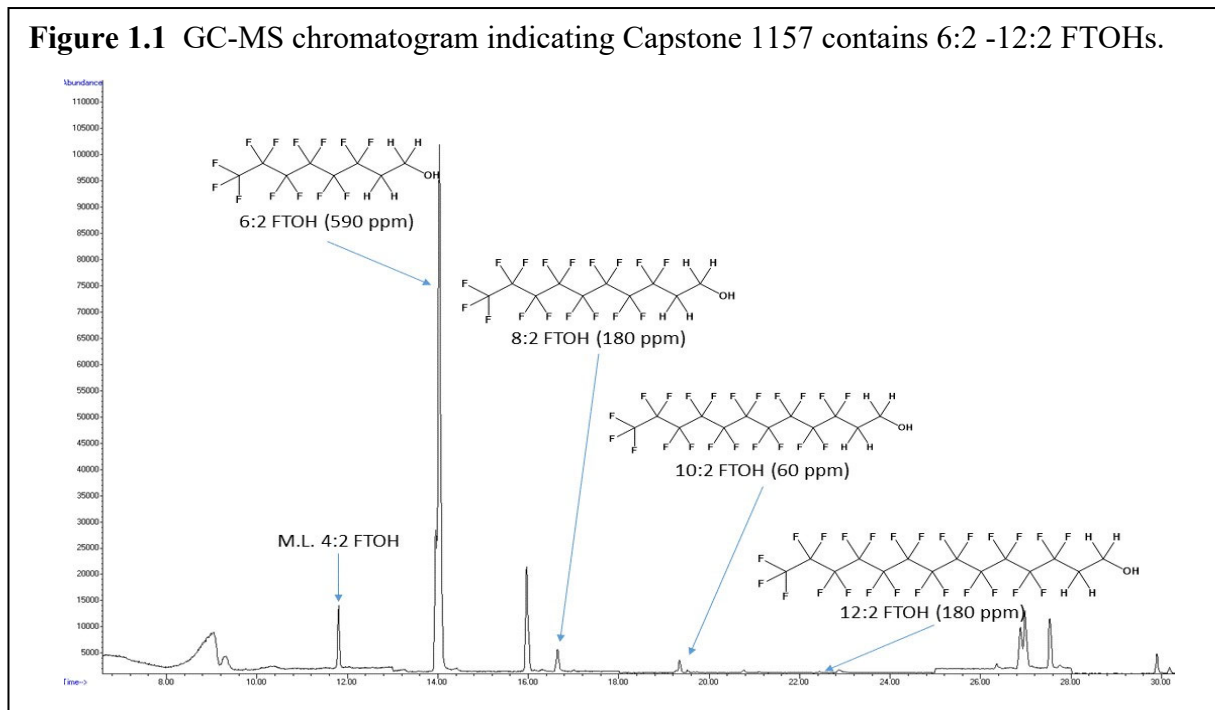
Table 1.1B. Target (T) and suspect (S) PFAS concentrations in Capstone 1157.

	N:2	Concentration (g/L)	Concentration (mmol F/L)
FtSaB	6:2 (T)	144	3280
	8:2 (S)	0.35	8.8
	10:2 (S)	N/A	N/A
	12:2 (S)	0.15	4.3
FtSaAm	6:2 (S)	26	655
	8:2 (S)	0.07	2.0
FtS	4:2 (T)	N/A	N/A
	6:2 (T)	14	425
	8:2 (T)	N/A	N/A
	10:2 (T)	N/A	N/A
FTOH	6:2 (T)	1.53	55
		Total	4,428

^aFtSaB = fluorotelomer sulfonamido betaine; FtSaAm = fluorotelomer sulfonamido amine; FtS= fluorotelomer sulfonates; FTOH = fluorotelomer alcohol; *NA= not analyzed; (T) = target compound; (S) = suspect compound

Volatile PFAS Analysis by GC-MS. Characterization of Capstone was performed by gas chromatography mass spectrometry (GC-MS) in positive chemical ionization and selected ion

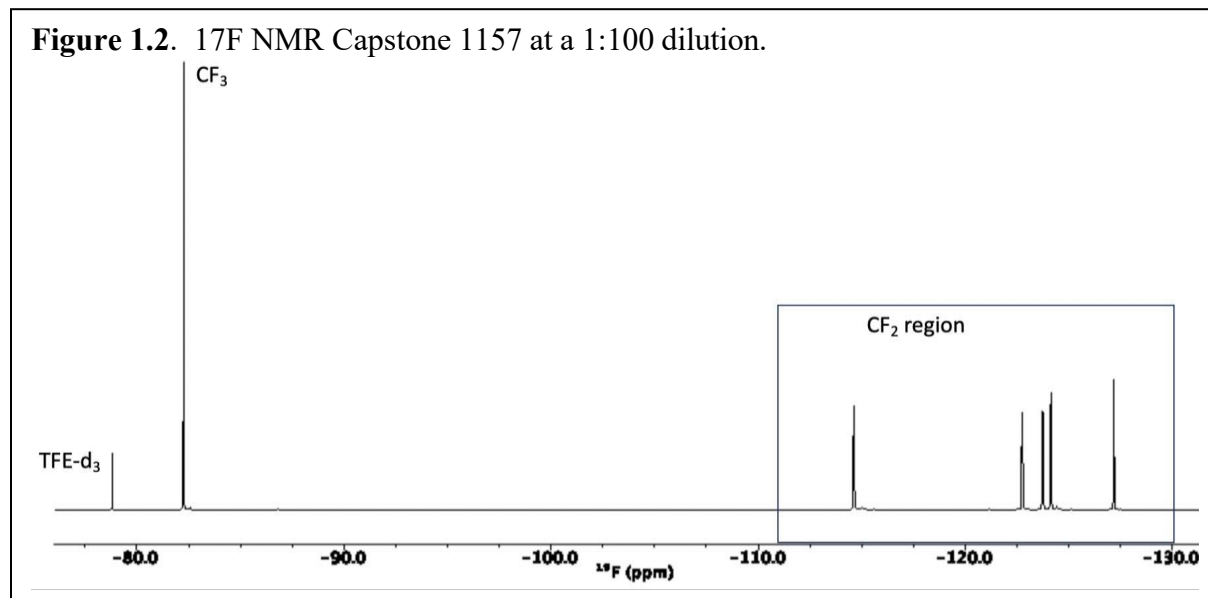
Figure 1.1 GC-MS chromatogram indicating Capstone 1157 contains 6:2 -12:2 FTOHs.



monitoring mode. Prior to analysis, Capstone 1157 was diluted 1:10 with methanol, spiked with 72 μL of 2.5 ng/ μL mass labeled (ML) standards. A 30 μL aliquot of the extract was added to an autosampler vial with 258 μL of methanol and spiked with 12 μL of 2.5 ng/ μL 7:1 fluorotelomer alcohol as the recovery (internal) standard for an additional 1:10 dilution. Analyses were performed via temperature programmed gas chromatography-large volume (10 μL) splitless injection on an Agilent 6890 Gas Chromatograph interfaced with an Agilent 5973N Mass Spectrometer. The GC-MS chromatograms indicated that all three commercial reference materials contain background concentrations of volatile PFAS. While Forafac 1157 and 1157N were comprised of primarily 6:2 FTOH, Capstone 1157 was comprised of 6:2 FTOH – 12:2 FTOH (**Figure 1.1**).

Total Fluorine Comparison to Fluorine Nuclear Magnetic Resonance (NMR). NMR measurements were performed to determine total fluorine in Capstone 1157. Capstone was diluted to factors of 1:50, 1:100, and 1:500 and spiked to give 10.1 mM trifluoroethanol- d_3 (internal standard). NMR experiments were carried out using a Bruker 800 MHz Avance IIIHD NMR spectrometer equipped with a 5 mm TCI cryogenic probe. For ^{19}F experiments, data were collected using a 90-degree pulse with a spectral window of 237.2 ppm, 65536 complex points, and a recycle delay of 15 s. Data collection was automated using a SampleCase and IconNMR software (Bruker). Data processing was automated using custom python scripts. Data were apodized, zero-filled, Fourier transformed, and phased using nmrPipe.³⁵ Baseline correction was performed using the python library pybaselines and the included doubly reweighted penalized least squares Whittaker smoothing function. Regions of interest were integrated using nmrglue.³⁶ Regions were defined as total fluorine, 0 - -200 ppm, $-\text{CF}_3$ -70 - -85 ppm, TFE -76.73 - -76.82 ppm. Spectra were referenced to CFCl_3 by adjusting the trifluoroethanol resonance to -76.76 ppm.³⁷

The total fluorine by ^{19}F NMR was 7,330 mmol F/L. The spectrum (**Figure 1.2**), was simple with few signals in the CF_2 region and only 23% of the total fluorine in the CF_3 group, which was consistent with the linear 6:2 FtSaB being the primary component of Capstone (**Table 1.1**).



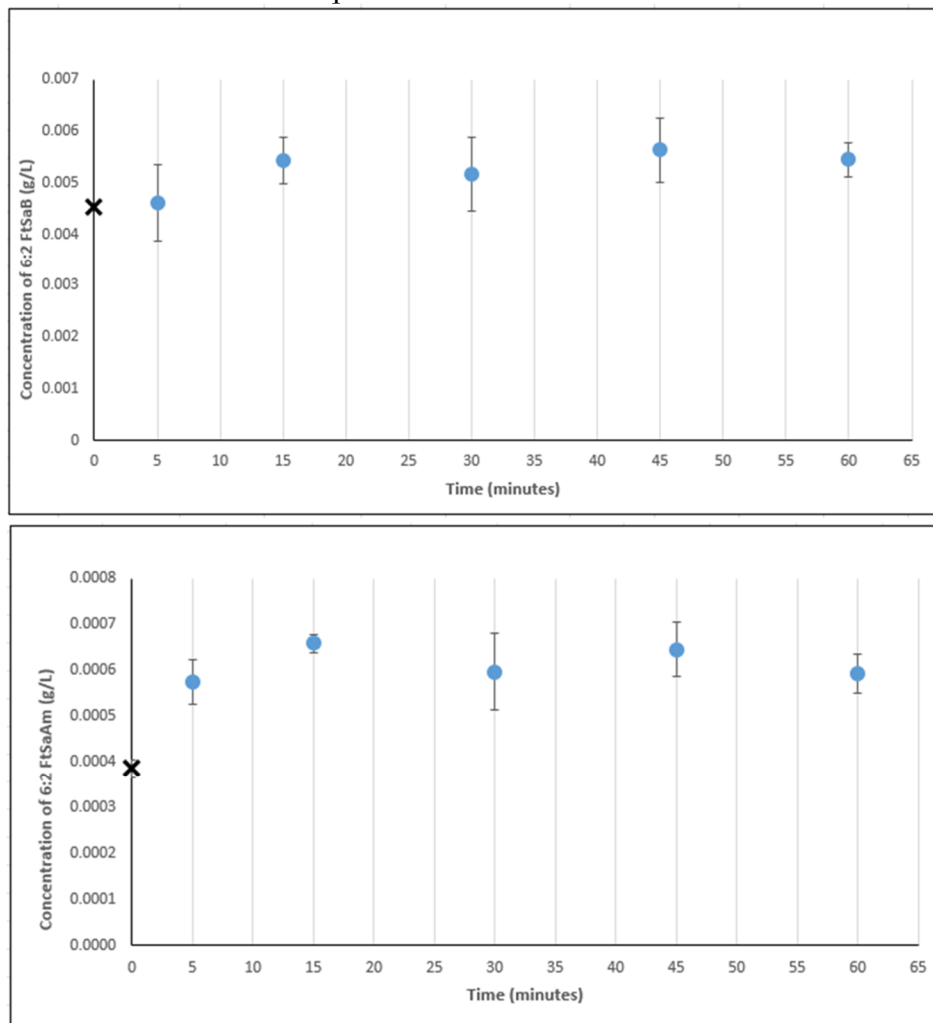
(ii) Microcosm Setup for mass balance of PFAS. A number of experiments were performed to determine that the material and chemicals (Capstone) selected did not produce negative (loss) or positive (background) artifacts.

PFAS Background Analysis on HDPE Bottles and Microcosm Media. The HDPE bottles used for the microcosms were analyzed to determine if there were any background non-volatile or volatile PFAS. The analysis included the whole bottle, the bottom of the caps that are in contact with the medium and solutions, and the medium itself. For blank analysis, three aliquots of 20 mL ACN were added to the HDPE bottles containing 10 mL of the medium mix and 50 mL Millipore water. The solution was stirred at 150 rpm at room temperature for 1 hour, making sure to rinse the cap to include it in the analysis. The step was repeated two more times for a total of 60 mL ACN. A 3 mL aliquot was removed and subjected to analysis. Extracts were analyzed directly and also at 1:1 K and 1:1 M. All non-volatile PFAS were below the LOD. All non-volatile and volatile PFAS were below the LOD for the whole bottle, the cap, and the medium used.

Capstone 1157 Recovery from HDPE bottles containing microcosm media. The recovery of Capstone 1157 components was evaluated in triplicate microcosms containing media. HDPE bottles were spiked with 100 mL of the 3 g/L Capstone 1157 by weight in methanol containing 10 mL of media. All bottles were stirred constantly at 150 rpm for 24 h. During the 24 h, 100 mL samples from the bottles were added to 900 mL of MeOH for a 1:10 dilution in polypropylene centrifuge tubes. Samples were taken in time increments of 5, 15, 30, 45, and 60 min and at 24 h. The bottles were then re-capped, wrapped in parafilm, and stored at 4 °C for 5 days. After 5 days, the bottles were removed and stirred at 150 rpm to room temperature and the same sample process ensued. The bottles were then re-capped and sealed with parafilm and stored at 4 °C for analysis.

Upon analysis via LC-QTOF, it was determined that there was no loss of 6:2 FtSaB or 6:2 FtSaAm onto the HDPE containers from the media (**Figure 1.3**). The data above supports the use of HDPE bottles for the microcosm time course study and dilution of aqueous samples for direct injection analysis as there was no apparent loss of any Capstone 1157 components from solution.

Figure 1.3. Concentrations of a) 6:2 FtSaB and b) FtSaAm in blank microcosms over 60 min period.



Volatile PFAS in Gas Phase Analysis. A series of experiments were performed to demonstrate capture of volatile PFAS onto C18 cartridges for headspace analysis. The volatile PFAS initially studies included 6:2 FTOH, which is the major FTOH in Capstone 1157, and 5:2 sFTOH, which is a suspected degradation product from 6:2 FTOH.

The volatilization and capture of 6:2 FTOH and 5:2 sFTOH on to a C18 cartridge were performed at TAMU. To a 120 mL HDPE bottle, 50 μ L of 50 mg/L 6:2 FTOH or 5:2 sFTOH in methanol were added. Replicates were not performed at this time due to C18 cartridge limitations from supply chain backorder. Standards were volatilized in the bottle at room temperature and the headspace of the bottle was purged with compressed air at flow rate of 0.5 L/min for 6 min into the C18 cartridge. Once completed, C18 cartridges were immediately wrapped in foil and stored at -15 $^{\circ}$ C. The C18 cartridges and HDPE bottles used in this study were then shipped on ice via overnight shipping from TAMU to OSU for analysis. At OSU, following the C18 SPE cartridge elution method in Zhao et. al.³⁸ and a solid phase extraction (SPE) manifold, 5 mL of methanol

was added to a reservoir above the cartridge. Solvent was then eluted through the cartridge and collected into a 15 mL polypropylene centrifuge tube. A 250 μ L volume of extract was added to an autosampler vial together with 32 μ L of methanol and spiked with 12 μ L of 2.5 ng/ μ L 7:1 fluorotelomer alcohol internal standard. In addition, to determine if there was significant mass of 6:2 FTOH or 5:2 sFTOH loss to the inside of original 120 mL HDPE bottle was washed with 5 mL of methanol. A 250 μ L aliquot of the wash was added to a second autosampler vial with 32 μ L of methanol and spiked with 12 μ L of 2.5 ng/ μ L 7:1 fluorotelomer alcohol internal standard. Analyses were then performed by GC-MS as described above.

The recoveries of 6:2 FTOH and 5:2 sFTOH were 116 and 52%, respectively (**Table 1.2**). It was speculated that the initial low recovery of the 5:2 sFTOH was due its higher volatility in comparison to the 6:2 FTOH. Additionally, it was determined that no 6:2 FTOH or 5:2 sFTOH remained in the HDPE bottles since the bottle wash aliquot was below the LOD for 6:2 FTOH and 5:2 sFTOH.

Table 1.2. Recovery of 6:2 FTOH and 5:2 sFTOH standards from C18 cartridge.

	Spiked Mass (ug)	Recovered Mass (ug)	Percent Recovery
6:2 FTOH	2.5	2.9	116%
5:2 sFTOH	2.5	1.3	52%

Additional C18 cartridges spiked with 50 pg/ μ L mass labeled surrogate were sent to TAMU to repeat this study. To consider the loss of the 5:2 sFTOH due to breakthrough, two cartridges were stacked in tandem with each other during the purging process and sent back to OSU for analysis. The “bottom cartridge” referred to the cartridge attached to the HDPE bottle and the “top cartridge” was the cartridge stacked on top. The study was done in duplicate; however, the spiked mass was now 1.25 ug due to the limited quantity of the 5:2 sFTOH standard. Using the same process as described above, the cartridges were eluted and analyzed with gas chromatography- mass spectrometry. Recoveries of 73 and 80% were observed for 5:2 sFTOH (**Table 1.3**. Breakthrough below the instrumental limit of quantification (LOQ) was observed on the second C18 cartridge.

Table 1.3. 5:2 sFTOH tandem C18 cartridge stacking recovery.

	Spiked Mass (ug)	Recovered Mass (ug)	Percent Recovery
5:2 sFTOH Bottom Cartridge R1		1.0	
5:2 sFTOH Top Cartridge R1	1.25	<LOQ	80%
5:2 sFTOH Bottom Cartridge R2	1.25	0.91	73%
5:2 sFTOH Top Cartridge R2		<LOQ	

Another set of experiments was performed to meet two objectives. The first was aimed towards understanding the potential loss of mass-labeled surrogates spiked onto C18 cartridges (used for headspace analysis of microcosms) during transit from OSU to TAMU, the purging process, and transit back to OSU. The second was to determine if volatile PFAS in Capstone could be detected in the headspace of microcosms containing Capstone 1157 and medium with or without soil before the addition of RHA1 at time zero.

Microcosms were fitted with C18 Maxi-Clean s*Pure cartridges in order to trap any potential gas-phase (volatile) PFAS. Prior to shipment to TAMU, the cartridge was washed with 5 mL of methanol on a SPE manifold. Immediately after, 120 µL of mass labeled volatile PFAS surrogates were spiked directly to the top of the cartridge. Cartridges were then wrapped in foil and stored in a -15 °C freezer. Cartridges were then shipped to TAMU on ice via overnight express shipping. To simulate sampling the headspace of a microcosm, at TAMU, the headspace of the bottle was purged with compressed air at flow rate of 0.5 L/min for 6 min into the C18 cartridge. Once completed, C18 cartridges were immediately wrapped in foil and stored at -15 °C. The C18 cartridges and HDPE bottles used in this study were then shipped on ice via overnight shipping from TAMU to OSU for analysis.

At OSU, following the C18 SPE cartridge elution method in Zhao et. al,³⁸ 5 mL of methanol was added to a reservoir above the cartridge. Solvent was then passed through the cartridge and collected into a 15 mL polypropylene centrifuge tube. A 250 µL volume of extract was added to an autosampler vial together with 32 µL of methanol and spiked with 12 µL of 2.5 ng/µL 7:1 fluorotelomer alcohol internal standard. In addition, to determine if there was significant mass of 6:2 FTOH or 5:2 sFTOH loss to the inside of original 120 mL HDPE bottle was washed with 5 mL of methanol. A 250 µL aliquot of the wash was added to a second autosampler vial with 32 µL of methanol and spiked with 12 µL of 2.5 ng/µL 7:1 fluorotelomer alcohol internal standard. The mass on the eluted from the cartridge was measured by GC-MS as described above.

Recoveries of the spiked mass-labelled standards fell within $\pm 30\%$ (**Table 1.4**), except for Et- and Me-FOSA, which were above 130%. The origin of high recovery for Et- and Me-FOSA are currently being investigated. Target PFAS (not labelled) were all below the instrumental LOD in the headspace of these microcosms.

Table 1.4. Average (from duplicates) percent recovery of mass-labelled surrogates of volatile PFAS from cartridges installed on microcosm with and without soil.

	4:2 FTOH	6:2 FTOH	8:2 FTOH	10:2 FTOH	Et FOSA	Me FOSA	Me FOSE	Et FOSE
AMS/ Capstone + Soil R1	80%	80%	71%	65%	170%	196%	81%	72%
AMS/ Capstone + Soil R2	97%	105%	86%	83%	194%	226%	85%	102%

AMS/ Capstone R1	100%	107%	87%	66%	196%	232%	104%	92%
AMS/ Capstone R2	88%	81%	81%	80%	168%	205%	77%	80%

Spiked cartridges were then attached to actual microcosms containing Capstone and a sample was taken at time zero. No volatile PFAS were detected in the headspace above Capstone in microcosms, which was consistent with the low levels in Capstone (Table 1.1) and the instrument's detection limit.

(ii) Biodegradation potential of Capstone by RHA1 strain under different nutrient conditions. Oxygen consumption and CO₂ production in the bottles receiving different nutrient treatment were monitored over time. As shown in **Figure 1.4** and **Figure 1.5**, except for the killed treatment, O₂ depleted sharply from 21% to 13-16 % and CO₂ produced quickly from 0.01% to 1.5-2.5% in first 72 hours (Day 3) for every treatment, and then slowly leveled until Day 9. Oxygen consumption was fast for nutrient rich conditions until Day 21, but much less for the rest. After additional 12 days of incubation (504 hour or Day 21), O₂ and CO₂ in nutrient-rich treatment changed significantly to from 15.3% to 2.3% and 2.8 % to 3.8%, respectively. However, the O₂ and CO₂ remain stable in other treatment. The results indicated that the growth of RHA1 might be limited by the available sulfur, nitrogen, or carbon under these test conditions.

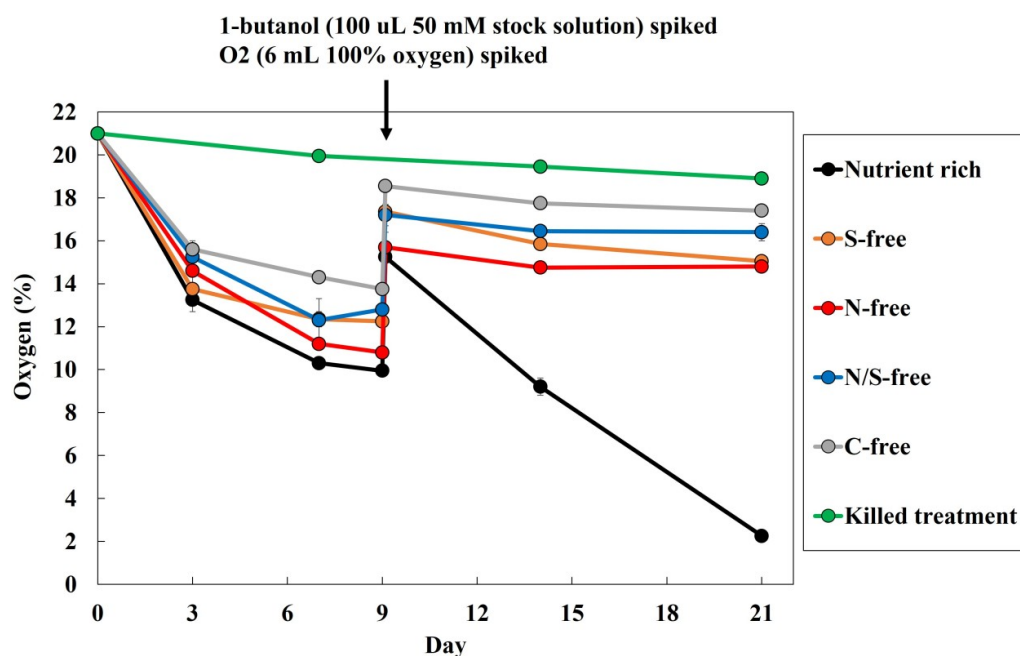


Figure 1.4. Oxygen consumption in each treatment over 21 days.

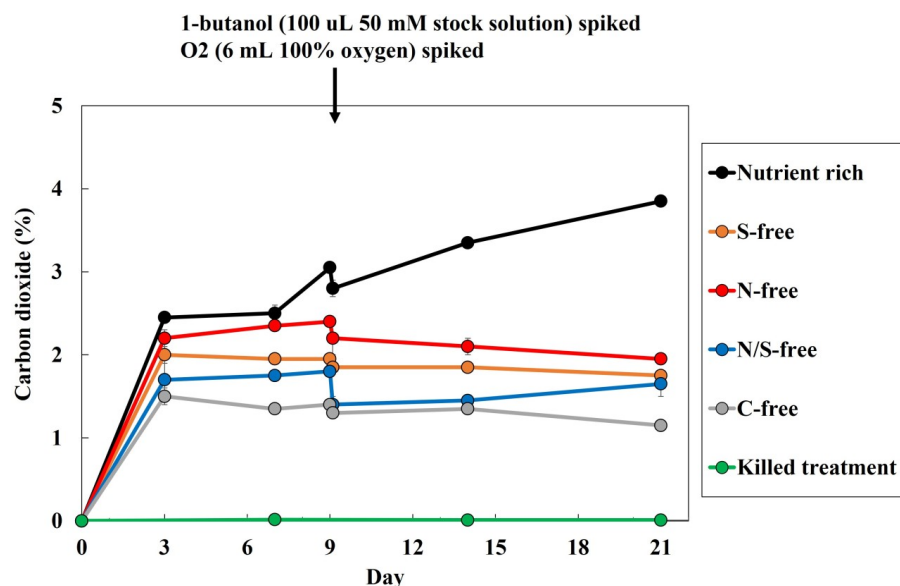


Figure 1.5. Carbon dioxide production in each treatment over 21 days.

Although FtSAB and FtSaAm did not decrease significantly in any of the treatments, 6:2 FtS (a major component also in Capstone 1157) did show significant decrease over the microcosm time course study in only the S-free medium condition. Two biodegradation products were also observed in the S-free medium condition where the 6:2 FtS decrease was observed. The 6:2 fluorotelomer unsaturated carboxylic acid (6:2 UFTCA) was found for all days (besides the time zero samples) at fairly high amounts (**Figure 1.6**). The 5:3 fluorotelomer carboxylic acid (5:3 FTCA) was only seen in the S-free condition on Day 14 at just above the instrumental LOQ. No other perfluoroalkyl carboxylates were observed in the microcosm samples.

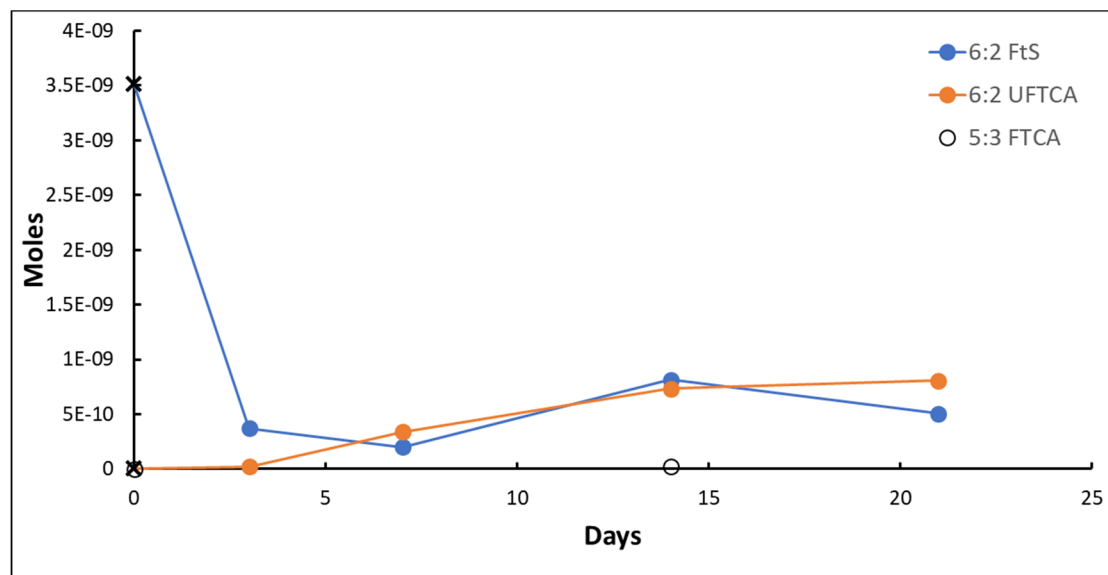


Figure 1.6. 6:2 FtS (moles) and two degradation products 6:2 UFTCA and 5:3FTA (at LOQ) in S- free microcosms; no decrease observed for other microcosm conditions (see Day 21).

(iii) **Negative and positive effects of hydrocarbon surfactants on 6:2 FtS degradation by RHA1 strain.** As shown in **Figure 1.7**, different O₂ consumption and CO₂ production by RHA1 were observed in the presence of different hydrocarbon surfactants, indicating that RHA1 remained active but metabolically inhibited by the presence of these hydrocarbon surfactants. The consumption of oxygen and production of carbon dioxide in PEG 400, SDS, DGBE, and hexylene glycol were greater than those in X-100, X102, and CPB.

Fluoride release was used as a quick assessment of the inhibitory effects of the surfactants on the defluorination ability of the 1-butanol-grown RHA1 (**Figure 1.8**). Compared to the live controls, higher oxygen consumption and carbon dioxide production were observed for those receiving DGBE and hexylene glycol (**Figure 1.7**). Interestingly, these sets also showed similar or higher fluoride release compared to those of the live controls (**Figure 1.8**). The higher metabolic activity (i.e., O₂ consumption and CO₂ production) along with the higher defluorination in the presence of DGBE and hexylene glycol, especially in their relatively low concentrations, suggested that RHA1 might be able to use these surfactants for growth or these surfactants might serve as inducers for unknown defluorination enzymes. Thus, more experiments are planned to better understand the underlying mechanisms triggered by the presence of these two surfactants.

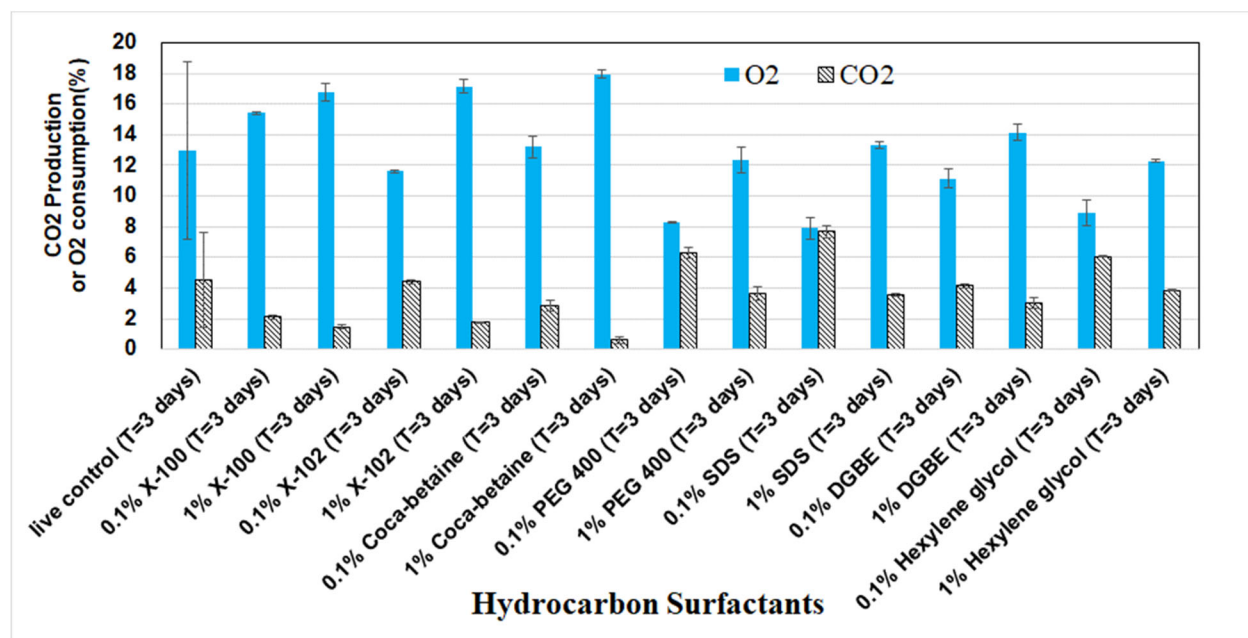


Figure 1.7. Consumption of oxygen and production of CO₂ by 1-butanol-grown RHA1 in the presence of seven different surfactants at two different concentrations.

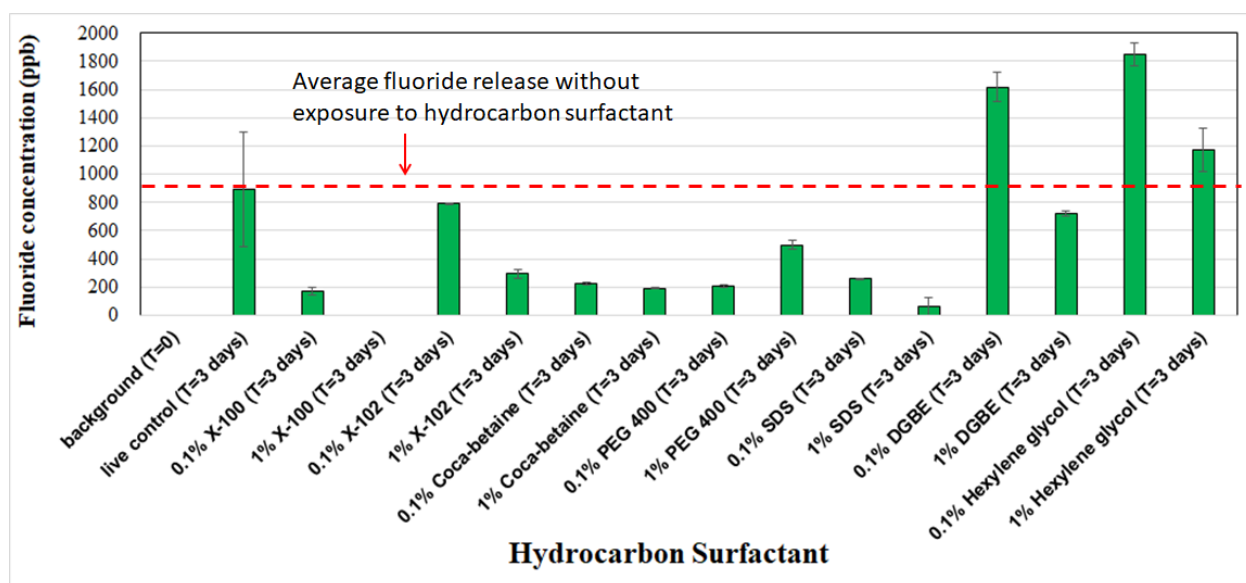


Figure 1.8 Fluoride release during 6:2 FtS degradation by 1-butanol-grown RHA1 in the presence of seven different surfactants at two different concentrations.

Task 2: Accelerate biodegradation of precursors via bioaugmentation

The goal of Task 2 in this limited-scope study was to collect data to determine if three different types of precursors, FTOHs, 6:2 FtS, and 6:2 FtSaB could be biodegraded by bioaugmenting with soil cultures that were enriched with different nutrient condition

(i) Establishment and characterization of stable enrichment cultures in SBRs. After days of incubation at room temperature, all enrichments showed significant growth based on optical density and CO₂ production in the headspace. Sequencing batch reactors (SBR) were set up in a series of large flasks with a working liquid volume of 50 mL using the established C2 enrichments as inocula with its corresponding carbon source. Enrichment cultures grown with 0.05% ethanol, 5mM 1-propanol, 5mM 1-butanol, 0.05% phenol, 0.05% CPB were operated at 1-day SRT and those grown with 0.05% hexane and 0.05% octane were operated at 2-day SRT.

(ii) Microbial community of the C2 soil enrichment cultures. Next generation sequences were performed to characterize the microbial community structure of each enrichment cultures as shown in **Figure 2.1**. The diversity indices of the enrichments were summarized in **Table 2.1**.

Not surprisingly, the diversity and abundance of C2 original soil culture were higher compared to those of the enrichment cultures that received different carbon sources over time. Based on the Shannon index values of enrichment cultures, from low to high, phenol- > ethanol- > CPB- > octane- > propanol- > hexane- > butanol-enriched cultures. The dominant species (Figure 2.2) in each of the enrichment cultures were different from each other, with high relative abundance of *Rhodococcus* in 1-BuOH-enriched cultures, high dominance of 2 genera of *Pseudomonas* and *Variovorax* in the CPB enrichment cultures, and the significant dominance of *Pandora* in the phenol enrichment cultures.

Table 2.1. Alpha diversity indices at sequence depth 33,000 of respective enrichment cultures and original C2 soil microbial communities

	C2 soil	EtOH	1-PrOH	1-BuOH	hexane	octane	phenol	CPB
Shannon's H	4.2±0.2	2.4±0.1	2.9±0.05	3.4±0.05	3.2±0.03	2.8±0.05	2.2±0.05	2.6±0.02
FaithPD	5.3±0.5	7.5±1.6	6.4±0.9	6.8±1.1	4.5±1.0	7.2±0.6	6.1±0.7	3.0±0.7
Observed features	87±10	35±3	36±3	45±4	37±2	44±2	30±2	14±1

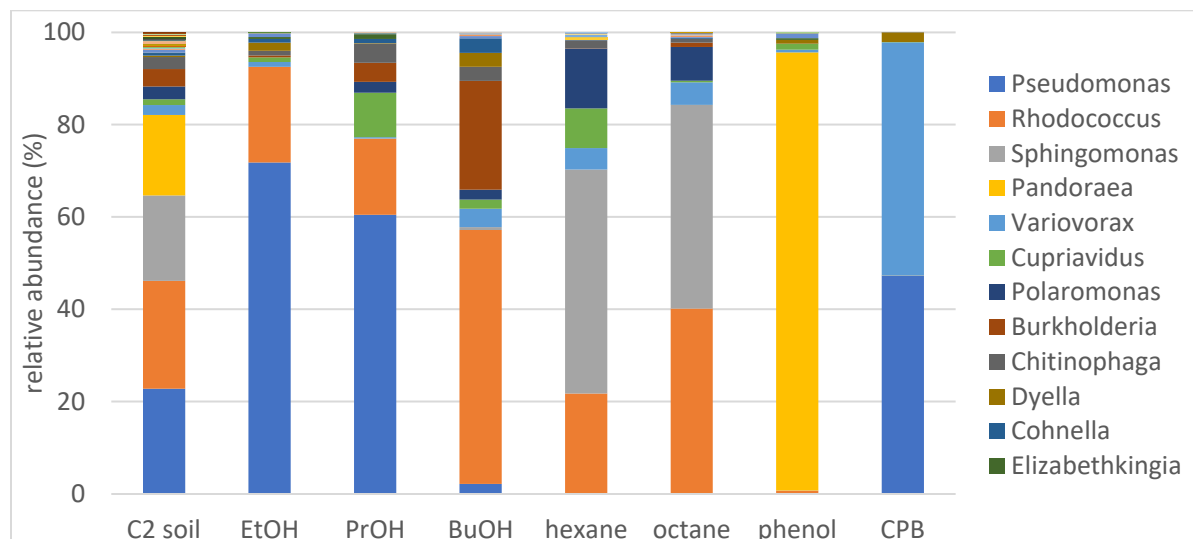


Figure 2.1. Microbial community composition of respective enrichment cultures used for the fluorotelomer-based PFAS degradation resting cell and growth experiments.

(iii) FTOH biodegradation by the cultures enriched with different carbon sources.

Defluorination of FTOHs. Overall, all enrichment cultures were able to defluorinate 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH, based on fluoride ion release in the media. 1-Butanol-enriched cultures showed high defluorination capacities as high fluoride concentrations were observed for all 3 different types of fluorotelomer alcohols (Figure 2.2). Degradation of 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH by 1-butanol-enriched culture resulted in release of fluoride concentrations of 616 ± 181 , 671 ± 50 , and 449 ± 132 ppb F⁻, respectively. Among all the enrichment cultures, ethanol-enriched cultures showed the least fluoride release from 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH degradation. For 4:2 FTOH, 1-propanol-enriched cultures showed the highest fluoride release (857 ± 159 ppb). Cultures enriched with CPB, hexane, and octane also have shown high defluorination ability toward 4:2 FTOH and 6:2 FTOH. Interestingly, cultures enriched with alcohols (i.e., ethanol, propanol, and butanol) showed a similar chain-length-dependent defluorination toward FTOHs from high to low, 4:2 FTOH > 6:2 FTOH > 8:2 FTOH.

Degradation metabolites of FTOHs. Various metabolites of FTOHs degradation by the resting enrichment cultures were observed (Figures 2.3, 2.4, 2.5). Two metabolites, 3:3 FTCA and PFBA, were detected in 4:2 FTOH to be the sole detected metabolites (Figure 2.3). More diverse biotransformation metabolites of 6:2 FTOH were observed, with 5:3 acid only detected in CPB- and propanol-enriched cultures and PFBA detected in all cultures except phenol-enriched culture (Figure 2.4). High amounts of 6:2 UFTCA (i.e., 1430, 1230, and 1588 $\mu\text{g/L}$, respectively) and 6:2 FTCA (i.e., 882, 1192, and 1676 $\mu\text{g/L}$, respectively) were detected from 1-BuOH, hexane, and octane enrichment cultures (Figure 2.4A.). High concentrations of PFHxA (i.e., 53, 54, and 80.4 $\mu\text{g/L}$, respectively) were also detected in same carbon source enrichment conditions (Figure 2.4B.). However, 5:3 FTCA was only detected from 1-PrOH (i.e., 4.98 $\mu\text{g/L}$ 5:2 FTCA) and CPB (i.e., 14.48 $\mu\text{g/L}$ 5:2 FTCA) enrichment conditions with trace PFHpA only being detected from hexane (i.e., 0.678 $\mu\text{g/L}$ PFHpA) and octane (i.e., 0.978 $\mu\text{g/L}$ PFHpA) enrichment resting cells. The major metabolites from 8:2 FTOH biotransformation were 8:2 UFTCA and 7:3 FTCA (Figure 2.5). The high fluoride release observed for 1-BuOH-, hexane-, and octane-enriched cultures concurrently showed the highest 8:2 UFTCA (i.e., 240, 165, and 234 $\mu\text{g/L}$, respectively) and 7:3 FTCA (i.e., 28.2, 3.72, and 3.42 $\mu\text{g/L}$, respectively), suggesting majority of the fluoride release might be due to the production of the corresponding 8:2 UFTCA. In addition, no 8:2 FTCA were detected in all resting cell conditions, indicating a potentially rapid transformation from 8:2 FTCA to 8:2 UFTCA.

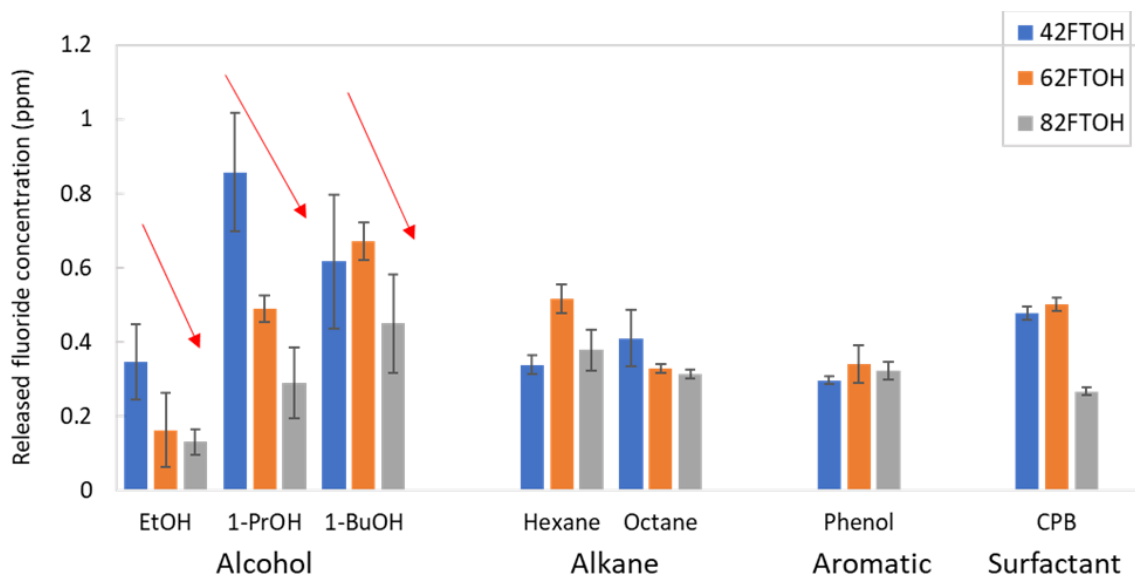


Figure 2.2. Released fluoride concentrations from 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH degraded by the enrichments resting cell experiments.

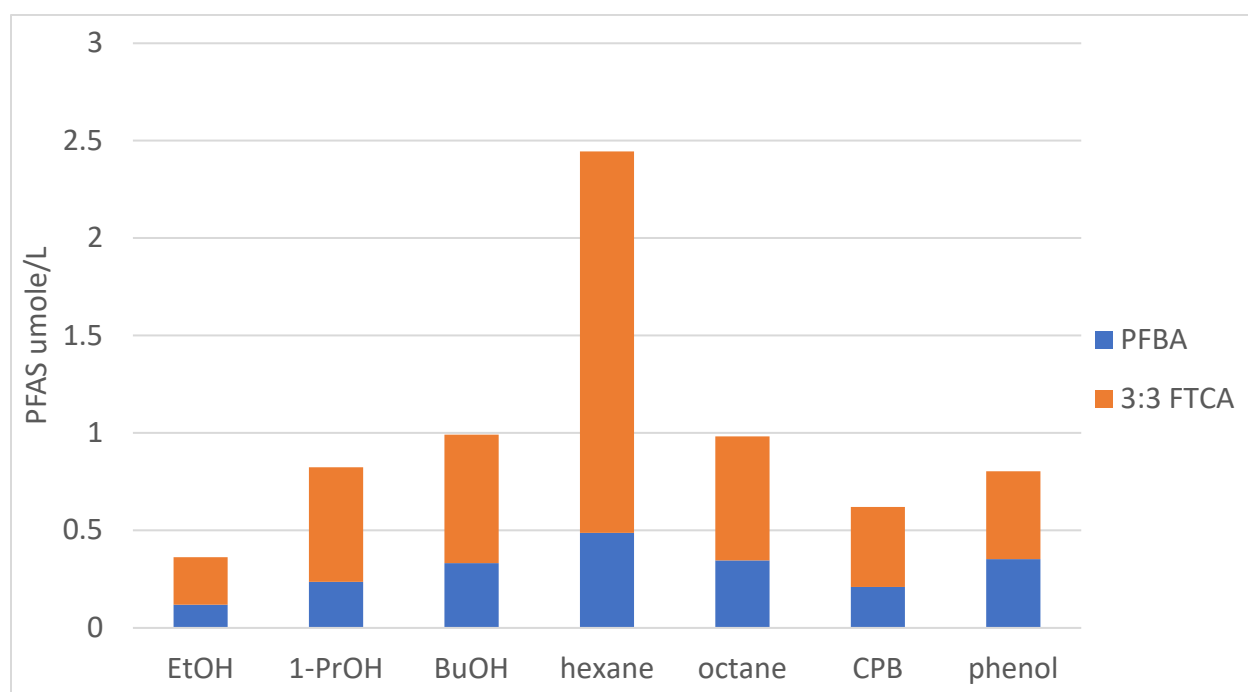
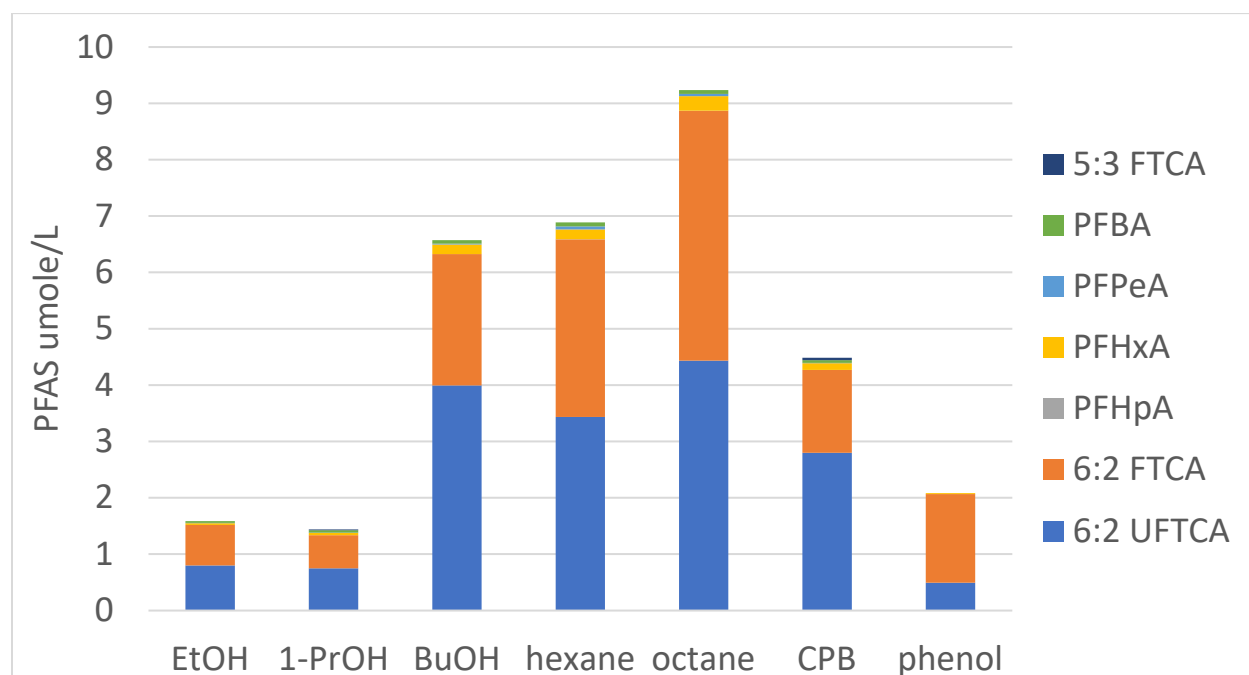


Figure 2.3. Biodegradation metabolites of 4:2 FTOH by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures.

A)



B)

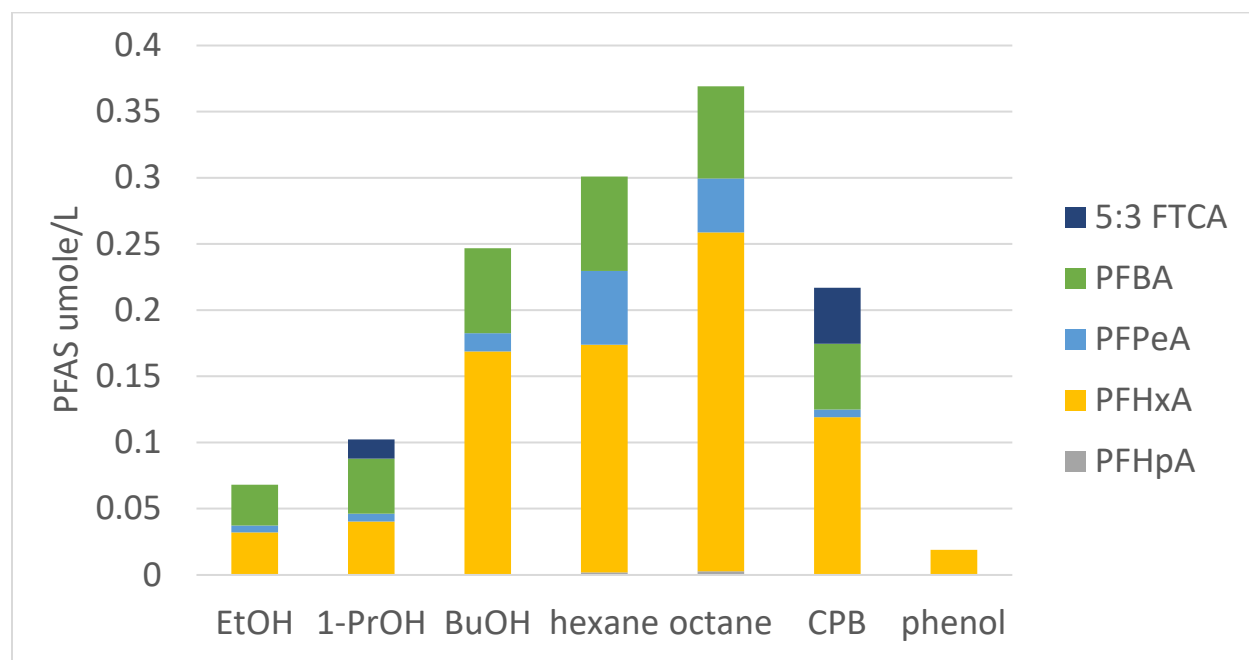


Figure 2.4. (A) PFAS metabolites of 6:2 FTOH degradation by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures, and (B) minor PFAS metabolite concentrations excluding 6:2 UFTCA and 6:2 FTCA from 6:2 FTOH degradation by the enrichment cultures.

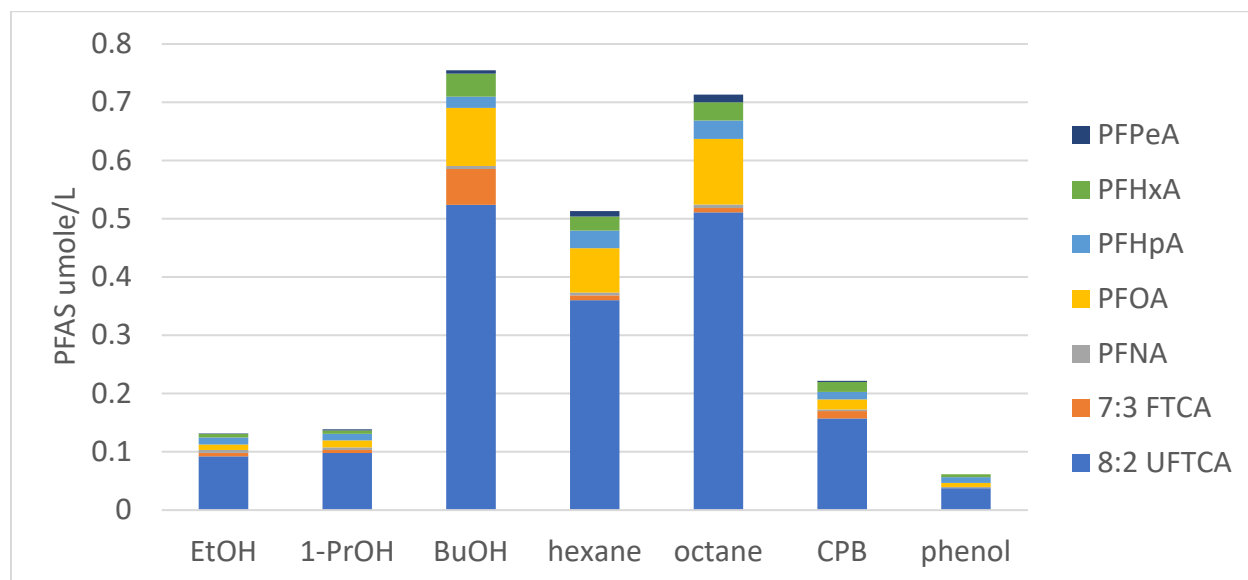
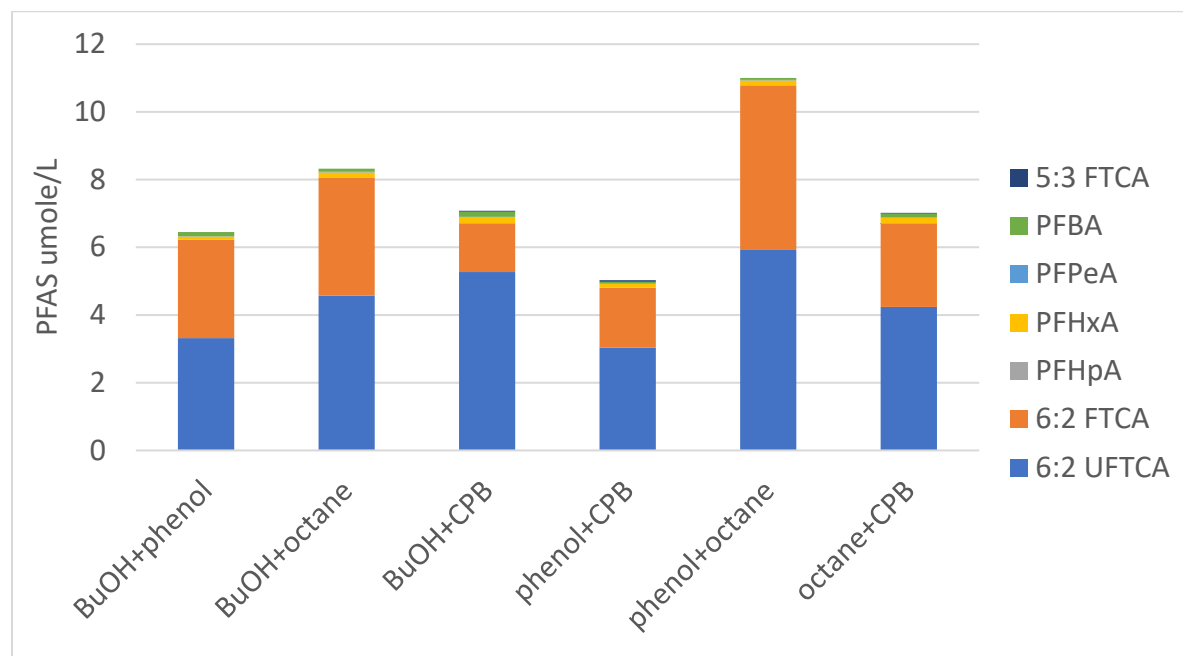


Figure 2.5. Biodegradation metabolites of 8:2 FTOH by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures.

(iv) Biodegradation of 6:2 FTOH by dual enrichments

Based on the fluoride release of FTOHs biodegradation by each enrichment cultures, new set of experiments was conducted to examine whether biodegradation of 6:2 FTOH be enhanced by two enrichments. As shown in **Figure 2.6**, increased metabolite concentrations were observed in the combination of using two enrichments compared to those using single enrichment. The combination of phenol and octane enrichment cultures produced highest concentrations of 6:2 FTUCA (2120 µg/L) and 6:2 FTCA (1838 µg/L). Interestingly, 5:3 FTCA was found in all conditions where the CPB-enriched culture was used as one of two enrichment cultures (i.e., BuOH+CPB, phenol+CPB, and octane+CPB). Note that the CPB enrichment resting cells experiment showed 14.48 µg/L 5:3 FTCA (Figure 2.4B). This indicated CPB enrichment cultures could potentially be responsible in the production of 5:3 FTCA.

A)



B)

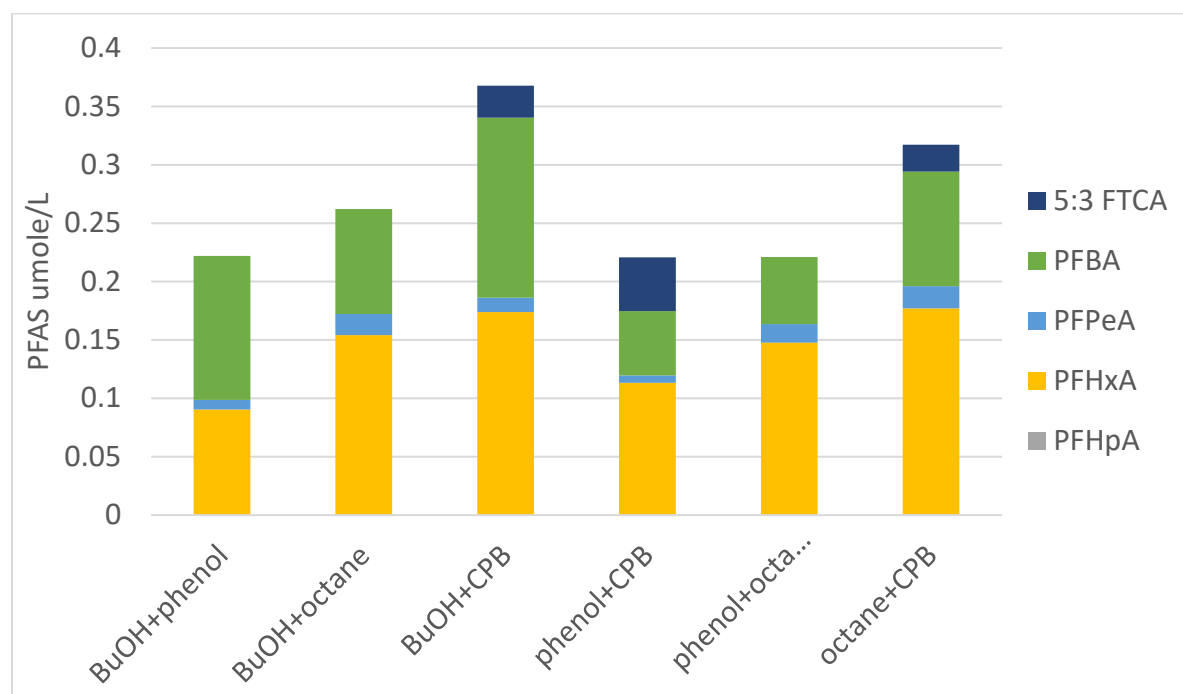


Figure 2.6 (A) All Biodegradation metabolites of 6:2 FTOH by two enrichment cultures, and (B) minor metabolites (excluding 6:2 UFTCA and 6:2 FTCA) of 6:2 FTOH biodegradation by two enrichments.

(v) 6:2 FtS biodegradation by the mixed cultures enriched with different carbon sources under S-free conditions.

All enrichments showed significant turbidity, indicating the enrichments could use 6:2 FtS (20 mg/L) as a sole sulfur source (**Figure 2.7**). High defluorination with 1-butanol was also observed. A much slower growth was observed when hexane was provided as the carbon source compared to other carbon sources. Also, the hexane-enriched culture released the lowest fluoride concentration, only 134 ± 5 ppb fluoride. All enrichment cultures were able to degrade 6:2 FtS (5 mg/L) with different degradation abilities (**Figure 2.8**). The 1-BuOH-grown culture showed near complete depletion of 6:2 FtS (i.e., $0.972 \mu\text{g/L}$) which indicated a high 6:2 FtS desulfonating and degrading capability of the corresponding enrichment culture.

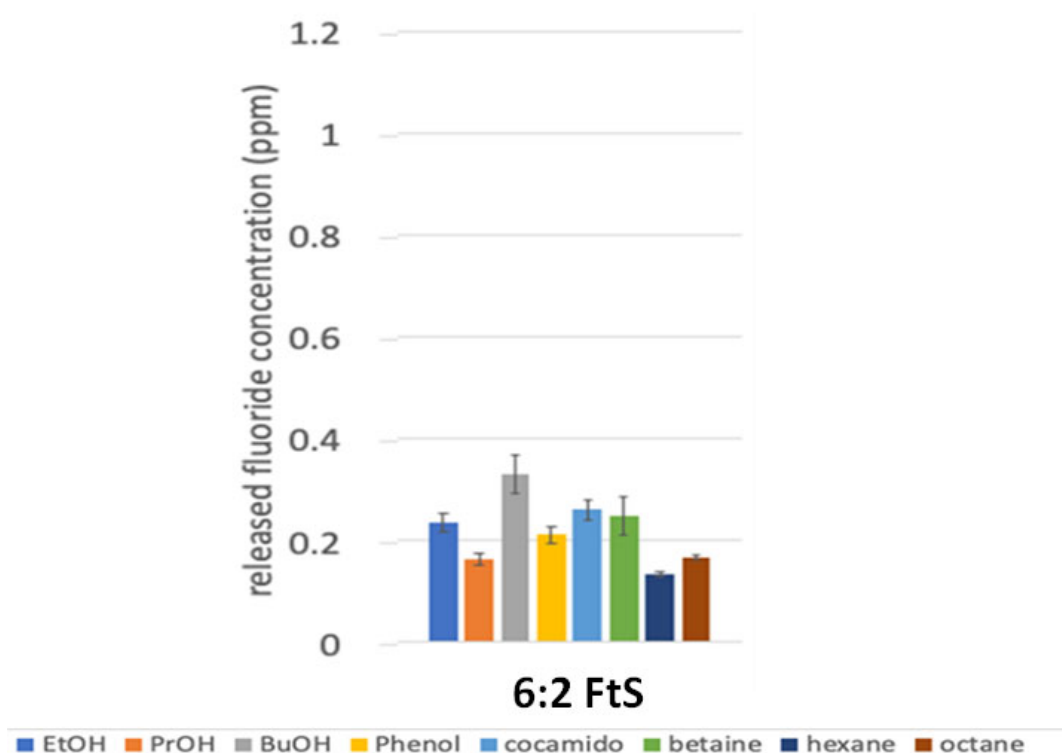
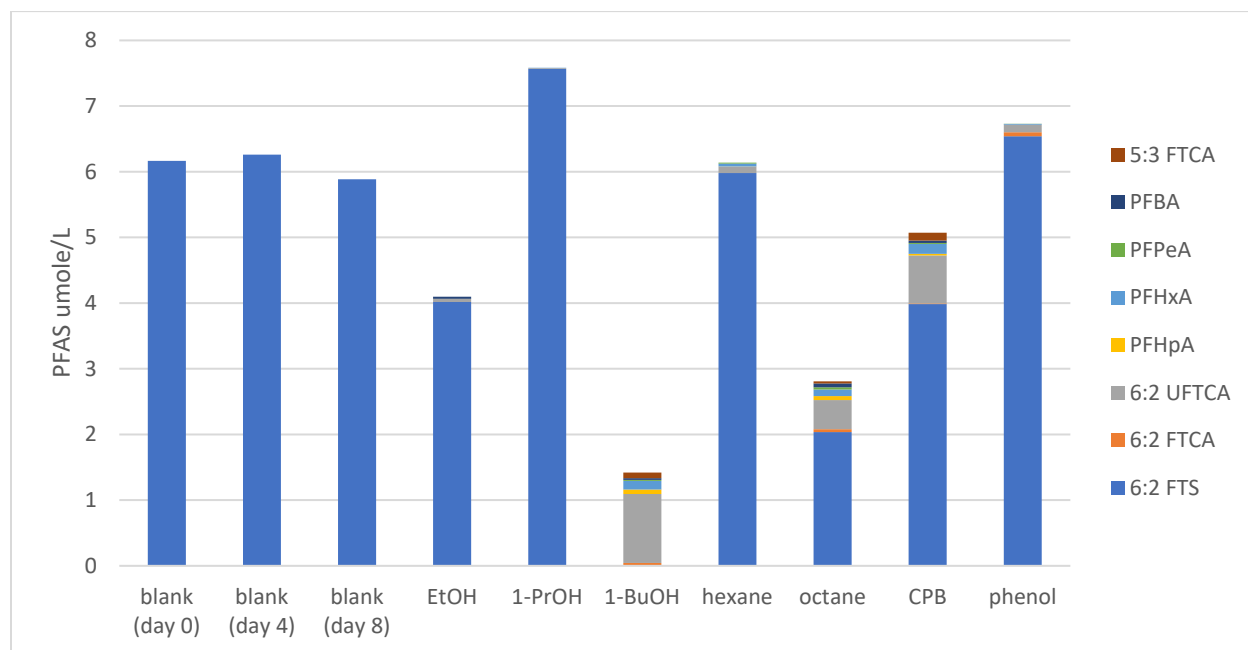


Figure 2.7. Released fluoride from 6:2 FtS when used as S-source for ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures.

A)



B)

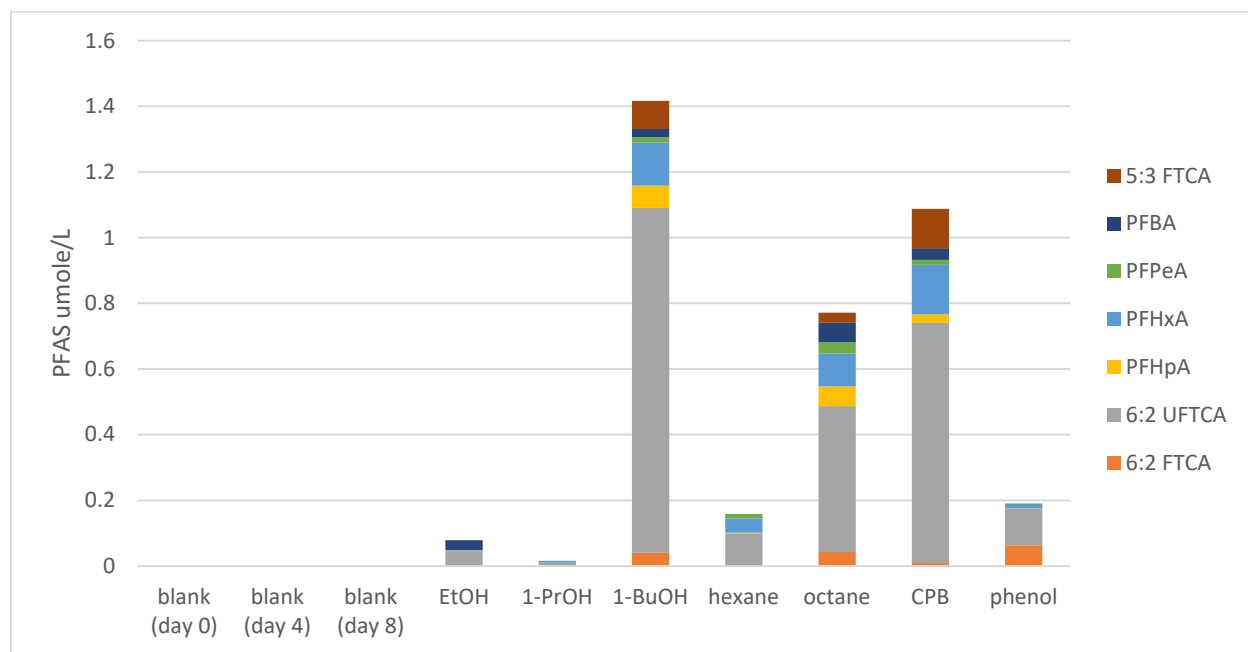


Figure 2.8. (A) All PFAS detected from 6:2 FtS degradation by ethanol-, propanol-, butanol-, hexane-, octane-, phenol-, and CPB-enriched cultures grown in S-free medium. (B) Metabolites (excluding 6:2 FtS) detected during 6:2 FtS degradation by the cultures.

(vi) 6:2 FtSaB biodegradation by the mixed cultures enriched with different carbon sources.

In the first degradation tests of 6:2 FtSaB, no metabolites of 6:2 FtSaB degradation were observed by the enrichment cultures in S-rich medium, suggesting that its high recalcitrance in the environments containing readily available sulfur sources. Thus, two sets of experiments using butanol-, phenol-, octane- and CPB-enrichment cultures was conducted under S-limited conditions. One set was provided with low initial carbon source at time zero, the other set was provided additional respective carbon sources during the course of experiments. As shown in Figure 2.10, no degradation metabolites of 6:2 FtSaB was detected from the octane-enrichment cultures. Interestingly, on day 7, one metabolite, 6:2 UFTCA was detected in both butanol- and phenol-enrichment cultures, with increased concentration of 6:2 UFTCA (on day 9) from both cultures when received extra carbon sources. Another metabolite, 5:3 FTCA, was only detected in CPB-enrichment culture receiving extra carbon sources. The results suggested that 6:2 FtSaB is biodegradable, but the degradation only observed under S-limited conditions. Also, the different microbial community in the enrichment cultures and the availability of carbon sources also affected the type of metabolites produced during 6:2 FtSaB degradation.

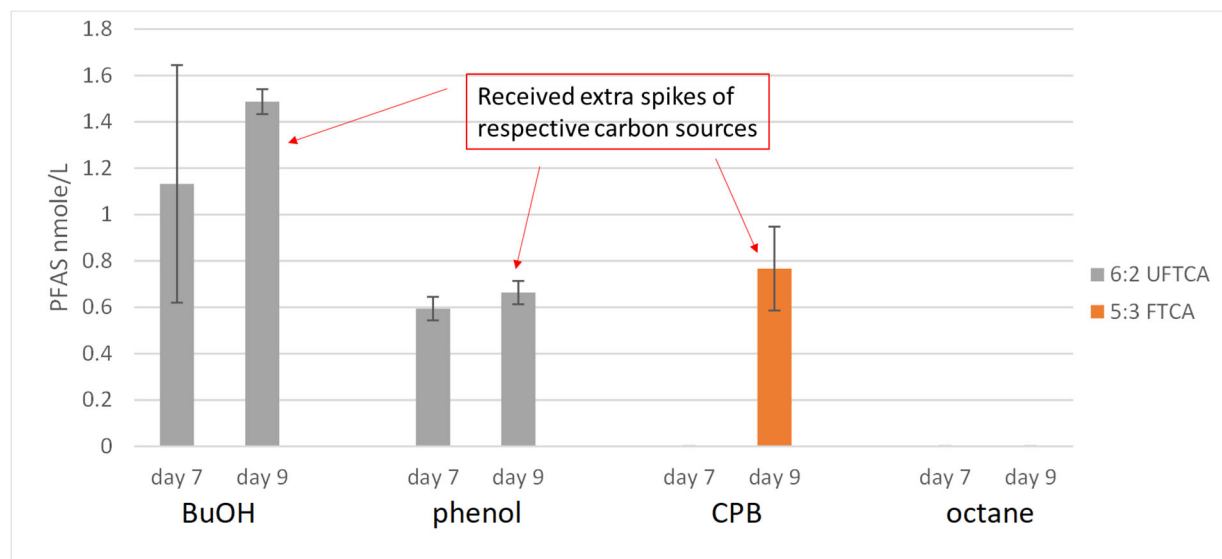
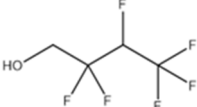
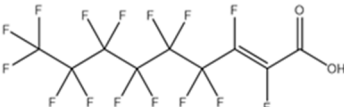
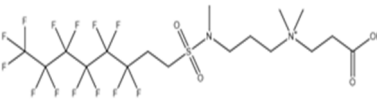
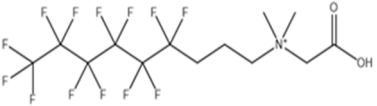
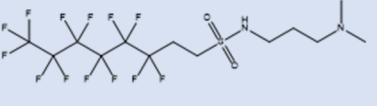


Figure 2.9. A metabolite, 6:2 UFTCA, was detected from biodegradation of 6:2 FtSaB by butanol (BuOH)- and phenol-enrichment cultures in S-limited medium. When received extra spikes of respective carbon sources, higher 6:2 UFTCA was observed in BuOH- and phenol-enrichment cultures, and 5:3 FTCA was detected only in the CPB-enrichment culture.

(vii) Potential suspects found in 6:2 FtS and 6:2 FtSaB microcosm (containing C2 enrichment cultures) time course studies

To date, all suspects found in both negative and positive modes are level 4 suspects, with the exception of one level 2 suspect in positive mode (Table 2.2).

Table 2.2. Potential suspects found in 6:2 FtS microcosm time course study. One suspect found in 6:2 FtSaB microcosm with 1-butanol enrichment culture on day 9 (Blue shaded).

NIST List ID	Ion Formula	Structure	Found at mass (Da)	Retention time (min)	Ionization mode	Confidence Level
2843	C ₄ H ₄ F ₆ O		181.009	4.7	[M-H] ⁻	4
4147	C ₉ HF ₁₅ O ₂		424.965	7.8	[M-H] ⁻	4
3925	C ₁₇ H ₂₄ F ₁₃ N ₂ O ₄ S		599.125	10.8	[M+H] ⁺	2
3795	C ₁₃ H ₁₅ F ₁₃ NO ₂		464.087	16.3	[M+H] ⁺	4
881	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₂ S		511.073	12.81	[M-H] ⁻	4

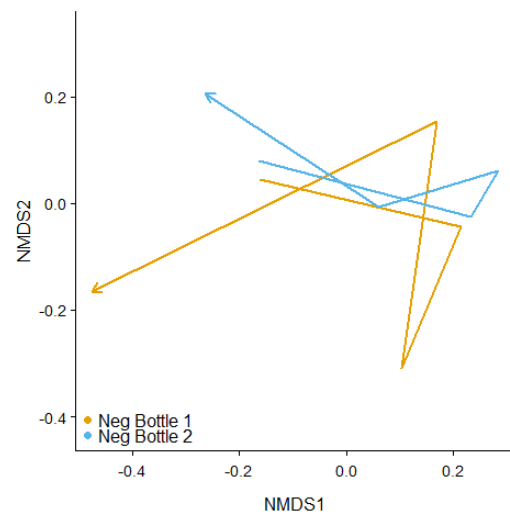
Non-Target Chemical Analysis for Microcosms. We used XCMS to process the HRMS data generated from the analysis (this needs to be linked with Inga's analysis methods). XCMS is an open-source framework for the processing and visualization of high-resolution mass spectral data for untargeted analyte profiling.³⁹ Data analysis focused on MS level 1, though SWATH data can also be analyzed in the future using this framework. The extracted ion chromatograms were inspected visually to develop setting parameters before peak detection was performed on the extracted ion chromatogram for each sample. This selection was then refined based on retention time range and intensities below a low threshold. Resultant chromatographic peaks were merged when possessing overlap in a second window per file, and further filtered by thresholding. Finally, calculated adjusted retention times for each spectrum and reported retention times of identified chromatographic peaks were used for alignment of the spectra, and results obtained. In all, 559 features were retained from 35 samples.

For purposes of visualization, non-metric multidimensional scaling (NMDS) analysis was performed using the results of XCMS processing (**Figures 2.10-2.12**). The goal of NMDS was to collapse information from multiple dimensions to fewer dimensions to visually interpret chemical

patterns within the data. While Principal Coordinates Analysis (PCA) assumes linear relationships among variables and multivariate normality, NMDS preserves rank orders, performs well with data that may be on irregular scales, and does not share the same data distortion as PCA with data possessing high beta diversity.^{40, 41} Transformation of the dataset is necessary to give no variable disproportionate weight.⁴¹ Thus, data was transformed using the general log transform formula: $\log_{10}(x+1)$.

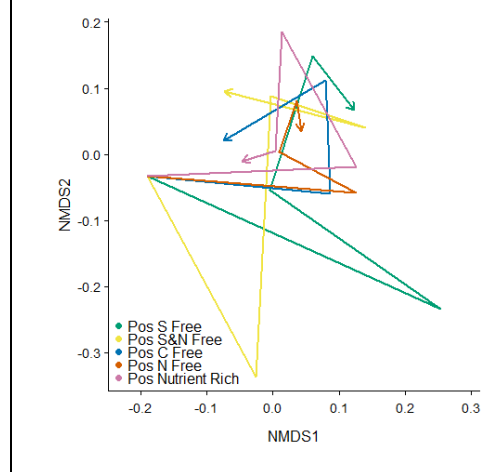
Bray-Curtis dissimilarity was chosen as the dissimilarity measure, with random runs with 999 permutations. In this process, data points were placed at random in ordination space at the start of each permutation, and placement was iteratively refined so that data point distances most closely match order of data point dissimilarities in the original distance matrix. As runs are randomized, the chance of only discovering local minima is reduced.⁴¹ The percentage of elements involved in ties was determined to be zero. Thus, weak ties were not penalized. In general, an NMDS reduced dimensional stress of less than 0.05 provides an excellent representation, less than 0.1 a great representation, less than 0.2 a good representation, and greater than 0.3 a poor representation.⁴¹

Fig. 2.10. NMDS ordination of microcosm data in negative mode with sulfur-free conditions in duplicate (Bottle 1, Bottle 2). Arrows begin at Day 0, and progress to Day 3, Day 7, Day 14, and Day 21. NMDS stress of 0.08 indicates a great representation.



Shifts in the overall chemical composition in the duplicate experiments (Bottle 1, Bottle 2) in sulfur-free conditions analyzed in negative-mode mass spectrometry follow similar patterns, though these patterns are not identical (**Figure 2.10**). At $t = 0$, both bottles have similar chemical composition, which is expected. The trajectory and magnitude of change is similar in the first time step. After the first time step, the overall magnitude and trajectory of change is similar on NMDS axis 1 but different on NMDS axis 2. These axes have not yet been interpreted, but it is possible these axes correspond to changes in microcosm conditions, giving further insight into the microcosm systems. While these results are preliminary, our ultimate goal was to understand how microbial communities drive changes in the target, suspect, and non-target PFAS and non-PFAS composition. In the future, we will examine changes in individual chemical features instead of

Fig 2.11. NMDS ordination of microcosm data in positive mode according to legend in sulfur free, sulfur and nitrogen free, carbon free, nitrogen free, and nutrient-rich conditions, respectively. Arrows begin at Day 0, and progress to Day 3, Day 7, Day 14, and Day 21. NMDS stress of 0.16 indicates a good representation.



examine overall chemical changes.

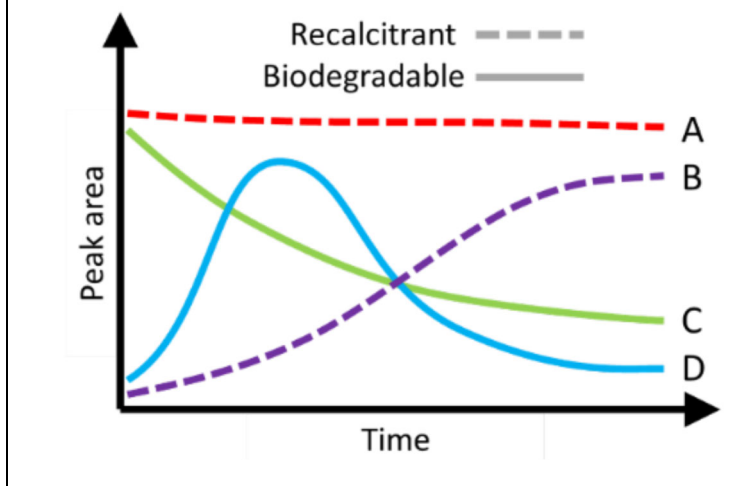
In nutrient samples, changes in the overall non-target chemical composition were similar in terms of both magnitude and direction (**Figure 2.11**). The largest changes in overall composition occurred within the first time step and are indicated by large positive changes in NMDS axis 1 for all bottles and large negative changes in NMDS axis 2 for the sulfur and nitrogen free bottle as well as the sulfur free bottle. During the second time step, all bottles exhibited relatively large positive changes in NMDS axis 2, but smaller compared to the first step. In most cases, the third step reverted back toward previous conditions. That is, the overall chemical composition became more similar to previous time steps. At this point, we have not examined specific chemical features to understand why the chemical composition or later periods are becoming more similar to the original composition.

Non-target chemical feature trend analysis. Our aim was to identify which non-target chemical features were recalcitrant and biodegradable. We considered four types of chemical features based on their expected peak area patterns: 1) recalcitrant parent features: peak areas are

constant throughout the experiment (A in **Figure 2.12**); 2) recalcitrant transformation products: peak areas are lowest initially and increase monotonically throughout the experiment (B in **Figure 2.12**); 3) biodegradable parent features: peak areas are greatest initially and decrease monotonically throughout the experiment (C in **Figure 2.12**); and 4) biodegradable transformation products: peak areas are unimodal and start low, peak in the middle, and end low (D in **Figure 2.12**).

For expected monotonic patterns (i.e., biodegradable parent features or recalcitrant transformation products), we used the Mann-Kendall test (MannKendall) in R, which statistically assess if there is a monotonic upward or downward trend of the variable of interest over time. If the observed monotonic trend is statistically negative or positive ($p < 0.05$), we will conclude that the feature is readily biodegradable or recalcitrant, respectively. For expected unimodal patterns (biodegradable transformation products), we used Hartigan's Dip Test (dip.test) in R, which statistically tests for unimodality. If the observed unimodal trend is statistically significant ($p < 0.05$), we will conclude that the feature is biodegradable. Lastly, for a constant peak area, we used the Augmented Dickey Fuller test (adf.test) in R, which tests for a stationary trend. If a trend with zero slopes (i.e., stationary) is statistically significant, we will conclude that the feature is recalcitrant. The statistical significance of the Mann-Kendall test, Hartigan's Dip Test, or Augmented Dickey Fuller tests will allow us to determine if a chemical feature is biodegradable or recalcitrant. These tests were all programmed in RStudio.

Fig 2.12. Recalcitrant chemical features are expected to remain constant (A) or increase monotonically (B) through time, while biodegradable chemical features are expected to decrease monotonically (C) or be unimodal (D) through time. Peak areas of parent compounds (A, C) are expected to be non-zero/high initially, while peak areas of transformation products (B, D) are expected to be zero/low.



Out of the 559 features generated from the XCMS analysis, only four features produced statistically significant results from the output of the Mann-Kendall test (**Table 2.3**), with three of them proposed to be biodegradable parent features as they trend negatively and one proposed to be a recalcitrant transformation product as it trends positively. The Hartigan's Dip test produced no results with P-values less than 0.05, thus making no features statistically significant under this test. P-values associated with all other trends were > 0.05 . After further examination, we determined that the sample size of our datasets was too small to elicit statistically significant trends. However, in the future, we will expand our analysis by considering trends < 0.1 to help identify possible chemical features that are biodegradable or recalcitrant.

Table 2.3 Statistically significant ($p < 0.05$) non-target chemical features that were identified using the Mann-Kendall test.

Feature ID/Mode	m/z	Retention Time (s)	Direction of change
FT345/Negative	466.3089	1228	Negative

FT494/Positive	685.4342	1166	Negative
FT236/Positive	388.2536	303	Negative
FT236/Positive	630.193	1121	Positive

The motivation for these statistical approaches was to use time series data in attempts to identify characterized PFASs. Time series analyses are useful because we can examine how things are changing. If we can see things that are coming and going (i.e., biodegradable) and couple that with relevant fragments (can we match a particular non-target feature to a likely loss in functional group from the parent), we might be able to identify features that are missing from the mass balance. Also, if there are hundreds of features, it becomes challenging to look at temporal trends manually, but with statistical tools, we can prioritize our analysis by looking at the strongest trends that match our hypothesized predictions (e.g., monotonically decreasing, unimodal, etc.). The ultimate goal was to prioritize features for further analysis in an effort to identify all intermediates, close the mass balance, and to simplify the search for features of interest by 1 orders of magnitude. However, due to the lack of activity for the main components in the microcosms, this part was not further pursued.

Task 3: Determine the efficiency of PFAS removal by MAC

The goal of Task 3 was to determine whether magnetic activated carbon (MAC) can be used to remediate PFAS-laden soils. To provide initial proof-of-concept data, MAC was applied to soils with different properties and organic contents, and with/without a history of PFAS contamination. Table 3.1 summarizes the soil properties. Figures 3.1-3.5 showed that MAC removed PFAS at different extents from the PFAS-spiked and AFFF-impacted soil

(i) Soil properties. The properties of the soils used in Task 3 are characterized and summarized in Table 3.1

Table 3.1 Properties of soils used in Task 3.

Analysis	Environmental C2 sandy soil	AFFF-impacted soil	commercial garden soil (Sunshine clean soil)	PFAS-impacted commercial garden soil
Water content % (w/w)	2.93	5.49	5	6.57
Particle Size distribution	250-500um (70.19%) Sandy	Sand 79 % Silt 13 % Clay 8 % Loamy Sand	Unable to determine due to high organic content	Sand 78 % Silt 14 % Clay 8 % Loamy Sand
pH	4.9	7.2	5.7	6.1
Conductivity (umho/cm)	50	2350	693	579
Nitrate-N (ppm)	3	25	119	134
Phosphorus (ppm)	21	52	9	25

Potassium (ppm)	4	386	22	96
Calcium (ppm)	48	7075	1288	3534
Magnesium (ppm)	12	190	91	241
Sulfur (ppm)	5	48	75	47
Sodium (ppm)	5	115	29	30
Organic matter (%)	0.05	9.94	56.93	7.15

*ppm=mg/kg

(ii) MAC removed PFAS from PFAS-spiked clean soils.

MAC achieved different PFAS removal efficiency from the PFAS-spiked soils, depending on PFAS types and soil properties (**Figures 3.1, 3.2, 3.3**). As shown in Figures 3.1 and 3.2, approximately 80-90% of PFAS (PFOS, or PFOA, or 6:2 FtS) in PFAS-spiked C2 soil and commercial sandy soil were removed. Dramatic PFAS reduction was observed after one month of incubation, with marginal removal of PFAS after three months of incubation, suggesting that new MAC addition might be needed after one month of incubation to remove more PFAS. However, low removal efficiency (0 to 20% PFAS removal) in the high-organic sunshine soils by MAC amendment (Figure 3.3).

(iii) MAC removed PFAS from AFFF-impacted and PFAS-contaminated garden soils.

Figures 3.4 and 3.5 show MAC ability to remove PFAS from PFAS-aged or AFFF-impacted aged soils. The San Antonio Soil samples, obtained from AFFF-contaminated aged soil from an Air Force base, are contaminated with a wide range of PFAS including 6:2 FtS, 8:2 FtS, PFBA, PFBS, PFHxA, PFHxS, PFOA, PFOS, PFDA, PFHpA, PFNA, PFPeA, PFTeDA, PFTrDA, PFUdA (see Appendix for Acronyms). A wide range of PFAS removal, ranging from 0 to 100 percentages, was observed after 3-month of MAC treatment. This could be due to the diverse PFAS types and concentrations in the San Antonio soil samples (where the initial concentrations of PFAS were PFOS > 200 ng/g, 6:2 FtS > 85 ng/g, 8:2 FtS > 120 ng/g, PFHxS > 70 ng/g in average). It was also possible that the applied MAC was quickly reached its adsorption capacity in the first month. Under such scenario, reapplication of MAC or using higher amount of MAC might be able to improve the removal efficiency.

In commercial organic soil, it showed a poor PFAS removal by MAC, leaving most aged PFAS in the soil ranging from 82% to 100% after 3-month MAC exposure (Figure 3.5). This could be due to the affinity of PFAS presenting in the organic matter, making MAC harder to compete with organics for PFAS. However, the better PFAS removal by the Sunshine PFAS-aged soil compared to PFAS-spiked Sunshine soil might be due to the decrease of organic contents in the Sunshine PFAS-aged soil. Good removals of GenX, PFBA, and PFBS at the first month of MAC treatment, but showed a poor removal rate of 6:2 FtS, PFOA, PFOS, and PFDA ranging from 77% to 100% after 3-month treatment. This could be the reason similar to the case in the air force PFAS-contaminated soils, because of the very high PFAS concentration in the soils as well (PFOA > 1100 ng/g, GenX > 600 ng/g, PFBA > 290 ng/g, PFBS > 130 ng/g).

Further LC/MS analysis of PFAS concentrations extracted from MAC could provide more straightforward evidence to show PFAS removal efficiency by MAC from soil and help calculate the overall mass balance.

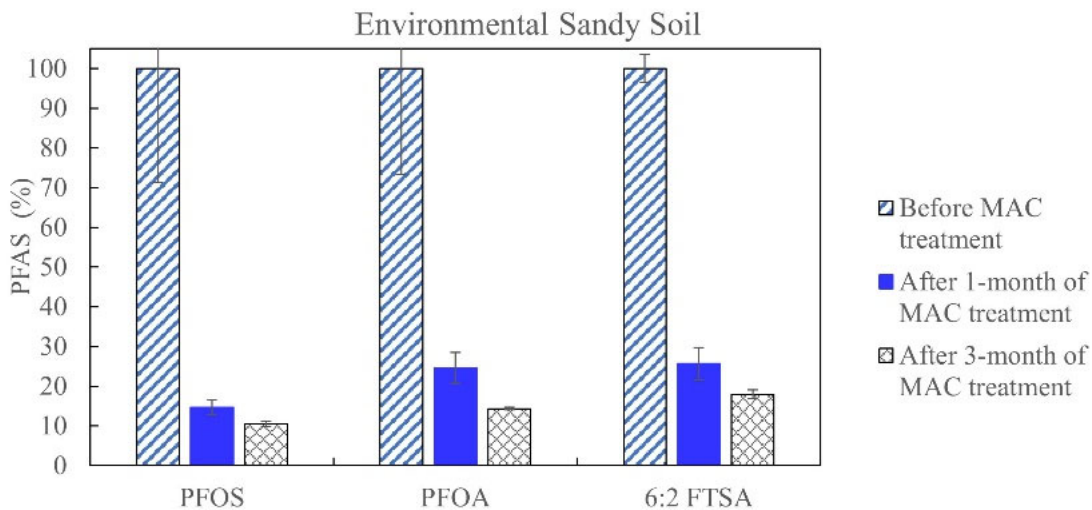


Figure 3.1. Removal of PFAS from PFAS-spiked environmental soil (C2 soil) after 3 months of incubation.

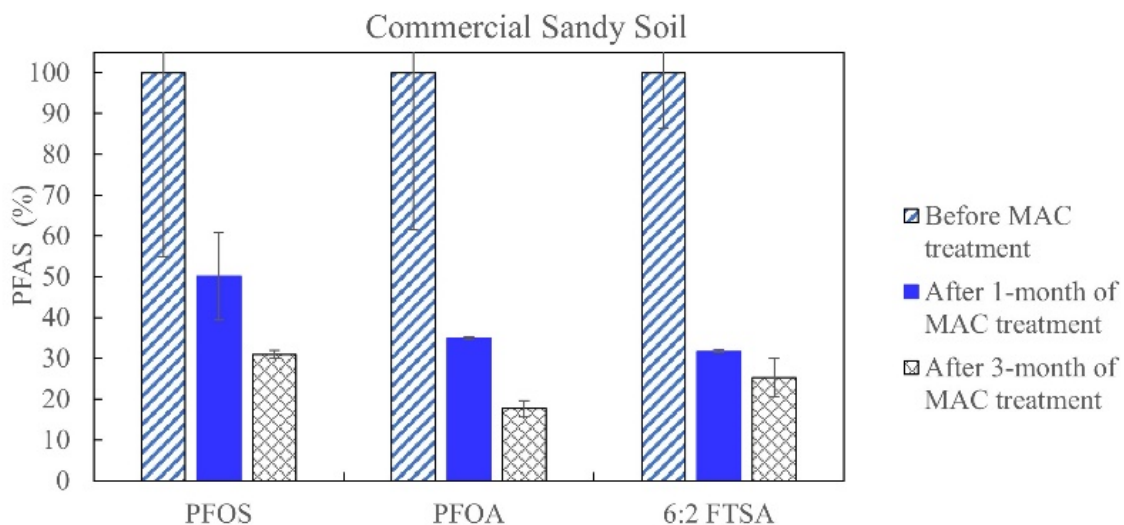


Figure 3.2. Removal of PFAS from PFAS-spiked commercial sandy soil after 3 months of incubation.

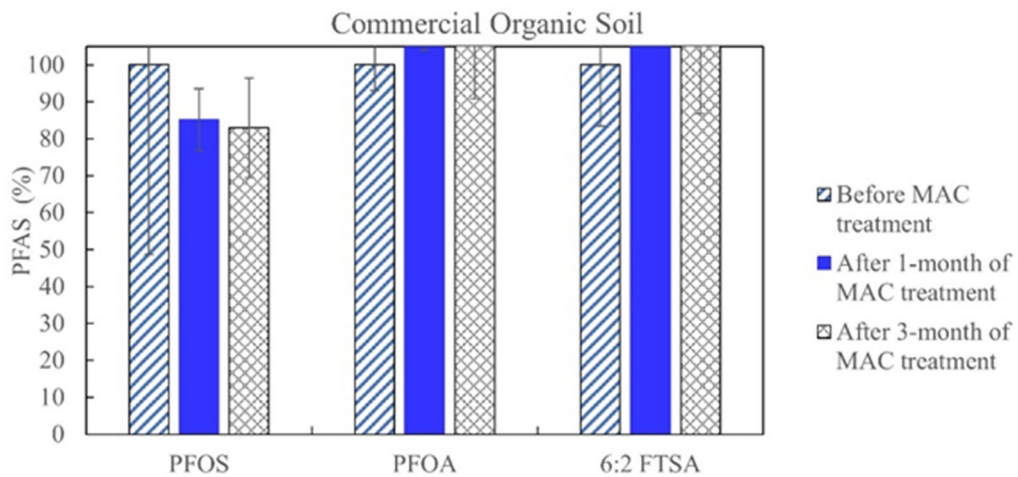


Figure 3.3. Removal of PFAS from PFAS-spiked commercial high-organic sunshine soil after 3 months of incubation.

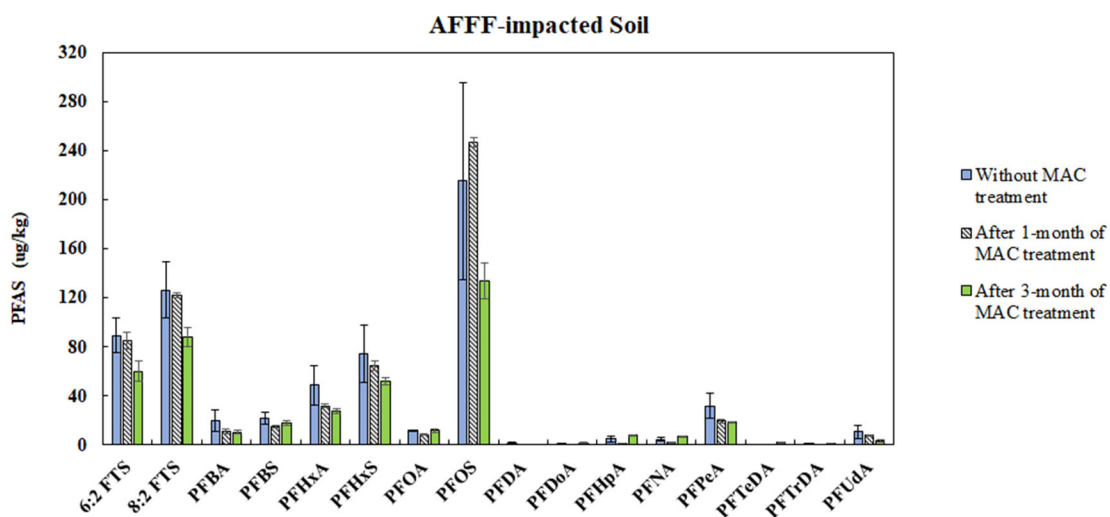


Figure 3.4. Removal of PFAS from AFFF-impacted soil after 3 months of incubation.

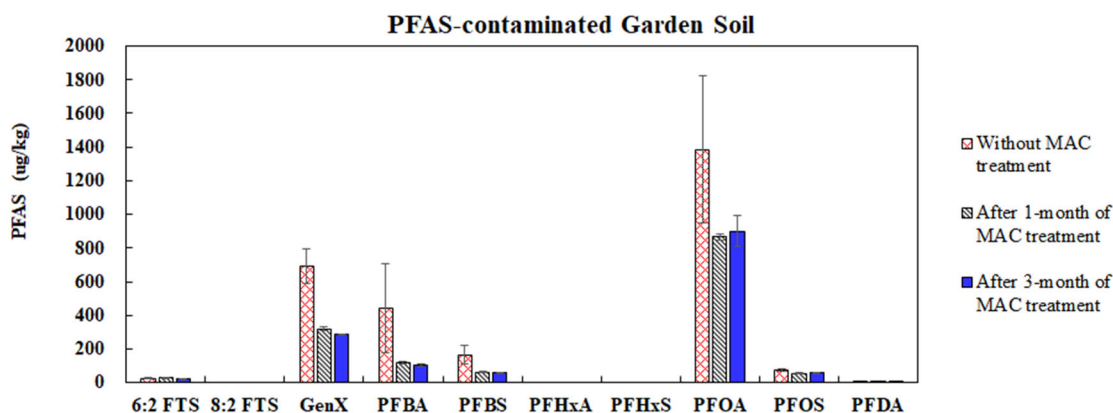


Figure 3.5. Removal of PFAS from PFAS-aged high-organic garden soil after 3 months of incubation.

Task 4: Assess if HALT-PFAS technology can destroy PFAS in spent MAC

The goal of Task 4 was to provide initial proof-of-concept data for the treatment of PFAS-laden MAC using the HALT technology pioneered at the Colorado School of Mines (CSM), leveraging work from Co-PI Strathmann's SERDP Project ER18-1501 (Advancement of Hydrothermal Alkaline Treatment Technology for On-Site Treatment of Per- and Polyfluoroalkyl Substance Wastes (HALT-PFAS)). Task 4 included 2 specific efforts: (A) conducting a fluorine mass balance analysis for destruction and defluorination of PFOS adsorbed on virgin MAC; and (B) evaluating the application of HALT for treatment of MAC recovered from PFAS-contaminated soil generated in T3

(i) Mass balance analysis of PFASs and fluorine during application of HALT-PFAS to MAC.

Results showed that application of HALT (350°C, 1 M NaOH) led to near-complete destruction of PFOS adsorbed onto MAC and stoichiometric generation of fluoride ion without detection of any fluoro-organic intermediates (**Figure 4.1**). In the data shown, the extent of PFOS destruction reached $97.3 \pm 0.2\%$, and the extent of defluorination reached $98 \pm 2\%$. These findings were consistent with what the Co-PI's group has found in tests with GAC, where HALT degrades adsorbed PFOS and releases a stoichiometric quantity of fluoride ion with no detectable organic intermediates.

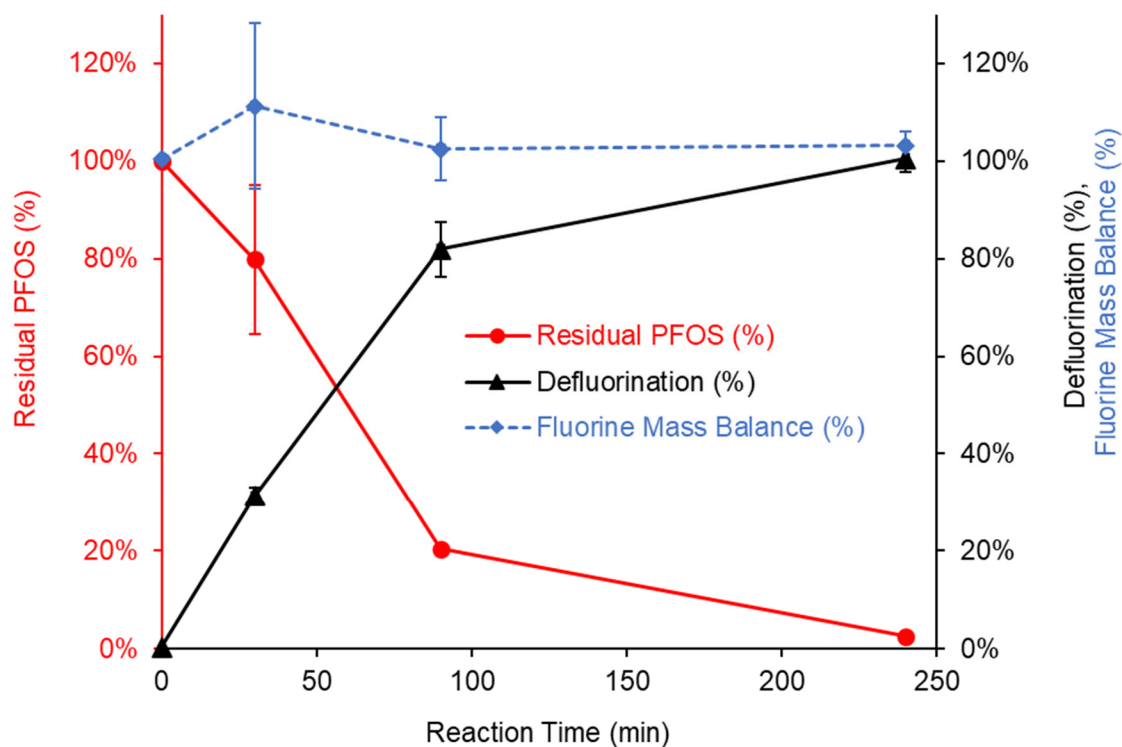


Figure 4.1. Degradation and defluorination of a known mass of PFOS adsorbed on MAC prior to treatment. Reaction conditions: 350°C, 1 M NaOH, 167 g L⁻¹ of virgin MAC initially loaded with 4.61 ± 0.04 mg g⁻¹ PFOS.

Furthermore, rates of MAC-adsorbed PFOS degradation by HALT are similar to those measured for PFOS dissolved in aqueous solution, demonstrating that the alkali reactants can readily access PFOS adsorbed within pores of MAC without significant mass transfer limitations. Based on the kinetics data presented in Figure 4.1, the estimated pseudo-first-order rate constant for PFOS destruction was estimated to be 0.0156 ± 0.0008 min⁻¹. This value compares to 0.015 ± 0.001 min⁻¹ measured in homogeneous aqueous solution and 0.014 ± 0.001 min⁻¹ measured for comparable granular activated carbon (GAC) suspensions at the same HALT reaction conditions (350°C, 1 M NaOH). Thus, rates of PFOS destruction are similar in MAC suspensions as aqueous solutions and GAC suspensions.

Tests also showed that the magnetic properties of MAC were retained after application of HALT reactions for up to 240 min (**Figure 4.2**). Although this was a qualitative test, it suggests that MAC may be re-usable as a magnetic adsorbent material following application of HALT. Future studies are suggested to evaluate repeated re-use of the MAC for adsorption following HALT treatment. Tests with GAC showed no significant change in specific surface area following repeated exposures to HALT reaction conditions.

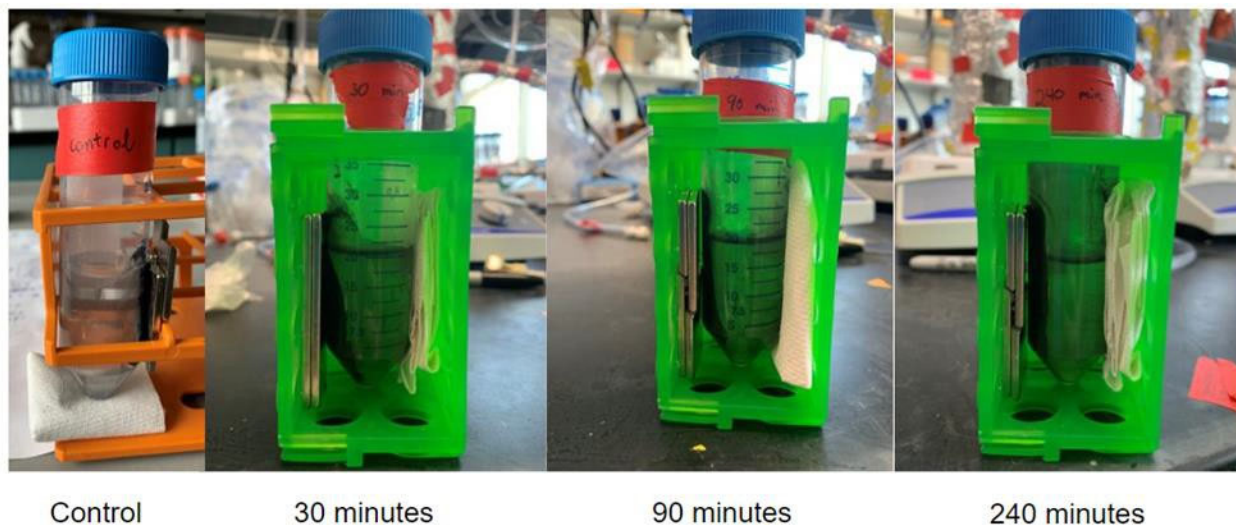


Figure 4.2. Attraction of MAC to a magnet before reaction and following HALT treatment for varying reaction times.

(ii) Evaluation of HALT-PFAS for MAC recovered from PFAS-contaminated soil.

Substantial degradation of PFASs on MAC derived from the AFFF-impacted soil was observed following 240 min reaction time. This data was shown in **Table 4.1** and **Figure 4.3**, which include PFASs with an initial concentration of $0.001 \mu\text{g g}^{-1}$ or greater. Background contamination of PFOA in HALT-treated MAC samples made quantification infeasible for the compound, which was present at comparatively low levels in the untreated MAC.

Compared to the PFOS-loaded samples from the mass balance experiments in Task 4a, removal of PFOS was significantly lower for the AFFF-impacted soil-derived MAC sample (81% as opposed to >97% in the PFOS-loaded MAC) at the same treatment residence time and alkali dose. This could be because of the incomplete separation of MAC from the soil, leading to the presence of soil particles in the reactor, which may contain silica or other base-neutralizing compounds. Previous work by Hao et. al.⁴² demonstrated that silica-rich soils, which can act to partially neutralize the added NaOH base, reduce the net rate of perfluoroalkyl sulfonic acid (PFSA) destruction compared to aqueous reaction systems. Whereas several PFASs were detected at relatively low concentrations, perfluoroalkyl carboxylates (PFCAs) were treated to values below the LOQ or LOD. The only PFCAs with measurable post-treatment sample concentrations (PFOA and PFDA) had similar concentrations to controls with no added PFASs, indicating that detection was due to background contamination of the samples analyzed.

Table 4.1. Concentrations of PFASs measured in untreated and HALT-treated MAC samples incubated with AFFF-impacted San Antonio, TX soil. Reaction conditions: 350°C, 1 M NaOH, 240 min, 67 g L⁻¹ MAC. LOQ = limit of quantification, ND = no detection

Compound	Pre-treatment sample concentration (µg g ⁻¹)	Post-treatment sample concentration (µg g ⁻¹)
PFOS	0.756	0.142
PFHxA	0.160	LOQ
6:2 FtS	0.138	LOQ
8:2 FtS	0.123	ND
PFHxS	0.081	0.009
PFPeA	0.063	LOQ
PFDoS	0.043	0.003
PFOA	0.034	-
PFBS	0.036	LOQ
10:2 FtS	0.031	LOQ
PFBA	0.026	ND
PFDS	0.023	0.002
PFUdA	0.017	ND
PFPeS	0.015	ND
PFHpA	0.010	ND
PFPrS	0.010	ND
PFNA	0.009	ND
FHxSA	0.008	ND
FOSA	0.007	ND
PFNS	0.007	0.001
PFHpS	0.006	0.001
PFTTrDA	0.006	ND
7:3 FTCA	0.005	ND
PFDA	0.005	0.001
MeFOSAA	0.004	ND
PFDoA	0.004	ND
Cl-PFOS	0.003	ND
PFTeDA	0.002	ND
EtFOSAA	0.001	ND

A different MAC sample previously incubated with AFFF-impacted soil was also treated, this time using a greater reaction residence time (350°C, 1 M NaOH, 600 min, 67 g L⁻¹ MAC). Data is shown in **Figure 4.4** alongside removal data from the 240 min reaction (also shown in **Figure 4.3**), demonstrating moderate improvements in destruction for certain compounds when residence time is increased from 240 min to 600 min. Notably, PFOS removal increased to >90% at the longer residence time as compared to 81% at 240 min. Removal of other PFASs, such as PFHxS, PFDS, and PFDoS also improved. The PFCAs shown were either treated to concentrations below the LOQ or LOD, or present at levels similar to a PFAS-free control.

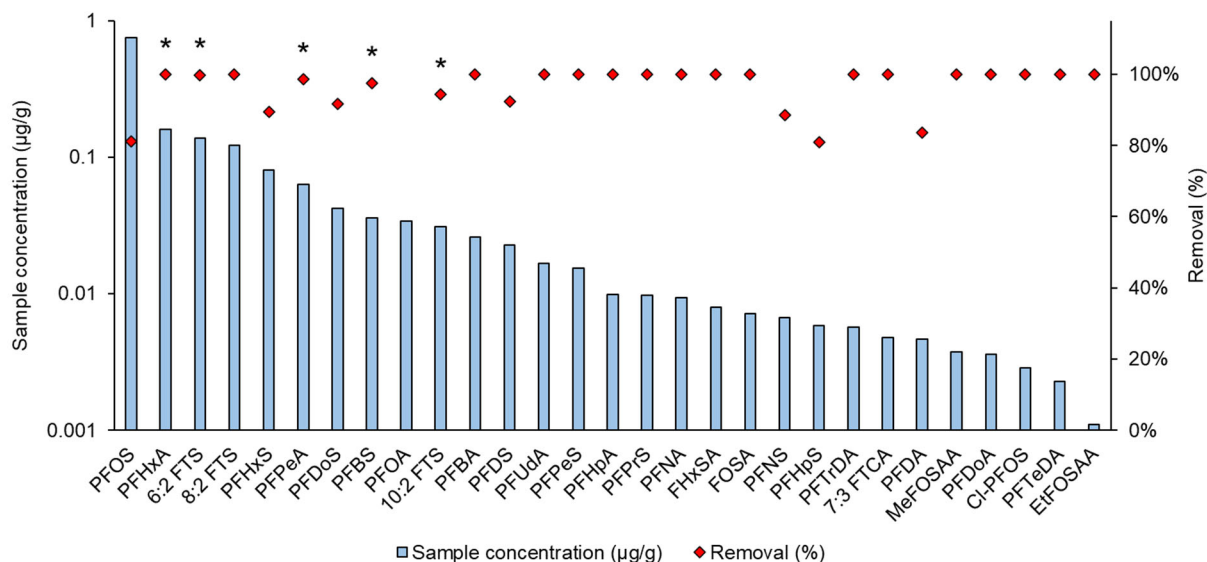


Figure 4.3. Concentrations of PFASs measured in untreated MAC samples incubated with AFFF-impacted San Antonio soil and removal following HALT. Reaction conditions: 350°C, 1 M NaOH, 240 min, 67 g L⁻¹ MAC. A “*” above the removal value indicates that the compound was treated to a value detected below the LOQ. The plotted removal is based on the LOQ, which gives a lower bound for the actual removal value.

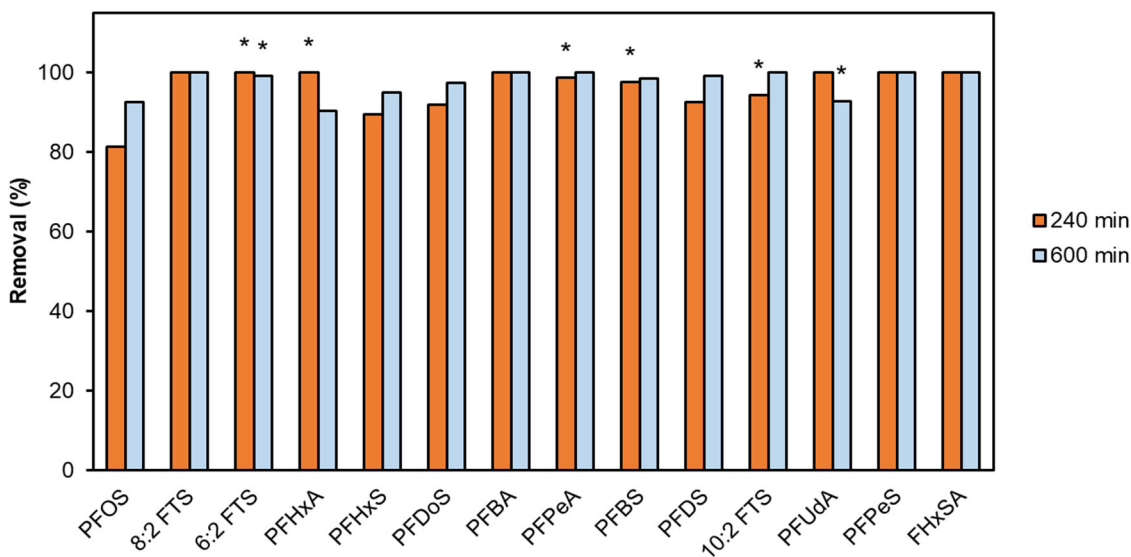


Figure 4.4. Removal of PFASs adsorbed to MAC incubated with AFFF-impacted soil following HALT. Reaction conditions: 350°C, 1 M NaOH, 67 g L⁻¹ MAC, 240 min (orange) and 600 min (light blue). The 240 min reaction is the same as the one for which data plotted in Figure 4.3. A “*” above the removal value indicates that the compound was treated to a value detected below the LOQ. The plotted removal, based on the LOQ, gives a lower bound for the actual removal value.

5. Conclusions and Implications for Future Research

5.1 Summary and Conclusions of Present Study

The findings of this Limited-Scope Project demonstrate the possibility to develop an integrated treatment system for the effective treatment of PFAS-laden soils in source zones based on the “Release-Capture-Destruction” concept. Precursors can be effectively converted to PFAAs, a process that is affected by available of nutrients and co-contaminants, and the PFAAs can be further captured by sorbents like magnetic activate carbon (MAC) and the spent MAC can be separated from the treated soils for further destructive treatment such as HALT-PFAS technology for final disposal. Below is a summary of major conclusions derived from this study:

- Based on non-volatile PFAS by LC-QTOF, three commercial reference materials Capstone 1157, Forafac 1157, and Forafac 1157N at four dilutions (1:1K, 1:1M, 1:10M, and 1:100M) were analyzed and determined to be similar in their PFAS composition.
- Capstone 1157 contains n:2 FtSaB (n= 6, 8, 12), n:2 FtSaAm (n= 6, 8), 6:2 FtS, and 6:2 FTOH.
- Upon analysis via LC-QTOF, it was determined that there was no loss of 6:2 FtSaB or 6:2 FtSaAm onto the HDPE containers from the media.
- Biodegradation of Capstone experiments using a known 6:2 FtS degrader, strain RHA1, showed that 6:2 FtS in Capstone was degraded and two metabolites, 6:2 UFTCA and 5:3FTCA, were detected under only S-limited conditions. However, no significant change of 6:2 FtSaB was observed, which, in part, might be due to the high initial concentration of Capstone was used.
- Based on fluoride release, the defluorination ability of 6:2 FtS by the 1-butanol-grown RHA1 was inhibited by four tested hydrocarbon surfactants (PEG 400, SDS, X-100, X102), but not by hexylene glycol and 0.1% DGBE.
- Environmental background soil (C2 soil) from a former air force base was a good inoculum source to enrich precursor-degrading cultures using a wide range of carbon sources. The diversity and abundance of C2 original soil culture were higher compared to those of the enrichment cultures that received different carbon sources over time. Based on the Shannon index values of enrichment cultures, from low to high, phenol- > ethanol- > CPB- > octane- > propanol- > hexane-> butanol-enriched cultures.
- All the enrichment cultures were able to defluorinate 4:2 FTOH, 6:2 FTOH, and 8:2 FTOH, based on fluoride ion release in the media. 1-Butanol-enriched cultures showed high defluorination capacities as high fluoride concentrations were observed for all 3 different types of fluorotelomer alcohols. The types and levels of metabolites from FTOH biodegradation were different among the enrichment cultures.
- Interestingly, cultures enriched with alcohols (i.e., ethanol, propanol, and butanol) showed a similar chain-length-dependent defluorination toward FTOHs from high to low, 4:2 FTOH > 6:2 FTOH > 8:2 FTOH.
- Enhanced fluoride release and metabolite concentrations of FTOHs biodegradation were observed when dual enrichment cultures were used. The combination of phenol and octane

enrichment cultures produced highest concentrations of 6:2 FTUCA (2120 µg/L) and 6:2 FTCA (1838 µg/L). Interestingly, 5:3 FTCA was found in all conditions where the CPB-enriched culture was used as one of two enrichment cultures (i.e., BuOH+CPB, phenol+CPB, and octane+CPB).

- All the enrichments could use 6:2 FtS as a sole sulfur source, with a high level of defluorination observed in the 1-butanol-enriched culture. A much slower growth was observed when hexane was provided as the carbon source compared to other carbon sources.
- Biodegradation of 6:2 FtSaB was observed by butanol-, phenol- and CPB-enrichment culture only under S-limited conditions over 9 days of incubation. 6:2 UFCA and 5:3 FTCA are two detected metabolites. The different microbial community in the enrichment cultures and the availability of carbon sources also affected the type of metabolites produced during 6:2 FtSaB degradation.
- MAC achieved different PFAS removal efficiency from the PFAS-spiked soils, depending on PFAS types and soil properties. Over 3 months of incubation, approximately 80-90% of PFAS (PFOS, or PFOA, or 6:2 FTS) in PFAS-spiked C2 soil and commercial sandy soil were removed. However, low removal efficiency (0 to 20% PFAS removal) in the high-organic sunshine soils by MAC amendment.
- MAC demonstrated an ability to remove PFAS from aged or AFFF-impacted soils, while the removal efficiency appears to be impacted by the presence of high organic contents and diverse types of PFAS in AFFF-impacted soil.
- Dramatic PFAS reduction was observed after one month of incubation, with marginal removal of PFAS after three months of incubation, suggesting that new MAC addition might be needed after one month of incubation to remove more PFAS.
- Application of HALT (350°C, 1 M NaOH) led to near-complete destruction of PFOS adsorbed onto MAC and stoichiometric generation of fluoride ion without detection of any fluoro-organic intermediates.
- Application of HALT did not negatively affect magnetic properties of the MAC material, and tests in a separate SERDP project with granular activated carbon (GAC) showed no reduction in surface area. Together, these findings suggest that HALT-treated MAC may be reused for further PFAS adsorption and treatment.
- Compared to the PFOS-loaded samples from the mass balance experiments in Task 4a, removal of PFOS was significantly lower for the AFFF-impacted soil-derived MAC sample (81% as opposed to >97% in the PFOS-loaded MAC) at the same treatment residence time and alkali dose. This could be because of the incomplete separation of MAC from the soil, leading to the presence of soil particles in the reactor, which may contain silica or other base-neutralizing compounds.

5.2 Potential Next Steps and Proposed Follow-On Research

The promising findings from this limited scope research project were highly supportive of the proposed “Release-Capture-Destruction” concept as an effective treatment train for PFAS-containing soil that can eliminate long-term PFAS sources due to the presence of precursors in soils. Still, this was a proof-of-concept study with only limited tasks. A number of fundamental and applied research questions need to be answered to achieve the overarching goal of this study, i.e., *to develop an integrated treatment system for the effective treatment of PFAS-laden soils in source zones*, and to transfer and apply this technology to the field. We would like to propose a follow-on SERDP research project to address the following important questions:

- Reduce the NMR detection limits with the goal of monitoring microcosms using NMR (total fluorine and monitor product formation).
- Set up semi-quantitative hydrocarbon surfactant methodology on LC-QTOF
- Determine biodegradation potential of Capstone that is sorbed in soils under different nutrient conditions.
- Determine Capstone degradation potential by dual or multiple enrichment cultures.
- Assess effects of co-contaminants like hydrocarbon surfactants on enrichment cultures with respects to their community structure and their functions focusing on their degradation ability toward precursors.
- Develop strategies to create sulfur-limited conditions to favor degradation of 6:2 FtS, 6:2 FtSaB and 6:2 FtSaAm.
- Isolation of CPB-degrading pure cultures from the CPB-enrichment cultures for defluorination pathway and enzymatic studies.
- Determine the optimal number of MAC applications to achieve desired acceptable treatment points over the shortest treatment time
- Evaluate MAC performance on Capstone.
- Evaluate performance of MAC over repeated adsorption-recovery-HALT treatment cycles to assess reusability of the material.
- Conduct a more in-depth analysis of the effects of HALT treatment on MAC properties, including chemical composition, iron content and mineralogy, specific surface area and porosity, surface functional groups.
- Evaluate fate of soil co-contaminants that co-adsorb on MAC during HALT treatment.
- Evaluate fate of natural soil organic matter that co-adsorbs on MAC during HALT treatment.
- Assess the effects of varying MAC properties on both adsorption and HALT-based destruction.

6. Literature Cited

1. Nickerson, A.; Rodowa, A. E.; Adamson, D. T.; Field, J. A.; Kulkarni, P. R.; Kornuc, J. J.; Higgins, C. P., Spatial Trends of Anionic, Zwitterionic, and Cationic PFASs at an AFFF-Impacted Site. *Environ Sci Technol* **2020**.
2. Adamson, D. T.; Nickerson, A.; Kulkarni, P. R.; Higgins, C. P.; Popovic, J.; Field, J.; Rodowa, A.; Newell, C.; DeBlanc, P.; Kornuc, J. J., Mass-Based, Field-Scale Demonstration of PFAS Retention within AFFF-Associated Source Areas. *Environ Sci Technol* **2020**, *54* (24), 15768-15777.
3. García, R. A.; Chiaia-Hernández, A. C.; Lara-Martin, P. A.; Loos, M.; Hollender, J.; Oetjen, K.; Higgins, C. P.; Field, J. A., Suspect Screening of Hydrocarbon Surfactants in AFFFs and AFFF-Contaminated Groundwater by High-Resolution Mass Spectrometry. *Environ Sci Technol* **2019**, *53* (14), 8068-8077.
4. Moe, M. K.; Huber, S.; Svenson, J.; Hagenaars, A.; Pabon, M.; Trümper, M.; Berger, U.; Knapen, D.; Herzke, D., The structure of the fire fighting foam surfactant Forafac®1157 and its biological and photolytic transformation products. *Chemosphere* **2012**, *89* (7), 869-75.
5. Backe, W. J.; Day, T. C.; Field, J. A., Zwitterionic, cationic, and anionic fluorinated chemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS. *Environ Sci Technol* **2013**, *47* (10), 5226-34.
6. D'Agostino, L. A.; Mabury, S. A., Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates. *Environmental Science & Technology* **2014**, *48* (1), 121-129.
7. Pabon, M.; Corpart, J. M., Fluorinated surfactants: synthesis, properties, effluent treatment. *Journal of Fluorine Chemistry* **2002**, *114* (2), 149-156.
8. Xu, Y.; Jang, J. H.; Gye, M. C., 4-Octylphenol induces developmental abnormalities and interferes the differentiation of neural crest cells in *Xenopus laevis* embryos. *Environ Pollut* **2021**, *274*, 116560.
9. Field, J. A.; Seow, J., Properties, occurrence, and fate of fluorotelomer sulfonates. *Critical Reviews in Environmental Science and Technology* **2017**, *47* (8), 643-691.
10. Barzen-Hanson, K. A.; Field, J. A., Discovery and Implications of C2 and C3 Perfluoroalkyl Sulfonates in Aqueous Film-Forming Foams and Groundwater. *Environmental Science & Technology Letters* **2015**, *2* (4), 95-99.
11. Barzen-Hanson, K. A.; Roberts, S. C.; Choyke, S.; Oetjen, K.; McAlees, A.; Riddell, N.; McCrindle, R.; Ferguson, P. L.; Higgins, C. P.; Field, J. A., Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater. *Environ Sci Technol* **2017**, *51* (4), 2047-2057.
12. Place, B. J.; Field, J. A., Identification of Novel Fluorochemicals in Aqueous Film-Forming Foams Used by the US Military. *Environmental Science & Technology* **2012**, *46* (13), 7120-7127.
13. Schaefer, C. E.; Nguyen, D.; Christie, E.; Shea, S.; Higgins, C. P.; Field, J. A., Desorption of Poly- and Perfluoroalkyl Substances from Soil Historically Impacted with Aqueous Film-Forming Foam. *Journal of Environmental Engineering* **2021**, *147* (2), 06020006.

14. Nickerson, A.; Maizel, A. C.; Kulkarni, P. R.; Adamson, D. T.; Kornuc, J. J.; Higgins, C. P., Enhanced Extraction of AFFF-Associated PFASs from Source Zone Soils. *Environmental Science & Technology* **2020**, *54* (8), 4952-4962.
15. Houtz, E. F.; Higgins, C. P.; Field, J. A.; Sedlak, D. L., Persistence of perfluoroalkyl acid precursors in AFFF-impacted groundwater and soil. *Environ Sci Technol* **2013**, *47* (15), 8187-95.
16. Mejia-Avendaño, S.; Munoz, G.; Sauvé, S.; Liu, J., Assessment of the Influence of Soil Characteristics and Hydrocarbon Fuel Cocontamination on the Solvent Extraction of Perfluoroalkyl and Polyfluoroalkyl Substances. *Analytical Chemistry* **2017**, *89* (4), 2539-2546.
17. Mejia-Avendaño, S.; Vo Duy, S.; Sauvé, S.; Liu, J., Generation of Perfluoroalkyl Acids from Aerobic Biotransformation of Quaternary Ammonium Polyfluoroalkyl Surfactants. *Environ Sci Technol* **2016**, *50* (18), 9923-32.
18. Liu, J.; Zhong, G.; Li, W.; Mejia Avendaño, S., Isomer-specific biotransformation of perfluoroalkyl sulfonamide compounds in aerobic soil. *Sci Total Environ* **2019**, *651* (Pt 1), 766-774.
19. Van Hamme, J. D.; Bottos, E. M.; Bilbey, N. J.; Brewer, S. E., Genomic and proteomic characterization of *Gordonia* sp. NB4-1Y in relation to 6 : 2 fluorotelomer sulfonate biodegradation. *Microbiology (Reading, England)* **2013**, *159* (Pt 8), 1618-28.
20. Zhang, S.; Lu, X.; Wang, N.; Buck, R. C., Biotransformation potential of 6:2 fluorotelomer sulfonate (6:2 FTSA) in aerobic and anaerobic sediment. *Chemosphere* **2016**, *154*, 224-30.
21. Liu, J.; Mejia Avendano, S., Microbial degradation of polyfluoroalkyl chemicals in the environment: a review. *Environment international* **2013**, *61*, 98-114.
22. Harding-Marjanovic, K. C.; Houtz, E. F.; Yi, S.; Field, J. A.; Sedlak, D. L.; Alvarez-Cohen, L., Aerobic Biotransformation of Fluorotelomer Thioether Amido Sulfonate (Lodyne) in AFFF-Amended Microcosms. *Environ Sci Technol* **2015**, *49* (13), 7666-74.
23. Rocha, L. S.; Pereira, D.; Sousa, E.; Otero, M.; Esteves, V. I.; Calisto, V., Recent advances on the development and application of magnetic activated carbon and char for the removal of pharmaceutical compounds from waters: A review. *Sci Total Environ* **2020**, *718*, 137272.
24. Arya, V.; Philip, L., Adsorption of pharmaceuticals in water using Fe₃O₄ coated polymer clay composite. *Microporous and Mesoporous Materials* **2016**, *232*, 273-280.
25. Lompe, K. M.; Vo Duy, S.; Peldszus, S.; Sauve, S.; Barbeau, B., Removal of micropollutants by fresh and colonized magnetic powdered activated carbon. *J Hazard Mater* **2018**, *360*, 349-355.
26. Meng, P.; Fang, X.; Maimaiti, A.; Yu, G.; Deng, S., Efficient removal of perfluorinated compounds from water using a regenerable magnetic activated carbon. *Chemosphere* **2019**, *224*, 187-194.
27. Xu, J.; Liu, Z.; Zhao, D.; Gao, N.; Fu, X., Enhanced adsorption of perfluorooctanoic acid (PFOA) from water by granular activated carbon supported magnetite nanoparticles. *Sci Total Environ* **2020**, *723*, 137757.
28. Wu, B.; Hao, S.; Choi, Y.; Higgins, C. P.; Deeb, R.; Strathmann, T. J., Rapid Destruction and Defluorination of Perfluorooctanesulfonate by Alkaline Hydrothermal Reaction. *Environmental Science & Technology Letters* **2019**, *6* (10), 630-636.

29. Yu, J.; Nickerson, A.; Li, Y.; Fang, Y.; Strathmann, T. J., Fate of per- and polyfluoroalkyl substances (PFAS) during hydrothermal liquefaction of municipal wastewater treatment sludge. *Environmental Science: Water Research & Technology* **2020**, 6 (5), 1388-1399.
30. Zhang, W.; Cao, H.; Mahadevan Subramanya, S.; Savage, P.; Liang, Y., Destruction of Perfluoroalkyl Acids Accumulated in Typha latifolia through Hydrothermal Liquefaction. *ACS Sustainable Chemistry & Engineering* **2020**, 8 (25), 9257-9262.
31. Choi, Y.; Wu, Y.; Sani, B.; Luthy, R. G.; Werner, D.; Kim, E., Performance of retrievable activated carbons to treat sediment contaminated with polycyclic aromatic hydrocarbons. *J Hazard Mater* **2016**, 320, 359-367.
32. Han, Z.; Sani, B.; Mroziak, W.; Obst, M.; Beckingham, B.; Karapanagioti, H. K.; Werner, D., Magnetite impregnation effects on the sorbent properties of activated carbons and biochars. *Water Res* **2015**, 70, 394-403.
33. Xiao, F.; Sasi, P. C.; Yao, B.; Kubátová, A.; Golovko, S. A.; Golovko, M. Y.; Soli, D., Thermal Stability and Decomposition of Perfluoroalkyl Substances on Spent Granular Activated Carbon. *Environmental Science & Technology Letters* **2020**, 7 (5), 343-350.
34. US National Institutes of Standards and Technology Suspect List of Possible Per- and Polyfluoroalkyl Substances (PFAS), <https://data.nist.gov/od/id/mds2-2387>.
35. Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A., NMRPIPE - A MULTIDIMENSIONAL SPECTRAL PROCESSING SYSTEM BASED ON UNIX PIPES. *Journal of Biomolecular Nmr* **1995**, 6 (3), 277-293.
36. Helmus, J. J.; Jaroniec, C. P., Nmrplug: an open source Python package for the analysis of multidimensional NMR data. *Journal of Biomolecular Nmr* **2013**, 55 (4), 355-367.
37. Thorarindottir, A. E.; Gaudette, A. I.; Harris, T. D., Spin-crossover and high-spin iron(II) complexes as chemical shift F-19 magnetic resonance thermometers. *Chemical Science* **2017**, 8 (3), 2448-2456.
38. Zhao, L.; McCausland, P. K.; Folsom, P. W.; Wolstenholme, B. W.; Sun, H.; Wang, N.; Buck, R. C., 6:2 Fluorotelomer alcohol aerobic biotransformation in activated sludge from two domestic wastewater treatment plants. *Chemosphere* **2013**, 92 (4), 464-70.
39. Smith, C. A.; Want, E. J.; O'Maille, G.; Abagyan, R.; Siuzdak, G., XCMS: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching, and Identification. *Analytical Chemistry* **2006**, 78 (3), 779-787.
40. Clarke, K. R.; Ainsworth, M., A method of linking multivariate community structure to environmental variables. *Marine Ecology Progress Series* **1993**, 92 (3), 205-219.
41. McCune, B.; Grace, J. B.; Urban, D. L., Analysis of ecological communities. **2002**. , 28, Gleneden Beach, OR: MjM software design.
42. Hao, S.; Choi, Y. J.; Deeb, R. A.; Strathmann, T. J.; Higgins, C. P., Application of Hydrothermal Alkaline Treatment for Destruction of Per- and Polyfluoroalkyl Substances in Contaminated Groundwater and Soil. *Environmental Science & Technology* **2022**, 56 (10), 6647-6657.